frontiers | Research Topics

What do we know about COVID-19 implications for cardiovascular disease?

Edited by
Hendrik Tevaearai Stahel, Masanori Aikawa, Shuyang Zhang, Mingxing Xie and Shuping Ge

Published in
Frontiers in Cardiovascular Medicine

ISSN 1664-8714
ISBN 978-2-8325-3810-4
DOI 10.3389/978-2-8325-3810-4

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

What do we know about COVID-19 implications for cardiovascular disease?

Topic editors

Hendrik Tevaearai Stahel — University Hospital of Bern, Switzerland
Masanori Aikawa — Brigham and Women's Hospital, Harvard Medical School, United States
Shuyang Zhang — Department of Cardiology, Peking Union Medical College Hospital (CAMS), China
Mingxing Xie — Huazhong University of Science and Technology, China
Shuping Ge — St. Christopher's Hospital for Children, United States

Citation

Stahel, H. T., Aikawa, M., Zhang, S., Xie, M., Ge, S., eds. (2023). *What do we know about COVID-19 implications for cardiovascular disease?* Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3810-4

Table of contents

frontiers in Cardiovascular Medicine

SYSTEMATIC REVIEW
published: 26 April 2021
doi: 10.3389/fcvm.2021.609857

Associations Between the Use of Renin–Angiotensin System Inhibitors and the Risks of Severe COVID-19 and Mortality in COVID-19 Patients With Hypertension: A Meta-Analysis of Observational Studies

Xiao-Ce Dai[1†], Zhuo-Yu An[2†], Zi-Yang Wang[2], Zi-Zhen Wang[3] and Yi-Ren Wang[2]*

[1] Department of Cardiology, Affiliated Hospital of Jiaxing University, Zhejiang, China, [2] Peking University People's Hospital, Beijing, China, [3] China-Japan Friendship Hospital, Beijing, China

OPEN ACCESS

Edited by:
Hendrik Tevaearai Stahel,
Bern University Hospital, Switzerland

Reviewed by:
Michel Burnier,
Centre Hospitalier Universitaire Vaudois (CHUV), Switzerland
Jane A. Leopold,
Brigham and Women's Hospital and Harvard Medical School, United States

***Correspondence:**
Xiao-Ce Dai
ielts_kris@163.com

[†]These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Hypertension, a section of the journal Frontiers in Cardiovascular Medicine

Received: 24 September 2020
Accepted: 22 February 2021
Published: 26 April 2021

Citation:
Dai X-C, An Z-Y, Wang Z-Y, Wang Z-Z and Wang Y-R (2021) Associations Between the Use of Renin–Angiotensin System Inhibitors and the Risks of Severe COVID-19 and Mortality in COVID-19 Patients With Hypertension: A Meta-Analysis of Observational Studies. Front. Cardiovasc. Med. 8:609857. doi: 10.3389/fcvm.2021.609857

Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) share a target receptor with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The use of ACEIs/ARBs may cause angiotensin-converting enzyme 2 receptor upregulation, facilitating the entry of SARS-CoV-2 into host cells. There is concern that the use of ACEIs/ARBs could increase the risks of severe COVID-19 and mortality. The impact of discontinuing these drugs in patients with COVID-19 remains uncertain. We aimed to assess the association between the use of ACEIs/ARBs and the risks of mortality and severe disease in patients with COVID-19. A systematic search was performed in PubMed, EMBASE, Cochrane Library, and MedRxiv.org from December 1, 2019, to June 20, 2020. We also identified additional citations by manually searching the reference lists of eligible articles. Forty-two observational studies including 63,893 participants were included. We found that the use of ACEIs/ARBs was not significantly associated with a reduction in the relative risk of all-cause mortality [odds ratio (OR) = 0.87, 95% confidence interval (95% CI) = 0.75–1.00; $I^2 = 57\%$, $p = 0.05$]. We found no significant reduction in the risk of severe disease in the ACEI subgroup (OR = 0.95, 95% CI = 0.88–1.02, $I^2 = 50\%$, $p = 0.18$), the ARB subgroup (OR = 1.03, 95% CI = 0.94–1.13, $I^2 = 62\%$, $p = 0.48$), or the ACEI/ARB subgroup (OR = 0.83, 95% CI = 0.65–1.08, $I^2 = 67\%$, $p = 0.16$). Moreover, seven studies showed no significant difference in the duration of hospitalization between the two groups (mean difference = 0.33, 95% CI = −1.75 to 2.40, $p = 0.76$). In conclusion, the use of ACEIs/ARBs appears to not have a significant effect on mortality, disease severity, or duration of hospitalization in COVID-19 patients. On the basis of the findings of this meta-analysis, there is no support for the cessation of treatment with ACEIs or ARBs in patients with COVID-19.

Keywords: angiotensin receptor blockers, angiotensin converting enzyme inhibitors, coronavirus disease 2019, hypertension, death

INTRODUCTION

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has initiated a global epidemic. SARS-CoV-2 uses the receptor angiotensin-converting enzyme 2 (ACE2) to gain entry into target cells (1–3). ACE2 is part of the renin–angiotensin system (RAS). Because RAS inhibitors, such as angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), increase the levels of ACE2, the protein that facilitates the entry of SARS-CoV-2 into cells, there are concerns that these drugs could increase the risks of severe COVID-19 and mortality (4). Evidence that ACEIs and ARBs might upregulate ACE2 in several organs, including the lungs and heart (5), supported the hypothesis widely reported by the press that their use might increase susceptibility to infection with SARS-CoV-2 and that their discontinuation might therefore be an appropriate preventive measure (6). Based on these facts and observations, the hypothesis has been developed that their use may affect human susceptibility to infection with SARS-CoV-2.

However, in animal models, ACEIs and ARBs are protective against acute lung injury, and pretreatment with ACEIs or ARBs may reduce the extent of experimentally induced lung injury and improve outcomes, an effect mediated by inhibition of the RAS (7).

Activation of the RAS can cause widespread endothelial dysfunction and varying degrees of injury to multiple organs (heart, kidney, and lung) (8). Thus, researchers have hypothesized that ACEIs/ARBs could theoretically be beneficial and reduce the risk of severe disease in patients with COVID-19.

These possibilities pose a dilemma for cardiologists in terms of whether they should recommend discontinuing treatment with ACEIs/ARBs. Therefore, we performed a large-scale meta-analysis to estimate the associations between ACEIs/ARBs use and the risk of severe COVID-19 and prolonged hospitalization due to COVID-19 (9).

METHODS

Literature Search

The present analysis was conducted in accordance with published PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and MOOSE (Meta-Analysis of Observational Studies in Epidemiology) guidelines (10). The meta-analysis was registered with the International Prospective Register of Systematic Reviews (PROSPERO identifier: CRD42020183921). Electronic searches were conducted in PubMed, EMBASE, Cochrane Library, and MedRxiv.org from December 1, 2019, to June 20, 2020. As of the date the searches were performed, no randomized controlled clinical trials had been published; therefore, only observational studies were included. We also identified additional citations by manually searching the reference lists of eligible studies.

We used the following medical subject headings and keywords to search for articles related to COVID-19: COVID-19, severe acute respiratory syndrome coronavirus 2, 2019-nCoV, and SARS-CoV-2; and the following search terms related to ACEIs/ARBs: renin–angiotensin system, angiotensin-converting enzyme inhibitor, and angiotensin II receptor blockers (**Supplementary Table 1**).

Eligibility Criteria

Two of the authors (ZA and ZW) independently analyzed the titles and abstracts of all articles retrieved from these searches to ascertain whether they met the inclusion criteria. We assessed the full texts of the initially eligible articles based on the PICOTS (Population, Intervention, Comparator, Outcome, Timing and Setting) framework, and articles were selected according to the following criteria: (1) articles reporting observational studies, including cohort studies and case-control studies; (2) articles that analyzed the effects of ACEIs/ARBs on COVID-19 in adult patients with hypertension; (3) articles that contained data on mortality, disease severity, and hospitalization durations in COVID-19 patients; and (4) articles that enrolled at least 50 patients (**Figure 1**).

Data Extraction

Two investigators (ZA and ZW) independently extracted the relevant data with a predetermined data collection table. Any discrepancies were settled by consensus or consultation with a third investigator (DXC). All the included data were aggregate data, and no patient-level data were available.

Quality Assessment

Study quality was assessed with the Newcastle–Ottawa Scale (NOS, maximum 9 points), which rates studies based on three parameters: the selection of groups, the comparability, and the ascertainment of outcome and exposure. The NOS can be used to evaluate the overall risk of bias in non-randomized studies.

Outcomes of Interest

Data for all-cause mortality, severity, and hospitalization duration in COVID patients were collected. Severe cases of COVID-19 were generally characterized by dyspnea, a respiratory rate >30 breaths/min, a blood oxygen saturation level $<93\%$ on room air, a PaO_2/FIO_2 ratio <300, and/or infiltration of $>50\%$ of the lung within 24–48 h, or according to the criteria defined in each included study (11).

Statistical Analysis

The adjusted odds ratios (ORs) and hazard ratios for all-cause mortality, severe disease, and prolonged hospitalization duration in COVID patients were reported in these studies. Both adjusted and unadjusted ORs were initially considered in the analysis. We pooled the adjusted ORs, which were derived from multivariate analyses. We used the I^2 statistic to assess the heterogeneity of the summary estimates, and a value $>50\%$ was considered evidence of significant heterogeneity (12). A random-effects model was used because the I^2 statistic was $>50\%$. To assess publication bias, we constructed a funnel plot and adopted the Begg rank correlation method ($p < 0.05$ indicated significant bias). We used Stata version 14.0 (Stata Corp., College Station, TX, USA) for all calculations. We used RevMan 5.3 (Nordic

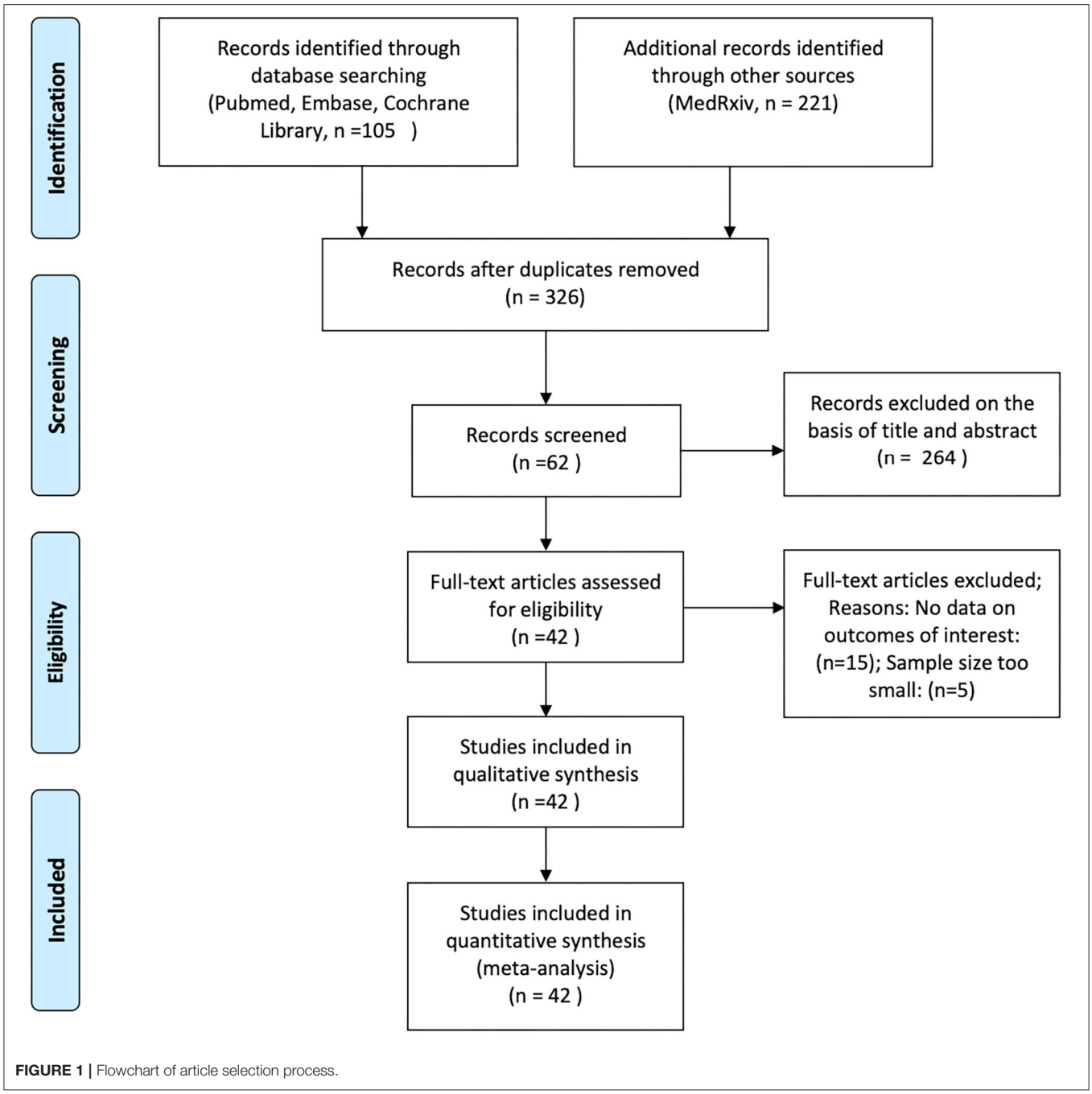


FIGURE 1 | Flowchart of article selection process.

Cochrane Centre, Cochrane Collaboration) to generate forest plots to show the results for the individual studies and the pooled analysis.

RESULTS

Characteristics and Quality of Included Studies

Among the 42 studies included, 14 were performed in Europe, 7 in the United States, 4 in Korea, 1 in Iran, and 16 in China. All studies were published within the past 6 months, and all were observational studies. We confirmed that all observational studies had adequate inclusion and exclusion criteria and an appropriate justification for the selection of the cohort. We collected and sorted the data on intervention measures and examination results obtained from the electronic medical records in all studies.

We summarized the baseline characteristics in each study in **Table 1** (8, 13–43, 45–48, 50, 52–56). The identified studies included 63,893 patients with COVID-19. Of these, 20,686 were taking ACEIs/ARBs. Thirty-five studies adjusted their analyses

TABLE 1 | Baseline characteristics of patients assessed in the studies included in the meta-analysis.

Study authors, year of publication, location	Total					ACEI/	Characteristics of controls	Confounding factors adjusted for in the analyses	NOS score (max = 9)
	Number	Deaths, *n* (%)	Age, mean ± SD or (range)	Gender (male, %)	Comorbidities, *n* (%)	ARB, *n*			
Andrea et al. (13), Italy	191	42 (28)	NA	68.6	CAD: 14.7% Heart Failure: 4.7% DM: 14.7% COPD: 5.2% CKD: 26.2%	69	Patients with hypertension and COVID-19 that were taking other anti-hypertension drugs.	Age, Heart failure, CKD.	7
Ashraf et al. (14), Iran	100	12	58 (48–68)	64%	DM: 26% CAD: 19%	19	Patients with hypertension and COVID-19 that were taking other anti-hypertension drugs.	The analyses were not adjusted for multiple comparisons.	6
Baker et al. (15), UK	316	81	75 (60–83)	55%	DM: 27% CAD: 21% CKD: 24%	311	Patients with hypertension and COVID-19 that were taking other anti-hypertension drugs.	The analyses were not adjusted for multiple comparisons.	6
Bean et al. (16), UK	205	53 (25.9%)	63 ± 20	52%	DM: 62 (30.2%) CVD: 30 (14.6%)	46	Patients with hypertension and COVID-19 that were taking other anti-hypertension drugs.	Age, gender, comorbidities (hypertension, DM, IHD, and heart failure)	7
Benelli et al. (17), Italy	411	72	66.8 ± 16.4	87%	DM: 16% CAD: 23%	135	Patients with hypertension and COVID-19 that were taking other anti-hypertension drugs.	Bonferroni correction was used to adjust for multiple testing.	8
Bravi et al. (18), Italy	543	129 (very severe/lethal)	NA	NA	NA	450	Patients with hypertension and COVID-19 that were taking other anti-hypertension drugs.	All estimates have been adjusted for age, gender, diabetes, major cardiovascular diseases, COPD, cancer. and renal diseases.	8
Chen et al. (19), China	123	31	57.7 ± 12.7	43%	DM: 11% CAD: (12%)	11	Patients with hypertension and COVID-19 that were taking other anti-hypertension drugs.	The analyses were not adjusted for multiple comparisons.	7
Choi et al. (20), Korea	1,585	192	66.5 ± 14	42.80%	DM: 44.9% Chronic lung diseases: 19.5%	892	Propensity score-matched hospitalized patients with COVID-19 that were taking other anti-hypertension drugs.	Adjusted for age, sex, region of hospitals, comorbidities (diabetes, chronic lung disease, and major neurologic diseases), Charlson comorbidity index, and treatment modalities.	7

(Continued)

TABLE 1 | Continued

Study authors, year of publication, location	Total					ACEI/ ARB, *n*	Characteristics of controls	Confounding factors adjusted for in the analyses	NOS score (max = 9)
	Number	Deaths, *n* (%)	Age, mean ± SD or (range)	Gender (male, %)	Comorbidities, *n* (%)				
Dauchet et al. (21), France	288	NA	NA	62%	DM: 40 (13.89%) CVD: 48 (16.67%) Pulmonary disease: 31 (10.76%) CKD: 9 (3.13%)	62	NA	Age, gender, weight, comorbidities (DM, pulmonary disease, kidney diseases, CVD)	7
De Spiegeleer et al. (22), Belgium	154	NA	86 ± 7	33%	DM: 18%	30	Residents at two elderly care homes with COVID-19 that were taking other anti-hypertension drugs.	Age, sex, functional status, diabetes mellitus, hypertension	7
Felice et al. (23), Italy	133	33	72.8 ± 12.3	64.70%	DM: 25.6% CAD: 42.1% COPD: 10.5%	82	Hospitalized patients with COVID-19 that were taking other anti-hypertension drugs.	Adjusted for age, gender, body mass index, days with symptoms prior to admission, previous cardiovascular events, diabetes, and cancer.	9
Feng et al. (24), China	476	38	53.0 (40.0–64.0)	56.90%	DM: 49 (10.29%) CVD: 38 (7.98%) Pulmonary disease: 22 (4.62%) CKD: 4 (0.84%)	35	Patients with hypertension hospitalized with COVID-19 that were taking other anti-hypertension drugs matched to the experimental group according to disease severity.	Age, sex, smoking, alcohol consumption	7
Fosbøl et al. (25), Denmark	4,480	478	54.7 (40.9–72.0)	47.90%	DM: 411 Heart Failure: 243 COPD: 634 CKD: 172	895	Hospitalized patients with COVID-19 with hypertension that were taking other anti-hypertension drugs.	Fully adjusted model includes the following covariates: age; sex; highest obtained educational level; medical history of myocardial infarction, heart failure, kidney disease, stroke, peripheral artery disease, atrial fibrillation, diabetes, chronic obstructive pulmonary disease, and malignancy; and use of the following concomitant medications: other antihypertensive drugs, lipid-lowering drugs, and anticoagulation.	8

(Continued)

TABLE 1 | Continued

Study authors, year of publication, location	Total					ACEI/	Characteristics of controls	Confounding factors adjusted for in the analyses	NOS score (max = 9)
	Number	Deaths, *n* (%)	Age, mean ± SD or (range)	Gender (male, %)	Comorbidities, *n* (%)	ARB, *n*			
Gao et al. (26), China	850	34	64.24 (11.2)	52.10%	DM: 26.8% CAD: 16.7%	183	Hospitalized patients with COVID-19 with hypertension that were taking other anti-hypertension drugs.	Adjusted for age, sex, medical history of diabetes, insulin-treated diabetes, myocardial infarction, underwent PCI/CABG, renal failure, stroke, heart failure, and COPD.	9
Giorgi et al. (27), Italy	2,653	217	63.2	50%	DM: 12% CAD: 7%	818	Symptomatic patients with COVID-19 that were taking other anti-hypertension drugs.	Adjused for age and comorbidities.	9
Guo et al. (28), China	187	43	58.5 ± 14.6	49%	DM: 15% CAD: 11.2%	19 (10%)	Patients with COVID-19 symptoms that required hospitalization that were taking other anti-hypertension drugs.	NA	8
Huang et al. (29), China	50	2	61.7 ± 12.9	54%	DM: 8% CAD: 2%	20	Patients with COVID-19 with hypertension that were taking other anti-hypertension drugs.	Unadjusted comparisons	8
Ip et al. (30). USA	1,129	399	NA	NA	NA	460	Patients with COVID-19 with hypertension that were taking other anti-hypertension drugs.	Adjusted for age, the effect of hypertension on mortality was greatly diminished, with a reduction in odds-ratio by over half; and completely disappeared when adjusted for other major covariates.	7
Jung et al. (31), Korea	5,179	84	44.6 ± 18	44%	DM: 17% CAD: 1% CKD: 5%	762	Patients with COVID-19 with hypertension that were taking other anti-hypertension drugs.	Adjusted for age, sex, Charlson Comorbidity Index, immunosuppression, and hospital type.	7
Jurado et al. (32), Spain	290	NA	NA	NA	NA	190	Patients with COVID-19 with hypertension that were not exposed to ACEI or ARB.	NA	7
Khera et al. (33), USA	10,196	1,128	NA	54%	DM: 48% CAD: 5% CKD: 27%	6,040	Patients with COVID-19 with hypertension that were not exposed to ACEI or ARB. Pairwise comparisons from propensity score matched cohorts. In hospital patient and outpatient were compared.	NA	7

(Continued)

TABLE 1 | Continued

Study authors, year of publication, location	Total					ACEI/	Characteristics of controls	Confounding factors adjusted for in the analyses	NOS score (max = 9)
	Number	Deaths, *n* (%)	Age, mean ± SD or (range)	Gender (male, %)	Comorbidities, *n* (%)	ARB, *n*			
Kim et al. (34), USA	2,491	420	62 (50–75)	53%	DM: 33% CAD: 14% CKD: 16%	573	Patients with COVID-19 with hypertension that were not exposed to ACEI or ARB	Adjusting for age group, sex, and race/ethnicity and underlying conditions.	8
Lee et al. (35), Korea	8,266	112	44.4 ± 19.1	38%	DM: 17% CAD: 6%	977	Hospitalized patients with COVID-19 with hypertension that were not exposed to ACEI or ARB	Adjusted for age, sex, the history of comorbidities (hypertension, diabetes mellitus, cancer, COPD, stroke, coronary artery disease, heart failure, and chronic kidney disease) before diagnosis of SARS-CoV-2.	7
Li et al. (36), China	362	77	66.0 (59.0–73.0)	52.20%	DM: 127 (35.1%) CVD: 62 (17.13%) CKD: 35 (9.67%)	118	Patients with hypertension hospitalized with COVID-19 that were taking other anti-hypertension drugs.	Age, gender, comorbidities (DM, cerebrovascular disease, coronary heart disease, digestive disorders, respiratory disease, neurological disease, solid tumor, CKD)	6
Liabeuf et al. (37), France	268	63	73 (61–84)	58%	DM: 21% CAD: 61% COPD: 10% CKD:7% Restrictive lung disease: 6%	96	Hospitalized patients with COVID-19 with hypertension that were not exposed to ACEI or ARB	Adjustment for age, sex, coronary heart disease, BMI.	8
Liu et al. (38), China	78	NA	65.2 ± 10.7	55%	NA	12	Patients with COVID-19 with hypertension that were not exposed to ACEI or ARB.	Adjustment was by multivariable logistic regression modeling with sex variable	7
Mancia et al. (39), Italy	6,272	NA	68 ± 13	63%	CVD: 1,891 (30.1%) CKD: 651 (10.4%)	2,896	30,759 beneficiaries of the Regional Health Service, matched to the experimental group according to sex, age, and municipality of residence	Drugs (antihypertensive drugs, oral antidiabetic drugs), comorbidities (CVD, respiratory disease, kidney disease, cancer), and chronic related conditions	7
Mehta et al. (40), USA	1,735	NA	NA	57%	DM: 46% CAD: 22%	214	Patients with COVID-19 with hypertension that were not exposed to ACEI or ARB.	Unadjusted comparisons	7

(Continued)

TABLE 1 | Continued

Study authors, year of publication, location	Total					ACEI/	Characteristics of controls	Confounding factors adjusted for in the analyses	NOS score (max = 9)
	Number	Deaths, *n* (%)	Age, mean ± SD or (range)	Gender (male, %)	Comorbidities, *n* (%)	ARB, *n*			
Meng et al. (41), China	417	NA	64.50 (55.80–69.00)	57.10%	DM: 5 (11.9%) CVD: 8 (19.0%) Pulmonary disease: 225 (8.5%)	17	Patients with COVID-19 that had hypertension comorbidity, based on treatment, but were taking non-ACEI/ARB anti-hypertension drugs.	Age, sex, symptoms, and signs	8
Peng et al. (42), China	112	17	62 (55–67)	47%	DM: 20% CAD: (55%)	22	Patients with COVID-19 symptoms that required hospitalization that have hypertension taking other anti-hypertension drugs.	NA	6
Rentsch et al. (43), USA	585	17	66.1 (60.4–71)	52%	DM: 30% CAD: 15%	263	Patients with symptoms that required hospitalization with COVID-19 that were taking other anti-hypertension drugs	Age, sex, race/ethnicity, residence type	7
Reynolds et al. (44), USA	5894	NA	NA	NA	NA	1,692	Patients with COVID-19 that had hypertension comorbidity, based on treatment, but were taking non-ACEI/ARB anti-hypertension drugs	Age, sex, race, ethnic group, BMI, smoking history, history of hypertension, myocardial infarction, heart failure, DM, CKD, obstructive lung disease, and other classes of medication	9
Rhee et al. (45), Korea	832	34	NA	53%	DM: 100% CAD: 27% CKD: 19%	327	Patients with COVID-19 that were taking non-ACEI/ARB anti-hypertension drugs	Adjustment for age, sex, comorbidity, and medication	8
Richardson et al. (46), USA	1,366	NA	63 (52–75)	60%	NA	456	Patients with COVID-19 with hypertension that were taking non-ACEI/ARB anti-hypertension drugs	Unadjusted comparisons	7
Tan et al. (8), China	100	11	NA	51%	DM: 28% CAD:(18%) CKD: (9%)	31	Patients with COVID-19 with hypertension that were taking non-ACEI/ARB anti-hypertension drugs.	Unadjusted comparisons	7
Tedeschi et al. (47), Italy	311	131	76 (67–83)	72%	CVD: 131 (42%) DM: 74 (24%) COPD: 49 (16%)	175	Patients with COVID-19 with hypertension that were taking non-ACEI/ARB anti-hypertension drugs.	Adjusted for age, gender, presence of CV comorbidities and COPD	8

(Continued)

TABLE 1 | Continued

Study authors, year of publication, location	Total					ACEI/ ARB, *n*	Characteristics of controls	Confounding factors adjusted for in the analyses	NOS score (max = 9)
	Number	Deaths, *n* (%)	Age, mean ± SD or (range)	Gender (male, %)	Comorbidities, *n* (%)				
Yan et al. (48), China	610	4	48.75 (±14.19)	51.10%	DM: 9.84% CVD: 2.62%	58	48,667 population-based controls from Zheijang, China with COVID-19 and hypertension that were taking non-ACEI/ARB anti-hypertension drugs.	Age, sex, BMI, and relevant comorbidities	8
Yang et al. (49), China	251	21	66.0 (60.0–73.0)	49%	DM: 55 (21.91%) CVD: 35 (13.94%) Pulmonary disease: 12 (4.78%) CKD: 4 (1.59%)	43	Patients with COVID-19 with hypertension that were taking non-ACEI/ARB anti-hypertension drugs.	Age, sex, BMI, complications (DM, pulmonary disease, hepatic disease, cardiopathy, neurological disease, immune diseases), other treatments (glucocorticoid, antiviral, antibiotic, immunoglobulin), and symptoms	8
Zeng et al. (50), China	274	21	NA	55%	DM: 42 (15%) CVD: 31 (11%)	28	Patients with COVID-19 with hypertension that were taking non-ACEI/ARB anti-hypertension drugs.	Age, sex, weight, BMI, comorbidities (obstructive pulmonary disease, CKD, CVD, DM, cerebrovascular disease, chronic liver disease, cancer), signs, and symptoms	7
Zhang et al. (51), China	522	NA	64 (56–69)	55.75%	DM: 126 (11.83%) CVD: 70 (13.41%) Pulmonary disease: 2 (0.38%) CKD: 18 (3.45%)	174	Patients with COVID-19 with hypertension that were taking non-ACEI/ARB anti-hypertension drugs.	Adjusted for age, gender, comorbidities (DM, coronary heart disease, cerebrovascular disease, and CKD), medication (antiviral drug and lipid lowering drug), symptoms, and signs.	9
Zhou et al. (52), China	36	7 (19.4%)	64.8 ± 10.1	53%	DM: 9 (25.0%) CAD:7 (19.4%)	15	Patients with COVID-19 with hypertension that were n taking non-ACEI/ARB anti-hypertension drugs.	age, sex, hospitalization time, time from onset to hospital admission	8
Zhou et al. (53), China	3,572	NA	66 (58–72)	51.10%	NA	989	Hospitalized patients with COVID-19 that were taking non-ACEI/ARB anti-hypertension drugs.	Adjustment for age, gender, disease severity, comorbidities, and CCB medication	7

BMI, body mass index; CKD, chronic kidney disease; CVD, cardiovascular disease; DM, diabetes mellitus; IHD, ischaemic heart disease; NOS, Newcastle-Ottawa Scale.

for comorbidities (including diabetes mellitus, cardiovascular disease, and chronic kidney disease). Seven studies did not describe the controls; however, the remaining studies described the controls as patients who had COVID-19 but had not been exposed to ACEIs/ARBs. Seven studies compared the hospitalization durations (days) between the ACEI/ARB and non-ACEI/ARB groups. Twenty-nine studies (69.04%) described mortality in the study populations.

We carefully evaluated the quality of each study with the NOS. Thirty-eight studies (90.5%) had 7 points or more, and the remaining four studies had 6 points. **Supplementary Table 2** shows the NOS scores of the included studies.

Outcome Measures

Effects of ACEIs/ARBs on All-Cause Mortality in Patients With COVID-19

Twenty-nine studies discussed the relationship between the use of ACEIs/ARBs and all-cause mortality in patients with COVID-19 (**Figure 2**). The use of ACEIs/ARBs was not significantly associated with a reduction in the relative risk of all-cause mortality [OR = 0.87, 95% confidence interval (95% CI) = 0.75–1.00; I^2 = 57%, p = 0.05]. The control groups generally included patients with COVID-19 who had taken other antihypertensive treatments. Most studies calculated the OR after adjusting for age, sex, and other factors to reduce the influence of confounding factors. To determine whether there was a difference between data from articles published in peer-reviewed journals and those posted on preprint servers, a subgroup analysis was conducted. There was no significant difference in the results for all-cause mortality between the two subgroups.

Effects of ACEIs and ARBs on the Severity of COVID-19

Twenty-five retrospective studies evaluated the effects of ACEIs and ARBs on the severity of COVID-19 (**Figure 3**). We found no significant reduction in disease severity in the ACEI subgroup (OR = 0.95, 95% CI = 0.88–1.02, I^2 = 50%, p = 0.18), in the ARB subgroup (OR = 1.03, 95% CI = 0.94–1.13, I^2 = 62%, p = 0.48), or in the ACEI/ARB subgroup (OR = 0.83, 95% CI = 0.65–1.08, I^2 = 67%, p = 0.16). Our meta-analysis demonstrated that there was no significant reduction in disease severity in patients taking ACEIs/ARBs (OR = 0.97, 95% CI = 0.92–1.03, I^2 = 58%, p = 0.38). These findings indicate that ACEIs and ARBs might not have either a protective or adverse effect on disease severity.

Effect of ACEIs/ARBs on the Duration of Hospitalization for COVID-19 Treatment

Seven included studies discussed the effects of ACEIs/ARBs on the duration of hospitalization required for the treatment of COVID-19. A meta-analysis of these studies showed that ACEIs/ARBs had no obvious effect on hospitalization duration (mean difference = 0.33, 95% CI = −1.75 to 2.40, p = 0.76). Because of the obvious limitation of the small number of included studies, we described these results qualitatively. In general, ACEIs/ARBs did not significantly shorten or prolong the hospitalization duration for patients with COVID-19 (**Figure 4**).

Sensitivity Analysis and Publication Bias

We performed a sensitivity analysis on the overall meta-analysis results. We performed sensitivity analyses for the effects of ACEIs/ARBs on the risks of mortality and severe disease by sequentially omitting one study at a time and investigating any changes in the findings. The results for all-cause mortality did not change significantly after excluding studies with low NOS scores, such as Li et al. (36). This finding indicated that the results were robust and reliable. Similarly, the pooled ORs for severe disease did not significantly change when we omitted studies one at a time.

We also evaluated publication bias with funnel plots. A visual inspection did not reveal any clear asymmetry (**Supplementary Figures 1–3**). Therefore, no significant publication bias was found among the included observational studies.

DISCUSSION

In the present meta-analysis, we found no significant association between the use of ACEIs/ARBs and the risks of mortality and severe disease in patients with COVID-19 after adjusting for baseline demographics and comorbidities (16, 36, 41, 44, 49, 57–59).

The concerns about the use of ACEIs or ARBs in patients with COVID-19 have mainly stemmed from arguments based on biologic plausibility, particularly the observation that ACEIs and ARBs have the potential to upregulate ACE2 receptors (which seem to be the mediators of the entry of SARS-CoV-2 into host cells) (60). However, it is also biologically plausible that ARBs may have beneficial effects in patients with COVID-19, although the findings have not been consistent across animal and human models (7). Therefore, ACE2 may act as a double-edged sword, depending on the phase of the disease. On the one hand, increased baseline ACE2 expression could potentially increase susceptibility of infection, making ACEI/ARB use a modifiable risk factor. On the other hand, once infected, the downregulation of ACE2 may be a hallmark of COVID-19 progression. Consequently, upregulation by preferentially blocking the RAS and replacing ACE2 in the acute respiratory syndrome phase may be beneficial. Our analysis supports that in the context of the current COVID-19 epidemic, the use of ACEIs/ARBs should not be restricted.

Several researchers found that the use of ACEIs/ARBs could worsen the prognosis of COVID-19 among patients with hypertension by promoting the expression of ACE2 (2–4, 61). These observational studies accounted for confounding factors, which is important because the factors that might indicate treatment with ACEIs or ARBs, such as comorbid cardiovascular conditions or diabetes, might also contribute to the development of severe COVID-19. We suspect that most of the patients taking RAS inhibitors had multiple comorbidities and cardiovascular risk factors, leading to a worse prognosis. Additionally, some

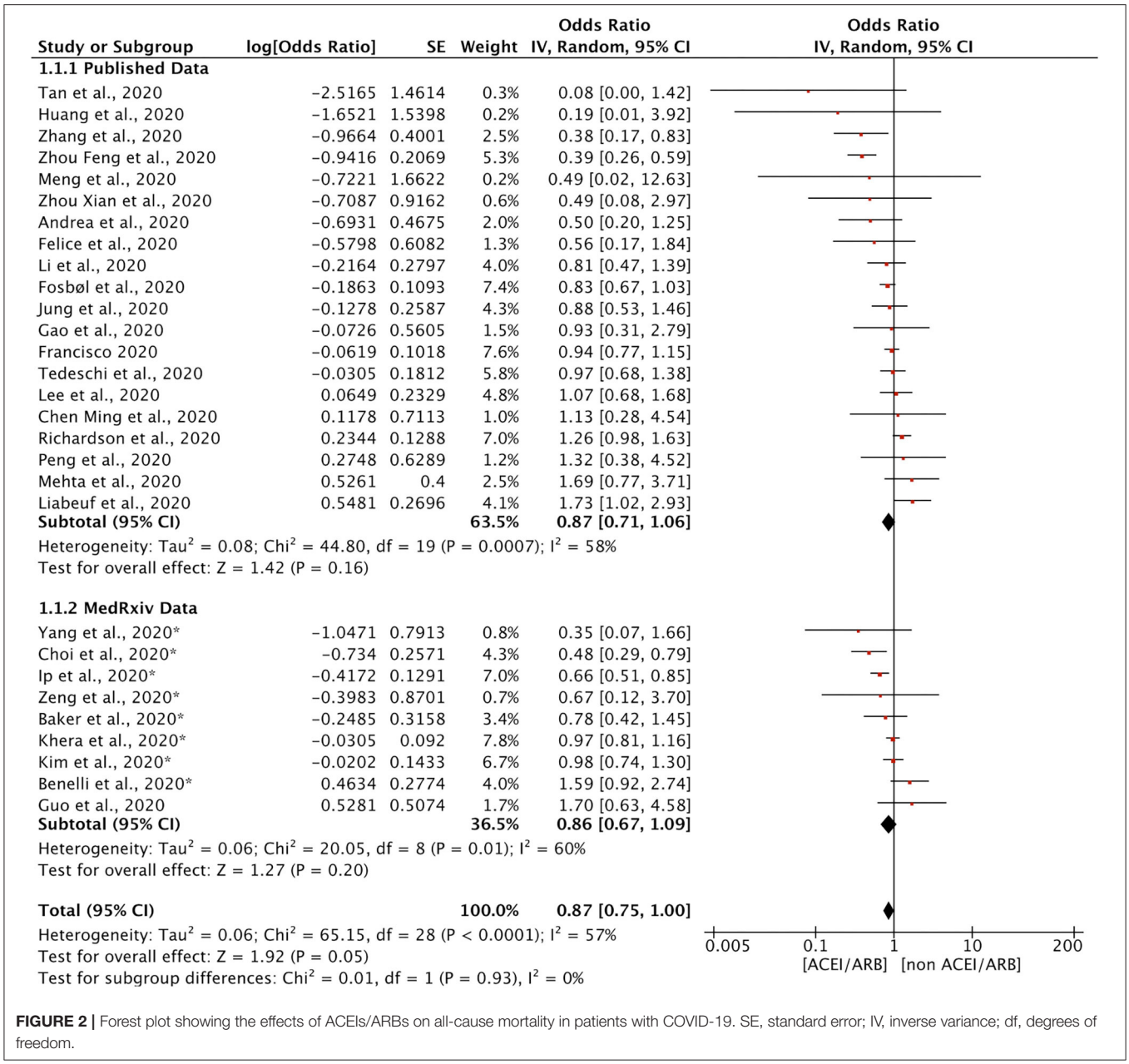


FIGURE 2 | Forest plot showing the effects of ACEIs/ARBs on all-cause mortality in patients with COVID-19. SE, standard error; IV, inverse variance; df, degrees of freedom.

of these studies' analyses were crude estimates that were not adjusted for confounding factors associated with hypertension, such as older age and cardiovascular disease. The adjustment of analyses is crucial for controlling for confounding factors, reducing bias, and increasing the reliability of the conclusions.

There have been three previous systematic reviews examining the effects of ACEI/ARB use in COVID-19 patients. Zhang et al. (51) found that ACEI/ARB exposure was not associated with a higher risk of severe disease or mortality. However, only 12 studies with unadjusted estimates were considered. Guo et al. (62) showed that ACEI/ARB use was associated with lower mortality in COVID-19 patients, although only six studies were included. Mackey et al. (63) conducted a narrative synthesis of 14 studies and concluded that there was no evidence of an association between ACEI/ARB use and severe COVID-19. In addition, Calderia et al. (64) and Barochiner and Martínez (65) drew similar conclusions, indicating that the use of ACEIs/ARBs does not increase the risk of severe COVID-19 or mortality; indeed, they suggested that the use of ACEIs/ARBs may have a protective effect. Our analysis included 48 studies and evaluated three outcomes. Additionally, to our knowledge, our review is the first to pool 35 adjusted effect estimates for mortality and severe COVID-19.

The results of the latest clinical trial, BRACE CORONA, have shown that the discontinuation of ACEIs/ARBs had no significant impact on the average survival duration or

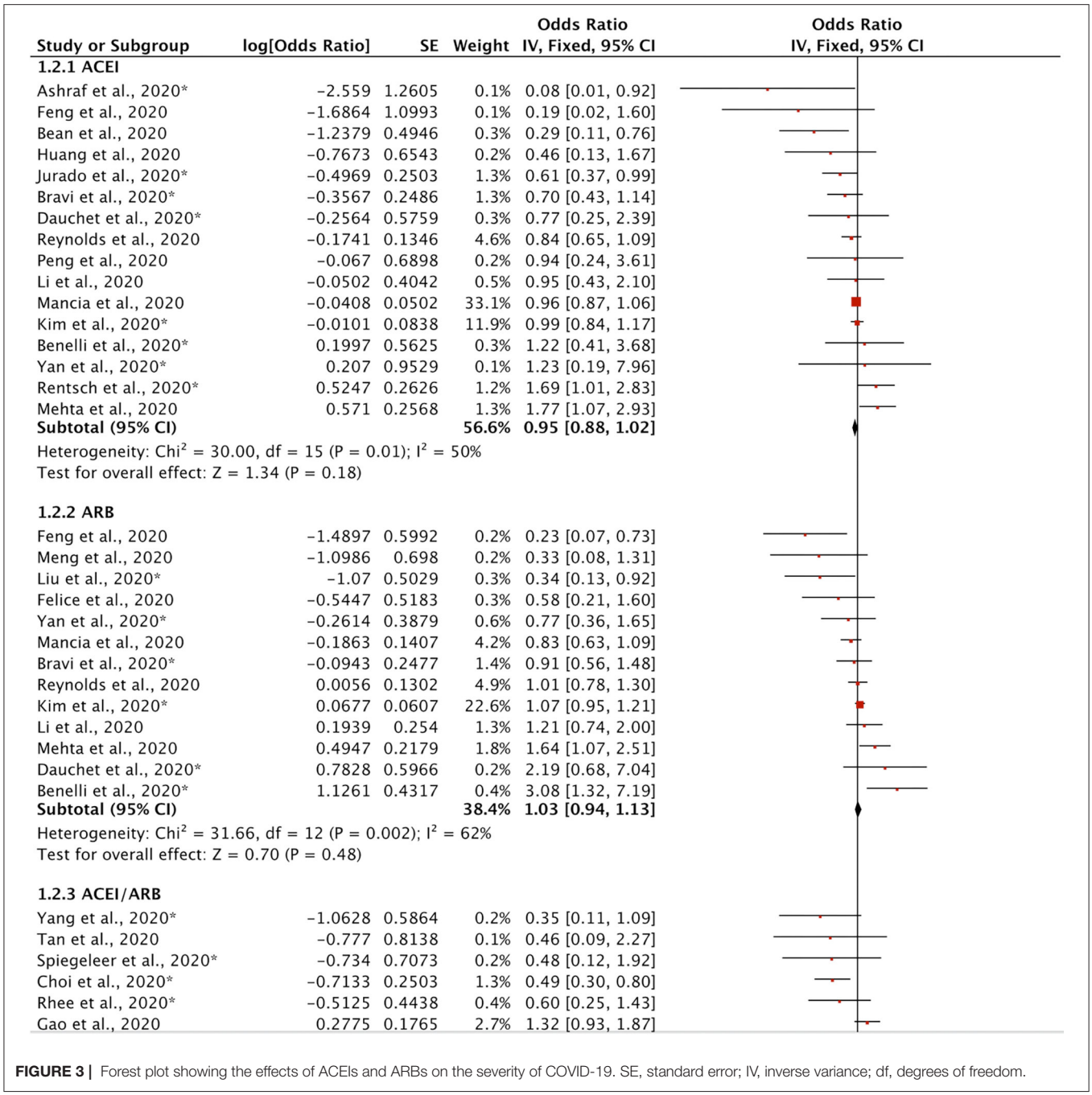


FIGURE 3 | Forest plot showing the effects of ACEIs and ARBs on the severity of COVID-19. SE, standard error; IV, inverse variance; df, degrees of freedom.

hospitalization duration (66). Currently, the European Society of Hypertension recommends continuing treatment with ACEIs/ARBs in patients with hypertension and COVID-19. These conclusions are consistent with those of our meta-analysis (67). We believe that the benefits of continuing treatment with ACEIs/ARBs outweigh the potential risks. Future well-designed randomized controlled trials and studies exploring the underlying mechanisms are needed to improve the level of evidence and determine whether the use of ACEIs/ARBs has an effect on the prognosis of patients with COVID-19.

LIMITATION

First, although most of the available studies included in this meta-analyses reported adjusted estimates, some of the studies did not adjust the models, leading to an increased risk of bias in the pooled effect measures. Second, the majority of the included studies were observational in nature; thus, causality cannot be concluded because of the methodological limitations of this design. Third, heterogeneity was high in most of the evaluated outcomes. Possible reasons for the heterogeneity were the

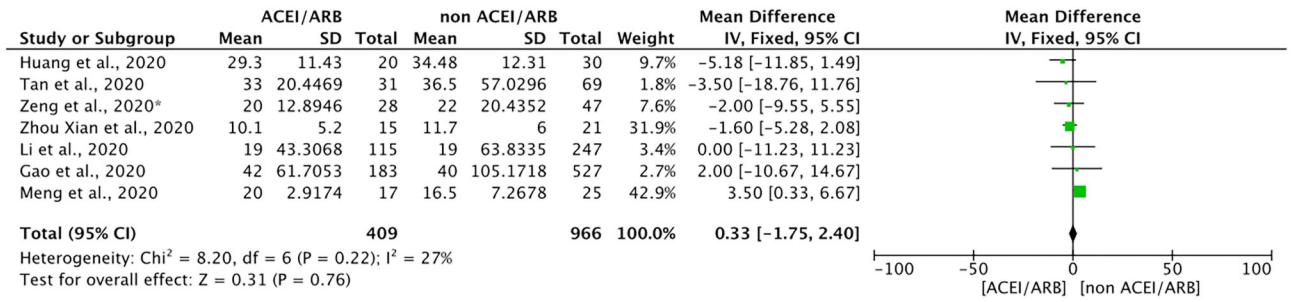


FIGURE 4 | Forest plot showing the effects of ACEIs/ARBs on hospitalization duration in patients with COVID-19. SE, standard error; IV, inverse variance; df, degrees of freedom.

sample sizes, differences in outcome definitions, heterogeneous population, etc. Finally, the inconsistency of reporting the discontinuation of ACEIs or ARBs during hospitalization across studies could have influenced the pooled estimates.

CONCLUSION

This meta-analysis suggested that ACEI/ARB use was not significantly associated with all-cause mortality in patients with hypertension who contracted COVID-19. In addition, ACEIs/ARBs had no significant effect on disease severity or the duration of hospitalization in COVID-19 patients with hypertension. This study provides additional evidence in favor of continuing antihypertension therapy after contracting COVID-19 unless the drugs cannot be tolerated because of hemodynamic instability.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Z-YA: analysis and interpretation of data, drafting the article, and reviewing and editing the article. X-CD, Z-YW, and Z-ZW: conception and design of the study, acquisition of data, and final approval of the version to be published. Z-YA and Y-RW: drafting the manuscript and checking the methodology and making the data curation. All authors: contributed to the article and approved the submitted version.

FUNDING

The study was supported by the Zhejiang Provincial Science Foundation of China under Grant No. LY20H020006 and the Zhejiang Provincial Basic Public Welfare Research Program of China under Grant No. LGF19H020007.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.609857/full#supplementary-material

REFERENCES

1. Kai H, Kai M. Interactions of coronaviruses with ACE2, angiotensin II, and RAS inhibitors-lessons from available evidence and insights into COVID-19. *Hypertens Res.* (2020) 43:985–6. doi: 10.1038/s41440-020-0455-8
2. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 Spike glycoprotein. *Cell.* (2020) 181:281–92.e6. doi: 10.1016/j.cell.2020.02.058
3. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell.* (2020) 181:271–80.e8. doi: 10.1016/j.cell.2020.02.052
4. Lopes RD, Macedo AVS, de Barros ESPGM, Moll-Bernardes RJ, Feldman A, D'Andréa Saba Arruda G, et al. Continuing versus suspending angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: impact on adverse outcomes in hospitalized patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–The BRACE CORONA Trial. *Am Heart J.* (2020) 226:49–59. doi: 10.1016/j.ahj.2020.05.002
5. Kreutz R, Algharably EAE, Azizi M, Dobrowolski P, Guzik T, Januszewicz A, et al. Hypertension, the renin-angiotensin system, and the risk of lower respiratory tract infections and lung injury: implications for COVID-19. *Cardiovasc Res.* (2020) 116:1688–99. doi: 10.1093/cvr/cvaa097
6. Esler M, Esler D. Can angiotensin receptor-blocking drugs perhaps be harmful in the COVID-19 pandemic? *J Hypertens.* (2020) 38:781–2. doi: 10.1097/HJH.0000000000002450
7. Vaduganathan M, Vardeny O, Michel T, McMurray JJV, Pfeffer MA, Solomon SD. Renin-Angiotensin-Aldosterone System Inhibitors in Patients with Covid-19. *N Engl J Med.* (2020) 382:1653–9. doi: 10.1056/NEJMsr2005760
8. Tan ND, Qiu Y, Xing XB, Ghosh S, Chen MH, Mao R. Associations between angiotensin-converting enzyme inhibitors and angiotensin ii receptor blocker use, gastrointestinal symptoms, and mortality among patients with COVID-19. *Gastroenterology.* (2020) 159:1170–2.e1. doi: 10.1053/j.gastro.2020.05.034
9. Ingraham NE, Barakat AG, Reilkoff R, Bezdicek T, Schacker T, Chipman JG, et al. Understanding the renin-angiotensin-aldosterone-SARS-CoV-axis: a comprehensive review. *Eur Respir J.* (2020) 56:2000912. doi: 10.1183/13993003.00912-2020

10. Hutton B, Salanti G, Caldwell DM, Chaimani A, Schmid CH, Cameron C, et al. The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. *Ann Intern Med*. (2015) 162:777–84. doi: 10.7326/M14-2385
11. Organization WH. *Clinical Management of Severe Acute Respiratory Infection when Novel Coronavirus (nCoV) Infection is Suspected*. Available online at: https://www.who.int/docs/default-source/coronaviruse/clinical-management-of-novel-cov.pdf
12. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Statist Med*. (2002) 21:1539–58. doi: 10.1002/sim.1186
13. Andrea C, Francesco M, Antonio N, Evgeny F, Marzia S, Fabio C, et al. Renin-angiotensin-aldosterone system inhibitors and outcome in patients with SARS-CoV-2 pneumonia: a case series study. *Hypertension*. (2020) 76:e10–2. doi: 10.1161/HYP.0000000000000185
14. Ashraf MA, Shokouhi N, Shirali E, Davari-tanha F, Memar O, Kamalipour A, et al. COVID-19 in Iran, a comprehensive investigation from exposure to treatment outcomes. *medRxiv [Preprint]*. (2020). doi: 10.21203/rs.3.rs-26339/v1
15. Baker KF, Hanrath AT, Schim van der Loeff I, Tee SA, Capstick R, Marchitelli G, et al. COVID-19 management in a UK NHS Foundation trust with a high consequence infectious diseases centre: a detailed descriptive analysis. *Med Sci (Basel)*. (2021) 9:6. doi: 10.3390/medsci9010006
16. Bean DM, Kraljevic Z, Searle T, Bendayan R, Kevin O, Pickles A, et al. Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers are not associated with severe COVID-19 infection in a multi-site UK acute hospital trust. *Eur J Heart Fail*. (2020) 22:967–74. doi: 10.1002/ejhf.1924
17. Gili T, Benelli G, Buscarini E, Canetta C, La Piana G, Merli G, et al. SARS-COV-2 comorbidity network and outcome in hospitalized patients in Crema, Italy. *PLoS One*. (2021) 16:e0248498. doi: 10.1371/journal.pone.0248498
18. Bravi F, Flacco ME, Carradori T, Volta CA, Cosenza G, De Togni A, et al. Predictors of severe or lethal COVID-19, including Angiotensin Converting Enzyme inhibitors and Angiotensin II Receptor Blockers, in a sample of infected Italian citizens. *PLoS One*. (2020) 15:e0235248. doi: 10.1371/journal.pone.0235248
19. Chen Y, Yang D, Cheng B, Chen J, Peng A, Yang C, et al. Clinical characteristics and outcomes of patients with diabetes and COVID-19 in association with glucose-lowering medication. *Diabetes Care*. (2020) 43:1399–407. doi: 10.2337/dc20-0660
20. Choi HK, Koo H-J, Seok H, Jeon JH, Choi WS, Kim DJ, et al. ARB/ACEI use and severe COVID-19: a nationwide case-control study. *medRxiv [Preprint]*. (2020). doi: 10.1101/2020.06.12.20129916
21. Dauchet L, Lambert M, Gauthier V, Poissy J, Faure K, Facon A, et al. ACE inhibitors, AT1 receptor blockers and COVID-19: clinical epidemiology evidences for a continuation of treatments. the ACER-COVID study. *medRxiv [Preprint]*. (2020). doi: 10.1101/2020.04.28.20078071
22. De Spiegeleer A, Bronselaer A, Teo JT, Byttebier G, De Tré G, Belmans L, et al. The effects of ARBs, ACEis, and statins on clinical outcomes of COVID-19 infection among nursing home residents. *J Am Med Dir Assoc*. (2020) 21:909–14.e2. doi: 10.1016/j.jamda.2020.06.018
23. Felice C, Nardin C, Di Tanna GL, Grossi U, Bernardi E, Scaldaferri L, et al. Use of RAAS inhibitors and risk of clinical deterioration in COVID-19: results from an Italian cohort of 133 hypertensives. *Am J Hypertens*. (2020) 33:944–8. doi: 10.1093/ajh/hpaa096
24. Feng Y, Ling Y, Bai T, Xie Y, Huang J, Li J, et al. COVID-19 with different severity: a multi-center study of clinical features. *Am J Respir Crit Care Med*. (2020) 201:1380–8. doi: 10.1164/rccm.202002-0445OC
25. Fosbol EL, Butt JH, Ostergaard L, Andersson C, Selmer C, Kragholm K, et al. Association of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker use with COVID-19 diagnosis and mortality. *JAMA*. (2020) 324:168–77. doi: 10.1001/jama.2020.11301
26. Gao C, Cai Y, Zhang K, Zhou L, Zhang Y, Zhang X, et al. Association of hypertension and antihypertensive treatment with COVID-19 mortality: a retrospective observational study. *Eur Heart J*. (2020) 41:2058–66. doi: 10.1093/eurheartj/ehaa433
27. Giorgi Rossi P, Marino M, Formisano D, Venturelli F, Vicentini M, Grilli R. Reggio emilia COVID-19 working group. Characteristics and outcomes of a cohort of COVID-19 patients in the Province of Reggio Emilia, Italy. *PLoS One*. (2020) 15:e0238281. doi: 10.1371/journal.pone.0238281
28. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease (2019). (COVID-19). *JAMA Cardiol*. (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017
29. Huang Z, Cao J, Yao Y, Jin X, Luo Z, Xue Y, et al. The effect of RAS blockers on the clinical characteristics of COVID-19 patients with hypertension. *Ann Transl Med*. (2020) 8:430. doi: 10.21037/atm.2020.03.229
30. Ip A, Parikh K, Parrillo JE, Mathura S, Hansen E, Sawczuk IS, et al. hypertension and renin-angiotensin-aldosterone system inhibitors in patients with Covid-19. *medRxiv [Preprint]*. (2020). doi: 10.1101/2020.04.24.20077388
31. Jung SY, Choi JC, You SH, Kim WY. Association of renin-angiotensin-aldosterone system inhibitors with COVID-19-related outcomes in Korea: a nationwide population-based cohort study. *Clin Infect Dis*. (2020) 71:2121–8. doi: 10.1093/cid/ciaa624
32. Jurado A, Martín MC, Abad-Molina C, Orduña A, Martínez A, Ocaña E, et al. COVID-19: age, Interleukin-6, C-reactive protein, and lymphocytes as key clues from a multicentre retrospective study. *Immun Ageing*. (2020) 17:22. doi: 10.1186/s12979-020-00194-w
33. Khera R, Clark C, Lu Y, Guo Y, Ren S, Truax B, et al. Association of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers with the risk of hospitalization and death in hypertensive patients with coronavirus disease-19. *J Am Heart Assoc*. (2021) 24:e018086. doi: 10.1161/JAHA.120.018086
34. Kim L, Garg S, O'Halloran A, Whitaker M, Pham H, Anderson EJ, et al. Interim analysis of risk factors for severe outcomes among a cohort of hospitalized adults identified through the U.S. Coronavirus Disease. 2019 (COVID-19)-Associated Hospitalization Surveillance Network (COVID-NET). *medRxiv [Preprint]*. (2020). doi: 10.1101/2020.05.18.20103390
35. Lee H-Y, Ahn J, Kang CK, Won S-H, Park J-H, Kang CH, et al. Association of Angiotensin II receptor association of Angiotensin II Receptor blockers and angiotensin-converting enzyme inhibitors on COVID-19-related outcome. *Lancet*. (2020). doi: 10.2139/ssrn.3569837. [Epub ahead of print].
36. Li J, Wang X, Chen J, Zhang H, Deng A. Association of renin-angiotensin system inhibitors with severity or risk of death in patients with hypertension hospitalized for coronavirus disease (2019). (COVID-19) infection in Wuhan, China. *JAMA Cardiol*. (2020) 5:825–30. doi: 10.1001/jamacardio.2020.1624
37. Liabeuf S, Moragny J, Bennis Y, Batteux B, Brochot E, Schmit JL, et al. Association between renin-angiotensin system inhibitors and COVID-19 complications. *Eur Heart J Cardiovasc Pharmacother*. (2020) doi: 10.1093/ehjcvp/pvaa062. [Epub ahead of print].
38. Liu Y, Huang F, Xu J, Yang P, Qin Y, Cao M, et al. Anti-hypertensive Angiotensin II receptor blockers associated to mitigation of disease severity in elderly COVID-19 patients. *medRxiv [Preprint]*. (2020). doi: 10.1101/2020.03.20.20039586
39. Mancia G, Rea F, Ludergnani M, Apolone G, Corrao G. Renin-angiotensin-aldosterone system blockers and the risk of Covid-19. *N Engl J Med*. (2020) 382:2431–40. doi: 10.1056/NEJMoa2006923
40. Mehta N, Kalra A, Nowacki AS, Anjewierden S, Han Z, Bhat P, et al. Association of use of angiotensin-converting enzyme inhibitors and Angiotensin II receptor blockers with testing positive for coronavirus disease 2019. (COVID-19). *JAMA Cardiol*. (2020) 5:1020–6. doi: 10.1001/jamacardio.2020.1855
41. Meng J, Xiao G, Zhang J, He X, Ou M, Bi J, et al. Renin-angiotensin system inhibitors improve the clinical outcomes of COVID-19 patients with hypertension. *Emerg Microb Infect*. (2020) 9:757–60. doi: 10.1080/22221751.2020.1746200
42. Peng YD, Meng K, Guan HQ, Leng L, Zhu RR, Wang BY, et al. Clinical characteristics outcomes of 112 cardiovascular disease patients infected by 2019-nCoV. *Zhonghua Xin Xue Guan Bing Za Zhi*. (2020) 48:450–5. doi: 10.3760/cma.j.cn112148-20200220-00105
43. Rentsch CT, Kidwai-Khan F, Tate JP, Park LS, King JT, Skanderson M, et al. Covid-19 testing, hospital admission, and intensive care among 2,026,227 United States veterans aged 54-75 years. *medRxiv [Preprint]*. (2020). doi: 10.1101/2020.04.09.20059964
44. Reynolds HR, Adhikari S, Pulgarin C, Troxel AB, Iturrate E, Johnson SB, et al. Renin-angiotensin-aldosterone system inhibitors and risk of Covid-19. *N Engl J Med*. (2020) 382:2441–8. doi: 10.1056/NEJMoa2008975

45. Rhee SY, Lee J, Nam H, Kyoung DS, Shin DW, Kim DJ. Effects of a DPP-4 inhibitor and RAS blockade on clinical outcomes of patients with diabetes and COVID-19. *Diabetes Metab J.* (2021) 45:251–9. doi: 10.4093/dmj.2020.0206
46. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. *JAMA*. (2020) 324:782–93. doi: 10.1001/jama.2020.6775
47. Tedeschi S, Giannella M, Bartoletti M, Trapani F, Tadolini M, Borghi C, et al. Clinical impact of renin-angiotensin system inhibitors on in-hospital mortality of patients with hypertension hospitalized for coronavirus disease 2019. *Clin Infect Dis*. (2020) 71:899–901. doi: 10.1093/cid/ciaa492
48. Yan H, Valdes AM, Vijay A, Wang S, Liang L, Yang S, et al. Role of drugs affecting the renin-angiotensin-aldosterone system on susceptibility and severity of COVID-19: a large case-control study from Zheijang Province, China. *medRxiv [Preprint]*. (2020). doi: 10.1101/2020.04.24.20077875
49. Yang G, Tan Z, Zhou L, Yang M, Peng L, Liu J, et al. Effects of ARBs and ACEIs on virus infection, inflammatory status and clinical outcomes in COVID-19 patients with hypertension: a single center retrospective study. *Hypertension*. (2020) 76:51–8. doi: 10.1161/HYPERTENSIONAHA.120.15143
50. Zeng Z, Sha T, Zhang Y, Wu F, Hu H, Li H, et al. Hypertension in patients hospitalized with COVID-19 in Wuhan, China: a single-center retrospective observational study. *medRxiv [Preprint]*. (2020). doi: 10.1101/2020.04.06.20054825
51. Zhang X, Yu J, Pan LY, Jiang HY. ACEI/ARB use and risk of infection or severity or mortality of COVID-19: a systematic review and meta-analysis. *Pharmacol Res*. (2020) 158:104927. doi: 10.1016/j.phrs.2020.104927
52. Zhou X, Zhu J, Xu T. Clinical characteristics of coronavirus disease 2019. (COVID-19) patients with hypertension on renin-angiotensin system inhibitors. *Clin Exp Hypertens*. (2020) 42:656–60. doi: 10.1080/10641963.2020.1764018
53. Zhou F, Liu YM, Xie J, Li H, Lei F, Yang H, et al. Comparative impacts of ACE (Angiotensin-Converting Enzyme) inhibitors versus Angiotensin II receptor blockers on the risk of COVID-19 mortality. *Hypertension*. (2020) 76:e15–7. doi: 10.1161/HYPERTENSIONAHA.120.15622
54. Regina J, Papadimitriou-Olivgeris M, Burger R, Le Pogam MA, Niemi T, Filippidis P, et al. Epidemiology, risk factors and clinical course of SARS-CoV-2 infected patients in a Swiss university hospital: An observational retrospective study. *PLoS One.* (2020) 15:e0240781. doi: 10.1371/journal.pone.0240781
55. Wang Y, Lu X, Li Y, Chen H, Chen T, Su N, et al. Clinical course and outcomes of 344 intensive care patients with COVID-19. *Am J Respir Crit Care Med*. (2020) 201:1430–4. doi: 10.1164/rccm.202003-0736LE
56. Yang G, Tan Z, Zhou L, Yang M, Peng L, Liu J, et al. Angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors usage is associated with improved inflammatory status and clinical outcomes in COVID-19 patients with hypertension. *medRxiv [Preprint]*. (2020). doi: 10.1101/2020.03.31.20038935
57. Guzik TJ, Mohiddin SA, Dimarco A, Patel V, Savvatis K, Marelli-Berg FM, et al. COVID-19 and the cardiovascular system: implications for risk assessment, diagnosis, and treatment options. *Cardiovasc Res.* (2020) 116:1666–87. doi: 10.1093/cvr/cvaa106
58. Sriram K, Insel PA. Risks of ACE inhibitor and ARB usage in COVID-19: evaluating the evidence. *Clin Pharmacol Ther*. (2020) 108:236–41. doi: 10.1002/cpt.1863
59. Rico-Mesa JS, White A, Anderson AS. Outcomes in patients with COVID-19 infection taking ACEI/ARB. *Curr Cardiol Rep*. (2020) 22:31. doi: 10.1007/s11886-020-01291-4
60. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *J Virol*. (2020) 94:e00127–20. doi: 10.1128/JVI.00127-20
61. Guo J, Huang Z, Lin L, Lv J. Coronavirus Disease 2019. (COVID-19) and cardiovascular disease: a viewpoint on the potential influence of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers on onset and severity of severe acute respiratory syndrome Coronavirus 2 infection. *J Am Heart Assoc*. (2020) 9:e016219. doi: 10.1161/JAHA.120.016219
62. Guo X, Zhu Y, Hong Y. Decreased mortality of COVID-19 with renin-angiotensin-aldosterone system inhibitors therapy in patients with hypertension: a meta-analysis. *Hypertension*. (2020) 76:e13–4. doi: 10.1161/HYPERTENSIONAHA.120.15572
63. Mackey K, King VJ, Gurley S, Kiefer M, Liederbauer E, Vela K, et al. Risks and impact of angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers on SARS-CoV-2 INFECTION IN ADULTS. *Ann Intern Med*. (2020) 173:195–203. doi: 10.7326/M20-1515
64. Caldeira D, Alves M, Gouveia EMR, Silvério António P, Cunha N, Nunes-Ferreira A, et al. Angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers and the risk of COVID-19 infection or severe disease: systematic review and meta-analysis. *Int J Cardiol Heart Vasc*. (2020) 31:100627. doi: 10.1016/j.ijcha.2020.100627
65. Barochiner J, Martínez R. Use of inhibitors of the renin-angiotensin system in hypertensive patients and COVID-19 severity: A systematic review and meta-analysis. *J Clin Pharm Ther*. (2020) 45:1244–52. doi: 10.1111/jcpt.13246
66. Lopes RD, Macedo AVS, de Barros ESPGM, Moll-Bernardes RJ, Dos Santos TM, Mazza L, et al. Effect of discontinuing vs continuing angiotensin-converting enzyme inhibitors and Angiotensin II receptor blockers on days alive and out of the hospital in patients admitted with COVID-19: a randomized clinical trial. *JAMA*. (2021) 325:254–64. doi: 10.1001/jama.2020.25864
67. Limbourg FP. [Hypertension and RAAS inhibition in times of COVID-19 - current recommendations of the European Society of Hypertension.]. *MMW Fortschr Med*. (2020) 162:67–9. doi: 10.1007/s15006-020-0483-y

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

CASE REPORT
published: 28 April 2021
doi: 10.3389/fcvm.2021.645135

Case Report: Are We Witnessing an Increase of Chronic Ascending Aortic Dissection as a Collateral Effect to the COVID-19 Pandemic?

Arnaud Lyon[1], Ziyad Gunga[2†], Lars Niclauss[2†], Valentina Rancati[3†] and Piergiorgio Tozzi[2]*

[1] School of Medicine, University of Lausanne, Lausanne, Switzerland, [2] Service de Chirurgie Cardiaque, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, [3] Département d'anesthésiologie, Hôpital Universitaire de Lausanne (CHUV), Lausanne, Switzerland

OPEN ACCESS

Edited by:
Robert Jeenchen Chen, The Ohio State University, United States

Reviewed by:
Antonino S. Rubino, University of Campania Luigi Vanvitelli, Italy
Teeranan Angkananard, Srinakharinwirot University, Thailand

***Correspondence:**
Arnaud Lyon
arnaud.lyon@unil.ch

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Heart Surgery, a section of the journal Frontiers in Cardiovascular Medicine

Received: *22 December 2020*
Accepted: *17 March 2021*
Published: *28 April 2021*

Citation:
Lyon A, Gunga Z, Niclauss L, Rancati V and Tozzi P (2021) Case Report: Are We Witnessing an Increase of Chronic Ascending Aortic Dissection as a Collateral Effect to the COVID-19 Pandemic? Front. Cardiovasc. Med. 8:645135. doi: 10.3389/fcvm.2021.645135

Background: The COVID-19 (coronavirus disease 2019) pandemic is reducing health care accessibility to non–life-threatening diseases, thus hiding their real incidence. Moreover, the incidence of potentially fatal conditions such as acute type A aortic dissection seems to have decreased since the pandemic began, whereas the number of cases of chronic ascending aortic dissections dramatically increased. We present two patients whose management has been affected by the exceptional sanitary situation we are dealing with.

Case report: A 70-year-old man with chest pain and an aortic regurgitation murmur had his cardiac workup delayed (4 months) because of sanitary restrictions. He was then diagnosed with chronic type A aortic dissection and underwent urgent replacement of ascending aorta and aortic root. The delay in surgical treatment made the intervention technically challenging because the ascending aorta grew up to 80 mm inducing strong adhesions and chronic inflammation. The second case report concerns a 68-year-old woman with right lower-limb pain who was diagnosed with deep vein thrombosis. However, a CT scan to exclude a pulmonary embolism could not be realized until 5 months later because of sanitary restrictions. When she eventually got the CT scan, it fortuitously showed a chronic dissection of the ascending aorta. She underwent urgent surgery, and the intervention was challenging because of adhesions and severe inflammation.

Conclusion: Delayed treatment due to sanitary restrictions related to COVID-19 pandemic is having a significant impact on the management of potentially life-threatening conditions including type A aortic dissection. We should remain careful to avoid COVID-19 also hitting patients who are not infected with the virus.

Keywords: ascending aorta, chronic aortic dissection, COVID−19, aortic surgery, delayed management

BACKGROUND

Acute Stanford type A aortic dissections (ATAADs) constitute critical emergencies that require immediate surgical treatment. This is due to the high risk of fatal complications, such as aortic rupture, severe aortic regurgitation, pericardial tamponade, and cerebral and coronary malperfusion (1), which are responsible for 33% mortality after 24 h and 50% mortality after 48 h (2). However, a very limited number of patients remain stable, with only mild to absent symptoms, and may thus survive the acute phase (1). After a 14-day period, the aortic dissection is defined as chronic (1). The global incidence of aortic dissection is 5 to 30 cases per 1 million people per year (3). We recently published our experience with ATAADs from 2014 to 2019. During the considered period, we treated 117 ATAADs in our center, which represents 3 to 5 ATAAD cases per month and had no chronic cases (4). However, between February and May 2020, the COVID-19 (coronavirus disease 2019) pandemic temporarily reduced health care accessibility. As such, these statistics were noticeably altered, with only three confirmed ATAADs, which represented a decrease of ¾, compared to the usual volume. Nevertheless, we experienced two cases of chronic type A aortic dissection (CTAAD) in July 2020, which is a pathology we usually see only once every 5 years (4).

This report illustrates the clinical implications of CTAAD that occurred in two patients shortly after the peak phase of the COVID-19 pandemic in our country.

FIRST CASE PRESENTATION

In February 2020, a 70-year-old man with a previously treated arterial hypertension consulted his general practitioner (GP) because of mild chest pain, without any other symptoms. The physical examination revealed a diastolic heart murmur that predominated in the aortic area. The patient's electrocardiogram (ECG) was normal, and the GP asked for cardiology advice. However, because of the sanitary restrictions due to the COVID-19 pandemic, the cardiologist examined the patient only 4 months later. The echocardiography revealed an aneurysm of the ascending aorta with signs of aortic dissection and severe aortic regurgitation.

A contrast medium thoracic computed tomography (CT) scan was immediately performed, which demonstrated an 82 × 87 mm aneurysm of the ascending aorta with a longitudinal tear of the intima that originated in a partially thrombosed circulating false lumen. The lesion began just above the ostium of the right coronary artery and extended up to the middle of the aortic arch with a 2.2-cm opening that was compatible with a Stanford type A aortic dissection. A 17-mm pericardial effusion was also identified (**Figures 1A,B**).

The patient underwent replacement of the ascending aorta and aortic root (Bentall procedure with a 25-mm Carpentier–Edwards biological valve mounted on a Valsalva-type 28-mm Dacron tube). The intervention was technically difficult, as the heart was totally displaced into the left chest, and there were strong adhesions due to chronic inflammatory reactions (**Figures 2A,B**). The perioperative echocardiography showed a thickened dissection flap localized just above the origin of the right coronary artery (**Figure 3**). The right coronary reimplantation was therefore challenging because the ostium was fragilized by the dissection.

The post-operative period was uneventful, and the patient quickly recovered.

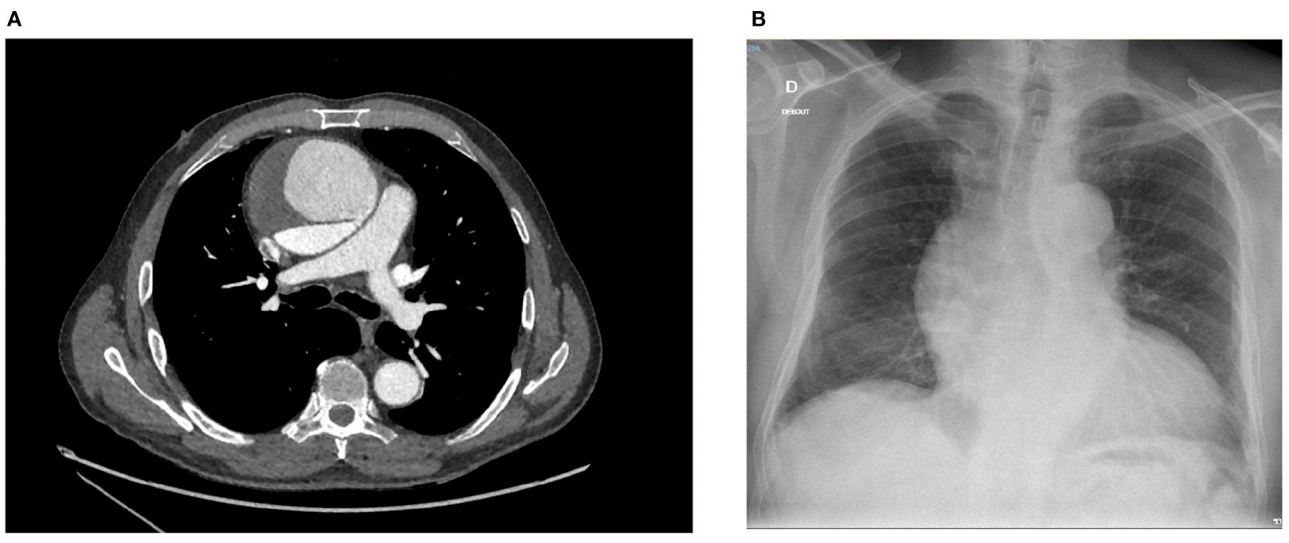


FIGURE 1 | (A) Contrast medium thoracic CT scan showing a CTAAD with a thick intimal tear (7 mm) and an enlarged ascending aorta (82 × 87 mm). The false lumen is partially thrombosed. **(B)** Chest X-ray showing an aortic aneurysm with a widening of the mediastinal silhouette, an enlargement of the aortic knob, and a displacement of the trachea from the midline. CT, computed tomography; CTAAD, chronic type A aortic dissection.

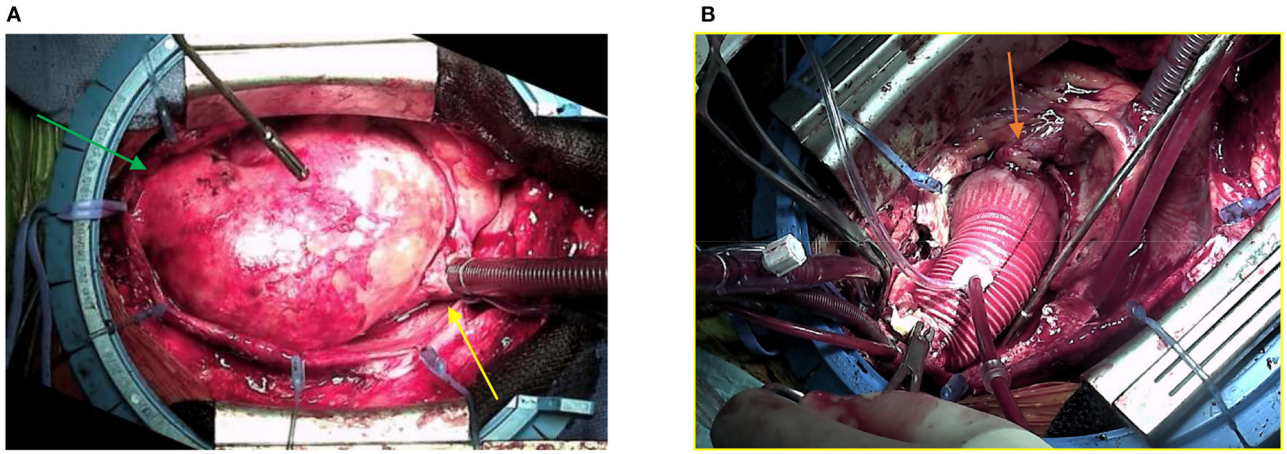


FIGURE 2 | (A) Surgical view of the mediastinum after sternotomy. The huge aneurysm of the ascending aorta (82 × 87 mm) filled the whole cavity, displacing the heart into the left chest. The yellow arrow indicates the right atrium, and the green arrow indicates the aortic arch. **(B)** The ascending aorta has been replaced by the Dacron tube, leaving a free space that was previously filled by the aneurysm. The orange arrow indicates the reimplantation of the right coronary ostium.

SECOND CASE PRESENTATION

A 68-year-old female active smoker with known hypercholesterolemia, who had been treated for arterial hypertension and had a history of stroke in 2010 and pulmonary embolism in 2016, consulted her GP on February 25, 2020. She complained about the spontaneous onset of acute right lower-limb pain. The patient did not present any chest pain or dyspnea. The clinical examination revealed a painful but mild pretibial edema on the left lower limb, whereas no heart murmur was documented. D-Dimer levels were 570 mg/L (reference, <500 mg/L). On the same day, the patient was referred to the angiology department where a diagnosis of unprovoked deep vein thrombosis (DVT) of the left lower limb was established. Her arterial pressure was 131/86 mm Hg in the right arm and 134/87 mm Hg in the left arm, and all peripheral pulses were palpable. The patient was discharged with a therapeutic anticoagulation treatment (rivaroxaban 20 mg, once daily), with a 3-month follow-up examination scheduled for May. The follow-up found a favorable development, so the rivaroxaban was stopped and replaced by cardioprotective aspirin.

In July, the patient's GP completed the diagnostic workup of her DVT with a CT scan. This examination unexpectedly showed an aneurysm of the ascending aorta (53 × 54 mm) with a chronic aortic dissection (**Figures 4A,B**). ECG was normal, and echocardiography found a ventricular function of 65% and a mild aortic insufficiency.

Based on the results of the CT scan, we performed an urgent repair of the dissected ascending aorta. We used a Gelweave 26-mm straight tube to replace the ascending aorta, just above the coronary ostia. The intervention was technically challenging, due to adhesions, but was ultimately uneventful, and the patient quickly recovered.

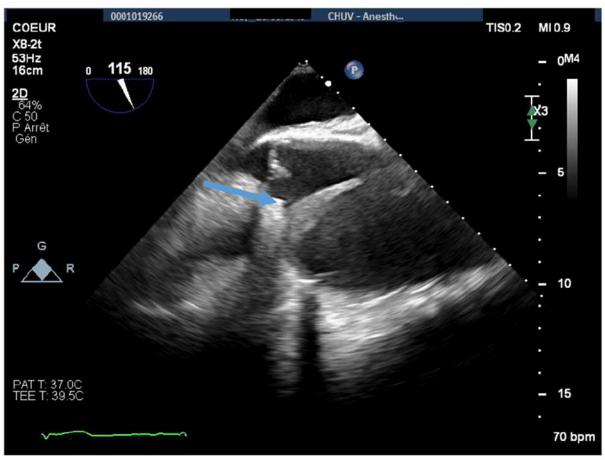


FIGURE 3 | Perioperative midesophageal long-axis view showing a thickened (5 mm) dissection flap localized in the proximal ascending aorta, immediately after the origin of the right coronary artery (blue arrow). The maximal diameter of the ascending aorta was 74 mm.

DISCUSSION

Delayed treatment of non–COVID-related diseases due to the COVID-19 pandemic is having a significant impact on patient safety even in developed countries such as Switzerland. Thousands of patients experience delayed management of potentially life-threatening conditions including type A aortic dissection. This is mainly due to a saturation of hospital capacity and patients' fear about becoming infected by the coronavirus in the hospital environment. We noticed a decrease in the number of ATAADs that were referred to our emergency department

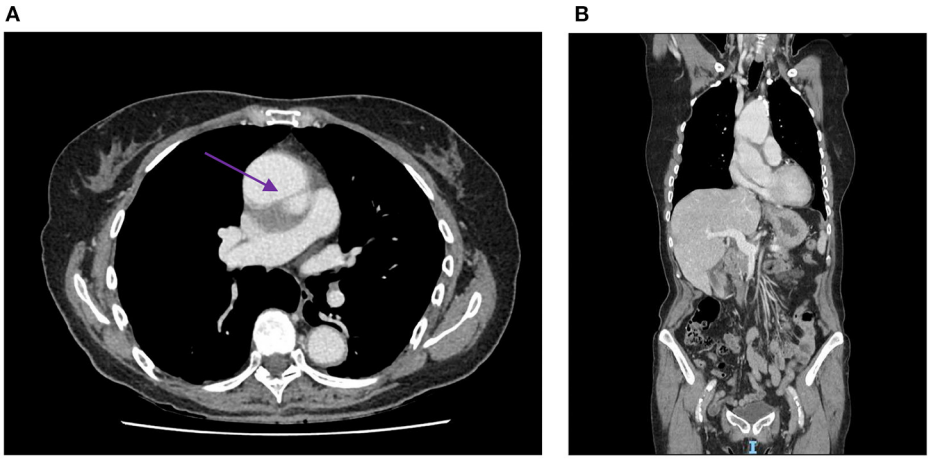


FIGURE 4 | (A) Contrast medium CT scan showing an aneurysm of the ascending aorta (53 × 54 mm) with a tear of the intima, creating a thick false lumen (4 mm), which is indicated by the purple arrow. **(B)** Sagittal section of the contrast medium CT scan, which also shows the aneurysm and thick tear of the intima. CT, computed tomography.

during the peak phase of the pandemic. This decrease of acute aortic syndromes was also highlighted by El-Hamamsy et al. who found the volume of ATAADs to be 76.5% lower than usual in New York City between March and April 2020 (5). Similar observations concerning myocardial infarctions and emergency department visits in general have also been reported (6, 7). Both of our patients exhibited atypical presentations with no specific symptoms or signs of ATAAD. We speculate that this played a role in their missed or delayed diagnoses. This may relate to the unexpected rise in CTAAD cases during the phase that followed the first peak of the pandemic. The absence of specific ATAAD symptoms in these patients was central to these delayed diagnoses. This phenomenon was reinforced by the pandemic, as patients altered their thresholds of symptoms that would normally compel them to seek medical advice. They waited longer before consulting a doctor than they would have before the pandemic.

CONCLUSION

The apparent decrease in acute aortic dissections during the COVID-19 pandemic does not appear to be real, and it only relied on many patients not consulting, and remaining unnoticed, thus preventing them to get the medical care they deserved. Most of these patients probably passed away due to the complications of their aortic dissections, while the acute aortic dissections of those with mild or atypical symptoms who survived may have evolved into a chronic state that was only discovered when normal accessibility to health care services resumed.

The surgical treatment of CTAADs is more challenging with respect to acute dissection because it is associated to strong adhesions and consistent inflammatory reaction, significantly increasing the surgical mortality and morbidity. Therefore, delayed diagnosis also impacts the prognosis of patients with mild to absent symptoms (8).

The example of aortic dissections also illustrates the fact that patients affected by a wide range of diseases are directly impacted by the sanitary restrictions related to the COVID-19 pandemic. We thus conclude that more attention should be paid to avoid COVID-19 also hitting patients who are not infected with the virus.

Limitations

Our case report is an observational study on a limited number of patients aimed at highlighting one of the possible consequences of the sanitary restrictions imposed by the authorities during the COVID-19 pandemic on the natural history of aortic dissection. By being a case report, it is not intended to clearly prove or bring statistical evidence of an association between the pandemic and an apparent increase of chronic aortic dissection cases. However, it shows a tendency in our center, which we believe is worth sharing with the medical community and which should be further investigated in a future larger epidemiological study.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

AL is responsible for the literature research as well as the writing of the manuscript. PT contributed to this work as senior author. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Hynes CF, Greenberg MD, Sarin S, Trachiotis GD. Chronic type A aortic dissection two cases and a review of current management strategies. *Aorta.* (2016) 4:16–21. doi: 10.12945/j.aorta.2015.15.016
2. Fournier Y, Moix PA, Hugli O. Dissection aortique aiguë: utilité diagnostique des D-dimères. *Rev Med.* (2008) 4:1759–63.
3. Levy D, Goyal A, Grigorova Y. Aortic dissection. In: Abai B, editor. *StatPearls.* Treasure Island, FL: StatPearls publishing (2020). p. 1–14.
4. Tozzi P, Gunga Z, Niclauss L, Delay D, Roumy A, Pfisher R, et al. Type A aortic dissection in aneurysms having modelled pre-dissection maximum diameter below 45 mm: should we implement current guidelines to improve the survival benefit of prophylactic surgery? *Eur J Cardiothorac Surg.* (2021) 59:473–78. doi: 10.1093/ejcts/ezaa351
5. El-Hamamsy I, Brinster DR, DeRose JJ, Girardi LN, Hisamoto K, Imam MN, et al. The COVID-19 pandemic and acute aortic dissections in New York a matter of public health. *J Am Coll Cardiol.* (2020) 76:227–9. doi: 10.1016/j.jacc.2020.05.022
6. Zitelny E, Newman N, Zhaob D. STEMI during the COVID-19 pandemic - an evaluation of incidence. *Cardiovasc Pathol.* (2020) 48:107232. doi: 10.1016/j.carpath.2020.107232
7. Hartnett K, Kite-Powell A, DeVies J, Coletta MA, Boehmer TK, Adjemian J, et al. Impact of the COVID-19 pandemic on emergency department visits — United States. *MMWR Morb Mortal Wkly Rep.* (2020) 69:699–704. doi: 10.15585/mmwr.mm6923e1
8. Jault F, Rama A, Lievre L, Bonnet N, Leprince P, Pavie A, et al. Chronic dissection of the ascending aorta: surgical results during a 20-year period. *Eur J Cardiothorac Surg.* (2006) 29:1041–5. doi: 10.1016/j.ejcts.2006.02.034

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 04 May 2021
doi: 10.3389/fcvm.2021.645172

In silico Exploration of Interactions Between Potential COVID-19 Antiviral Treatments and the Pore of the hERG Potassium Channel—A Drug Antitarget

Ehab Al-Moubarak[1], *Mohsen Sharifi*[2] *and Jules C. Hancox*[1]*

[1] School of Physiology, Pharmacology and Neuroscience, University of Bristol, Biomedical Sciences Building, University Walk, Bristol, United Kingdom, [2] Independent Scientist, Indianapolis, IN, United States

OPEN ACCESS

Edited by:
Shuping Ge,
St. Christopher's Hospital for Children, United States

Reviewed by:
Xiaohu Li,
First Affiliated Hospital of Anhui Medical University, China
Luis Alberto Gonano,
National University of La Plata, Argentina
Anna Weinzinger,
University of Vienna, Austria

***Correspondence:**
Jules C. Hancox
jules.hancox@bristol.ac.uk

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *22 December 2020*
Accepted: *30 March 2021*
Published: *04 May 2021*

Citation:
Al-Moubarak E, Sharifi M and Hancox JC (2021) In silico Exploration of Interactions Between Potential COVID-19 Antiviral Treatments and the Pore of the hERG Potassium Channel—A Drug Antitarget. Front. Cardiovasc. Med. 8:645172. doi: 10.3389/fcvm.2021.645172

Background: In the absence of SARS-CoV-2 specific antiviral treatments, various repurposed pharmaceutical approaches are under investigation for the treatment of COVID-19. Antiviral drugs considered for this condition include atazanavir, remdesivir, lopinavir-ritonavir, and favipiravir. Whilst the combination of lopinavir and ritonavir has been previously linked to prolongation of the QT_c interval on the ECG and risk of *torsades de pointes* arrhythmia, less is known in this regard about atazanavir, remdesivir, and favipiravir. Unwanted abnormalities of drug-induced QT_c prolongation by diverse drugs are commonly mediated by a single cardiac anti-target, the hERG potassium channel. This computational modeling study was undertaken in order to explore the ability of these five drugs to interact with known determinants of drug binding to the hERG channel pore.

Methods: Atazanavir, remdesivir, ritonavir, lopinavir and favipiravir were docked to *in silico* models of the pore domain of hERG, derived from cryo-EM structures of hERG and the closely related EAG channel.

Results: Atazanavir was readily accommodated in the open hERG channel pore in proximity to the S6 Y652 and F656 residues, consistent with published experimental data implicating these aromatic residues in atazanavir binding to the channel. Lopinavir, ritonavir, and remdesivir were also accommodated in the open channel, making contacts in a model-dependent fashion with S6 aromatic residues and with residues at the base of the selectivity filter/pore helix. The ability of remdesivir (at 30 μM) to inhibit the channel was confirmed using patch-clamp recording. None of these four drugs could be accommodated in the closed channel structure. Favipiravir, a much smaller molecule, was able to fit within the closed channel and could adopt multiple binding poses in the open channel, but with few simultaneous interactions with key binding residues. Only favipiravir and remdesivir showed the potential to interact with lateral pockets below the selectivity filter of the channel.

Conclusions: All the antiviral drugs studied here can, in principle, interact with components of the hERG potassium channel canonical binding site, but are likely to differ in their ability to access lateral binding pockets. Favipiravir's small size and relatively paucity of simultaneous interactions may confer reduced hERG liability compared to the other drugs. Experimental structure-function studies are now warranted to validate these observations.

Keywords: hERG, human ether-à-go-go–related gene, antiviral, atazanavir, lopinavir-ritonavir, remdesivir, favipiravir

INTRODUCTION

The coronavirus disease of 2019 (COVID-19), caused by the SARS-CoV-2 virus, poses an unprecedented challenge to modern healthcare systems. Although vaccines are now emerging [e.g., (1–4)], logistical challenges in production and global administration of billions of vaccine doses and the potential for incomplete vaccine take-up and efficacy mean that therapeutic treatments are also needed. Since the emergence of SARS-CoV-2, considerable effort has been invested to identify existing drugs that may be successfully repurposed for treatment of the illness. The antimalarial agents chloroquine and hydroxychloroquine were initially reported to be effective against SARS-CoV-2 *in vitro* (5, 6). However, whilst some studies have reported potential clinical benefit of these drugs [e.g., (7–9)], others are inconsistent with benefit [e.g., (10–12)] and there is a risk of QT interval prolongation and ventricular arrhythmia, particularly at higher concentrations (12–14). Other potential Covid-19 repurposed treatments include antivirals originally developed for other conditions (15, 16). The use of an *in silico* drug target deep-learning model has suggested a number of antiviral agents to be able to inhibit the 3C-like proteinase of SARS-CoV-2, including the antiretrovirals atazanavir and lopinavir and the broad spectrum antiviral agent remdesivir (17). Lopinavir is used in combination with ritonavir (which increases lopinavir half-life through inhibition of cytochrome P450) to treat human immunodeficiency virus and there is some evidence of efficacy against SARS-CoV-1 and MERS-CoV (15, 16). Initial randomized control trial data have not provided evidence for a benefit of the lopinavir-ritonavir combination beyond standard care, in patients hospitalized with severe COVID-19 (18). Remdesivir is a broad spectrum antiviral that has been found to be effective against diverse types of β coronaviruses (19). Intravenous remdesivir is undergoing clinical investigation in patients hospitalized with COVID-19 and initial trial data have shown a trend toward a reduction in time to clinical improvement (20, 21), warranting further study. Favipiravir is a broad spectrum antiviral agent shown to inhibit replication of a substantial number of RNA viruses (22). Favipiravir's efficacy against SARS-Cov-2 has been demonstrated pre-clinically in a Syrian Hamster model in which the drug reduced lung viral titers and alleviated disease (23). A recent open label study of its use in COVID-19 has reported an association between favipiravir and a shorter viral clearance time and, following adjustment for confounders, improved chest imaging (24). Whilst further study is needed, a recent scoping review has concluded that both remdesivir and favipiravir may be promising treatments for COVID-19 (25).

A proportion of COVID-19 patients have cardiac damage and concerns have been expressed regarding the risk of proarrhythmic effects of potential COVID-19 treatments, particularly in relation to producing prolongation of the rate corrected QT (QT_c) interval and associated *torsades de pointes* (TdP) arrhythmia (26–28). Whilst the emerging clinical data clearly support such a risk for chloroquine/hydroxychloroquine (12–14), it may also occur for some antivirals. TdP requiring resuscitation has been reported for a critically ill COVID-19 patient treated with remdesivir (29). Lopinavir/ritonavir and atazanavir have previously been associated with QT prolongation and TdP in the absence of COVID-19 (30–32). Favipiravir has been reported not to affect the QT/QT_c interval in healthy adults (33), although mild QT_c interval prolongation has been reported in a patient infected by Ebola-virus (34). Nearly all drugs associated with QT_c interval prolongation and TdP inhibit the cardiac hERG (*human Ether-à-go-go Related Gene*) potassium channel, which mediates the rapid delayed rectifier K^+ current, I_{Kr}; I_{Kr} is a key determinant of ventricular repolarisation (35, 36). The association between TdP/QT_c interval prolongation and pharmacological inhibition of hERG channels is sufficiently strong that testing for pharmacological inhibition of hERG channels is a key component of safety testing of candidate pharmaceuticals (36, 37). The consequences of pharmacological blockade of hERG may be exacerbated in hyperinflammatory states, since interleukin-6 can inhibit I_{Kr}/hERG via the Janus Kinase pathway (38) and the risk of arrhythmia may increase with severity of infection/inflammation (27). Lopinavir, ritonavir, and atazanvir have been reported to be able to inhibit hERG channel current (30, 39). However, at the time of writing, there are no peer reviewed studies of the ability of remdesivir or favipiravir to interact with the hERG channel. Whilst such information would be valuable, the SARS-CoV-2 pandemic has interfered with much laboratory-based experimental activity. The recent availability of a cryo-EM structure of the hERG channel (40) provides a means to investigate *in silico* the ability of drugs to interact with known molecular determinants of drug binding to the channel (41). Accordingly, this computational modeling study was undertaken to probe interactions between each of atazanavir, ritonavir, lopinavir, remdesivir, favipiravir, and constituents of the canonical drug binding site within the hERG channel pore. Our findings suggest that all of these agents can,

in principle, interact with components of the hERG potassium channel canonical binding site, but with some drug-specific differences in the observed interactions.

MATERIALS AND METHODS

Docking simulations used the recent Cryo-EM structure of the open pore state of hERG channel (40), PDB: 5VA2 and two closely related open pore models. These models were developed to predict more favorable hERG pore conformations for drug binding using molecular dynamics (MD) simulations starting from the available Cryo-EM hERG structure with the aim of presenting important F656 side chains into a pore-facing conformation to interact with drugs. The F656 residue is well-known to be an important determinant of drug-hERG channel interactions and its position relative to pore varies between models (41). The published Dickson model was obtained from MD simulations in the presence of hERG inhibitors (42). The in-house model was obtained from a short MD simulation in which the F656 side chain of one of the four hERG subunits was found to reorient toward the pore—this subunit was then replicated around all four pore subunits to produce a model with all four F656 side chains facing the pore. Molecular dynamics simulation of the hERG membrane domain, which underpinned the in-house model, was conducted using the cryoEM structure of hERG (PDB:5VA2) with several extracellular loops of missing atom density modeled into the structure using Modeler 9.17 (43) and the N- and C-terminal cytoplasmic domains removed. Unrestrained MD of the hERG membrane domain model was run in a POPC bilayer patch (385 lipids: 127 × 133 angstroms) with water layers (150 mM NaCl) above and below the membrane resulting in a total depth of the periodic boundary system of 120 angstroms. MD simulations were carried out at 310 K as described in (44), using Gromacs 5.1.4 with the amber99sb-ildn force field for protein and the SLipids force field for POPC (45, 46). The use of two MD-based open hERG models along with the Cryo EM structure was anticipated to give the opportunity to explore antiviral binding in different conformations of the canonical binding site (and in particular the position of the F656 with respect to the pore). A series of docking simulations was also performed with a hERG closed pore model based on rat EAG closed pore cryo-EM structure as described previously (47). Antiviral structures were converted from SMILE representation (obtained from PubChem database) to 3D structures and then hydrogens added and energy minimized using Molecular Operating Environment (MOE).

Antiviral molecules were docked in each of the hERG structures and models using GOLD (version v2020.1; Cambridge Crystallographic Data Centre, Cambridge, UK). The central pore cavity was chosen as a binding site where a radius of 10 angstrom extended from the centre of the cavity and in a level with a middle point between the canonical aromatic residues F656 and Y652. The side chains of these aromatic residues were allowed to be freely flexible during docking simulations. The antiviral ligands were also fully flexible during the molecular docking studies. Rotamer sampling was maximally set to 300,000 generations. Docking was scored by Goldscore and rescored with ChemScore scoring function. Two-hundred docking runs were made in each case and the low-energy-score poses were retained and inspected. Antiviral molecules were also docked within a side pocket under the selectivity filter in the open pore F656-rotated hERG model. This binding pocket was centred above the β-carbon of Y652 and encompassed a volume having a radius of 7 angstroms. Amino acid side chains that comprise the putative canonical binding site and binding pocket were allowed to rotate freely during docking runs to accommodate the drug. Thus, the side chains for the following residues from chain A were allowed to rotate freely: F557, L622, T623, S624, L650, M651, Y652, I655. F656 from chains A and B were also allowed to rotate. Similar settings and parameters were used as above where also 200 docking repeats for each drug were generated and low energy poses were considered.

A further independent set of docking simulations was performed using MOE suite using the Cryo-EM structure and the two open pore models. Fifty docking repeats were performed for each potential antiviral compound in the central cavity binding site. The hERG channel structures were prepared and 3D-protonated followed by performing tethered energy minimization prior to commencement of docking. Docking regions were biased by selection of key residues in the canonical and lateral binding site (namely F656, Y652, T623, S624; and additionally, the following residues: F557, M651) with a further nine angstroms from selection. Energy-minimized (using an all-atom forcefield combining Amber12 and parameterized for small molecules using 2D Extended-Hückel-Theory method). Antiviral ligands were then docked in each of three hERG structures. The GBVI/WSA ΔG scoring function was used which is a forcefield-based scoring function that estimates the free energy of binding of the ligand from a given pose.

The results are visualized using PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.

Functional hERG channels are comprised of four identical protein subunits (designated here A, B, C, D; see **Supplementary Figure 1**). As some drug-channel interactions involved residues from different subunits in places the results text refers to the Chain ID when identifying amino acid and in such cases, the subunit ID is given before the residue ID, (e.g., C:F557). Details of the interactions are described in **Supplementary Figure 1** (see online supplement).

Patch clamp experiments to investigate remdesivir inhibition of hERG ionic current (I_{hERG}) were performed on HEK 293 cells stably expressing WT hERG. Remdesivir (purchased from Medkoo Biosciences) was dissolved in dimethyl sulfoxide (DMSO) to produce a stock solution of 30 mM and was applied at a 1/1,000 dilution (30 μM) in Tyrode's solution. Recordings were made at 37°C (whole cell patch clamp) using an Axopatch 200B amplifier (Molecular Devices) with a CV-4/100 headstage and data acquisition via a Digidata 1320 interface (Molecular Devices). The extracellular superfusate was a standard Tyrode's solution containing (in mM): 140 NaCl, 4 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 5 HEPES (titrated to pH 7.4 with NaOH) (48–50). Patch pipettes (AM-systems Inc, USA) had resistances

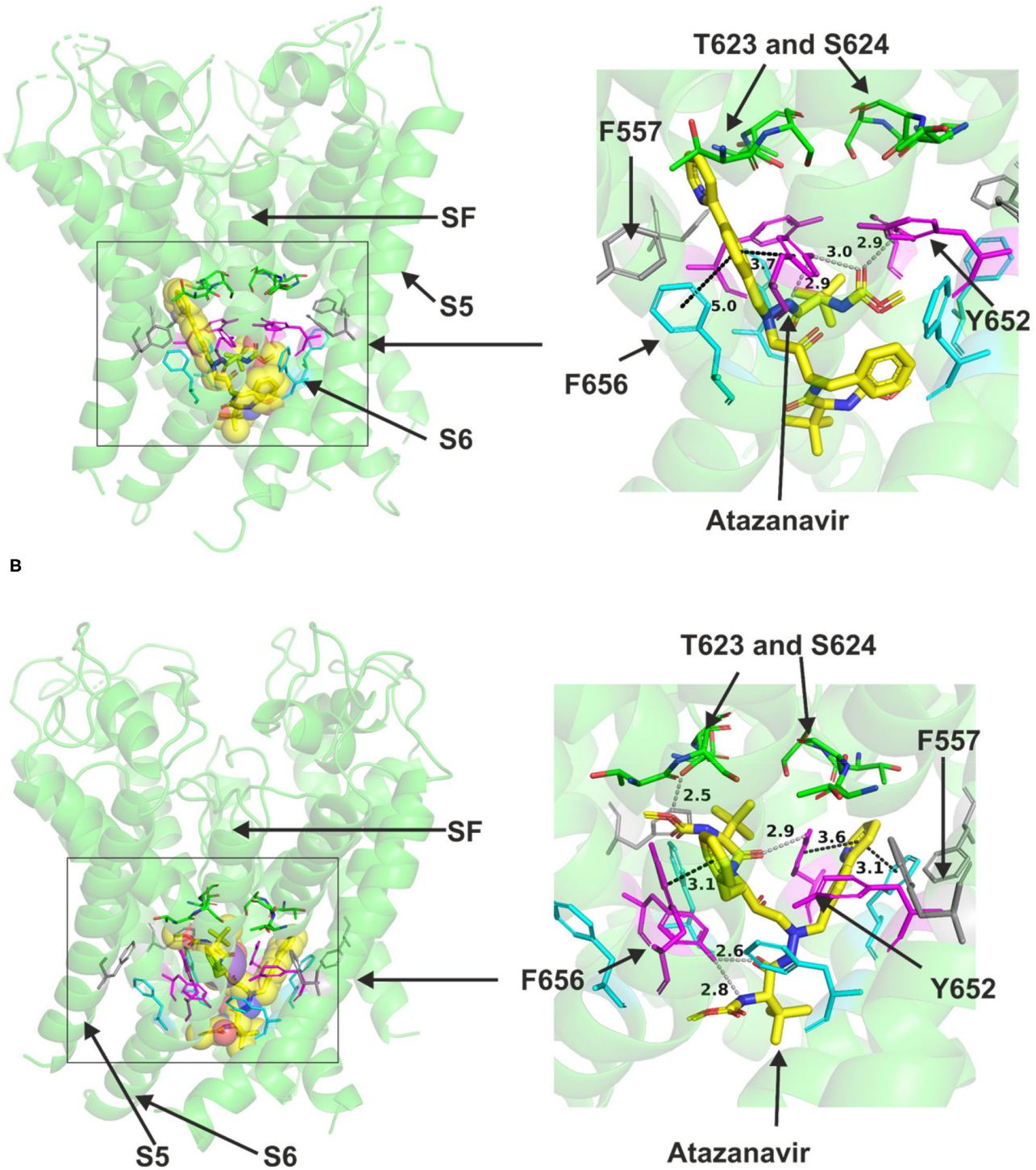


FIGURE 1 | Location of the assigned binding site of atazanavir in the hERG cryo-EM structure and the closely related Dickson et al. model with the drug docked in the configuration shown in the methods section. The complete structure in **(A)** and **(B)** illustrates the location of segment 6 (S6) lining the pore where key residues such as Y652 and F656 are located and the outer S5 segment where F557, a major residue of the side pocket, is located. The selectivity filter region (SF) is also annotated where T623 and S624 sit at the base of the pore helix attached to it. Atazanavir is represented as a space-filling structure in which carbon atoms are represented in yellow, nitrogen in blue, oxygen in red. Major amino acid constituents of the binding sites were shown as sticks. In all structure figures, the hERG pore amino acid side

(Continued)

FIGURE 1 | chains are colored as follows: Phe-557, *gray*; Thr-623 and Ser-624, *green*; Tyr-652, *pink*; and Phe-656, *blue*. Atazanavir is shown in *yellow*. **(A,B)** show low-energy-score pose for atazanavir docked into the hERG pore with docking biased to promote occupation of canonical binding site. Annotations (dotted lines) define potential interactions between drug and amino acid side chains, distances in angstroms between the drug molecule and key residues were written adjacent to each dotted line. **(A)** shows the atazanavir docking in the hERG cryo-EM structure. **(B)**, shows the atazanavir docking in the Dickson et al. model based on hERG cryo-EM structure. This run in the Dickson et al. model is particularly important since rotamers of at least one of Phe-656 side chains was selected to orient the side chain Cα-Cβ bond toward the pore.

of 2–4 MΩ and were filled with a solution containing (in mM): 130 KCl, 1 $MgCl_2$, 5 EGTA, 5 MgATP, and 10 HEPES (titrated to pH 7.2 with KOH) (48–50). Series resistance was typically compensated 60–80%. Currents were filtered at 2 kHz and were digitized at 10 kHz. Data are presented as mean ± SEM of the number of independent experiments indicated (*n*) after analysis.

RESULTS

Atazanavir

Atazanavir could readily be accommodated in the canonical central cavity binding site in hERG open pore structure (**Figure 1**); however, due to its size, it did not fit in hERG closed pore model in which the central cavity became significantly smaller compared to that in the open state. Docking the drug to both the cryo EM structure and the closely related MD-based model of hERG by Dickson et al. suggested that direct binding interactions occur between the molecule and the channel (**Figure 1**). In low energy poses, atazanavir was found in proximity to canonical aromatic residues F656 and Y652 in both models (**Figure 1B**) illustrates this for the Dickson et al. model. This is in good agreement with experimental observations for atazanavir (39). The drug also approached T623 and S624 near the base of the selectivity filter/pore helix. Atazanavir was also able to contact a serine residue (S660) one turn lower than F656 toward the cytoplasmic opening of the channel. Further details of predicted interactions are described in **Supplementary Figure 2**.

Lopinavir and Ritonavir

Lopinavir could be accommodated in the central cavity of the hERG open pore structure and models (**Figure 2**). The drug interacted with the channel mainly via hydrophobic interactions. F656 and Y652 in S6 were able to interact with the drug in the low energy poses in the cryo-EM structure and in the open pore in-house model. However, docking the drug molecule to the open channel model by Dickson et al. showed the possibility that a part of the drug molecule extended toward the side pocket and interact with F557 in S5 (**Figure 2B**). Despite the ability of part of the drug molecule to stretch further to the side pocket, it was still able to contact key residues in the canonical binding site (**Supplementary Figure 3**). The docking also showed the possibility of the drug to form strong hydrogen bonds, mainly with S624, F656, and Y652 residues (**Supplementary Figure 2**).

Ritonavir was also readily accommodated in the canonical binding site of hERG when docked to the Cryo-EM structure or the Dickson et al. model, both representing the open pore state of the channel (**Figure 3**). Docking ritonavir to the cryo-EM structure resulted in association with several central cavity residues including Y652, S660 in S6 and S624 in the pore helix (details are presented in **Supplementary Figure 4**). Docking the drug in the Dickson et al. model revealed slightly different pose with a part of the drug molecule able to advance near the peripheral residue 557 in S5 like lopinavir. In this pose, Y652 was also able to interact with ritonavir. T623, S624 in the pore helix and F656 in S6 could also interact with the drug via hydrogen bonds. Both binding models indicated the ability of Y652 to interact with the sulfur atom in a thiazole group within the ritonavir molecule.

Neither lopinavir nor ritonavir could be docked to the closed pore model of hERG. Attempts were also made to dock each of the two drugs to a side pocket under the selectivity filter in the in-house open pore model but could not be accommodated. However, as introduced, small part of these structures could advance to this binding pocket while the majority parts of the molecules were still in the canonical binding site. Collectively, the docking simulations suggest that both lopinavir and ritonavir can be accommodated in the central cavity of hERG and directly binding to the channel via hydrogen bonds and hydrophobic interactions. The dockings also revealed the possibility that a phenyl group from any of the two drugs might enter a pocket under the selectivity filter and bind to F557.

Remdesivir

Remdesivir could fit into both the canonical site in the central cavity and the side binding pocket in open channel hERG models (**Figure 4**). Docking the drug into the hERG cavity revealed potential binding of the drug to the channel protein via several hydrogen bonds and some hydrophobic interactions. The three docking runs in the channel open conformation (Cryo-EM structure, Dickson et al. and the in-house models) showed the drug can reside in the central cavity and mainly interact with Y652 in S6, L622, and S624 residues near the selectivity filter. The details of docking remdesivir in the cavity of either the open hERG represented by Dickson et al. model or the cryo EM structure are largely similar (details are in **Supplementary Figure 5**). However, distinct from the EM structure, docking to the Dickson et al. model showed the possibility of F656 in S6 interacting with remdesivir. More importantly, docking to the Dickson model showed the potential that part of the remdesivir molecule can advance toward the side pocket and interact with F557 (S5) and M651 (S6) and L622 from the pore helix. These residues are key amino acids in the side pocket (details are shown in **Supplementary Figure 5**.

Remdesivir was also docked to the in-house made open hERG model. When docked to the central cavity binding site, the molecule -as the above- described could be fitted in the cavity.

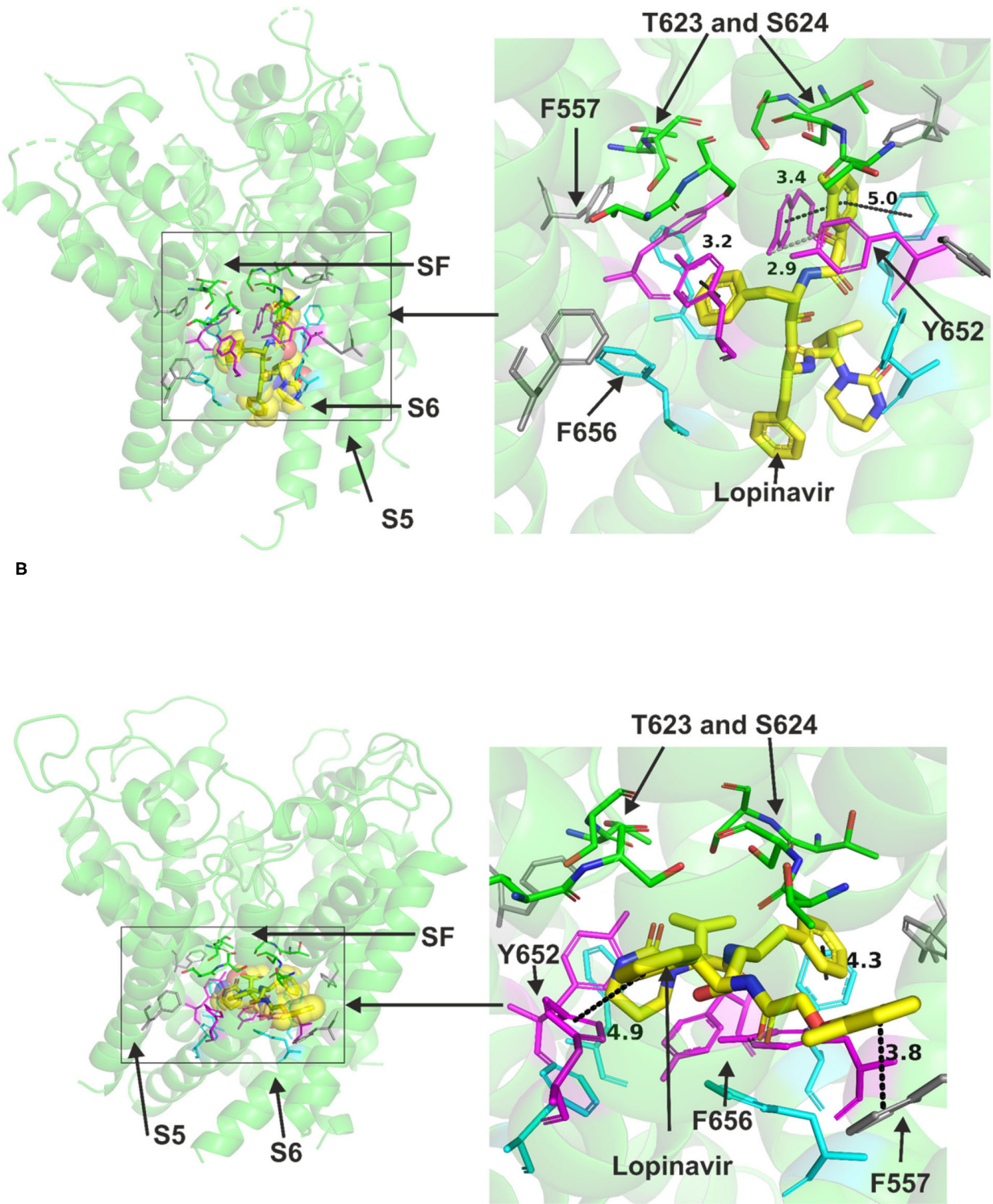


FIGURE 2 | Location of the assigned pore binding site of lopinavir in the hERG cryo-EM structure and the closely related Dickson et al. model with the drug docked in the configuration shown in the methods section. The complete structure in **(A,B)** shows the location of segment 6 (S6) lining the pore where key residues such as

(Continued)

FIGURE 2 | Y652 and F656 are located and the outer S5 segment where F557, a major residue of the side pocket, is located. The selectivity SF was also annotated where T623 and S624 sit at the base of the pore helix attached to it. Lopinavir is represented as a space-filling structure. Major amino acid constituents of the binding sites were shown as sticks. Binding residues and atoms of the drug molecule colored as for **Figure 1**. **(A,B)** show low-energy-score pose for lopinavir docked into the hERG pore with docking biased to promote occupation of the canonical binding site. **(A)** shows the lopinavir docking in the hERG cryo-EM structure. **(B)** shows the lopinavir docking in the Dickson et al. model based on hERG cryo-EM structure. Annotations (dotted lines) define potential interactions between drug and amino acid side chains, distances in **(A)** between the drug molecule and key residues were written adjacent to each dotted line. This run in the Dickson et al. model is particularly important since rotamers of at least one of F656 side chains was selected to orient the side chain Cα-Cβ bond toward the pore.

Interestingly, remdesivir was successfully docked in the side pocket in the in-house open model of hERG. The major aromatic parts of the structure which in previously described runs were residing in the cavity could access the binding pocket in this docking setting and able to interact with F557, L622, and Y652 (further details are in **Supplementary Figure 6**). This pose showed the possibility for remdesivir to be accommodated in and make interactions with the side pocket binding site while the other above three dockings to the open pore structure and models showed the potential interactions of remdesivir with key binding determinant in the central cavity. Like atazanavir, lopinavir, and ritonavir, remdesivir could not be accommodated in the closed hERG channel. In the period following initial submission/review of this report an independent study was published in which an acute inhibitory effect of remdesivir on hERG channels was reported to be absent (51). Therefore, a limited experimental series was conducted here to evaluate the effect of acute application of remdesivir on I_{hERG}. The response to remdesivir was measured using the protocol shown in **Figure 4D**. This was comprised of a 2 s depolarization from −80 to +20 mV, followed by repolarization to −40 mV, at which the resurgent tail current that is typical of hERG was observed (52). I_{hERG} tail magnitude was measured as described previously (48–50, 52). Exemplar traces are shown in **Figure 4D**. 30 μM remdesivir inhibited hERG current by 38 ± 2% ($n = 6$).

Favipiravir

Favipiravir is a very small molecule (MW of 157 g/mol) compared to the other antivirals studied. Due to its small size, favipiravir was readily accommodated within the central cavity of open pore structure and models of hERG and the closed model of the channel (**Figure 5**). It could also fit to the side pocket of the in-house open pore model of hERG. However, favipiravir could only make relatively few binding contacts with the channel in all these dockings. The molecule also appeared relatively distant from key residues (**Supplementary Figure 6**). The residues involved in different poses include T623, S624, and Y652. The binding was slightly improved when docked to the side pocket which involved interaction with F557 (further details are in **Supplementary Figure 7**). Favipiravir also interacted weakly with the channel when docked in the closed model of hERG with the potential to interact with Y652, T623 and S624 residues (see also **Supplementary Figure 7**).

DISCUSSION

Implications of the Findings of This Study

The results of this *in silico* study demonstrate that despite their comparatively large size, atazanavir, lopinavir, ritonavir, and remdesivir can interact with the canonical binding site on the hERG potassium channel. At present there are no *in vitro* mutagenesis data available for lopinavir, ritonavir, and remdesivir to verify that these drugs interact predominantly or solely with the pore binding site on hERG. However, the fact that our simulation data for atazanavir are consistent with experimental data that implicate the aromatic Y652 and F656 residues in hERG channel current (I_{hERG}) blockade (39) provides confidence in the approach adopted here. Furthermore, to ensure the docking performance was consistent across software platforms, we also ran the docking procedure in the MOE suite (with a similar setting to Gold) where we found the docking energy and poses of the top poses were correlated with those in Gold (results not shown).

Our *in silico* data enable predictions to be made that can be addressed in future experimental studies. First, the inability of atazanavir, lopinavir, ritonavir, and remdesivir to interact with pore binding determinants in the closed channel state is consistent with a requirement for gating to occur for these agents to be able to interact with aromatic binding residues. Atazanavir has been reported not to alter voltage dependent activation or inactivation of wild-type (WT) hERG current (I_{hERG}), but protocols to interrogate a requirement for channel opening were not applied (39). Similarly, detailed interrogation of the kinetics of lopinavir/ritonavir inhibition was not conducted (30). The results of our docking simulations suggest that it is likely that, with the potential exception of favipiravir, the drugs studied here can only access key binding determinants on channel gating; this should manifest in a measurable time-dependence of inhibition on channel opening.

The reported hERG current IC_{50} for atazanavir inhibition of I_{hERG} is 5.7 μM (39), whilst those for lopinavir and ritonavir are similar, being, respectively, 8.6 and 6.2 μM (30). In documents considered by the European Medicines Agency (EMA) early during the Covid-19 pandemic, for the compassionate licensing of remdesivir, its hERG IC_{50} is given as 28.9 μM, which is 26-fold the estimated free drug concentration (C_{max}) of 1.1 μM at the proposed maximal clinical dose (53). However, after submission of this study, an independent report was published claiming that remdesivir does not produce an acute inhibition of I_{hERG} at 10 or 50 μM (51). In the same study, chronic application of remdesivir led to increased hERG expression and I_{hERG} amplitude, consistent with a potential for the drug to promote hERG channel trafficking (51). The ability of drugs to rescue misprocessed mutant hERG channels has previously been linked to hydrophobic interactions within the pore-cavity

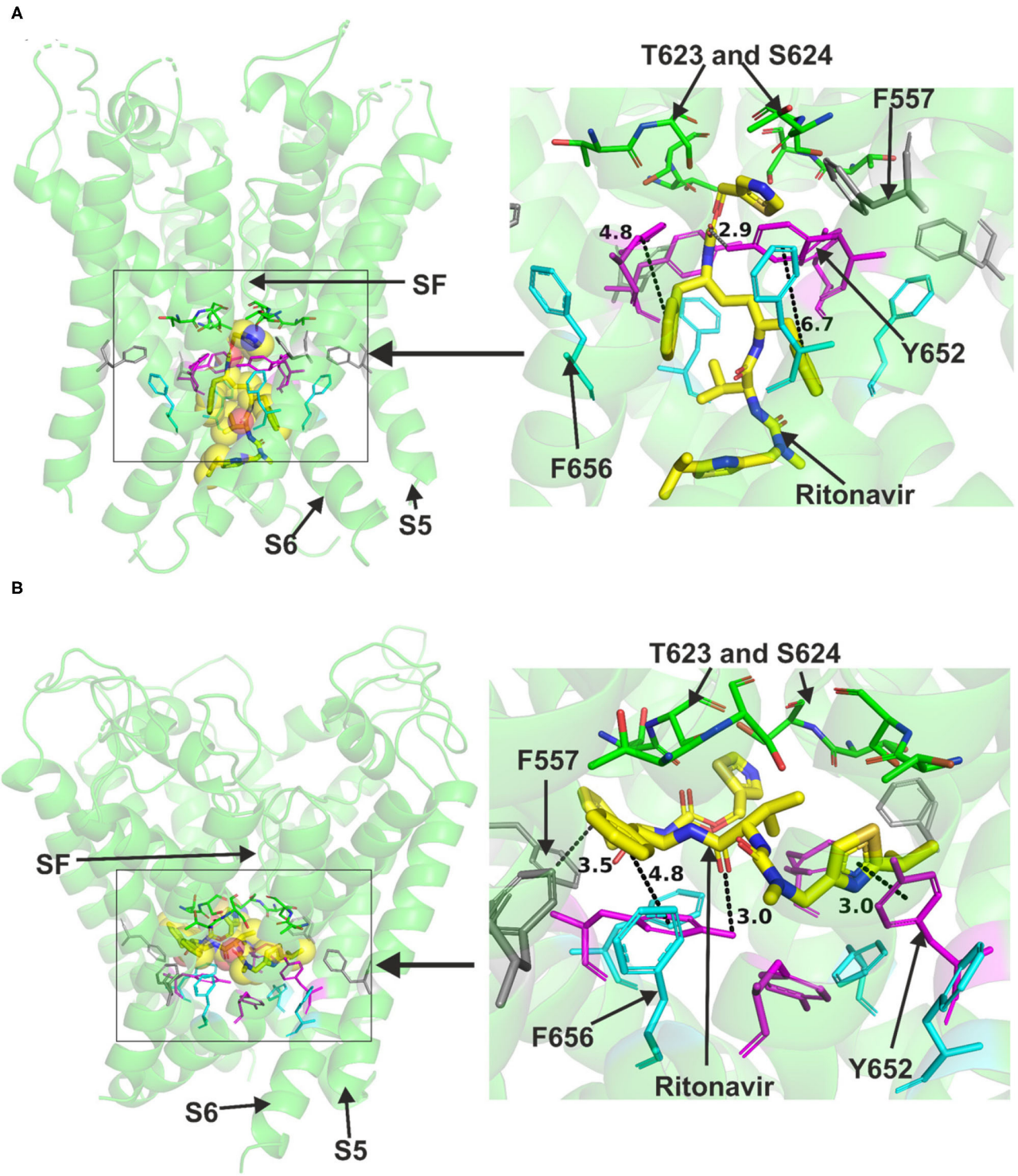


FIGURE 3 | Location of the assigned pore binding site of ritonavir in the hERG cryo-EM structure and the closely related Dickson et al. model with the drug docked in the configuration shown in the methods section. The complete structure in **(A,B)** shows the location of segment 6 (S6) lining the pore where key residues such as Y652 and F656 are located and the outer S5 segment where F557, a major residue of the side pocket, is located. The selectivity SF was also annotated where T623 and S624 sit at the base of the pore helix attached to it. Ritonavir is represented as a space-filling *yellow* surface. Major amino acid constituents of the binding sites were shown as sticks in the box. Binding residues and atoms of drug molecule colored as for **Figure 1**. **(A,B)** show low-energy-score pose for ritonavir docked into the hERG pore with docking biased to promote occupation of canonical binding site. Annotations (dotted lines) define potential interactions between drug and amino

(Continued)

FIGURE 3 | acid side chains, distances in **(A)** between the drug molecule and key residues were written adjacent to each dotted line. **(A)** Shows the ritonavir docking in the hERG cryo-EM structure. *B* Shows the ritonavir docking in the Dickson et al. model based on hERG cryo-EM structure. This run in the Dickson et al. model is particularly important since rotamers of at least one of F656 side chains was selected to orient the side chain Cα-Cβ bond toward the pore.

(54); thus, trafficking promotion by remdesivir without an ability to produce acute block would be highly notable. In our experiments, we observed 38% inhibition of I_{hERG} by 30 μM remdesivir, which is in fair agreement with the inhibitory potency in documents submitted to the EMA (53) and is inconsistent with a lack of acute I_{hERG} inhibition reported in (51). I_{hERG} inhibitory potencies of drugs can vary significantly depending on experimental temperature and stimulus waveform [e.g., (55, 56)]. Our measurements were made at 37°C, whilst those in (51) were made at room temperature, although whether or not this may account for the differences in respect of remdesivir is unclear. On the basis of comparison of I_{hERG} IC_{50} values and therapeutic C_{max} values of a broad range of drugs in relation to TdP risk, Redfern et al. proposed in 2003 a 30-fold safety margin for drugs undergoing clinical evaluation (57). A recent re-evaluation of the hERG safety margin for QT_c prolongation suggested an optimal margin of 50-fold (58). The safety margin for remdesivir may not exceed this value.

The single Ebola patient who experienced mild QT interval prolongation on favipiravir received multiple other drug treatments and experienced cardiac effusion (34); the factors that may have sensitized this patient to QT prolongation following favipiravir administration are unclear. In adult healthy volunteers, subjects oral dosing with 1,200 or 2,400 mg of favipiravir did not affect QT or QT_c intervals (33). There are currently no peer reviewed, published data on I_{hERG} inhibition by favipiravir. However, publicly available information at the Japanese Pharmaceuticals and Medical Devices Agency (PDMA) suggests no inhibitory effects of favipiravir on I_{hERG} at 40 or 200 μM and only an ∼8% reduction at 1,000 μM (which concentration was described as ∼3 times the human C_{max}) (59). Although no experimental details are available for this information, it is suggestive of a low propensity of favipiravir to produce a pharmacological block of hERG channels, which is borne out by the docking simulation results in the present study. Drug size has previously been observed to be a significant determinant of inhibitory potency when comparing drugs of different sizes that share structural similarity. Thus, in a direct comparison, the I_{hERG} IC_{50} value of the antianginal and antiarrhythmic agent ranolazine was ∼16 fold lower than that of structurally similar, but smaller lidocaine (48); the difference was attributable to the fact that ranolazine was able to form a greater range of interactions with hERG pore residue side chains than was lidocaine (48). Whilst it is important that the effects of favipiravir on I_{hERG} are established under a known, standardized set of conditions and compared with other candidate antivirals, it seems likely that the small size of favipiravir may be advantageous in conferring comparatively low hERG liability.

Relevance to Interrogation of Interactions of Drug Molecules With the hERG Pore Structure Determined With Cryo-EM

The publication of the cryo-EM structure revealed two unexpected structural features of the hERG channel: first, the central pore cavity of the channel was found to have a smaller volume compared to that assumed from homology modeling; second, four deep hydrophobic pockets surrounding the cavity were identified that could provide drug interaction sites (40, 41, 60). However, the cryo-EM structure represents a single, fixed hERG conformation and, at least for some drug molecules it has been difficult to recapitulate aspects of experimental mutagenesis data using the original cryo-EM structure (47, 61). For example, high potency I_{hERG} inhibition by the minimally structured hERG inhibitor "Cavalli-2" showed a strong sensitivity to mutation of F566, but in the cryo-EM structure the aromatic side chain of this residue was oriented away from the cavity (47). Reconciliation of docking with mutagenesis results required a small clockwise rotation of the S6 helix to optimize F656 residue orientations compatible with high affinity inhibition block (47). Here we employed both the original and modified cryo-EM structures. The use of different models produced a common outcome in that they all supported the ability of the antivirals studied to interact with the pore binding site; however, some drug- and model-specific observations were made. For example, of the larger antiviral molecules studied only remdesivir showed a propensity to interact with the lateral binding pockets surrounding the central cavity and there was a marked difference between interactions with residues in this region observed using the MD based Dickson model (42) and the original cryo-EM structure (40). For ritonavir, the use of the Dickson model allowed the drug to be in close proximity to F557 [a residue implicated in binding of a number of drugs (47, 62–64)]. The future experimental investigation of pore cavity and lateral binding pocket residue mutants should be able to identify which of the different binding modes predicted here most accurately describes drug-channel interactions and whether or not any particular channel structure is used for the docking here outperforms the others in matching experimental observations. Moreover, the inability of atazanavir, lopinavir, and ritonavir to reside in the lateral pockets of the cryo-EM structure, should make these drugs valuable for comprehensive (alanine-scanning) mapping of binding to the channel pore, with a general lack of responsiveness to mutation of residues predicted to line the lateral pockets.

Limitations and Conclusions

This study was conducted almost entirely *in silico* and was designed to investigate potential interactions between the selected drugs and hERG only with the canonical drug binding site and lateral pockets that can inform future experimental

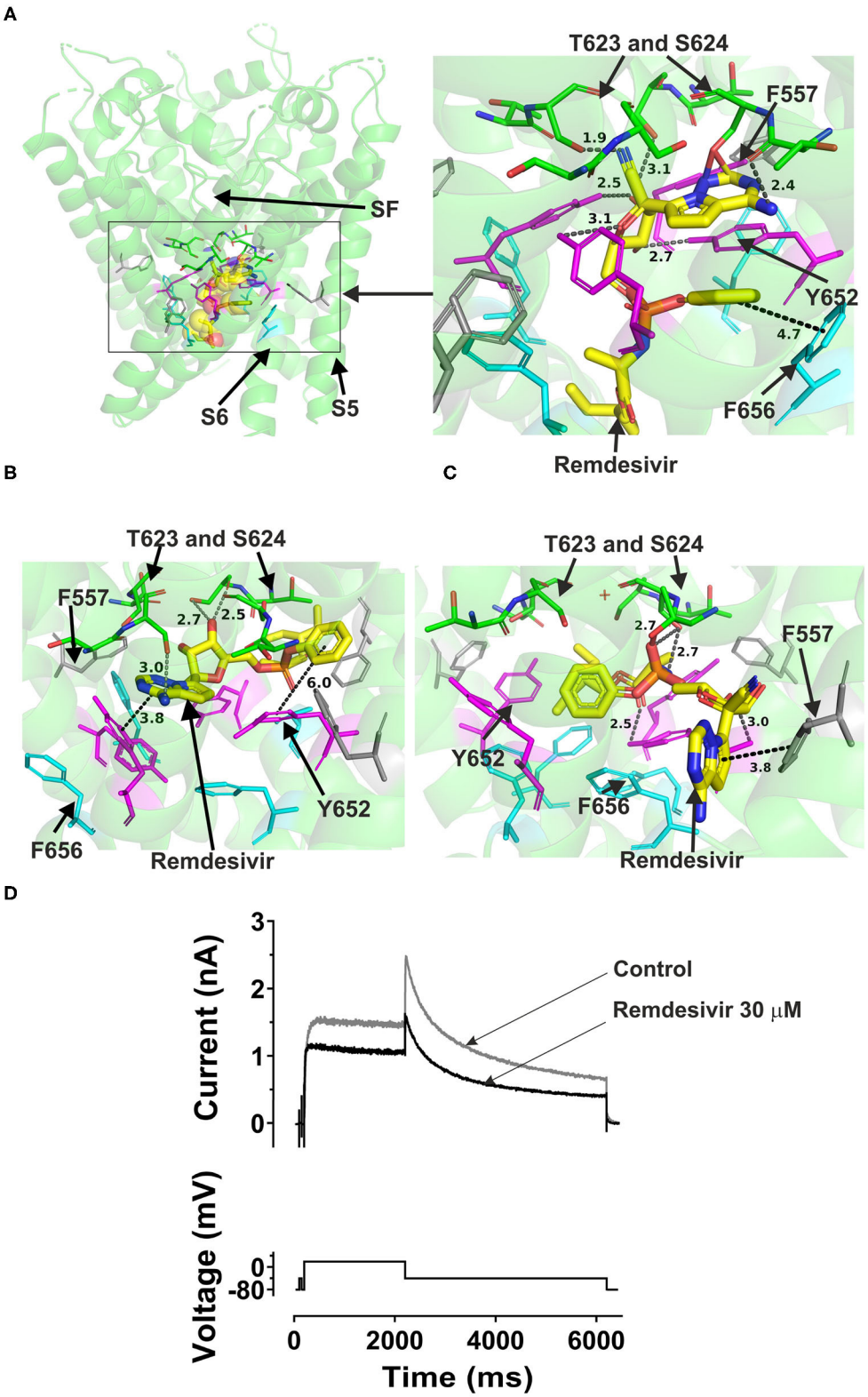


FIGURE 4 | Location of the assigned pore binding site of remdesivir in the hERG cryo-EM structure and the closely related Dickson et al. model and the house MD model with the drug docked in the configuration shown in the methods section. The complete structure in **(A)** shows the location of segment 6 (S6) lining the pore

(Continued)

FIGURE 4 | where key residues such as Y652 and F656 are located and the outer S5 segment where F557, a major residue of the side pocket, is located. The selectivity SF was also annotated where T623 and S624 sit at the base of the pore helix attached to it. Remdesivir is represented as a space-filling *yellow* surface. Major amino acid constituents of the binding sites were shown as sticks. Binding residues and atoms of drug molecule colored as for **Figure 1**. **(A,B)** show low-energy-score pose for remdesivir docked into the hERG pore with docking biased to promote occupation of canonical binding site. Annotations (dotted lines) define potential interactions between drug and amino acid side chains, distances in A between the drug molecule and key residues were written adjacent to each dotted line. **(C)** shows the low-energy-score pose for remdesivir docked into the hERG pore with docking biased to promote occupation of the side pocket. **(A)** Shows the remdesivir docking in the hERG cryo-EM structure. **(B)** shows the remdesivir docking in the Dickson et al. model based on hERG cryo-EM structure. This run in the Dickson et al. model is particularly important since rotamers of at least one of Phe-656 side chains was selected to orient the side chain Cα-Cβ bond toward the pore. **(C)** shows the remdesivir docking in the in house MD hERG model where the drug was docked in the region of the side pocket. **(D)** I_{hERG} during superfusion with control (Tyrode's) solution and during application of 30 μM of remdesivir. I_{hERG} was elicited by a voltage protocol shown as lower traces, comprised of a 2 s depolarizing pulse to +20 mV, followed by repolarization to −40 mV. Thirty micomolar remdesivir inhibited I_{hERG} tails, producing a fractional block 0.38 ± 0.02, (i.e., a mean tail current amplitude reduction of 38%; $n = 6$).

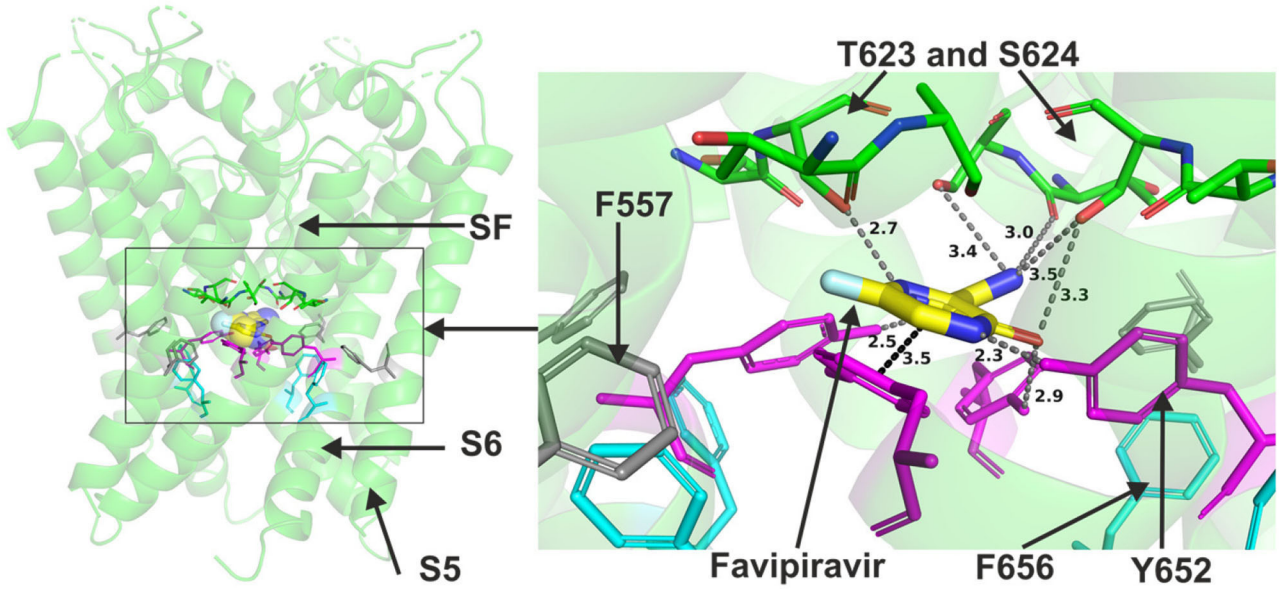


FIGURE 5 | Location of the assigned binding site of favipiravir in the hERG cryo-EM structure. The complete structure shows the location of segment 6 (S6) lining the pore where key residues such as Y652 and F656 are located and the outer S5 segment where F557, a major residue of the side pocket, is located. The selectivity SF was also annotated where T623 and S624 sit at the base of the pore helix attached to it. Favipiravir is represented as a space-filling surface. Major amino acid constituents of the binding sites were shown as sticks in the box. Binding residues and atoms of drug molecule colored as for **Figure 1**. The figure shows a low-energy-score pose for favipiravir docked into the hERG pore with docking biased to promote occupation of canonical binding site. Annotations (dotted lines) define potential interactions between drug and amino acid side chains, distances in A between the drug molecule and key residues were written adjacent to each dotted line.

studies. Given the comparatively large size of most of the drugs studied, we cannot preclude the potential for (additional) interactions outside the channel pore, as may occur for macrolide antibiotics (65).

hERG liability is a very important consideration but not the only one in the evaluation of pro-arrhythmic risk with clinically used drugs. Potential drug effects on other channels that might mitigate the effects of hERG block need to be considered for an overall evaluation of cardiac risk (36, 37, 66). Whilst it is important to acknowledge these limitations, the strengths of the present study are that it: (i) highlights the potential for all the drugs studied here to interact with hERG; (ii) provides specific observations that can form the basis for experimental hypothesis formation and testing; and consequently (iii) provides a valuable basis from which future experimental investigation of both hERG inhibition and overall cardiac arrhythmia liability can be tested. This may be particularly important for remdesivir and favipiravir, given their potential as COVID-19 treatments. Indeed, the present study usefully complements a recent independent investigation that has used a combination of predictive indices for drug-induced LQTS (though not structural modeling as conducted here) to evaluate risks with potential COVID-19 treatments, on the basis of which it has recommended close monitoring of QT/QT_c intervals in patients receiving both drugs (67). On the basis of our observations, we suggest that a direct *in vitro* experimental comparison would be informative of I_{hERG} inhibitory potency between remdesivir, atazanavir, lopinavir, and ritonavir and favipiravir under a standardized set of conditions; this would aid further evaluation of likely I_{hERG} safety margin. Those data could usefully be combined with further acute and

chronic channel assays employing additional key ventricular ion channels and action potential repolarization measurements to arrive at an integrated preclinical risk evaluation. Finally, it should be noted that whilst the motivation for this study arose from ongoing efforts toward the repurposing of the drugs studied here for COVID-19, any implications for cardiac safety also have wider relevance for the use of these agents in the treatment of other infectious conditions.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

JH and EA-M conceived and designed the study and drafted the manuscript. EA-M and MS conducted and analyzed the docking simulations. EA-M conducted patch clamp recording. All authors revised the manuscript.

FUNDING

The authors thank the British Heart Foundation for funding (PG/17/77/33125, PG/17/89/33414, and PG/20/10252).

ACKNOWLEDGMENTS

The authors thank Dr. Christopher Dempsey for invaluable discussion and training.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.645172/full#supplementary-material

REFERENCES

1. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet.* (2020) 397:99–111. doi: 10.1016/S0140-6736(20)32661-1
2. Singh JA, Upshur REG. The granting of emergency use designation to COVID-19 candidate vaccines: implications for COVID-19 vaccine trials. *Lancet Infect Dis.* (2020) 21:e103–9. doi: 10.1016/S1473-3099(20)30923-3
3. Mahase E. Covid-19: moderna applies for US and EU approval as vaccine trial reports 94.1% efficacy. *BMJ.* (2020) 371:m4709. doi: 10.1136/bmj.m4709
4. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med.* (2020) 383:2603–15. doi: 10.1056/NEJMoa2034577
5. Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection *in vitro*. *Cell Discov.* (2020) 6:16. doi: 10.1038/s41421-020-0156-0
6. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) *in vitro*. *Cell Res.* (2020) 30:269–71. doi: 10.1038/s41422-020-0282-0
7. Gao J, Tian Z, Yang X. Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends.* (2020) 14:72–3. doi: 10.5582/bst.2020.01047
8. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents.* (2020) 56:105949. doi: 10.1016/j.ijantimicag.2020.105949
9. Million M, Lagier JC, Gautret P, Colson P, Fournier PE, Amrane S, et al. Full-length title: early treatment of COVID-19 patients with hydroxychloroquine and azithromycin: A retrospective analysis of 1061 cases in Marseille, France. *Travel Med Infect Dis.* (2020) 35:101738. doi: 10.1016/j.tmaid.2020.101738
10. Geleris J, Sun Y, Platt J, Zucker J, Baldwin M, Hripcsak G, et al. Observational study of hydroxychloroquine in hospitalized patients with Covid-19. *N Engl J Med.* (2020) 382:2411–8. doi: 10.1056/NEJMoa2012410
11. Magagnoli J, Narendran S, Pereira F, Cummings T, Hardin JW, Sutton SS, et al. Outcomes of hydroxychloroquine usage in United States veterans hospitalized with Covid-19. *medRXiv [Preprint].* (2020). doi: 10.1101/2020.04.16.20065920
12. Mehra MR, Desai SS, Ruschitzka F, Patel AN. Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis. *Lancet.* 395:1820. doi: 10.1016/S0140-6736(20)31324-6
13. Szekely Y, Lichter Y, Shrkihe BA, Bruck H, Oster HS, Viskin S. Chloroquine-induced torsade de pointes in a COVID-19 patient. *Heart Rhythm.* (2020) 17:1452–5. doi: 10.1016/j.hrthm.2020.04.046
14. Borba MGS, Val FFA, Sampaio VS, Alexandre MAA, Melo GC, Brito M, et al. Effect of high vs low doses of chloroquine diphosphate as adjunctive therapy for patients hospitalized with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection: a randomized clinical trial. *JAMA Netw Open.* (2020) 3:e208857. doi: 10.1001/jamanetworkopen.2020.8857
15. Martinez MA. Compounds with therapeutic potential against novel respiratory 2019. coronavirus. *Antimicrob Agents Chemother.* (2020) 64:e00399-20. doi: 10.1128/AAC.00399-20
16. Lu CC, Chen MY, Chang YL. Potential therapeutic agents against COVID-19: What we know so far. *J Chin Med Assoc.* (2020) 83:534–6. doi: 10.1097/JCMA.0000000000000318
17. Beck BR, Shin B, Choi Y, Park S, Kang K. Predicting commercially available antiviral drugs that may act on the novel coronavirus (SARS-CoV-2) through a drug-target interaction deep learning model. *Comput Struct Biotechnol J.* (2020) 18:784–90. doi: 10.1016/j.csbj.2020.03.025
18. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, et al. A trial of lopinavir-ritonavir in adults hospitalized with severe Covid-19. *N Engl J Med.* (2020) 382:1787–99. doi: 10.1056/NEJMc2008043
19. Jorgensen SC, Kebriaei R, Dresser LD. Remdesivir: review of pharmacology, pre-clinical data and emerging clinical experience for COVID-19. *Pharmacotherapy.* (2020) 40:659–71. doi: 10.1002/phar.2429
20. Wang Y, Zhang D, Du G, Du R, Zhao J, Jin Y, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet.* (2020) 395:1569–78. doi: 10.1016/S0140-6736(20)31022-9
21. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the treatment of Covid-19 - preliminary report. *N Engl J Med.* (2020) 383:1813–26. doi: 10.1056/NEJMoa2007764
22. McKee DL, Sternberg A, Stange U, Laufer S, Naujokat C. Candidate drugs against SARS-CoV-2 and COVID-19. *Pharmacol Res.* (2020) 157:104859. doi: 10.1016/j.phrs.2020.104859
23. Driouich JS, Cochin M, Lingas G, Moureau G, Touret F, Petit PR, et al. Favipiravir antiviral efficacy against SARS-CoV-2 in a hamster model. *Nat Commun.* (2021) 12:1735. doi: 10.1038/s41467-021-21992-w
24. Cai Q, Yang M, Liu D, Chen J, Shu D, Xia J, et al. Experimental treatment with favipiravir for COVID-19: an open-label control study. *Engineering.* (2020) 6:1192–8. doi: 10.1016/j.eng.2020.03.007
25. Wadaa-Allah A, Emhamed MS, Sadeq MA, Ben Hadj DN, Ullah I, Farrag NS, et al. Efficacy of the current investigational drugs for the

treatment of COVID-19: a scoping review. *Ann Med.* (2021) 53:318–34. doi: 10.1080/07853890.2021.1875500
26. Giudicessi JR, Noseworthy PA, Friedman PA, Ackerman MJ. Urgent guidance for navigating and circumventing the QTc-prolonging and torsadogenic potential of possible pharmacotherapies for coronavirus disease 19 (COVID-19). *Mayo Clin Proc.* (2020) 95:1213–21. doi: 10.1016/j.mayocp.2020.03.024
27. Carpenter A, Chambers OJ, El HA, Bond R, Hanington O, Harmer SC, et al. COVID-19 management and arrhythmia: risks and challenges for clinicians treating patients affected by SARS-CoV-2. *Front Cardiovasc Med.* (2020) 7:85. doi: 10.3389/fcvm.2020.00085
28. Naksuk N, Lazar S, Peeraphatdit TB. Cardiac safety of off-label COVID-19 drug therapy: a review and proposed monitoring protocol. *Eur Heart J Acute Cardiovasc Care.* (2020) 9:215–21. doi: 10.1177/2048872620922784
29. Durante-Mangoni E, Andini R, Bertolino L, Mele F, Florio LL, Murino P, et al. Early experience with remdesivir in SARS-CoV-2 pneumonia. *Infection.* (2020) 48:779–82. doi: 10.1007/s15010-020-01448-x
30. Anson BD, Weaver JG, Ackerman MJ, Akinsete O, Henry K, January CT, et al. Blockade of HERG channels by HIV protease inhibitors. *Lancet.* (2005) 365:682–6. doi: 10.1016/S0140-6736(05)70936-3
31. Ly T, Ruiz ME. Prolonged QT interval and torsades de pointes associated with atazanavir therapy. *Clin Infect Dis.* (2007) 44:e67–8. doi: 10.1086/511875
32. Atazanavir: new indication. First-line treatment: fewer gastrointestinal disorders but more cases of jaundice and a risk of torsades de pointes. *Prescrire Int*. (2009). 18:104.
33. Kumagai Y, Murakawa Y, Hasunuma T, Aso M, Yuji W, Sakurai T, et al. Lack of effect of favipiravir, a novel antiviral agent, on QT interval in healthy Japanese adults. *Int J Clin Pharmacol Ther.* (2015) 53:866–74. doi: 10.5414/CP202388
34. Chinello P, Petrosillo N, Pittalis S, Biava G, Ippolito G, Nicastri E. QTc interval prolongation during favipiravir therapy in an Ebolavirus-infected patient. *PLoS Negl Trop Dis.* (2017) 11:e0006034. doi: 10.1371/journal.pntd.0006034
35. Sanguinetti MC, Tristani-Firouzi M. hERG potassium channels and cardiac arrhythmia. *Nature.* (2006) 440:463–9. doi: 10.1038/nature04710
36. Hancox JC, McPate MJ, El Harchi A, Zhang YH. The hERG potassium channel and hERG screening for drug-induced torsades de pointes. *Pharmacol Therap.* (2008) 119:118–32. doi: 10.1016/j.pharmthera.2008.05.009
37. Gintant GA. Preclinical Torsades-de-Pointes screens: advantages and limitations of surrogate and direct approaches in evaluating proarrhythmic risk. *Pharmacol Ther.* (2008) 119:199–209. doi: 10.1016/j.pharmthera.2008.04.010
38. Aromolaran AS, Srivastava U, Ali A, Chahine M, Lazaro D, El-Sherif N, et al. Interleukin-6 inhibition of hERG underlies risk for acquired long QT in cardiac and systemic inflammation. *PLoS ONE.* (2018) 13:e0208321. doi: 10.1371/journal.pone.0208321
39. Han SN, Sun XY, Zhang Z, Zhang LR. The protease inhibitor atazanavir blocks hERG K^+ channels expressed in HEK293 cells and obstructs hERG protein transport to cell membrane. *Acta Pharmacol Sin.* (2015) 36:454–62. doi: 10.1038/aps.2014.165
40. Wang W, MacKinnon R. Cryo-EM structure of the open human ether-a-go-go-related K^+ channel hERG. *Cell.* (2017) 169:422–30. doi: 10.1016/j.cell.2017.03.048
41. Butler A, Helliwell MV, Zhang Y, Hancox JC, Dempsey CE. An update on the structure of hERG. *Front Pharmacol.* (2019) 10:1572. doi: 10.3389/fphar.2019.01572
42. Dickson CJ, Velez-Vega C, Duca JS. Revealing molecular determinants of hERG blocker and activator binding. *J Chem Inf Model.* (2020) 60:192–203. doi: 10.1021/acs.jcim.9b00773
43. Webb B, Sali A. Comparative protein structure modeling using MODELLER. *Curr Protoc Bioinformatics.* (2016) 54:5. doi: 10.1002/cpbi.3
44. Colenso CK, Sessions RB, Zhang YH, Hancox JC, Dempsey CE. Interactions between voltage sensor and pore domains in a hERG K^+ channel model from molecular simulations and the effects of a voltage sensor mutation. *J Chem Inf Model.* (2013) 53:1358–70. doi: 10.1021/ci4000739
45. Lindorff-Larsen K, Piana S, Palmo K, Maragakis P, Klepeis JL, Dror RO, et al. Improved side-chain torsion potentials for the Amber ff99SB protein force field. *Proteins.* (2010) 78:1950–8. doi: 10.1002/prot.22711
46. Jambeck JP, Lyubartsev AP. An extension and further validation of an all-atomistic force field for biological membranes. *J Chem Theory Comput.* (2012) 8:2938–48. doi: 10.1021/ct300342n
47. Helliwell MV, Zhang Y, El Harchi A, Du C, Hancox JC, Dempsey CE. Structural implications of hERG K^+ channel block by a high affinity minimally-structured blocker. *J Biol Chem.* (2018) 293:7040–57. doi: 10.1074/jbc.RA117.000363
48. Du C, Zhang Y, El Harchi A, Dempsey CE, Hancox JC. Ranolazine inhibition of hERG potassium channels: drug-pore interactions and reduced potency against inactivation mutants. *J Mol Cell Cardiol.* (2014) 74C:220–30. doi: 10.1016/j.yjmcc.2014.05.013
49. Melgari D, Brack KE, Zhang C, Zhang Y, El Harchi A, Mitcheson JS, et al. hERG potassium channel blockade by the HCN channel inhibitor bradycardic agent ivabradine. *J Am Heart Assoc.* (2015) 4:e001813. doi: 10.1161/JAHA.115.001813
50. Zhang Y, Colenso CK, El Harchi A, Cheng H, Witchel HJ, Dempsey CE, et al. Interactions between amiodarone and the hERG potassium channel pore determined with mutagenesis and in silico docking. *Biochem Pharmacol.* (2016) 113:24–35. doi: 10.1016/j.bcp.2016.05.013
51. Szendrey M, Guo J, Li W, Yang T, Zhang S. COVID-19 drugs chloroquine and hydroxychloroquine, but not azithromycin and remdesivir, block hERG potassium channels. *J Pharmacol Exp Ther.* (2021) 377:265–72. doi: 10.1124/jpet.120.000484
52. Al Moubarak E, Zhang Y, Dempsey CE, Zhang H, Harmer SC, Hancox JC. Serine mutation of a conserved threonine in the hERG K^+ channel S6-pore region leads to loss-of-function through trafficking impairment. *Biochem Biophys Res Comm*. (2020) 526:1085–91. doi: 10.1016/j.bbrc.2020.04.003
53. Human Medicines Division EMA *Summary on Compassionate Use.* (2020). Available online at: https://www.ema.europa.eu/en/documents/other/summary-compassionate-use-remdesivir-gilead_en.pdf EMEA/H/K/5622/CU, 1-43 (accessed April 16, 2021).
54. Ficker E, Obejero-Paz CA, Zhao S, Brown AM. The binding site for channel blockers that rescue misprocessed human long QT syndrome type 2 ether-a-gogo-related gene (HERG) mutations. *J Biol Chem.* (2002) 277:4989–98. doi: 10.1074/jbc.M107345200
55. Kirsch GE, Trepakova ES, Brimecombe JC, Sidach SS, Erickson HD, Kochan MC, et al. Variability in the measurement of hERG potassium channel inhibition: effects of temperature and stimulus pattern. *J Pharmacol Toxicol Methods.* (2004) 50:93–101. doi: 10.1016/j.vascn.2004.06.003
56. Milnes JT, Witchel HJ, Leaney JL, Leishman DJ, Hancox JC. Investigating dynamic protocol-dependence of hERG potassium channel inhibition at 37 degrees C: Cisapride versus dofetilide. *J Pharmacol Toxicol Methods.* (2010) 61:178–91. doi: 10.1016/j.vascn.2010.02.007
57. Redfern WS, Carlsson L, Davis AS, Lynch WG, MacKenzie I, Palethorpe S, et al. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovas Res.* (2003) 58:32–45. doi: 10.1016/S0008-6363(02)00846-5
58. Leishman DJ, Abernathy MM, Wang EB. Revisiting the hERG safety margin after 20 years of routine hERG screening. *J Pharmacol Toxicol Methods.* (2020) 105:106900. doi: 10.1016/j.vascn.2020.106900
59. Anon. *Report on the Deliberation Results*. Available online at: https://www.pmda.go.jp/files/000210319.pdf, 1-172. 4-3-2014 (accessed April 16, 2021).
60. Robertson GA, Morais-Cabral JH. hERG function in light of structure. *Biophys J.* (2019) 118:790–7. doi: 10.1016/j.bpj.2019.10.010
61. Cernuda B, Fernandes CT, Allam SM, Orzillo M, Suppa G, Chia CZ, et al. The molecular determinants of R-roscovitine block of hERG channels. *PLoS ONE.* (2019) 14:e0217733. doi: 10.1371/journal.pone.0217733
62. Saxena P, Zangerl-Plessl EM, Linder T, Windisch A, Hohaus A, Timin E, et al. New potential binding determinant for hERG channel inhibitors. *Sci Rep.* (2016) 6:24182. doi: 10.1038/srep24182
63. Duff HJ, Noskov SY, Muruve D, Perlovic G, Ol KM, Sharapova A, et al. The pore-lipid interface: role of amino acid determinants of lipophilic access by ivabradine to the hERG1 pore domain. *Mol Pharmacol.* (2019) 96:259–71. doi: 10.1124/mol.118.115642
64. Cheng H, Du C, Zhang Y, James AF, Dempsey CE, Abdala AP, et al. Potent hERG channel inhibition by sarizotan, an investigative treatment for Rett Syndrome. *J Mol Cell Cardiol.* (2019) 135:22–30. doi: 10.1016/j.yjmcc.2019.07.012

65. Duncan RS, Ridley JM, Dempsey CE, Leishman DJ, Leaney JL, Hancox JC, et al. Erythromycin block of the HERG K^+ channel: accessibility to F656 and Y652. *Biochem Biophys Res Comm.* (2006) 341:500–6. doi: 10.1016/j.bbrc.2006.01.008
66. Vicente J, Zusterzeel R, Johannesen L, Ochoa-Jimenez R, Mason JW, Sanabria C, et al. Assessment of multi-ion channel block in a Phase I Randomized study design: results of the CiPA phase I ECG biomarker validation study. *Clin Pharmacol Ther.* (2019) 105:943–53. doi: 10.1002/cpt.1303
67. Michaud V, Dow P, Al Rihani SB, Deodhar M, Arwood M, Cicali B, et al. Risk assessment of drug-induced long QT syndrome for some COVID-19 repurposed drugs. *Clin Transl Sci.* (2021) 14:20–8. doi: 10.1111/cts.12882

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

CASE REPORT
published: 12 May 2021
doi: 10.3389/fcvm.2021.671669

Case Report: Changes of Vascular Reactivity and Arterial Stiffness in a Patient With Covid-19 Infection

Philipp Jud[1]*, *Harald H. Kessler*[2] *and Marianne Brodmann*[1]

[1] Division of Angiology, Department of Internal Medicine, Medical University of Graz, Graz, Austria, [2] Diagnostic and Research Institute of Hygiene, Microbiology, and Environmental Medicine, Medical University of Graz, Graz, Austria

Covid-19 infection may be associated with a higher incidence developing cardiovascular complications, however, the underlying mechanisms contributing to cardiovascular complications are largely unknown, while endothelial cell damage may be present. We want to report a 24-year-old woman with Covid-19 infection who had undergone measurements of vascular reactivity and arterial stiffness, including flow-mediated dilation (FMD), nitroglycerin-mediated dilation (NMD), aortic pulse wave velocity (PWV), augmentation index and carotid intima-media-thickness (cIMT) at the time when Covid-19 was diagnosed. Reduced FMD of 0.0% and NMD of 15.5% were observed, while PWV (5.9 m/s), Aix (27%) and cIMT with 0.4 mm of both common carotid arteries were unremarkable. Repeated measurements of FMD, NMD, PWV, Aix, and cIMT 6 weeks after Covid-19 infection revealed persistently reduced FMD (0.0%), while NMD (17.24%), PWV (5.6 m/s) and augmentation index (13%) ameliorated. This case suggests potential impact of Covid-19 infection on endothelial function, also in young Covid-19 patients without any co-morbidity.

Keywords: Covid-19, endothelial dysfunction, vascular reactivity, arterial stiffness, vasculopathy

OPEN ACCESS

Edited by:
Shuyang Zhang,
Peking Union Medical College Hospital, China

Reviewed by:
Fabio Mangiacapra,
Campus Bio-Medico University, Italy
Zhichao Zhou,
Karolinska Institutet (KI), Sweden

***Correspondence:**
Philipp Jud
philipp.jud@medunigraz.at

Specialty section:
This article was submitted to Cardiovascular Biologics and Regenerative Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *24 February 2021*
Accepted: *13 April 2021*
Published: *12 May 2021*

Citation:
Jud P, Kessler HH and Brodmann M (2021) Case Report: Changes of Vascular Reactivity and Arterial Stiffness in a Patient With Covid-19 Infection. Front. Cardiovasc. Med. 8:671669. doi: 10.3389/fcvm.2021.671669

INTRODUCTION

Covid-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) affecting primarily the respiratory system. Patients with cardiovascular comorbidities have an increased risk of in-hospital death and Covid-19 infection may lead to a higher risk of cardiovascular complications like heart failure, venous thromboembolism or stroke (1–4). Although prior data suggested a direct viral infection of the endothelial cell and diffuse endothelial inflammation which may promote to cardiovascular changes in Covid-19, a recent study assumed that direct endothelial infection by SARS-CoV-2 *via* angiotensin-converting enzyme 2 (ACE2) receptors is unlikely as there is a lack of ACE2 in human endothelial cells (5, 6). Furthermore, other pathways have been suggested contributing also to endothelial changes in Covid-19 (7–9). We report a 24-year-old woman with Covid-19 infection who had undergone measurements of vascular reactivity and arterial stiffness on the day of proven Covid-19 infection and 6 weeks after infection.

CASE REPORT

A 24-year-old woman underwent measurements of flow-mediated dilation (FMD), nitroglycerin-mediated dilation (NMD), pulse wave velocity (PWV), and carotid

intima-media-thickness (cIMT) due to a preventive medical check-up at the beginning of December 2020. She was otherwise healthy, had a body-mass-index of 23.8 kg/m² without any known atherosclerotic risk factor and worked as a secretary for a medical office in a hospital. Additionally, she was a non-smoker without a family history of cardiovascular disease and did not take any medications. Due to governmental initiated shutdown in Austria from November 3rd, 2020 to December 23rd, 2020, the patient refrained from sports activities during that shutdown, but she was active with regular sport activity of 30 min three times a week prior to that shutdown.

Measurements of FMD, NMD, cIMT, and pulse-wave analysis were performed in the morning between 7:00 a.m. and 9:00 a.m. after overnight fasting in a temperature-controlled (22–24°C) and quiet room by one trained technician. At the beginning of the FMD measurement, a blood pressure cuff was placed on the right forearm below the antecubital fossa and the baseline diameter of the right brachial artery was examined in a longitudinal plane between 2 and 7 centimeters proximal to the antecubital fossa in the patient. Three end-diastolic diameters between two intimal layers were measured ECG-gated during image acquisition in a one-centimeter-long segment of the brachial artery. Subsequently, the cuff was inflated >50 mmHg above the resting systolic pressure for 5 min, then deflated and 60 s after cuff release, the post-ischemic diameter of the brachial artery was measured. During a rest of 15 min, pulse-wave analysis including measurement of the aortic PWV and augmentation index was performed on the left arm and calculated *via* the oscillometric device Mobil-O-Graph® (I.E.M. Mobil-O-Graph, I.E.M., Cockerillstr., Stolberg, Germany) by an automated analysis. A size-adjusted cuff was placed on the patient's left upper arm about 2-4 centimeters above the antecubital fossa in supine position and subsequent pulse-wave analysis was performed, while the patient did not to speak or move over the whole pulse-wave analysis. Also, during the same rest of 15 min, the patient underwent measurement of the cIMT of both common carotid artery in supine position using a high-resolution linear array probe with 8–13 MHz (Siemens ACUSON S2000™, Siemens Healthcare Corp., Henkelstr., Erlangen, Germany). The thickness of the intimal and medial layers of the common carotid wall was measured on frozen longitudinal images in at least one-centimeter-long segment of the artery. After that rest of 15 min, the diameter of the right brachial artery was recorded similar to the technique described for FMD before and 5 min after sublingual administration of 0.4 mg glyceryl trinitrate spray. FMD and NMD measurements were performed with an 8–13 MHz linear array transducer using a conventional ultrasound scanner (Siemens ACUSON S2000™, Siemens Healthcare Corp., Henkelstr., Erlangen, Germany). Most recommendations for the measurement of FMD and NMD were fulfilled according to recent guidelines (10). The measurements revealed a reduced FMD of 0.0% and a reduced NMD of 15.5% according to proposed reference values (11). Pulse-wave analysis revealed a PWV of 5.9 m/s and an augmentation index of 27% while ultrasonography revealed a cIMT of 0.4 mm of both common carotid arteries.

The patient was asymptomatic at the time of the respective measurements without potential symptoms of Covid-19 infection or any other infection. One hour after the respective measurements, testing for Covid-19 by polymerase chain reaction (PCR) was performed in that patient due to a routine testing for hospital staff which confirmed an acute Covid-19 infection with a cycle threshold of 22. The initial physical examination including auscultation was unremarkable with a body temperature of 36.6°C and a blood pressure of 127/88 mmHg. Measurement of oxygen level and chest x-ray were not performed as the patient was asymptomatic without respiratory symptoms. There was only a slightly elevated C-reactive protein (8.4 mg/L, reference value 0–5 mg/L) without lymphopenia and lipid parameters were also normal. The patient was subsequently home-isolated and was advised to monitor her health. During home-quarantine, the patient developed headache and myalgia within the first 3 days, which were treated by acetaminophen on demand and resolved afterwards, followed by loss of taste and smell as well as by mild dyspnea on exertion after the fifth and seventh day of home quarantine, respectively. On the tenth day of quarantine, Covid-19 PCR was performed again with a cycle threshold of 26. The patient was asymptomatic after 20 days of initial Covid-19 PCR and repeated PCR testing for Covid-19 was negative on the 21st day after initial Covid-19 PCR. A timeline

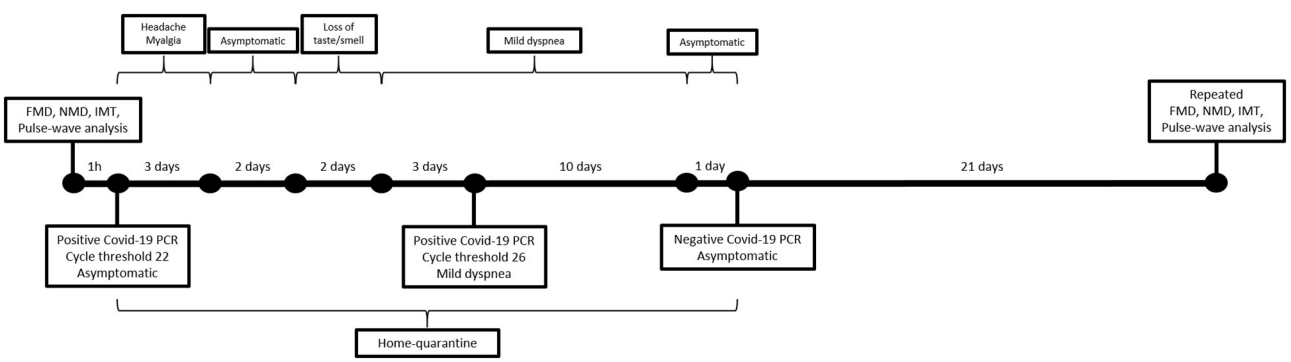


FIGURE 1 | Timeline of patient's Covid-19 infection with symptoms and performance of laboratory and clinical measurements.

of patient's Covid-19 infection with symptoms and performance of Covid-19 PCR and clinical measurements are shown in **Figure 1**.

Six weeks after initial Covid-19 testing, measurements for FMD, NMD, cIMT, and pulse-wave analysis were repeated by the same measurement methods as describes above evaluating changes of the respective parameters. FMD remained unchanged with 0.0% while NMD ameliorated to 17.24%. Furthermore, also PWV with 5.6% and augmentation index with 13% decreased while cIMT was unchanged.

DISCUSSION

We demonstrated with our case a potential impact of Covid-19 infection on endothelial dysfunction. Prior investigations of endothelial changes in Covid-19 infection have demonstrated direct viral infection of endothelial cells and endothelial inflammation with microthrombi and microangiopathy (5, 12). As the vascular endothelium is essential for the maintenance of vascular homoeostasis, dysfunction of the endothelium may result in cardiovascular changes. So far, there are only limited data about the pathophysiological mechanisms how SARS-CoV-2 contributes to endothelial dysfunction. While potential interactions of SARS-CoV-2 with ACE2 receptors have been suggested initially, recent data indicate that there is lacking evidence of ACE2 receptors expression on human endothelial cells assuming thus that direct infection of endothelial cells by SARS-CoV-2 is unlikely (5, 6, 13). Besides potential microvascular damage, also macrovascular damage may be promoted by Covid-19 infection since low values of FMD and NMD of the brachial artery were present in our patient. Additionally, amelioration of NMD, aortic PWV and Aix were observed after Covid-19 infection which indicates that Covid-19 infection may influence vascular homeostasis also in large arteries. Brachial FMD and NMD as well as aortic PWV are proven predictors of cardiovascular events and mortality and changes of those parameters are also associated cardiovascular events and mortality (14, 15). So far, data evaluating vascular reactivity or arterial stiffness in Covid-19 infection are very limited. Only one study investigated FMD and PWV in young adults 4 weeks after positive testing for SARS-CoV-2 revealing significantly lower values of FMD and higher values of PWV in the group of subjects with a suffered SARS-CoV-2 (16). However, data about vascular reactivity and arterial stiffness in acute Covid-19 infection are still lacking and follow-up changes of these parameters during a Covid-19 infection have not been investigated yet.

Underlying pathways by which SARS-CoV-2 may contribute to endothelial dysfunction are yet unknown. Our case and previous data suggest that both, direct cytotoxicity and indirect endothelial injury promote to endothelial dysfunction. Besides a potential but unlikely pathway of SARS-CoV-2 with ACE2 receptors, other pathways promoted by inflammatory mediators including interleukin-6 and prothrombotic mediators, like von Willebrand factor and neutrophil extracellular traps, may result in widespread inflammation and also in endothelial dysfunction (5–8, 13, 17, 18). As acetaminophen has only a weak anti-inflammatory effect, potential interaction of acetaminophen on inflammatory mediators which may affect endothelial dysfunction can be excluded (19). Additionally, as FMD and NMD indicates bioavailability of nitric oxide and PWV and augmentation index are parameters of arterial elasticity, we hypothesize that SARS-CoV-2 exhibits also an influence on nitric oxide metabolism and morphological changes of the arterial wall.

One limitation of our measurements was that we did not fulfill all recent recommendations for the assessment of FMD and NMD according to recent guidelines (10). Recommendations regarding subject preparation, operator-dependent factors and protocol were fulfilled, except for the recommended dose of sublingual glyceryl trinitrate. In our case, 0.4 mg glyceryl trinitrate was used instead of recommended 25 μg glyceryl trinitrate. Additionally, all other recommendations for technique and analysis were fulfilled, except for continuous measurement of velocity and diameter using simultaneous live duplex ultrasound, the use of continuous edge-detection and wall tracking software and calculating peak diameter and shear rate stimulus, since such a software was not available. Instead, offline analysis by a blinded observer was performed. Other limitations are that we conducted measurements of vascular reactivity and arterial stiffness only in one patient with Covid-19 infection and the lacking comparison of the results to a potential healthy, sex- and age-matched control subject.

Our case demonstrated that endothelial dysfunction may be present at a very early stage of Covid-19 infection and seems to be partly persistent even if SARS-CoV-2 is not detectable anymore. Our patient was asymptomatic at the time of verified Covid-19 infection when measurements of vascular reactivity and arterial stiffness were performed and symptoms occurred a few days later. It needs to be elucidated if parameters differ between asymptomatic and symptomatic patients as well as between patients with a different severity of symptoms. Furthermore, it needs to be elucidated if parameters of vascular reactivity and arterial stiffness remain altered as a long-term consequence of Covid-19 or if these changes may be present only in the acute phase of this infection. Moreover, studies evaluating parameters of vascular reactivity and arterial stiffness as potential predictors for cardiovascular events and mortality need to be performed.

In conclusion, we could demonstrate that infection by SARS-CoV-2 may alter different parameters of vascular reactivity and arterial stiffness probably by causing direct and indirectly endothelial dysfunction, which may promote to cardiovascular complications in patients with Covid-19 infection. Further studies evaluating parameters of endothelial dysfunction are urgently needed.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because all data are listed within the article. Requests to access the datasets should be directed to philipp.jud@medunigraz.at.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

PJ and MB contributed to conception and design of the study. HK contributed to data analysis. PJ wrote the first draft of the manuscript. All authors contributed to manuscript revision.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.671669/full#supplementary-material

REFERENCES

1. Figliozzi S, Masci PG, Ahmadi N, Tondi L, Koutli E, Aimo A, et al. Predictors of adverse prognosis in COVID-19: a systematic review and meta-analysis. *Eur J Clin Invest*. (2020) 50:e13362. doi: 10.1111/eci.13362
2. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3
3. Klok FA, Kriup MJHA, van der Meer NJM, Arbous MS, Gommers D, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res*. (2020) 191:145–47. doi: 10.1016/j.thromres.2020.04.013
4. Diener HC, Berlit P, Masjuan J. COVID-19: patients with stroke or risk of stroke. *Eur Heart J Suppl*. (2020) 22(Suppl. Pt t):P25–8. doi: 10.1093/eurheartj/suaa174
5. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet*. (2020) 395:1417–18. doi: 10.1016/S0140-6736(20)30937-5
6. McCracken IR, Saginc G, He L, Huseynov A, Daniels A, et al. Lack of evidence of angiotensin-converting enzyme 2 expression and replicative infection by SARS-CoV-2 in human endothelial cells. *Circulation*. (2021) 143:865–8. doi: 10.1101/2020.12.02.391664
7. Wang K, Gheblawi M, Oudit GY. Angiotensin converting enzyme 2: a double-edged sword. *Circulation*. (2020) 142:426–8. doi: 10.1161/CIRCULATIONAHA.120.047049
8. Panigada M, Bottino N, Tagliabue P, Grasselli G, Novembrino C, Chantarangkul V, et al. Hypercoagulability of COVID-19 patients in intensive care unit: a report of thromboelastography findings and other parameters of hemostasis. *J Thromb Haemost*. (2020) 18:1738–42. doi: 10.1111/jth.14850
9. Dou Q, Wei X, Zhou K, Yang S, Jia P. Cardiovascular manifestations and mechanisms in patients with COVID-19. *Trends Endocrinol Metab*. (2020) 31:893–904. doi: 10.1016/j.tem.2020.10.001
10. Thijssen DHJ, Bruno RM, van Mil ACCM, Holder SM, Faita F, Greyling A, et al. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *Eur Heart J*. (2019) 40:2534–47. doi: 10.1093/eurheartj/ehz350
11. Maruhashi T, Kajikawa M, Kishimoto S, Hashimoto H, Takaeko Y, Yamaji T, et al. Diagnostic criteria of flow-mediated vasodilation for normal endothelial function and nitroglycerin-induced vasodilation for normal vascular smooth muscle function of the brachial artery. *J Am Heart Assoc*. (2020) 9:e013915. doi: 10.1161/JAHA.119.013915
12. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med*. (2020) 383:120–8. doi: 10.1056/NEJMoa2015432
13. Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong JC, Turner AJ, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. *Circ Res*. (2020) 126:1456–74. doi: 10.1161/CIRCRESAHA.120.317015
14. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *Int J Cardiovasc Imaging*. (2010) 26:631–40. doi: 10.1007/s10554-010-9616-1
15. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol*. (2010) 55:1318–27. doi: 10.1016/j.jacc.2009.10.061
16. Ratchford SM, Stickford JL, Province VM, Stute N, Augenreich MA, Koontz LK, et al. Vascular alterations among young adults with SARS-CoV-2. *Am J Physiol Heart Circ Physiol*. (2021) 320:H404–10. doi: 10.1152/ajpheart.00897.2020
17. Pathan N, Hemingway CA, Alizadeh AA, Stephens AC, Boldrick JC, Oragui EE, et al. Role of interleukin 6 in myocardial dysfunction of meningococcal septic shock. *Lancet*. (2004) 363:203–9. doi: 10.1016/S0140-6736(03)15326-3
18. Middleton EA, Hue XY, Denorme F, Campbell RA, Ng D, Salvatore SP, et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. *Blood*. (2020) 136:1169–79. doi: 10.1182/blood.2020007008
19. Flower R, Gryglewski R, Herbaczyńska-Cedro K, Vane JR. Effects of anti-inflammatory drugs on prostaglandin biosynthesis. *Nat New Biol*. (1972) 238:104–6. doi: 10.1038/newbio238104a0

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 13 May 2021
doi: 10.3389/fcvm.2021.652298

Personal Protective Equipment and Donning and Doffing Techniques in the Cardiac Catheterization Laboratory During the COVID-19 Pandemic: Insights From an Internet Search for Protocols

*Justin Haloot[1], Omar Sheikh[1], Fatima Dollar[1], Heta Javeri[2], Hendre Jeannetta Fichardt[1], Gail Fernandes[1], Marlene Garcia[1] and Anand Prasad[1]**

[1] Division of Cardiology, Department of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX, United States, [2] Division of Infectious Disease, Department of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX, United States

OPEN ACCESS

Edited by:
Hendrik Tevaearai Stahel,
Bern University Hospital, Switzerland

Reviewed by:
Xiaohu Li,
First Affiliated Hospital of Anhui Medical University, China
Owais Bhat,
Virginia Commonwealth University, United States

***Correspondence:**
Anand Prasad
prasada@uthscsa.edu

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: 12 January 2021
Accepted: 30 March 2021
Published: 13 May 2021

Citation:
Haloot J, Sheikh O, Dollar F, Javeri H, Fichardt HJ, Fernandes G, Garcia M and Prasad A (2021) Personal Protective Equipment and Donning and Doffing Techniques in the Cardiac Catheterization Laboratory During the COVID-19 Pandemic: Insights From an Internet Search for Protocols. Front. Cardiovasc. Med. 8:652298. doi: 10.3389/fcvm.2021.652298

Background: Due to the ongoing coronavirus disease 2019 (COVID-19) pandemic, a need for precise donning and doffing protocols for personal protective equipment (PPE) among healthcare infrastructures is paramount. Procedures involving the cardiac catheterization laboratory (CCL) are routinely non-aerosolizing but have the potential for rapid patient deterioration, creating the need for aerosolizing generating procedures. Multiple societal and governmental guidelines on the use of PPE during medical procedures are available on Internet websites; however, there is limited literature available in peer-reviewed formats in this context. This study aims to provide an overview of current PPE donning and doffing protocols specific to the catheterization laboratory.

Methods: A series of internet searches regarding donning and doffing of PPE in the CCL including published articles and internet protocols were compiled and compared using Pubmed.gov, Google.com, www.twitter.com, and www.youtube.com.

Results: Most institutions used N95 masks, shoe covers, at least one head covering, face shield or goggles, two pairs of gloves, and inner and outer gowns. Doffing variation was greater than donning. Doffing has the potential to contaminate the healthcare worker (HCW), and therefore, this step of PPE management requires further study. Common steps in temporal priority included cleaning of gloved hands, removal of outer (or only) gown, removal of outer gloves, repeat gloved hand cleaning, removal of facial PPE last, and a final non-gloved hand cleaning.

Conclusions: This analysis provides a summary of commonly used practices that may be considered when designing CCL-specific PPE protocols. Analysis of consistent steps from the literature led the authors to formulate a suggested protocol for CCL HCWs when performing procedures on patients with confirmed or suspected/unknown COVID-19.

Keywords: COVID-19, personal protective equipment, cardiac cath lab, donning and doffing process, protocol

INTRODUCTION

As the coronavirus disease 2019 (COVID-19) pandemic continues to evolve, there is an increased need for healthcare systems to manage personal protective equipment (PPE) resources (1). In addition, the highly contagious nature of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus requires healthcare workers (HCWs) to follow protocol driven use and removal of PPE. Although present for many years in the medical lexicon, "donning" and "doffing" have now entered more commonly into daily use with the COVID-19 pandemic. The terms "don" and "doff" are combinations of the English words "do" "on" and "do" "off" and trace their origins to the fourteenth century. Proper donning and doffing of PPE is paramount to reducing HCW exposure to the SARS-CoV-2 virus. Current data suggest that person-to-person transmission *via* respiratory droplets is the most common mode of infection. Surface contamination is also a concern with this virus. Several studies have now linked infections in HCWs to hospital-based exposure (2, 3). Data from a hospital experience in China found that inadequate use of hand washing and PPE was the most likely cause of nosocomial HCW infection (4).

The intersection of COVID-19 and cardiovascular disease is multifaceted, and these interactions have been outlined previously (5). HCWs in the cardiac catheterization laboratory (CCL) are at possible risk of viral exposure. Although routine cardiac catheterization is a non-aerosolizing procedure, the potential for clinical deterioration in critically ill patients makes this environment important to consider for level of recommended PPE use. Cardiopulmonary instability with need for non-invasive mechanical ventilation, intubation, or cardiopulmonary resuscitation (all aerosolizing treatments) can occur in patients with acute coronary syndromes—particularly with ST segment elevation myocardial infarction or cardiogenic shock. In these scenarios, time delays are suboptimal, making viral test results unavailable prior to arrival to the CCL.

Multiple societal guidelines and governmental agencies such as the Centers for Disease Control and Prevention in the United States have recommended the use of PPE when performing medical procedures. Hospital systems are commonly left to develop their own individual set of guidelines—often based on resource availability. These institutional protocols are generally not published in peer-reviewed formats but rather viewable or disseminated on Internet-based sites. The purpose of this study was to perform an Internet-based search for PPE protocols relevant to the protection of HCWs in the CCL and to provide a descriptive overview of current practices.

METHODS

Both published articles and protocols available on the Internet were included in the present study. For published papers, PubMed was utilized using the following terms in combinations: "cardiac catheterization," "personal protective equipment," and "COVID-19." For the broader Internet search, these terms were inputted into Google.com, www.twitter.com, and www.youtube.com. The search date range for PubMed/LITCOVID was for relevant articles from January 1, 2020 through July 15, 2020, and the Internet searches were performed on October 20, 2020. Relevant articles were screened through initial PubMed review of the title, abstract, and, as needed, the full manuscript. For protocols obtained through the Internet searches, the retrieved documents were included if they contained information about both donning and doffing with associated PPE equipment delineated. The outline of the data collection searches is shown in **Figure 1**. The full description of each protocol from the initial web search is listed in **Supplementary Table 1**.

RESULTS

The search revealed 5 relevant articles from PubMed, 12 from Google.com searches, 1 from www.twitter.com, and 6 from www.youtube.com. Seven protocols were from individuals, and 17 were from institutions/hospitals.

PPE Equipment

Of the protocols listed above, 24 provided granular details on equipment use, donning, and doffing. These protocols included the Society of Cardiovascular Angiography and Interventions (SCAI), Italian Society of Interventional Cardiology (GISE), European Society of Cardiology (6), Stellenbosch University and Tygerberg Academic Hospital, Indiana Chapter of ACC, Spanish Society of Cardiology, New York University (NYU), and University Health System in San Antonio, TX (UHS). We began our data analysis by looking at the equipment recommended by these protocols. The results are seen in **Table 1**. All protocols recommended the use of standard lead apron protection, N95 mask or equivalent, goggles and/or face shield, and two pairs of sterile gloves. One hundred percent of all protocols recommended the use of at least one head covering, with 18 (75%) recommending one head covering and 6 (20.8%) of protocols recommending two head coverings. Eight (33%) of the protocols recommended use of a surgical mask in addition to an N95.

Donning

Figure 2 demonstrates the most common steps involved in the donning of PPE before a procedure in the CCL. The particular sequence of steps in the donning process was variable among the listed protocols; therefore, instead of including a sequence, we instead constructed a figure demonstrating the most common steps involved. The x-axis shows the various steps in donning, and the y-axis demonstrates the percentage (%) of protocols that included that specific step. If a protocol required only one gown, the step was counted in the "outer gown" since there is no other gown. Similarly, if only one head covering was required in the protocol, it was counted in the "outer head cover" category. In the donning process, only 25% of protocols had a designed donning area for PPE, which is a stark difference compared to the doffing process. Seventy-four percent of protocols designated steps inside the CCL lab and outside CCL (**Supplementary Table 2**). In comparison, the majority of the doffing process occurs in the CCL with a few remaining steps outside the CCL.

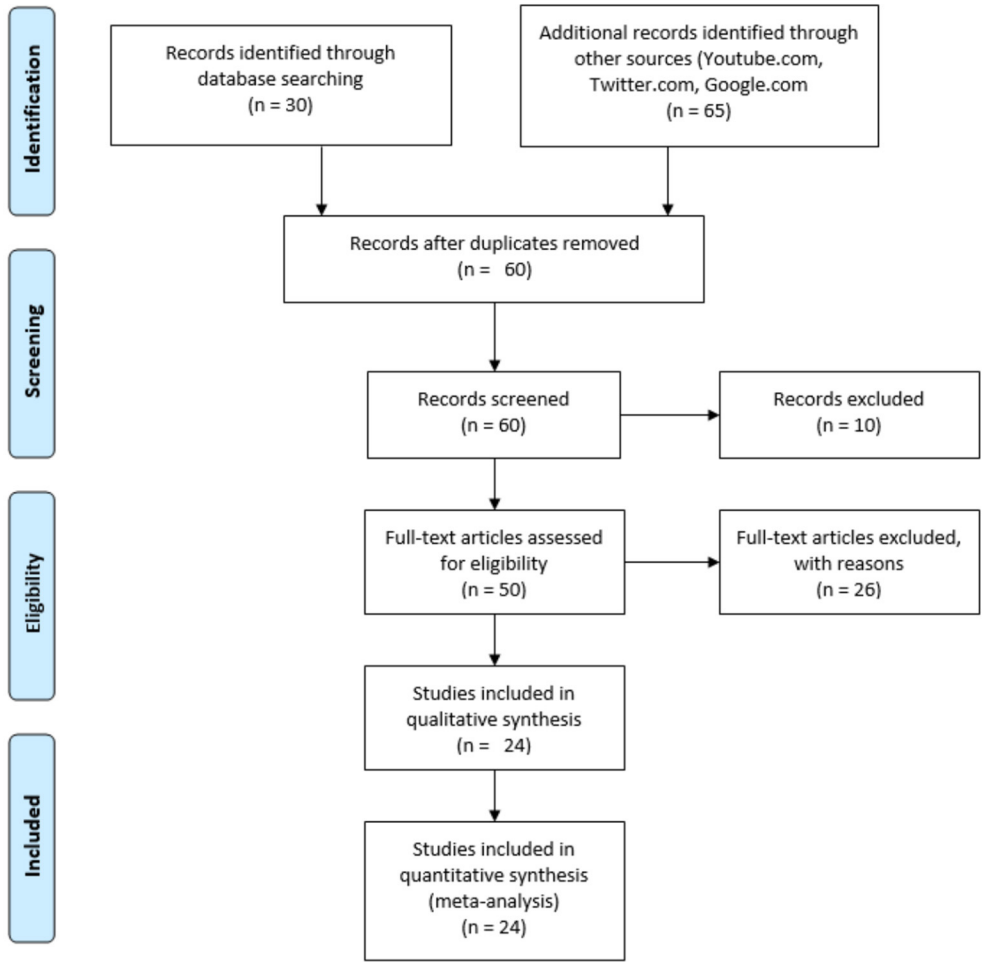


FIGURE 1 | Study retrieval flow diagram.

TABLE 1 | Most recommended equipment for doffing/donning PPE in cardiac cath lab.

Equipment	% Recommended (%)
Lead protection	100
N95 mask or equivalent (FFP2)	100
Goggles/face shield	100
2 pairs of sterile gloves	100
Shoe covers	83.3
1 head covering	75
2 gowns	65.2
Designated donning area	41.2
1 gown	37.5
Surgical mask (in addition to N95)	33.3
2 head coverings	20.8
1 pair of sterile gloves	0

The majority of the steps involved includes hand washing, placement of the outer gown, placement of N95 or equivalent, placement of goggles and/or face shield, placement of an outer head cover, and placement of an inner and outer pair of gloves. Forty-one percent of protocols recommended patients wear surgical masks. The remaining 59% of protocols did not specify if patients should wear masks or not; notably, no institution recommended against the patient wearing a mask. Protocols had various recommendations regarding patients getting tested, including depending on specific indications, i.e., testing in non-critical situations. The details of each protocol are specified in **Supplementary Table 3**.

Doffing

Doffing of PPE carries a higher risk of exposure of SARS-CoV-2 to healthcare workers after interactions with COVID-19 patients. The first four steps of the doffing process were determined to be the most crucial and noted that these steps had the most consistency (**Figure 3**). Across institutions, it was found that donning protocols were generally more homogeneous in regard to equipment used, whereas greater variation existed among doffing protocols. Our search indicated that hand washing was the first step in 60% of the protocols included, followed by removing outer gown (50%) as the

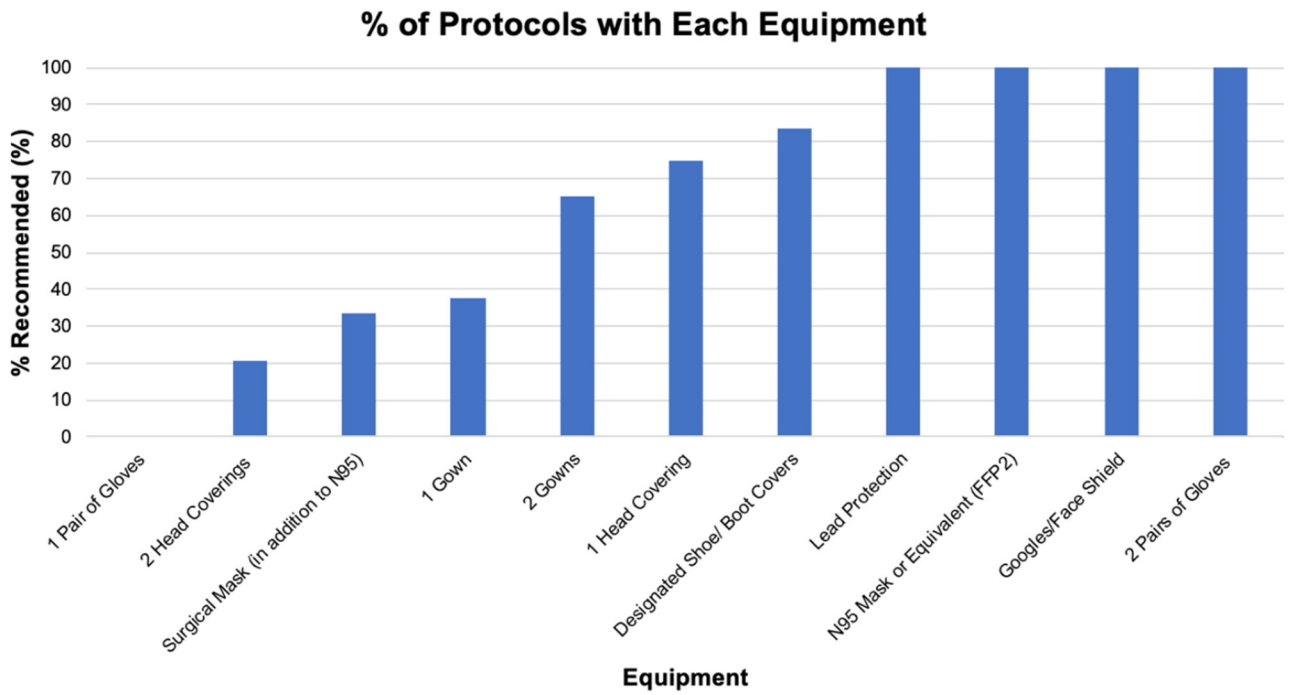


FIGURE 2 | Most commonly involved steps in donning PPE.

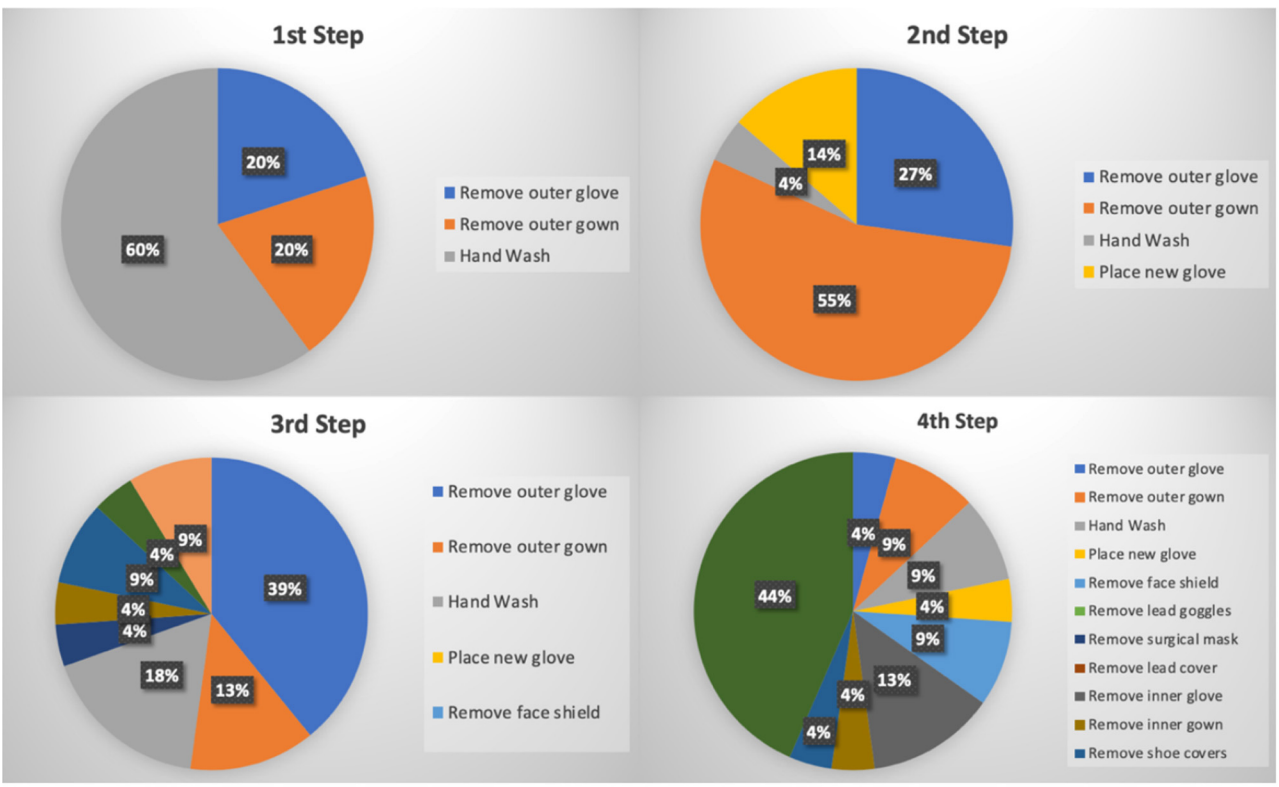


FIGURE 3 | Pie chart of the first four steps recommended for doffing of PPE. Wuhan Asia Heart Hospital did not provide a doffing protocol, so the number of donning protocols = 24, and the number of doffing protocols = 23.

second step. The SCAI Emerging Leader Mentorship (ELM) protocol gave a specification to use a soap and water hand wash, while the UHS protocol gave a specification of hand sanitizer (**Supplementary Table 4**). Other protocols did not appear to designate a specific type of hand wash. The Spanish Society of Cardiology and UHS recommended at least two hand washings—one after the removal of the outer gloves and one after the removal of the other PPE. The protocol(s) of NYU and Stellenbosch University and Tygerberg Academic Hospital had seven steps of hand washing that was done after the removal of each individual PPE (i.e., hand wash after the removal of the outer gown, hand wash after removal of the goggles/face mask, etc.). Variations began to arise starting the third step and beyond where removal of outer glove (39%), hand washing (18%), outer gown (13%), placement of a new glove (9%), and removal of the outer head cover/face shield (9%) were considered the third step. Likewise, the fourth step was also heterogeneous among institutions with removal of lead googles (44%), removal of inner glove (13%), removal of outer gown (9%), hand wash (9%), removal of face shield (9%), and placement of a new glove (4%). Variations continued to exist in the subsequent steps; however, 100% of protocols indicated removal of the lead apron to be the second to last step and additional hand washing to be the final step. Seventy-five percent of the protocols have designated steps inside the CCL and steps outside the CCL (**Table 2**).

DISCUSSION

There has been no established, unified CCL protocol based on evidence-based medicine to protect against transmission of SARS-CoV-2. Even prior to the COVID-19 pandemic, recommendations for sterile techniques within the CCL are not supported by robust prospective clinical trials (7). Our main objective was to examine the variation in PPE used and the donning and doffing protocols available through formal and informal published sources.

General PPE use as described by the CDC first begins with proper hand hygiene before patient contact for all healthcare workers. It is recommended to use an alcohol-based hand rub with 60–95% alcohol or to wash hands with soap and water for at least 20 s, the latter being the preferred method. Following that, the isolation gown, filtering facepiece respirator or higher, face shield or goggles, and reperforming hand hygiene before putting on gloves is performed in that order. The appropriate sequence for donning and doffing PPE can be found on the CDC website: (https://www.cdc.gov/hai/pdfs/ppe/ppe-sequence.pdf).

PPE and Infection Control Specific to the CCL

The CCL requires the use of additional radiation-specific PPE including a one- or two-piece lead/lead equivalent apron, thyroid collar, and often goggles. As noted earlier, many of the patients coming to the CCL may be unstable or may become unstable due to cardiopulmonary compromise. Conversion from contact and droplet-only PPE to aerosol protection may be impractical while managing patients in extremis. The CCL environment often includes multiple HCWs interacting with the patient including physicians, nurses, technologists, and respiratory therapists. Having optimally protective PPE for each staff member at the outset of the case is therefore helpful when dealing with suspected and confirmed COVID-19 patients.

Furthermore, although not specifically addressed in the present analysis, the risk of aerosol-mediated contamination of CCL surfaces is a concern. For this possibility, we have developed a number of interventions at our own institution that can be considered. These include using a COVID-19-specific room for procedures, minimizing extraneous equipment or supplies in the room, covering equipment with protective drapes if they cannot be relocated, decreasing unnecessary traffic into or out of the room, wiping off lead aprons with disinfectant wipes at the end of the case, use of lead PPE specific for COVID-19/potential cases, use of ultraviolet-light-based cleaning robots (Xenex, San Antonio, Texas), and a full terminal cleaning of the room. CCLs are typical divided into two primary sections: the actual procedural room and a control room. The infection control protocols described in the literature focus largely on the actual procedure room. Given that air flow is generally in continuity between the two rooms, droplet protection at a minimum with surgical masks is reasonable while in the control room. Factors such as proximity of the HCWs in the control room to the patient, airflow patterns/handling, and aerosol status of the patient may dictate the use of more protective masks (N95 respirators). The cleaning of non-disposable PPE, procedure and control rooms, and other equipment was not consistently reported in the sources from our survey. Although beyond the scope of the present analysis, HCW to HCW spread in the CCL is also a concern. Use of general hand washing, face covering, and social distancing is advised. This latter component is particularly important in break rooms where masks might be removed during mealtimes. Staggering breaks and finding additional locations for meals are often required in this context.

Summary Recommendations

It should be emphasized that the efficacy and validity of many of the interventions described in this paper remain to be confirmed from a microbiological standpoint. The specific protocols used by individual hospitals are generally based on local infection control departments with reference to published CDC recommendations. The present analysis provides a summary of commonly used practices across multiple institutions that should be considered when designing a CCL PPE protocol. The majority of institutions used N95 masks, shoe covers, at least one head covering, face shield or goggles, two pairs of gloves, and inner and outer gowns. Doffing variation was greater than donning. Doffing has the potential to contaminate the HCW, and therefore, this step of PPE management requires further study. Common steps in temporal priority included cleaning of gloved hands, removal of outer (or only) gown, removal of outer gloves, repeat gloved hand cleaning, removal of facial PPE last, and a final non-gloved hand cleaning.

TABLE 2 | Suggested donning/doffing protocol.

Donning:	
Step 1:	Apply shoe cover
Step 2:	First hand wash
Step 3:	Wear head cover
Step 4:	Wear surgical mask
Step 5:	Wear goggles
Step 6:	Wear face shield
Step 7:	Wear lead apron
Step 8:	Sterile hand wash/solution
Step 9:	Wear sterile gown
Step 10:	Wear inner gloves
Step 11:	Wear outer gloves
Doffing:	
Step 1:	Hand wash with solution
Step 2:	Remove outer glove
Step 3:	Remove gown with inner gloves and then remove inner gloves
Step 4:	Wear non-sterile gloves
Step 5:	Remove lead
Step 6:	Remove goggles and face shield
Step 7:	Remove shoe covers
Step 8:	Dispose of gloves
The following occurs in COVID-19 free area	
Step 9:	Hand wash
Step 10:	Wear non-sterile gloves
Step 11:	Remove head cover
Step 12:	Remove mask and dispose or recycle
Step 13:	Hand wash

Using many consistent items and steps from the literature, a suggested protocol by the authors can be found in **Table 2**. Of note, after initially using two sets of gowns—outer sterile and an inner non-sterile "bunny suit"—we have adopted a single sterile outer gown strategy. This modification was done after consultation with our local infection control team due to concerns that removal of the inner full body gown would increase doffing contamination risk. In our institution, the protocol we describe is invoked for patients with COVID-19, suspected COVID-19, or those who have an unknown status of infection (suspected or not suspected). Provided that adequate PPE supplies remain, this approach affords defined protection for our CCL HCWs. We further augment our safety with universal testing of outpatients prior to catheterization procedures. Inpatients also are universally tested; however, urgent cases are done with PPE protection even if results are not available. In addition, we recommend involved HCWs to undergo simulation training per their institution's protocol to avoid or minimize exposure to infectious material.

With this study, we present a review of the current methods utilized for donning and doffing of PPE in the CCL. To date, there has been no established study formally examining the best method of donning and doffing of PPE in the CCL for the protection of HCW. This study provides an initial analysis and evidence of current practices.

LIMITATIONS

One major limitation of this study is the utilization of atypical research methods. COVID-19 is a rapidly growing entity creating a need for rapid development of guidelines and protocols for the treatment of these patients. Our research depended on protocols published by the various institutions in multiple platforms on the Internet including YouTube and Twitter. The amount of information is dependent on the amount shared by each institution, which makes it difficult to compare. It was noted that some institutions also promoted similarly used content. For example, cardiovascular innovations utilized similar material from GISE. We counted the protocol as a protocol for cardiovascular innovations, as this was what they recommended publicly. Therefore, there may be some duplicate protocols due to being similar to other published information.

Currently, there is a lack of randomized studies to determine which specific protocol would provide the best protection for healthcare workers. Our study is mainly descriptive in nature. This is largely in part due to the diverse nature of the protocols utilized at the different institutions. This would make it difficult to allow for comparison. We recommend that institutions develop a trial to study their specific protocol and examine the rate of COVID-19 transmission to HCWs in the CCL and compare their results with other institutions with the ultimate goal of identifying practices that decrease COVID-19 transmission.

Despite these limitations, media outlets have allowed for greater communication of protocols between institutions. Rather than waiting for a unified guideline, institutions can share their experiences in real time. Shared information has allowed for the formation of more formal statements/guidelines as seen by SCAI, GISE, European Society of Cardiology (ESC), and the Spanish Society of Cardiology.

The fight against COVID-19 and PPE continues to evolve, which provides a temporal limitation to our study. As the pandemic continues, the medical community innovates in PPE utilization. One current new method is the use of antibacterial and antiviral agents for the reuse of PPE. The most promising methods include ultraviolet germicidal irradiation, vaporous hydrogen peroxide, and moist heat. The CDC recommends utilization of these methods mainly when there is a shortage of PPE or filtering facepiece respiratory (FFR) (8). More studies will be needed to determine the efficacy of these methods.

CONCLUSIONS

As the COVID-19 pandemic evolves, the protection of healthcare workers has become crucial. HCWs can significantly reduce their risk of acquiring the virus by adhering to society/guideline recommendations, specifically during the donning and doffing of PPE. The steps involved in the donning

and doffing vary across institutions. From our analysis, we found 24 protocols from multiple databases including PubMed, Google.com, www.twitter.com, and www.youtube.com, listing the number of steps involved and the amount of PPE used per protocol. We have a suggested protocol that we developed at our home institution, University Health System, San Antonio. With time and more analysis, our hope is that a unified protocol that carries the lowest risk of contamination for HCWs can be adopted among many institutions.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was supported by the Freeman Heart Association Endowment in Cardiovascular Disease.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.652298/full#supplementary-material

REFERENCES

1. Tarantini G. *COVID-19 Cath Lab Implications: Frontline View From Italy*.
2. Bielicki JA, Duval X, Gobat N, Goossens H, Koopmans M, Tacconelli E, et al. Monitoring approaches for health-care workers during the COVID-19 pandemic. *Lancet Infect Dis*. (2020) 20:e261–7. doi: 10.1016/S1473-3099(20)30458-8
3. International Council of Nurses High proportion of healthcare workers with COVID-19 in Italy is a stark warning to the world: protecting nurses and their colleagues must be the number one priority (2020).
4. Wong SCY, Kwong RT, Wu TC, Chan JWM, Chu MY, Lee SY, et al. Risk of nosocomial transmission of coronavirus disease 2019: an experience in a general ward setting in Hong Kong. *J Hosp Infect*. (2020) 105:119–27. doi: 10.1016/j.jhin.2020.03.036
5. Prasad A, Panhwar S, Hendel RC, Sheikh O, Mushtaq Z, Dollar F, et al. COVID-19 and the cardiovascular system: a review of current data, summary of best practices, outline of controversies, and illustrative case reports. *Am Heart J*. (2020) 226:174–87. doi: 10.1016/j.ahj.2020.06.009
6. Luigi Palmieri XA, Bella A, Bellino S, Boros S, Canevelli M, Castrucci MR, et al. *COVID-19 Surveillance Group. Characteristics of COVID-19 Patients Dying in Italy: Report Based on Available Data on Rome, Italy*. Instituto Superiore Di Sanita (2020).
7. Chambers CE, Eisenhauer MD, McNicol LB, Block PC, Phillips WJ, Dehmer GJ, et al. Infection control guidelines for the cardiac catheterization laboratory: society guidelines revisited. *Catheter Cardiovasc Interv*. (2006) 67:78–86. doi: 10.1002/ccd.20589
8. *Implementing Filtering Facepiece Respirator (FFR) Reuse, Including Reuse after Decontamination, When There Are Known Shortages of N95 Respirators*. Available online at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/ppe-strategy/decontamination-reuse-respirators.html (accessed March 15, 2020).

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 13 May 2021
doi: 10.3389/fcvm.2021.654405

Cardiac Involvement in Recovered Patients From COVID-19: A Preliminary 6-Month Follow-Up Study

Xiaoyan Wu[1†], Ke-Qiong Deng[1†], Chenze Li[1†], Zhaoxia Yang[2], Huijuan Hu[2], Huanhuan Cai[1], Chao Zhang[1], Tao He[1], Fang Zheng[3], Hairong Wang[1], Xin A. Zhang[4], Antoine Caillon[5], Yufeng Yuan[6], Xinghuan Wang[7], Haibo Xu[2]* and Zhibing Lu[1]**

[1] Department of Cardiology, Zhongnan Hospital of Wuhan University, Wuhan, China, [2] Department of Radiology, Zhongnan Hospital of Wuhan University, Wuhan, China, [3] Department of Clinical Laboratory, Zhongnan Hospital of Wuhan University, Wuhan, China, [4] University of Oklahoma Health Science Center, Oklahoma City, OK, United States, [5] Hypertension and Vascular Research Unit, Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, Montreal, QC, Canada, [6] Department of Hepatobiliary and Pancreatic Surgery, Zhongnan Hospital of Wuhan University, Wuhan, China, [7] Center for Evidence-Based and Translational Medicine, Zhongnan Hospital of Wuhan University, Wuhan, China

Background: Accumulating evidence has revealed that coronavirus disease 2019 (COVID-19) patients may be complicated with myocardial injury during hospitalization. However, data regarding persistent cardiac involvement in patients who recovered from COVID-19 are limited. Our goal is to further explore the sustained impact of COVID-19 during follow-up, focusing on the cardiac involvement in the recovered patients.

Methods: In this prospective observational follow-up study, we enrolled a total of 40 COVID-19 patients (20 with and 20 without cardiac injury during hospitalization) who were discharged from Zhongnan Hospital of Wuhan University for more than 6 months, and 27 patients (13 with and 14 without cardiac injury during hospitalization) were finally included in the analysis. Clinical information including self-reported symptoms, medications, laboratory findings, Short Form 36-item scores, 6-min walk test, clinical events, electrocardiogram assessment, echocardiography measurement, and cardiac magnetic resonance imaging was collected and analyzed.

Results: Among 27 patients finally included, none of patients reported any obvious cardiopulmonary symptoms at the 6-month follow-up. There were no statistically significant differences in terms of the quality of life and exercise capacity between the patients with and without cardiac injury. No significant abnormalities were detected in electrocardiogram manifestations in both groups, except for nonspecific ST-T changes, premature beats, sinus tachycardia/bradycardia, PR interval prolongation, and bundle-branch block. All patients showed normal cardiac structure and function, without any statistical differences between patients with and without cardiac injury by echocardiography. Compared with patients without cardiac injury, patients with cardiac injury exhibited a significantly higher positive proportion in late gadolinium enhancement sequences [7/13 (53.8%) vs. 1/14 (7.1%), $p = 0.013$], accompanied by the elevation of circulating ST2 level [median (interquartile range) = 16.6 (12.1, 22.5) vs. 12.5 (9.5, 16.7); $p = 0.044$].

OPEN ACCESS

Edited by:
Mingxing Xie,
Huazhong University of Science and Technology, China

Reviewed by:
Roberto Latini,
Istituto di Ricerche Farmacologiche Mario Negri (IRCCS), Italy
Bastiaan Geelhoed,
University Medical Center Groningen, Netherlands

***Correspondence:**
Xinghuan Wang
wangxinghuan@whu.edu.cn
Haibo Xu
xuhaibo1120@hotmail.com
Zhibing Lu
luzhibing222@163.com

[†]These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Cardiovascular Epidemiology and Prevention, a section of the journal Frontiers in Cardiovascular Medicine

Received: *27 January 2021*
Accepted: *25 March 2021*
Published: *13 May 2021*

Citation:
Wu X, Deng K-Q, Li C, Yang Z, Hu H, Cai H, Zhang C, He T, Zheng F, Wang H, Zhang XA, Caillon A, Yuan Y, Wang X, Xu H and Lu Z (2021) Cardiac Involvement in Recovered Patients From COVID-19: A Preliminary 6-Month Follow-Up Study. Front. Cardiovasc. Med. 8:654405. doi: 10.3389/fcvm.2021.654405

Patients with cardiac injury presented higher levels of aspartate aminotransferase, creatinine, high-sensitivity troponin I, lactate dehydrogenase, and N-terminal pro–B-type natriuretic peptide than those without cardiac injury, although these indexes were within the normal range for all recovered patients at the 6-month follow-up. Among patients with cardiac injury, patients with positive late gadolinium enhancement presented higher cardiac biomarker (high-sensitivity troponin I) and inflammatory factor (high-sensitivity C-reactive protein) on admission than the late gadolinium enhancement–negative subgroup.

Conclusions: Our preliminary 6-month follow-up study with a limited number of patients revealed persistent cardiac involvement in 29.6% (8/27) of recovered patients from COVID-19 after discharge. Patients with cardiac injury during hospitalization were more prone to develop cardiac fibrosis during their recovery. Among patients with cardiac injury, those with relatively higher cardiac biomarkers and inflammatory factors on admission appeared more likely to have cardiac involvement in the convalescence phase.

Keywords: cardiac magnetic resonance imaging, fibrosis, follow-up, cardiac injury, COVID-19

INTRODUCTION

The novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has developed into an unprecedented global pandemic (1). To date, more than 140 million confirmed cases have been identified in more than 200 countries around the world, according to the latest data from the World Health Organization. The clinical presentation of COVID-19 is mostly characterized by respiratory symptoms, and the lung is the major organ involved, causing complications related to pneumonia and acute respiratory distress syndrome (2). Accumulating evidence has revealed that COVID-19 affects multiple organs including the cardiovascular system (3–6). In our previous report, we have demonstrated that myocardial injury with troponin elevation is significantly associated with fatal outcomes of COVID-19 patients, which has been confirmed by other studies (7–12). However, it is unknown whether patients with cardiac injury during hospitalization suffer from a sustained myocardial impairment, cardiac sequelae during their convalescence, and the implications of persistent cardiac involvement on the consequence are not clear.

Recent follow-up studies have demonstrated that patients in the early convalescence may suffer from impairment of pulmonary function, symptoms of fatigue, and physical and psychological damage after their discharge for 1 month (13–16). Some studies have also reported short-term cardiac involvement in convalescent patients (17, 18). Nevertheless, longer follow-up studies are needed to fully evaluate the long-term cardiac impact of COVID-19. In this study, the patients with COVID-19 who were discharged from our hospital for more than 6 months were enrolled and analyzed. The purpose of our study was to further observe the persistent impact of COVID-19 during follow-up, focusing on the cardiac involvement in the recovered patients.

METHODS

Study Design and Participants

A prospective observational study was designed to investigate the long-term prognosis of COVID-19 patients with or without cardiac injury during hospitalization. The study protocol was approved by the ethics committees of Zhongnan Hospital of Wuhan University and conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. Written informed consent was obtained from all participants.

The participants were consecutively recruited at Zhongnan Hospital of Wuhan University from March 1 to April 1, 2020. Initially, 32 patients with cardiac injury during hospitalization were screened for eligibility. The enrolled patients had to meet the following eligibility requirements: (1) patients were older than 18 years old; (2) patients were diagnosed as COVID-19 based on a positive real-time polymerase chain reaction (RT-PCR) for SARS-CoV-2 in association with clinical symptoms according to the diagnosis and treatment guideline published by the National Health Commission of China during hospitalization; (3) patients had cardiac injury during hospitalization. Cardiac troponin I (cTnI) was measured using a high-sensitivity cTnI (hs-cTnI) assay (Abbott ARCHITECT). The assay's limit of detection is 1.9 pg/mL, the 99th percentile upper reference limit (URL) is 26.2 pg/mL, and the coefficient of variation at 26.2 pg/mL is <5%. Cardiac injury was defined as at least one cTn concentration is above the 99th percentile URL (>26.2 pg/mL); (4) patients who recovered from SARS-CoV-2 infection and discharged from the hospital for more than 6 months at follow-up; (5) patients consented to participate in the follow-up study. Patients were discharged if a combination of the following criteria was satisfied according to the guideline of the Chinese Center for Disease Control and Prevention: the absence of fever for at least 3 days, clinical remission of respiratory symptoms, substantial improvement in acute

exudative lesions on chest computed tomography scan, and two consecutive throat swabs negative RT-PCR test results for SARS-CoV-2 RNA obtained at least 24 h apart. The baseline clinical information, including demographic characteristics, coexisting diseases, laboratory findings, and clinical treatments during hospitalization, was collected using a standardized case report form at the time of patients' enrollment via electronic medical charts. Besides, the patients with the following conditions were excluded from this study: (1) patients had contraindications to the examination of cardiac magnetic resonance (CMR) imaging; (2) patients had a history of coronary heart disease or cardiomyopathy before admission; (3) patients had a malignant tumor for which life expectancy was <6 months; (4) liver or kidney dysfunction unrelated to COVID-19 [aspartate aminotransferase (AST) or alanine aminotransferase (ALT) is 2 times higher than the upper threshold; creatinine >90 μmol/L]. Finally, 20 patients with cardiac injury were included in the schedule for routine follow-up appointments in the outpatient clinic 6 months after discharge. For comparisons, 20 age- and gender-matched COVID-19 patients without cardiac injury were included as controls in this prospective study. Their inclusion and exclusion criteria were consistent with that in patients with cardiac injury, except for the requirements of elevated hs-cTnI level during hospitalization.

The Clinical Follow-Up

Investigators were instructed to contact patients and perform face-to-face interviews at predesignated times after discharge, to collect relevant clinical information. The information included self-reported symptoms, medications, laboratory findings (biomarkers for liver and renal function, coagulation, inflammation, and myocardial injury), quality-of-life scores [Short Form 36-item questionnaire (SF-36)], 6-min walk test, clinical events, electrocardiogram (ECG), echocardiography, and CMR imaging. Also, the serum level of soluble ST2 was quantified using a commercial enzyme-linked immunosorbent assay kit (R&D Systems, DST200). The observation window of follow-up was defined as the interval range from the date of discharge to the date of the last contact. If a patient was only contacted by physicians via telephone follow-up and refused to undergo laboratory and imaging examinations, the patient was then not included in the analysis. The last follow-up date was October 17, 2020.

Quality-of-Life Assessment

The quality of life of the patients at follow-up was evaluated by the SF-36 survey. The SF-36 scale was composed of 36 items, which can be divided into eight dimensions, including physical functioning, role limitation due to physical problems (role physical), bodily pain, general health, vitality, social functioning, and role limitation due to emotional health problems (role emotional). Each dimension was scored separately from 0 to 100, with high values representing better functional status.

Exercise Tolerance Test

Exercise endurance of patients was performed by a 6-min walk test without supplemental oxygen. Measurements of heart rate (HR), systolic/diastolic blood pressure, and percutaneous oxygen saturation (SpO_2) were measured. Rating of perceived exertion with Borg scale in patients was also scored after the 6-min walk test.

Transthoracic Echocardiography

The cardiac structure and function of patients were evaluated by sequential transthoracic echocardiography scans using a P4-2S ultrasound scanner (Mindray; Shenzhen, China). From the parasternal long-axis view, we obtained the systolic and diastolic measurements, including left atrial (LA) dimension, left ventricular (LV) dimension, right atrial (RA) dimension, right ventricular (RV) dimension, interventricular septum thickness (IVS), and LV posterior wall thickness (LVPW). Tricuspid annular plane systolic excursion (TAPSE) was measured as the systolic displacement of the tricuspid lateral annulus on M-mode imaging. We used M-mode echocardiography to calculate LV ejection fraction (LVEF).

CMR Imaging

A 3.0-T MR scanner (Prisma, Siemens Healthcare, Germany) was applied to obtain CMR imaging in all patients. The data were collected through an 18-channel phased-array body coil combined with 12 channels from the spine coil. Patients with a HR higher than 75 beats/min were administered with β-blocker (metoprolol, 25–50 mg). We first used the conventional CMR scan protocol, including long- and short-axis cine and late gadolinium enhancement (LGE) to obtain images, and then native T1 mapping and extracellular volume (ECV) were quantitatively evaluated. T1 myocardial mapping was collected in three locations covering the base, midventricle, and apex of the short-axis LV by a modified Look-Locker inversion recovery pulse sequence before contrast administration. After patients were administered with 0.10–0.15/kg gadoterate meglumine (Dotarem; Guerbet AG, Paris, France), LGE sequences were obtained approximately for 10–15 min. Postcontrast T1 mapping was obtained approximately for 15–20 min after gadoterate meglumine administration, and postcontrast T1 mapping was collected using the same imaging plane as the pre-contrast T1 mapping. Two experienced radiologists blindly analyzed all CMR images using a commercial software cvi 42, v.5.3 (Circle Cardiovascular Imaging, Calgary, Alberta, Canada). If there are any discrepancies between the two radiologists, another senior radiologist adjudicated the CMR imaging.

Statistical Analysis

Patients were divided into two groups based on hs-cTnI level throughout hospitalization, and their baseline characteristics and follow-up findings were then compared. The continuous data were presented as median [interquartile range (IQR)], and their comparisons between groups were performed by Mann–Whitney *U* tests. For categorical variables, data were expressed as frequency (percentage) and compared by Fisher exact test. R version 3.4.0 (Vienna, Austria) was used to perform statistical analysis. All comparisons were two-sided, and a $p < 0.05$ was considered statistically significant.

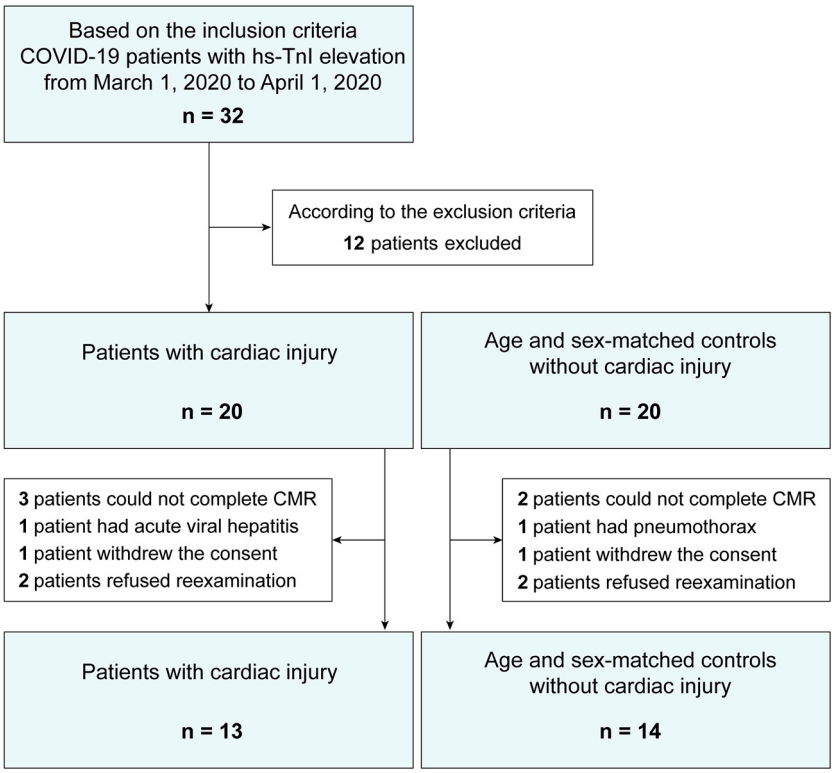


FIGURE 1 | A flowchart of patient recruitment.

RESULTS

Patient Characteristics

A flowchart for patient recruitment is illustrated in **Figure 1**. Based on the inclusion and exclusion criteria, 40 consecutive patients admitted to our hospital from March 1 to April 1, 2020, were initially followed up. No patients were readmitted for cardiopulmonary reasons or died during follow-up. Five patients could not complete the examination of CMR for allergic reaction to contrast media (two patients), claustrophobia (one patient), unwillingness (one patient), and presence of metal implants during follow-up (one patient). One patient had severe liver dysfunction for acute viral hepatitis. One patient suffered from pneumothorax caused by thoracic trauma. Two patients dropped out of the study because of withdrawal of consent. Four patients refused to return to the hospital for reexamination because of fearing reinfection. At last, a total of 27 patients who recovered from COVID-19 for at least 6 months were enrolled for analysis, of which myocardial injury with positive troponin (hs-TnI >26.2 pg/mL) throughout hospitalization was confirmed in 13 patients (exclusion of acute coronary syndrome), and 14 age- and gender-matched patients without cardiac injury were included as controls.

The details of baseline characteristics are presented in **Table 1**. The median ages were 63 years (IQR = 59–70 years) in patients with cardiac injury and 63 years (IQR = 57–70 years) in those without cardiac injury, respectively. Of the 27 patients, 16 were diagnosed with moderate-type COVID-19, 8 with severe-type, and 3 with critical type, according to the Diagnosis and Treatment Protocol of Novel Coronavirus issued by the National Health Commission of the People's Republic of China (fifth version). Patients with cardiac injury were tended to be identified with more severe and critical types (8/13 vs. 3/14; **Table 1**). The median duration of hospital stay was 11 days (IQR = 8–18 days). Comorbidities were presented in nine patients, with a history of diabetes in five patients (18.5%) and hypertension in four patients (14.8%). During COVID-19 hospitalization, 81.5% of patients underwent antiviral therapy (19/27), antibiotics (12/27), corticosteroids (5/27), immunoglobulin (1/27), oxygen inhalation (16/27), and mechanical ventilation (2/27). Angiotensin-converting enzyme inhibitor (1/27), β-blocker (2/27), calcium-channel blocker (4/27), and statin (2/27) were applied in these patients after discharge. There were no statistical differences in age, gender, underlying commodities, therapeutic history, and medication after discharge between the patients with and without cardiac injury. For the laboratory values on admission, patients with cardiac injury showed significantly higher levels of lactate dehydrogenase (LDH), creatinine, and N-terminal pro–B-type natriuretic peptide (NT-proBNP) compared with those without (all $p < 0.05$). However, the elevation of hs-cTnI was marginally

TABLE 1 | Baseline characteristics of study subjects.

Characteristics	Overall ($n = 27$)	Patients with cardiac injury ($n = 13$)	Patients without cardiac injury ($n = 14$)	*p*
Age (years)	63 [58, 70]	63 [59, 70]	63 [57, 70]	0.697
Male *n* (%)	8 (29.6)	4 (30.8)	4 (28.6)	0.999
Illness classification *n* (%)				0.054
Mild	16 (59.3)	5 (38.5)	11 (78.6)	
Severe/critical	11 (40.7)	8 (61.5)	3 (21.4)	
Length of hospital stay (days)	11 [8, 18]	11 [9, 24]	10 [8, 13]	0.205
Presence of comorbidities				
History of hypertension *n* (%)	4 (14.8)	1 (7.7)	3 (21.4)	0.596
History of diabetes mellitus *n* (%)	5 (18.5)	2 (15.4)	3 (21.4)	0.999
History of coronary heart disease *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)	–
History of heart failure *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)	–
History of atrial fibrillation *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)	–
History of cardiomyopathy *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)	–
Laboratory values on admission				
WBC (10^9/L)	5.7 [4.6, 6.7]	5.9 [4.5, 8.1]	5.5 [4.7, 6.6]	0.607
Hb (10^{12}/L)	121 [114, 130]	123 [112, 129]	120 [115, 130]	0.797
PLT (10^9/L)	203 [169, 253]	192 [178, 238]	216 [165, 253]	0.738
ALT (U/L)	32 [20, 45]	32 [24, 42]	28 [17, 45]	0.425
AST (U/L)	27 [19, 35]	32 [24, 48]	22 [16, 30]	0.057
LDH (U/L)	258 [171, 338]	332 [227, 422]	185 [156, 274]	0.046
Creatinine (umol/L)	52 [46, 67]	64 [51, 76]	51 [44, 59]	0.027
hs-cTnI (pg/mL)	6.3 [2.2, 14.6]	9.9 [5.6, 20.6]	5.3 [2.0, 6.8]	0.061
NT-proBNP (pg/mL)	278 [121, 388]	390 [324, 530]	123 [116, 274]	0.020
hs-CRP (mg/L)	10.8 [2.6, 74.7]	29.0 [5.5, 81.4]	3.6 [2.3, 33.9]	0.219
IL-6 (pg/mL)	12.6 [4.8, 20.3]	9.2 [5.2, 12.9]	17.4 [8.3, 24.3]	0.418
D-dimer (ng/mL)	337 [292, 847]	332 [313, 912]	346 [222, 749]	0.554
Treatments during hospitalization				
Antibiotics *n* (%)	12 (44.4)	6 (46.2)	6 (42.9)	0.870
Corticosteroids *n* (%)	5 (18.5)	3 (23.1)	2 (14.3)	0.557
Antiviral drugs *n* (%)	19 (70.4)	10 (76.9)	9 (64.3)	0.472
Immunoglobulin *n* (%)	1 (3.7)	0 (0.0)	1 (7.1)	0.326
Oxygen inhalation *n* (%)	16 (59.3)	9 (69.2)	7 (50.0)	0.310
Mechanical therapy *n* (%)	2 (7.4)	2 (15.4)	0 (0.0)	0.127

WBC, white blood cell; Hb, hemoglobin; PLT, platelets; ALT, glutamic-pyruvic transaminase; AST, glutamic-oxalacetic transaminease; LDH, lactate dehydrogenase; hs-cTnI, high-sensitivity cardiac troponin I; NT-proBNP, N-terminal pro-B-type natriuretic peptide; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6.

significant ($p = 0.061$) on admission, although hs-cTnI levels were confirmed to be significantly elevated in all patients with cardiac injury during their hospitalization.

Quality-of-Life Assessments and Exercise Capacity Test

At a 6-month follow-up, none of the patients reported any obvious cardiopulmonary symptoms such as chest distress, chest pain, palpitation, and anhelation. These patients presented normal HR and blood pressure, and there were no significant differences between patients with and without myocardial injury. The SF-36 questionnaire was performed to assess the quality of life of the patients. No significant difference was observed in the SF-36 mean scores for eight specific dimensions between the patients with cardiac injury and those without, as illustrated in **Table 2**. All patients were also instructed to undergo a 6-min walk test to evaluate the exercise tolerance, except for two with leg pain. The walk distance median was 334 (IQR = 315–358), and oxygen saturation did not decrease after exercise. Although two patients (one in each group) reported obvious fatigue, the blood pressure and dyspnea on the Borg scale after the test did not show any abnormity (**Table 2**). There was no significant difference in exercise capacity between the patients with and without cardiac injury (**Table 2**).

ECG Findings

In patients without cardiac injury, changes of T-wave morphology and ST segment were the most common ECG manifestations (7/14, 50%), especially in older patients.

TABLE 2 | SF-36 questionnaire and 6-min walk test at 6-month follow-up.

Measurements	Overall ($n = 27$)	Patients with cardiac injury ($n = 13$)	Patients without cardiac injury ($n = 14$)	p
SF-36 questionnaire				
Physical functioning	85 [73.8, 91.2]	75 [62, 89]	90 [78, 94]	0.193
Role-physical	75 [0, 100]	75 [0, 100]	88 [0, 100]	0.976
Bodily pain	84 [62, 100]	100 [74, 100]	84 [54, 96]	0.161
General health	55 [35, 70]	55 [38, 72]	52 [35, 68]	0.804
Vitality	65 [45, 80]	65 [45, 75]	62 [46, 85]	0.660
Social functioning	63 [25, 63]	62 [50, 75]	62 [25, 62]	0.352
Role-emotional	100 [67, 100]	100 [84, 100]	100 [42, 100]	0.306
Mental health	76 [56, 88]	76 [64, 84]	80 [56, 88]	0.934
Reported health transition	2 [1, 3]	2 [1, 3]	2 [1, 3]	0.908
6-min walk test				
Distance (m)	334 [315, 358]	344 [324, 358]	334 [301, 358]	0.624
HR before test (bpm)	75 [68, 85]	75 [69, 88]	75 [67, 84]	0.742
Systolic pressure before test (mmHg)	121 [110, 138]	136 [105, 142]	116 [111, 134]	0.412
Diastolic pressure before test (mmHg)	78 [74, 83]	79 [71, 87]	78 [75, 82]	0.945
Spo_2 before test (%)	98 [97, 99]	98 [97, 99]	97 [96, 99]	0.505
HR after test (bpm)	75 [68, 85]	75 [69, 88]	75 [67, 84]	0.742
Systolic pressure after test (mmHg)	129 [122, 143]	130 [115, 144]	127 [122, 143]	0.999
Diastolic pressure after test (mmHg)	79 [73, 86]	80 [71, 86]	79 [73, 86]	0.950
Spo_2 after test (%)	99 [98, 100]	99 [98, 100]	98 [98, 99]	0.356
Borg score after test	0.0 [0.0, 0.4]	0.0 [0.0, 0.0]	0.0 [0.0, 0.5]	0.742

HR, heart rate.

Premature beat (1/14, 7.1%) was also observed. In patients with cardiac injury, ST-segment change (1/13, 7.7%), premature beat (1/13, 7.7%), sinus tachycardia (1/13, 7.7%), sinus bradycardia (1/13, 7.7%), prolongation of PR interval (1/13, 7.7%), and bundle-branch block (2/13, 15.4%) were identified.

Echocardiography Findings

Echocardiographic characteristics of patients who recovered from COVID-19 are summarized in **Table 3**. Compared with reference values, no patients showed abnormalities in cardiac structure, as indicated by normal LA dimension, LV dimension, RA dimension, RV dimension, IVS, and LVPW. The functions of LV and RV were preserved, as evidenced by LVEF and TAPSE, respectively. All echocardiographic parameters were statistically comparable between the patients with and without cardiac injury (**Table 3**).

CMR Findings

Most of the morphological and functional parameters were within the reference range, as indicated by LVEF, cardiac output, cardiac index, end-diastolic volume index, end- systolic volume index, stroke volume index, and myocardial mass index. There were no significant differences among these parameters between the two groups (**Table 3**). A total of eight patients (8/27, 29.6%) were observed with positive LGE, indicating the existence of myocardial fibrosis. The median of the LGE volume to the total LV myocardium volume ratio was 8.4% (IQR = 7.2%−9.2%; range from 5.5 to 9.9%). Most LGEs (7/8, 87.5%) were located at LV septal segments, followed by RV insertion points (4/8, 50%). Importantly, compared with patients without cardiac injury, patients with cardiac injury exhibited a much higher positive proportion in LGE sequences [7/13 (53.8%) vs. 1/14 (7.1%), $p = 0.013$]. Representative CMR images with LGE positive are shown in **Figure 2**. There were no significant differences for native T1 and ECV measurements between the two groups.

Laboratory Findings

Laboratory findings are presented in **Table 3**. Most of the serum biochemical indexes were within the normal range for recovered patients at the time of 6-month follow-up. However, patients with and without cardiac injury still differed significantly concerning multiple indexes of organ function including the liver, kidney, and heart. Patients with cardiac injury showed significantly higher levels of AST, LDH, creatinine, hs-cTnI, and NT-proBNP, compared with patients without cardiac injury. Notably, consistent with CMR findings, ST2, a recommended indicator of myocardial fibrosis, was higher in patients with cardiac injury (19, 20). No significant differences were observed in the levels of inflammatory factors, interleukin 6, and high-sensitivity C-reactive protein (hs-CRP). The levels of coagulation index D-dimer were also comparable between the two groups.

TABLE 3 | Echocardiography, cardiac magnetic resonance imaging and laboratory findings at 6-month follow-up.

Measurements	Overall ($n = 27$)	Patients with cardiac injury ($n = 13$)	Patients without cardiac injury ($n = 14$)	p
ECHO parameters				
LA (mm)	34 [30, 38]	31 [30, 34]	35 [34, 38]	0.094
LV (mm)	45 [43, 47]	44 [42, 47]	46 [44, 48]	0.135
RA (mm)	38 [35, 41]	38 [35, 41]	38 [36, 40]	0.897
RV (mm)	25 [21, 29]	23 [20, 29]	26 [22, 29]	0.340
IVS (mm)	10 [9, 11]	10 [9, 10]	11 [9, 11]	0.155
LVPW (mm)	10 [9, 11]	10 [9, 10]	11 [9, 11]	0.134
LVEF (%)	60 [56, 66]	61 [53, 66]	60 [57, 66]	0.700
TAPSE	27 [26, 30]	26 [22, 29]	27 [26, 30]	0.187
CMR parameters				
LGE *n* (%)	8 (29.6)	7 (53.8)	1 (7.1)	0.013
LVEF (%)	56 [54, 59]	55 [53, 58]	57 [54, 59]	0.537
EDV (mL)	94 [89, 106]	93 [88, 100]	95 [90, 109]	0.498
ESV (mL)	43 [37, 49]	42 [34, 46]	43 [39, 50]	0.878
Myo mass (g)	65 [57, 73]	63 [51, 70]	68 [60, 75]	0.230
CO (L/min)	3.5 [3.1, 3.9]	3.2 [2.7, 3.6]	3.8 [3.3, 3.9]	0.196
CI (L/min/m^2)	2.1 [1.8, 2.3]	2.1 [1.8, 2.3]	2.0 [1.8, 2.3]	0.805
EDVI (mL/m^2)	57 [52, 63]	60 [54, 66]	54 [50, 61]	0.206
ESVI (mL/m^2)	25 [22, 31]	26 [23, 31]	24 [22, 29]	0.422
SVI (mL/m^2)	33 [29, 37]	33 [29, 38]	32 [28, 34]	0.371
Myo mass index (g/m^2)	38 [35, 43]	40 [33, 43]	38 [35, 42]	0.975
T1 mapping	1211.7 [1185.2, 1247.1]	1242.6 [1202.6, 1265.3]	1205.1 [1179.0, 1236.5]	0.196
ECV	0.28 [0.26, 0.31]	0.28 [0.25, 0.30]	0.29 [0.27, 0.31]	0.618
Laboratory findings				
ALT (U/L)	20 [13, 29]	23 [16, 36]	16 [12, 28]	0.152
AST (U/L)	22 [16, 29]	29 [19, 30]	17 [14, 24]	0.004
LDH (U/L)	198 [164, 218]	217 [212, 224]	168 [151, 187]	0.001
Creatinine (umol/L)	58 [50, 68]	65 [57, 78]	52 [47, 60]	0.016
hs-cTnI (pg/mL)	2.9 [2.0, 4.4]	4.3 [2.0, 6.7]	2.5 [1.8, 2.9]	0.041
NT-proBNP (pg/mL)	73 [49, 100]	99 [78, 138]	47 [36, 61]	0.004
hs-CRP (mg/L)	0.9 [0.6, 2.1]	0.8 [0.5, 2.0]	1.5 [0.8, 2.2]	0.356
IL-6 (pg/mL)	1.5 [1.5, 1.9]	1.5 [1.5, 1.5]	1.7 [1.5, 3.0]	0.117
D-dimer (ng/mL)	146 [103, 230]	152 [138, 213]	120 [90, 230]	0.227
ST2 (ng/mL)	14.1 [9.8, 17.9]	16.6 [12.1, 22.5]	12.5 [9.5, 16.7]	0.044

ECHO, echocardiography; CMR, Cardiac Magnetic Resonance; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle; IVS, interventricular septum; LVPW, left ventricular posterior wall; SV, stroke volume; LGE, late gadolinium enhancement; LVEF, left ventricular ejection fraction; TAPSE, tricuspid annular plane systolic excursion; EDV, end-diastolic volume; ESV, end-systolic volume; CO, cardiac output; CI, cardiac index; EDVI, end-diastolic volume index; ESVI, end-systolic volume index; SVI, stroke volume index; ECV, extracellular volume; ALT, glutamic-pyruvic transaminase; AST, glutamic-oxalacetic transaminease; LDH, lactate dehydrogenase; hs-cTnI, high-sensitivity cardiac troponin I; NT-proBNP, N-terminal pro-B-type natriuretic peptide; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6.

Comparison Between Patients Detected With and Without Positive LGE

According to the presence or absence of positive LGE, patients with cardiac injury were further divided into two subgroups (**Table 4**). Patients in the LGE-positive group were diagnosed with more severe and critical types (6/7 vs. 2/6; **Table 4**), and more patients were subjected to oxygen inhalation (7/7 vs. 2/6; **Table 4**) than those with negative LGE. Although the number of patients was limited, compared with the LGE-negative patients, patients with positive LGE presented higher cardiac biomarker (hs-cTnI, median [IQR]: 20.6 [12.6, 27.8] vs. 3.6 [2.8, 4.3], $p = 0.011$), and inflammatory factor (hs-CRP, median [IQR]: 84.2 [73.1, 159.8] vs. 5.2 [1.8, 6.3], $p = 0.009$) on admission. There were no significant differences in these indexes at the 6-month follow-up between the two groups.

DISCUSSION

To the best of our knowledge, this is the first prospective study reporting 6-month follow-up data of patients who recovered

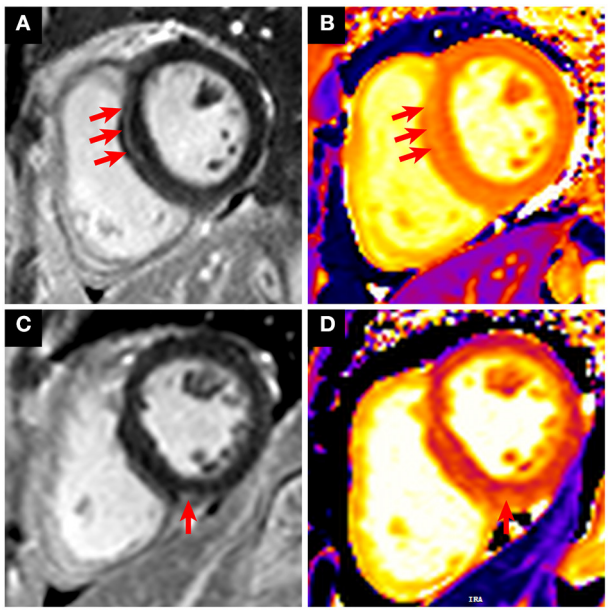


FIGURE 2 | Representative CMR images with LGE positive in patients who recovered from COVID-19. **(A,B)** A 31-year-old male patient with cardiac injury underwent CMR 6 months after recovery from COVID-19. The short-axis LGE sequence showed enhancement in the LV septal segment (**A**, red arrows). Increased native T1 was shown in the corresponding location of focal LGE (**B**, red arrows). **(C,D)** A 63-year-old female patient with cardiac injury underwent CMR 6 months after recovery from COVID-19. The short-axis LGE sequence showed enhancement in the right ventricular insertion point (**C**, red arrow). Increased native T1 was shown in the corresponding location of focal LGE (**D**, red arrow).

from COVID-19. Of the 27 patients enrolled, no patients reported any obvious cardiopulmonary symptoms at the 6-month follow-up, and there were no statistically significant differences in terms of the quality of life and exercise capacity between the patients with and without cardiac injury, as demonstrated by the SF-36 and 6-min walk tests, respectively. Echocardiography and ECG measurements did not exhibit any obvious abnormalities in these 27 patients, after their recovery from COVID-19 for 6 months. However, comparing the groups of patients with and without cardiac injury, a much higher proportion of positive LGE was found in patients with cardiac injury [7 of 13 (53.8%) vs. 1 of 14 (7.1%), $p = 0.013$], accompanied by the elevation of circulating ST2 level, a recommended indicator of myocardial fibrosis. Patients with cardiac injury presented higher levels of AST, LDH, creatinine, hs-cTnI, and NT-proBNP than those without cardiac injury, which suggested that COVID-19 patients with cardiac injury during hospitalization needed a long-term recovery from cardiac events associated with COVID-19. Nevertheless, these indexes were within the normal range for all recovered patients at the 6-month follow-up.

Previous studies revealed that impaired RV function was detected in patients who recovered from COVID-19 who demonstrated cardiac involvement in the early stage (less than 3 months) of recovery (17, 18). In a recent echocardiographic study in patients with COVID-19, 39% showed RV dilatation dysfunction during hospitalization (21). Compared with patients with normal troponin, patients with elevated troponin presented no significant difference in LV function, but they were identified with worse RV function. However, in our study, recovered patients showed both normal functions of LV and RV, without significant difference between the patients with and without myocardial damage. Longer follow-up duration may be a plausible explanation accounting for this discrepancy. The median duration between discharge and echocardiography was as long as 188 days (IQR = 182–210 days) in our current study. The cardiac function had returned to normal at the 6-month follow-up, although these patients may have suffered from cardiac dysfunction during early convalescence.

An important finding of the CMR assessment is that more than half of patients (7/13, 53.8%) in the cardiac injury group were identified with LGE positive, whereas only one patient (1/14, 7.1%) was observed with LGE positive in patients without cardiac injury. LGE imaging is currently recognized as the gold standard for non-invasive assessment of localized myocardial fibrosis (22). Consistent with the CMR findings, patients with cardiac injury were also detected with higher circulating levels of ST2, a recommended indicator of myocardial fibrosis (19, 20). These results suggest a more frequent existence of cardiac fibrosis in patients with cardiac injury during their convalescence from COVID-19. Further analysis indicated that patients with positive LGE exhibited higher troponin and hs-CRP on admission, although these biomarkers did not differ significantly between the LGE-positive and LGE-negative subgroups in the convalescence stage. These findings provided important insights into the association of myocardial injury in hospitalized patients and cardiac involvement during their recovery from COVID-19. Patients with cardiac injury appeared to be more prone to develop cardiac fibrosis after their recovery. Among these patients, it seemed that more attention should be paid to those with relative higher cardiac biomarkers and inflammatory factors on admission in their convalescence phase. Because of the limited number of included patients, correlation analyses were not conducted. Even so, our results may suggest a possible predictive value of cardiac biomarkers and inflammatory factors in cardiac fibrosis in patients who recovered from COVID-19.

It was reported that cardiac remodeling may occur following viral infection-induced myocardial damage (23). Consistent with this, although LVEF was preserved in all patients who recovered from COVID-19, myocardial fibrosis was detected using CMR, especially in those with elevated troponin during hospitalization. Nevertheless, myocardial fibrosis induced by aging and preexisting cardiac conditions cannot be completely excluded. In our cohort, we noted that a 31-year-old male patient without cardiac comorbidities and a family history of heart disease suffered from myocardial damage followed by COVID-19 infection and was identified with cardiac fibrosis at a 6-month follow-up (**Figure 2**). It is plausible to believe that cardiac fibrosis may occur in patients during the recovery phase due to COVID-19–triggered myocardial damage, which

TABLE 4 | Comparison between patients with and without positive LGE.

Characteristics	Patients with positive LGE ($n = 7$)	Patients without positive LGE ($n = 6$)	*p*
Age (years)	63 [62, 73]	61 [58, 65]	0.352
Male *n* (%)	3 (42.9)	1 (16.7)	0.559
Illness classification *n* (%)			0.103
Mild	1 (14.3)	4 (66.7)	
Severe/critical	6 (85.7)	2 (33.3)	
Length of hospital stay (days)	11 [11, 19]	16 [8, 24]	0.667
Presence of comorbidities			
History of hypertension *n* (%)	0 (0.0)	1 (16.7)	0.462
History of diabetes mellitus *n* (%)	2 (28.6)	0 (0.0)	0.462
History of coronary heart disease *n* (%)	0 (0.0)	0 (0.0)	-
History of heart failure *n* (%)	0 (0.0)	0 (0.0)	-
History of arrhythmias *n* (%)	0 (0.0)	0 (0.0)	-
History of cardiomyopathy *n* (%)	0 (0.0)	0 (0.0)	-
Laboratory values on admission			
WBC (10^9/L)	4.9 [4.4, 7.2]	6.6 [6.1, 7.8]	0.372
Hb (10^{12}/L)	122 [112, 128]	124 [115, 130]	0.685
PLT (10^9/L)	187 [138, 237]	196 [187, 229]	0.515
ALT (U/L)	32 [28, 45]	33 [23, 35]	0.685
AST (U/L)	34 [28, 48]	29 [19, 46]	0.515
LDH (U/L)	377 [303, 422]	246 [208, 335]	0.465
Creatinine (umol/L)	69 [64, 81]	52 [47, 53]	0.062
hs-cTnI (pg/mL)	20.6 [12.6, 27.8]	3.6 [1.9, 5.6]	0.011
NT-proBNP (pg/mL)	492 [390, 820]	252 [197, 308]	0.165
hs-CRP (mg/L)	84.2 [73.1, 159.8]	5.2 [1.8, 6.3]	0.009
IL-6 (pg/mL)	12.6 [11.3, 14.1]	5.6 [4.8, 6.3]	0.180
D-dimer (ng/mL)	339 [312, 955]	326 [314, 708]	0.685
Treatments during hospitalization			
Antibiotics *n* (%)	4 (57.1)	2 (33.3)	0.592
Corticosteroids *n* (%)	2 (28.6)	1 (16.7)	>0.999
Antiviral drugs *n* (%)	5 (71.4)	5 (83.3)	>0.999
Immunoglobulin *n* (%)	0 (0.0)	0 (0.0)	-
Oxygen inhalation *n* (%)	7 (100.0)	2 (33.3)	0.009
Mechanical therapy *n* (%)	1 (14.3)	1 (16.7)	0.906
ECHO parameters at 6-month follow-up			
LA (mm)	33 [31, 37]	30 [29, 31]	0.113
LV (mm)	44 [44, 47]	43 [41, 46]	0.385
RA (mm)	40 [37, 42]	35 [34, 37]	0.151
RV (mm)	29 [22, 30]	21 [19, 27]	0.195
IVS (mm)	10 [10, 11]	10 [9, 10]	0.289
LVPW (mm)	10 [10, 10]	10 [9, 10]	0.512
LVEF (%)	61 [54, 65]	60 [54, 66]	0.721
TAPSE	26 [24, 30]	25 [22, 29]	0.886
CMR parameters at 6-month follow-up			
LVEF (%)	53 [51, 55]	58 [56, 62]	0.059
EDV (mL)	92 [88, 104]	96 [88, 100]	0.85
ESV (mL)	45 [38, 52]	36 [31, 42]	0.257
Myo mass (g)	67 [62, 83]	48 [42, 56]	0.059
CO (L/min)	3.2 [2.6, 3.7]	3.5 [3.3, 3.6]	0.570
CI (L/min/m^2)	1.9 [1.7, 2.2]	2.3 [2.1, 2.4]	0.257
EDVI (mL/m^2)	55 [54, 62]	64 [59, 68]	0.449

(Continued)

TABLE 4 | Continued

Characteristics	Patients with positive LGE ($n = 7$)	Patients without positive LGE ($n = 6$)	*p*
ESVI (ml/m^2)	26 [25, 32]	26 [21, 30]	0.506
SVI (mL/m^2)	29 [29, 35]	38 [36, 40]	0.107
Myo mass index (g/m^2)	40 [37, 49]	33 [30, 38]	0.086
T1 mapping	1248.5 [1243.8, 1287.0]	1202.6 [1192.5, 1215.1]	0.089
ECV	0.28 [0.27, 0.34]	0.27 [0.24, 0.29]	0.562
Laboratory findings at 6-month follow-up			
ALT (U/L)	23 [21, 34]	21 [16, 34]	0.774
AST (U/L)	29 [27, 30]	24 [17, 40]	0.566
LDH (U/L)	212 [212, 231]	218 [196, 223]	0.829
Creatinine (umol/L)	70 [61, 81]	61 [53, 69]	0.199
hs-cTnI (pg/mL)	5.7 [3.8, 9.4]	2.8 [1.9, 4.3]	0.317
NT-proBNP (pg/mL)	92 [84, 131]	100 [73, 136]	0.855
hs-CRP (mg/L)	0.8 [0.5, 4.5]	0.7 [0.5, 0.9]	0.391
IL-6 (pg/mL)	1.5 [1.5, 1.5]	1.5 [1.5, 1.5]	0.562
D-dimer (ng/mL)	171 [144, 290]	146 [135, 163]	0.423
ST2 (ng/mL)	14.1 [10.5, 21.2]	18.5 [16.3, 22.0]	0.475

LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle; IVS, interventricular septum; LVPW, left ventricular posterior wall; SV, stroke volume; LVEF, left ventricular ejection fraction; TAPSE, tricuspid annular plane systolic excursion; EDV, end-diastolic volume; ESV, end-systolic volume; CO, cardiac output; CI, cardiac index; EDVI, end-diastolic volume index; ESVI, end-systolic volume index; SVI, stroke volume index; LGE, late gadolinium enhanced; ECV, extracellular volume fraction; WBC, white blood cell; Hb, hemoglobin; PLT, platelets; ALT, glutamic-pyruvic transaminase; AST, glutamic-oxalacetic transaminease; LDH, lactate dehydrogenase; hs-cTnI, high-sensitivity cardiac troponin I; NT-proBNP, N-terminal pro-B-type natriuretic peptide; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6.

represents a repair process. Detection of cardiac fibrosis may indicate that these patients were in a relatively early stage of cardiac involvement, and whether it would progress to cardiac dysfunction or electrophysiological disturbance is a potential concern. Further follow-up will be valuable to confirm the long-term clinical implication of cardiac fibrosis in these LGE-positive patients who recovered from COVID-19. Moreover, it would be important to evaluate whether positive LGE and high plasmatic concentration of ST2 could be long-term predictors of cardiac outcomes in a large cohort of recovered COVID-19 patients.

It has been demonstrated that 8–12% of unselected COVID-19 cases were identified with cardiac injury (24). Studies from our group and others have demonstrated that cardiac injury is significantly associated with fatal outcomes of hospitalized patients with COVID-19 (7–12). In the present study, our results implied that cardiac fibrosis during convalescence may be a direct consequence of myocardial damage induced by COVID-19. However, the exact pathophysiological mechanisms underlying myocardial injury induced by COVID-19 remain to be fully elucidated. The possible mechanisms include the direct damage to cardiomyocytes of SARS-CoV-2 infection through angiotensin-converting enzyme 2, cytokine storm precipitated by overactivation of the immune response, dysregulation of the renin–angiotensin–aldosterone system, and disturbances of coagulation and microcirculation-induced hypoxia (3, 5, 25). A previous study revealed a critical role of SARS-CoV in transforming growth factor β signaling, which is a predominant regulator of cardiac fibrosis (26). Given the high homology of the two viruses, SARS-CoV-2 may share a similar mechanism for the contribution to cardiac fibrosis. More mechanism studies are needed to investigate the association between myocardial injury during hospitalization and cardiac fibrosis at recovery in COVID-19 patients.

LIMITATIONS

There are several limitations that should be highlighted. First, in the present study, the sample size of enrolled patients is limited, possibly due to the strict criteria of inclusion and exclusion and the early stage of this outbreak. The findings in this report should be interpreted with caution and warrant further large-scale prospective studies to validate. However, patients in our cohort were recruited from the same hospital, which had detailed clinical data and was confirmed with homogeneity of diagnosis and treatment. Second, we did not observe any major cardiovascular events for now, although cardiac fibrosis was manifested in COVID-19 patients with cardiac injury in our medium-term follow-up. Long-term observation is still needed to further investigate the prognosis of those with cardiac injury after the infection of SARS-CoV-2. Third, pulmonary evaluation such as chest computed tomography and lung function test was not performed in the current study. However, all patients enrolled did not report any pulmonary symptoms at follow-up, and no patients showed abnormity of oxygen saturation and respiration

after a 6-min walk test. Finally, even if we have accounted for variables associated with the prognosis of patients with COVID-19 as much as possible, undetected factors might still appear.

CONCLUSIONS

In summary, our preliminary follow-up data with a limited number of patients revealed persistent cardiac involvement in 29.6% (8/27) of recovered patients from COVID-19 up to 6 months after discharge. Patients with cardiac injury during hospitalization were more prone to develop cardiac fibrosis during their recovery. Among patients with cardiac injury, it seemed that those with relatively higher cardiac biomarkers and inflammatory factors were more likely to have cardiac involvement in the convalescence phase. More studies are needed to investigate the association between myocardial injury during hospitalization and cardiac fibrosis in recovered patients from COVID-19.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committees of Zhongnan Hospital of Wuhan University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ZL, HX, and XWa had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, and contributed to the conception and design of the work. XWu, K-QD, and CL acquired and analyzed the data. K-QD and CL performed the statistical analysis. ZY, HH, HC, CZ, TH, and FZ acquired the data and provide technical support. K-QD, CL, HX, and ZL drafted the manuscript. HW, XZ, AC, YY, and XWa provided critical revision of the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Excellent Doctoral Program of Zhongnan Hospital of Wuhan University (ZNYB2019001).

REFERENCES

1. Han E, Tan MMJ, Turk E, Sridhar D, Leung GM, Shibuya K, et al. Lessons learnt from easing COVID-19 restrictions: an analysis of countries and regions in Asia Pacific and Europe. *Lancet*. (2020) 396:1525–34. doi: 10.1016/S0140-6736(20)32007-9
2. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. (2020) 579:270–3. doi: 10.1038/s41586-020-2951-z
3. Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, et al. Extrapulmonary manifestations of COVID-19. *Nat Med*. (2020) 26:1017–32. doi: 10.1038/s41591-020-0968-3
4. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential Effects of coronaviruses on the cardiovascular system: a review. *JAMA Cardiol*. (2020) 5:831–40. doi: 10.1001/jamacardio.2020.1286
5. Nishiga M, Wang DW, Han Y, Lewis DB, Wu JC. COVID-19 and cardiovascular disease: from basic mechanisms to clinical perspectives. *Nat Rev Cardiol*. (2020) 17:543–58. doi: 10.1038/s41569-020-0413-9
6. Driggin E, Madhavan MV, Bikdeli B, Chuich T, Laracy J, Biondi-Zoccai G, et al. Cardiovascular considerations for patients, health care workers, and health systems during the COVID-19 pandemic. *J Am Coll Cardiol*. (2020) 75:2352–71. doi: 10.1016/j.jacc.2020.03.031
7. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol*. (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017
8. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol*. (2020) 5:802–10. doi: 10.1001/jamacardio.2020.0950
9. Nie SF, Yu M, Xie T, Yang F, Wang HB, Wang ZH, et al. Cardiac troponin I is an independent predictor for mortality in hospitalized patients with COVID-19. *Circulation*. (2020) 142:608–10. doi: 10.1161/CIRCULATIONAHA.120.048789
10. Lala A, Johnson KW, Januzzi JL, Russak AJ, Paranjpe I, Richter F, et al. Prevalence and impact of myocardial injury in patients hospitalized with COVID-19 infection. *J Am Coll Cardiol*. (2020) 76:533–46. doi: 10.1101/2020.04.20.20072702
11. Giustino G, Croft LB, Stefanini GG, Bragato R, Silbiger JJ, Vicenzi M, et al. Characterization of myocardial injury in patients with COVID-19. *J Am Coll Cardiol*. (2020) 76:2043–55. doi: 10.1016/j.jacc.2020.08.069
12. Shi S, Qin M, Cai Y, Liu T, Shen B, Yang F, et al. Characteristics and clinical significance of myocardial injury in patients with severe coronavirus disease 2019. *Eur Heart J*. (2020) 41:2070–9. doi: 10.1093/eurheartj/ehaa408
13. van den Borst B, Peters JB, Brink M, Schoon Y, Bleeker-Rovers CP, Schers H, et al. Comprehensive health assessment three months after recovery from acute COVID-19. *Clin Infect Dis*. (2020). doi: 10.1093/cid/ciaa1750. [Epub ahead of print].
14. Daher A, Balfanz P, Cornelissen C, Muller A, Bergs I, Marx N, et al. Follow up of patients with severe coronavirus disease 2019 (COVID-19): pulmonary and extrapulmonary disease sequelae. *Respir Med*. (2020) 174:106197. doi: 10.1016/j.rmed.2020.106197
15. Huang Y, Tan C, Wu J, Chen M, Wang Z, Luo L, et al. Impact of coronavirus disease 2019 on pulmonary function in early convalescence phase. *Respir Res*. (2020) 21:163. doi: 10.1186/s12931-020-01429-6
16. Chen K-Y, Li T, Gong F-H, Zhang J-S, Li X-K. Predictors of health-related quality of life and influencing factors for COVID-19 patients, a follow-up at one month. *Front Psychiatry*. (2020) 11:668. doi: 10.3389/fpsyt.2020.00668
17. Huang L, Zhao P, Tang D, Zhu T, Han R, Zhan C, et al. Cardiac involvement in patients recovered from COVID-2019 identified using magnetic resonance imaging. *JACC Cardiovasc Imaging*. (2020) 13:2330–9. doi: 10.1016/j.jcmg.2020.05.004
18. Puntmann VO, Carerj ML, Wieters I, Fahim M, Arendt C, Hoffmann J, et al. Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19). *JAMA Cardiol*. (2020) 5:1265–73. doi: 10.1001/jamacardio.2020.3557
19. de Boer RA, Daniels LB, Maisel AS, Januzzi JL, Jr. State of the Art: newer biomarkers in heart failure. *Eur J Heart Fail*. (2015) 17:559–69. doi: 10.1002/ejhf.273

20. Bayes-Genis A, de Antonio M, Vila J, Penafiel J, Galan A, Barallat J, et al. Head-to-head comparison of 2 myocardial fibrosis biomarkers for long-term heart failure risk stratification: ST2 versus galectin-3. *J Am Coll Cardiol*. (2014) 63:158–66. doi: 10.1016/j.jacc.2013.07.087
21. Szekely Y, Lichter Y, Taieb P, Banai A, Hochstadt A, Merdler I, et al. Spectrum of cardiac manifestations in COVID-19: a systematic echocardiographic study. *Circulation*. (2020) 142:342–53. doi: 10.1161/CIRCULATIONAHA.120.047971
22. Ambale-Venkatesh B, Lima JA. Cardiac MRI: a central prognostic tool in myocardial fibrosis. *Nat Rev Cardiol*. (2015) 12:18–29. doi: 10.1038/nrcardio.2014.159
23. Kraft L, Erdenesukh T, Sauter M, Tschope C, Klingel K. Blocking the IL-1beta signalling pathway prevents chronic viral myocarditis and cardiac remodeling. *Basic Res Cardiol*. (2019) 114:11. doi: 10.1007/s00395-019-0719-0
24. Tomasoni D, Italia L, Adamo M, Inciardi RM, Lombardi CM, Solomon SD, et al. COVID-19 and heart failure: from infection to inflammation and angiotensin II stimulation. Searching for evidence from a new disease. *Eur J Heart Fail*. (2020) 22:957–66. doi: 10.1002/ejhf.1871
25. Dou Q, Wei X, Zhou K, Yang S, Jia P. Cardiovascular manifestations and mechanisms in patients with COVID-19. *Trends Endocrinol Metab*. (2020) 31:893–904. doi: 10.1016/j.tem.2020.10.001
26. Zhao X, Nicholls JM, Chen Y-G. Severe acute respiratory syndrome-associated coronavirus nucleocapsid protein interacts with Smad3 and modulates transforming growth factor-beta signaling. *J Biol Chem*. (2008) 283:3272–80. doi: 10.1074/jbc.M708033200

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 13 May 2021
doi: 10.3389/fcvm.2021.633878

Preexisting Oral Anticoagulant Therapy Ameliorates Prognosis in Hospitalized COVID-19 Patients

Guido Iaccarino[1†‡], Guido Grassi[2‡], Claudio Borghi[3‡], Davide Grassi[4], Costantino Mancusi[1], Maria Lorenza Muiesan[5], Massimo Salvetti[5], Massimo Volpe[6,7‡] and Claudio Ferri[4†] on behalf of the SARS-RAS Investigators*

[1] Department of Advanced Biomedical Sciences, Federico II University, Naples, Italy, [2] Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy, [3] Department of Medicine and Surgery Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy, [4] Department of Clinical Medicine, Public Health, Life and Environment Sciences, University of L'Aquila, L'Aquila, Italy, [5] Department of Clinical and Experimental Sciences, University of Brescia, Medicina 2, ASST Spedali Civili Brescia, Brescia, Italy, [6] Clinical and Molecular Medicine Department, Sapienza University, Rome, Italy, [7] Sant'Andrea Hospital, Rome, Italy

Objective: Altered coagulation parameters in COVID-19 patients is associated with a poor prognosis. We tested whether COVID-19 patients on chronic oral anticoagulants (cOACs) for thromboembolism prophylaxis could receive protection from developing more severe phenotypes of the disease.

Approach and Results: We searched the database of the SARS-RAS study (Clinicaltrials.gov: NCT04331574), a cross-sectional observational multicenter nationwide survey in Italy designed by the Italian Society of Hypertension. The database counts 2,377 charts of Italian COVID-19 patients in 26 hospitals. We calculated the Charlson comorbidity index (CCI), which is associated with death in COVID-19 patients. In our population (n = 2,377, age 68.2 ± 0.4 years, CCI: 3.04 ± 0.04), we confirm that CCI is associated with increased mortality [OR: 1.756 (1.628-1.894)], admission to intensive care units [ICU; OR: 1.074 (1.017-1.134)], and combined hard events [CHE; OR: 1.277 (1.215-1.342)]. One hundred twenty-five patients were on cOACs (age: 79.3 ± 0.9 years, CCI: 4.35 ± 0.13); despite the higher CCI, cOACs patients presented with a lower risk of admissions to the ICU [OR 0.469 (0.250-0.880)] but not of death [OR: 1.306 (0.78-2.188)] or CHE [OR: 0.843 (0.541-1.312)]. In multivariable logistic regression, cOACs confirmed their protective effect on ICU admission and CHE. The CCI remains the most important risk factor for ICU admission, death, and CHE.

Conclusions: Our data support a mechanism for the continuation of cOAC therapy after hospital admission for those patients who are on chronic treatment. Our preliminary results suggest the prophylactic use of direct cOACs in patients with elevated CCI score at the time of the COVID-19 pandemic even in absence of other risks of thromboembolism.

Keywords: multimorbidity, atrial fibrillation, prophylaxis, death, intensive care admissions, COVID-19 outcomes, hypertension, thrombosys

OPEN ACCESS

Edited by:
Mingxing Xie,
Huazhong University of Science and Technology, China

Reviewed by:
Antonio Cano,
University of Valencia, Spain
Yani Liu,
Huazhong University of Science and Technology, China

***Correspondence:**
Guido Iaccarino
guiaccar@unina.it

† These authors have contributed equally to this work

‡ORCID:
Guido Iaccarino
orcid.org/0000-0002-8997-835X
Guido Grassi
orcid.org/0000-0003-1922-6547
Claudio Borghi
orcid.org/0000-0001-8039-8781
Massimo Volpe
orcid.org/0000-0002-9642-8380

Specialty section:
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

Received: *26 November 2020*
Accepted: *19 March 2021*
Published: *13 May 2021*

Citation:
Iaccarino G, Grassi G, Borghi C, Grassi D, Mancusi C, Muiesan ML, Salvetti M, Volpe M and Ferri C (2021) Preexisting Oral Anticoagulant Therapy Ameliorates Prognosis in Hospitalized COVID-19 Patients. Front. Cardiovasc. Med. 8:633878. doi: 10.3389/fcvm.2021.633878

INTRODUCTION

The current epidemic of COVID-19 has put the world population and health care systems under enormous stress, acting as an accelerator for death in the older population and anticipating the failure of hospitalocentric administration of health care in light of the increased request for hospital admissions. The scientific community has to, therefore, identify how to protect the high-risk population from the development of critical conditions that would increase the request for high-intensity care. It is now clear from the available literature that older and multimorbid patients are at risk of worse outcomes of COVID-19 (1, 2). Emerging evidence, though, proposes that the more severe outcomes of COVID-19 are also associated with alteration in coagulation patterns. The evidence that altered coagulation parameters in COVID-19 patients is associated with poor prognosis (3, 4) and has led us to hypothesize that the virus can cause an endothelial disease with systemic manifestation due to increased thrombosis (5). Low-molecular-weight heparin anticoagulation in the intensive care unit is associated with better prognosis in severe COVID-19 patients (6). Indeed, in this scenario, anticoagulant treatments are indicated by the majority as pivotal for the management of COVID-19 (7, 8). We explore the possibility that COVID-19 patients on chronic oral anticoagulants (cOACs) for a concomitant condition (i.e., atrial fibrillation, mechanic valvular replacement, pulmonary thromboembolism prophylaxis) before admission receive protection from more severe outcomes.

METHODS

We designed a cross-sectional, multicenter, observational study involving 26 hospitals approached through the Italian Society of Hypertension network in 14 regions of Italy to achieve a nationally representative population sample (SARS-RAS Study) (9). The study is based on an online questionnaire to be filled in with data collected from the hospital charts of COVID-19 patients (see **Supplementary Material**). The patient cohort included 2,377 patients aged 18-101 years who were referred to the hospital for symptoms of COVID-19. All patients included in the questionnaire were diagnosed with COVID-19 according to World Health Organization interim guidance (10). The observation period started March 9 and ended May 9, 2020. The study was performed following article 89 of General Data Protection and Regulation (https://gdpr-info.eu). The SARS-RAS study is registered on Clinicaltrials.gov with the accession number NCT04331574. The online questionnaire was distributed among the centers to collect reviewed epidemiological, clinical, and outcomes data from hospital emergency rooms and regular and intensive care wards. Each center designated one or more physicians who were tasked with the acquisition and review of the requested information. Patients were pseudonymized by assigning a deidentified identification code. The questionnaire

TABLE 1 | Clinical Characteristics of study population.

	Population n = 2,377	cOACs n = 125	No cOACs n = 2,252	Significance
Age (years)	68.21 ± 0.38	79.35 ± 0.86	67.59 ± 0.39	<0.01
Female	37.3% (888)	45.6% (57)	36.9% (831)	<0.05
CCI	3.04 ± 0,04	4.35 ± 0.13	2.97 ± 0.04	<0.01
Hypertension	59% (1,402)	70.4% (88)	57.86% (1,303)	<0.01
Diabetes	18% (428)	16.8% (21)	18.29% (412)	n.s.
Obesity	7% (166)	4.8% (6)	6.75% (152)	n.s.
BPCO	8% (190)	8% (10)	8.48% (191)	n.s.
CKD	6% (143)	4% (5)	5.68% (128)	n.s.
HF	12% (285)	28.8% (36)	11.14% (251)	<0.01
Death	12% (285)	14.4% (18)	11.41% (257)	n.s.
ICU	17% (404)	8.8% (11)	17.05% (384)	<0.02
Atrial Fibrillation	4.7% (111)	88.8% (111)		
Venous Thrombosis	0.5% (14)	11.2% (14)		

CCI, Charlson comorbidity index; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; HF, heart failure; ICU, patients in intensive care unit; cOACS, chronic oral anticoagulants. Significance refers to cOACs vs. no cOACS; data are expressed in mean ± SEM or %; in brackets, the absolute numbers; statistical analysis was conducted using ANOVA for continuous variables and chi-squared for distribution.

TABLE 2 | Univariate and multivariate analysis.

	Wald	Sign.	Exp(B)	95% CI, Lower	95% CI, Upper
Death, univariate analysis					
CCI	211.48	**0.00**	1.76	1.63	1.89
Age	123.16	**0.00**	1.07	1.05	1.08
Female sex	0.80	0.37	0.89	0.68	1.15
cOACs	1.03	0.31	1.31	0.78	2.19
Death, multivariate analysis					
CCI	212.80	**0.00**	1.76	1.63	1.90
Female sex	1.17	0.28	0.86	0.65	1.14
cOACS	1.08	0.30	1.34	0.77	2.30
ICU admission, univariate analysis					
Age	0.64	0.42	1.00	1.00	1.01
Female sex	24.14	**0.00**	0.55	0.43	0.70
CCI	6.50	**0.01**	1.07	1.02	1.13
cOACs	5.56	**0.02**	2.13	1.14	3.99
ICU admission, multivariate analysis					
CCI	9.16	**0.00**	1.09	1.03	1.15
Female sex	23.74	**0.00**	0.55	0.43	0.70
cOACs	6.48	**0.01**	2.28	1.21	4.31
Combined hard events, univariate analysis					
CCI	92.16	**0.00**	1.28	1.21	1.34
Age	40.27	**0.00**	1.02	1.01	1.03
Female sex	22.33	**0.00**	0.61	0.50	0.75
cOACs	0.57	0.45	1.19	0.76	1.85
Combined hard events, multivariate analysis					
CCI	98.16	**0.00**	1.29	1.23	1.36
Female sex	24.21	**0.00**	0.59	0.48	0.73
cOACs	3.86	**0.05**	1.58	1.00	2.48

CCI, Charlson comorbidity index; cOACS, chronic oral anticoagulants; CI, confidence intervals.
Bold p values indicate statistical significance.

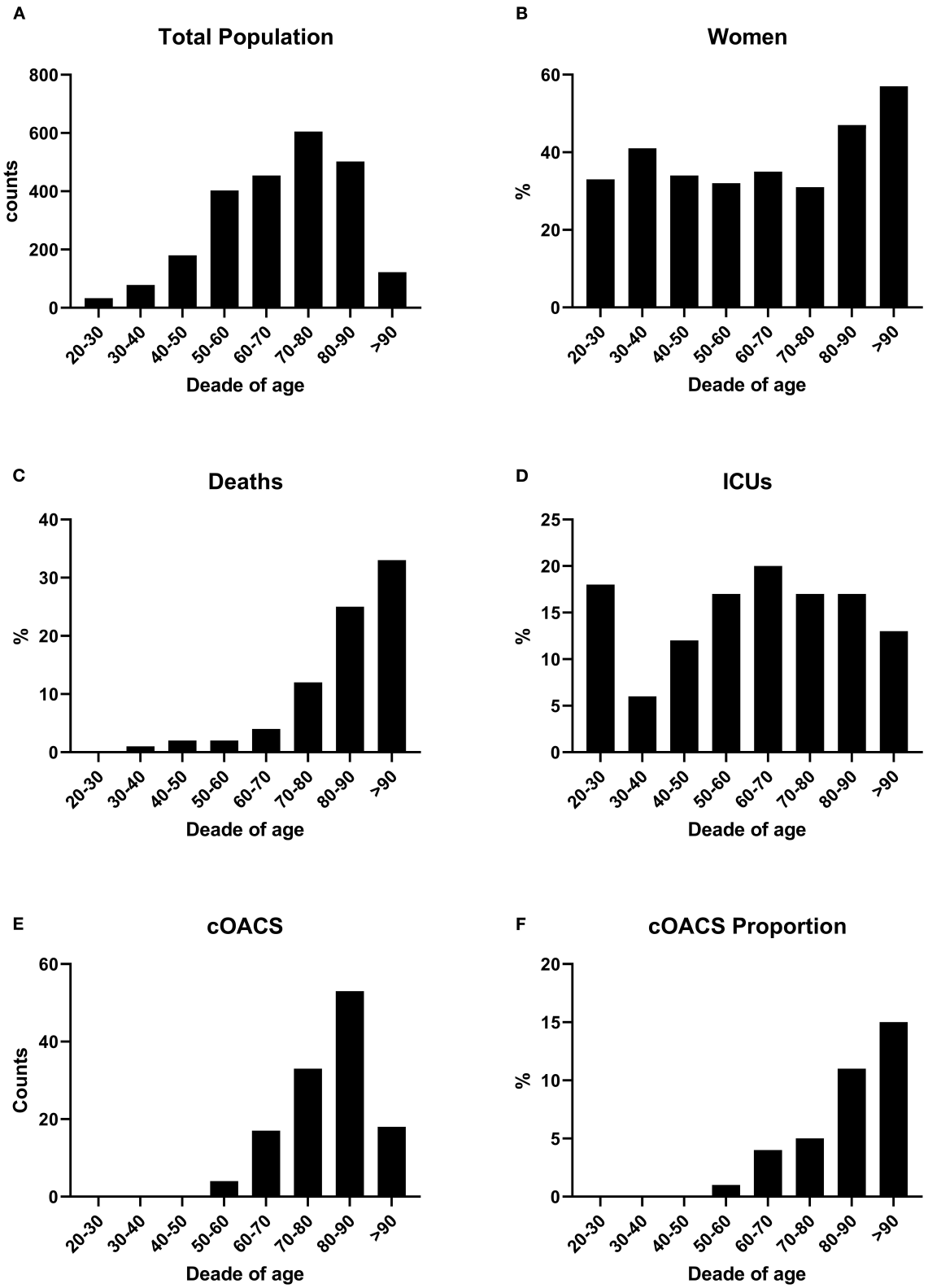


FIGURE 1 | (A) COVID-19 patients were grouped by age decades to show the impact of the disease according to age. Patient numbers increased by age decades. **(B)** Women were stably below 40% of total cases up to the age of 80 years. **(C)** Death rates increased with age. **(D)** ICU admissions were stable along all ages. **(E)** cOACs were administered increasingly with age. **(F)** The percentage of patients on cOACs increased with age.

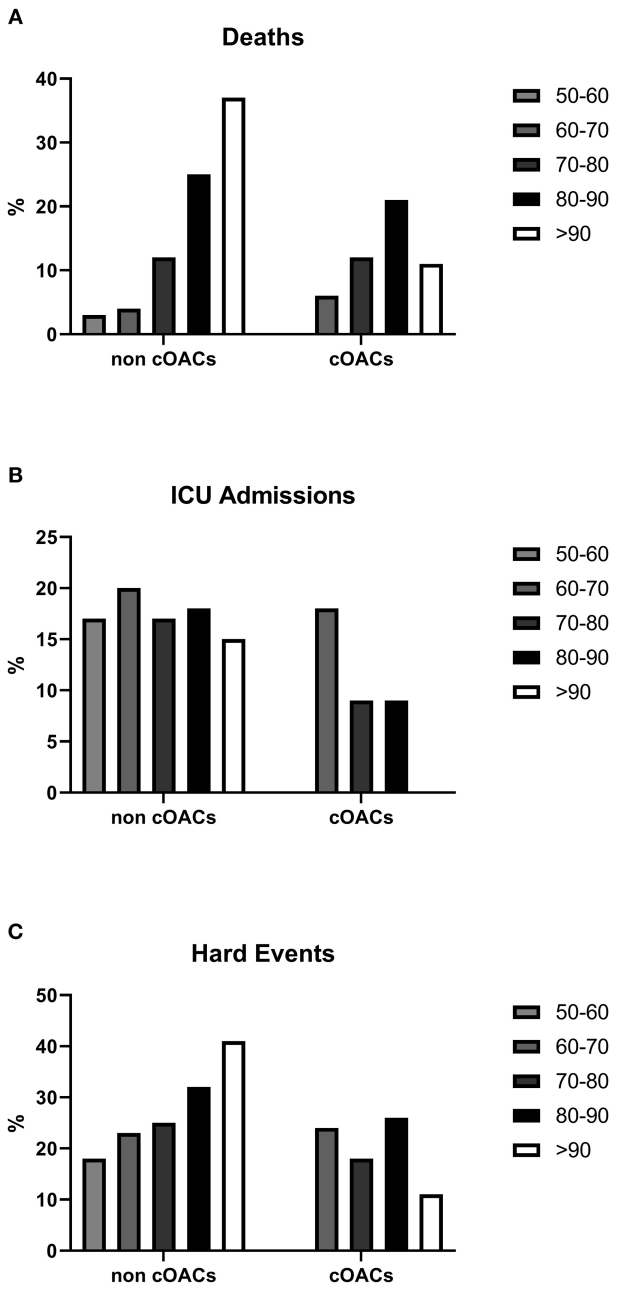


FIGURE 2 | (A) Death rates in non-cOACs and cOACs COVID-19 patients. **(B)** ICU admission rates were lower in non-cOACs than cOACs COVID-19 patients. **(C)** CHE (death and ICU admission) rates were similar in non-cOACs and cOACs COVID-19 patients.

collected information regarding the center and the age, sex, nationality (Italian or other), and city of origin of the patient. From the anamnesis, we collected whether the patient had a known diagnosis of hypertension, coronary artery disease (history of myocardial infarction, PCI, or CABG), heart failure (based on clinical history), atrial fibrillation, diabetes, chronic kidney disease (anamnestic estimated glomerular filtration rate below 60 ml/min/kg), chronic obstructive pulmonary disease (according to GOLD 2019), obesity (body mass index > 30 kg/m^2), history of blood and solid tumors, liver disease, or other comorbidities; we annotated the presence of prescribed antihypertensive, anticoagulant, and antidiabetic therapy.

The severity of the disease was classified according to the Chinese Center for Disease Control (11) into three groups: asymptomatic or with light symptoms, moderate symptoms, and severe intensity.

We collected also the outcomes (hospital dismission or exitus). All patients for which the course of the disease was in an active state were classified as such (10).

For each patient, we calculated the Charlson comorbidity index (CCI) based on the available data and according to the original description of the score (12). Descriptive analyses of the variables were expressed as mean and standard errors or frequencies expressed in absolute numbers and percentages. Continuous variables were analyzed by ANOVA; categorical data were compared using the χ^2 test. Regression analyses, odds ratio, and confidence intervals were tested on the interest variables grouped by outcomes; multivariable regression analyses were performed on the significant and clinically relevant continuous and categorical variables.

RESULTS

We collected charts of 2,569 patients. We excluded from the analysis 192 patients for incomplete or discordant data. The analysis was then performed on 2,377 patients. The clinical features of our population are indicated in **Table 1**. Women were less frequently affected than men, and the mean age indicates that the disease was prevalent among the senior population (**Figure 1**). We counted 285 deaths and more than 400 patients admitted to intensive care units (ICUs) (**Figure 2**). We confirm that CCI is associated with increased mortality [**Table 2**, OR: 1.756 (1.628-1.894)], admission to ICUs [OR: 1.074 (1.017-1.134)], and combined hard events [CHE, OR: 1.277 (1.215-1.342)]. One hundred twenty-five patients were on cOACs for thromboembolic prevention for atrial fibrillation and venous thromboembolism for at least 6 months before the diagnosis of COVID-19. Compared with non-cOACs, cOACs patients were older, included more women, and had a larger CCI (**Table 1**). Despite the larger CCI, cOACs patients were less likely to be referred to the ICU [**Table 2**, OR 0.469 (0.250-0.880)], but with a similar risk of death [OR: 1.306 (0.78-2.188)] or CHE [OR: 0.843 (0.541-1.312)]. To ascertain the role of age, multimorbidity (combined in the CCI score), sex, and cOACs on the outcome, we performed a multivariable logistic regression analysis on ICU admission, death, and CHE. cOACs confirmed the protective effect on admission to the ICU and CHE (**Figures 3A,C**) but not on death (**Figure 3B**). Sex and CCI remain significant risk factors for ICU access and CHE in COVID-19 patients (**Figures 3A-C**). In particular, CCI represents the most important risk factor for death in COVID-19 patients.

DISCUSSION

Our study shows that cOACs modify the risk of admission to the ICU and CHE even independently from the major determinants

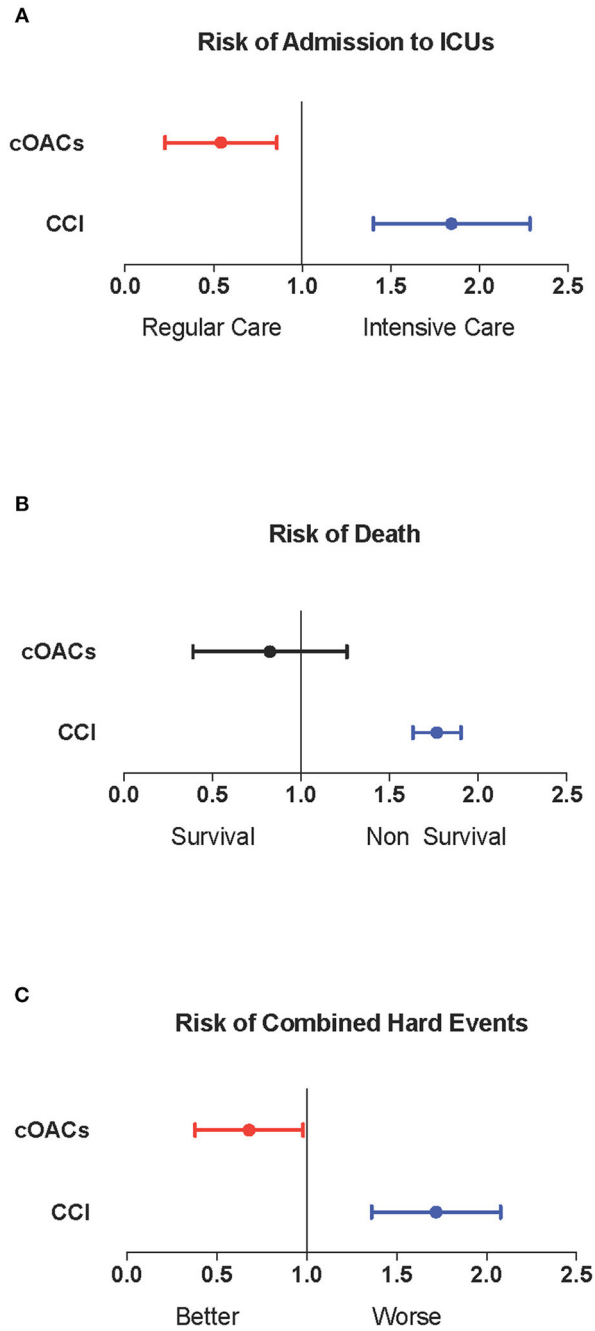


FIGURE 3 | (A) Odds ratio and confidence intervals for admission to intensive care units; cOACs: $T = -0.825$, $p < 0.01$; CCI: $T = 0.085$, $p < 0.003$; male sex: $T = 0.602$; $p < 0.001$. **(B)** Odds ratio and confidence intervals for deaths; CCI: $T = 0.567$, $p < 0.001$. **(C)** Odds ratio and confidence intervals for combined hard events (deaths and ICU admissions); cOACs: $T = -0.455$; $p < 0.05$; male sex: $T = 0.527$; $p < 0.001$; CCI:$T = 0.257$; $p < 0.001$. CCI, Charlson comorbidity index; cOACs, chronic oral anticoagulants; ICU, intensive care units.

of outcomes in COVID-19, which are age, comorbidity, and sex. This result is well in agreement with the proposed empiric use of anticoagulants for the treatment of severely ill COVID-19 patients (13). So far, this use is based mainly on evidence gathered on a small number of patients of the subgroup analysis from a single retrospective, poorly controlled study (4). The largest number of patients, the multicenter design, and the possibility to perform a multivariable statistic approach confer our study larger statistical soundness.

COVID-19 patients are prone to venous thromboembolism (3) and present with abnormal coagulation parameters, such as D-dimer and APTT (7). The underlying mechanisms include possibly a direct action of the virus on coagulation-competent tissues and cells, such as endothelium (5) and platelets as well as the indirect immune activation and further potentiated hyper-coagulable state, which leads to the development of thromboembolic complications in patients (14). In this scenario, cOACS cannot prevent the infection and the development of COVID-19 but might provide protection toward the consequences of the hypercoagulability state caused by the disease.

Our study also proposes oral anticoagulant therapy as a strategic therapy, in particular, for the early treatment of patients before they become severely ill, as an alternative to the use of parental anticoagulants.

Overall, our data support the proposed use of anticoagulant therapy to prevent a mechanistic approach for the prophylactic use of direct OACs in patients with elevated CCI score at the time of the COVID-19 pandemic to reduce the risk of more severe clinical disease.

In contrast to our findings, a very small study conducted in the United Kingdom indicates a non-significant death reduction in patients treated with either warfarin or DOACs with a paradoxical—although, again, non-significant—increment in ICU admission in patients on OACs (15). Further, progression to an acute respiratory distress syndrome was increased by OACs in 192 hospitalized Italian patients (RR = 1.24, 95% CI 0.56–2.08). However, due to the small sample size, the influence of OACs on disease severity was again non-significant ($p = 0.465$) (16). Thus, our findings seem to represent the only data available in a large population indicating that preexisting OAC therapy can reduce ICU admission in hospitalized patients. Further data from prospective studies could help better our understanding of the prophylactic strategy to choose between different OACs.

LIMITATIONS

The study has a cross-sectional observational design, which could affect the results. For this reason, our research was never intended to be conclusive but rather hypothesis generating. Finally, we cannot discriminate against the role of different anticoagulants because we did not collect the names of the active principles.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and

accession number(s) can be found below: doi: 10.6084/m9.figshare.12622208.

ETHICS STATEMENT

Ethical approval was not provided for this study on human participants because the study is performed following the article 89 of the General Data Protection and Regulation (https://gdpr-info.eu). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

THE SARS-RAS INVESTIGATOR GROUP IS COMPOSED OF

Arrigo F. G. Cicero[1], Claudia Agabiti Rosei[14], Michele Bevilacqua[10], Valeria Bisogni[9], Michele Bombelli[22], Luca Bulfone[16], Flaminia Canichella[18], Giovanni Carpani[22], Massimo Catanuso[17], Carmine Savoia[2], Damiano Rizzoni[15], Giulia Chiarini[15], Fernando Chiumiento[21], Giuseppe Mulè[4], Rosario Cianci[6], Giuliano Tocci[2], Franco Cipollini[23], Antonio Concistrè[6], Andrea Dalbeni[10], Roberto Alberto De Blasi[2], Riccardo Sarzani[19], Carolina De Ciuceis[14], Raffaella Dell'Oro[22], Antonino Di Guardo[17], Stefano Perlini[5], Santo Di Lorenzo[2], Monica Di Norcia[25], Giacomo Pucci[9], Roberto Ervo[11], Elisabetta Eula[20], Davide Fabbricatore[14], Pietro Minuz[10], Elvira Fanelli[20], Claudio Letizia[6], Cristiano Fava[10], Enzo Grasso[12], Stefano Carugo[13], Alessandro Grimaldi[25], Maddalena Illario[3], Claudio Invernizzi[22], Maria Lorenza Muiesan[14], Elena Iraca[1], Federica Liegi[1], Francesca Magalini[26], Franco Veglio[20], Paolo Malerba[15], Leonardo Sechi[16], Alessandro Maloberti[12], Costantino Mancusi[3], Martina Mezzadri[6], Francesco Fallo[7], Giulia Molinari[22], Roberta Mussinelli[5], Anna Paini[14], Paola Pellimassi[2], Paolo Mulatero[20], Ornella Piazza[8], Davide Grassi[25], Luigi Pietramala[6], Roberto Pontremoli[24], Fosca Quarti Tevano[22], Franco Rabbia[20], Monica Rocco[2], Anna Sabena[5], Francesco Salinaro[5], Paola Schiavi[19], Maria Chiara Sgariglia[6], Francesco Spannella[19], Cristina Giannattasio[12], Sara Tedeschi[1], Pierluigi Viale[1] and the COVID19 Niguarda group.

The SARS-RAS centers are the following:
[1]AO Policlinico Sant'Orsola-Malpighi, Bologna; [2]AOU Sant'Andrea, Roma; [3]AOU Federico II, Napoli; [4]AOU Policlinico Paolo Giaccone, Palermo; [5]AOU Policlinico San Matteo, Pavia; [6]AOU Policlinico Umberto I, Roma; [7]AOU Policlinico Universitario, Padova; [8]AOU San Giovanni di Dio e Ruggi d'Aragona, PO "Dell'Olmo" Cava de' Tirreni; [9]AOU Santa Maria, Terni; [10]AOUI Verona, Italy; [11]ASL 1 Imperiese, Ventimiglia; [12]ASST Grande Ospedale Metropolitano Niguarda, Milano; [13]ASST Santi Paolo e Carlo, Milano; [14]ASST SPEDALI CIVILI BRESCIA; [15]ASST SPEDALI CIVILI. PO Montichiari; [16]ASUI Friuli Centrale, Udine; [17]Centro Ipertensione Mascalucia, Catania; [18]INMI Lazzaro Spallanzani, Roma; [19]INRCA, Ancona, Italy; [20]Ospedale "Le Molinette", Torino; [21]Ospedale di Eboli, Salerno; [22]Ospedale San Gerardo, Monza; [23]Ospedale San Jacopo, Pistoia; [24]Ospedale San Martino, Genova; [25]PO San Salvatore, L'Aquila; [26]Ospedale Maggiore, Parma.

AUTHOR CONTRIBUTIONS

GI: study design, statistical analysis, and paper writing. GG, CB, MM, and MV: study design and paper editing. DG and MS: data collection and elaboration. CM: data collection and elaboration, statistical analysis, and paper writing. CF: study design and paper writing. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.633878/full#supplementary-material

REFERENCES

1. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York city area. *JAMA*. (2020) 323:2052–59. doi: 10.1001/jama.2020.6775
2. Emami A, Javanmardi F, Pirbonyeh N, Akbari A. Prevalence of underlying diseases in hospitalized patients with COVID-19: a systematic review and meta-analysis. *Arch Acad Emerg Med*. (2020) 8:e35.
3. Cui S, Chen S, Li X, Liu S, Wang F. Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. *J Thromb Haemost*. (2020) 18:1421–4. doi: 10.1111/jth.14830
4. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost*. (2020) 18:844–7. doi: 10.1111/jth.14768
5. Sardu C, Gambardella J, Morelli MB, Wang X, Marfella R, Santulli G. Hypertension thrombosis, kidney failure, and diabetes: is COVID-19 an endothelial disease? a comprehensive evaluation of clinical and basic evidence. *J Clin Med*. (2020) 9:1417. doi: 10.3390/jcm9051417
6. Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost*. (2020) 18:1094–99. doi: 10.1111/jth.14817
7. Kollias A, Kyriakoulis KG, Dimakakos E, Poulakou G, Stergiou GS, Syrigos K. Thromboembolic risk and anticoagulant therapy in COVID-19 patients: emerging evidence and call for action. *Br J Haematol*. (2020) 189:846–7. doi: 10.1111/bjh.16727
8. Barnes GD, Burnett A, Allen A, Blumenstein M, Clark NP, Cuker A, et al. Thromboembolism and anticoagulant therapy during the COVID-19 pandemic: interim clinical guidance from the anticoagulation forum. *J Thromb Thrombolysis*. (2020) 50:72–81. doi: 10.1007/s11239-020-02138-z
9. Iaccarino G, Borghi C, Cicero AFG, Ferri C, Minuz P, Muiesan ML, et al. Renin-angiotensin system inhibition in cardiovascular patients at the time of COVID19: much ado for nothing? A Statement of Activity from the Directors of the Board and the Scientific Directors of the Italian Society of Hypertension. *High Blood Press Cardiovasc Prev*. (2020) 27:105–8. doi: 10.1007/s40292-020-00380-3

10. World Health Organization. *Clinical Management Of Severe Acute Respiratory Infection When Novel Coronavirus (Ncov) Infection Is Suspected: Interim Guidance*. World Health Organization (2020).
11. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. *JAMA*. (2020) 323:1239–42. doi: 10.1001/jama.2020.2648
12. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis*. (1987) 40:373–83. doi: 10.1016/0021-9681(87)90171-8
13. Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, et al. Right ventricular: COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. *J Am Coll Cardiol*. (2020) 75:2950–73. doi: 10.1016/j.jacc.2020.04.031
14. Page EM, Ariens RAS. Mechanisms of thrombosis and cardiovascular complications in COVID-19. *Thromb Res*. (2021) 200:1–8. doi: 10.1016/j.thromres.2021.01.005
15. Sivaloganathan H, Ladikou EE, Chevassut T. COVID-19 mortality in patients on anticoagulants and antiplatelet agents. *Br J Haematol*. (2020) 190:e192–5. doi: 10.1111/bjh.16968
16. Russo V, Di Maio M, Attena E, Silverio A, Scudiero F, Celentani D, et al. Clinical impact of pre-admission antithrombotic therapy in hospitalized patients with COVID-19: a multicenter observational study. *Pharmacol Res*. (2020) 159:104965. doi: 10.1016/j.phrs.2020.104965

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

MINI REVIEW
published: 20 May 2021
doi: 10.3389/fcvm.2021.644095

COVID-19 in Relation to Hyperglycemia and Diabetes Mellitus

Hayder M. Al-kuraishy[1], Ali I. Al-Gareeb[1], M. Alblihed[2], Susana G. Guerreiro[3,4,5], Natália Cruz-Martins[3,5,6] and Gaber El-Saber Batiha[7]**

[1] Department of Clinical Pharmacology and Therapeutic Medicine, College of Medicine, ALmustansiriyiah University, Baghdad, Iraq, [2] Department of Microbiology, College of Medicine, Taif University, Taif, Saudi Arabia, [3] Faculty of Medicine, University of Porto, Porto, Portugal, [4] Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal, [5] Institute for Research and Innovation in Health (i3S), University of Porto, Porto, Portugal, [6] Laboratory of Neuropsychophysiology, Faculty of Psychology and Education Sciences, University of Porto, Porto, Portugal, [7] Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt*

Coronavirus disease 2019 (COVID-19), triggered by the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), may lead to extrapulmonary manifestations like diabetes mellitus (DM) and hyperglycemia, both predicting a poor prognosis and an increased risk of death. SARS-CoV-2 infects the pancreas through angiotensin-converting enzyme 2 (ACE2), where it is highly expressed compared to other organs, leading to pancreatic damage with subsequent impairment of insulin secretion and development of hyperglycemia even in non-DM patients. Thus, this review aims to provide an overview of the potential link between COVID-19 and hyperglycemia as a risk factor for DM development in relation to DM pharmacotherapy. For that, a systematic search was done in the database of MEDLINE through Scopus, Web of Science, PubMed, Embase, China National Knowledge Infrastructure (CNKI), China Biology Medicine (CBM), and Wanfang Data. Data obtained underline that SARS-CoV-2 infection in DM patients is more severe and associated with poor clinical outcomes due to preexistence of comorbidities and inflammation disorders. SARS-CoV-2 infection impairs glucose homeostasis and metabolism in DM and non-DM patients due to cytokine storm (CS) development, downregulation of ACE2, and direct injury of pancreatic β-cells. Therefore, the potent anti-inflammatory effect of diabetic pharmacotherapies such as metformin, pioglitazone, sodium-glucose co-transporter-2 inhibitors (SGLT2Is), and dipeptidyl peptidase-4 (DPP4) inhibitors may mitigate COVID-19 severity. In addition, some antidiabetic agents and also insulin may reduce SARS-CoV-2 infectivity and severity through the modulation of the ACE2 receptor expression. The findings presented here illustrate that insulin therapy might seem as more appropriate than other anti-DM pharmacotherapies in the management of COVID-19 patients with DM due to low risk of uncontrolled hyperglycemia and diabetic ketoacidosis (DKA). From these findings, we could not give the final conclusion about the efficacy of diabetic pharmacotherapy in COVID-19; thus, clinical trial and prospective studies are warranted to confirm this finding and concern.

Keywords: COVID-19, SARS-CoV-2, diabetes mellitus, hyperglycemia, cardiometabolic disturbances

OPEN ACCESS

Edited by:
Hendrik Tevaearai Stahel, Bern University Hospital, Switzerland

Reviewed by:
Ye Qiu, Hunan University, China
Muneeb A. Faiq, New York University, United States

****Correspondence:***
Natália Cruz-Martins
ncmartins@med.up.pt
Gaber El-Saber Batiha
gaberbatiha@gmail.com

Specialty section:
This article was submitted to Cardiovascular Epidemiology and Prevention, a section of the journal Frontiers in Cardiovascular Medicine

Received: *19 December 2020*
Accepted: *29 March 2021*
Published: *20 May 2021*

Citation:
Al-kuraishy HM, Al-Gareeb AI, Alblihed M, Guerreiro SG, Cruz-Martins N and Batiha GE-S (2021) COVID-19 in Relation to Hyperglycemia and Diabetes Mellitus. Front. Cardiovasc. Med. 8:644095. doi: 10.3389/fcvm.2021.644095

INTRODUCTION

Novel coronavirus disease 2019 (nCov19) or coronavirus disease 2019 (COVID-19) is a recent viral infectious disease that emerged in December 2019; it was first identified in Wuhan, Hubei Province, China and presented as clusters of pneumonia, formally known as a pneumonia cluster of unknown etiology. COVID-19 is triggered by severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) (1). Some years ago, namely, in 2003 and 2012, respectively, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) led to fatal pneumonia, acute lung injury (ALI), and acute respiratory distress syndrome (ARDS). SARS-CoV, MERS-CoV, and SARS-CoV-2 are positive-sense, enveloped single-strand RNA *Betacoronaviruses* with some phylogenetic similarities. As a matter of fact, SARS-CoV-2 presents 79% similarity with SARS-CoV and 96% with bat CoV. In addition, it has been shown that SARS-CoV-2 has a higher ability of transmission and a lower fatality rate compared to SARS-CoV and MERS-CoV (2).

Declared on 30 January 2020 as a Public Health Emergency of International Concern by the World Health Organization (WHO), COVID-19 was quickly renamed to a pandemic on 11 March 2020, and on 3 November 2020, 47,705,405 confirmed cases were officially reported in more than 200 countries with 1,217,347 deaths globally (3).

The incubation period of SARS-CoV-2 is 2–14 days; however, some studies have reported that COVID-19 symptoms are developed in 97.5% of cases within 4–5 days. SARS-CoV-2 is mainly transmitted by respiratory droplets up to a distance of 6 ft. This virus remains viable for about 3 h in the aerosols and can be transmitted in closed environments. Nonetheless, viable SARS-CoV-2 has also been detected in fecal swabs; thereby, transmission of SARS-CoV-2 through the fecal-oral route might be an important route, mainly in patients on drugs that upregulate intestinal angiotensin receptor type 2 (ACE2) (4). The age group most affected by COVID-19 is mainly between 47 and 59 years, where men are more prone to the disease. Fewer COVID-19 cases have been reported in infants and children; as disclosed by a large cohort study in China, only 2% of COVID-19 cases were below the age of 20 (5). Regarding clinical presentation, the clinical spectrum of COVID-19 presents mainly as asymptomatic or mild flu-like illness in 85%, mostly in children and adults, although in 10% of cases, a severe disease state may be present, along with an increased risk of developing ARDS. In addition, in severe cases, COVID-19 may trigger extrapulmonary manifestations like acute cardiac injury, arrhythmias, acute kidney injury, acute brain injury, endocrine failure, multiple organ failure, and even death (6).

Diabetes mellitus (DM) is an endocrine disorder characterized by hyperglycemia, polyuria, polydipsia, and weight loss due to a defect in insulin secretion and/or action. DM is commonly associated with metabolic, macrovascular, and microvascular complications that increase morbidity and mortality in different viral infections (7). It has been reported that DM and reactive hyperglycemia are regarded as predictors of severity in SARS-CoV and MERS-CoV infected patients (8). However, little is known about the association between SARS-CoV-2 and DM; nevertheless, different recent studies observed the link between hyperglycemia and SARS-CoV-2 even in non-DM patients (9). Therefore, this review aims to provide an overview of the potential link between COVID-19 and hyperglycemia as a risk factor for DM development.

METHOD AND SEARCH STRATEGY

In order to review the report of ethical considerations in these papers, we proposed a protocol for a systematic review of the COVID-19 articles. The search criteria proposed for the review were based on what would be a reasonable search conducted by a lay member of the public with access to PubMed.gov. It was proposed to publish the findings of the review as a summary of the institutional Research Ethics Committee's response to the challenges of reviewing and approving clinical research proposals in times of a pandemic.

To accomplish that, a systematic search in MEDLINE through Scopus, Web of Science, PubMed, Embase, China National Knowledge Infrastructure (CNKI), China Biology Medicine (CBM), and Wanfang Data was done using the following terms and keywords: [COVID-19] OR [SARS-CoV-2] OR [2019-nCov] OR [Wuhan virus] AND [DM] OR [hyperglycemia] OR [Pancreatic injury]. There were no limitations for language and type of published articles as well as preprinted data.

COVID-19 AND HYPERGLYCEMIA

It has been stated that COVID-19 is associated with hyperglycemia, actually considered a direct predictor of the poor prognosis of the disease and to an increased risk of death (10). Briefly, the binding site and entry point of SARS-CoV-2 is the ACE2 receptor, which is highly expressed in the lung, liver, brain, placenta, and pancreas. SARS-CoV-2 infects the pancreas through ACE2, being highly expressed there when compared to other organs, leading to pancreatic damage with subsequent impairment of insulin secretion and development of hyperglycemia even in non-DM patients. Similarly, SARS-CoV-2-induced pancreatic injury may worsen a preexistent DM (11). Previous data have shown that SARS-CoV, which is closely related to SARS-CoV-2, triggers transient hyperglycemia and impairment of pancreatic β-cell function during epidemic-derived pneumonia (12). Moreover, the COVID-19-induced inflammation and cytokine storm (CS), which are characterized by profound elevations in the levels of tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6, lead to peripheral insulin resistance (IR) (13). Besides, high TNF-α and IL-6 in CS impair pancreatic β-cell function and inhibit insulin secretion. Taken together, both IR and impairment of pancreatic β-cell function contribute to a vicious cycle in the development and progression of hyperglycemia in COVID-19 patients (14). Furthermore, hyperglycemia and induced oxidative stress and gluco-lipotoxicity contribute to the development of IR and impairment of pancreatic β-cell function (15). In addition,

prolonged hyperglycemia could worsen the course of COVID-19 via glycation of pancreatic ACE2, which facilitates the SARS-CoV-2 binding and entry at the pancreatic β-cell (16).

Different reports have shown that an abnormal expression of cell ACE2 receptors in different tissues reduces the protective effect against viral entry and, consequently, exacerbates the severity and poor outcomes of SARS-CoV-2 infection (2). On the other hand, the systemic renin-angiotensin system (RAS) regulates pancreatic β-cell function, while local RAS of pancreatic β-cell function controls β-cell apoptosis, cell proliferation, and oxidative stress (17). Angiotensin II (AngII) through AT1R leads to DM induction in experimental models, while inhibiting the glucose-stimulated insulin secretion. Therefore, blockade of AT1R improves pancreatic β-cell function and increases the pro-insulin and insulin biosynthesis. Besides, upregulated local pancreatic AngII induces oxidative stress that triggers β-cell damage by NADPH oxidase induction (18). Indeed, it has been shown that hyperglycemia upregulates AT1R leading to β-cell function impairment and insulin secretion (19).

In COVID-19, ACE2 dysregulation by SARS-CoV-2 leads to marked elevation of vasoconstrictor AngII with a reduction in the vasodilator Ang1-7 (**Figure 1**), which *per se* leads to pancreatic β-cell dysfunction, inhibition of insulin secretion, and hyperglycemia, which might be transient even in non-DM patients (20). Furthermore, elevated AngII leads to pulmonary vasoconstriction, ALI, and ARDS with the induction of inflammation cascade and oxidative stress, which together participate in the induction of pancreatic β-cell function and hyperglycemia (21). Then, hyperglycemia in COVID-19 leads to ALI through the induction of pulmonary sodium-potassium-chloride co-transporter 1(NKCC1), involved in the regulation of the transport of water and ions to alveolar cells. Thus, untreated and long-standing hyperglycemia may lead to ALI through ischemic-reperfusion injury (22). Also noteworthy is the fact that hyperglycemia is associated with oxidative stress' induction and inflammatory mediators' overproduction, which together partake in the development of endothelial dysfunction and thrombosis due to alterations in both function and generation of antithrombin III (23). Taken together, these findings reveal that both COVID-19 and hyperglycemia interact in a vicious cycle leading to more complications and worse metabolic outcomes.

COVID-19 AND PANCREATIC INJURY

Pancreatic injury (PI) is often presented as acute pancreatitis, which is rarely reported in COVID-19 and may be misdiagnosed by the general signs and symptoms of acute viral infections (24). Different studies reported that COVID-19-induced PI is diagnosed through detailed medical history, physical examination, and ultrasonography imaging with elevation in serum lipase levels. However, in severe cases, abdominal CT scan imaging is recommended (25). In COVID-19, PI may occur either by direct invasion of SARS-CoV-2 or indirectly through the induction of CS (26). Previously, SARS-CoV was detected in the pancreatic tissue suggesting binding of this virus to ACE2, highly expressed in the pancreatic tissue mainly in β-cell and exocrine ducts (27). These findings suggest that the direct cytopathic effect of SARS-CoV-2 may be linked with the development of PI.

In SARS-CoV-2, the systemic inflammatory response and CS may be the cause of PI as part of multiorgan failure. SARS-CoV-2-induced PI increases the release of pancreatic lipase causing lipolysis and the release of unsaturated fatty acids that ultimately trigger pancreatic mitochondrial damage and overproduction of pro-inflammatory mediators similar to that of CS (24). Recent reports disclosed that SARS-CoV-2 affects both pancreatic lipase and peripheral adipose tissue leading to PI and lipotoxicity that contribute to CS induction (28). Also, postmortem studies in both SARS-CoV and SARS-CoV-2 patients illustrated a higher proliferation of these viruses in the pancreatic tissues (29). Therefore, SARS-CoV-2 may lead to PI directly or indirectly with subsequent endocrine and exocrine dysfunctions that are presented as acute pancreatitis and transient hyperglycemia (30, 31) (**Figure 2**).

Also, IL-6 is regarded as a potential link between ALI and PI in mice since PI-induced inflammations activate myeloid cells to secrete IL-6. In COVID-19-induced CS, IL-6 is the main cytokine involved in ALI and ARDS development (32). The binding of SARS-CoV-2 to the pancreatic β-cell ACE2 receptor stimulates A disintegrin and metalloprotease-17 (ADAM-17), which activate the ACE2 receptor shedding and TNF-α production (33). Therefore, SARS-CoV-2 infection is linked with the downregulation of ACE2 and the dysregulation of systemic and local pancreatic β-cell RAS. In this sense, the administration of recombinant soluble ACE2 neutralizes SARS-CoV-2 and prevents further viral entry with significant amelioration of pancreatic function (34).

It has been shown that ACE2 deficiency alters glucose homeostasis and metabolism since ACE2 knockout mice illustrated AngII-independent pancreatic β-cells dysfunction, suggesting a direct protective role of the β-cell ACE2 receptor (35). Furthermore, overexpression of the β-cell ACE2 receptor may improve glucose homeostasis and β-cell sensitivity, while the downregulation of the peripheral ACE2 receptor is linked to the development of IR through the reduction of glucose transporter 4 (GLUT4) and Ang1-7, which increases peripheral insulin sensitivity (36). Liu et al. (37) illustrated that ACE2 polymorphism is associated with the development of type 2 DM. Also, ACE2 receptors have been reported to act as a compensatory mechanism against hyperglycemia induced-RAS activation since hyperglycemia activates ADAM-17 and ACE2 renal shedding that are common in patients with T2DM and IR. Also, ADAM-17 activation by SARS-CoV-2 leads to hyperglycemia. These changes augment the action of AngII on AT1R leading to vasoconstriction, hypertension, endothelial dysfunction, and hypercoagulation status (38). Previously, SARS-CoV infection was associated with 50% of acute DM cases due to a reduction in the pancreatic β-cell ACE2 receptors by direct viral invasion; nevertheless, only 10% of them developed chronic DM after 3 years (39). Besides, an augmented ADAM-17 during SARS-CoV-2 infection activates the release of pro-inflammatory cytokines, including IL-6 and TNF-α, which are correlated with a higher risk of ARDS and ALI (40). Hence, a rapid management

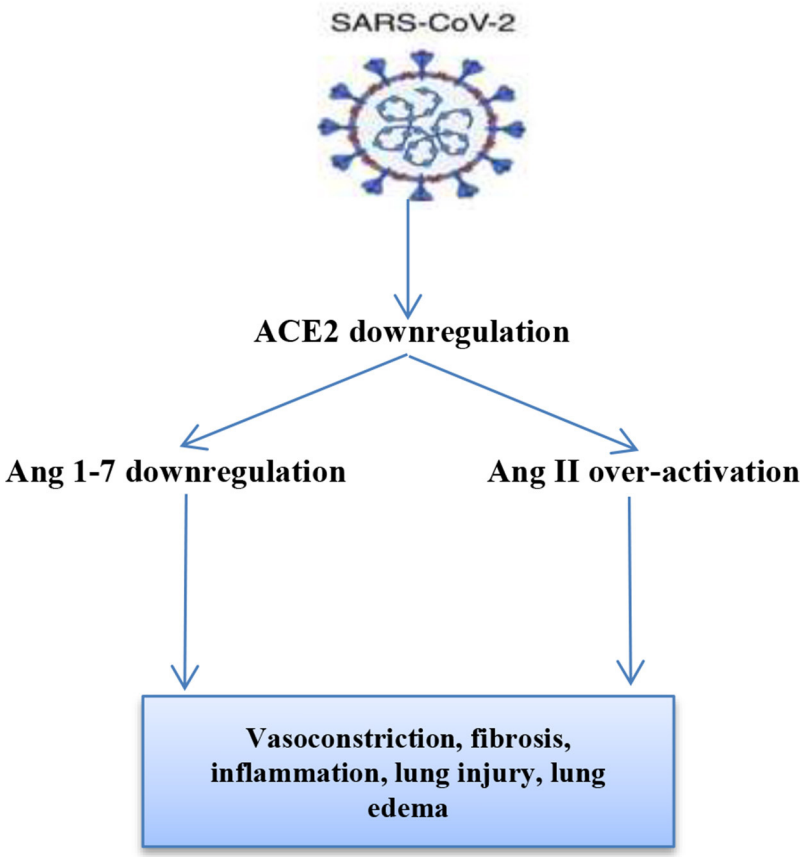


FIGURE 1 | SARS-CoV-2 and renin-angiotensin system (RAS) interaction: SARS-CoV-2 down-regulates ACE2 leading to over-activation of AngII and reduction of Ang 1-7. Ang II through angiotensin receptor type 1 (AT1R) leads to lung injury, inflammation, and vasoconstriction. Ang 1-7 through Mas receptor leads to vasodilation and lung protection.

of PI in COVID-19 patients may mitigate and attenuate the associated ALI. Therefore, the interaction between SARS-CoV-2 and pancreatic ACE2 not only causes PI but also may extend to cause systemic inflammatory changes (**Figure 3**).

COVID-19 AND DIABETES MELLITUS

DM accounted for about 20% of the intensive care unit (ICU) admission due to COVID-19 according to cohort reports (41). However, data from Italy illustrated that more than two-thirds of COVID-19 patients who died had DM, with the mortality rate of DM patients with COVID-19 being similar to that of SARS and MERS (42).

During the previous SARS-CoV infection, non-DM patients may develop hyperglycemia on the 3rd day of acute infection that was reversed within 2 weeks (10% of them developed DM 3 years later). These finding are not observed in other viral pneumonia, suggesting the involvement of the pancreatic axis in coronavirus infection (43). In COVID-19, DM patients presented with preprandial and postprandial hyperglycemia as well as diabetic ketoacidosis, being higher compared to non-infected DM patients (44).

It has been accounted that any acute disease, as occurs in viral diseases, triggers stress and higher inflammatory responses that augment the sympathetic outflow with the release of catchecholamines, growth hormones, cortisol, and cytokines that together increase the frequency and severity of DM complications. However, in cases of coronavirus infection, the severity of such complications is also linked with the development of PI (45). Conversely, hypoglycemia may develop in SARS-CoV due to hepatic and pancreatic alpha cell injury, despite the fact that alpha cell dysfunction and lower glucagon serum levels were not confirmed in COVID-19 patients (46).

On the other hand, it was stated that DM increases the risk of COVID-19 progression and worsens the outcomes of other coronaviruses and H1N1 infections. The mortality rate of DM patients with COVID-19 is about 16% due to associated comorbidities and hidden presentation of mild disease. In fact, an underestimation of these signs and symptoms in DM patients may even worsen the outcomes in suspected SARS-CoV-2 infection (47).

Also, DM is linked to low-grade chronic inflammation, which may facilitate CS induced by COVID-19 pneumonia. The levels of IL-6, CRP, and D-dimer appear to be higher in COVID-19

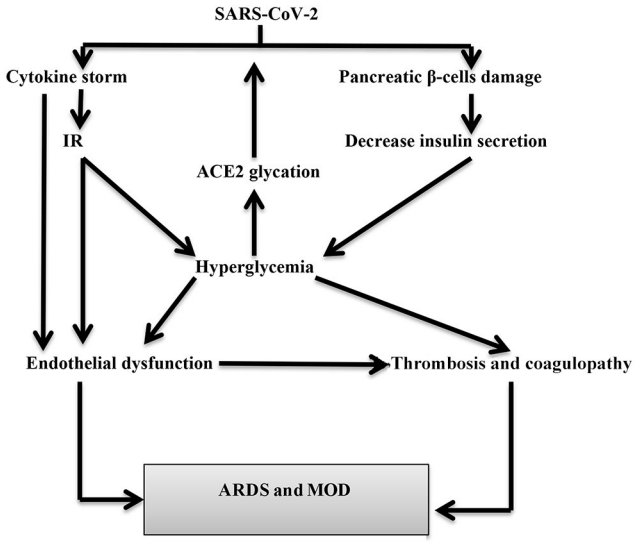


FIGURE 2 | SARS-CoV-2-induced hyperglycemia in COVID-19 patients. SARS-CoV-2 leads to hyperglycemia either directly through pancreatic β-cells injury or indirectly through cytokine storm-induced insulin resistance (IR). Hyperglycemia increased SARS-CoV-2 entry through glycation of ACE2. Hyperglycemia may cause acute respiratory distress syndrome (ARDS) and multiorgan damage (MOD) through the induction of endothelial dysfunction and coagulopathy.

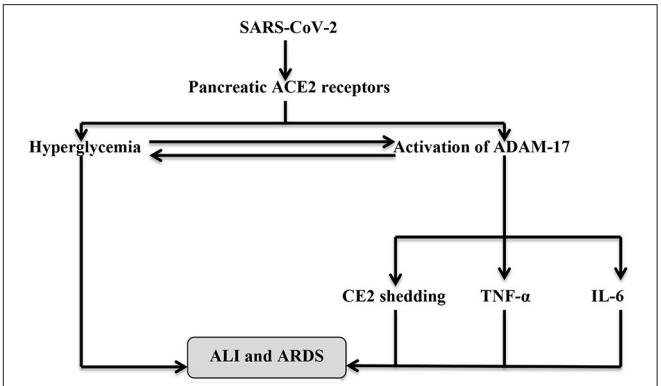


FIGURE 3 | The interaction between SARS-CoV-2 and pancreatic ACE2. Binding of SARS-CoV-2 to the pancreatic ACE2 leads to pancreatic injury (PI)-induced hyperglycemia and activation of A disintegrin and metalloprotease-17 (ADAM-17), which activate shedding of ACE2 receptors and production of TNF-α and IL-6. Hyperglycemia activates ADAM-17 and vice versa. These changes together participate in the development of ALI and ARDS.

pneumonia patients with DM. Of note, in DM patients with COVID-19, IL-6 links to associated metabolic disorders and cardiovascular complications, so IL-6 antagonist tocilizumab may attenuate the clinical course and outcomes in DM patients with COVID-19-induced pneumonia (48).

The interaction between DM and COVID-19 could be bi-directional, as SARS-CoV-2 infection may potentially deteriorate the preexisting DM and even predispose to frank DM in non-DM patients. In addition, pancreatic β-cell invasion by SARS-CoV-2 triggers β-cell autoimmunity in the susceptible subjects with subsequent development of type 1 DM (T1DM) (49).

The potential mechanisms that increase the risk of SARS-CoV-2 infection in DM patients are related to different metabolic pathways. It has been shown that DM patients with high body mass index, hypertension, and microvascular complications have a higher severity and mortality due to COVID-19-derived pneumonia (50). Also, hyperglycemia in DM individuals, independently, or secondarily to the presence of diabetic complications, increases the risk of SARS-CoV-2 infection in different ways, including an increased affinity from SARS-CoV-2 to ACE2, reduction of viral clearance, T cell-mediated immunity dysfunction, and CS induction (51).

In DM, there is a noteworthy disorder in the innate, adaptive, and acquired immunity with delay in the activation of Th1 cell-mediated immunity and late hyper-inflammation due to an abnormal cytokine response and alterations in $CD4^+$ T cell counts. These abnormal immunological responses appear to be responsible for the blunted antiviral response in DM (52). Moreover, in DM, there is an overexpression of ACE2 in lung, kidney, heart, and pancreas that favors SARS-CoV-2 binding and entry (52, 53). Besides, the associated anti-DM pharmacotherapy and other administrated drugs in DM patients may affect the expression of ACE2 (53). However, insulin therapy reduces the expression of ACE2, while metformin, glucagon-like peptide-1 agonist, thiazolidinediones, statins, and ACE inhibitors upregulate the expressions of ACE2 (54).

Nonetheless, the causal relationship between DM and ALI in COVID-19 cases, to what concerns to the expression of ACE2, is not yet clear. However, a recent study confirms that long-standing DM is linked to an overexpression of pulmonary ACE2 (55). Also, the susceptibility of DM patients to SARS-CoV-2 infection is also related to a higher furin serum level, which is engaged in the S-domain cleaving of spike protein and increasing the SARS-CoV-2 binding to the ACE2 receptors (56). Interestingly, the pulmonary ACE/ACE2 ratio is increased in DM, which favors the generation of vasoconstrictor AngII, involved in the induction of ALI. Also, in DM patients, high AngII levels and SARS-CoV-2 infection interact mutually at a vascular endothelial bed causing endothelial dysfunction, inhibition of fibrinolytic system, and activation of coagulation cascades that trigger thromboembolic disorders (57). Thus, there is a mutual interaction between DM and SARS-CoV-2 in COVID-19 (**Figure 4**).

ANTI-COVID-19 MEDICATIONS AND BLOOD GLUCOSE VARIABILITY

Currently used drugs for COVID-19 treatment may affect blood glucose variability in both DM and non-DM patients. For example, chloroquine and hydroxychloroquine have

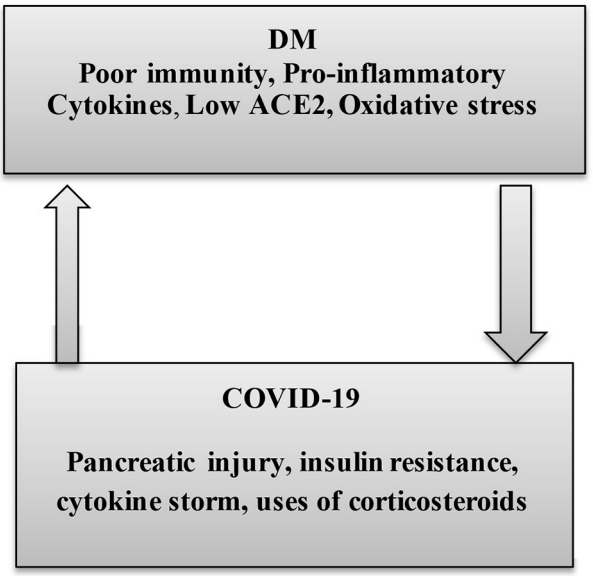


FIGURE 4 | The interaction between diabetes mellitus and COVID-19. In diabetes mellitus (DM), poor immunity, high pro-inflammatory cytokine, low ACE2, and oxidative stress increase susceptibility for COVID-19. Below, COVID-19-induced pancreatic injury, insulin resistance (IR), cytokine storm, and uses of corticosteroids in the management of COVID-19 collectively participate in the induction of DM.

been shown to be effective in controlling SARS-CoV-2 replications and in modulating COVID-19-induced CS due to their potent anti-inflammatory and immunomodulating effects (58). It has been reported that hydroxychloroquine improves glycemic indices, β-cell function, and insulin secretion and can be effectively used in the management of uncontrolled T2DM. Thus, hydroxychloroquine therapy in COVID-19 may lead to hypoglycemia since this drug reduces insulin degradation and improves insulin storage with augmentation of peripheral glucose metabolism (59). Therefore, cautions should be regarded in the use of hydroxychloroquine for treating COVID-19 patients with DM.

Corticosteroids, such as dexamethasone, are approved drugs since a long time ago that have shown to be effective in COVID-19 patients, namely, reducing the exaggerated immune response-induced ALI and ARDS. Despite this beneficial effect, dexamethasone blocks both viral clearance and immune response (60). In addition, administration of corticosteroids in COVID-19 patients is associated with hyperglycemia even in non-DM patients. In clinical practice, short-term therapy of low-dose methylprednisolone (30–80 mg/day for 3–5 days) is ineffective for COVID-19 management; however, a high-dose methylprednisolone (80–160 mg/day for 7 days) triggers an effective action in suppressing CS, and this high dose may aggravate hyperglycemia in DM (61, 62). Therefore, a strict glucose monitoring is crucial for COVID-19 patients who receive corticosteroids to prevent hyperglycemia-induced complications.

DIABETIC PHARMACOTHERAPY AND COVID-19

Diabetes pharmacotherapy may affect the clinical course and outcomes in DM patients with COVID-19 through modulation of ACE2 expression and potential anti-inflammatory effects.

Metformin

Metformin improves IR and peripheral glucose uptake through the activation of AMP-dependent protein kinase. Also, metformin exerts pleiotropic effects through the AMP-independent pathway including anti-inflammatory and immunomodulatory effects (63); it inhibits the synthesis and release of CRP, IL-1β-induced IL-6, and ferritin from macrophages, endothelial cells, smooth muscle vascular cells, and hepatocytes (64). An observational study illustrated that metformin reduces the mortality rate in DM patients with severe COVID-19, more evident in women than men, through the suppression of TNF-α synthesis and release (65). It has been reported that up to 88% of T2DM patients receive metformin as first-line therapy, and since COVID-19 is common in DM, metformin may affect this pandemic (66). Specifically what concerns COVID-19 is that metformin upregulates ACE2 with increases in its stability and acts synergistically with ACEIs in the overexpression of pulmonary ACE2 receptors. As a consequence, the overexpression of pulmonary ACE2 receptors may attenuate the deleterious effect of SARS-CoV-2 invasion in alveolar cells while restoring the RAS balance (67). Metformin also reduces the binding of SARS-CoV-2 to ACE2 through inducing functional changes in the transmembrane enzyme by AMP-dependent phosphorylation (68). In addition, metformin blocks the mammalian target of rapamycin (mTOR) signaling, which is an important signaling pathway involved in viral pathogenesis and replication, such as influenza, SARS, MERS, and SARS-CoV-2; thus, metformin may attenuate viral replication through preventing the interaction of the viral protein complex (69). It has been documented that metformin therapy in DM patients inhibits the Zika virus replication through the activation of AMP signaling, which might be applied against SARS-CoV-2 (70). Add to this the fact that metformin therapy in DM patients with COVID-19 improves insulin sensitivity, and so it prevents the IR-induced overexpression of pancreatic ACE2. It is well-known that IR is linked to the development of cardiometabolic disorders that favor COVID-19 complications in DM (71).

Different substantial data have shown that metformin leads to a decrease in the generation of reactive oxygen species (ROS) through the inhibition of the mitochondrial respiratory chain and 3-kinase phosphoinosis (PI3K)-Akt-dependent inflammatory response in lung tissue (71). Furthermore, it also inhibits nitric oxide (NO), prostaglandin E2 (PGE-2), and NF-kB in lung macrophages during SARS and MERS infection. However, administration of metformin in COVID-19 should be weighed against the risk of lactic acidosis and kidney impairment, which are commonly associated with COVID-19 pneumonia (72). Thus, regardless of its glucose-lowering abilities, metformin is recommended in COVID-19 pneumonia due to its potent anti-inflammatory effects. Al-kuraishy et al. (73) observed that

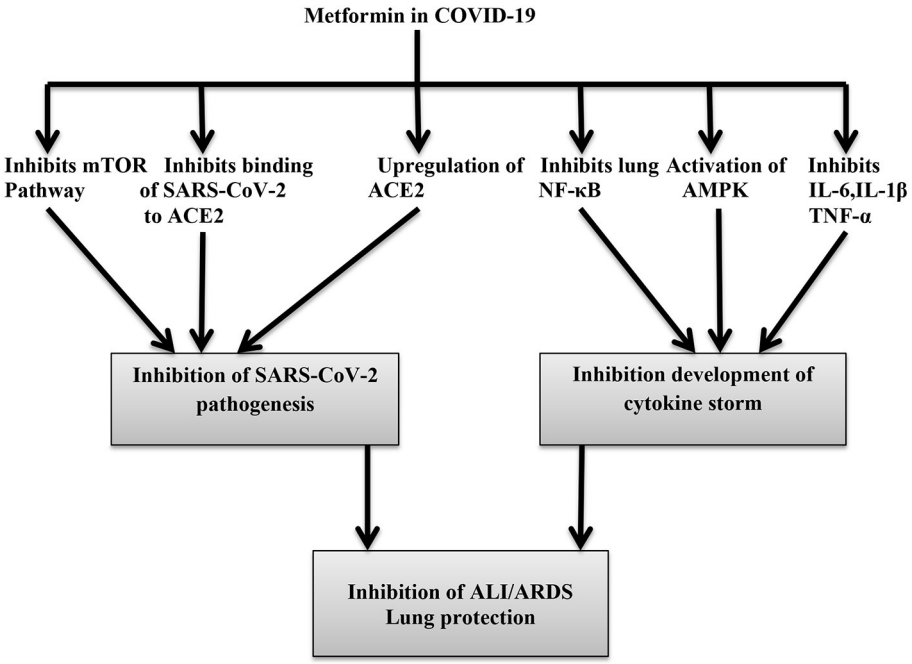


FIGURE 5 | Metformin inhibits pathogenesis of COVID-19 through inhibition of pro-inflammatory cytokines, inflammatory signaling pathway, and binding to ACE2.

metformin is effective in the reduction of COVID-19 severity and associated complications, such as ALI and acute ischemic stroke (AIS), through the modulation of SARS-CoV-2-induced inflammatory reactions in COVID-19 patients with T2DM. Nonetheless, its use is contraindicated in COVID-19 patients with lactic acidosis, multiorgan failure, severe gastrointestinal disorders, and hypoxia (74). The potential benefit of metformin therapy in DM patients with COVID-19 is illustrated in **Figure 5**.

Thiazolidinediones

Thiazolidinediones, such as pioglitazone and rosiglitazone, are classes of antidiabetic drugs that act through the activation of peroxisome proliferators-activated receptor-gamma (PPAR-γ) leading to the reduction of IR, the suppression of lipolysis, and the activation of lipogenesis with improvement of insulin sensitivity (75). Exactly, pioglitazone improves the peripheral glucose uptake and increases the pancreatic β-cell sensitivity, while also exerting an important anti-inflammatory effect through the suppression of monocytes and IL-6 release. Besides, pioglitazone reduces serum ferritin, CRP, and other pro-inflammatory cytokines in T2DM, thus reducing the likelihood of CS when COVID-19 is developed (76). Pioglitazone also reduces the SARS-CoV-2-induced IR and hyperglycemia in non-DM patients via the attenuation of ACE2 glycation and the dysregulation of RAS. Therefore, pioglitazone and other thiazolidinediones may have potential roles in the management of COVID-19-related complications (77).

Moreover, thiazolidinediones attenuate pulmonary fibrosis and ALI by suppressing pulmonary myofibroblast differentiation and TGF-β signaling (78). For this reason, SARS-CoV-2 pathophysiology is related to its interaction with adipocytes and adipose-like cells that favor the differentiation of lung lipofibroblasts into myofibroblasts (79). Pioglitazone also increases ACE2 expression in insulin-sensitive tissues, normalizing blood glucose and attenuating acute kidney injury through the amelioration of the expression of renal ADAM17 (80). Therefore, thiazolidinediones can reduce the interaction between SARS-CoV-2 and adipocytes with subsequent reduction of COVID-19 severity (**Figure 6**).

Dipeptidyl Peptidase-4 Inhibitors

Dipeptidyl peptidase-4 (DPP4) is a transmembrane glycoprotein type II expressed in different tissues and immune cells and plays an important role in the metabolism of glucagon-like peptide (GLP-1). DPP4 expression is higher in the visceral adipose tissue and involved in visceral inflammation and IR progression through enzymatic cleavage of cytokines and chemokines (81).

DPP4 inhibitors (DPP4Is) are oral hypoglycemic agents used in the management of T2DM acting through inhibiting the DPP4 enzyme, thereby increasing the incretin levels, which, in turn, increase insulin secretion with the reduction of glucagon secretion and blood glucose. Briefly, DPP4Is enhance the insulin secretion in a glucose-dependent manner (82).

Different studies have shown that DPP4Is exert anti-inflammatory and immunoregulatory effects in both autoimmune and inflammatory diseases (83). Among such drugs, sitagliptin, linagliptin, and vildagliptin reduce the CRP markers in T2DM patients within 12 weeks of treatment (76). However, there are no available data for other types of DPP4Is regarding their effects on CRP and ferritin serum levels in T2DM (84).

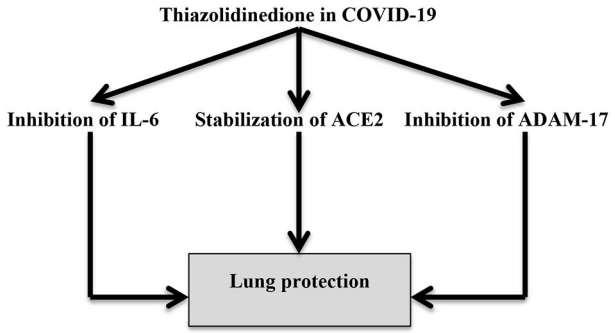


FIGURE 6 | Thiazolidinedione attenuates the pathogenesis of COVID-19 through inhibition of IL-6 and ADAM-17 with the modulation of ACE2, as thiazolidinediones can regulate the AngII-mediated effects.

Concerning the viral infections, it has been confirmed that the DPP4 receptor is a recognized receptor for MERS-CoV that induces T-cell-dependent inflammatory reactions. So, antibodies directed against the DPP4 receptor inhibit MERS-CoV proliferation (85). In the context of the COVID-19 outbreak, DPP4Is and GLP-1 analog exert anti-adipogenic and anti-inflammatory effects that may reduce macrophage polarization and differentiation (86). Mirani et al. (51) showed that DM patients on DPP4I therapy developed a less severe pneumonia, with a lower need of mechanical ventilation and a lower mortality rate when developing COVID-19. For this reason, DPP4Is reduce COVID-19 virulence through the suppression of DPP4/CD26-dependent inflammatory signaling with subsequent inhibition of CS and disease progression. Recent evidences also suggest that SARS-CoV-2 interacts with both DPP4/CD26 and ACE2; besides, SARS-CoV-2 interacts with 293T-cells expressing DPP4 (87). Vankadari and Wilce (88) confirmed that sitagliptin triggers a marked inhibition of SARS-CoV-2 proliferation through binding to the F357 residue, causing conformational changes that prevent its binding with DPP4 receptors. These finding suggest that DPP4Is may attenuate SARS-CoV-2-induced ARDS by suppressing DPP4/CD26 signaling interactions. Therefore, the anti-inflammatory effects of DPP4Is may mitigate DM and coexisting COVID-19-induced IR, hyperglycemia, and inflammation (89).

On the other hand, the GLP-1 receptor analog (GLP-1RA), such as exenatide, has also potent anti-inflammatory and antiproliferative effects and plays a role in the attenuation of ALI. Exenatide improves the anti-inflammatory interleukin, IL-10, and inhibits pro-inflammatory cytokines, TNF-α and IL-1β, in the macrophage's/monocyte's axis (90). Also, the GLP-1 agonist, such as liraglutide, increases the ACE2 expression in the lungs and might have a protective role against the development of ALI in COVID-19-induced pneumonia (91). However, large prospective studies are recommended to confirm the potential role of DPP4Is and GLP-1RA in COVID-19. Therefore, DPP4Is have potential effects against SARS-CoV-2-induced ARDS through the modulation of anti-inflammatory and pro-inflammatory cytokines (**Figure 7**).

Sodium-glucose Co-transporter-2 Inhibitors

Sodium-glucose co-transporter-2 inhibitors (SGLT2Is), also called gliflozins, which include canagliflozin, dapagliflozin, and empagliflozin, are a class of anti-DM drugs that inhibit SGLT2 at renal tubules and prevent glucose reabsorption (92). In addition, SGLT2Is reduce body weight and blood pressure, and exert anti-inflammatory effects through the reduction of IL-6, CRP, ferritin, and oxidative stress, thus being effective in mitigating ALI in T2DM (76). SGLT2Is play a role in COVID-19 management through the upregulation of protective ACE2, the attenuation of CS through the inhibition of IL-6 release, cytoprotective effect through the improvement of cell oxygenation, and reduction of lactate formation (93).

On the other hand, an elevated lactate level in COVID-19 reflects a status of hypoxia and anaerobic metabolism and is correlated with CS induction and multiorgan injury (94). Briefly, SARS-CoV-2 can induce anaerobic metabolism via the disruption of cell oxygenation and the induction of anaerobic glycolysis (95). As cell pH is controlled by Na^+/H^+ and lactate/H^+ exchangers and symporters, respectively, high lactate serum levels in SARS-CoV-2 raise the activity of the lactate/H^+ symporter with subsequent cell acidosis (96). Dapagliflozin inhibits cell Na^+/H^+ exchangers, thus reducing cell acidosis and SARS-CoV-2 activation at acidic pH. Similarly, other SGLT2Is also reduce lactate serum levels through the inhibition of lactate production and release, and increase urinary lactate excretion and LDH-dependent lactate formation (97). However, the risk of euglycemic DM ketoacidosis with SGLT2Is is very low (about 1%), but the risk of this side effect should be counterbalanced with the beneficial use of SGLT2Is in DM patients with COVID-19 (98). Hence, the net effect of SGLT2Is in COVID-19 is mainly related to the maintenance of cell pH with the reduction of the viral load. Into the bargain, SGLT2Is reduce IR and hyperglycemia-induced inflammatory reactions and ACE2 glycation, thereby reducing the risk of CS and AngII that are augmented in COVID-19 (99).

Sulfonylureas

Sulfonylureas (SU), such as glibenclamide, glipizide, and glimepiride, are a class of anti-DM agents that increase insulin secretion from pancreatic β-cells (100). SU has potent anti-inflammatory effects through the inhibition of IL-1β and nod-like receptor pyrine 3 NLRP3 inflammasome with antiplatelet effects and the reduction of thromboxane A2 activation (101). Platelets' aggregation and activation of both IL-1β and inflammasome are involved in CS generation in COVID-19 patients (102). SU blocks NLRP3 inflammasome-induced ALI through the suppression of the K^+-ATP channel, K^+ outflow block, the inhibition of Ca^{2+} entry and of oxidative stress, and the improvement of endogenous antioxidant capacity (103). However, the use of glibenclamide in DM patients with COVID-19 has not been evaluated since most DM patients with COVID-19 are switched to insulin therapy (104). Nonetheless, glibenclamide therapy in T2DM patients increases the risk of hypoglycemia, which might occur in COVID-19 patients (105, 106).

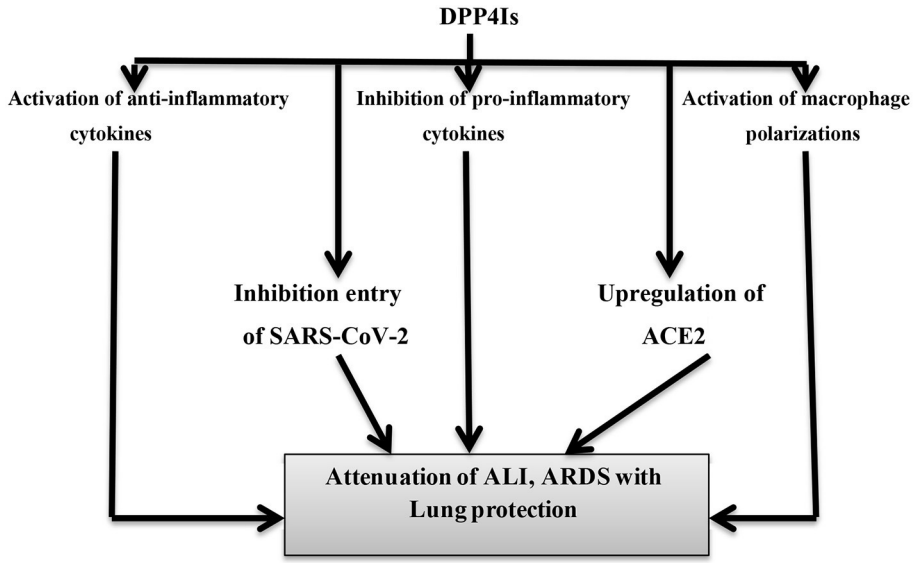


FIGURE 7 | Potential effects of DPP4 inhibitors on the pathogenesis of COVID-19. Dipeptidyl peptidase-4 inhibitors (DPP4Is) have anti-inflammatory effects through the activation of anti-inflammatory cytokines, the activation of macrophage polarizations, and the inhibition of pro-inflammatory cytokines. DPP4Is inhibit the entry of SARS-CoV-2 through blocking of DPP4 receptors. The net effects of DPP4Is are lung protection with improvement in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS).

Insulin

Recent data have shown that insulin requirements are increased and correlated with high CRP serum levels and COVID-19 severity (107). For this reason, COVID-19-induced hyperglycemia, IR, and associated inflammatory disorders can increase the pancreatic β-cell burden in DM and non-DM patients (19). However, at the ICU, insulin requirements are higher in DM patients compared to controls due to the preexistence of an inflammatory status and cardiometabolic comorbidities (108). In COVID-19 patients, the direct interaction with pancreatic β-cells by SARS-CoV-2 leads to a significant reduction in insulin release. However, C-peptide is raised in COVID-19, suggesting that SARS-CoV-2 may cause transient pancreatic β-cell toxicity (109).

Thus, as hyperglycemia is commonly reported in critical illness, to ensure a proper control of hyperglycemia through insulin therapy, it is crucial to prevent the occurrence of cardiometabolic complications (110); therefore, early insulin therapy in critical illnesses, as occur in cases of COVID-19-induced hyperglycemia, may improve the clinical outcomes while reducing the mortality rate through different ways: (a) insulin inhibits pro-inflammatory cytokine linked to ARDS (111); (b) insulin promotes a restoration of pancreatic and renal ACE2 and ADAM-17 activity, and RAS balance (112); (c) insulin therapy reduces the risk of hyperglycemia and DKA that are associated with high mortality rates in COVID-19 patients. In addition, insulin therapy exerts a protective role against SARS-CoV-2-induced ALI and ARDS (113). To this effect, any COVID-19 patient should be monitored for blood glucose and HbA1c, along with a strict blood glucose monitoring, where insulin therapy should be properly administered. Besides, long-term evaluation of pancreatic β-cell function is recommended to ascertain potential β-cell damage and future DM development.

Moreover, higher CRP serum levels and neutrophil count reveal a humoral immune response in DM patients with COVID-19 (114). In fact, hyperglycemia affects antibody response during viral infection through the impairment of lymphocytes, macrophages, and neutrophil functions, as well as complement response (115). Therefore, the antibody response for SARS-CoV-2 vaccine may be impaired in DM due to hyperglycemia and IR (116). For these reasons, a strict insulin therapy is advisable to control the cell and humoral immune impairments in DM patients with COVID-19.

CONCLUSIONS

Data obtained underlined that SARS-CoV-2 infection in DM patients is more severe and associated with poor clinical outcomes due to preexistent comorbidities and pro-inflammatory phenotype. SARS-CoV-2 infection impairs glucose homeostasis and metabolism in DM and non-DM patients due to cytokine storm (CS) development, downregulation of ACE2, and direct injury of pancreatic β-cells. Therefore, the potent anti-inflammatory effect of diabetic pharmacotherapies such as metformin, pioglitazone, sodium-glucose co-transporter-2 inhibitors (SGLT2Is), and dipeptidyl peptidase-4 (DPP4) inhibitors may mitigate the COVID-19 severity. In addition, some antidiabetic agents, including insulin, can reduce the SARS-CoV-2 infectivity and severity by modulating the expression of ACE2 receptors. Taken together, the data presented here illustrate that insulin therapy may seem as more appropriate than other anti-DM pharmacotherapies in the management of COVID-19 patients with DM due to the lower risk of uncontrolled hyperglycemia and reduced propensity to develop diabetic ketoacidosis (DKA). However, based on these findings,

it is not yet possible to conclude decisively on the efficacy of diabetic pharmacotherapy in COVID-19, so thorough clinical trials are warranted.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

NC-M acknowledges the Portuguese Foundation for Science and Technology under the Horizon 2020 Program (PTDC/PSI-GER/28076/2017). HA-k acknowledges medical staff members of Al-Shiffa Medical Center, Baghdad, Iraq for their participations. This work was supported by Taif University Researchers Supporting Program (project number: TURSP-2020/93), Taif University, Saudi Arabia.

REFERENCES

1. Al-Kuraishy HM, Al-Gareeb AI. From SARS-CoV to nCoV-2019: ruction and argument. *Arch Clin Infect Dis.* (2020) 15:e102624. doi: 10.5812/archcid.10262
2. Al-Kuraishy HM, Al-Naimi MS, Lungnier CM, Al-Gareeb AI. Macrolides and COVID-19: an optimum premise. *Biomed Biotechnol Res J.* (2020) 4:189. doi: 10.4103/bbrj.bbrj_103_20
3. Sohrabi C, Alsafi Z, O'Neill N, Khan M, Kerwan A, Al-Jabir A, et al. World Health Organization declares global emergency: a review of the 2019 novel coronavirus (COVID-19). *Int J Surg.* (2020) 76:71–6. doi: 10.1016/j.ijsu.2020.02.034
4. Kumar A, Faiq MA, Pareek V, Raza K, Narayan RK, Prasoon P, et al. Relevance of SARS-CoV-2 related factors ACE2 and TMPRSS2 expressions in gastrointestinal tissue with pathogenesis of digestive symptoms, diabetes-associated mortality, and disease recurrence in COVID-19 patients. *Med Hypothes.* (2020) 144:110271. doi: 10.1016/j.mehy.2020.110271
5. Alon TM, Doepke M, Olmstead-Rumsey J, Tertilt M. The impact of COVID-19 on gender equality. *Natl Bureau Econ Res.* (2020). doi: 10.3386/w26947
6. Al-Kuraishy HM, Al-Gareeb AI, Al-Niemi MS, Al-Buhadily AK, Al-Harchan NA, Lugnier C. COVID-19 and phosphodiesterase enzyme type 5 inhibitors. *J Microscopy Ultrastruct.* (2020) 8:141. doi: 10.4103/JMAU.JMAU_63_20
7. Rasheed HA, Al-Kuraishy HM, Al-Gareeb AI, Hussien NR, Al-Nami MS. Effects of diabetic pharmacotherapy on prolactin hormone in patients with type 2 diabetes mellitus: Bane or Boon. *J Adv Pharmaceut Technol Res.* (2019) 10:163. doi: 10.4103/japtr.JAPTR_65_19
8. Meo SA, Alhowikan AM, Al-Khlaiwi T, Meo IM, Halepoto DM, Iqbal M, et al. Novel coronavirus 2019-nCoV: prevalence, biological and clinical characteristics comparison with SARS-CoV and MERS-CoV. *Eur Rev Med Pharmacol Sci.* (2020) 24:2012–9. doi: 10.26355/eurrev_202002_20379
9. Ilias I, Jahaj E, Kokkoris S, Zervakis D, Temperikidis P, Magira E, et al. Clinical study of hyperglycemia and SARS-CoV-2 infection in intensive care unit patients. *In Vivo.* (2020) 34:3029–32. doi: 10.21873/invivo.12136
10. Somasundaram NP, Ranathunga I, Ratnasamy V, Wijewickrama PS, Dissanayake HA, Yogendranathan N, et al. The impact of SARS-Cov-2 virus infection on the endocrine system. *J Endocrine Soc.* (2020) 4:bvaa082. doi: 10.1210/jendso/bvaa082
11. Wang A, Zhao W, Xu Z, Gu J. Timely blood glucose management for the outbreak of 2019 novel coronavirus disease (COVID-19) is urgently needed. *Diabetes Res Clin Pract.* (2020) 162:108118. doi: 10.1016/j.diabres.2020.108118
12. Michalakis K, Ilias I. SARS-CoV-2 infection and obesity: Common inflammatory and metabolic aspects. *Diabetes Metab Syndrome.* (2020) 14:469–71. doi: 10.1016/j.dsx.2020.04.033
13. Al-kuraishy HM, Al-Maiahy TJ, Al-Gareeb AI, Musa RA, Ali ZH. COVID-19 pneumonia in an Iraqi pregnant woman with preterm delivery. *Asian Pacific J Reproduct.* (2020) 9:156. doi: 10.4103/2305-0500.282984
14. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet.* (2020) 395:1033–4. doi: 10.1016/S0140-6736(20)30628-0
15. Abdul-Hadi MH, Naji MT, Shams HA, Sami OM, Al-Harchan NA, Al-Kuraishy HM, et al. Oxidative stress injury and glucolipotoxicity in type 2 diabetes mellitus: The potential role of metformin and sitagliptin. *Biomed Biotechnol Res J.* (2020) 4:166. doi: 10.4103/bbrj.bbrj_7_20
16. Albulescu R, Dima SO, Florea IR, Lixandru D, Serban AM, Aspritoiu VM, et al. COVID-19 and diabetes mellitus: unraveling the hypotheses that worsen the prognosis. *Experi Therap Med.* (2020) 20:194. doi: 10.3892/etm.2020.9324
17. Al-kuraishy HM, Al-Gareeb AI, Abdullah SM, Cruz-Martins N, Batiha GE. Case report: hyperbilirubinemia in gilbert syndrome attenuates Covid-19-induced metabolic disturbances. *Front Cardiovasc Med.* (2021) 8:e642181. doi: 10.3389/fcvm.2021.642181
18. Lugnier C, Al-Kuraishi HM, Rousseau E. PDE4 inhibition as a therapeutic strategy for improvement of pulmonary dysfunctions in Covid-19 and cigarette smoking. *Biochem Pharmacol.* (2021) 28:114431. doi: 10.1016/j.bcp.2021.114431
19. Singh AK, Singh R. Hyperglycemia without diabetes and new-onset diabetes are both associated with poorer outcomes in COVID-19. *Diabetes Res Clin Pract.* (2020) 167:108382. doi: 10.1016/j.diabres.2020.108382
20. Göbölös L, Rácz I, Hogan M, Remsey-Semmelweis E, Atallah B, AlMahmeed W, et al. The role of renin-angiotensin system activated phagocytes in the SARS-CoV-2 coronavirus infection. *J Vasc Surg.* (2020). doi: 10.1016/j.jvs.2020.12.056
21. Dwi N, Netra WI. The analysis of blood glucose level and blood pressure on hypertension patients in mersi village, East Purwokerto, Central Java. In: *1st International* Conference *on Community Health (ICCH 2019)*. Purwokerto: Atlantis Press (2020). doi: 10.2991/ahsr.k.200204.014
22. Ceriello A. Hyperglycemia and COVID-19: what was known and what is really new?. *Diabetes Res Clin Pract.* (2020) 167:108383. doi: 10.1016/j.diabres.2020.108383
23. Al-Nami MS, Al-Kuraishy HM, Al-Gareeb AI, Al-Mamoori F. Metabolic profile and prolactin serum levels in men with type 2 diabetes mellitus: old-new rubric. *Int J Criti Illness Injury Sci.* (2019) 9:120. doi: 10.4103/IJCIIS.IJCIIS_40_19
24. Alves AM, Yvamoto EY, Marzinotto MA, Teixeira AC, Carrilho FJ. SARS-CoV-2 leading to acute pancreatitis: an unusual presentation. *Brazil J Infect Dis.* (2020) 24:561–4. doi: 10.1016/j.bjid.2020.08.011
25. Ilahi O, Bochicchio GV, Scalea TM, Aaland MO. Efficacy of computed tomography in the diagnosis of pancreatic injury in adult blunt trauma patients: a single-institutional study/discussion. *Am Surg.* (2002) 68:704. doi: 10.1515/hmbci-2019-0066
26. Wang F, Wang H, Fan J, Zhang Y, Wang H, Zhao Q. Pancreatic injury patterns in patients with coronavirus disease 19 pneumonia. *Gastroenterology.* (2020) 159:367–70. doi: 10.1053/j.gastro.2020.03.055
27. Zhang QL, Ding YQ, Hou JL, He L, Huang ZX, Wang HJ, et al. Detection of severe acute respiratory syndrome (SARS)-associated coronavirus RNA in autopsy tissues with in situ hybridization. *Acad J First Med College PLA.* (2003) 23:1125–7.
28. Taneera J, El-Huneidi W, Hamad M, Mohammed AK, Elaraby E, Hachim MY. Expression profile of SARS-CoV-2 host receptors in human pancreatic islets revealed upregulation of ACE2 in diabetic donors. *Biology.* (2020) 9:215. doi: 10.3390/biology9080215
29. Gomes IC, Karmirian K, Oliveira J, Pedrosa C, Rosman FC, Chimelli L, et al. SARS-CoV-2 infection in the central nervous system of a 1-year-old infant. *Preprints.* (2020). doi: 10.20944/preprints202009.0297.v1

30. Coate KC, Cha J, Shrestha S, Wang W, Gonçalves LM, Almaça J, et al. SARS-CoV-2 cell entry factors ACE2 and TMPRSS2 are expressed in the microvasculature and ducts of human pancreas but are not enriched in β cells. *Cell Metabo.* (2020) 32:1028–40. doi: 10.1016/j.cmet.2020.11.006
31. Caballero AE, Ceriello A, Misra A, Aschner P, McDonnell ME, Hassanein M, et al. COVID-19 in people living with diabetes: an international consensus. *J Diabetes Compli.* (2020) 34:107671. doi: 10.1016/j.jdiacomp.2020.107671
32. Liu B, Li M, Zhou Z, Guan X, Xiang Y. Can we use interleukin-6 (IL-6) blockade for coronavirus disease 2019 (COVID-19)-induced cytokine release syndrome (CRS)? *J Autoimmun*. (2020) 10:102452. doi: 10.1016/j.jaut.2020.102452
33. Huang W, Meng Y, Wu P, Li X. Rebalance of the RAAS axes: the therapeutic strategy of COVID-19?. *Authorea Preprints.* (2020). doi: 10.22541/au.159050479.95287268
34. Chan KK, Dorosky D, Sharma P, Abbasi SA, Dye JM, Kranz DM, et al. Engineering human ACE2 to optimize binding to the spike protein of SARS coronavirus 2. *Science.* (2020) 369:1261–5. doi: 10.1126/science.abc0870
35. Sharma RK, Stevens BR, Obukhov AG, Grant MB, Oudit GY, Li Q, et al. ACE2 (Angiotensin-Converting Enzyme 2) in cardiopulmonary diseases: ramifications for the control of SARS-CoV-2. *Hypertension.* (2020) 76:651–61. doi: 10.1161/HYPERTENSIONAHA.120.15595
36. Kawabe Y, Mori J, Morimoto H, Yamaguchi M, Miyagaki S, Ota T, et al. ACE2 exerts anti-obesity effect via stimulating brown adipose tissue and induction of browning in white adipose tissue. *Am J Physiol Endocrinol Metabo.* (2019) 317:E1140–9. doi: 10.1152/ajpendo.00311.2019
37. Liu C, Li Y, Guan T, Lai Y, Shen Y, Zeyaweiding A, et al. ACE2 polymorphisms associated with cardiovascular risk in Uygurs with type 2 diabetes mellitus. *Cardiovasc Diabetol.* (2018) 17:1–1. doi: 10.1186/s12933-018-0771-3
38. Fadnavis R. Effect of hyperglycemia and thiazolidinediones on cardiac angiotensin converting enzyme 2 (ACE2) and neprilysin (NEP) in db/db diabetic mice (Doctoral dissertation) Wright State University, Columbus, OH, United States.
39. Yang JK, Lin SS, Ji XJ, Guo LM. Binding of SARS coronavirus to its receptor damages islets and causes acute diabetes. *Acta Diabetol.* (2010) 47:193–9. doi: 10.1007/s00592-009-0109-4
40. Domingo P, Mur I, Pomar V, Corominas H, Casademont J, de Benito N. The four horsemen of a viral Apocalypse: The pathogenesis of SARS-CoV-2 infection (COVID-19). *EBioMedicine.* (2020) 58:102887. doi: 10.1016/j.ebiom.2020.102887
41. Pranata R, Lim MA, Huang I, Raharjo SB, Lukito AA. Hypertension is associated with increased mortality and severity of disease in COVID-19 pneumonia: a systematic review, meta-analysis and meta-regression. *J Renin-Angiotensin-Aldosterone Syst.* (2020) 21:1470320320926899. doi: 10.1177/1470320320926899
42. Li Y, Han X, Alwalid O, Cui Y, Cao Y, Liu J, et al. Baseline characteristics and risk factors for short-term outcomes in 132 COVID-19 patients with diabetes in Wuhan China: a retrospective study. *Diabetes Res Clin Pract.* (2020) 166:108299. doi: 10.1016/j.diabres.2020.108299
43. Fadini GP, Morieri ML, Longato E, Avogaro A. Prevalence and impact of diabetes among people infected with SARS-CoV-2. *J Endocrinol Investig.* (2020) 43:867–9. doi: 10.1007/s40618-020-01236-2
44. Zhu L, She ZG, Cheng X, Qin JJ, Zhang XJ, Cai J, et al. Association of blood glucose control and outcomes in patients with COVID-19 and pre-existing type 2 diabetes. *Cell Metabo.* (2020) 31:1068–77. doi: 10.1016/j.cmet.2020.04.021
45. Raoult D, Zumla A, Locatelli F, Ippolito G, Kroemer G. Coronavirus infections: epidemiological, clinical and immunological features and hypotheses. *Cell Stress.* (2020) 4:66. doi: 10.15698/cst2020.04.216
46. Caruso I, Di Molfetta S, Guarini F, Giordano F, Cignarelli A, Natalicchio A, et al. Reduction of hypoglycaemia, lifestyle modifications and psychological distress during lockdown following SARS-CoV-2 outbreak in type 1 diabetes. *Diabetes Metabo Res Rev.* (2020) 1:e3404. doi: 10.1002/dmrr.3404
47. Roncon L, Zuin M, Rigatelli G, Zuliani G. Diabetic patients with COVID-19 infection are at higher risk of ICU admission and poor short-term outcome. *J Clin Virol.* (2020) 127:104354. doi: 10.1016/j.jcv.2020.104354
48. Huang I, Lim MA, Pranata R. Diabetes mellitus is associated with increased mortality and severity of disease in COVID-19 pneumonia–a systematic review, meta-analysis, and meta-regression. *Diabetes Metabol Syndrome: Clin Res Rev.* (2020) 14:395–403. doi: 10.1016/j.dsx.2020.04.018
49. Maddaloni E, Buzzetti R. Covid-19 and diabetes mellitus: unveiling the interaction of two pandemics. *Diabetes Metabol Res Rev.* (2020) 36:e33213321. doi: 10.1002/dmrr.3321
50. Sathish T, Cao Y, Kapoor N. Newly diagnosed diabetes in COVID-19 patients. *Primary Care Diabetes.* (2020) 15:194. doi: 10.1016/j.pcd.2020.08.014
51. Mirani M, Favacchio G, Carrone F, Betella N, Biamonte E, Morenghi E, et al. Impact of comorbidities and glycemia at admission and dipeptidyl peptidase 4 inhibitors in patients with type 2 diabetes with COVID-19: a case series from an academic hospital in Lombardy, Italy. *Diabetes Care.* (2020) 43:3042–9. doi: 10.2337/dc20-1340
52. Takeda Y, Shimomura T, Wakabayashi I. Immunological disorders of diabetes mellitus in experimental rat models. Nihon eiseigaku zasshi. *Japan J Hygiene.* (2014) 69:166–76. doi: 10.1265/jjh.69.166
53. Pal R, Bhansali A. COVID-19, diabetes mellitus and ACE2: the conundrum. *Diabetes Res Clin Pract.* (2020) 162:108132. doi: 10.1016/j.diabres.2020.108132
54. Sinha S, Cheng K, Schäffer AA, Aldape K, Schiff E, Ruppin E. *In vitro* and *in vivo* identification of clinically approved drugs that modify ACE 2 expression. *Mol Syst Biol.* (2020) 16:e9628. doi: 10.15252/msb.20209628
55. Gupta R, Hussain A, Misra A. Diabetes and COVID-19: evidence, current status and unanswered research questions. *Eur J Clin Nutr.* (2020) 74:864–70. doi: 10.1038/s41430-020-0652-1
56. Klimstra WB, Tilston-Lunel NL, Nambulli S, Boslett J, McMillen CM, Gilliland T, et al. SARS-CoV-2 growth, furin-cleavage-site adaptation and neutralization using serum from acutely infected hospitalized COVID-19 patients. *J Gene Virol.* (2020) 101:1156–69. doi: 10.1099/jgv.0.001481
57. John TJ, John K. What is the role of angiotensin-converting enzyme 2 (ACE2) in COVID-19 infection in hypertensive patients with diabetes?. *Canad J Cardiol.* (2020) 36:969–e3. doi: 10.1016/j.cjca.2020.03.049
58. Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection *in vitro*. *Cell Disc.* (2020) 6:1–4. doi: 10.1038/s41421-020-0156-0
59. Wondafrash DZ, Desalegn TZ, Yimer EM, Tsige AG, Adamu BA, Zewdie KA. Potential effect of hydroxychloroquine in diabetes mellitus: a systematic review on preclinical and clinical trial studies. *J Diabetes Res.* (2020) 2020:5214751. doi: 10.1155/2020/5214751
60. Hasan SS, Capstick T, Ahmed R, Kow CS, Mazhar F, Merchant HA, et al. Mortality in COVID-19 patients with acute respiratory distress syndrome and corticosteroids use: a systematic review and meta-analysis. *Expert Rev Respirat Med.* (2020) 14:1149–63. doi: 10.1080/17476348.2020.1804365
61. Saad AF, Chappell L, Saade GR, Pacheco LD. Corticosteroids in the management of pregnant patients with coronavirus disease (COVID-19). *Obstetr Gynecol.* (2020) 136:823–6. doi: 10.1097/AOG.0000000000004103
62. Singh AK, Majumdar S, Singh R, Misra A. Role of corticosteroid in the management of COVID-19: a systemic review and a clinician's perspective. *Diabetes Metabol Syndr.* (2020) 14:971–8. doi: 10.1016/j.dsx.2020.06.054
63. Al-Nami MS, Al-Kuraishy HM, Al-Gareeb AI. Impact of thioctic acid on glycemic indices and associated inflammatory-induced endothelial dysfunction in patients with type 2 diabetes mellitus: a case control study. *Int J Criti Illness Injury Sci.* (2020) 10:21. doi: 10.4103/IJCIIS.IJCIIS_62_19
64. Al-Hashem F, Al-Humayed S, Amin SN, Kamar SS, Mansy SS, Hassan S, et al. Metformin inhibits mTOR–HIF-1α axis and profibrogenic and inflammatory biomarkers in thioacetamide-induced hepatic tissue alterations. *J Cell Physiol.* (2019) 234:9328–37. doi: 10.1002/jcp.27616
65. Rajpal A, Rahimi L, Ismail-Beigi F. Factors leading to high morbidity and mortality of COVID-19 in patients with type 2 diabetes. *J Diabetes.* (2020) 12:895–908. doi: 10.1111/1753-0407.13085
66. Crouse AB, Grimes T, Li P, Might M, Ovalle F, Shalev A. Metformin use is associated with reduced mortality in a diverse population with COVID-19 and diabetes. *Front Endocrinol.* (2021) 11:1081. doi: 10.3389/fendo.2020.600439
67. Scheen AJ. Metformin and COVID-19: From cellular mechanisms to reduced mortality. *Diabetes Metabol.* (2020) 46:423–6. doi: 10.1016/j.diabet.2020.07.006

68. Sharma S, Ray A, Sadasivam B. Metformin in COVID-19: a possible role beyond diabetes. *Diabetes Res Clin Pract.* (2020) 164:108183. doi: 10.1016/j.diabres.2020.108183
69. Abdel-Maksoud MS, El-Gamal MI, Benhalilou DR, Ashraf S, Mohammed SA, Oh CH. Mechanistic/mammalian target of rapamycin: recent pathological aspects and inhibitors. *Med Res Rev.* (2019) 39:631–64. doi: 10.1002/med.21535
70. Singh S, Singh PK, Suhail H, Arumugaswami V, Pellett PE, Giri S, et al. AMP-activated protein kinase restricts Zika virus replication in endothelial cells by potentiating innate antiviral responses and inhibiting glycolysis. *J Immunol.* (2020) 204:1810–24. doi: 10.4049/jimmunol.1901310
71. Wu K, Tian R, Huang J, Yang Y, Dai J, Jiang R, et al. Metformin alleviated endotoxemia-induced acute lung injury via restoring AMPK-dependent suppression of mTOR. *Chem Biol Interact.* (2018) 291:1–6. doi: 10.1016/j.cbi.2018.05.018
72. Cheng X, Liu YM, Li H, Zhang X, Lei F, Qin JJ, et al. Metformin is associated with higher incidence of acidosis, but not mortality, in individuals with COVID-19 and pre-existing type 2 diabetes. *Cell Metabo.* (2020) 32:537–47. doi: 10.1016/j.cmet.2020.08.013
73. Al-kuraishy HM, Al-Gareeb AI, Alblihed M, Cruz-Martins N, Batiha GE. COVID-19 and risk of acute ischemic stroke and acute lung injury in patients with type ii diabetes mellitus: the anti-inflammatory role of metformin. *Front Med.* (2021) 8:110. doi: 10.3389/fmed.2021.644295
74. Hariyanto TI, Kurniawan A. Metformin use is associated with reduced mortality rate from coronavirus disease 2019 (COVID-19) infection. *Obesity Med*. (2020) 19:100290. doi: 10.1016/j.obmed.2020.100290
75. Tahlan S, Verma PK. Biological potential of thiazolidinedione derivatives of synthetic origin. *Chem Central J.* (2017) 11:1–29. doi: 10.1186/s13065-017-0357-2
76. Kalavalapalli S, Bril F, Koelmel JP, Abdo K, Guingab J, Andrews P, et al. Pioglitazone improves hepatic mitochondrial function in a mouse model of nonalcoholic steatohepatitis. *Am J Physiol Endocrinol Metabo.* (2018) 315:E163–73. doi: 10.1152/ajpendo.00023.2018
77. Carboni E, Carta AR, Carboni E. Can pioglitazone be potentially useful therapeutically in treating patients with COVID-19?. *Med Hypothes.* (2020) 140:109776. doi: 10.1016/j.mehy.2020.109776
78. Kökény G, Calvier L, Legchenko E, Chouvarine P, Mózes MM, Hansmann G. PPARγ is a gatekeeper for extracellular matrix and vascular cell homeostasis: beneficial role in pulmonary hypertension and renal/cardiac/pulmonary fibrosis. *Curr Opin Nephrol Hyperten.* (2020) 29:171–9. doi: 10.1097/MNH.0000000000000580
79. Kruglikov IL, Scherer PE. The role of adipocytes and adipocyte-like cells in the severity of COVID-19 infections. *Obesity.* (2020) 28:1187–90. doi: 10.1002/oby.22856
80. Subir R. Use of pioglitazone in people with type 2 diabetes mellitus with coronavirus disease 2019. (COVID-19): Boon or bane?. *Diabetes Metabo Syndr.* (2020) 14:829–31. doi: 10.1016/j.dsx.2020.06.015
81. Al-Kuraishy HM, Sami OM, Hussain NR, Al-Gareeb AI. Metformin and/or vildagliptin mitigate type II diabetes mellitus induced-oxidative stress: the intriguing effect. *J Adv Pharm Technol Res.* (2020) 11:142. doi: 10.4103/japtr.JAPTR_18_20
82. Gallwitz B. Clinical use of DPP-4 inhibitors. *Front Endocrinol.* (2019) 10:389. doi: 10.3389/fendo.2019.00389
83. Deacon CF. A review of dipeptidyl peptidase-4 inhibitors. Hot topics from randomized controlled trials. *Diabetes Obesity Metabo.* (2018) 20:34–46. doi: 10.1111/dom.13135
84. Li G, Crowley MJ, Tang H, Yang JY, Sandler RS, Wang T. Dipeptidyl peptidase 4 inhibitors and risk of inflammatory bowel disease among patients with type 2 diabetes: a meta-analysis of randomized controlled trials. *Diabetes Care.* (2019) 42:e119–21. doi: 10.2337/dc18-1578
85. Kawaguchi Y, Shimauchi R, Nishibori N, Kawashima K, Oshitani S, Fujiya A, et al. Dipeptidyl peptidase-4 inhibitors-associated bullous pemphigoid: a retrospective study of 168 pemphigoid and 9,304 diabetes mellitus patients. *J Diabetes Investig.* (2019) 10:392–8. doi: 10.1111/jdi.12877
86. Li Y, Zhang Z, Yang L, Lian X, Xie Y, Li S, et al. The MERS-CoV receptor DPP4 as a candidate binding target of the SARS-CoV-2 spike. *Iscience.* (2020) 23:101160. doi: 10.1016/j.isci.2020.101160
87. Valencia I, Peiró C, Lorenzo Ó, Sánchez-Ferrer CF, Eckel J, Romacho T. DPP4 and ACE2 in diabetes and COVID-19: therapeutic targets for cardiovascular complications? *Front Pharmacol.* (2020) 11:1161. doi: 10.3389/fphar.2020.01161
88. Vankadari N, Wilce JA. Emerging COVID-19 coronavirus: glycan shield and structure prediction of spike glycoprotein and its interaction with human CD26. *Emerg Microbes Infect.* (2020) 9:601–4. doi: 10.1080/22221751.2020.1739565
89. Bassendine MF, Bridge SH, McCaughan GW, Gorrell MD. COVID-19 and comorbidities: A role for dipeptidyl peptidase 4 (DPP4) in disease severity?. *J Diabetes.* (2020) 12:649–58. doi: 10.1111/1753-0407.13052
90. Tawfik MK, Mohamed MI. Exenatide suppresses 1, 2-dimethylhydrazine-induced colon cancer in diabetic mice: Effect on tumor angiogenesis and cell proliferation. *Biomed Pharm.* (2016) 82:106–16. doi: 10.1016/j.biopha.2016.05.005
91. Longo M, Caruso P, Maiorino MI, Bellastella G, Giugliano D, Esposito K. Treating type 2 diabetes in COVID-19 patients: the potential benefits of injective therapies. *Cardiovasc Diabetol.* (2020) 19:1–5. doi: 10.1186/s12933-020-01090-9
92. Bersoff-Matcha SJ, Chamberlain C, Cao C, Kortepeter C, Chong WH. Fournier gangrene associated with sodium–glucose cotransporter-2 inhibitors: a review of spontaneous postmarketing cases. *Ann Internal Med.* (2019) 170:764–9. doi: 10.7326/M19-0085
93. Patoulias D, Papadopoulos C, Katsimardou A, Toumpourleka M, Doumas M. Sodium–glucose cotransporter 2 inhibitors and major COVID-19 outcomes: promising mechanisms, conflicting data, and intriguing clinical decisions. *Diabetes Ther.* (2020) 11:3003–5. doi: 10.1007/s13300-020-00942-7
94. Velavan TP, Kieu Linh LT, Kreidenweiss A, Gabor J, Krishna S, Kremsner PG. Longitudinal monitoring of lactate in hospitalized and ambulatory COVID-19 patients. *Am J Trop Med Hygiene.* (2021) 11:tpmd201282. doi: 10.4269/ajtmh.20-1282
95. Li C, Ye J, Chen Q, Hu W, Wang L, Fan Y, et al. Elevated lactate dehydrogenase (LDH) level as an independent risk factor for the severity and mortality of COVID-19. *Aging.* (2020) 12:15670. doi: 10.18632/aging.103770
96. Jimenez L, Codo AC, Sampaio VS, Oliveira AE, Ferreira LK, Davanzo GG, et al. The influence of pH on SARS-CoV-2 infection and COVID-19 severity. *MedRxiv.* (2020). doi: 10.1101/2020.09.10.20179135
97. Chatterjee S. SGLT-2 inhibitors for COVID-19—A miracle waiting to happen or just another beat around the bush?. *Primary Care Diabetes.* (2020) 14:564–5. doi: 10.1016/j.pcd.2020.05.013
98. Oriot P, Hermans MP. Euglycemic diabetic ketoacidosis in a patient with type 1 diabetes and SARS-CoV-2 pneumonia: case report and review of the literature. *Acta Clin Belgica.* (2020) 18:1–5. doi: 10.1080/17843286.2020.1780390
99. Koufakis T, Metallidis S, Zebekakis P, Ajjan RA, Kotsa K. Sodium-glucose cotransporter 2 inhibitors in the Era of COVID-19 pandemic: is the benefit to risk ratio still favorable? *J Diabetes Sci Technol.* (2020) 14:745–7. doi: 10.1177/1932296820932155
100. Hussien NR, Al-Naimi MS, Rasheed HA, Al-Kuraishy HM, Al-Gareeb AI. Sulfonylurea and neuroprotection: the bright side of the moon. *J Adv Pharm Technol Res.* (2018) 9:120–3. doi: 10.4103/japtr.JAPTR_317_18
101. Yaribeygi H, Atkin SL, Butler AE, Sahebkar A. Sodium–glucose cotransporter inhibitors and oxidative stress: an update. *J Cell Physiol.* (2019) 234:3231–7. doi: 10.1002/jcp.26760
102. de Rivero Vaccari JC, Dietrich WD, Keane RW, de Rivero Vaccari JP. The inflammasome in times of COVID-19. *Front Immunol.* (2020) 11:2474. doi: 10.3389/fimmu.2020.583373
103. Hill JR, Coll RC, Sue N, Reid JC, Dou J, Holley CL, et al. Sulfonylureas as concomitant insulin secretagogues and NLRP3 inflammasome inhibitors. *ChemMedChem.* (2017) 12:1449–57. doi: 10.1002/cmdc.201700270
104. Maddaloni E, Coraggio L, Pieralice S, Carlone A, Pozzilli P, Buzzetti R. Effects of COVID-19 lockdown on glucose control: continuous glucose monitoring data from people with diabetes on intensive insulin therapy. *Diabetes Care.* (2020) 43:e86–7. doi: 10.2337/dc20-0954
105. Hanif S, Ali SN, Hassanein M, Khunti K, Hanif W. Managing people with diabetes fasting for ramadan during the COVID-19 pandemic: A

South Asian health foundation update. *Diabetic Med.* (2020) 37:1094–102. doi: 10.1111/dme.14312
106. Kawahara Y, Kaneko T, Yoshinaga Y, Arita Y, Nakamura K, Koga C, et al. Effects of sulfonylureas on periodontopathic bacteria-induced inflammation. *J Dental Res.* (2020) 99:830–8. doi: 10.1177/00220345209 13250
107. Lockhart S, Griffiths H, Petrisor B, Usman A, Calvo-Latorre J, Heales L, et al. The excess insulin requirement in severe COVID-19 compared to non-COVID-19 viral pneumonitis is related to the severity of respiratory failure and pre-existing diabetes. *medRxiv.* (2020). doi: 10.1101/2020.10.03.20206284
108. Attri B, Goyal A, Gupta Y, Tandon N. Basal-bolus insulin regimen for hospitalised patients with COVID-19 and diabetes mellitus: a practical approach. *Diabetes Therapy.* (2020) 11:2177–94. doi: 10.1007/s13300-020-00873-3
109. Muniangi-Muhitu H, Akalestou E, Salem V, Misra S, Oliver NS, Rutter GA. Covid-19 and diabetes: a complex bidirectional relationship. *Front Endocrinol.* (2020) 11:758. doi: 10.3389/fendo.2020.582936
110. Becker CD, Sabang RL, Cordeiro MF, Hassan IF, Goldberg MD, Scurlock CS. Hyperglycemia in medically critically ill patients: risk factors and clinical outcomes. *Am J Med.* (2020) 133:e568–74. doi: 10.1016/j.amjmed.2020.03.012
111. Ballak DB, Li S, Cavalli G, Stahl JL, Tengesdal IW, van Diepen JA, et al. Interleukin-37 treatment of mice with metabolic syndrome improves insulin sensitivity and reduces pro-inflammatory cytokine production in adipose tissue. *J Biol Chem.* (2018) 293:14224–36. doi: 10.1074/jbc.RA118. 003698
112. Wang Y, Rijal B, Xu M, Li Z, An Y, Zhang F, et al. Renal denervation improves vascular endothelial dysfunction by inducing autophagy via AMPK/MTOR signaling activation in a rat model of type 2 diabetes mellitus with insulin resistance. *Acta Diabetol.* (2020) 57:1227–43. doi: 10.1007/s00592-020-01532-6
113. Ren H, Yang Y, Wang F, Yan Y, Shi X, Dong K, et al. Association of the insulin resistance marker TyG index with the severity and mortality of COVID-19. *Cardiovasc Diabetol.* (2020) 19:1–8. doi: 10.1186/s12933-020-01035-2
114. Vandercam G, Simon A, Scohy A, Belkhir L, Kabamba B, Rodriguez-Villalobos H, et al. Clinical characteristics and humoral immune response in healthcare workers with COVID-19 in a teaching hospital in Belgium. *J Hospital Infect.* (2020) 106:713–20. doi: 10.1016/j.jhin.2020.09.018
115. Hu R, Xia CQ, Butfiloski E, Clare-Salzler M. Effect of high glucose on cytokine production by human peripheral blood immune cells and type I interferon signaling in monocytes: Implications for the role of hyperglycemia in the diabetes inflammatory process and host defense against infection. *Clin Immunol.* (2018) 195:139–48. doi: 10.1016/j.clim.2018.06.003
116. Erener S. Diabetes, infection risk and COVID-19. *Mol Metabo.* (2020) 23:101044. doi: 10.1016/j.molmet.2020.101044

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

REVIEW
published: 21 May 2021
doi: 10.3389/fcvm.2021.663864

Utility of Non-invasive Cardiac Imaging Assessment in Coronavirus Disease 2019

Sandeep S. Hothi[1,2]*, *Jin Jiang*[1], *Richard P. Steeds*[2,3] *and William E. Moody*[2,3]

[1] Heart and Lung Centre, New Cross Hospital, Wolverhampton, United Kingdom, [2] Institute of Cardiovascular Sciences, University of Birmingham, Birmingham, United Kingdom, [3] Department of Cardiology, Queen Elizabeth Hospital Birmingham, University of Birmingham NHS Foundation Trust, Birmingham, United Kingdom

Coronavirus disease 2019 (COVID-19) was initially regarded as a disease of the lungs, which manifests as an acute respiratory illness and pneumonia, although more recently cardiac complications have been well-characterised. Serological cardiac biomarkers have been used to define acute myocardial injury, with significant elevation of high-sensitivity cardiac troponin (hs-cTn) associated with poor prognosis. Accordingly, 20–25% patients with acute myocardial injury (as defined by an elevated hs-cTn greater than the 99th percentile) have clinical signs of heart failure and increased mortality. An important outstanding clinical question is how best to determine the extent and nature of cardiac involvement in COVID-19. Non-invasive cardiac imaging has a well-established role in assessing cardiac structure and function in a wide range of cardiac diseases. It offers the potential to differentiate between direct and indirect COVID-19 effects upon the heart, providing incremental diagnostic and prognostic utility beyond the information yielded by elevated cardiac biomarkers in isolation. This review will focus on the non-invasive imaging assessment of cardiac involvement in COVID-19.

Keywords: COVID-19, echocardiography, cardiac MRI, cardiac CT, prognosis

OPEN ACCESS

Edited by:
Mingxing Xie,
Huazhong University of Science and Technology, China

Reviewed by:
Karin Wildi,
Critical Care Research Group (CCRG), Australia
Guo-wei Tu,
Fudan University, China

***Correspondence:**
Sandeep S. Hothi
s.hothi@nhs.net

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *03 February 2021*
Accepted: *29 March 2021*
Published: *21 May 2021*

Citation:
Hothi SS, Jiang J, Steeds RP and Moody WE (2021) Utility of Non-invasive Cardiac Imaging Assessment in Coronavirus Disease 2019. Front. Cardiovasc. Med. 8:663864. doi: 10.3389/fcvm.2021.663864

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by the novel RNA beta coronavirus, severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) (1). Initially, lung disease that manifests as an acute pneumonia was recognised as the dominant feature of this pandemic-causing disease; but in some cases, potentially due to cytokine storm (2), there is progression to acute respiratory distress syndrome (ARDS), multi-organ failure and death (3–5). Cardiac sequelae have, however, also been widely reported in association with multi-systemic involvement, which can include gastrointestinal, hepatic and nervous systems (6). Elevation in high-sensitivity cardiac troponin (hs-cTn) greater than the 99th percentile, whether troponin I (7) or T (8), defines myocardial injury and has been associated with poor prognosis: up to a third of patients presenting to hospital demonstrate elevated hs-cTn (7), which confers an increased risk of mortality and incident heart failure (8, 9). In a meta-analysis of 44 studies including 14,866 patients hospitalised with COVID-19, acute cardiac injury was present in 15% of patients (10), while a US study from New York reported 36% of hospitalised patients had acute cardiac injury, with even small elevations of hs-cTn associated with an increased risk of death (11).

An increase in hs-cTn may result from one or more of a wide range of aetiologies (12). An elegant pathophysiological scheme for COVID-19-related cardiac injury has only recently been put forward (13) and outlines numerous co-existing factors: indirect myocardial injury via a cytokine storm; organ failure due to systemic inflammatory response syndrome (SIRS); oxygen supply and demand mismatch due to acute respiratory failure; cardiotoxicity from treatments; coronary thrombosis due to plaque rupture caused by shear stress; arrhythmia; and embolic complications due to SIRS. Post-mortem studies have demonstrated microthrombi within the pulmonary vasculature (14). In addition, direct myocardial injury may be caused by inflammation following direct viral entry via ACE-2 receptor binding and cellular entry. Finally, it is likely that the balance of effects described in the above paradigm may result in differing degrees and patterns of cardiac involvement, with respect to the extent of ventricular dysfunction, left vs. right heart involvement, and ischaemic vs. non-ischaemic patterns of myocardial injury.

A key question still debated in clinical practise is how best to define the extent and nature of cardiac involvement in COVID-19. Non-invasive cardiac imaging has a well-established role in assessing cardiac structure and function in a wide range of cardiac diseases. It also offers the potential to elucidate COVID-19 effects upon the heart, beyond information yielded by elevated biomarkers *per se*, which may result from indirect as well as direct myocardial injury.

In this review, our aim is to summarise the available studies of non-invasive cardiac imaging assessment among patients with COVID-19. We acknowledge that this is a relatively new disease and that our understanding will continue to evolve, but a timely appraisal of the latest literature is important to help inform current clinical and research strategies.

CARDIAC INVOLVEMENT IN CORONAVIRUS DISEASE 2019

The cardiac abnormalities reported to date among patients with COVID-19 are wide ranging and include the following: acute coronary syndromes (15), Takotsubo cardiomyopathy (16, 17), myocarditis (18), right heart dysfunction/acute cor pulmonale (19–22), left ventricular (LV) dysfunction (23), pericardial effusion (24), and arrhythmias (25). For all of these sequelae, the first-line non-invasive cardiac imaging modality of choice remains to be echocardiography.

Abbreviations: AT, acceleration time; BAME, Black, Asian and minority ethnic; CTA, computed tomography angiography; CMR, cardiac magnetic resonance; CRP, C-reactive protein; COVID-19, coronavirus disease 2019; D-dimer, fibrin degradation products; E, early transmitral peak Doppler velocity; e′, early tissue Doppler peak velocity; ECV, extracellular volume; hs-cTn, high-sensitivity cardiac troponin; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; LA, left atrium; LAA, left atrial appendage; LV, left ventricle; LVEF, left ventricular ejection fraction; LGE, late gadolinium enhancement; LVGLS, left ventricular global longitudinal strain; LVSD, left ventricular systolic dysfunction; RV, right ventricle; RVEF, right ventricular ejection fraction; RVEDA, right ventricular end-diastolic area; RVESA, right ventricular end-systolic area; RVEDV, right ventricular end-diastolic volume; RVESV, right ventricular end-systolic volume; RVFWLS, right ventricular free-wall longitudinal strain; RVGLS, right ventricular global longitudinal strain; S′, peak systolic tissue Doppler velocity; STE, speckle tracking echocardiography; PASP, pulmonary artery systolic pressure; PADP, pulmonary artery diastolic pressure; RVSD, right ventricular systolic dysfunction; T2 STIR, short tau inversion recovery; TAPSE, tricuspid annular plane peak systolic excursion; TTE, transthoracic echocardiography; TOE, transoesophageal echocardiography; TAVI, transcutaneous aortic valve intervention; TMVR, transcutaneous mitral valve intervention; TR Vmax, tricuspid regurgitant peak velocity.

ECHOCARDIOGRAPHY

Transthoracic Echocardiography

Transthoracic echocardiography (TTE) is the most widely available form of cardiac imaging for the assessment of cardiac structure and function in a range of clinical settings, indications and pathologies (26). It can be performed with high-end, high-specification machines, with portable laptop-type systems or with handheld devices (27). It is relatively quick, although study durations depend on the extent of data collected and can be performed on a stable or critically unwell patient, without any known side effects (thermal heating is a theoretical concern not encountered in normal clinical practise). Accordingly, this lends itself to performance in the outpatient echo laboratory, or by the inpatient bedside (**Table 1**). High-quality TTE does, however, require highly trained staff, whichever modality of echo imaging, analysis or system is used. Different specifications of systems determine the types of acquisition and analyses that are possible. For instance, Doppler imaging, 3-D and speckle tracking deformation imaging are not available on all devices, especially smaller, handheld devices. Bedside TTE requires the close proximity of sonographer and patient, which increases the potential for coronavirus transmission from patient to staff or vice versa, whether via surface contact or droplet spread. Appropriate protection to mitigate this risk is strongly advised, as has been recommended by both the American and British Societies of Echocardiography (28, 29). Guidance has been issued, which focuses on balancing the risk of infection vs. clinical demand. Considerations include the need for experienced practitioners, appropriate personal protective equipment (PPE), appropriate case selection (i.e., performance in patients where knowledge is most likely to be of clinical utility) and abbreviating the study appropriately to reduce exposure duration while still answering the clinical question.

Point-of-Care Echocardiography

Focused echocardiography protocols use variously shortened imaging protocols, such as point-of-care ultrasound (POCUS), level 1 echo or focused cardiac ultrasound (FOCUS), performed at the bedside using normal or handheld echo devices. These can be used in a range of settings with the advantage of being portable and quick, particularly shortening sonographer–patient contact time (27). This qualitative approach has been recommended in COVID-19 patients with guidance from the American Society of Echocardiography and widely proposed for echo assessment in the COVID-19 patient and pandemic (28, 30, 31). Reports have described such utility in COVID-19 to assess LV and right ventricular (RV) size and systolic function, interventricular septal flattening, signs of pulmonary embolism and pulmonary

TABLE 1 | Transthoracic echocardiography and cardiovascular magnetic resonance imaging—relative merits and limitations.

	Echocardiography		Cardiovascular magnetic resonance	
	Advantages	**Limitations**	**Advantages**	**Limitations**
Portability	Highly portable			Not portable—fixed systems
Ionisation	Non-ionising		Non-ionising	
Image quality	Highly variable—from excellent to poor; dependent upon sonographer skills, intrinsic patient echo window factors and patient cooperation		More consistently excellent image quality	Image quality degraded by arrhythmia, poor breath-holding and motion
Speed of scanning	Rapid, tailored approach			Longer protocols relative to echo
Myocardial characterisation	Strain assessment allows good contractile function assessment		Range of tissue characterisation parameters that yield data regarding oedema, inflammation, extracellular volume and scarring (fibrosis/infarct)	Quantitative myocardial strain analysis not yet in clinical practise
Volumetric assessment	Variable depending on image quality for left ventricle	Limited for right ventricle	Excellent left and right ventricular volumetric assessment	
Diastolic left ventricular assessment	Superior by echo			Not yet validated for clinical CMR use
Valve assessment	Superior characterisation of blood flow velocity and gradients		Superior assessment of valvular regurgitation volumes	
Pulmonary pressure assessments	Quantitative approaches to pulmonary pressure estimates (PASP and PADP) in addition to visual assessment of septal motion and pulmonary artery diameter	Requires measurable TR jet	Qualitative assessment of septal motion and pulmonary artery calibre only	No quantitative measures
Temporal resolution	Superior temporal resolution			Inferior temporal resolution
Staff factors		Highly trained sonographers required		Highly trained radiographers required
Availability	Widely available			Availability limited to fixed locations in certain hospitals/medical facilities
Patient factors	Claustrophobia is not a concern			Unattractive to claustrophobic patients
	Can scan patients with orthopnoea			Patient must be able to lie flat for ≥40 min
	Patient can be scanned in echo lab or a portable machine taken to the bedside			Difficult logistics transporting critically unwell patients to the scanner
	Generally scanned in a semi-recumbent position; can also obtain at least some data if lying flat			Patients must be able to comfortably hold their breath while lying flat
	Kidney function not an issue with echo with or without echo contrast agents			Caution in patients with poor renal function if using gadolinium-based contrast, although lesser concerns with modern agents
Magnetic materials	No concern			Patients or equipment with ferromagnetic materials cannot enter the scanner room
Cost	Relatively cheap equipment			Much more expensive than echo systems
Infection control considerations		Close proximity of sonographer and patient	Distance between patient and radiographer	

CMR, cardiac magnetic resonance; PASP, pulmonary artery systolic pressure; PADP, Pulmonary artery diastolic pressure; TR, tricuspid regurgitant.

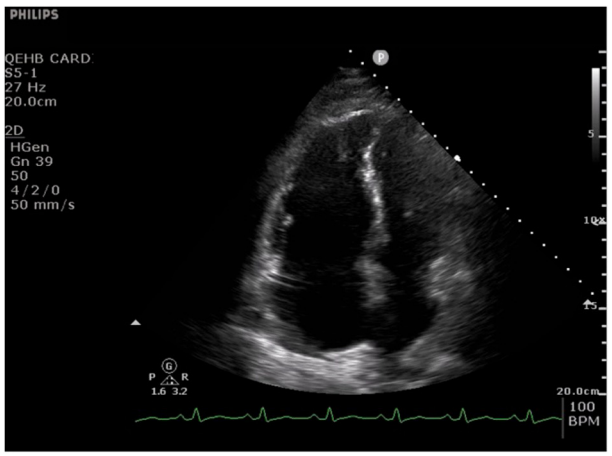


FIGURE 1 | Apical four chamber echocardiographic still image from a cine typical of many patients with acute severe COVID-19 pneumonia. The cine (**Video 1**) shows a dilated right ventricle and severe impairment of radial right ventricle systolic function with relative preservation of long axis function. The left ventricle is small and hyperdynamic. There is also paradoxical septal wall motion and a thin rim of pericardial effusion adjacent to the right atrium.

hypertension, inferior vena cava (IVC) calibre and inspiratory collapse, pericardial effusion, monitoring for changes in cardiac function, as well as guiding ventricular intravascular volume assessment, and aid triage decisions for intensive care (32). The impact on patient outcomes, infection transmission rates and diagnostic yield compared with those of complete echo studies remain unknown. The pandemic has resulted in system pressure with clinical resource constraints of echo provision during the COVID-19 pandemic. This has been cited as an additional reason for focused echo, potentially by personnel not usually performing echocardiography, to meet clinical demands for acute echo. Further assessment is needed of the potential positive or negative implications of using focused methodology, handheld technology and its application by practitioners with limited echo training.

Transoesophageal echocardiography (TOE) permits superior imaging quality of certain cardiac structures due to position of the ultrasound probe within the oesophagus and stomach. In a patient without a cuffed endotracheal tube, however, oesophageal intubation, and extubation can lead to coughing and aerosol generation. This makes for a high-risk study because of the potential for airborne and saliva-borne transmission of coronavirus from the patient to the operator and supporting staff. Full PPE with a face visor, eye protection, gown, gloves, head cover and FFP3/N-95 type mask is essential, although local policies differ in their advice especially with regard to mask type. TOE studies should, therefore, only be performed if the information is critical and cannot be obtained by another method (28, 29). In an already tracheal-intubated patient, the same precautions should be taken, although the additional infection risk is speculated to be reduced by the closed respiratory circuit.

ECHOCARDIOGRAPHIC FINDINGS IN CORONAVIRUS DISEASE 2019

Right Ventricular Size and Systolic Function

The RV has received great attention in COVID-19 patients primarily from clinical analogies drawn from patients suffering ARDS. Right ventricular dilatation and systolic dysfunction are highly prevalent and have been identified in COVID-19 patients in several cohort studies and one multicentre study (please see **Figure 1**, **Video 1**, and **Table 2**).

One of the original echocardiographic studies performed in Israel included 100 consecutive patients hospitalised with mild-to-severe COVID-19 (33). TTE was performed within 24 h of admission, and notably, the most common echocardiographic abnormality involved the RV, with dilatation with or without systolic dysfunction in 39% when measured by fractional area change (FAC) and S′. In contrast, LV systolic dysfunction (LVSD) was observed in only 10%, of whom two patients (2%) already had known ischaemic heart disease.

These findings were in keeping with our UK single-centre, retrospective observational cohort study of 74 critically unwell adults hospitalised with COVID-19 (41). In a sick cohort in whom the majority needed mechanical ventilation and over half vasopressor support, the primary abnormalities were dilatation of the RV in nearly half (41%) and RV systolic dysfunction (RVSD) in nearly a third (27%) (19). These changes correlated with elevated D-dimer and C-reactive protein (CRP). RVSD was predominantly related to reduced radial function, reflected by abnormal FAC, in the face of relatively well-preserved longitudinal function as measured by tricuspid annular plane peak systolic excursion (TAPSE). Of those with RVSD, 20% were diagnosed with pulmonary embolism, but the true figure could be higher because not all patients underwent CT pulmonary angiography (CTPA). Although a large proportion had mechanical ventilation and/or vasopressors, the study showed no associated between either of these with the right heart abnormalities. In addition to the small sample size, many COVID-19 PCR-positive patients admitted to the study centre did not undergo echocardiography, in line with the clinical guidelines in place at the time, thus limiting the study to the critically unwell. In a subsequent multicentre, retrospective study of 164 patients hospitalised for COVID-19, we demonstrated a similarly high prevalence of RV dilatation and RV systolic dysfunction (20). Reduction in RVSD was more frequent when measured by RV FAC than by TAPSE, although reduced TAPSE was significantly associated with increased mortality. LV abnormalities were uncommon, with LV ejection fraction (LVEF) being normal or supranormal in 83%, and the LV dilated in only 1%. The study group comprised 36% patients from Black, Asian and minority ethnic (BAME) groups, but no significant difference in echo findings or mortality was seen between white and BAME patients; this might reflect the size of the study group, and a larger investigation exploring for the presence or absence of ethnic effects upon cardiac sequelae of COVID-19 is merited.

TABLE 2 | Contrasting left and right ventricular findings in COVID-19 vs. controls or defined subgroup comparison.

Study	Study design	Size (N)	RV systolic function							LV systolic function			LV diastolic function
			RV size	RVEF	TAPSE	FAC	AT	S′	Tei index	Longitudinal strain	LV size	LVEF	Longitudinal strain	
Mahmoud-Elsayed et al. (19)	Single centre, retrospective	74	↑			↓					↔	Mainly ↔ or ↑		
Moody et al. (20)	Multicentre, retrospective	164	↑		↓	↓					↔	Mainly ↔ or ↑		
Szekely et al. (33)	Single centre, prospective	100	↑		↔	↓	↓	↓	↔			Mainly ↔, ↓ in 10%		↑
Rothschild et al. (21)	Consecutive cohort	100	↔		↓	↔			↓	↓	↔	Mainly ↔, ↓ in 11%	↓	↔
Argulian et al. (34)	Single centre, retrospective	105	↑								↔	↔		
Barman et al. (24)	Single centre, retrospective	90	↑		↔	↑						↑	↑	
Zeng et al. (23)	Single centre, retrospective	57									↔	↓		↔
Vasudev et al. (16)	Single centre, retrospective	45	↓	↓								↓		
Kim et al. (35)	Multicentre, retrospective	510	↑		↓			↓			↔	↓ in patients with RV remodelling		
Baycan et al. (13)	Single centre, prospective	100	↑		↔	↔		↔		↓	↔	↔	↓	↔
Li et al. (36)	Single centre, observational	120	↑		↓	↓		↔			↓	↔	↔	
Schott et al. (37)	Single centre, retrospective	66	↑									Mainly ↔, ↓ in 3%		
Churchill et al. (38)	Single centre, retrospective	125	ns	ns	ns	ns	ns	ns	ns	ns		Variable: ↑ or ↔; ↓ in 26%		
Brito et al. 2020(39)	Single centre, cross-sectional observational	54	↓	↓		↓		↔		↔	↔	↔	↔	
Pagnesi et al. (40)	Single centre, cross-sectional	200			↓			↓						

ns, not stated; COVID-19, coronavirus disease 2019; RV, right ventricle; LV, left ventricle; RVEF, right ventricular ejection fraction; TAPSE, tricuspid annular plane peak systolic excursion; FAC, fractional area change; AT, acceleration time.

The high prevalence of RV abnormalities was mirrored in a multicentre study [largely intensive care unit (ICU) based] involving eight hospitals in Michigan, USA; in 66 out of 1,780 hospitalised patients undergoing TTE, 71% had a dilated RV (37). While 70% of patients had increased LV wall thickness, reduced LVEF was uncommon (3%). In this study, RV dilatation was defined by the ratio of RV:LV basal diameter, rather than the absolute basal RV size and unlike most other studies to date, RV systolic function was assessed visually rather than quantitatively, which are important limitations.

A further study using focused echocardiographic protocols in a single-centre, retrospective study in New York, USA, assessed RV and LV size and systolic function in hospitalised patients (34). The group comprised 105 consecutive patients of whom 30% were intubated and mechanically ventilated. The RV was dilated in 31% of patients and was the only independent predictor of mortality on a multivariate analysis. Abnormality of RV systolic function was far more common in those with RV dilatation. LV size and LVEF did not differ between patients with or without RV dilatation. In 10 patients with RV dilatation, CTPA was performed and identified pulmonary embolism in half of this small subset. RV dilation might relate to numerous concomitant factors including hypoxic pulmonary vasoconstriction related to ARDS, ventilator parameters or direct myocardial injury.

In contrast to the above two studies that assessed RV and LV systolic functions using standard parameters, a Chinese group used 2-D speckle tracking to examine changes in RV longitudinal strain (RVLS) in 120 consecutive patients admitted with COVID-19. Patients with known cardiomyopathy, previous myocardial infarction, or poor image quality were excluded (36). In this study, non-survivors had greater RV dilatation and elevated pulmonary artery systolic pressure (PASP). Furthermore, RVLS

was a strong predictor of mortality and superior to RV FAC or TAPSE, with an optimum cut-off value of RVLS for detection of increased mortality of 23%, with 94% sensitivity and 65% specificity. While RVLS was predictive of mortality independent of LVEF, LV systolic function was not assessed by LV strain analysis. The authors speculate that RVLS may identify RVSD earlier than conventional markers of RV systolic function such as FAC and S′, due to it incorporating the entire RV free wall rather than only the basal free wall and tracking motion through the cardiac cycle. Furthermore, unlike TAPSE and FAC, RVLS measurement is not limited by a dependence on the angle of insonation or plane. Limitations of the study include image quality requisites for speckle tracking echocardiography (STE), which are highlighted by the exclusion of 24 patients from the original cohort of 150 due to insufficient image quality, the single-centre nature of the study and sample size.

In a small study of 54 college student athletes comprising a spectrum from asymptomatic to mild-to-moderate COVID-19 symptoms when tested for SARS-CoV-2 by TTE, a median of 27 days after the positive test revealed no change in LVEF, mass or LV volumes. RV systolic function as measured using FAC was reduced among symptomatic athletes and asymptomatic athletes compared with COVID-19-negative athletic controls (FAC, 23.2% and 26.4% vs. 43.0%) but not according to RV free-wall longitudinal strain (RVFWLS) (−26.8% and −28.0% vs. −26.9%) or RV S′ (14.0 and 13.9 vs. 14.0 cm/s) (data in parentheses are mean values for symptomatic athletes, asymptomatic COVID-19-positive athletes and COVID-19-negative athletic controls) (39).

Biventricular Involvement

Initial case reports demonstrated significant LV systolic dysfunction in patients with COVID-19 (42, 43). In contrast, and as noted above, in the larger studies that followed, LV abnormalities were infrequently observed with abnormalities primarily confined to the RV (19, 20, 33, 34). Indeed, LVEF was often hyperdynamic (19). Biventricular abnormalities have, however, been documented in other case reports (44) and studies (8). Indeed, a study of 45 patients from New Jersey, USA, reported a greater incidence of LVEF (31% of patients) than RVSD (11%) (16) in hospitalised patients with COVID-19 pneumonia. Among 125 predominantly critically ill COVID-19 patients, one unit has reported that LVEF was normal or hyperdynamic in the majority of patients but impaired in 26% (38); RV findings were not reported. Another study compared small numbers of ICU with non-ICU patients and discovered greater RV dilatation and RVSD in the ICU patients, when measured by TAPSE, S′ or FAC, making it difficult to identify a single best parameter for RV function (23). Alongside this, there was a high incidence of LV wall thickening and reduced LVEF in the ICU cohort. Among COVID-19 patients with elevated hs-cTnI, a study from Turkey reported greater rates of biventricular dilatation and biventricular systolic dysfunction [measured as LVEF and RV ejection fraction (RVEF)] in the severe vs. non-severe groups (24). The definitions of severity of COVID-19 across studies can differ, but for this report, severe COVID-19 was defined as a respiratory rate ≥30 breaths/min; oxygen saturation ≤93% at rest; partial pressure of arterial oxygen: fractional concentration of inspired oxygen ≤300 mmHg; critical complication (septic shock, multiple organ dysfunction/failure requiring ICU admission); or any type of respiratory failure that required mechanical ventilation.

While the study by Li et al. investigated RVLS strain (RVFWLS), it did not assess RV global longitudinal strain (RVGLS) or LV strain (36). A single-centre study subsequently analysed both parameters by 2-D STE in 100 consecutive hospitalised, COVID-19 patients comprising mild-to-severe disease (21). Strain analysis showed reduced LV global longitudinal strain (LVGLS) and RVFWLS in 42% and 38%, respectively, while LVEF was reduced in a smaller proportion (11%) of patients. Both strain indices were prognostic, with LVGLS predicting mortality and RVFWLS predicting the combination of intubation or death.

A role for STE-derived longitudinal strain was also investigated to seek subclinical ventricular dysfunction in COVID-19 in patients with preserved LVEF and preserved RVEF (13). LVGLS and RVLS and conventional 2-D echo were measured in 100 hospitalised COVID-19 patients with LVEF ≥50% over a consecutive 2-week period from a centre in Turkey. Patients were divided into severe and non-severe COVID-19 groups and compared with a control group free of COVID-19. Severe COVID-19 was defined as per the definition above. RV size was the greatest in the severe COVID-19 group vs. the other two groups. The study was inherently limited by its small sample and single-centre nature and an absence of premorbid echo data. Both LVGLS and RVLS were reduced in the severe group compared with the non-severe and control groups, and both independently associated with in-hospital mortality by a multivariate analysis. In fact, both LVGLS and RVGLS were significantly different between the three groups, being the greatest in controls and the lowest in the severe group [LVGLS: -14.5 ± 1.8 vs. -16.7 ± 1.3 vs. -19.4 ± 1.6, respectively ($p < 0.001$); RVLS: -17.2 ± 2.3 vs. -20.5 ± 3.2 vs. -27.3 ± 3.1, respectively ($p < 0.001$)]. Although there was no difference in LVEF between groups, this is unsurprising given that the inclusion criteria required a normal LVEF.

In the largest detailed TTE study to date, Kim and colleagues focused on RV abnormalities among 510 patients admitted to three hospitals in New York, USA (35). RV size was measured by 2-D echo (using a cut-off of >4.1 cm for the definition of RV dilation), while systolic function was measured by TAPSE or S′ (both needed to be abnormal to diagnose RVSD). RV dilation was present in 35% of patients and RV dysfunction in 15% of patients. In patients with RV dilation and preserved systolic function, the basal diameter was 4.8 ± 0.5 cm with RV S′ 12.3 ± 4.6 cm/s and TAPSE 1.8 ± 0.6 cm. In patients with RV systolic dysfunction (S′ 8.4 ± 1.3 cm/s, TAPSE 1.3 ± 0.2 cm), RV size was 4.3 ± 1.0 cm. The authors demonstrated a robust association between RV adverse remodelling (defined as RV dysfunction and/or dilatation) and early mortality. Moreover, the presence of adverse RV remodelling provided incremental prognostic utility over and above biomarker and standard clinical markers. Interestingly, both markers of RV remodelling were

associated with LVSD measured by reduced LVEF, although LVEF did not correlate with mortality.

An international registry led by the European Society of Cardiology assessed qualitative but not quantitative echo findings in confirmed or suspected COVID-19 patients in 1,216 patients from 69 countries. They found abnormalities of LV or RV dysfunction in 39 and 33% of patients, respectively. Abnormalities variably included ventricular chamber dilatation, systolic dysfunction and features of pulmonary hypertension. Echocardiography was followed by a change in management in one third of cases (45). Within this subgroup, changes in disease-specific therapy were made in 42% including altering treatment for heart failure, acute coronary syndrome, tamponade, pulmonary embolism or endocarditis; TTE was less frequently used to titrate haemodynamic support (13%) and determine changes in the level of patient care (8%). Limitations of this study included its dependence on voluntary data submission, a lack of detailed echocardiographic quantitative data and incomplete data on changes in clinical management following echocardiography in 151 patients.

Contrasting Effects of Coronavirus Disease 2019 Upon Different Parameters of Right Ventricular Systolic Function

As evident from the studies detailed in this review, a range of methods of quantification of RV systolic function have been used across different studies (**Table 2**). As not all studies have measured the same parameters, it is difficult to compare their relative utility. RV FAC is often reduced in COVID-19 patients (20, 33), and this correlates with the degree of diminished RVLS (36). In contrast, while RV S′ was reduced in one study (33), this parameter did not correlate with the degree of RV dysfunction as quantified by RVLS (36). This difference might reflect the fact that RV S′ measures basal segment longitudinal function rather than the entire RV free wall or, indeed, RV global longitudinal function. The effects of COVID-19 on longitudinal RV function as measured by TAPSE appear variable, having been reported as unchanged in some studies (24, 33) but reduced in others (20, 36). Longitudinal function as measured by more sensitive measures such as RVLS (variably characterised as either RVFWLS or RVGLS) tends to be reduced in COVID-19 (21, 36). A descriptive study demonstrated a graded reduction in RVLS according to the severity of COVID-19 pneumonia compared with controls (13). Although limited by the absence of ECG gating, pulmonary AT may be shortened reflecting increased pulmonary pressures, although the myocardial performance index (Tei index) is not always affected (33).

Contrasting Right vs. Left Ventricular Findings in Coronavirus Disease 2019

While the effect of COVID upon RV size and systolic function is generally the most common abnormality among the studies to date, the difference in LV findings is striking. There are several potential explanations. Firstly, the sample sizes are relatively small in all studies to date, such that the different outcomes may reflect incomplete representations of the more widespread effects of COVID-19 upon LV function, appearing more clearly in some studies and then mildly or almost not at all in others. Secondly, the study populations differ with respect to geography, co-morbidities and disease severities. Thirdly, the method of assessment of LV function differs significantly, ranging from visual LVEF assessment to quantitative biplane LVEF and, in some cases, more advanced analysis by STE to determine LVGLS. Fourthly, the definition of ventricular dysfunction, whether left or right, varies. In some studies, a reduced LVEF is considered dysfunction, whether visually or quantitatively determined. In others, LVEF may be normal, but LV function is considered abnormal if longitudinal strain is abnormal. Acknowledging differences in terminology and definitions of abnormality is key when interpreting these studies.

The importance of the LVGLS findings in two of the studies assessing longitudinal strain (13, 21) suggest that subclinical LVSD, not sufficient to detect by 2-D echo alone, may exist in COVID-19, can be detected by STE and is associated with elevated mortality. Indeed, both LVGLS and RVLS were predictors of mortality, as were hs-cTnI, D-dimer, and SaO_2 (13). LVGLS and RVLS measure long axis fibre function; and because the responsible fibres run in the subendocardium and are susceptible to early injury and fibrosis, long axis function may decline before LVEF falls. Longitudinal strain could, therefore, offer more sensitive, early prognostic utility in COVID-19 as it has done in other disease cohorts. In addition, LVGLS and RVLS measured by STE as opposed to tissue Doppler imaging (TDI) is superior by being angle-independent and having greater reproducibility (46). The analyses can be performed away from the bedside and therefore do not prolong scanning duration, although the requirement for good images might (47). It would be interesting to know whether RV and LV longitudinal functions are normal or abnormal in those studies in which EF was normal but strain was not measured.

In any measurement of LV systolic function, the presence or absence of inotropic drugs and loading conditions should be noted. Adjusting for this is challenging and makes it difficult to compare patients within and between studies.

Finally, different outcomes may be reported due to the use of different thresholds for the definition of abnormal. For instance, in most studies, reduced FAC and TAPSE are defined as <0.35 and <17 mm, respectively. However, in the study from Wuhan, thresholds different from those adopted in consensus guidelines were used (36). Furthermore, datasets used to define normality themselves have intrinsic limitations when applied to populations with different characteristics. Thus, the NORRE dataset used by the latest British Society of Echocardiography normal values was derived from Caucasian Europeans, and furthermore, RV dimensions from these data differ to those of the joint American Society and European Society of Cardiology consensus guidelines (48).

Pulmonary Artery Systolic Pressure

Many studies have demonstrated abnormalities of echo-derived estimates of PASP. A single-centre study of 200 non-ICU

patients showed that PASP was higher in more severe COVID-19 pneumonia and that it correlated with mortality, in contrast to RVSD (reduced TAPSE or S′) (40). Other studies also identified PASP and its importance in COVID-19 patients. It was shown to be higher among COVID-19 patients who subsequently died than in survivors (36), in those with greater impairment of RVLS and in those with greater severity of COVID-19 disease (13), in severely ill COVID-19 patients with normal biventricular ejection fractions (13) and in those with ARDS (22) and occurs with either RVSD or RV dilatation (35). The aetiology of the rise in pulmonary pressure, for instance, a direct consequence of COVID-19 pneumonia, or left or right heart dysfunction, remains unknown.

Inferior Vena Cava Diameter and Inspiratory Collapse

Assessment for dilatation and loss of inspiratory collapse can help identify patients with a higher likelihood of elevated right atrial pressure. However, in mechanically ventilated patients, this correlation is unreliable (49). Nevertheless, trends in IVC size and degree of distensibility could potentially be of utility. The IVC diameter was increased in the severely ill COVID-19 patients in one study (24). The implications for such measurements in COVID-19 require further research.

Left Ventricular Diastolic Function

There was an absence of diastolic functional differences in COVID-19 patients with preserved biventricular ejection fractions, in whom measures of LV diastolic function, LVEF, LV size, LV mass and left atrial size were similar across groups in a study of patients with COVID-19 and normal LVEF (13). Similarly, no change in LV diastolic parameters was observed between patients with varying degrees of RVLS impairment nor between survivors and non-survivors (36). While LV filling pressure and left atrial volume might be greater in COVID-19 patients vs. controls in a study from Israel, the majority (80%) did not meet criteria for significant diastolic dysfunction ($E/E' \geq 14$) (33). Average E/e′ was 10.5 ± 0.8, 10.6 ± 0.4 and 9.0 ± 0.4, across the three clinical grades of presentation with no significant difference between clinical groups. Yet in the same study, a sub-study of patients with hs-cTnI elevated above the 99th percentile (above 28 ng/L) had increased E/e′, suggesting higher LV filling pressures and impaired diastolic function [average E/E' 11.3 ± 6 vs. 9.8 ± 6; $p = 0.003$, hs-cTnI $>$ 28 ng/L ($n = 20$), vs. hs-cTnI $<$ 28 ng/L ($n = 80$)]. No difference in LV diastolic function was detected among ICU compared with non-ICU patients (23) similar to a study by Rothschild et al., which showed no significant difference between COVID-19 hospitalised patients and controls (21).

Diastolic dysfunction is often identifiable by non-invasive imaging earlier than systolic dysfunction across a range of cardiac pathologies, and its assessment is an integral part of a complete echo study (48, 50). The absence of significant changes in diastolic dysfunction in COVID-19 echo studies reported to date should be interpreted with caution and will require assessment in larger and more detailed studies because of several limitations. These include, firstly, incomplete measurement of required diastolic functional parameters, which should include spectral Doppler-based transmitral E and A velocities, E wave deceleration time and mitral annular tissue Doppler e′ velocities, derived E/e′ ratio and pulmonary venous systolic and diastolic flow rates. Secondly, patients sick with COVID-19 are often tachycardic, making measurement of some of these parameters impossible due to E and A wave fusion. Finally, in the presence of tachycardia, all time intervals need adjustment for heart rate (51).

Pericardial Effusion

Pericardial effusions have been identified, particularly so in severely unwell patients [(23, 24) Zeng et al.; Barman], although these are not common (20).

Echocardiographic Changes During Coronavirus Disease 2019 Illness vs. Premorbid Status

There are little data comparing echo findings before and after COVID-19. The multicentre study from New York included a subset of patients (35). Out of 73 patients with pre-existing TTEs, RV dilatation was more common following COVID-19 than before, and there was a trend toward greater RVSD.

Prognostic Value of Echocardiographic Indices

Among the echocardiographic indices so far investigated, a prognostic role for several has been identified (**Table 3**). As the studies are relatively small, verification in large studies and other populations will be needed.

Nevertheless, the data so far demonstrate prognostic roles for RV dilatation in some studies (33–36) but not all (20). RV systolic function has been measured in various ways with prognostic value in many studies. Thus, receiver operating curve analyses show prognostic value of systolic function in decreasing order when assessed by RVLS, FAC, or TAPSE (36). RV assessment is also prognostic when measured by the Tei index (33). Strain analysis of RVLS (13) and LVGLS (13, 21) has been shown to be prognostic as has RV strain quantified as RVFWLS (21). Pulmonary hypertension, estimated by TR Vmax, has also been shown to have a potential prognostic role (36, 40). Low LVEF and elevated LV E/e′ are associated with increased mortality (33).

Biomarkers and Their Relationship With Echo Findings

Elevated troponin has been associated with RV size (24), RVSD measured by FAC (24) and RVSD measured by FAC, S′, PA AT and TAPSE (20, 33, 35). Hs-cTn has also been associated with LVSD in some studies when measured by LVGLS (13) or by LVEF (24), and also with increased E/E′, suggesting increased left heart filling pressures (33). Elevations in D-dimer have been associated with RV size (24, 35) and RVSD according to reduced TAPSE (20), and also correlated with LVSD measured by LVGLS (13) or

TABLE 3 | Prognostic echo findings in COVID-19—parameters associated with increased mortality.

Study	RV systolic function								PHTN	LV systolic function		LV diastolic function
	RV size	RVEF	TAPSE	FAC	S′	AT	Tei index	Longitudinal strain		LV size	LVEF	Longitudinal strain	
Mahmoud-Elsayed et al. (19)													
Moody et al. (20)	−		+	+									
Szekely et al. (33)	+						+				+		+
Rothschild et al. (21)								+				+	
Argulian et al. (34)	+												
Kim et al. (35)	+		+		+								
Baycan et al. (13)								+				+	
Li et al. (36)			+	+				+	+				
Pagnesi et al. (40)									+				

COVID-19, coronavirus disease 2019; RV, right ventricle; PHTN, pulmonary hypertension; LV, left ventricle; RVEF, right ventricular ejection fraction; TAPSE, tricuspid annular plane peak systolic excursion; FAC, fractional area change; AT, acceleration time.

by LVEF (24). While ferritin has been correlated with RVSD, this likely relates to its role as an acute phase reactant rather than as a reflection of iron stores (35). Elevated D-dimer, troponin, CRP, and troponin-I have all been associated with reduced RV AT (33). With the exception of E/E′, correlations with diastolic indices have not been reported, although diastolic characterisation in COVID-19 remains limited to date.

Serial Echocardiographic Changes

Appreciation of longitudinal cardiac changes during the acute phase of COVID-19, and in the medium and longer terms after the acute illness, is limited.

A subset analysis performed in 20 hospitalised patients who suffered clinical deterioration following their first echo study yields some potential insight. In these patients, the most common finding was a deterioration of RV parameters, including shortened acceleration time (AT) and increased RV end-diastolic area (RVEDA); however, there was no significant deterioration in LVEF or LV E/e′, except in an even smaller subset of five patients who showed reduction in LVEF alongside elevated troponin and a reduction in AT (33). The authors speculated that deterioration in these patients reflects increased pulmonary vascular resistance and thus increased RV afterload in a form of acute cor pulmonale and suggested research into echo-guided anticoagulation strategies guided by estimates of pulmonary pressure (33). While this mechanistic explanation is physiologically sound, it is not known what changes would have been identified in the patients who did not clinically deteriorate and therefore did not have a second echo study. The findings are also limited by the small sample size, and verification in a large group would be informative.

In the study by Mahmoud-El-Sayed et al., a subset of patients had follow-up TTE but limited to 31% of the original 74 patients, and at the median interval of 8 days, no significant changes in LV or RV size or systolic function were evident (19). On the other hand, in hospitalised COVID-19 patients with elevated troponin, LV dysfunction improved in nine out of 11 patients who underwent repeat echo assessment at a median of 14 days, although 22 patients with LVSD were not restudied (38).

In a multicentre, prospective, observational study of 79 adults hospitalised with COVID-19 pneumonia, echo was performed during admission and at 3 months follow-up (52). At baseline, 41% had a normal echo. Of those with abnormal findings, most had RV remodelling (41%) rather than LV (6%) or biventricular (8%) remodelling, with RV dilatation more common than RV dysfunction. At follow-up, 71% had a normal echo. Although most patients underwent reverse remodelling reflected by a reduction in mean basal RV dimension and an increase in FAC, adverse RV remodelling persisted in 20% despite the normalisation of cardiac biomarkers.

In a small subgroup of hospitalised patients having a repeat study due to clinical deterioration in haemodynamics or need for intubation, RVFWLS was more often reduced in the mid-free wall segment, with relative apical sparing, reminiscent of McConnell's sign (21). This regional reduction in RV wall motion might explain why TAPSE or RV S′ could offer less sensitivity in detecting RVSD among COVID-19 patients, and this may be important when considering serial evaluation.

Finally, in a small case series of COVID-19 patients with ARDS, increased RV wall thickness was also reported in association with acute cor pulmonale, while in those who survived to discharge, PASP decreased compared with elevated baseline values (22).

Limitations of Echocardiographic Studies

Most of the aforementioned studies have been small, retrospective and heavily subject to selection bias, having been performed in hospitalised patients at the severe end of the COVID-19 disease spectrum, many of whom required ventilatory and/or circulatory support. The effects of COVID-19 upon cardiac function as assessed by echocardiography in asymptomatic patients or with only mild-to-moderate disease

not requiring hospitalisation remain unknown. Studies are also limited by their cross-sectional design or short duration of follow-up. Indeed, the medium- and long-term effects in patients with moderate and severe acute COVID-19 disease have yet to be fully characterised.

A further limitation relates to differences in echo protocols. Some departments have used a relatively standard or abbreviated "level 2" (53) approach, while others employed a level 1 or modified level 1 (41) approach in the interest of reducing study duration, as guided by consensus guidelines (28, 29). These intrinsic differences in imaging protocols will have influenced the results reported in the studies.

Finally, there have been differences between studies relating to post-processing analyses, namely, STE and strain analysis. Discrepancy in the method of RVLS measurement is notable; some studies measured RVFWLS, while others have assessed RVGLS. This may be further compounded by differences from the use of different versions of analysis software and different software vendors (54, 55). Heart rate and sampling frame rate present significant limitations in the application of STE to patients with tachycardia, as is commonly observed in patients with significant illness from COVID-19, potentially degrading the reliability of derived data. None of the studies to date have presented 3D echo analytical techniques.

CARDIAC MAGNETIC RESONANCE IMAGING

Cardiac magnetic resonance (CMR) imaging has the advantage of being able to provide structural, functional and tissue characterisation (**Table 1**).

An early CMR case report demonstrated subepicardial apical and inferolateral late gadolinium enhancement (LGE) in a patient acutely infected with COVID-19 in whom there was a significant troponin elevation and anterior T wave inversion and no coronary disease at angiography (18). Subsequent reports have also demonstrated LGE in a non-ischaemic pattern, and normal or increased signal on T2 short tau inversion recovery (STIR) imaging, and normal or increased values on parametric T1 and T2 mapping, consistent with previous or active myocarditis (56, 57).

A single-centre study of 26 patients who recovered from COVID-19 and had outpatient symptoms suggesting a cardiac origin were evaluated by CMR in Wuhan, China (58). The CMR was performed a median of 47 days from the onset of the cardiac symptoms. Conventional and quantitative mapping sequences were applied. Out of the 26 patients, 15 had myocardial oedema and/or focal LGE. The increased T2 STIR signal was mainly found in the interventricular septum, anterior, anterolateral and inferior wall segments in either the basal or mid-myocardial level. The authors contrasted these findings from the more common position of basal to mid-inferior and inferolateral wall segments for many other viruses yet acknowledged that the LGE distribution tended to be similar to that of common viral myocarditis, present in the subepicardial inferior and inferolateral wall. Conventional imaging demonstrated reduced RVEF in those with oedema and/or LGE, while LVEF was normal in all bar one patient, irrespective of the presence of oedema or fibrosis. Tissue mapping demonstrates increased T1, T2 and extracellular volume (ECV) in those patients with oedema and or LGE, but not in patients with normal conventional CMR imaging. RV analyses showed reduced RVEF, but no significant change in end-diastolic volume, in patients with abnormalities by conventional CMR imaging, but not in those without. The most important limitation of this study is clearly the very small sample size and, following this, the fact that most patients had had moderate rather than severe or mild COVID-19 disease.

A larger study of 100 patients with recent COVID-19 patient has since reported CMR findings in a German population of unselected volunteers (59). Of note, this group comprised 67 who recovered at home and 33 who had been hospitalised and in so doing included patients who had a mixed severity of COVID-19 illness. CMR-specific findings in the recovered patients vs. controls included, in order of frequency, elevated native T1, elevated native T2, myocardial LGE or pericardial LGE. LV volumes were mildly increased and LVEF mildly reduced in the COVID-19 patients compared with controls, however, with a relatively broad overlap of values. Interestingly, 12% of the patients had an ischaemic pattern of myocardial LGE. However, despite the parametric mapping abnormalities, there were no overt functional abnormalities based on LVEF and RVEF, leaving questions over the significance of these findings.

Native T1 and T2 mapping correlated with high-sensitivity troponins measured at the time of imaging. Comparing patients who recovered at home vs. in the hospital, native T1 was slightly but statistically higher in the hospitalised recovery group, and indeed, there was a similar difference in high-sensitivity troponin levels at the time of CMR imaging. ECV was not measured in this study. In three patients with severe CMR findings, myocardial biopsy revealed active lymphocytic inflammation. These findings were recorded at a median of 71 days from COVID-19 diagnosis and demonstrate ongoing cardiac abnormality beyond the phase of acute illness, consistent with ongoing myocarditis, pericarditis or myopericarditis, occurring independently of the severity, or time from onset, of the original COVID-19 illness.

Among 148 hospitalised patients with severe COVID-19 infection and elevated troponin, outpatient CMR was performed ∼2 months following diagnosis or discharge (60). This showed normal LVEF in 89% of patients, LGE or ischaemia in 54% of patients, of whom 26% had a non-ischaemic pattern and 22% had an ischaemic pattern, and dual pathology was seen in 6%. Active myocarditis was present in 30%. RVEF was lower in the COVID-19 group compared with the controls (61 vs. 64%, respectively). The study is, however, limited by the absence of matched premorbid CMR data to compare against. In addition, the significance of LGE in the presence of normal LVEF is uncertain.

A small series of 26 competitive athletes were assessed by CMR after COVID-19 without hospitalisation (61). All

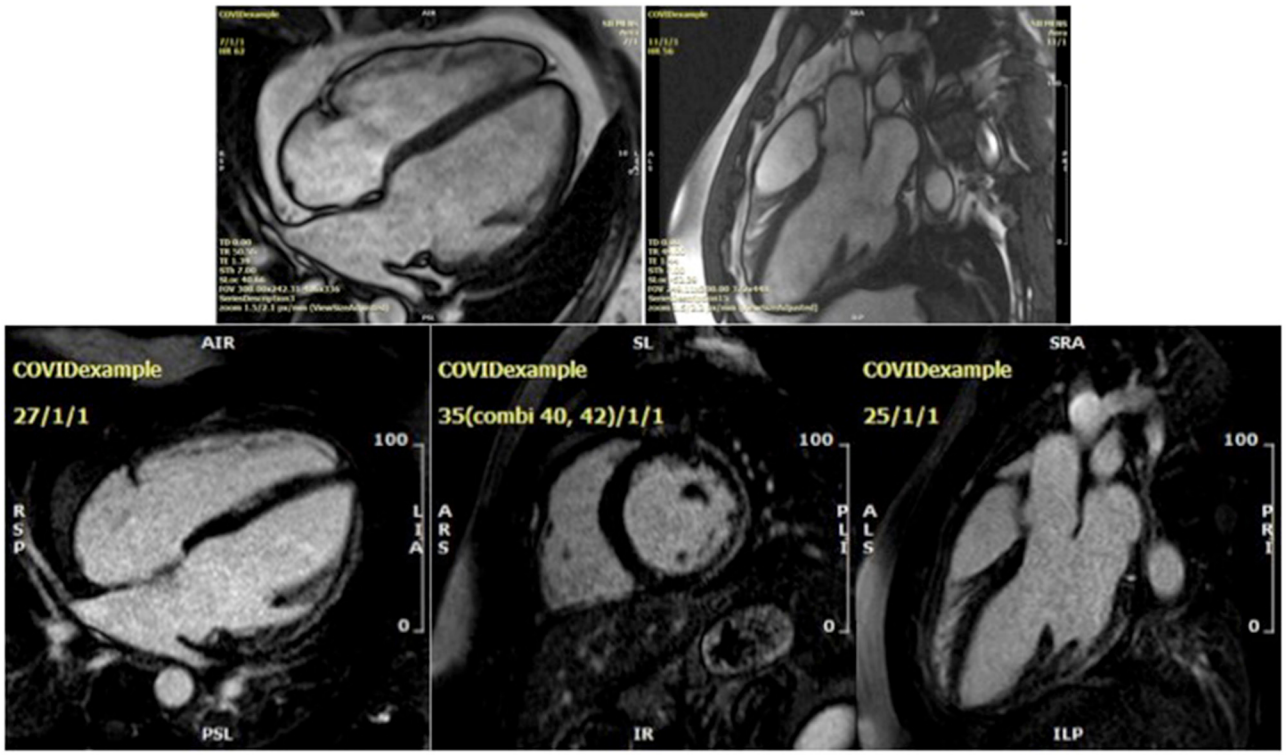


FIGURE 2 | Cardiac magnetic resonance findings for a 32-year-old male presenting 6 months after an initial diagnosis of severe COVID-19 pneumonia. Steady-state free precession cine imaging demonstrates a dilated left ventricle with severe global impairment of systolic function (upper two figures, see **Video 2**). Subepicardial late gadolinium enhancement involving the basal and mid lateral wall is non-ischaemic in aetiology and in keeping with a prior COVID-19 myocarditis (lower three figures, showing 4 chamber, short axis and LVOT1 views, respectively).

TABLE 4 | Cardiac computed tomography in COVID-19.

Potential role	Scenarios	Comment
Coronary assessment (epicardial)	Differential myocardial injury vs. obstructive coronary disease	CMR has a clear role here; cardiac CT might permit sufficient coronary assessment before a patient is able to undergo CMR for myocardial assessment
	First assessment of non-ST elevation acute coronary syndromes	Instead of ICA first
	Prior to non-coronary cardiac surgery	Already being used in some patients and centres prior to COVID-19
	Prior to structural heart interventions: LAA occlusion, TMVR, TAVI	May reduce need for ICA, especially in patients with fewer coronary risk factors
Left atrial appendage thrombus assessment	In patients requiring DC cardioversion of atrial arrhythmia, or prior to atrial fibrillation/flutter ablation, where sufficient anticoagulation has not been present, or there is higher than average thrombus risk	Reduces need for TOE An early and delayed image phase helps distinguish contrast stasis from thrombus. Further data on sensitivity and specificity vs. TOE will be important here
Myocarditis	Potential role through use of delayed contrast imaging to distinguish myocardial infarction with unobstructed coronaries from myocarditis	CMR is the gold standard in assessment of myocarditis by non-invasive imaging and has a larger evidence base. Further data will be needed
Structural cardiology interventions	Established role in pre-procedural planning in LAA, TMVR, and TAVI	May further reduce need for TOE where this is used

COVID-19, coronavirus disease 2019; CMR, cardiac magnetic resonance; ICA, invasive coronary angiography; LAA, left atrial appendage; TMVR, transcutaneous mitral valve intervention; TAVI, transcutaneous aortic valve intervention; TOE, transoesophageal echocardiography.

had normal ventricular volumes and function. Twelve of these had non-ischaemic LGE, comprising eight without and four with T2 elevation, suggesting previous and current myocarditis, respectively. However, some of the subjects had been asymptomatic from COVID-19, and there was no control group for comparison.

In contrast, among 48 college student athletes comprising a spectrum from asymptomatic to mild/moderate COVID-19, CMR performed at a median 27 days after the positive SARS-CoV-2 test showed the predominant abnormality was pericardial LGE and small pockets of pericardial effusion but no signs of active myocarditis (39). The CMR findings from a young male who was readmitted with dyspnoea and an elevated hs-cTnI of 80 ng/L (normal range < 14 ng/L) 6 months following an initial diagnosis of severe COVID-19 pneumonitis are shown in **Figure 2** and **Video 2**.

As with echocardiographic assessment, appreciation of longitudinal cardiac changes as measured by CMR during the acute phase of COVID-19, and in the medium and long term after the acute illness, is limited.

CARDIAC COMPUTED TOMOGRAPHY

Cardiac computed tomography angiography (CTA) provides another non-invasive diagnostic modality for a range of cardiac presentations. It has the advantage of rapid acquisition and relevant to the COVID clinical environment can be performed with less personal contact than traditional stress testing. This test offers an alternative to traditional methods that require longer contact time between staff and patient and those with

TABLE 5 | Factors to consider in the application of non-invasive imaging in COVID-19.

Factor	Considerations	Comment
Who to scan?	Biomarkers (troponin, D-dimer, ferritin, potentially BNP); ECG changes; cardiac symptoms; known cardiac disease	Biomarker cut-offs are unclear—the general trend and overall picture are likely to be the deciding factor until further data guide further ECG changes can be non-specific, and the entire clinical picture must be taken into consideration
	Critically and seriously unwell patients (abnormal haemodynamics and oxygen requirements)	Echo is likely to be the most available imaging modality in the critically unwell
	Prognostication and triage decisions for escalation to critical care level 2 care where resources are limited	This is a topic of medical ethics. Imaging may guide requirements for higher care and may inform probability of survival, although on a population rather than individual level. Echo can provide sufficient data
How to scan?	Echo, CMR, and CT are all considerations from the cardiovascular perspective	See **Table 1** for advantages vs. disadvantages of echo vs. CMR. See **Table 4** for potential uses of cardiac CT. CMR is likely best reserved for those with ongoing symptoms after recovery from acute COVID or in those with abnormal echocardiography
	Diagnostic considerations	Echo may be indicated to guide diagnosis of hypotension and differentiate septic shock vs. cardiogenic shock (thus guide inotropic, mechanical support decisions, maybe even transplant decisions). Cardiac CT offers a potential "quadruple rule-out" for assessment of aortic, pulmonary, coronary and myocardial pathology. See text for other considerations
When to scan?	Acute Outpatient—early vs. mid vs. long term Monitoring progress	These are factors that will require further exploration. Echo clearly permits accessible, convenient and serial follow-up whether as an inpatient or outpatient CMR may be a good pre-discharge assessment of cardiac status and, if abnormal, might be repeated as an outpatient to track longitudinal change. Where this is not practicable, an early outpatient CMR may be performed. Progress may be monitored by serial echo, especially in those who are severely ill and those with abnormalities on a baseline echo, with response to treatments including proning, steroids, oxygen, and novel therapies
Resource availability	Scanning systems (echo, CMR or CT); scanner time and availability; sonographer/radiographer expertise and availability; reporting clinician availability	Availability of all these factors will vary between units and countries. At a pragmatic level, these factors must be balanced against the considerations above to create locally achievable processes, while constraints are tackled to permit wider access
Safety considerations	Infection prevention	Strict considerations to mitigate risks of infection transmission during echo, CMR and CT studies are essential. Appropriate PPE and timing of the study are critical here, to balance the infection risk vs. potential improvements in clinical outcomes afforded by the data revealed by the study in question TTE should be the echo modality of choice rather than TOE—and TOE reserved for very highly selective cases due to its aerosol-generating nature—to cases where the TOE finding will change management. This is likely to be a very small proportion of cases, such as ICU cases where TTE windows are non-diagnostic
	Study duration? Role for abbreviated echo studies	Focused echo (level 1 echo or modified level 1 echo) will certainly provide useful data; tailoring what to truncate is a fine art and better applied by more senior practitioners than junior staff
Treatment	A role for imaging guided changes in treatment is not yet defined.	Potentially, imaging findings of right ventricular dysfunction, dilatation or pulmonary hypertension might trigger earlier initiation of advanced therapies ads they become identified

COVID-19, coronavirus disease 2019; BNP, brain natriuretic peptide; CMR, cardiac magnetic resonance; PPE, personal protective equipment; TTE, transthoracic echocardiography; TOE, transoesophageal echocardiography; ICU, intensive care unit.

actual or potential aerosol generation such as TOE and invasive coronary angiography (ICA) (**Table 4**) (62). It permits analysis of epicardial coronary arterial calcification, coronary atheroma, coronary wall characteristics, valvular calcification, intracardiac masses and pericardial assessment. However, it requires radiation exposure, although with modern scanners the doses are significantly lower than before, iodinated contrast administration with potential nephrotoxicity and contrast allergy, and good heart rate control with an ability to breath-hold, except for calcium scoring alone.

Cardiac CTA assesses for obstructive coronary disease in the following conditions: evaluation of myocardial infarction vs. myocardial injury; coronary assessment in patients with more typical non-ST elevation acute coronary syndromes, rather than an ICA approach; investigation of patients with newly impaired LV systolic function; preoperative assessment for heart valve surgery; and pre-procedural coronary assessment prior to percutaneous structural interventions. Secondly, it can investigate for left atrial appendage (LAA) thrombus, thereby avoiding TOE, prior to electrical cardioversion of atrial arrhythmia or atrial fibrillation ablation. Thirdly, it has been applied to assess for infective endocarditis in hearts with native and prosthetic valves, with the additional advantage of assessing for extra-valvular cardiac infection. Finally, while CT can provide useful information on COVID lung infection, by using a delayed enhancement CT protocol, it might also be able to offer detection of myocarditis (63), although CMR remains the gold standard test for this diagnosis.

FUTURE RESEARCH DIRECTIONS

To date, there has been a significant variation in the quantity and quality of cardiac imaging performed between centres. Collectively, cardiac imaging tests have been aimed at aiding diagnosis, prognostication, triaging decisions for escalation and monitoring of progress. In the future, imaging tests may be used for informed decisions related to initiation of novel COVID-19-specific treatments, anti-heart failure medication and duration of anticoagulation. The proposed timing of cardiac imaging and its modality [acute, subacute, pre-discharge, and outpatient (medium and long term)] is likely to depend on the clinical status of the patient guided by the results of biomarkers. The utility of biomarkers is likely to be in the risk stratification of patients by identifying those with cardiac injury who may benefit from cardiac imaging (12), rather than specifying the aetiology of cardiac injury. Prognostication could clearly be aided by imaging with RV, LV and pulmonary pressure findings from echo, and LGE findings on CMR provide potential as prognostic markers. Larger multicentre studies, such as COVID-HEART (54) and an imaging-based study of the Post-Hospitalisation COVID-19 study, PHOSP-COVID (55), are needed in the medium and long term, across the spectrum of disease severity, and should help to answer many of the following outstanding questions.

Characterisation

- Can cardiac imaging be used to help explain the differential response to COVID-19 reflected by patient demographic and established risk factors such as age, sex, race and BMI?
- What are the genetic determinants of adverse outcome in COVID-19 and how do they relate to the presence of adverse cardiac remodelling defined by cardiac imaging?
- Which patients are likely to benefit from imaging in the acute setting and who warrants follow-up cardiac imaging? When, where and how do we image (choice of modality)? (**Table 5**)
- What is the role of handheld echocardiography in acute COVID-19 patients? Anecdotally, this is still being widely used in many units.
- What is the role for imaging in guiding patients in their return to "normal activity" and for athletes returning to competitive sport. Is a CMR required or will echo/biomarker data suffice?
- Does strain (echo or CMR) offer clinical utility? There is a suggestion that RVGLS is superior to RVFWLS in severe heart failure (64); does this require further assessment with regard to assessment of RV function and prognosis in COVID-19? Does adding RVLS or RVFWLS to echo studies provide incremental prognostic information beyond standard indices of RV function?
- In patients with advanced respiratory failure, the prone position is often used. How do echo findings in prone patients compare with those in the same patient in a supine position? How do different software analysis packages affect results of 2D STE echo data, whether LV or RV?
- Can cardiac CT reliably expand beyond its more established role in coronary assessment to provide routine assessment of LAA thrombus and myocarditis?
- Long COVID refers to patients with ongoing symptoms beyond the acute illness (65)—what proportion of these have clinical or subclinical cardiac dysfunction?
- Finally, will the emergence of and infection by different SARS-CoV-2 strains result in differential cardiac effects (66)?

Treatment

- Is there a role for echo-guided anticoagulation strategies to prevent pulmonary hypertension, RV afterload and acute pulmonale (33)?
- What is the effect of novel treatments, including dexamethasone or antibody therapy on the cardiac response to severe COVID-19 infection?
- Do all patients with reduced LVEF related to COVID-19 benefit from conventional anti-heart failure medication?

Risks

- How does RV/LV dilatation/dysfunction progress? Does it resolve, or does it worsen? Is RV or LV abnormality the stronger determinant of adverse prognosis in the acute setting and at follow-up?
- Does LVEF remain normal even if normal at baseline, especially in those with oedema and/or LGE and/or persistent elevation in biomarkers? Does oedema resolve in all and in what time frame, or will we see some patients develop replacement fibrosis? How do the findings on CMR tissue characterisation relate to arrhythmogenic risk?
- Risks to the patient and to health care staff—Do longer echo studies lead to more nosocomial infection spread? Do more CMR studies lead to increased nosocomial infection spread?

DISCUSSION

In less than a year, the world has seen a pandemic caused by a novel coronavirus, and the resulting COVID-19 disease has resulted in millions of deaths and widespread short and-medium-term morbidity, with long-term effects yet to be

realised. While initially considered a respiratory disease, it is now apparent that cardiac involvement is an important potential phenomenon in COVID-19.

While biomarkers, particularly hs-cTn and D-dimer, ECG changes and cardiac symptoms and signs, may identify patients with cardiac injury, non-invasive cardiac imaging has a growing and powerful role in the assessment of cardiac structure and function in these patients. Beyond this diagnostic role, imaging can reveal prognostic data, can guide treatment and response to treatment, may aid decision-making when triaging limited resources among patients and can provide serial monitoring, for instance, of RV function and pulmonary hypertension. Cardiac phenotyping can be made possible using minimally invasive methods and when incorporated into clinical data mining will enhance and maintain patient safety. Herein lies the power of non-invasive imaging. RV dilatation and dysfunction in COVID-19 appears to be the dominant, although clearly not the only cardiac abnormality based on the echocardiographic data. Possible explanations include effects of COVID-19 upon pulmonary vascular resistance and lung parenchyma, either of which could result in increased pulmonary arterial pressure, RV afterload, RV dilatation and dysfunction.

Echocardiography, CMR and cardiac CT have been considered in this review of non-invasive cardiac imaging in COVID-19. It is often said that CMR is the "gold standard" for various aspects of cardiac assessment, and for volumetric assessment and tissue characterisation, and this holds true for compliant subjects with ideal or near-ideal scanning conditions. For health care systems with limited resource, echocardiography will likely remain the mainstream imaging modality for assessment of COVID-19 patients and offers a pragmatic alternative in the acute setting when MRI and CT scanning conditions, related to patient factors predominantly, are often suboptimal. In particular, CMR would appear more suitable for patients who recover from COVID-19 and acute cardiac injury, rather than during their acute phase, where the patient may often be too sick to transfer, imaging quality may be significantly compromised and resources are limited.

Recommendations for Cardiac Imaging in COVID 19

We propose TTE as the first-line imaging modality for hospitalised COVID-19 patients with critical illness, haemodynamic instability, significantly elevated hs-cTn or clinical features consistent with cardiac dysfunction. Outpatient follow-up TTE should be considered in those where the inpatient echocardiogram was abnormal or for patients with long COVID. This may be supplemented or superseded with cardiac MRI dependent on local accessibility and patient status. These initial recommendations will need refining as further evidence becomes available.

AUTHOR CONTRIBUTIONS

SH, RS, and WM contributed to conception and design of the review. SH and JJ organised the initial literature review. SH wrote the first draft of the manuscript. RS, JJ, and WM critically evaluated the first draft and suggested alterations to the manuscript. WM contributed the images and videos. All authors approved the final version.

FUNDING

The Institute of Cardiovascular Sciences is a recipient of a British Heart Foundation Accelerator Award (AA/18/2/34218).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.663864/full#supplementary-material

REFERENCES

1. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of Novel Coronavirus–Infected Pneumonia. *N Engl J Med.* (2020) 382:1199–207. doi: 10.1056/NEJMoa2001316
2. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet.* (2020) 395:1033–4. doi: 10.1016/S0140-6736(20)30628-0
3. World Health Organization. *WHO Director-General's opening remarks at the media briefing on COVID-19*. (2020). Available from: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19 (accessed on March 11, 2020).
4. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* (2020) 395:507–13. doi: 10.1016/S0140-6736(20)30211-7
5. Wu Z, McGoogan JM. Characteristics of and Important Lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases from the Chinese Center for Disease Control and Prevention. *J Am Med Assoc.* (2020) 323:1239–42. doi: 10.1001/jama.2020.2648
6. Feng Y, Ling Y, Bai T, Xie Y, Huang J, Li J, et al. COVID-19 with different severities: a multicenter study of clinical features. *Am J Respir Crit Care Med.* (2020) 201:1380–8. doi: 10.1164/rccm.202002-0445OC
7. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. *Clin Chem Lab Med.* (2020) 57:1131–4. doi: 10.1515/cclm-2020-0198
8. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular Implications of Fatal Outcomes of Patients with Coronavirus Disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017
9. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of Cardiac Injury with Mortality in Hospitalized Patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* (2020) 5:802–10. doi: 10.1001/jamacardio.2020.0950
10. Potere N, Valeriani E, Candeloro M, Tana M, Porreca E, Abbate A, et al. Acute complications and mortality in hospitalized patients with coronavirus disease 2019: a systematic review and meta-analysis. *Crit Care.* (2020) 24:1–12. doi: 10.1186/s13054-020-03022-1
11. Lala A, Johnson KW, Januzzi JL, Russak AJ, Paranjpe I, Richter F, et al. Prevalence and impact of myocardial injury in patients hospitalized with COVID-19 infection. *J Am Coll Cardiol.* (2020) 76:533–46. doi: 10.1016/j.jacc.2020.06.007
12. Sandoval Y, Januzzi JL Jr, Jaffe AS. Cardiac troponin for assessment of myocardial injury in COVID-19. *J Am Coll Cardiol.* (2020) 76:1244–58. doi: 10.1016/j.jacc.2020.06.068
13. Baycan OF, Barman HA, Atici A, Tatlisu A, Bolen F, Ergen P, et al. Evaluation of biventricular function in patients with COVID-19 using

speckle tracking echocardiography. *Int J Cardiovasc Imaging*. (2020) 142. doi: 10.1161/circ.142.suppl_3.14937
14. Porembskaya O, Lobastov K, Pashovkina O, Tsaplin S, Schastlivtsev I, Zhuravlev S, et al. Thrombosis of pulmonary vasculature despite anticoagulation and thrombolysis: the findings from seven autopsies. *Thromb Updat*. (2020) 1:100017. doi: 10.1016/j.tru.2020.100017
15. Bangalore S, Sharma A, Slotwiner A, Yatskar L, Harari R, Shah B, et al. ST-segment elevation in patients with Covid-19 — A Case Series. *N Engl J Med*. (2020) 385:2478–80. doi: 10.1056/NEJMc2009020
16. Vasudev R, Guragai N, Habib H, Hosein K, Virk H, Goldfarb I, et al. The utility of bedside echocardiography in critically ill COVID-19 patients: early observational findings from three Northern New Jersey hospitals. *Echocardiography*. (2020) 37:1362–5. doi: 10.1111/echo.14825
17. Fujiyoshi K, Ako J, Ishida K, Ishida M, Minami Y, Inomata T. Takotsubo-like left ventricular dysfunction in a patient with COVID-19 demonstrated by non-invasive multi-modality imaging. *J Nucl Cardiol*. (2020). doi: 10.1007/s12350-020-02367-y. [Epub ahead of print].
18. Doyen D, Moceri P, Ducreux D, Dellamonica J. Myocarditis in a patient with COVID-19: a cause of raised troponin and ECG changes. *Lancet*. (2020) 395:1516. doi: 10.1016/S0140-6736(20)30912-0
19. Mahmoud-Elsayed HM, Moody WE, Bradlow WM, Khan-Kheil AM, Senior J, Hudsmith LE, et al. Echocardiographic findings in patients with COVID-19 pneumonia. *Can J Cardiol J Cardiol*. (2020) 36:1203–7. doi: 10.1016/j.cjca.2020.05.030
20. Moody WE, Mahmoud-Elsayed HM, Senior J, Gul U, Khan-Kheil AM, Horne S, et al. Impact of right ventricular dysfunction on mortality in patients hospitalized with COVID-19 according to race. *CJC Open*. (2021) 3:91–100. doi: 10.1016/j.cjco.2020.09.016
21. Rothschild E, Baruch G, Szekely Y, Lichter Y, Kaplan A, Taieb P, et al. The predictive role of left and right ventricular speckle-tracking echocardiography in COVID-19. *Cardiovasc Imaging Imaging*. (2020) 13:2471–4. doi: 10.1016/j.jcmg.2020.07.026
22. Lazzeri C, Bonizzoli M, Batacchi S, Cianchi G, Franci A, Fulceri GE, et al. Cardiac involvement in COVID-19 – Related Acute Respiratory Distress Syndrome. *Am J Cardiol*. (2020) 132:147–9. doi: 10.1016/j.amjcard.2020.07.010
23. Zeng JH, Wu WB, Qu JX, Wang Y, Dong CF, Luo YF, et al. Cardiac manifestations of COVID-19 in Shenzhen, China. *Infection*. (2020) 48:861–70. doi: 10.1007/s15010-020-01473-w
24. Barman HA, Atici A, Tekin EA, Baycan OF, Alici G, Meric BK, et al. Echocardiographic features of patients with COVID-19 infection: a cross-sectional study. *Int J Cardiovasc Imaging*. (2020) 37:825–34. doi: 10.22541/au.158921502.21778694
25. Enea L Pietro, Mohamed B, Leopoldo CP. COVID-19, arrhythmic risk, and inflammation. *Circulation*. (2020) 142:7–9. doi: 10.1161/CIRCULATIONAHA.120.047293
26. Oh JK, Kane G, Seward J, Tajik AJ. *The Echo Manual*. 4th ed. Philadelphia, PA: Lippincott Williams and Wilkins (2018). p. 528.
27. Hothi SS, Sprigings D, Chambers J. Point-of-care cardiac ultrasound in acute medicine – the quick scan. *Clin Med*. (2014) 14:608–11. doi: 10.7861/clinmedicine.14-6-608
28. Kirkpatrick JN, Mitchell C, Taub C, Kort S, Hung J, Swaminathan M. ASE statement on protection of patients and echocardiography service providers during the 2019 novel coronavirus outbreak: endorsed by the American college of cardiology. *J Am Soc Echocardiogr*. (2020) 33:648–53. doi: 10.1016/j.echo.2020.04.001
29. BSE. Clinical guidance regarding provision of echocardiography during the COVID-19 pandemic. *Brit Soc Echocardiogr*. (2020). Available online at: https://bsecho.org/covid19
30. Johri AM, Galen B, Kirkpatrick JN, Lanspa M, Mulvagh S. ASE Statement on Point-of-Care Ultrasound during the 2019 Novel Coronavirus Pandemic. *J Am Soc Echocardiogr*. (2020) 33:670–3. doi: 10.1016/j.echo.2020.04.017
31. Picard MH, Weiner RB. Echocardiography in the time of COVID-19 Michael. *J*. (2020) 33:674–5. doi: 10.1016/j.echo.2020.04.011
32. Huang G, Vengerovsky A MA, J T, D C, Y K. Development of a COVID-19 point-of-care ultrasound protocol. *J Am Soc Echocardiogr*. (2020) 33:903–5. doi: 10.1016/j.echo.2020.04.023
33. Szekely Y, Lichter Y, Taieb P, Banai A, Hochstadt A, Merdler I, et al. Spectrum of cardiac manifestations in COVID-19: a systematic echocardiographic study. *Circulation*. (2020) 142:342–53. doi: 10.1161/CIRCULATIONAHA.120.047971
34. Argulian E, Sud K, Vogel B, Bohra C, Garg VP, Talebi S, et al. Right Ventricular Dilation in Hospitalized Patients With COVID-19 Infection. *JACC Cardiovasc Imaging*. (2020) 13:2459–61. doi: 10.1016/j.jcmg.2020.05.010
35. Kim J, Volodarskiy A, Sultana R, Pollie MP, Yum B, Nambiar L, et al. Prognostic utility of right ventricular remodeling over conventional risk stratification in patients with COVID-19. *J Am Coll Cardiol*. (2020) 76:1965–77. doi: 10.1016/j.jacc.2020.08.066
36. Li Y, Li H, Zhu S, Xie Y, Wang B, He L, et al. Prognostic value of right ventricular longitudinal strain in patients with COVID-19. *JACC Cardiovasc Imaging*. (2020) 13:2287–99. doi: 10.1016/j.jcmg.2020.04.014
37. Schott JP, Mertens AN, Bloomingdale R, O'Connell TF, Gallagher MJ, Dixon S, et al. Transthoracic echocardiographic findings in patients admitted with SARS-CoV-2 infection. *Echocardiography*. (2020) 37:1551–6. doi: 10.1111/echo.14835
38. Churchill TW, Bertrand PB, Bernard S, Namasivayam M, Churchill J, Crousillat D, et al. Echocardiographic features of COVID-19 Illness and Association with Cardiac Biomarkers. *J Am Soc Echocardiogr*. (2020) 33:1053–4. doi: 10.1016/j.echo.2020.05.028
39. Brito D, Meester S, Yanamala N, Patel HB, Balcik BJ, Casaclang-Verzosa G, et al. High prevalence of pericardial involvement in College Student Athletes Recovering From COVID-19. *JACC Cardiovasc Imaging*. (2020) 14:541–55. doi: 10.1016/j.jcmg.2020.10.023
40. Pagnesi M, Baldetti L, Beneduce A, Calvo F, Gramegna M, Pazzanese V, et al. Pulmonary hypertension and right ventricular involvement in hospitalised patients with COVID-19. *Heart*. (2020) 19:1324–31. doi: 10.1136/heartjnl-2020-317355
41. Hindocha R, Garry D, Short N, Ingram TE, Steeds RP, Colebourn CL, et al. A minimum dataset for a Level 1 echocardiogram: a guideline protocol from the British Society of Echocardiography. *Echo Res Pract*. (2020) 7:G51–8. doi: 10.1530/ERP-19-0060
42. Zeng J-H, Liu Y-X, Yuan J, Wang F-X, Wu W-B, Li J-X, et al. First case of COVID-19 complicated with fulminant myocarditis: a case report and insights. *Infection*. (2020) 48:773–7. doi: 10.1007/s15010-020-01424-5
43. Hu H, Ma F, Wei X, Fang Y. Coronavirus fulminant myocarditis treated with glucocorticoid and human immunoglobulin. *Eur Hear J*. (2020) 42:191. doi: 10.1093/eurheartj/ehaa190
44. Inciardi RM, Lupi L, Zaccone G, Italia L, Raffo M, Tomasoni D, et al. Cardiac involvement in a Patient With Coronavirus Disease 2019 (COVID-19). *JAMA Cardiol*. (2020) 5:819–24. doi: 10.1001/jamacardio.2020.1096
45. Dweck MR, Bularga A, Hahn RT, Bing R, Lee KK, Chapman AR, et al. Global evaluation of echocardiography in patients with COVID-19. *Eur Heart J Cardiovasc Imaging*. (2020) 21:949–58. doi: 10.1093/ehjci/jeaa178
46. Kalam K, Otahal P, Marwick TH. Prognostic implications of global LV dysfunction: a systematic review and meta-analysis of global longitudinal strain and ejection fraction. *Heart*. (2014) 100:1673–80. doi: 10.1136/heartjnl-2014-305538
47. Lazzeri C, Bonizzoli M, Batacchi S, Peris A. Echocardiographic assessment of the right ventricle in COVID -related acute respiratory syndrome. *Intern Emerg Med*. (2020) 16:1–5. doi: 10.1007/s11739-020-02494-x
48. Lang RM, Badano LP, Mor-avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults : an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr*. (2015) 28:1–39. doi: 10.1016/j.echo.2014.10.003
49. Lang R, Badano L, Tsang W, Adams D, Agricola E, Buck T, et al. American Society of Echocardiography; European Association of Echocardiography. EAE/ASE recommendations for image acquisition and display using three-dimensional echocardiography. *J Am Soc Echocardiogr*. (2012) 25:3–46. doi: 10.1016/j.echo.2011.11.010
50. Flachskampf, FA, Biering-Sørensen T, Solomon SD, Duvernoy O, Bjerner OAS. Cardiac imaging to evaluate left ventricular diastolic function. *JACC Cardiovasc Imaging*. (2015) 8:1071–93. doi: 10.1016/j.jcmg.2015.07.004

51. Henein MY, Lindqvist P. Diastolic function assessment by echocardiography: a practical manual for clinical use and future applications. *Echocardiography*. (2020) 37:1908–18. doi: 10.1111/echo.14698
52. Moody WE, Liu B, Mahmoud-elsayed HM, Senior J, Lalla S, Khan-kheil AM, et al. Persisting adverse ventricular remodeling in COVID-19 survivors: a longitudinal echocardiographic study. *J Am Soc Echocardiogr*. (2021). doi: 10.1016/j.echo.2021.01.020. [Epub ahead of print].
53. Wharton G, Steeds R, Allen J, Phillips H, Jones R, Kanagala P, et al. A minimum dataset for a standard adult transthoracic echocardiogram: a guideline protocol from the British Society of Echocardiography. *Echo Res Pr*. (2015) 2:G9–24. doi: 10.1530/ERP-14-0079
54. Anwar S, Negishi K, Borowszki A, Gladding P, Popović ZB, Erenberg F, et al. Comparison of two-dimensional strain analysis using vendor-independent and vendor-specific software in adult and pediatric patients. *JRSM Cardiovasc Dis*. (2017) 6:2048004017712862. doi: 10.1177/2048004017712862
55. Edvardsen T, Haugaa KH. Strain echocardiography: from variability to predictability. *JACC Cardiovasc Imaging*. (2018) 11:35–7. doi: 10.1016/j.jcmg.2017.03.012
56. Caballeros Lam M, de la Fuente Villena A, Hernández Hernández A, García de Yébenes M BAG. Cardiac magnetic resonance characterization of COVID-19 myocarditis. *Rev Esp Cardiol*. (2020) 73:863–4. doi: 10.1016/j.rec.2020.06.018
57. Beşler MS, Arslan H. Acute myocarditis associated with COVID-19 infection. *Am J Emerg Med*. (2020) 38:2489.e1-2489.e2. doi: 10.1016/j.ajem.2020.05.100
58. Huang L, Zhao P, Tang D, Zhu T, Han R, Zhan C, et al. Cardiac involvement in patients recovered from COVID-2019 identified using magnetic resonance imaging. *JACC Cardiovasc Imaging*. (2020) 13:2330–9. doi: 10.1016/j.jcmg.2020.05.004
59. Puntmann VO, Carerj ML, Wieters I, Fahim M, Arendt C, Hoffmann J, et al. Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from Coronavirus Disease 2019 (COVID-19). *JAMA Cardiol*. (2020) 5:1265–73. doi: 10.1001/jamacardio.2020.3557
60. Kotecha T, Knight DS, Razvi Y, Kumar K, Vimalesvaran K, Thornton G, et al. Patterns ofmyocardial injury in recovered troponin-positive COVID-19 patients assessed by cardiovascularmagnetic resonance. *Eur Heart J*. (2021). doi: 10.1093/eurheartj/ehab075. [Epub ahead of print].
61. Rajpal S, Tong MS, Borchers J, Zareba KM, Obarski TP, Simonetti OP, et al. Cardiovascular magnetic resonance findings in competitive athletes recovering from COVID-19 infection. *JAMA Cardiol*. (2021) 6:116–8. doi: 10.1001/jamacardio.2020.4916
62. Singh V, Choi AD, Leipsic J, Aghayev A, Earls JP, Blanke P, et al. Use of cardiac CT amidst the COVID-19 pandemic and beyond: North American perspective. *J Cardiovasc Comput Tomogr*. (2021) 15:16–26.
63. Pontone G, Baggiano A, Conte E, Teruzzi G, Cosentino N, Campodonico J, et al. "Quadruple Rule-Out" with computed tomography in a COVID-19 patient with equivocal acute coronary syndrome presentation. *JACC Cardiovasc Imaging*. (2020) 13:1854–6. doi: 10.1016/j.jcmg.2020.04.012
64. Cameli M, Bernazzali S, Lisi M, Tsioulpas C, Croccia MG, Lisi G, et al. Right ventricular longitudinal strain and right ventricular stroke work index in patients with severe heart failure: left ventricular assist device suitability for transplant candidates. *Transpl Proc*. (2012) 44:2013–5. doi: 10.1016/j.transproceed.2012.06.018
65. Iacobucci G. Long Covid: damage to multiple organs presents in young, low risk patients. *BMJ*. (2020) 371:m4470. doi: 10.1136/bmj.m4470
66. Wise J. Covid-19: new coronavirus variant is identified in UK. *BMJ*. (2020) 371:m4857. doi: 10.1136/bmj.m4857

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 25 May 2021
doi: 10.3389/fcvm.2021.633539

Lung Ultrasound Score as a Predictor of Mortality in Patients With COVID-19

Zhenxing Sun[1,2†], Ziming Zhang[1,2†], Jie Liu[1,2†], Yue Song[1,2], Shi Qiao[1,2], Yilian Duan[1,2], Haiyan Cao[1,2], Yuji Xie[1,2], Rui Wang[1,2], Wen Zhang[1,2], Manjie You[1,2], Cheng Yu[1,2], Li Ji[1,2], Chunyan Cao[1,2], Jing Wang[1,2], Yali Yang[1,2], Qing Lv[1,2], Hongbo Wang[3‡], Haotian Gu[4*‡] and Mingxing Xie[1,2*‡]*

[1] Department of Ultrasound Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [2] Hubei Province Key Laboratory of Molecular Imaging, Wuhan, China, [3] Department of Gynecology and Obstetrics, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [4] British Heart Foundation Centre of Research Excellence, King's College London, London, United Kingdom

OPEN ACCESS

Edited by:
Matteo Cameli,
University of Siena, Italy

Reviewed by:
Maria Concetta Pastore,
Università del Piemonte Orientale, Italy
Ciro Santoro,
Federico II University Hospital, Italy

***Correspondence:**
Haotian Gu
haotian.gu@kcl.ac.uk
Hongbo Wang
drwanghb69@sina.com
Mingxing Xie
xiemx@hust.edu.cn

[†]These authors share first authorship

[‡]These authors share senior authorship

Specialty section:
This article was submitted to Cardiovascular Imaging, a section of the journal Frontiers in Cardiovascular Medicine

Received: 25 November 2020
Accepted: 22 March 2021
Published: 25 May 2021

Citation:
Sun Z, Zhang Z, Liu J, Song Y, Qiao S, Duan Y, Cao H, Xie Y, Wang R, Zhang W, You M, Yu C, Ji L, Cao C, Wang J, Yang Y, Lv Q, Wang H, Gu H and Xie M (2021) Lung Ultrasound Score as a Predictor of Mortality in Patients With COVID-19. Front. Cardiovasc. Med. 8:633539. doi: 10.3389/fcvm.2021.633539

Background: Lung injury is a common condition among hospitalized patients with coronavirus disease 2019 (COVID-19). However, whether lung ultrasound (LUS) score predicts all-cause mortality in patients with COVID-19 is unknown. The aim of the present study was to explore the predictive value of lung ultrasound score for mortality in patients with COVID-19.

Methods: Patients with COVID-19 who underwent lung ultrasound were prospectively enrolled from three hospitals in Wuhan, China between February 2020 and March 2020. Demographic, clinical, and laboratory data were collected from digital patient records. Lung ultrasound scores were analyzed offline by two observers. Primary outcome was in-hospital mortality.

Results: Of the 402 patients, 318 (79.1%) had abnormal lung ultrasound. Compared with survivors ($n = 360$), non-survivors ($n = 42$) presented with more B2 lines, pleural line abnormalities, pulmonary consolidation, and pleural effusion (all $p < 0.05$). Moreover, non-survivors had higher global and anterolateral lung ultrasound score than survivors. In the receiver operating characteristic analysis, areas under the curve were 0.936 and 0.913 for global and anterolateral lung ultrasound score, respectively. A cutoff value of 15 for global lung ultrasound score had a sensitivity of 92.9% and specificity of 85.3%, and 9 for anterolateral score had a sensitivity of 88.1% and specificity of 83.3% for prediction of death. Kaplan–Meier analysis showed that both global and anterolateral scores were strong predictors of death (both $p < 0.001$). Multivariate Cox regression analysis showed that global lung ultrasound score was an independent predictor (hazard ratio, 1.08; 95% confidence interval, 1.01–1.16; $p = 0.03$) of death together with age, male sex, C-reactive protein, and creatine kinase-myocardial band.

Conclusion: Lung ultrasound score as a semiquantitative tool can be easily measured by bedside lung ultrasound. It is a powerful predictor of in-hospital mortality and may play a crucial role in risk stratification of patients with COVID-19.

Keywords: COVID-19, SARS-CoV-2, lung ultrasound score, mortality, prognosis

BACKGROUND

The coronavirus disease 2019 (COVID-19) is a newly recognized infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although chest computed tomography (CT) has been regarded as an important diagnostic tool for COVID-19 diagnosis (1), it is limited by high cost, radiation exposure, infection control challenges, and lack of continuous monitoring, particularly for critically ill patients (2). Lung ultrasound (LUS), with the advantage of being non-invasive, low cost, and radiation free, has been increasingly used as a bed-side tool for evaluation and monitoring of lung diseases, particularly in the intensive care unit (ICU) (2, 3). It was found to have high accuracy in diagnosing viral community-acquired pneumonia with 94% sensitivity and 89% specificity for the detection of viral pneumonia in symptomatic patients (4). Global LUS score, a semiquantitative numerical score of lung aeration across 12 lung regions, has been shown as a useful tool to diagnose acute respiratory distress syndrome (ARDS) (5).

We therefore hypothesized that LUS score may play an important role in detecting lung lesions and optimizing risk stratification in patients with COVID-19. To test this hypothesis, LUS images in patients prospectively recruited from three hospitals in Wuhan, China were analyzed to evaluate the prognostic value of LUS score for in-hospital mortality in patients with COVID-19.

MATERIALS AND METHODS

Patient Population

Patients with confirmed COVID-19 who underwent lung ultrasound were consecutively recruited from the West Branch of Wuhan Union Hospital, Cancer Centre of Union Hospital, and Jianghan Mobile Cabin Hospital Wuhan, China between February 6, 2020 and March 15, 2020. The study was approved by the ethics committee, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (No. 20200021). Written informed consent was waived because of the unprecedented nature of COVID-19 pandemic.

Inclusion criteria were age ≥18 years and confirmed COVID-19. Exclusion criteria were incomplete image acquisition, missing clinical data, and cardiac failure causing cardiogenic pulmonary oedema.

Demographic, clinical history, comorbidities, laboratory data, and outcomes of all patients were obtained from electronic medical records (Dthealth Medical Systems CO, Tianjin, China). Primary outcome was all-cause mortality. All patients were followed up until April 7, 2020 when the last patient in the study was discharged.

Lung Ultrasound

LUS examinations were performed by nine qualified ultrasound doctors using Mindray M9 potable ultrasound machines (Mindray Bio-medical electronics Co, Shenzhen, China) with 1- to 5- MHz convex probes. LUS consisted of 12 different regions (two anterior, two lateral, and two posterior thoracic regions) (**Supplementary Figure 1**) as previously described (6). All video files were recorded in a hospital local archive and were interpreted and scored offline by two experienced observers within 24 h of LUS examinations who were blinded to the clinical data and outcomes. In case of disagreement between observers, the two observers agreed by consensus on the LUS score.

Examples of ultrasound findings including the patterns of B lines, consolidations, pleural line abnormalities, pleural effusion, and the lesion distribution are shown in **Figure 1**.

Lung Ultrasound Score

LUS score was determined based on four lung patterns (**Supplementary Table 1**): N = 0, B1 = 1, B2 = 2, and C = 3 as described previously (7):

a. N pattern—normal aeration: A lines or <3 isolated B lines;
b. B1 pattern—moderate loss of lung aeration: a clear number of multiple visible B lines with horizontal spacing between adjacent B lines ≤7 mm (B1 lines);
c. B2 pattern—severe loss of lung aeration: multiple B lines fused together with horizontal spacing between adjacent B lines ≤3 mm, including "white lung" (B2 lines); and
d. C pattern—complete loss of aeration: pulmonary consolidation, presence of tissue pattern accompanied by static or dynamic air bronchograms.

Global LUS score was calculated by summing the scores of all 12 lung regions (ranging from 0 to 36). An adjusted composite score, antero-lateral score, was also derived by summing the anterior and lateral regional scores (range from 0 to 24) (5, 7).

Repeatability and Reproducibility of Lung Ultrasound Score

Intra- and interobserver variability of global LUS score was assessed in 30 randomly selected subjects by repeat measurements on the same images 1 month apart by two observers. Bland–Altman plots were produced.

Statistical Analysis

Demographic, clinical, and outcome variables were presented as percentages for categorical variables and as medians with interquartile ranges (IQRs) for continuous variables. The Mann–Whitney *U*-test was used to compare LUS scores between survivors and non-survivors.

Receiver operating characteristic (ROC) curves for death were drafted for global and anterolateral score. The area under the receiver operating characteristic curves (AUCs) was calculated to determine the diagnostic accuracy for death. The optimal cutoffs were determined as the highest Youden's index (sensitivity + specificity – 1).

Kaplan–Meier curves were used to examine cumulative death rate, and differences between groups were tested using a log rank test. Univariate and multivariate Cox regression analysis was performed to identify potential predictors of death. Multivariate models were constructed to assess the prognostic utility of global and anterolateral scores, incorporating covariables that were significant ($p < 0.05$) in the univariate analysis. All statistical analyses were performed using SPSS version 25 (SPSS Inc. Chicago, Illinois).

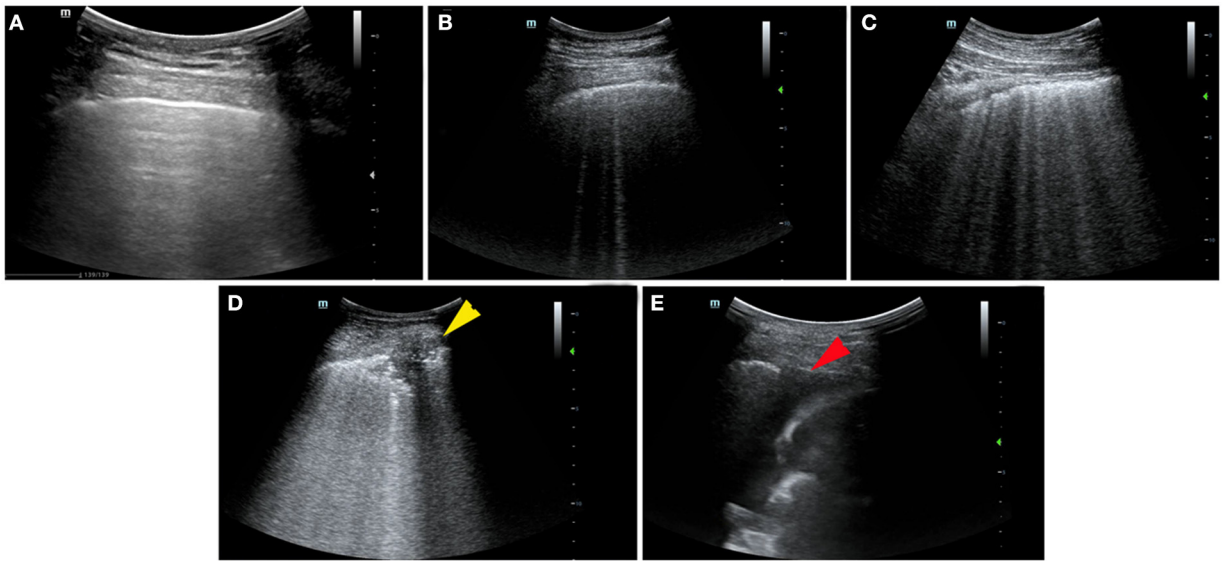


FIGURE 1 | Ultrasonographic features and lung ultrasound (LUS) score in patients with coronavirus disease 2019 (COVID-19). **(A)** Normal: the presence of A lines beyond the pleural line characterizes mornal pulmonay aeration, LUS score: 0. **(B)** B1 line: the presence of multiple vertical B lines (comet tails) with well-defined spacing regularly spaced B lines 7 mm apart, LUS score: 1. **(C)** B2 line: the presence of coalescent B lines <3 mm apart, LUS score: 2. **(D)** Lung consolidation: the presence of a tissue pattern (yellow arrowhead), LUS score: 3. **(E)** Pleural effusion at costophrenic angle (red arrowhead).

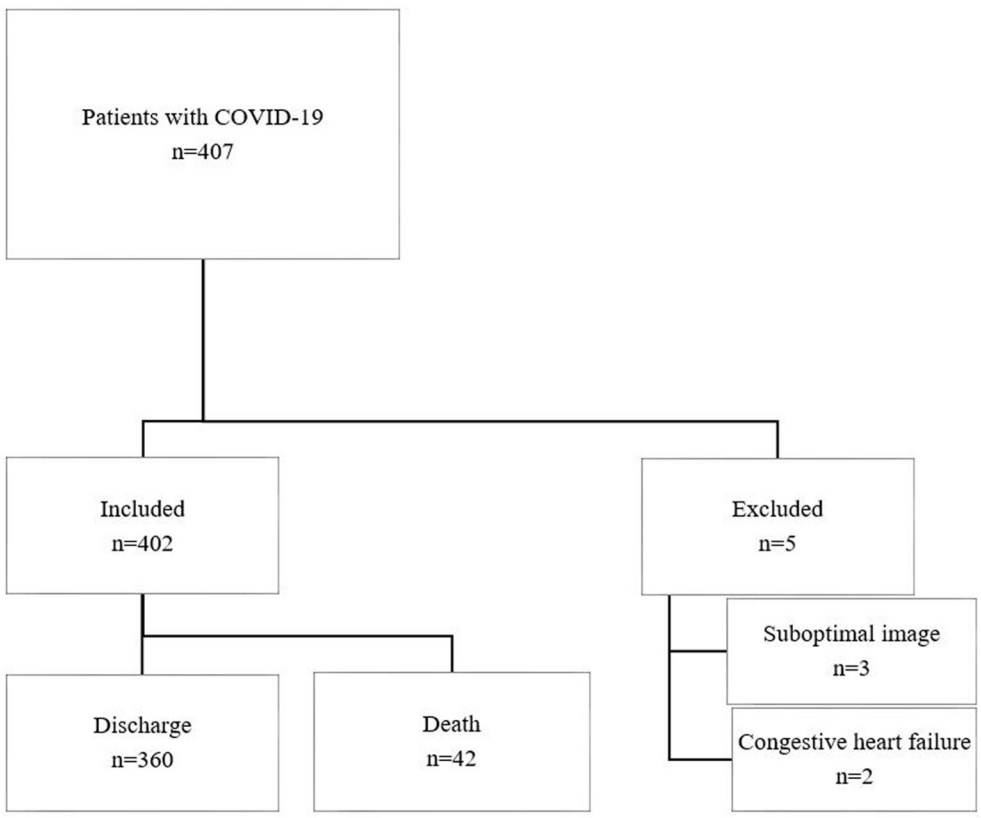


FIGURE 2 | Study flow chart.

TABLE 1 | Patient characteristics.

	No. (%)			*p*-value	No. (%)		*p*-value
	Total (*N* = 402)	**Survivors (*N* = 360)**	**Non-survivors (*N* = 42)**		**Global LUS Score <15 (*N* = 310)**	**Global LUS Score ≥15 (*N* = 92)**	
Age, median (IQR), years	63 (52–70)	62 (52–69)	69 (61–77)	<0.001	61 (51–68)	69 (61–77)	<0.001
Age distribution	–	–	–	<0.001	–	–	<0.001
20–40 years	39 (9.7)	39 (10.8)	0		36 (11.6)	4 (4.3)	
40–60 years	125 (31.1)	124 (34.5)	1 (2.4)		112 (36.1)	18 (19.6)	
≥60 years	238 (59.2)	197 (54.7)	41 (97.6)		162 (52.3)	70 (76.1)	
Sex	–	–	–	0.002	–	–	0.002
Female	210 (52.2)	199 (55.3)	11 (26.2)		175 (56.5)	35 (38.0)	
Male	192 (47.8)	161 (44.7)	31 (73.8)		135 (43.5)	57 (62.0)	
Clinical presentation							
Fever	395 (98.2)	353 (98.0)	42 (100)	0.36	304 (98.1)	91 (98.9)	0.93
Dry cough	279 (69.4)	246 (68.3)	33 (78.6)	0.17	209 (67.4)	70 (76.1)	0.11
Headache	23 (5.7)	18 (5.0)	5 (11.9)	0.14	14 (4.8)	9 (9.8)	0.06
Sore throat	45 (11.1)	42 (11.7)	3 (7.1)	0.53	31 (10.0)	14 (15.3)	0.16
Myalgia	135 (33.6)	116 (32.2)	19 (45.2)	0.09	97 (31.3)	38 (41.3)	0.07
Fatigue	131 (32.6)	115 (31.9)	16 (38.1)	0.42	100 (32.3)	31 (33.7)	0.80
Dyspnea	124 (30.8)	104 (23.2)	20 (34.8)	0.01	72 (23.2)	32 (34.8)	0.03
Rhinorrhea	43 (10.7)	35 (9.4)	8 (21.4)	0.11	32 (18.6)	11 (16.8)	0.66
Nausea and vomiting	26 (6.5)	24 (6.7)	2 (4.8)	0.89	23 (7.4)	3 (3.3)	0.15
Diarrhea	51 (12.7)	47 (13.1)	4 (9.5)	0.52	37 (11.9)	14 (15.2)	0.41
Comorbidities							
Hypertension	97 (24.1)	80 (22.2)	17 (40.5)	0.009	64 (20.6)	33 (35.9)	0.003
Coronary heart disease	50 (12.4)	35 (9.7)	15 (35.7)	<0.001	30 (9.7)	20 (21.7)	0.002
Arrhythmia	10 (2.5)	9 (2.5)	1 (2.4)	1.00	8 (2.6)	2 (2.2)	1.00
Diabetes	40 (10.0)	36 (10.0)	4 (9.5)	1.00	27 (8.7)	13 (14.1)	0.13
Cerebrovascular disease	12 (3.0)	9 (2.5)	3 (7.1)	0.23	6 (1.9)	6 (6.5)	0.06
Chronic pulmonary Disease	15 (3.7)	11 (3.1)	4 (9.5)	0.01	9 (2.9)	6 (6.5)	0.11
Chronic liver disease	17 (4.2)	15 (4.2)	2 (4.8)	1.00	12 (3.9)	5 (5.4)	0.72
Chronic kidney disease	5 (1.2)	4 (1.1)	1 (2.4)	1.00	3 (1.0)	2 (2.2)	0.70
Malignancy	25 (6.2)	18 (5.0)	7 (16.7)	0.009	14 (4.5)	11 (12.0)	0.01
Clinical outcome	–	–	–	<0.001	–	–	<0.001
Discharged	360 (89.6)	360 (100)	0		305 (98.4)	55 (59.8)	
Died	42 (10.4)	0	42 (100)		5 (1.6)	37 (40.2)	
ARDS	85 (21.1)	43 (11.9)	42 (100)	<0.001	17 (5.5)	68 (73.9)	<0.001
ICU admission	79 (19.7)	38 (10.5)	41 (97.6)	<0.001	15 (4.8)	64 (69.6)	<0.001
Mechanical Ventilation	76 (18.9)	36 (10.0)	40 (95.2)	<0.001	13 (4.2)	63 (68.5)	<0.001
Days from admission to ultrasonic examination, median (IQR), days	3 (2–5)	3 (2–5)	3 (1–4)	0.44	3 (2–5)	3 (2–5)	0.32
Length of hospital stay, median (IQR), days	27 (20–39)	28 (21–40)	23 (15–31)	0.002	27 (20–40)	27 (19–37)	0.88

Global LUS score: summing the scores of all 12 lung regions (two anterior, two lateral, and two posterior thoracic regions) (ranging from 0 to 36).
ARDS, acute respiratory distress syndrome; ICU, intensive care unit.

RESULTS

Patient Characteristics

A total of 407 patients with COVID-19 meeting the inclusion criteria were recruited, of whom 5 were excluded due to suboptimal LUS images ($n = 3$) and congestive heart failure ($n = 2$) (**Figure 2**). Four hundred two patients were included in the final analysis, of whom 42 died with median time to death 21 (IQR, 14–29) days. Cause of death was recorded as multiorgan failure (42.9%), respiratory failure (26.1%), cardiac (9.5%), septic shock (9.5%), unknown (7.1%), and stroke (4.8%). Baseline characteristics are summarized in **Table 1**. Non-survivors were older and more male gender compared to survivors. There was a higher prevalence of

TABLE 2 | Laboratory findings.

	Median (IQR)			*p*-value	Median (IQR)		*p*-value
	Total (*N* =402)	**Survivors (*N* = 360)**	**Non-survivors (*N* = 42)**		**Global LUS Score <15 (*N* = 310)**	**Global LUS Score ≥15 (*N* = 92)**	
Blood count							
WBC count, ×109/L	5.94 (4.73–7.56)	5.85 (4.62–6.87)	6.99 (4.98–10.51)	0.045	5.85 (4.62–6.87)	6.99 (4.98–10.51)	<0.001
Lymphocyte count, ×109/L	1.49 (1.11–1.87)	1.45 (1.09–1.85)	0.45 (0.28–0.78)	<0.001	1.60 (1.25–1.96)	0.97 (0.45–1.38)	<0.001
Platelet count, ×109/L	205 (160–250)	210 (167–255)	140 (92–208)	<0.001	211 (168–256)	179 (139–223)	<0.001
Hemoglobin, g/dl	120 (107–132)	121 (109–132)	104 (92–124)	0.001	122 (112–134)	107 (95–124)	<0.001
Coagulation function							
PT, s, (*n* = 384)	13.0 (12.4–13.8)	12.9 (12.4–13.6)	15.8 (13.9–18.4)	<0.001	12.9 (12.4–13.6)	13.8 (12.8–16.2)	<0.001
APTT, s, (*n* = 384)	37.1 (34.5–41.7)	36.7 (34.2–40.4)	47.9 (39.3–58.4)	<0.001	36.5 (34.2–40.3)	40.5 (35.1–49.5)	<0.001
D-dimer, mg/L, (*n* = 384)	0.44 (0.22–1.22)	0.39 (0.21–0.93)	3.08 (1.36–8.00)	<0.001	0.37 (0.20–0.84)	1.10 (0.39–3.01)	<0.001
Blood biochemistry							
TP, g/L	66.3 (62.7–70.2)	66.7 (63.5–70.6)	59.5 (54.8–65.4)	<0.001	66.7 (63.6–70.5)	64.3 (57.7–68.4)	0.003
Albumin, g/L	38.6 (35.0–41.5)	39.2 (36.3–41.8)	26.9 (24.4–30.1)	<0.001	39.6 (365. –41.9)	33.7 (27.0–38.2)	<0.001
ALT, U/L	28 (19–47)	29 (19–46)	37.0 (22–70)	0.06	29.0 (19.5–46.0)	26.0 (18.0–47.0)	0.08
AST, U/L	24 (19–32)	23 (19.0–31)	42 (29–75)	0.01	23.0 (18.0–30.5)	31 (22.0–45.0)	0.02
TB, μmol/L	10.4 (7.8–13.7)	10.0 (7.7–13.1)	14.7 (9.5–28.8)	0.002	10.2 (7.8–13.2)	11.0 (7.5–15.4)	0.05
Sodium, mmol/L	139.8 (138.5–141.6)	139.7 (138.5–141.3)	141.5 (138.6–144.3)	0.05	139.8 (138.7–141.4)	139.8 (137.5–142.5)	0.98
Potassium, mmol/L	4.15 (3.90–4.37)	4.16 (3.93–4.37)	3.96 (3.55–4.39)	0.73	4.17 (3.94–4.37)	4.10 (3.79–4.40)	0.58
BUN, mmol/L, (*n* = 382)	4.92 (3.90–6.01)	4.75 (3.84–5.69)	10.61 (6.85–18.48)	<0.001	4.70 (3.87–5.70)	5.65 (4.23–10.52)	<0.001
Creatinine, μmol/L	64.3 (53.8–77.0)	63.8 (53.9–75.5)	76.9 (50.7–140.3)	0.024	63.5 (54.3–75.7)	68.7 (50.3–91.0)	0.05
hs-cTnI, pg/mL, (*n* = 382)	3.3 (1.7–12.1)	2.6 (1.6–6.5)	100.6 (29.3–407.4)	<0.001	2.50 (1.53–5.18)	15.4 (4.12–98.05)	<0.001
LDH, U/L	180 (153–228)	174 (151–206)	393 (278–670)	<0.001	174 (150–206)	216 (166–365)	0.001
CK-MB, U/L (*n* = 347)	0.9 (0.4–9.0)	0.8 (0.4–7.0)	21.6 (9.0–34.3)	0.008	0.8 (0.4–8.0)	1.9 (0.6–21.1)	0.03
Infection-related biomarkers							
CRP, mg/L, (*n* = 370)	3.03 (0.72–10.4)	2.43 (0.62–5.92)	90.19 (53.7–125.8)	<0.001	2.37 (0.59–5.8)	24.93 (2.21–105.6)	<0.001
PCT, ng/ml, (*n* = 370)	0.06 (0.04–0.13)	0.06 (0.04–0.11)	0.38 (0.14–1.51)	<0.001	0.06 (0.04–0.10)	0.07 (0.07–0.43)	0.03

WBC, white blood cell; PT, prothrombin time; APTT, activated partial thromboplastin time; TP, total protein; ALT, alanine transaminase; AST, aspartate aminotransferase; TB, total bilirubin; BUN, blood urea nitrogen; hs-cTnI, hypersensitive troponin I; LDH, lactate dehydrogenase; CK-MB, creatine kinase–MB; CRP, hypersensitive C-reactive protein; PCT, procalcitonin.

preexisting conditions including hypertension, coronary heart disease (CHD), and malignancy in non-survivors compared to survivors.

Laboratory Findings

Laboratory data on hospital admission are summarized in **Table 2**. Overall, non-survivors had significant worse

TABLE 3 | Lung ultrasound findings.

	No. (%)			*p*-value
	Total (*N* = 402)	**Survivors (*N* = 360)**	**Non-survivors (*N* = 42)**	
Normal baseline lung ultrasound	84 (20.9)	84 (23.3)	0	<0.001
Abnormal baseline lung ultrasound	318 (79.1)	276 (76.7)	42 (100)	
Characteristics of lung ultrasound				
B line	318 (79.1)	276 (76.7)	42 (100)	<0.001
B1 line	236 (58.7)	210 (58.3)	26 (61.9)	0.66
B2 line	213 (51.5)	171 (45.8)	42 (100)	<0.001
Pleural line abnormalities	137 (31.8)	103 (26.4)	34 (78.6)	<0.001
Pulmonary consolidation	117 (25.6)	83 (20.6)	34 (69.0)	<0.001
Pleural effusion	36 (8.2)	18 (4.4)	18 (40.5)	<0.001
Distribution at baseline ultrasound				<0.001
Right lung	63 (15.7)	63 (17.5)	0	
Left lung	30 (7.5)	30 (8.3)	0	
Bilateral lungs	223 (55.5)	181 (50.3)	42 (100)	
Abnormalities at lung region				
Left anterior superior	129 (32.1)	93 (25.8)	36 (85.7)	<0.001
Left anterior inferior	112 (28.4)	74 (20.6)	38 (90.5)	<0.001
Left lateral superior	128 (31.8)	100 (27.8)	28 (66.7)	<0.001
Left lateral inferior	153 (38.1)	112 (31.1)	41 (97.6)	<0.001
Left posterior superior	111 (27.6)	81 (22.5)	30 (71.4)	<0.001
Left posterior inferior	156 (38.8)	125 (34.7)	31 (73.8)	<0.001
Right anterior superior	139 (34.5)	108 (30.0)	31 (73.8)	<0.001
Right anterior inferior	138 (34.3)	102 (28.3)	36 (85.7)	<0.001
Right lateral superior	129 (32.1)	103 (28.6)	26 (61.9)	<0.001
Right lateral inferior	160 (39.8)	120 (33.3)	40 (95.2)	<0.001
Right posterior superior	142 (35.3)	107 (29.7)	35 (83.3)	<0.001
Right posterior inferior	150 (37.3)	130 (36.1)	20 (47.6)	0.14
Global LUS score, median (IQR)	4 (1–13)	3 (1–9)	20 (18–23)	<0.001
Anterolateral LUS score, median (IQR)	2 (0–8)	5 (0–9)	14 (11–15)	0.001

laboratory results, including increased white blood cell count, prothrombin time, activated partial thromboplastin time, D-dimer, aspartate aminotransferase, total bilirubin, blood urea nitrogen, creatinine, hypersensitive troponin I (hs-Tnl), lactate dehydrogenase (LDH), creatine kinase–myocardial band (CK-MB), hypersensitive C-reactive protein (CRP), and procalcitonin and decreased lymphocyte count, platelet count, hemoglobin, total protein, and albumin (all $p < 0.05$) compared to survivors. Patients with a higher global LUS score (>15) had significant worse laboratory results, in particular, significantly increased D-dimer and CRP compared to those with a global LUS score <15.

Lung Ultrasound Findings and Lung Ultrasound Score

Lung ultrasound was performed within a median of 3 (IQR, 2–5) days from hospital admission. Lung ultrasound findings are shown in **Table 3**. Eighty-four patients (20.9%) had normal LUS. The presence of B lines was the most common finding (318/402, 79.1%), followed by pleural line abnormalities (137/402, 31.8%) and consolidation (117/402, 25.6%). Pleural effusions were detected in 36 (8.2%) patients. Compared to survivors, non-survivors were more likely to have B2 lines, pleural line abnormalities, pulmonary consolidation, and pleural effusion, but there was no difference in the presence of B1 lines. All non-survivors had bilateral involvement. Survivors had significantly lower global and anterior–lateral LUS scores compared to non-survivors (**Figure 3**). Findings of each of 12 lung regions are shown in **Supplementary Figure 2**. Regional LUS scores including anterior, lateral, and posterior scores are presented in **Supplementary Figure 3**. Bland–Altman plots for intra- and interobserver variability of global LUS score are shown in **Supplementary Figure 4**. All repeated measures were within 1.96 × standard deviation of the mean, which suggested a good reproducibility of global LUS score.

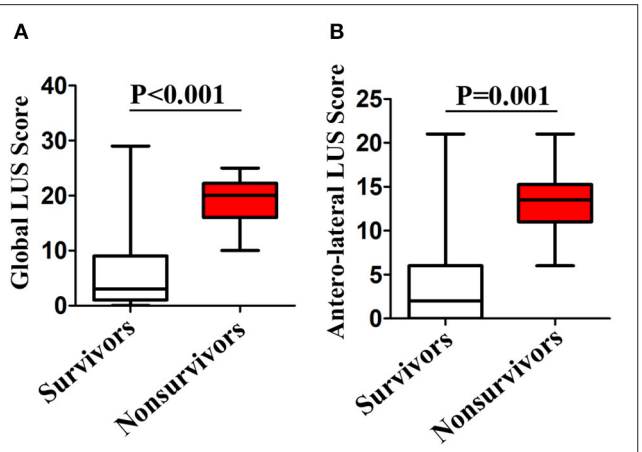


FIGURE 3 | Box plots representation of **(A)** global and **(B)** anterolateral lung ultrasound (LUS) scores in survivors and non-survivors.

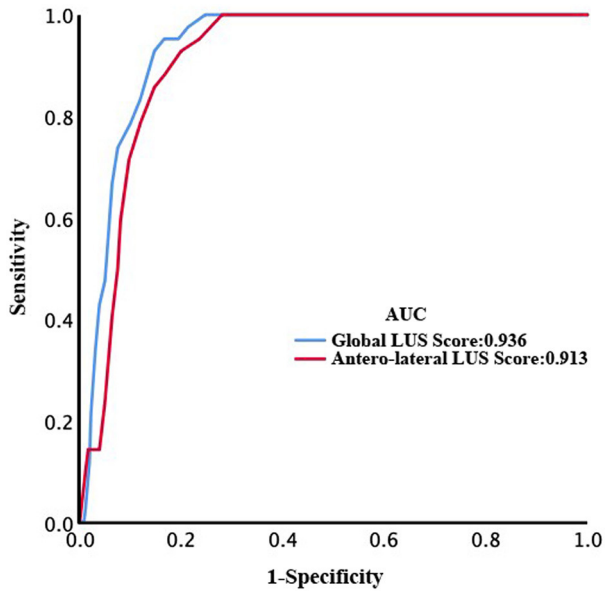


FIGURE 4 | Receiver operating characteristic (ROC) curves of global and anterolateral lung ultrasound (LUS) score for prediction of death.

Prediction of Mortality by LUS Global and Anterolateral Score

After a median of 27 (IQR, 20–39) days of follow-up, 42 patients died. ROC curve analyses of global and anterolateral LUS score for predicting mortality are shown in **Figure 4**. The area under the curve were 0.936 and 0.913 for global and anterolateral LUS score, respectively. A cutoff value of 15 for global LUS score had a sensitivity of 92.9% and specificity of 85.3% for prediction of death, and a cutoff value of 9 for anterolateral LUS score had a sensitivity of 88.1% and specificity of 83.3%. Clinical characteristics and laboratory findings dichotomized according to global LUS score optimal value of 15 are shown in **Tables 1**, **2**.

Kaplan–Meier analysis showed that both global and anterolateral LUS scores were strong predictors of death (**Figure 5**). When global LUS score was >15, 37/92 (40.2%) patients died compared to only 5/310 (1.6%) death in those with a global LUS score <15. When patients were dichotomized by anterolateral LUS score of 9, there were 36/97 (37.1%) deaths in patients with a high score compared to 6/305 (2.0%) deaths in those with a low anterolateral score.

On univariate Cox regression analysis, age, male gender, malignancy, CHD, CRP, hs-cTnl, CK-MB, D-dimer, global LUS score, and anterolateral LUS score were significantly associated with mortality (**Table 4**). In multivariate model 1, considering global LUS score together with other significant predictors in the univariate model, age, male sex, CRP, CK-MB, and global LUS score [hazard ratio (HR), 1.08; 95%CI, 1.01–1.16, $p = 0.03$) remained as a significant predictor. In multivariate model 2, when anterolateral LUS score was tested with other variables, the predictive power of anterolateral LUS score did not remain significant.

DISCUSSION

Our data suggested that global LUS score was a predictor of in-hospital mortality independent of age, gender, comorbidities, and biochemical markers and was superior to LUS anterolateral score. The optimal threshold of 15 for global LUS score and 9 for anterolateral LUS score were in line with those derived from previous investigations (5, 8). These findings supported the clinical utility of LUS in patients with COVID-19 (7, 9) given its ease of use at point of care, low cost, lack of radiation exposure, and ready combination with other components of critical care ultrasonography (10, 11).

LUS features in patients with COVID-19 in our study manifested as multiple lesions, various types of B lines, irregularly pleural lines, and subpleural consolidations. B lines presented in 79.1% patients. B2 lines and consolidations were more common in non-survivors than in survivors. Pleural effusion, pleural thickening, and pneumothorax were less common in COVID-19 patients, which were consistent with the latest autopsy report (12) that COVID-19 patients presented with acute interstitial lung disease.

Bass et al. showed that LUS had high sensitivity for detection of interstitial and alveolar–interstitial lung disease with peripheral distribution (13). Consistent with these features, our findings suggested that global LUS score was highly predictive of death in COVID-19 and independent of other previously identified predictors. Non-survivors in our study were older and more male with higher prevalence of preexisting conditions including hypertension, CHD, and malignancy and higher levels of cardiac injury and systematic inflammation markers than survivors, which were in consistence with previous studies (14).

Another interesting finding of our study was that when the posterior regions were excluded, the predictive power of anterolateral LUS score disappeared in the multivariate cox regression model. This finding was consistent with chest CT findings that the most commonly involved lung segments in patients with COVID-19 were the dorsal segment of the right

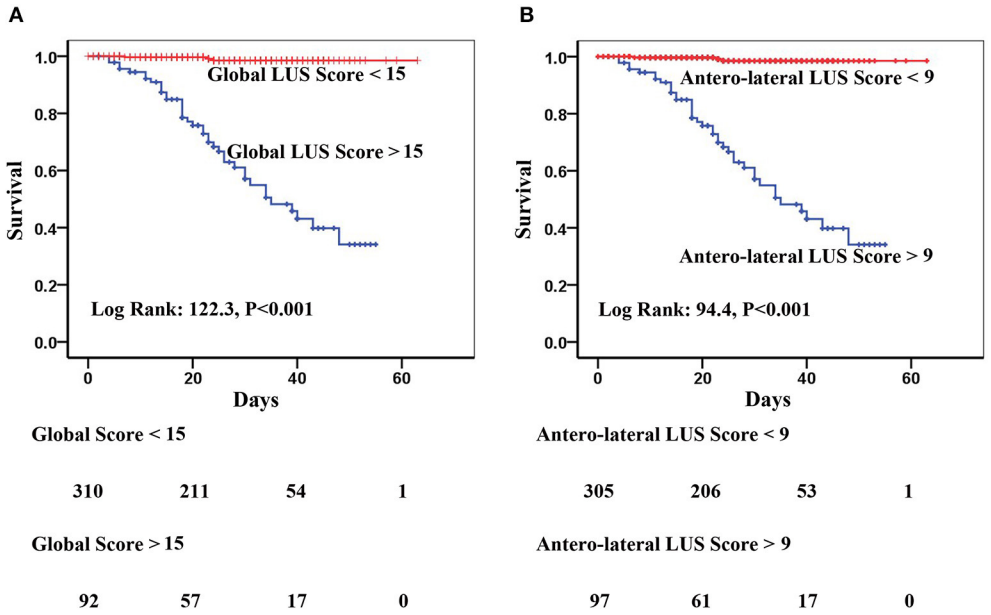


FIGURE 5 | Kaplan–Meier curves of **(A)** global lung ultrasound (LUS) score with optimal cutoff value of 15 and **(B)** anterolateral LUS score with optimal cutoff value of 9 for prediction of in-hospital mortality.

TABLE 4 | Univariate and multivariate Cox regression.

	HR	CI (95%)	*p*	HR	CI (95%)	*p*	HR	CI (95%)	*p*
		Univariate			Model 1			Model 2	
Age	1.04	1.01–1.07	**0.005**	1.05	1.00–1.10	**0.04**	1.05	1.00–1.09	**0.05**
Male sex	0.33	0.17–0.67	**0.002**	0.31	0.11–0.89	**0.03**	0.34	0.12–0.92	**0.03**
Hypertension	0.55	0.30–1.03	0.06						
Malignancy	0.32	0.14–0.72	**0.006**	0.57	0.18–1.81	0.34	0.57	0.18–1.82	0.34
CHD	0.28	0.15–0.52	**<0.001**	0.99	0.45–2.18	0.99	0.93	0.43–2.02	0.85
CRP	2.58	1.99–3.35	**<0.001**	1.60	1.17–2.20	**0.004**	1.69	1.23–2.31	**0.001**
hs-cTnI	1.82	1.61–2.04	**<0.001**	1.11	0.90–1.37	0.34	1.17	0.95–1.44	0.14
CK-MB	2.11	1.75–2.54	**<0.001**	1.47	1.09–1.99	**0.01**	1.54	1.13–2.08	**0.006**
D-Dimer	2.75	2.08–3.65	**<0.001**	1.19	0.83–1.69	0.34	1.17	0.82–1.66	0.38
Global LUS score	1.20	1.15–1.26	**<0.001**	1.08	1.01–1.16	**0.03**			
Anterolateral LUS score	1.23	1.17–1.29	**<0.001**				1.04	0.96–1.13	0.34
C-index					**0.995**			**0.994**	

CHD, coronary heart disease; CRP, C-reactive protein; hs-cTnI, hypersensitive troponin I; CK-MB, creatine kinase–myocardial band.
Global LUS score: summing the scores of all 12 lung regions (two anterior, two lateral, and two posterior thoracic regions) (ranging from 0 to 36). The values in bold represent statistical differences in data.

lower lobe, the posterior basal segment of the right lower lobe, the lateral basal segment of the right lower lobe, and the dorsal segment and the posterior basal segment of the left lower lobe (15). Despite some studies showing that the posterior regions had the lowest diagnostic accuracy (5), scores from these regions could play an important role in risk stratification. In the present study, lung lesions were mainly located in the right lateral inferior area (39.8%), left lateral inferior area (38.1%), left posterior inferior area (38.8%), and right posterior inferior area (37.3%) (the lower posterior and lateral segments of the lungs). This finding also supported that the potential benefit of prone position in patients affected with COVID-19 acute respiratory distress syndrome (ARDS) due to a more even distribution of the gas–tissue ratios along the dependent–non-dependent axis and a more homogeneous distribution of lung stress and strain (16).

Although anterolateral LUS score had less predive power compared to global LUS score, it may still play an important role particularly in patients on ICU.

Clinical Implications

COVID-19 as a global pandemic imposes a huge burden on medical systems. Early quantification of patients with severe lung

involvement may be critical for optimization of treatment and management. LUS as a non-invasive and cost-effective diagnostic tool can be performed rapidly, particularly in ICU. Severe studies have also demonstrated that echocardiography is a crucial tool in detecting cardiovascular complications (in particular on assessment of left and right ventricular function) and predicts poor prognosis in patients COVID-19 (17, 18). Combining LUS with echocardiography may add additional value to identify patients at higher risk of poor outcomes.

Limitations

Our study has several limitations. First, mortality rate was relatively low, which limits the strength of our conclusion. Low mortality rate may be due to the fact that majority of patients in the present study were not in ICU, while this rate was similar to previously published data (19). Second, the follow-up period was relatively short, as majority of patients were discharged within 28 days from admission.

Although our findings suggested that LUS may add additional value in risk stratification, the strength of our conclusion may be limited by the nature of an observational study. There are several other limitations of LUS that cannot be ignored such as the requirement of special training to perform high-quality LUS, lack of evidence-based guidelines, the high risk of infection when performing LUS examination in patients with COVID-19 (20, 21).

Patients included in this study were recruited from three hardest-hit hospitals in Wuhan, and these patients may not represent the population in other areas. Finally, the relationship between LUS and lung CT was not explored, as the majority of patients did not have lung CT due to limited availabilities and the nature of infectious disease.

CONCLUSION

Global LUS score as a semiquantitative measure of lung conditions is a powerful predictor of in-hospital mortality in patients with COVID-19 and may add additional value in patient monitoring and risk stratification.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

ZS, HW, HG, and MX conceived and designed the study. ZZ and JL contributed to the literature search. ZS, CC, SQ, YS, YD, WZ, MY, and LJ contributed to data collection. ZZ, CY, and HG contributed to data analysis. JW, YY, QL, and HG contributed to data interpretation. YX and RW contributed to the figures. ZS, HG, ZZ, and JL drafted the article. All authors contributed to the article and approved the submitted version.

FUNDING

We acknowledge the financial support from the National Natural Science Foundation of China (grant 81922033 to Li Zhang; grant 81727805 to MX; and grant 81701716 to ZS) and National Institute for Health Research, UK (ICA-CL-2018-04-ST2-012) to HG and by British Heart Foundation, UK (PG/19/23/34259) to HG.

ACKNOWLEDGMENTS

We are grateful for all our colleagues, especially Dr. Li Zhang and Hongliang Yuan, for their support to the present study. We are also grateful to the many frontline medical staffs for their dedication in the face of this outbreak despite the potential threat to their own lives and the lives of their families.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.633539/full#supplementary-material

REFERENCES

1. Shi H, Han X, Jiang N, Cao Y, Alwalid O, Gu J, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. *Lancet Infect Dis.* (2020) 20:425–34. doi: 10.1016/S1473-3099(20)30086-4
2. Lepri G, Orlandi M, Lazzeri C, Bruni C, Hughes M, Bonizzoli M, et al. The emerging role of lung ultrasound in COVID-19 pneumonia. *Eur J Rheumatol.* (2020) 7:129–33. doi: 10.5152/eurjrheum.2020.2063
3. Volpicelli G, Lamorte A, Villen T. What's new in lung ultrasound during the COVID-19 pandemic. *Intensive Care Med.* (2020) 46:1445–48. doi: 10.1007/s00134-020-06048-9
4. Zhang YK, Li J, Yang JP, Zhan Y, Chen J. Lung ultrasonography for the diagnosis of 11 patients with acute respiratory distress syndrome due to bird flu H7N9 infection. *Virol J.* (2015) 12:176. doi: 10.1186/s12985-015-0406-1
5. Pisani L, Vercesi V, van Tongeren PSI, Lagrand WK, Leopold SJ, Huson MAM, et al. The diagnostic accuracy for ARDS of global versus regional lung ultrasound scores - a post hoc analysis of an observational study in invasively ventilated ICU patients. *Intensive Care Med Exp.* (2019) 7:44. doi: 10.1186/s40635-019-0241-6
6. Bouhemad B, Mongodi S, Via G, Rouquette I. Ultrasound for "lung monitoring" of ventilated patients. *Anesthesiology.* (2015) 122:437–47. doi: 10.1097/ALN.0000000000000558

7. Volpicelli G, Elbarbary M, Blaivas M, Lichtenstein DA, Mathis G, Kirkpatrick AW, et al. International evidence-based recommendations for point-of-care lung ultrasound. *Intensive Care Med.* (2012) 38:577–91. doi: 10.1007/s00134-012-2513-4
8. Tierney DM, Boland LL, Overgaard JD, Huelster JS, Jorgenson A, Normington JP, et al. Pulmonary ultrasound scoring system for intubated critically ill patients and its association with clinical metrics and mortality: a prospective cohort study. *J Clin Ultrasound.* (2018) 46:14–22. doi: 10.1002/jcu.22526
9. Nazerian P, Volpicelli G, Vanni S, Gigli C, Betti L, Bartolucci M, et al. Accuracy of lung ultrasound for the diagnosis of consolidations when compared to chest computed tomography. *Am J Emerg Med.* (2015) 33:620–5. doi: 10.1016/j.ajem.2015.01.035
10. Peng QY, Wang XT, Zhang LN. Findings of lung ultrasonography of novel corona virus pneumonia during the 2019-2020 epidemic. *Intensive Care Med.* (2020) 46:849–50. doi: 10.1007/s00134-020-05996-6
11. Vetrugno L, Bove T, Orso D, Barbariol F, Bassi F, Boero E, et al. Our Italian experience using lung ultrasound for identification, grading and serial follow-up of severity of lung involvement for management of patients with COVID-19. *Echocardiography.* (2020) 37:625–27. doi: 10.1111/echo.14664
12. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med.* (2020) 8:420–22. doi: 10.1016/S2213-2600(20)30076-X
13. Bass CM, Sajed DR, Adedipe AA, West TE. Pulmonary ultrasound and pulse oximetry versus chest radiography and arterial blood gas analysis for the diagnosis of acute respiratory distress syndrome: a pilot study. *Crit Care.* (2015) 19:282. doi: 10.1186/s13054-015-0995-5
14. Luo X, Zhou W, Yan X, Guo T, Wang B, Xia H, et al. Prognostic value of C-reactive protein in patients with COVID-19. *Clin Infect Dis.* (2020) 71:2174–9. doi: 10.1093/cid/ciaa641
15. Wu J, Wu X, Zeng W, Guo D, Fang Z, Chen L, et al. Chest CT findings in patients with coronavirus disease 2019 and its relationship with clinical features. *Invest Radiol.* (2020) 55:257–61. doi: 10.1097/RLI.0000000000000670
16. Guérin C, Albert RK, Beitler J, Gattinoni L, Jaber S, Marini JJ, et al. Prone position in ARDS patients: why, when, how and for whom. *Intensive Care Med.* (2020) 46:2385–96. doi: 10.1007/s00134-020-06306-w
17. Moody WE, Mahmoud-Elsayed HM, Senior J, Gul U, Khan-Kheil AM, Horne S, et al. Impact of right ventricular dysfunction on mortality in patients hospitalized with COVID-19, according to race. *CJC Open.* (2021) 3:91–100. doi: 10.1016/j.cjco.2020.09.016
18. Cameli M, Pastore MC, Soliman Aboumarie H, Mandoli GE, D'Ascenzi F, et al. Usefulness of echocardiography to detect cardiac involvement in COVID-19 patients. *Echocardiography.* (2020) 37:1278–86. doi: 10.1111/echo.14779
19. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* (2020) 395:507–13. doi: 10.1016/S0140-6736(20)30211-7
20. Gargani L, Soliman-Aboumarie H, Volpicelli G, Corradi F, Pastore MC, Cameli M. Why, when, and how to use lung ultrasound during the COVID-19 pandemic: enthusiasm and caution. *Eur Heart J Cardiovasc Imaging.* (2020) 21:941–8. doi: 10.1093/ehjci/jeaa163
21. Di Serafino M, Notaro M, Rea G, Iacobellis F, Delli Paoli V, Acampora C, et al. The lung ultrasound: facts or artifacts? In the era of COVID-19 outbreak. *Radiol Med.* (2020) 125:738–53. doi: 10.1007/s11547-020-01236-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

MINI REVIEW
published: 26 May 2021
doi: 10.3389/fcvm.2021.681948

The Notch Pathway: A Link Between COVID-19 Pathophysiology and Its Cardiovascular Complications

Randa M. Breikaa[1,2] *and Brenda Lilly*[1,3]*

[1] Center for Cardiovascular Research and The Heart Center, Nationwide Children's Hospital, Columbus, OH, United States,
[2] Molecular, Cellular and Developmental Biology Program, The Ohio State University, Columbus, OH, United States,
[3] Department of Pediatrics, The Ohio State University, Columbus, OH, United States

COVID-19 is associated with a large number of cardiovascular sequelae, including dysrhythmias, myocardial injury, myocarditis and thrombosis. The Notch pathway is one likely culprit leading to these complications due to its direct role in viral entry, inflammation and coagulation processes, all shown to be key parts of COVID-19 pathogenesis. This review highlights links between the pathophysiology of SARS-CoV2 and the Notch signaling pathway that serve as primary drivers of the cardiovascular complications seen in COVID-19 patients.

Keywords: notch signaling pathway, COVID-19, cardiovascular, vascular biology, cardiovascular disease

OPEN ACCESS

Edited by:
Masanori Aikawa, Brigham and Women's Hospital and Harvard Medical School, United States

Reviewed by:
Jun-ichiro Koga, Kyushu University, Japan
Yafeng Li, Shanxi Provincial People's Hospital, China

***Correspondence:**
Brenda Lilly
brenda.lilly@nationwidechildrens.org

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *17 March 2021*
Accepted: *06 May 2021*
Published: *26 May 2021*

Citation:
Breikaa RM and Lilly B (2021) The Notch Pathway: A Link Between COVID-19 Pathophysiology and Its Cardiovascular Complications. Front. Cardiovasc. Med. 8:681948. doi: 10.3389/fcvm.2021.681948

INTRODUCTION

Beginning in December 2019, the world faced a challenging nemesis presented by a member of the coronaviruses family, SARS-CoV2, later known as Coronavirus Disease 2019 or COVID-19 (1–3). First feared for its aggressive attack on the respiratory system (4, 5), it is now recognized for its severe cardiovascular complications (6–8). These range from hemodynamic instabilities, dysrhythmias, and thromboembolic events, to myocarditis, acute heart failure and cardiac arrest (9, 10). Analyzing patient data from several countries, cardiovascular disease appears in two contexts associated with COVID-19. First, studies have shown that pre-existing cardiovascular disease increases the risk of COVID-19 infection and is indeed present in a high number of cases (11–13). Second, COVID-19 patients develop cardiovascular complications during the course of the disease (14, 15). Despite a clear connection with COVID-19 and the cardiovascular system, we understand little about this relationship.

Notch signaling is a master regulator of cardiovascular function in both health and disease, and has been linked to several biological processes mediating viral infections (16, 17). A recent study by Rosa et al., characterized transcriptional signatures induced in a rhesus macaque model of SARS-CoV2 and showed an increase in Notch signaling in the lungs of the macaques (18). Another group studying human protein interactions with SARS-CoV2 using computational models, showed that proteins interacting with the 5'-region of SARS-CoV2 RNA were associated with Notch2 receptor signaling (19). The Notch pathway is also implicated in the hypoxic response and in coagulopathic processes, both of which are present in COVID-19 patients. These known roles of the Notch pathway make this signaling pathway a likely player in the COVID-19-driven cardiovascular complications.

THE BEGINNING (VIRAL ENTRY)

The angiotensin converting enzyme 2 (ACE2) has been established to play a significant role in SARS-CoV viruses infectivity, including COVID-19, by binding to the viral spike protein and facilitating entry into the host cell (20, 21). ACE2 has distinct roles in the body, ranging from amino acid transportation and catalytic activities, to serving as functional receptors for viruses like the coronaviruses. In the heart, it is localized to cardiomyocytes, cardiac fibroblasts, epicardial adipose tissue, and the coronary vascular endothelium. In the lungs, it is expressed on the cell surface of the inner respiratory tract, protecting against lung injury. This protective effect stems from its negative regulation of the renin-angiotensin system which leads to the inhibition of the vasoconstrictive, pro-inflammatory angiotensin II (ANGII)—ANGII type 1 receptor (AT1) axis (22–24). Its unique location in both organs combined with its function make it a pivotal player in the pulmonary pathogenicity of the virus and its associated cardiovascular complications. Thus, ACE2 on one hand offers protection against injury, while on the other hand facilitates viral entry. Furthermore, upon binding of ACE2 to the viral particle, the receptor itself becomes endocytosed by the cells causing depletion of cell surface ACE2 and its mediated tissue protection (25, 26). This dilemma and the realization of the importance of ACE2 in maintaining cardiovascular homeostasis drove attempts to manipulate the ACE2/ANGII axis to mitigate virus-induced injury, while minimizing the negative effects on the protective functions of ACE2 (20, 23). One solution for this problem and an attractive target for vaccine development are the viral S-proteins, which when targeted make the enzyme unable to bind, preventing viral entry (21, 27).

Notch signaling has been known to interact with many viral particles facilitating their infectivity (**Table 1**). Given that Notch regulates various proliferative and differentiation events in cells, it is no surprise that the pathway is an attractive target for viruses, which are dependent on the cell cycle machinery of the cell. Those viruses tap into the Notch pathway to ensure their own survival (60–62). The first evidence that demonstrated Notch pathway-viral interactions was reported for the Epstein-Barr virus, which targets RBPJ (mouse)/CBF1 (human), the nuclear effector of Notch (28, 63). Other examples include the human papilloma virus (HPV), hepatitis B virus (HBV), and hepatitis C virus (HCV). In the case of HCV, the Notch1 receptor has been shown to facilitate nuclear localization of p65 in response to tumor necrosis factor-alpha (TNF-α) in human hepatocytes, leading to increased pathogenicity of the virus (64). Additionally, the influenza virus has been shown to block the Notch ligand Delta-like 1 (DLL1) causing a heightened inflammatory response and decreased interferon-c levels, which leads to compromised immunity against the virus. In contrast, macrophages were found to enhance their DLL1 production during the course of infection to protect against the same virus (32, 33, 65). In the case of COVID-19, an interesting enzyme that could be linking Notch and COVID-19 activation is FURIN. FURIN is a member of the protein convertases family and is both an activator and a direct target of Notch activity (66, 67). Its enzymatic activity has been proven to be exploited by a variety of bacteria and viruses, including measles, yellow fever, ebola, and avian influenza, thereby facilitating their virulence and spread (68, 69). To discern the potential role of FURIN in COVID-19, understanding the structure of the viral S-glycoprotein is important. The S-protein has two functional domains: one for receptor binding and the other for mediating fusion of the viral particle with the cell membrane. The S-protein must be cleaved by the protease to expose these fusion sequences and allow cell entry. FURIN takes on this role in coronaviruses including COVID-19 (70–72). Since Notch1 has been shown to transcriptionally induce FURIN, Notch signaling may indirectly lead to enhanced viral entry via enhanced FURIN expression (73, 74).

In addition to having effects on viral infectivity, interestingly both the Notch receptors and ACE2 receptor share a common mechanism of activation through cleavage by the A disintegrin and metalloproteinase (ADAM) family of enzymes, specifically ADAM17 (75, 76). ADAM17 mediates ectodomain shedding of ACE2 which can facilitate viral entry (77, 78). ADAM17 also activates the Notch signaling pathway via receptor cleavage leading to increased viral infectivity through regulation of FURIN. Therefore, Notch activity is indirectly involved in COVID-19 infectivity through FURIN induction and shared activation axis of ACE2, both of which aid in viral entry.

THE CYTOKINE STORM

A balanced innate and adaptive host immunity is key for an effective antiviral response, including activation of T cells, macrophages, and production of various pro-inflammatory cytokines. However, in case of COVID-19, this response becomes heightened, causing a hyperinflammatory reaction known as "The Cytokine Storm Syndrome" (14, 27). The cytokine storm is one of the key factors causing cardiovascular complications in COVID-19 patients. This is attributed to the resulting inflammation-induced vascular injury, myocarditis, arrhythmia, and destabilization of coronary artery plaques leading to myocardial infarcts (79, 80). The common profile of a COVID-19 patient with cytokine storm syndrome includes elevated interleukin-6 (IL-6), IL-2 receptor, TNF-α, granulocyte-colony stimulating factor, among others. IL-6 is secreted by activated leukocytes, promotes differentiation of B lymphocytes and production of acute phase proteins, and is important for thermoregulation (14, 81).

The role of the Notch pathway in inflammation is well-documented, where it has been shown to promote the pro-inflammatory microenvironment (82–84). It is implicated in macrophage polarization and contributes to amplification of the inflammatory loop by promoting the M1 phenotype of macrophages over the M2 phenotype (17, 85). Furthermore, in macrophages, Notch1 directly binds the IL-6 promoter and activates IL-6 transcription in response to interferon-γ (81, 86). Additionally, IL-6 in turn increases the expression of the Notch ligand DLL1, amplifying the Notch signal. This works as a positive feedback loop that further drives the production of

TABLE 1 | Reported link of the Notch signaling pathway to common viral infections.

Viral infection	Link to notch	References
Epstein-barr virus	The Epstein-Barr virus nuclear antigen 2 (EBNA2) is tethered to promoters by targeting RBPJ, the nuclear effector of Notch. Since EBNA2 has been proven to be partly interchangeable with Notch intracellular domain in activation of target genes modulating differentiation processes, it is seen as a biological equivalent of an activated Notch receptor. The Epstein-Barr virus-encoded latent membrane protein 2A (LMP2A) promotes cellular migration mediated by Notch signaling by altering mitochondrial dynamics.	(28–31)
Influenza virus	Macrophages are reported to enhance their Notch ligand DLL1 production in response to the viral infection to protect against the virus. Blocking DLL1 caused heightened inflammatory response and decreased interferon-c levels, leading to a compromised immunity against the virus.	(32–34)
Respiratory syncytial virus (RSV)	Notch signaling has been reported to contribute to the production of inflammatory cytokines induced by the virus in alveolar macrophages. Notch signaling communicates with the Toll-like receptor (TLR) pathway to fine-tune the innate inflammatory responses. In studies where TLR pathway was activated, while Notch signaling was inhibited, RSV-enhanced respiratory disease (ERD) was prevented.	(35, 36)
Human papilloma virus (HPV)	Notch inhibition impairs epithelial differentiation, which is suggested to contribute to HPV replication and viral oncogenesis. HPV8E6 protein inhibits Notch transcriptional activator complexes involving RBPJ and MAML at the Notch target genes, decreasing Notch activity during keratinocyte differentiation. HPV16E6 protein increases Notch levels in keratinocytes. HPV16E6 potentiates Notch activation and differentiation without activating cellular arrest, entirely uncoupling cellular arrest from increased differentiation.	(37–42)
Human T-cell leukemia virus type 1 (HTLV-I)	Notch signaling promotes proliferation and tumor formation of HTLV-I-associated adult T-cell leukemia.	(43, 44)
Hepatitis C virus (HCV)	Notch signaling regulates T Helper 22 Cells in chronic HCV patients. Notch1 receptor has been shown to facilitate nuclear localization of p65 in human hepatocytes in response to TNF-α, leading to increased pathogenicity of the virus. HCV NS3 protein leads to Notch activation by binding to SRCAP transcription factor. HCV causes Notch-dependent modulation in miRNA-449a levels, leading to differential expression of the inflammatory biomarker YKL40.	(45–48)
Hepatitis B virus (HBV)	HBV increases Notch1 and TGF-β levels on intrahepatic T cells in cirrhosis, promoting fibrogenesis and disease progression. HBV X protein activates Notch signaling by increasing DLL4 and Notch1, promoting the growth of hepatocellular carcinoma, in addition to increasing CREB-mediated activation of miR-3188. HBV X protein causes Notch-dependent decrease in nuclear factor-kappa B (NF-κB) signaling. Notch signaling contributes to hepatic inflammation in HBV infection by regulating IL-22-producing cells. Notch signaling aids in transcription of HBV covalently closed circular DNA by a mechanism involving cAMP response element-binding protein and E3 ubiquitin ligase-modulation. In acute hepatitis B (AVH-B) infection, a complementary association between Notch1 and Hes1 in $CD8^+$T cells was reported. In chronic hepatitis B (CHB) infection, repression of the Notch receptors mediates the immune response regulation in patients who progress to cirrhosis and hepatocellular carcinoma.	(49–55)
Human immunodeficiency virus (HIV)	Notch signaling is activated in HIV-associated nephropathy, where Notch ligands (Jagged-1, Jagged-2, DLL1, and DLL4) are all up in kidney tubules, while glomeruli show minimal ligand expression. Notch1 and 4 receptors are up in glomeruli, and only Notch4 is expressed in tubules. Notch inhibition results in improvement of kidney injury scores and renal functions, and blocks podocyte proliferation induced by HIV proteins Nef and Tat.	(56–59)

more IL-6 (87, 88). Nitric Oxide Synthase (iNOS) expression is linked to manifestation of the cytokine storm (89, 90). Direct interaction between the Notch Intracellular Domain (NICD) and TNF-α on the iNOS promoter has also been documented, indicating multiple avenues by which Notch signaling drives hyper-inflammation (91). Further, TNF-α itself has been shown to induce expression of Notch1 and Notch4, in addition to regulating NICD nuclear translocation, which leads to the activation of Notch downstream mediators (92, 93).

This interplay between Notch and pro-inflammatory processes makes the Notch pathway an attractive target for reversing inflammatory events. Indeed, genetic and pharmacological inhibition of Notch signaling was reported to ameliorate disease progression in many inflammatory disease models. These include rheumatoid arthritis, autoimmune encephalomyelitis, and several models of infectious disease (94, 95). In the case of COVID-19, the recommendation to use corticosteroids was discouraged due to controversial efficacy and reports showing exacerbation of patient symptoms. Potentially targeting the Notch pathway to specifically block the inflammatory loop re-enforced by IL-6 and TNF-α may present a viable therapy for these cases (96–98).

THE HYPOXIC RESPONSE

The hypoxic events in COVID-19 patients have been a mystery to medical caretakers and physicians. This is due to the fact that the patients display minimal visible distress, although clinical oxygen levels are remarkably low (99). Its presentation defies its pathophysiology, which initially led to its description of "Happy Hypoxia" (100). Hypoxia is also linked to the thrombotic events seen in these patients, which spirals quickly into more severe cardiovascular complications such as myocarditis and myocardial infarction (101–103).

The Notch pathway plays a significant role in hypoxic events (104). Notch3 is induced under hypoxic conditions in the lungs and vasculature. Notch3 deletion has been shown to protect against the development of pulmonary arterial hypertension in response to hypoxic stimulation (105, 106). Further, Notch3 was found to cooperate with the hypoxia-inducible factor-1 alpha (HIF-1α) (105, 107), a transcription factor upregulated in hypoxia and inflammatory microenvironments and a master regulator of oxygen homeostasis (108). HIF-1α also induces the expression of two of the Notch ligands, DLL4 and Jagged-1 (109–111). Another link between Notch signaling and HIF-1α is through Notch1 receptor. As mentioned previously, Notch1 receptor has been shown to promote M1 macrophage polarization and switching of macrophage metabolism to glycolysis. This is followed by induction of M1 gene transcription, coupled with an increase in mitochondrial oxidative phosphorylation and generation of reactive oxygen species (112). This in turn activates HIF-1α to induce M1 macrophage activation, in a type of positive feedback loop (111).

Additionally, enhanced Notch signaling has been linked to structural changes in air sacs in the lungs that include decreased septation of terminal alveoli, emphysematous patterns and progressive fibrotic changes (113). Furthermore, Notch3 plays a critical role in regulating alveolar epithelium and increased levels of Notch3 are associated with disruption of differentiation processes and altered lung morphology (114). Interestingly in COVID-19-associated hypoxia, the air sacs do not fill up with fluid like in pneumonia, but also show structural changes in the sacs that lead them to collapse (115, 116). Hence, Notch activation in COVID-19 patients is likely directly exacerbating the hypoxic events by cooperating with HIF-1α in addition to promoting structural defects in the air sacs.

THE COAGULOPATHIC RESPONSE

The realization that COVID-19 causes hypercoagulopathy poses more questions than answers, with studies showing severe thrombotic manifestations, while others show postmortem lung sections with extensive bleeding (117, 118). In a recent study by Boonyawa et al. a 28% incidence of venous thromboembolism was reported in COVID-19 patients in the intensive care unit (119). Another study by Klok et al. found a 31% incidence of combined deep vein thrombosis, pulmonary embolism, and arterial thrombosis in critically ill patients (120). Thus, there is an urgent need to understand the rate of bleeding and thrombotic events associated with COVID-19 coagulopathy.

Hypercoagulopathy is an important hallmark of inflammation. In fact, pro-inflammatory cytokines are directly involved in accelerating platelet hyperactivation and driving thrombotic events, while impairing crucial physiological anticoagulation pathways including antithrombin III, tissue factor pathway, and the protein C system (121, 122). The mechanisms involved in COVID-19 coagulopathy have not been fully elucidated yet, but crosstalk between the coagulation and the inflammatory systems is evident, with at least four factors seeming to contribute to this condition (123). First, the pro-inflammatory mediators such as IL-6 and IL-1β produced during the cytokine storm stimulate the production of tissue factor on immune cells. This in turn initiates the activation of the extrinsic coagulation cascade (81, 124). Secondly, those pro-inflammatory mediators directly activate the platelets themselves (125). Thirdly, a decrease in plasminogen activator coupled with an increase in plasminogen activator inhibitor suppresses the fibrinolytic system (126, 127). Lastly, the damage caused to the endothelial cells by the inflammatory reaction results in vascular homeostatic imbalances, causing accelerated local thrombotic events in addition to systemic coagulation defects. Of note is that this damaged endothelium also binds platelets more readily due to enhanced platelet-vessel wall interaction caused by the large von Willebrand factor multimers released by damaged endothelial cells (128).

Despite efforts by the scientific community to understand COVID-19-associated coagulopathy, there is still a lot to clarify regarding mechanisms involved and how to reverse the resulting homeostatic imbalances. Previous studies by Duarte et al. and Gough beautifully demonstrated a link between the Notch pathway and the coagulation pathway through fibroblast growth factor 1 (FGF1) (129, 130). These studies utilized a soluble form of the Notch ligand Jagged-1 to show the effect of Notch inhibition on FGF1 and the coagulation cascade. This link between Notch signaling and coagulation is supported by several previous findings. First, the activation of the coagulation cascade by damaged tissue generates thrombin, which activates the protease-activated receptor 1 (PAR1) and PAR1-dependent FGF1 expression and release. Released FGF1 subsequently promotes angiogenesis and induces Jagged-1 expression in the damaged tissue (131). Second, Alagille syndrome patients, who primarily have mutations in Jagged-1, show bleeding disorders (132). Consistent with this, Jagged-1 null mice show hemorrhage during their embryonic development (133, 134). Lastly, Jagged-1 was found to be the FGF1 response gene responsible for FGF1-dependent endothelial cell differentiation on fibrin matrices (131, 135). Taken together, these studies indicate that there is a Notch-dependent mechanism by which thrombin can regulate FGF1 secretion, which in turn contributes to thrombin's activity, the key protease of the coagulation cascade. Thus, through its established role in both inflammation and coagulation, Notch signaling seems likely responsible for exacerbating COVID-19-associated coagulopathy.

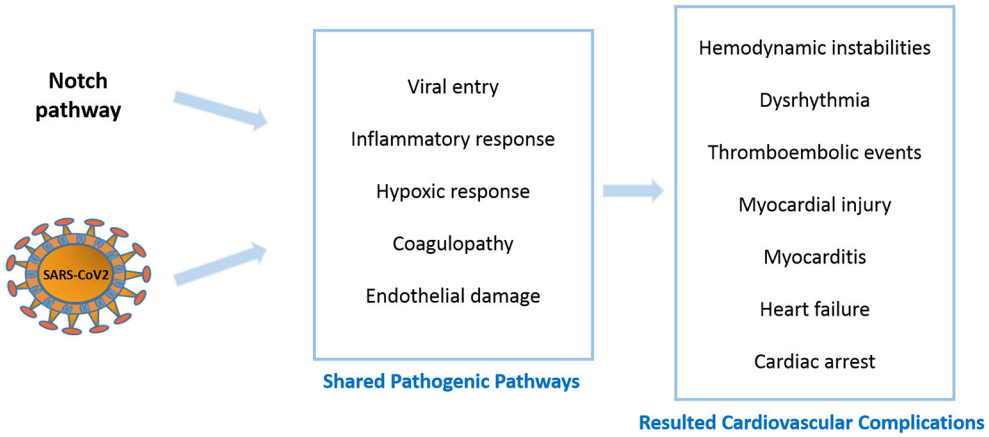


FIGURE 1 | Notch signaling and COVID-19 shared pathogenic events and resulted cardiovascular complications.

ENDOTHELIAL CELL INVOLVEMENT

The endothelium is a single layer of cells lining blood vessels, constituting a barrier between the circulation and the rest of the blood vessel wall. In addition, it is the source for several vasoreactive substances responsible for blood vessel contraction and relaxation such as endothelin and nitric oxide (136). Thus, it is a key regulator of vascular homeostasis, and damage to this layer can lead to loss of the homeostatic state and exacerbation of disease conditions. Indeed, endothelial dysfunction shifts the vascular equilibrium toward an inflammatory, pro-coagulant state (137, 138). Coordination of leukocyte trafficking in particular is critical for the inflammatory response. Under physiological conditions, the endothelial cells prevent binding and extravasation of leukocytes from the blood. However, under disease conditions such as the case in COVID-19 patients, the endothelial junctions are weakened and leaky, resulting in facilitated exit of the leukocytes from the circulation into the tissues (82, 93, 139). Interestingly, immunostaining studies have shown the confinement of Notch4 receptors on endothelial cells at the apical membrane. This localization makes Notch4 ideal for receptor/ligand communication between the endothelial and the inflammatory cells in the blood stream (140). In addition, the Notch ligand DLL4 on the endothelium has been shown to trigger a bidirectional Notch signaling between endothelial cells and monocytes (93, 141).

Several reports have linked endothelial cells to SARS-CoV2 pathology, where histological sections through hearts, kidneys and lungs showed accumulation of both inflammatory cells and viral particles within the endothelium (81, 142, 143). This COVID-19-associated endotheliitis could explain the impaired circulatory function in the various vascular beds and the clinical complications in COVID-19 patients (144–146). Furthermore, endothelial cells express the ACE2 receptor, the entry portal for the virus (147–150). This, coupled with previous reports of development of autoantibodies against endothelial cells after SARS-CoV1 infection, suggests that CoV2 infection of endothelial cells and their subsequent damage is a prominent step in the pathogenesis of COVID-19 (151, 152). Important to consider here is the discrepancy that exists between current studies, where some advocate for the endothelial cell hypothesis of COVID-19 pathology, which reinforces the idea that endothelial cells are the origin for COVID-19-associated cardiovascular impairments. In contrast, others promote a pericyte-COVID-19 hypothesis, where pericytes are the main contributors to disease progression. The studies that propose the pericyte hypothesis are based on the fact that ACE2 expression in the heart is highest in pericytes, and in the brain vasculature ACE2 is on vascular smooth muscle cells and pericytes and not on the endothelium (121, 153). Although the endothelial cell hypothesis seems to be more plausible according to consequences of endotheliitis in COVID-19 patients, these discrepancies highlight the importance of considering tissue type in disease pathology.

CONCLUSIONS

COVID-19 is associated with a large number of cardiovascular sequelae, including dysrhythmias, myocardial injury, myocarditis and thrombosis. Many of these complications seem to be linked to compromised signaling pathways in the patients, including the Notch pathway (**Figure 1**). Notch signaling can indirectly enhance viral entry through inducing FURIN, the protease responsible for exposing the fusion sequences of the viral S-protein. The established role of Notch signaling in both inflammation and coagulation suggests its involvement in COVID-19 cytokine storm and hypercoagulopathy, both of which are main contributors to the cardiovascular complications. Furthermore, Notch activation is known to exacerbate hypoxic events by cooperating with HIF-1α in addition to enhancing structural defects in the air sacs in the lungs, which together may contribute to enhanced lung pathology in COVID-19 patients. Lastly, the suggested role of the endothelium in COVID-19 cardiovascular impairments coupled with the specific localization of Notch4 receptors on the apical membrane of

the endothelium reinforces the idea that the Notch pathway serves as a communication channel between endothelial and inflammatory cells.

In summary, several scenarios can be considered regarding the link between the Notch pathway and COVID-19-associated cardiovascular events. COVID-19 may act upstream to increase Notch signaling, leading to enhanced viral entry and associated pathogenic processes. Alternatively, maladaptive responses of Notch signaling due to COVID-19 infection may contribute to the enhanced inflammatory, coagulopathic, and hypoxic events. Both scenarios eventually lead to exacerbation of cardiovascular impairments in COVID-19 patients that are Notch-associated. Gamma-secretase inhibitors, which inhibit Notch receptor cleavage have been used to attenuate Notch signaling in cancer and Alzheimer disease (154, 155). These compounds, however, are associated with significant toxicity. Alternatives include Notch-specific antibodies and decoys. Antibodies allow blockade of individual Notch components, thus are not associated with complications seen with the pan inhibitors (156–158). Notch decoys also selectively block Notch receptors by a unique mechanism that involves mimicking the Notch extracellular domain of a specific Notch ligand or receptor (159, 160). Finally, uncovering new aspects of a Notch-COVID-19 relationship might help mitigate cardiac and pulmonary complications caused by the SARS-CoV family of viruses.

AUTHOR CONTRIBUTIONS

RB wrote, edited, and conceived of topic. BL edited, wrote, and provided topic guidance. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by NIH R01-HL132801 (BL) and NIH-5T32-HL134616 predoctoral fellowship (RB).

REFERENCES

1. Masters PS. The molecular biology of coronaviruses. *Adv Virus Res.* (2006) 66:193–292. doi: 10.1016/S0065-3527(06)66005-3
2. Chen Y, Liu Q, Guo D. Emerging coronaviruses: genome structure, replication, and pathogenesis. *J Med Virol.* (2020) 92:418–23. doi: 10.1002/jmv.25681
3. Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. *Acta biomed.* (2020) 91:157–60. doi: 10.23750/abm.v91i1.9397
4. Menter T, Haslbauer JD, Nienhold R, Savic S, Hopfer H, Deigendesch N, et al. Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. *Histopathology.* (2020) 77:198–209. doi: 10.1111/his.14134
5. Wu J, Li X, Huang B, Su H, Li Y, Luo D, et al. Pathological changes of fatal coronavirus disease 2019 (COVID-19) in the lungs: report of 10 cases by postmortem needle autopsy. *Zhonghua Bing Li Xue Za Zhi.* (2020) 49: 568–75. doi: 10.3760/cma.j.cn112151-20200405-00291
6. Lang JP, Wang X, Moura FA, Siddiqi HK, Morrow DA, Bohula EA. A current review of COVID-19 for the cardiovascular specialist. *Am Heart J.* (2020) 226:29–44. doi: 10.1016/j.ahj.2020.04.025
7. Long B, Brady WJ, Koyfman A, Gottlieb M. Cardiovascular complications in COVID-19. *Am J Emerg Med.* (2020) 3:1504–7. doi: 10.1016/j.ajem.2020.04.048
8. Matsushita K, Marchandot B, Jesel L, Ohlmann P, Morel O. Impact of COVID-19 on the cardiovascular system: a review. *J Clin Med.* (2020) 9:1407. doi: 10.3390/jcm9051407
9. Liu PP, Blet A, Smyth D, Li H. The science underlying COVID-19: implications for the cardiovascular system. *Circulation.* (2020) 142:68–78. doi: 10.1161/CIRCULATIONAHA.120.047549
10. Zheng YY, Ma YT. COVID-19 and the cardiovascular system. *Nat Rev Cardiol.* (2020) 17:259–60. doi: 10.1038/s41569-020-0360-5
11. Boureau AS, De Decker L, Berrut G, Hanon O. COVID-19 and cardiovascular diseases: viewpoint for older patients. *Geriatr Psychol Neuropsychiatr Vieil.* (2020) 18:141–9. doi: 10.1684/pnv.2020.0864
12. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* (2020) 395:507–13. doi: 10.1016/S0140-6736(20)30211-7
13. Ganatra S, Dani SS, Shah S, Asnani A, Neilan TG, Lenihan D, et al. Management of cardiovascular disease during coronavirus disease (COVID-19) pandemic. *Trends Cardiovasc Med.* (2020) 30:315–25. doi: 10.1016/j.tcm.2020.05.004
14. Ashraf O, Young M, Malik KJ, Cheema T. Systemic complications of COVID-19. *Crit Care Nurs Q.* (2020) 43:390–9. doi: 10.1097/CNQ.0000000000000324
15. Li G, Saguner AM, An J, Ning Y. Cardiovascular disease during the COVID-19 pandemic: think ahead, protect hearts, reduce mortality. *Cardiol J.* (2020) 27:616–24. doi: 10.5603/CJ.a2020.0101
16. Aster JC. In brief: notch signalling in health and disease. *J Pathol.* (2014) 232:1–3. doi: 10.1002/path.4291
17. Shang Y, Smith S, Hu X. Role of Notch signaling in regulating innate immunity and inflammation in health and disease. *Protein Cell.* (2016) 7:159–74. doi: 10.1007/s13238-016-0250-0
18. Rosa BA, Ahmed M, Singh DK, Choreño-Parra JA, Cole J, Jiménez-Álvarez LA, et al. IFN signaling and neutrophil degranulation transcriptional signatures are induced during SARS-CoV-2 infection. *Commun Biol.* (2021) 4:1–14. doi: 10.1038/s42003-021-01829-4
19. Vandelli A, Monti M, Milanetti E, Armaos A, Rupert J, Zacco E, et al. Structural analysis of SARS-CoV-2 genome and predictions of the human interactome. *Nucleic Acids Res.* (2020) 48:11270–83. doi: 10.1093/nar/gkaa864
20. Bourgonje AR, Abdulle AE, Timens W, Hillebrands JL, Navis GJ, Gordijn SJ, et al. Angiotensin-converting enzyme-2 (ACE2), SARS-CoV-2 and pathophysiology of coronavirus disease 2019 (COVID-19). *J Pathol.* (2020) 251:228–48. doi: 10.1002/path.5471
21. Wu J, Deng W, Li S, Yang X. Advances in research on ACE2 as a receptor for 2019-nCoV. *Cell Mol Life Sci.* (2021). 78:531–44. doi: 10.1007/s00018-020-03611-x
22. Eguchi S, Kawai T, Scalia R, Rizzo V. Understanding angiotensin II type 1 receptor signaling in vascular pathophysiology. *Hypertension.* (2018) 71:804–10. doi: 10.1161/HYPERTENSIONAHA.118.10266
23. Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong, J-C, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. *Circ Res.* (2020) 126:1456–74. doi: 10.1161/CIRCRESAHA.120.317015
24. Murray E, Tomaszewski M, Guzik TJ. Binding of SARS-CoV-2 and angiotensin-converting enzyme 2: clinical implications. *Cardiovasc Res.* (2020) 116:e87–9. doi: 10.1093/cvr/cvaa096
25. Alsibai KD. Expression of angiotensin-converting enzyme 2 and proteases in COVID-19 patients: a potential role of cellular FURIN in the pathogenesis of SARS-CoV-2. *Med Hypotheses.* (2020) 143:109893. doi: 10.1016/j.mehy.2020.109893
26. Wang Q, Qiu Y. Receptor utilization of angiotensin converting enzyme 2 (ACE2) indicates a narrower host range of SARS-CoV-2 than that

of SARS-CoV. *Transbound Emerg Dis*. (2020) 1–8. doi: 10.1111/tbed.13792
27. Barillà F, Bassareo PP, Calcaterra G, Romeo F, Mehta JL. Focus on clinical practice: angiotensin-converting enzyme 2 and corona virus disease 2019: pathophysiology and clinical implications. *J Cardiovasc Med (Hagerstown)*. (2020) 21:630–3. doi: 10.2459/JCM.0000000000001071
28. Robertson ES, Lin J, Kieff E. The amino-terminal domains of Epstein-Barr virus nuclear proteins 3A, 3B, and 3C interact with RBPJ (kappa). *J Virol*. (1996) 70:3068–74. doi: 10.1128/JVI.70.5.3068-3074.1996
29. Hsieh J, Hayward SD. Masking of the CBF1/RBPJ kappa transcriptional repression domain by Epstein-Barr virus EBNA2. *Science*. (1995) 268:560–3. doi: 10.1126/science.7725102
30. Zimber-Strobl U, Strobl LJ. EBNA2 and Notch signalling in Epstein-Barr virus mediated immortalization of B lymphocytes. *Semin Cancer Biol*. (2001) 11:423–34. doi: 10.1006/scbi.2001.0409
31. Pal AD, Basak NP, Banerjee AS, Banerjee S. Epstein–Barr virus latent membrane protein-2A alters mitochondrial dynamics promoting cellular migration mediated by Notch signaling pathway. *Carcinogenesis*. (2014) 35:1592–601. doi: 10.1093/carcin/bgu069
32. Ito T, Allen RM, Carson Iv WF, Schaller M, Cavassani KA, Hogaboam CM, et al. The critical role of Notch ligand Delta-like 1 in the pathogenesis of influenza A virus (H1N1) infection. *PLoS Pathog*. (2011) 7:e1002341. doi: 10.1371/journal.ppat.1002341
33. Ito T, Matsukawa A. Notch system in influenza A/H1N1 virus infection. *Inflammation and Regeneration*. (2012) 32:132–136. doi: 10.2492/inflammregen.32.132
34. Yang J, Huang X, Liu Y, Zhao D, Han K, Zhang L, et al. Analysis of the microRNA expression profiles of chicken dendritic cells in response to H9N2 avian influenza virus infection. *Vet Res*. (2020) 51:132. doi: 10.1186/s13567-020-00856-z
35. Zhang L, Li H, Hai Y, Yin W, Li W, Zheng B, et al. CpG in combination with an inhibitor of Notch signaling suppresses formalin-inactivated respiratory syncytial virus-enhanced airway hyperresponsiveness and inflammation by inhibiting Th17 memory responses and promoting tissue-resident memory cells in lungs. *J Virol*. (2017) 91:e02111–16. doi: 10.1128/JVI.02111-16
36. Buonvino S, Melino S. New Consensus pattern in Spike CoV-2: potential implications in coagulation process and cell–cell fusion. *Cell Death Discovery*. (2020) 6:134. doi: 10.1038/s41420-020-00372-1
37. Chakrabarti O, Veeraraghavalu K, Tergaonkar V, Liu Y, Androphy EJ, Stanley MA, et al. Human papillomavirus type 16 E6 amino acid 83 variants enhance E6-mediated MAPK signaling and differentially regulate tumorigenesis by notch signaling and oncogenic Ras. *J Virol*. (2004) 78:5934–45. doi: 10.1128/JVI.78.11.5934-5945.2004
38. Tan MJA, White EA, Sowa ME, Harper JW, Aster JC, Howley PM. Cutaneous β-human papillomavirus E6 proteins bind Mastermind-like coactivators and repress Notch signaling. *Proc Natl Acad Sci USA*. (2012) 109:E1473–80. doi: 10.1073/pnas.1205991109
39. Meyers JM, Spangle JM, Munger K. The human papillomavirus type 8 E6 protein interferes with NOTCH activation during keratinocyte differentiation. *J Virol*. (2013) 87:4762–7. doi: 10.1128/JVI.02527-12
40. Vliet-Gregg PA, Hamilton JR, Katzenellenbogen RA. NFX1-123 and human papillomavirus 16E6 increase Notch expression in keratinocytes. *J Virol*. (2013) 87:13741–50. doi: 10.1128/JVI.02582-13
41. Das T, Zhong R, Spiotto MT. Notch signaling and human papillomavirus–associated oral tumorigenesis. *Adv Exp Med Biol*. (2020) 1287:105–22. doi: 10.1007/978-3-030-55031-8_8
42. Vliet-Gregg PA, Hamilton JR, Katzenellenbogen RA. Human papillomavirus 16E6 and NFX1-123 potentiate Notch signaling and differentiation without activating cellular arrest. *Virology*. (2015) 478:50–60. doi: 10.1016/j.virol.2015.02.002
43. Pancewicz J, Taylor JM, Datta A, Baydoun HH, Waldmann TA, Hermine O, et al. Notch signaling contributes to proliferation and tumor formation of human T-cell leukemia virus type 1–associated adult T-cell leukemia. *Proc Natl Acad Sci USA*. (2010) 107:16619–24. doi: 10.1073/pnas.10107 22107
44. Kamdje AHN, Krampera M. Notch signaling in acute lymphoblastic leukemia: any role for stromal microenvironment? *Blood*. (2011) 118:6506–14. doi: 10.1182/blood-2011-08-376061
45. Wurmbach E, Chen YB, Khitrov G, Zhang W, Roayaie S, Schwartz M, et al. Genome-wide molecular profiles of HCV-induced dysplasia and hepatocellular carcinoma. *Hepatology*. (2007) 45:938–47. doi: 10.1002/hep.21622
46. Iwai A, Takegami T, Shiozaki T, Miyazaki T. Hepatitis C virus NS3 protein can activate the notch-signaling pathway through binding to a transcription factor, SRCAP. *PLoS ONE*. (2011) 6:e20718. doi: 10.1371/journal.pone.0020718
47. Jiang BC, Liu X, Liu XH, Li ZS, Zhu GZ. Notch signaling regulates circulating T helper 22 cells in patients with chronic hepatitis C. *Viral Immunol*. (2017) 30:522–32. doi: 10.1089/vim.2017.0007
48. Qin L, Zhou YC, Wu HJ, Zhuo Y, Wang YP, Si CY, et al. Notch signaling modulates the balance of regulatory T cells and T helper 17 cells in patients with chronic hepatitis C. *DNA Cell Biol*. (2017) 36:311–20. doi: 10.1089/dna.2016.3609
49. Wang F, Zhou H, Xia X, Sun Q, Wang Y, Cheng B. Activated Notch signaling is required for hepatitis B virus X protein to promote proliferation and survival of human hepatic cells. *Cancer Lett*. (2010) 298:64–73. doi: 10.1016/j.canlet.2010.06.003
50. Trehanpati N, Shrivastav S, Shivakumar B, Khosla R, Bhardwaj S, Chaturvedi J. Analysis of Notch and TGF-β signaling expression in different stages of disease progression during hepatitis B virus infection. *Clin Transl Gastroenterol*. (2012) 3:e23. doi: 10.1038/ctg.2012.17
51. Luo J, Zhou H, Wang F, Xia X, Sun Q, Wang R, et al. The hepatitis B virus X protein downregulates NF-κB signaling pathways through decreasing the Notch signaling pathway in HBx-transformed L02 cells. *Int J Oncol*. (2013) 42:1636–43. doi: 10.3892/ijo.2013.1842
52. Kongkavitoon P, Tangkijvanich P, Hirankarn N, Palaga T. Hepatitis B virus HBx activates Notch signaling via delta-like 4/Notch1 in hepatocellular carcinoma. *PLoS ONE*. (2016) 11:e0146696. doi: 10.1371/journal.pone.0146696
53. Wei X, Wang JP, Hao CQ, Yang F, Wang LX, Huang CX, et al. Notch Signaling contributes to liver inflammation by regulation of interleukin-22-producing cells in hepatitis B virus infection. *Front Cell Infect Microbiol*. (2016) 6:132. doi: 10.3389/fcimb.2016.00132
54. Zhou SJ, Deng YL, Liang HF, Jaoude JC, Liu FY. Hepatitis B virus X protein promotes CREB-mediated activation of miR-3188 and Notch signaling in hepatocellular carcinoma. *Cell Death Differ*. (2017) 24:1577–87. doi: 10.1038/cdd.2017.87
55. Wang Z, Kawaguchi K, Honda M, Hashimoto S, Shirasaki T, Okada H, et al. Notch signaling facilitates hepatitis B virus covalently closed circular DNA transcription via cAMP response element-binding protein with E3 ubiquitin ligase-modulation. *Sci Rep*.. (2019) 9:1–12. doi: 10.1038/s41598-018-38139-5
56. Shoham N, Cohen L, Yaniv A, Gazit A. The Tat protein of the human immunodeficiency virus type 1 (HIV-1) interacts with the EGF-like repeats of the Notch proteins and the EGF precursor. *Virus Res*. (2003) 98:57–61. doi: 10.1016/j.virusres.2003.08.016
57. Sharma M, Callen S, Zhang D, Singhal PC, Vanden Heuvel GB, Buch S. Activation of Notch signaling pathway in HIV-associated nephropathy. *AIDS*. (2010) 24:2161–70. doi: 10.1097/QAD.0b013e32833dbc31
58. Fan Y, Gao X, Chen J, Liu Y, He JJ. HIV tat impairs neurogenesis through functioning as a notch ligand and activation of notch signaling pathway. *J Neurosci*. (2016) 36:11362–73. doi: 10.1523/JNEUROSCI.1208-16.2016
59. Yan Q, Zhao R, Shen C, Wang F, Li W, Gao J, et al. Upregulation of MicroRNA 711 mediates HIV-1 Vpr promotion of kaposi's sarcoma-associated herpesvirus latency and induction of pro-proliferation and pro-survival cytokines by targeting the Notch/NF-κB-signaling axis. *J Virol*. (2018) 92:e00580–18. doi: 10.1128/JVI.00580-18
60. Schaller MA, Neupane R, Rudd BD, Kunkel SL, Kallal LE, Lincoln P, et al. Notch ligand Delta-like 4 regulates disease pathogenesis during respiratory viral infections by modulating Th2 cytokines. *J Exp Med*. (2007) 204:2925–34. doi: 10.1084/jem.20070661
61. Penton AL, Leonard LD, Spinner NB. Notch signaling in human development and disease. *Semin Cell Dev Biol*. (2012) 23:450–7. doi: 10.1016/j.semcdb.2012.01.010
62. Hayward SD. Viral interactions with the Notch pathway. *Semin Cancer Biol*. (2004) 14:387–96. doi: 10.1016/j.semcancer.2004.04.018

63. Kovall RA, Hendrickson WA. Crystal structure of the nuclear effector of Notch signaling, CSL bound to DNA. *EMBO J.* (2004) 23:3441–51. doi: 10.1038/sj.emboj.7600349
64. Sarma NJ, Tiriveedhi V, Subramanian V, Shenoy S, Crippin JS, Chapman WC, et al. Hepatitis C virus mediated changes in miRNA-449a modulates inflammatory biomarker YKL40 through components of the NOTCH signaling pathway. *PLoS ONE.* (2012) 7:e50826. doi: 10.1371/journal.pone.0050826
65. Duval F, Mathieu M, Labrecque N. Notch controls effector CD8+ T cell differentiation. *Oncotarget.* (2015) 6:21787. doi: 10.18632/oncotarget.4886
66. Ma YC, Shi C, Zhang YN, Wang G, Liu H, Jia HT, et al. The tyrosine kinase c-Src directly mediates growth factor-induced Notch-1 and Furin interaction and Notch-1 activation in pancreatic cancer cells. *PLoS ONE.* (2012) 7:e33414. doi: 10.1371/journal.pone.0033414
67. Qiu H, Tang X, Ma J, Shaverdashvili K, Zhang K, Bedogni B. Notch1 autoactivation via transcriptional regulation of furin, which sustains Notch1 signaling by processing Notch1-activating proteases ADAM10 and membrane type 1 matrix metalloproteinase. *Mol Cell Biol.* (2015) 35:3622–32. doi: 10.1128/MCB.00116-15
68. Thomas G. Furin at the cutting edge: from protein traffic to embryogenesis and disease. *Nat Rev Mol Cell Biol.* (2002) 3:753–66. doi: 10.1038/nrm934
69. Braun E, Sauter D. Furin-mediated protein processing in infectious diseases and cancer. *Clin Translational Immunol.* (2019) 8:e1073–e1073. doi: 10.1002/cti2.1073
70. Bradding P, Richardson M, Hinks TS, Howarth PH, Choy DF, Arron JR, et al. ACE2, TMPRSS2, and furin gene expression in the airways of people with asthma—implications for COVID-19. *J Allergy Clin Immunol.* (2020) 146:208–11. doi: 10.1016/j.jaci.2020.05.013
71. Li X, Duan G, Zhang W, Shi J, Chen J, Chen S, et al. A furin cleavage site was discovered in the S protein of the 2019 novel coronavirus. *Chinese J Bioinformatics*. (2020) 18. doi: 10.12113/202002001
72. Wu C, Zheng M, Yang Y, Gu X, Yang K, Li M, et al. Furin: a potential therapeutic target for COVID-19. *Iscience.* (2020) 23:101642. doi: 10.1016/j.isci.2020.101642
73. Bestle D, Heindl MR, Limburg H, Van Lam Van T. TMPRSS2 and furin are both essential for proteolytic activation of SARS-CoV-2 in human airway cells. *bioRxiv [Preprint]*. (2020) 3. doi: 10.1101/2020.04.15.042085
74. Xi J, Xu K, Jiang P, Lian J, Hao S, Jia H, et al. Virus strain of a mild COVID-19 patient in Hangzhou representing a new trend in SARS-CoV-2 evolution related to Furin cleavage site. *medRxiv*. (2020). doi: 10.1101/2020.03.10.20033944
75. Bozkulak EC, Weinmaster G. Selective use of ADAM10 and ADAM17 in activation of Notch1 signaling. *Mol Cell Biol.* (2009) 29:5679–95. doi: 10.1128/MCB.00406-09
76. Parr-Sturgess CA, Rushton DJ, Parkin ET. Ectodomain shedding of the Notch ligand Jagged1 is mediated by ADAM17, but is not a lipid-raft-associated event. *Biochem J.* (2010) 432:283–94. doi: 10.1042/BJ20100321
77. Lambert DW, Yarski M, Warner FJ, Thornhill P, Parkin ET, Smith AI, et al. Tumor necrosis factor-α convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). *J Biol Chem.* (2005) 280:30113–9. doi: 10.1074/jbc.M505111200
78. Rizzo P, Dalla Sega FV, Fortini F, Marracino L, Rapezzi C, Ferrari R. COVID-19 in the heart and the lungs: could we "Notch" the inflammatory storm? *Basic Res Cardiol.* (2020) 115:31. doi: 10.1007/s00395-020-0791-5
79. Collins SD. Excess mortality from causes other than influenza and pneumonia during influenza epidemics. *Public Health Reports.* (1932) 47:2159–79. doi: 10.2307/4580606
80. Corrales-Medina VF, Madjid M, Musher DM. Role of acute infection in triggering acute coronary syndromes. *Lancet Infect Dis.* (2010) 10:83–92. doi: 10.1016/S1473-3099(09)70331-7
81. Zhang C, Wu Z, Li, J-W, Zhao H, Wang Q. The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality. *Int J Antimicrob Agents*. (2020) 55:105954. doi: 10.1016/j.ijantimicag.2020.105954
82. Pabois A, Devallière J, Quillard T, Coulon F, Gérard N, Laboisse C, et al. The disintegrin and metalloproteinase ADAM10 mediates a canonical Notch-dependent regulation of IL-6 through Dll4 in human endothelial cells. *Biochem Pharmacol.* (2014) 91:510–21. doi: 10.1016/j.bcp.2014.08.007
83. Fazio C, Ricciardiello L. Inflammation and Notch signaling: a crosstalk with opposite effects on tumorigenesis. *Cell Death Dis.* (2016) 7:e2515. doi: 10.1038/cddis.2016.408
84. Lu Y, Zhang Y, Xiang X, Sharma M, Liu K, Wei J, et al. Notch signaling contributes to the expression of inflammatory cytokines induced by highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) infection in porcine alveolar macrophages. *Dev Comp Immunol.* (2020) 108:103690. doi: 10.1016/j.dci.2020.103690
85. Keewan E, Naser SA. The role of Notch signaling in macrophages during inflammation and infection: implication in rheumatoid arthritis? *Cells.* (2020) 9:111. doi: 10.3390/cells9010111
86. Wongchana W, Palaga T. Direct regulation of interleukin-6 expression by Notch signaling in macrophages. *Cell Mol Immunol.* (2012) 9:155–62. doi: 10.1038/cmi.2011.36
87. Guo Y, Xu F, Lu T, Duan Z, Zhang Z. Interleukin-6 signaling pathway in targeted therapy for cancer. *Cancer Treat Rev.* (2012) 38:904–10. doi: 10.1016/j.ctrv.2012.04.007
88. Yang Z, Guo L, Liu D, Sun L, Chen H, Deng Q, et al. Acquisition of resistance to trastuzumab in gastric cancer cells is associated with activation of IL-6/STAT3/Jagged-1/Notch positive feedback loop. *Oncotarget.* (2015) 6:5072. doi: 10.18632/oncotarget.3241
89. Adusumilli NC, Zhang D, Friedman JM, Friedman AJ. Harnessing nitric oxide for preventing, limiting and treating the severe pulmonary consequences of COVID-19. *Nitric Oxide*. (2020) 103:4–8. doi: 10.1016/j.niox.2020.07.003
90. Cespuglio R, Strekalova T, Spencer PS, Román GC, Reis J, Bouteille B, et al. SARS-CoV-2 infection and sleep disturbances: nitric oxide involvement and therapeutic opportunity. *Sleep*. (2021) 44:zsab009. doi: 10.1093/sleep/zsab009
91. Wang R, Li Y, Tsung A, Huang H, Du Q, Yang M, et al. iNOS promotes CD24+ CD133+ liver cancer stem cell phenotype through a TACE/ADAM17-dependent Notch signaling pathway. *Proc Natl Acad Sci USA*. (2018) 115:E10127–36. doi: 10.1073/pnas.1722100115
92. Bell JH, Herrera AH, Li Y, Walcheck B. Role of ADAM17 in the ectodomain shedding of TNF-α and its receptors by neutrophils and macrophages. *J Leukoc Biol.* (2007) 82:173–6. doi: 10.1189/jlb.0307193
93. Quillard T, Devallière J, Coupel S, Charreau B. Inflammation dysregulates Notch signaling in endothelial cells: implication of Notch2 and Notch4 to endothelial dysfunction. *Biochem Pharmacol.* (2010) 80:2032–41. doi: 10.1016/j.bcp.2010.07.010
94. Dees C, Tomcik M, Zerr P, Akhmetshina A, Horn A, Palumbo K, et al. Notch signalling regulates fibroblast activation and collagen release in systemic sclerosis. *Ann Rheum Dis.* (2011) 70:1304–10. doi: 10.1136/ard.2010.134742
95. Liu S, Liu D, Chen C, Hamamura K, Moshaverinia A, Yang R, et al. MSC transplantation improves osteopenia via epigenetic regulation of notch signaling in lupus. *Cell Metab.* (2015) 22:606–18. doi: 10.1016/j.cmet.2015.08.018
96. Russell B, Moss C, Rigg A, Van Hemelrijck M. COVID-19 and treatment with NSAIDs and corticosteroids: should we be limiting their use in the clinical setting? *Ecancermedicalscience.* (2020) 14:1023. doi: 10.3332/ecancer.2020.1023
97. Theoharides T, Conti P. Dexamethasone for COVID-19? Not so fast. *J Biol Regul Homeost Agents.* (2020) 34:1241–3. doi: 10.23812/20-EDITORIAL_1-5
98. Zha L, Li S, Pan L, Tefsen B, Li Y, French N, et al. Corticosteroid treatment of patients with coronavirus disease 2019 (COVID-19). *Med J Australia.* (2020) 212:416–20. doi: 10.5694/mja2.50577
99. Herrmann J, Mori V, Bates JH, Suki B. Can hyperperfusion of nonaerated lung explain COVID-19 hypoxia? *Res Sq. [Preprint].* (2020). doi: 10.21203/rs.3.rs-32949/v1
100. Couzin-Frankel J. The mystery of the pandemic's 'happy hypoxia'. *Science.* (2020) 368:455–6. doi: 10.1126/science.368.6490.455
101. Savla JJ, Levine BD, Sadek HA. The effect of hypoxia on cardiovascular disease: friend or foe? *High Alt Med Biol.* (2018) 19:124–30. doi: 10.1089/ham.2018.0044

102. Kashani KB. Hypoxia in COVID-19: sign of severity or cause for poor outcomes. *Mayo Clin Proc.* (2020) 95:1094–6. doi: 10.1016/j.mayocp.2020.04.021
103. Thachil J. Hypoxia-an overlooked trigger for thrombosis in COVID-19 and other critically ill patients. *J Thromb Haemost*. (2020) 18:3109–10. doi: 10.1111/jth.15029
104. Gustafsson MV, Zheng X, Pereira T, Gradin K, Jin S, Lundkvist J, et al. Hypoxia requires notch signaling to maintain the undifferentiated cell state. *Dev Cell.* (2005) 9:617–28. doi: 10.1016/j.devcel.2005.09.010
105. Li X, Zhang X, Leathers R, Makino A, Huang C, Parsa P, et al. Notch3 signaling promotes the development of pulmonary arterial hypertension. *Nat Med.* (2009) 15:1289–97. doi: 10.1038/nm.2021
106. Thistlethwaite PA, Li X, Zhang X. Notch signaling in pulmonary hypertension. *Adv Exp Med Biol.* (2010) 661:279–98. doi: 10.1007/978-1-60761-500-2_18
107. Liu H, Zhang W, Kennard S, Caldwell RB, Lilly B. Notch3 is critical for proper angiogenesis and mural cell investment. *Circ Res.* (2010) 107:860–70. doi: 10.1161/CIRCRESAHA.110.218271
108. Chun YS, Kim MS, Park JW. Oxygen-dependent and-independent regulation of HIF-1alpha. *J Korean Med Sci.* (2002) 17:581. doi: 10.3346/jkms.2002.17.5.581
109. Sainson RC, Harris AL. Hypoxia-regulated differentiation: let's step it up a Notch. *Trends Mol Med.* (2006) 12:141–3. doi: 10.1016/j.molmed.2006.02.001
110. Fung E, Tang, S-MT, Canner JP, Morishige K, Arboleda-Velasquez JF, et al. Delta-like 4 induces notch signaling in macrophages. *Circulation.* (2007) 115:2948–56. doi: 10.1161/CIRCULATIONAHA.106.675462
111. Zheng X, Linke S, Dias JM, Zheng X, Gradin K, Wallis TP, et al. Interaction with factor inhibiting HIF-1 defines an additional mode of cross-coupling between the Notch and hypoxia signaling pathways. *Proc Natl Acad Sci USA.* (2008) 105:3368–73. doi: 10.1073/pnas.0711591105
112. Xu J, Chi F, Guo T, Punj V, Lee WP, French SW, et al. NOTCH reprograms mitochondrial metabolism for proinflammatory macrophage activation. *J Clin Invest.* (2015) 125:1579–90. doi: 10.1172/JCI76468
113. Jespersen K, Liu Z, Li C, Harding P, Sestak K, Batra R, et al. Enhanced Notch3 signaling contributes to pulmonary emphysema in a Murine Model of Marfan syndrome. *Sci Rep*. (2020) 10:1–11. doi: 10.1038/s41598-020-67941-3
114. Guseh JS, Bores SA, Stanger BZ, Zhou Q, Anderson WJ, Melton DA, et al. Notch signaling promotes airway mucous metaplasia and inhibits alveolar development. *Development.* (2009) 136:1751–9. doi: 10.1242/dev.029249
115. Teo J. Early detection of silent hypoxia in COVID-19 pneumonia using Smartphone pulse oximetry. *J Med Syst*. (2020) 44:1–2. doi: 10.1007/s10916-020-01587-6
116. Wilkerson RG, Adler JD, Shah NG, Brown R. Silent hypoxia: a harbinger of clinical deterioration in patients with COVID-19. *Am J Emerg Med*. (2020) 38:2243.e5–2243.e6. doi: 10.1016/j.ajem.2020.05.044
117. Al-Samkari H, Karp Leaf RS, Dzik WH, Carlson JCT, Fogerty AE, Waheed A, et al. COVID-19 and coagulation: bleeding and thrombotic manifestations of SARS-CoV-2 infection. *Blood*. (2020) 136:489–500. doi: 10.1182/blood.2020006520
118. Chan NC, Weitz JI. COVID-19 coagulopathy, thrombosis, and bleeding. *Blood.* (2020) 136:381–383. doi: 10.1182/blood.2020007335
119. Boonyawat K, Chantrathammachart P, Numthavaj P, Nanthatanti N, Phusanti S, Phuphuakrat A, et al. Incidence of thromboembolism in patients with COVID-19: a systematic review and meta-analysis. *Thromb J.* (2020) 18:34. doi: 10.1186/s12959-020-00248-5
120. Klok FA, Kruip M, Van Der Meer NJM, Arbous MS, Gommers D, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res.* (2020) 191:145–7. doi: 10.1016/j.thromres.2020.04.013
121. He L, Mae MA, Sun Y, Muhl L, Nahar K, Liebanas EV, et al. Pericyte-specific vascular expression of SARS-CoV-2 receptor ACE2-implications for microvascular inflammation and hypercoagulopathy in COVID-19 patients. *bioRxiv*. (2020). doi: 10.1101/2020.05.11.088500
122. McGonagle D, O'donnell JS, Sharif K, Emery P, Bridgewood C. Immune mechanisms of pulmonary intravascular coagulopathy in COVID-19 pneumonia. *Lancet Rheumatol.* (2020) 2:e437–45. doi: 10.1016/S2665-9913(20)30121-1
123. Colling ME, Kanthi Y. COVID-19-associated coagulopathy: an exploration of mechanisms. *Vasc Med.* (2020) 25:471–8. doi: 10.1177/1358863X20932640
124. Gemmati D, Bramanti B, Serino ML, Secchiero P, Zauli G, Tisato V. COVID-19 and individual genetic susceptibility/receptivity: role of ACE1/ACE2 genes, immunity, inflammation and coagulation. Might the double X-chromosome in females be protective against SARS-CoV-2 compared to the single X-chromosome in males? *Int J Mol Sci.* (2020) 21:3474. doi: 10.3390/ijms21103474
125. Martín-Rojas RM, Pérez-Rus G, Delgado-Pinos VE, Domingo-González A, Regalado-Artamendi I, Alba-Urdiales N, et al. COVID-19 coagulopathy: an in-depth analysis of the coagulation system. *Eur J Haematol.* (2020) 105:741–50. doi: 10.1111/ejh.13501
126. Cesari M, Pahor M, Incalzi RA. Plasminogen activator inhibitor-1 (PAI-1): a key factor linking fibrinolysis and age-related subclinical and clinical conditions. *Cardiovasc Ther.* (2010) 28:e72–e91. doi: 10.1111/j.1755-5922.2010.00171.x
127. Medcalf RL, Keragala CB, Myles PS. Fibrinolysis and COVID-19: a plasmin paradox. *J Thromb Haemost.* (2020) 18:2118–22. doi: 10.1111/jth.14960
128. O'sullivan JM, Mc Gonagle D, Ward SE, Preston RJ, O'donnell JS. Endothelial cells orchestrate COVID-19 coagulopathy. *Lancet Haematol.* (2020) 7:e553–5. doi: 10.1016/S2352-3026(20)30215-5
129. Duarte M, Kolev V, Kacer D, Mouta-Bellum C, Soldi R, Graziani I, et al. Novel cross-talk between three cardiovascular regulators: thrombin cleavage fragment of Jagged1 induces fibroblast growth factor 1 expression and release. *Mol Biol Cell.* (2008) 19:4863–74. doi: 10.1091/mbc.e07-12-1237
130. Gough NR. Thrombin targets notch signaling. *Sci Signal.* (2008) 1:ec375. doi: 10.1126/scisignal.144ec375
131. Zimrin AB, Pepper MS, Mcmahon GA, Nguyen F, Montesano R, Maciag T. An antisense oligonucleotide to the notch ligand jagged enhances fibroblast growth factor-induced angiogenesis *in vitro*. *J Biol Chem.* (1996) 271:32499–502. doi: 10.1074/jbc.271.51.32499
132. Joutel A, Tournier-Lasserve E. Notch signalling pathway and human diseases. In: *Seminars in Cell and Developmental Biology*. Elsevier (1998). p. 619–25. doi: 10.1006/scdb.1998.0261
133. Xue Y, Gao X, Lindsell CE, Norton CR, Chang B, Hicks C, et al. Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged1. *Hum Mol Genet.* (1999) 8:723–30. doi: 10.1093/hmg/8.5.723
134. Crosnier C, Attié-Bitach T, Encha-Razavi F, Audollent S, Soudy F, Hadchouel M, et al. JAGGED1 gene expression during human embryogenesis elucidates the wide phenotypic spectrum of Alagille syndrome. *Hepatology.* (2000) 32:574–81. doi: 10.1053/jhep.2000.16600
135. Zimrin AB, Villeponteau B, Maciag T. Models of *in vitro* angiogenesis: endothelial cell differentiation on fibrin but not matrigel is transcriptionally dependent. *Biochem Biophys Res Commun.* (1995) 213:630–8. doi: 10.1006/bbrc.1995.2178
136. Sumpio BE, Riley JT, Dardik A. Cells in focus: endothelial cell. *Int J Biochem Cell Biol.* (2002) 34:1508–12. doi: 10.1016/S1357-2725(02)00075-4
137. Rubanyi GM. The role of endothelium in cardiovascular homeostasis and diseases. *J Cardiovasc Pharmacol.* (1993) 22:S1–14. doi: 10.1097/00005344-199322004-00002
138. Kazmi RS, Boyce S, Lwaleed BA. Homeostasis of hemostasis: the role of endothelium. *Semin Thromb Hemost.* (2015) 41:549–55. doi: 10.1055/s-0035-1556586
139. Quillard T, Devalliere J, Chatelais M, Coulon F, Séveno C, Romagnoli M, et al. Notch2 signaling sensitizes endothelial cells to apoptosis by negatively regulating the key protective molecule survivin. *PLoS ONE.* (2009) 4:e8244. doi: 10.1371/journal.pone.0008244
140. Lizama CO, Zovein AC. Polarizing pathways: balancing endothelial polarity, permeability, lumen formation. *Exp Cell Res.* (2013) 319:1247–54. doi: 10.1016/j.yexcr.2013.03.028
141. Pabois A, Pagie S, Gérard N, Laboisse C, Pattier S, Hulin P, et al. Notch signaling mediates crosstalk between endothelial cells and macrophages via Dll4 and IL6 in cardiac microvascular inflammation. *Biochem Pharmacol.* (2016) 104:95–107. doi: 10.1016/j.bcp.2016.01.016
142. Yang X, Chang Y, Wei W. Endothelial dysfunction and inflammation: immunity in rheumatoid arthritis. *Mediators Inflamm.* (2016) 2016:6813016. doi: 10.1155/2016/6813016

143. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med.* (2020) 383:120–8. doi: 10.1056/NEJMoa 2015432
144. Fox SE, Lameira FS, Rinker EB, Vander Heide RS Cardiac Endotheliitis and multisystem inflammatory syndrome after COVID-19. *Ann Intern Med.* (2020) 173:1025–7. doi: 10.7326/L20-0882
145. Endotheliitis and Endothelial Dysfunction in Patients with COVID-19: Its Role in Thrombosis and Adverse Outcomes. *J Clin Med.* (2020) 9:1862. doi: 10.3390/jcm9061862
146. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet.* (2020) 395:1417–8. doi: 10.1016/S0140-6736(20)30937-5
147. Hamming I, Timens W, Bulthuis M, Lely A, Navis GV, Van Goor. H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol.* (2004) 203:631–7. doi: 10.1002/path.1570
148. Sluimer J, Gasc J, Hamming I, Van Goor H, Michaud A, Van Den Akker L, et al. Angiotensin-converting enzyme 2 (ACE2) expression and activity in human carotid atherosclerotic lesions. *J Pathol.* (2008) 215:273–79. doi: 10.1002/path.2357
149. Muus C, Luecken MD, Eraslan G, Waghray A, Heimberg G, Sikkema L, et al. Integrated analyses of single-cell atlases reveal age, gender, and smoking status associations with cell type-specific expression of mediators of SARS-CoV-2 viral entry and highlights inflammatory programs in putative target cells. *BioRxiv.* (2020). doi: 10.1101/2020.04.19.049254
150. Ratajczak MZ, Bujko K, Ciechanowicz A, Sielatycka K, Cymer M, Marlicz W, et al. SARS-CoV-2 entry receptor ACE2 is expressed on very small CD45– precursors of hematopoietic and endothelial cells and in response to virus spike protein activates the Nlrp3 inflammasome. *Stem Cell Rev Reports.* (2020) 16:1–2. doi: 10.1007/s12015-019-09951-x
151. Rahimi FS, Afaghi S, Tarki FE, Goudarzi K, Alamdari NM. Viral outbreaks of SARS-CoV1, SARS-CoV2, MERS-CoV, influenza H1N1, and ebola in 21st Century; a comparative review of the pathogenesis and clinical characteristics. *School Med Stud J.* 151. (2020). doi: 10.22037/smsj.v2i3. 30455
152. Iba T, Levy JH, Levi M, Thachil J. Coagulopathy in COVID-19. *J Thromb Haemost.* (2020) 18:2103–9. doi: 10.1111/jth.14975
153. Chen L, Li X, Chen M, Feng Y, Xiong C. The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. *Cardiovasc Res.* (2020) 116:1097–100. doi: 10.1093/cvr/cvaa078
154. Miele L, Miao H, Nickoloff B. NOTCH signaling as a novel cancer therapeutic target. *Curr Cancer Drug Targets.* (2006) 6:313–23. doi: 10.2174/156800906777441771
155. Woo HN, Park JS, Gwon AR, Arumugam TV, Jo DG. Alzheimer's disease and Notch signaling. *Biochem Biophys Res Commun.* (2009) 390:1093–7. doi: 10.1016/j.bbrc.2009.10.093
156. Li K, Li Y, Wu W, Gordon WR, Chang DW, Lu M, et al. Modulation of Notch signaling by antibodies specific for the extracellular negative regulatory region of NOTCH3. *J Biol Chem.* (2008) 283:8046–54. doi: 10.1074/jbc.M800170200
157. Falk R, Falk A, Dyson MR, Melidoni AN, Parthiban K, Young JL, et al. Generation of anti-Notch antibodies and their application in blocking Notch signalling in neural stem cells. *Methods.* (2012) 58:69–78. doi: 10.1016/j.ymeth.2012.07.008
158. Valcourt DM, Dang MN, Scully MA, Day ES. Nanoparticle-mediated co-delivery of Notch-1 antibodies and ABT-737 as a potent treatment strategy for triple-negative breast cancer. *ACS Nano.* (2020) 14:3378–88. doi: 10.1021/acsnano.9b09263
159. Kangsamaksin T, Murtomaki A, Kofler NM, Cuervo H, Chaudhri RA, Tattersall IW, et al. NOTCH decoys that selectively block DLL/NOTCH or JAG/NOTCH disrupt angiogenesis by unique mechanisms to inhibit tumor growth. *Cancer Discovery.* (2015) 5:182–97. doi: 10.1158/2159-8290.CD-14-0650
160. Mitra A, Shanthalingam S, Sherman HL, Singh K, Canakci M, Torres JA, et al. CD28 signaling drives notch ligand expression on CD4 T cells. *Front Immunol.* (2020) 11:735. doi: 10.3389/fimmu.2020. 00735

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 28 May 2021
doi: 10.3389/fcvm.2021.645587

Early vs. Late Onset Cardiac Injury and Mortality in Hospitalized COVID-19 Patients in Wuhan

Wei Sun[1,2,3†], *Yanting Zhang*[1,2,3†], *Chun Wu*[1,2,3†], *Shuyuan Wang*[1,2,3†], *Yuji Xie*[1,2,3], *Danqing Zhang*[1,2,3], *Hongliang Yuan*[1,2,3], *Yongxing Zhang*[1,2,3], *Li Cui*[1,2,3], *Meng Li*[1,2,3], *Yiwei Zhang*[1,2,3], *Yuman Li*[1,2,3], *Jing Wang*[1,2,3], *Yali Yang*[1,2,3], *Qing Lv*[1,2,3], *Li Zhang*[1,2,3*], *Philip Haines*[4*], *Wen-Chih Wu*[5*] *and Mingxing Xie*[1,2,3*]

[1] Department of Ultrasound, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [2] Hubei Province Clinical Research Center for Medical Imaging, Wuhan, China, [3] Hubei Province Key Laboratory of Molecular Imaging, Wuhan, China, [4] Rhode Island Hospital, Warren Alpert Medical School of Brown University, Providence, RI, United States, [5] Department of Medicine, Providence VA Medical Center, Brown University Warren Alpert Medical School, Providence, RI, United States

OPEN ACCESS

Edited by:
Pier Leopoldo Capecchi, University of Siena, Italy

Reviewed by:
Carlos Egea, Araba University Hospital, Spain
Nicola Mumoli, ASST Ovest Milanese, Italy

***Correspondence:**
Mingxing Xie
xiemx@hust.edu.cn
Wen-Chih Wu
wen-chih_wu@brown.edu
Philip Haines
philip_haines@brown.edu
Li Zhang
zli429@hust.edu.cn

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: 23 December 2020
Accepted: 29 March 2021
Published: 28 May 2021

Citation:
Sun W, Zhang Y, Wu C, Wang S, Xie Y, Zhang D, Yuan H, Zhang Y, Cui L, Li M, Zhang Y, Li Y, Wang J, Yang Y, Lv Q, Zhang L, Haines P, Wu W-C and Xie M (2021) Early vs. Late Onset Cardiac Injury and Mortality in Hospitalized COVID-19 Patients in Wuhan. Front. Cardiovasc. Med. 8:645587. doi: 10.3389/fcvm.2021.645587

Background: Increasing evidence points to cardiac injury (CI) as a common coronavirus disease 2019 (COVID-19) related complication. The characteristics of early CI (occurred within 72 h of admission) and late CI (occurred after 72 h of admission) and its association with mortality in COVID-19 patients is unknown.

Methods: This retrospective study analyzed patients confirmed with COVID-19 in Union Hospital (Wuhan, China) from Jan 29th to Mar 15th, 2020. Clinical outcomes (discharge, or death) were monitored to April 15, 2020, the latest date of follow-up. Demographic, clinical, laboratory, as well as treatment and prognosis were collected and analyzed in patients with early, late CI and without CI.

Results: A total of 196 COVID-19 patients were included for analysis. The median age was 65 years [interquartile range (IQR) 56–73 years], and 112 (57.1%) were male. Of the 196 COVID-19 patients, 49 (25.0%) patients had early and 20 (10.2%) patients had late CI, 56.6% developed Acute-Respiratory-Distress-Syndrome (ARDS) and 43 (21.9%) patients died. Patients with any CI were more likely to have developed ARDS (87.0 vs. 40.2%) and had a higher in-hospital mortality than those without (52.2 vs. 5.5%, $P < 0.001$). Among CI subtypes, a significantly higher risk of in-hospital death was found in patients with early CI with recurrence [19/49 patients, adjusted odds ratio (OR) = 7.184, 95% CI 1.472–35.071] and patients with late CI (adjusted OR = 5.019, 95% CI 1.125–22.388) compared to patients with early CI but no recurrence.

Conclusions: CI can occur early on or late after, the initial 72 h of admission and is associated with ARDS and an increased risk of in-hospital mortality. Both late CI and recurrent CI after the initial episode were associated with worse outcomes than patients with early CI alone. This study highlights the importance of early examination and periodical monitoring of cardiac biomarkers, especially for patients with early CI or at risk of clinical deterioration.

Keywords: COVID-19, cardiac injury, early, late, mortality

INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected over 200 countries (1). With the increasing number of confirmed cases, the cardiovascular manifestations associated by this highly contagious viral infection have gained more and more attention. Several observational studies have found that between 7.2 and 37.5% of COVID-19 patients had cardiac injury (CI) which was associated with higher mortality in COVID-19 patients (2–11). However, a portion of CI does not occur on admission and the association of the timing of CI with prognosis is unknown. Furthermore, the clinical features and risk factors associated with early or late onset CI in COVID-19 patients have not been formally evaluated. The clinical sequence preceding and following CI at the time of admission may provide additional understanding of the pathogenesis associated with CI in COVID-19. Therefore, this study compared the clinical characteristics, risk factors and prognostic value of early vs. late onset of CI in COVID-19 patients.

METHODS

Study Design and Participants

We performed this retrospective study at Union Hospital (Affiliated Tongji Medical College, Huazhong University of Science and Technology) Wuhan, China. The West Branch of Union Hospital was one of the major designated hospitals for critically ill COVID-19 patients. We enrolled 429 consecutive patients with confirmed COVID-19, according to the WHO interim guidance criteria (12), who were either discharged alive or died during hospitalization from Jan 29th to April 15th, 2020. Only participants who had high-sensitivity troponin I (hs-TNI) measured before and after 72 h from admission during their hospitalization were included in the study (233 patients excluded). The study was approved by the ethics committee of the Union hospital, Tongji Medical College, Huazhong University of Science and Technology. Per institutional policy, written informed consent was waived for all participants with emerging infectious diseases.

Data Collection

Data were extracted from the electronic medical records including demographic information and clinical characteristics (i.e., vital signs, symptoms, laboratory findings, medical history, underlying comorbidities, treatments, complications, and outcomes) of the participants on admission and during hospitalization. The date of illness onset was defined as the day when symptoms of COVID-19 as defined by the World Health Organization (12) were appreciated. Laboratory measurements within and after 72 h of admission were collected. If multiple measurements were available, the patient's first abnormal measurements, both within and after 72 h, were recorded for the determination of the timing of the CI. The duration from the onset of admission to the onset of clinical complications and death in the hospital were also recorded. Clinical outcomes (discharge and mortality) were monitored up to April 15, 2020, the last date of follow-up. Complete hospitalization data was available in all patients included in the study.

Timing of CI

The hs-TNI data for each patient were collected from admission to discharge or death. COVID-19 related CI was defined as the serum levels of cardiac high-sensitivity troponin I (hs-TNI) above the 99th percentile upper reference limit in a patient diagnosed with COVID-19 per Huang et al. and Shi et al. (2, 7–10). Early CI was defined as CI that occurred within 72 h of admission, whereas late CI was defined as occurring after 72 h of admission. We also defined a subgroup of recurrent CI within the early CI group as a second rise of hs-TNI value of >20% from its previous value after 72 h of admission.

Non-cardiac Complications

Acute respiratory distress syndrome (ARDS) was defined according to the World Health Organization interim guidance criteria (13). Acute kidney injury was identified according to the KDIGO clinical practice guidelines as an increase in serum creatinine by ≥0.3 mg/dl (≥26.5 μmol/l) within 48 h or by 1.5 times of the baseline values (14). Coagulation dysfunction was defined as a >3-s prolongation of prothrombin time (PT) or a 5-s prolongation of activated partial thromboplastin time (APTT). Thrombocytopenia was characterized by a platelet count $<125 \times 10^9$/L (15).

Statistical Analysis

Categorical variables were expressed as number (%), and continuous variables were expressed as mean ± standard deviation (SD) or median [interquartile range (IQR)]. The normality of the distribution was tested with the Shapiro-Wilk normality test. Differences among the three groups (without CI, early CI or late CI) were assessed by ANOVA for normally distributed and Kruskal–Wallis *H*-test for non-normally distributed continuous variables. Categorical variables were compared by Chi-square or Fisher exact test where applicable. In-hospital survival curves of four groups of patients with early but no recurrent CI, early with recurrent CI, late CI, and no CI were estimated with the Kaplan-Meier method and the groups compared with the log-rank test. Univariate and multivariate logistic regression analyses were used to determine the independent risk associated with each of the four groups (early but no recurrent CI, early with recurrent CI, late CI, and no CI) of patients with in-hospital death, adjusted by known risk factors of COVID-19 mortality in the literature [age, sex, respiratory rate, heart rate, SpO_2, temperature, mean arterial pressure, coma, hypertension history, Lymphocyte count, C-reactive protein (CRP), and lactate dehydrogenase (LDH)] (16–18). All statistical analyses were performed with SPSS version 24.0 (Statistical Package for the Social Sciences, Chicago, Illinois), and STATA software version 10 (StataCorp, Texas, USA). A two-tailed *P*-value of <0.05 was considered statistically significant.

RESULTS

Clinical Characteristics, Laboratory Findings, and Treatments Within 72 h of Admission

Among the 429 patients, 100 patients were excluded due to missing hs-TNI data during the entire hospitalization, 133 patients were excluded due to missing hs-TNI data within 72 h of admission, the remaining 196 patients were included for analysis. The median age was 65.0 years (IQR: 56.0–73.0 years), and 112 (57.1%) were men. Of 196 COVID-19 patients, 69/196 (35.2%) had evidence of CI during hospitalization: 49/196 (25.0%) patients had early CI and 20/196 (10.2%) patients had late CI. In addition, 19/49 (38.8%) patients with early CI had recurrent CI after 72 h of admission. Compared with patients without CI, patients with early and late CI were more often older, and had lower SpO_2 on admission. They also had more comorbidities such as hypertension and underlying cardiac disease.

Compared with non-CI group, patients with early and late CI presented with more abnormal laboratory findings within 72 h of admission including lower lymphocyte and platelet counts, higher inflammation-related indices [CRP, procalcitonin (PCT)] and further elevations in liver and renal function indices.

Concerning the treatment of the 196 patients within 72 h of admission, there was no difference in the antiviral (P = 0.551) or antibiotic therapy (P = 0.235) among these three groups. However, compared with the non-CI group, more patients with early and late CI received glucocorticoid therapy (18.9 vs. 38.8% and 30.0%, P = 0.021), high-flow oxygen (18.1 vs. 69.4% and 80.0%, P <0.001), invasive mechanical ventilation (0.8 vs. 4.1% and 15.0%; P = 0.005), non-invasive mechanical ventilation (0.0 vs. 8.2% and 15.0%; P <0.001), and more subjects were transferred to the intensive care unit (ICU) (0.8 vs. 10.2% and 5.0%; P = 0.007) (**Table 1**).

Timing of CI and Non-cardiac Complications After 72 h of Admission

Major complications after 72 h included ARDS [111/196 (56.6%)], coagulation dysfunction [57/193 (29.5%)], late CI [20/196 (10.2%)] and acute kidney injury [33/193 (17.1%)]. Patients with early and late CI were more likely to have developed ARDS, ICU transfer and receive invasive mechanical ventilation (IMV) during their hospitalization compared to the non-CI group (**Table 2**). A majority, 87.0% (60/69) of patients with CI vs. 40.2% (51/127) without CI, developed ARDS. Overall, the median time from admission to ARDS was 4 days, to acute kidney injury was 7 days, to late CI was 11 days, and to coagulation dysfunction was 11 days for all patients.

For the early CI group (n = 49), the median time from admission to CI was 1 day (IQR 0–1 day), to the onset of ARDS (81.6%) was 2 days (IQR 1–8 days), and to the onset of recurrent CI [19/49 (38.8%)] was 7 days (IQR 5–16 days) for affected patients (**Figure 1A**). For the late CI group [20/196 (10.2%)], the median time from admission to the onset of ARDS (100%) was 7 days (IQR 2–8 days) and the onset of late CI was 11 days (IQR 5–22 days) (**Figure 1B**). Conversely, for the non-CI group [127/196 (64.8%)], the median time from admission to the onset of ARDS (40.2%) was 6 days (IQR 3–12 days) (**Figure 1C**).

Using the onset of ARDS as the temporal reference point, 15/20 (75%) patients with late CI had ARDS before their CI (**Figure 2**), with a median time between ARDS and CI of 4 days (IQR 3–17 days).

Cardiac Injury and Mortality

As of April 15, 2020, 153 patients (78.1%) were discharged and 43 patients (21.9%) died in the hospital. Patients with any CI had significantly higher in-hospital mortality than those without (52.2 vs. 5.5%, P < 0.001) (**Figure 3**). Among CI subtypes, multivariable regression modeling showed that compared to patients with early CI but no recurrence, a significantly higher risk of in-hospital death was found in patients with early CI with recurrence [odds ratio (OR) = 7.184, P = 0.015] and patients with late CI (OR = 5.019, P = 0.034) (**Table 3**).

DISCUSSION

In this cohort of 196 hospitalized COVID-19 patients from Wuhan, China, we found 35.2% had evidence of CI during hospitalization that included 25.0% of patients with early CI and 10.2% patients with late CI. In addition, there were 38.8% of patients with early CI who also had recurrent CI. Patients with any CI had significantly higher incidence of ARDS and in-hospital mortality than those without. Moreover, among CI subtypes, a significantly higher risk of in-hospital death was found in patients with early CI with recurrence and patients with late CI compared to patients with early CI and no recurrence.

Increasingly, researchers are reporting CI in COVID-19 patients, with the prevalence varying from 7.2 to 37.5% (4, 7, 8, 11). In our study, a remarkable 35.2% of patients had any CI. The pathogenesis of CI associated with COVID-19 is still unknown partly due to a dearth of autopsy or biopsy reported in these patients. The following potential mechanisms for CI have been proposed. The first possibility is direct myocardial damage by the virus, because angiotensin-converting enzyme 2 has been identified as a functional receptor for coronaviruses, which is also expressed abundantly in the myocardium (19, 20). Mixed literature from autopsy and biopsy case series were reported without conclusive evidence yet (21–25). The second mechanism is presumably the systemic inflammatory cytokine response, namely cytokine storm (26, 27). It can cause the proliferation of highly pro-inflammatory CCR4+CCR6+Th17 cells amongst the CD4+T cells, the expression of high concentrations of cytotoxic particles in CD8+T cells and over activation of T cells in general, all of which lead to a stronger inflammatory response in return (3, 21). Inflammation can in turn lead to thromboembolic complications (28). Our study showed that inflammation-related indices (CRP, PCT) were higher

TABLE 1 | Comparisons of demographics, clinical characteristics and laboratory examinations on admission within 72 h among the three groups.

	Total population ($n = 196$)	Without CI ($n = 127$)	Early CI ($n = 49$)	Late CI ($n = 20$)	*P*-value
Age (years)	65.0 (56.0, 73.0)	61.0 (49.0, 69.0)	71.0 (66.0, 77.0)*	69.0 (59.3, 72.8)	<0.001
Gender					0.458
Male, *n* (%)	112 (57.1)	70 (51.1)	28 (57.1)	14 (70.0)	
Female, *n* (%)	84 (42.9)	57 (44.9)	21 (42.9)	6 (30.0)	
Smoking, *n* (%)	22 (11.2)	16 (12.6)	5 (10.2)	1 (5.0)	0.537
Vital signs					
Temperature (°C)	38.0 (36.7, 38.7)	38.0 (36.7, 38.7)	37.9 (36.7, 38.5)	38.9 (38.1, 39.5)*#	0.004
Respiratory rate (breaths/min)	20.0 (20.0, 25.0)	20.0 (20.0, 24.0)	23.0 (20.0, 30.0)*	22.0 (20.0, 25.0)	0.008
Heart rate (bpm)	89.0 (80.0, 101.0)	87.0 (80.0, 98.0)	95.0 (81.0, 110.5)	92.0 (83.0, 106.5)	0.068
SBP (mmHg)	133.6 ± 19.9	132.7 ± 18.8	135.3 ± 22.6	135.5 ± 20.7	0.547
DBP (mmHg)	80.4 ± 13.1	81.3 ± 12.0	78.7 ± 15.5	79.0 ± 18.8	0.182
Mean arterial pressure (mmHg)	98.2 ± 13.9	98.4 ± 12.9	97.6 ± 16.2	98.6 ± 15.0	0.806
SpO_2 (%)	97.0 (94.0, 99.0)	98.0 (95.0, 99.0)	95.0 (89.5, 98.0)*	94.0 (87.5, 98.0)*	<0.001
Common initial symptoms					
Fever, *n* (%)	151 (77.0)	101 (79.5)	33 (67.3)	17 (85.0)	0.162
Cough, *n* (%)	113 (57.7)	78 (61.4)	23 (46.9)	12 (60.0)	0.214
Fatigue, *n* (%)	89 (45.4)	52 (40.9)	28 (57.1)	9 (45.0)	0.154
Dyspnea, *n* (%)	87 (44.4)	50 (39.4)	29 (59.2)	8 (40.0)	0.055
Chest tightness/chest pain, *n* (%)	75 (38.3)	50 (39.4)	17 (34.7)	8 (40.0)	0.837
Diarrhea, *n* (%)	21 (10.7)	17 (13.4)	4 (8.2)	0 (0.0)	0.056
Headache, *n* (%)	11 (5.6)	8 (6.3)	2 (4.1)	1 (5.0)	0.893
Coma, *n* (%)	9 (4.6)	2 (1.6)	6 (12.2)*	1 (5.0)	0.011
Comorbidities					
Hypertension, *n* (%)	87 (44.4)	48 (37.8)	30 (61.2)*	9 (45.0)	0.020
Diabetes mellitus, *n* (%)	29 (14.8)	14 (11.0)	10 (20.4)	5 (25.0)	0.130
Cardiac disease, *n* (%)	32 (16.3)	12 (9.4)	16 (32.7)*	4 (20.0)	0.001
Cerebral infarction, *n* (%)	15 (7.7)	7 (5.5)	6 (12.2)	2 (10.0)	0.234
Malignancy, *n* (%)	9 (4.6)	5 (3.9)	2 (4.1)	2 (10.0)	0.417
Chronic liver disease, *n* (%)	4 (2.0)	1 (0.8)	1 (2.0)	2 (10.0)*	0.037
Chronic kidney disease, *n* (%)	6 (3.1)	2 (1.6)	4 (8.2)	0 (0.0)	0.056
Chronic obstructive pulmonary disease, *n* (%)	8 (4.1)	3 (2.4)	4 (8.2)	1 (5.0)	0.173
Symptom onset to hospital admission (days)	14.0 (8.0, 20.0)	15.0 (10.0, 21.0)	10.0 (5.0, 14.5)*	10.0 (7.0, 15.0)*	<0.001
Laboratory findings on admission (within 72 h)					
White blood cells ($\times 10^9$/L)	7.0 (5.5, 9.2) 195/196	6.4 (5.2, 7.9) 126/127	8.9 (6.8, 10.9)* 49/49	7.0 (5.2, 9.6) 20/20	<0.001
Lymphocyte (%)	15.9 (7.4, 25.9) 195/196	22.1 (13.8, 29.3) 126/127	6.6 (4.6, 15.9)* 49/49	10.4 (6.0, 17.4)* 20/20	<0.001
Neutrophil ($\times 10^9$/L)	5.3 (3.7, 7.4) 195/196	4.3 (3.1, 6.0) 126/127	7.2 (5.9, 10.1)* 49/49	5.8 (4.5, 7.7) 20/20	<0.001
Platelets ($\times 10^9$/L)	205.0 (152.0, 262.0) 195/196	214.0 (174.5, 273.5) 126/127	179.0 (117.5, 262.5)* 49/49	167.5 (108.5, 245.0) 20/20	0.008
Hemoglobin (g/L)	124.0 (112.0, 135.0) 195/196	125.0 (110.8, 135.3) 126/127	121.0 (101.0, 135.0) 49/49	128.5 (116.3, 133.3) 20/20	0.452
CRP (mg/L)	32.0 (3.4, 75.9) 195/196	6.5 (1.9, 55.2) 126/127	62.4 (36.3, 109.9)* 49/49	71.0 (33.8, 114.6)* 20/20	<0.001
PCT (ng/ml)	0.11 (0.05, 0.24) 176/196	0.06 (0.04, 0.12) 114/127	0.40 (0.14, 0.60)* 42/49	0.15 (0.11, 0.28)* 20/20	<0.001
Coagulation function index					
D-dimer (μg/mL)	1.11 (0.35, 4.27) 186/196	0.75 (0.29, 2.39) 120/127	2.73 (1.28, 8.00)* 46/49	1.05 (0.46, 6.35) 20/20	<0.001

(Continued)

TABLE 1 | Continued

	Total population (*n* = 196)	Without CI (*n* = 127)	Early CI (*n* = 49)	Late CI (*n* = 20)	*P*-value
PT (s)	13.4 (12.6, 14.3) 186/196	13.1 (12.4, 14.0) 120/127	14.2 (13.2, 15.1)* 46/49	13.5 (13.0, 14.2) 20/20	<0.001
APTT (s)	37.4 (33.1, 42.2) 186/196	36.0 (32.5, 39.9) 120/127	38.7 (33.9, 44.9) 46/49	38.1 (32.4, 44.1) 20/20	0.274
Liver function index					
Total protein (g/L)	64.1 (58.9, 67.6) 196/196	64.4 (58.8, 67.7) 127/127	60.7 (56.2, 64.5)* 49/49	62.1 (60.0, 66.8) 20/20	0.002
Albumin (g/L)	31.7 (26.8, 37.4) 196/196	32.1 (27.3, 37.7) 127/127	28.2 (24.4, 32.1)* 49/49	27.4 (25.3, 31.8)* 20/20	<0.001
AST (U/L)	31.5 (23.0, 48.0) 196/196	28.0 (20.0, 43.0) 127/127	39.0 (29.0, 59.5)* 49/49	47.0 (33.8, 76.8)* 20/20	<0.001
ALT (U/L)	36.0 (23.0, 54.8) 196/196	32.5 (21.0, 50.0) 127/127	38.0 (23.0, 56.5) 49/49	38.5 (30.3, 78.8) 20/20	0.262
Total bilirubin (μmol/L)	11.2 (8.7, 16.9) 196/196	10.9 (8.3, 15.2) 127/127	14.2 (9.9, 21.3) 49/49	10.5 (7.8, 18.7) 20/20	0.110
Direct bilirubin (μmol/L)	3.8 (2.7, 5.6) 196/196	3.4 (2.5, 5.1) 127/127	4.8 (3.2, 7.6)* 49/49	4.3 (2.7, 5.9) 20/20	0.003
LDH (U/L)	250.5 (182.3, 403.0) 196/196	227.0 (174.3, 352.5) 127/127	388.0 (250.0, 597.0)* 49/49	492.0 (320.0, 665.3)* 20/20	<0.001
Kidney function index					
BUN (mmol/L)	5.2 (4.1, 7.3) 196/196	4.8 (3.7, 6.3) 127/127	7.0 (5.0, 11.2)* 49/49	5.7 (4.1, 7.5) 20/20	<0.001
Serum creatinine (μmol/L)	68.4 (56.4, 82.5) 196/196	64.3 (54.4, 75.2) 127/127	75.3 (61.4, 98.1)* 49/49	68.6 (62.5, 82.2) 20/20	0.008
K+ (mmol/L)	4.0 (3.5, 4.3) 195/196	3.9 (3.5, 4.2) 126/127	4.0 (3.5, 4.4) 49/49	4.0 (3.5, 4.6) 20/20	0.427
Na+ (mmol/L)	138.9 (136.9, 141.1) 195/196	139.0 (137.3, 141.2) 126/127	139.9 (136.1, 144.0) 49/49	138.8 (135.8, 140.5) 20/20	0.558
Cardiac injury index					
hs-TNI (ng/L)	8.3 (2.7, 27.0) 196/196	3.9 (2.0, 9.8) 127/127	86.4 (44.9, 378.8)* 49/49	10.8 (7.5, 16.2)*# 20/58	<0.001
CK-MB (U/L)	12.0 (10.0, 17.0) 170/196	11.0 (9.0, 15.0) 108/127	16.0 (10.0, 26.3)* 42/49	16.0 (10.3, 21.8)* 20/20	<0.001
BNP (pg/ml)	40.7 (15.0, 128.0) 152/196	30.1 (10.5, 84.1) 91/127	153.4 (46.0, 431.6)* 44/49	40.6 (22.9, 122.4)# 17/20	<0.001
Treatments on admission (within 72 h)					
Antiviral therapy, *n* (%)	147 (75.0)	94 (74.0)	36 (73.5)	17 (85.0)	0.551
Antibiotic therapy, *n* (%)	107 (54.6)	61 (48.0)	31 (63.3)	15 (75.0)	0.235
Glucocorticoid therapy, *n* (%)	49 (25.0)	24 (18.9)	19 (38.8)*	6 (30.0)	0.021
Immunoglobulin, *n* (%)	21 (10.7)	10 (7.9)	6 (12.2)	5 (25.0)	0.104
ACEI/ARB, *n* (%)	8 (4.1)	4 (3.1)	4 (8.2)	0 (0.0)	0.258
Oxygen therapy, *n* (%)	132 (67.3)	73 (57.5)	42 (85.7)*	17 (85.0)	<0.001
High-flow oxygen, *n* (%)	73 (37.2)	23 (18.1)	34 (69.4)*	16 (80.0)*	<0.001
IMV, *n* (%)	6 (3.1)	1 (0.8)	2 (4.1)	3 (15.0)*	0.005
NIMV, *n* (%)	7 (3.6)	0 (0.0)	4 (8.2)*	3 (15.0)*	<0.001
ICU transfer, *n* (%)	7 (3.6)	1 (0.8)	5 (10.2)*	1 (5.0)	0.007

**P < 0.05, vs. without CI; #P < 0.05, vs. early CI; ACE-I, angiotensin-converting enzyme inhibitors; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; ARB, angiotensin II receptor blockers; AST, aspartate aminotransferase; BNP, B-type natriuretic peptide; BUN, blood urea nitrogen; CI, cardiac injury; CK-MB, creatine kinase muscle-brain; CRP, C-reactive protein; DBP, diastolic blood pressure; hs-TNI, hypersensitive troponin I; ICU, intensive care unit; IMV, invasive mechanical ventilation; IQR, interquartile range; LDH, lactate dehydrogenase; NIMV, non-invasive mechanical ventilation; PCT, procalcitonin; PT, prothrombin time; SBP, systolic blood pressure; SD, standard deviation.*

in the CI group compared to the non-CI group. Another mechanism is CI related to hypoxia. The balance between the oxygen demand and supply of the myocardium is disrupted during acute hypoxia. A cascade of cellular, biochemical and inflammatory reactions can occur during hypoxia, eventually causing myocardial apoptosis (29). Acute severe hypoxia can

TABLE 2 | Comparisons of additional treatment, complications, and prognosis after 72h of admission among the three groups.

Variables	Total population (*n* = 196)	Without CI (*n* = 127)	Early CI (*n* = 49)	Late CI (*n* = 20)	*P*-value
Additional treatment after admission					
Antiviral therapy, *n* (%)	36 (18.4)	24 (18.9)	9 (18.4)	3 (15.0)	1.000
Antibiotic therapy, *n* (%)	50 (25.5)	31 (24.4)	14 (28.6)	5 (25.0)	0.882
Glucocorticoid therapy, *n* (%)	48 (24.5)	26 (20.5)	11 (22.4)	11 (55.0)*#	0.007
Immunoglobulin, *n* (%)	49 (25.0)	22 (17.3)	14 (28.6)	13 (65.0)*#	<0.001
ACEI/ARB, *n* (%)	17 (8.7)	12 (9.4)	3 (6.1)	2 (10.0)	0.733
Oxygen therapy, *n* (%)	34 (17.3)	26 (20.5)	5 (10.2)	3 (15.0)	0.273
High-flow oxygen, *n* (%)	51 (26.0)	42 (33.1)	5 (10.2)*	4 (20.0)	0.006
IMV, *n* (%)	33 (16.8)	8 (6.3)	13 (26.5)*	12 (60.0)*#	<0.001
NIMV, *n* (%)	16 (8.2)	8 (6.3)	5 (10.2)	3 (15.0)	0.234
ICU transfer, *n* (%)	25 (12.8)	6 (4.7)	7 (14.3)	12 (60.0)*#	<0.001
Complications					
Cardiac injury (CI)					
Early CI, *n* (%)	49/196 (25.0)	/	49/49(100)	0/20 (0)	/
Recurrent CI, *n* (%)	19/49 (38.8)	/	19/49 (38.8)	/	/
Late CI, *n* (%)	20/196 (10.2)	/	/	20/20 (100)	/
ARDS, *n* (%)	111/196 (56.6)	51/127 (40.2)	40/49 (81.6)*	20/20 (100)*	<0.001
Coagulation dysfunction, *n* (%)	57/193 (29.5)	21/124 (16.9)	22/49 (44.9)*	14/20 (70.0)*	<0.001
Acute kidney injury, *n* (%)	33/193 (17.1)	8/125 (6.4)	17/48 (35.4)*	8/20 (40.0)*	<0.001
Time from admission to complications onset					
Cardiac injury (CI)					
Early CI (days)	1 (0, 1)	/	1 (0, 1)	/	/
Recurrent CI (days)	7 (5, 16)	/	7 (5, 16)	/	/
Late CI (days)	11 (5, 22)	/	/	11 (5, 22)	/
ARDS (days)	4 (2, 9)	6 (3, 12)	2 (1, 8)*	7 (2, 8)	0.014
Coagulation dysfunction (days)	11 (1, 20)	11 (1, 19)	8 (1, 15)	23 (8, 25)#	0.02
Acute kidney injury (days)	7 (3, 14)	10 (3, 17)	4 (2, 7)	12 (6, 21)	0.065
Prognosis					
Death, *n* (%)	43 (21.9)	7 (5.5)	23 (46.9)*	13 (65.0)*	<0.001

*Data are median (IQR) or n (%).*P < 0.05, vs. without CI; #P < 0.05, vs. early CI; ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; ARDS, acute respiratory distress syndrome; ICU, intensive care unit; IMV, invasive mechanical ventilation; IQR, interquartile range; NIMV, non-invasive mechanical ventilation.*

also trigger a systemic inflammatory response (30). In this study, patients with early or late CI had lower level of SpO_2 on admission and higher incidence of ARDS compared to the non-CI group. There was a large timing overlap between CI and ARDS in the early CI group and 75% of patients with late CI were preceded by ARDS which supports a strong relationship between CI and hypoxia. Lastly, antiviral drugs can cause cardiac insufficiency, arrhythmia or other cardiovascular disorders with variable individual susceptibility (4, 31). In the present study, almost all patients (93.4% of COVID-19 patients) were administrated with antiviral drugs either on admission or after admission for which the opportunity of individual susceptibility and/or interaction with the underlying comorbid conditions could contribute to onset of late CI or recurrence of the initial one. In addition, patients in the CI group were more likely to have received glucocorticoid therapy. The relationship between glucocorticoid therapy and cardiac injury remains controversial and is under investigation (32). On the other hand, it is also likely that patients with any CI had higher disease severity for which more treatment was administered. This may suggest that the CI after admission was a sign of disease severity and/or progression.

Recent studies have demonstrated that CI was associated with increased mortality in COVID-19 patients (7, 8, 33). However, to the best of our knowledge, this is the first study to depict the clinical characteristics of both early CI and late CI and their association with in-hospital mortality. Our study showed that early CI was an independent predictor of death in COVID-19 patients even after accounting for variables that have proven prognostic value in the risk stratification of acutely ill patients, such as respiratory rate, heart rate, SpO_2, temperature, mean arterial pressure, and coma (16, 34). Cardiac injury remained significant after accounting for potential confounding laboratory variables with proven prognostic value in COVID hospitalizations such as lymphocyte count, LDH, CRP (17). This is important because clinicians should not consider patients

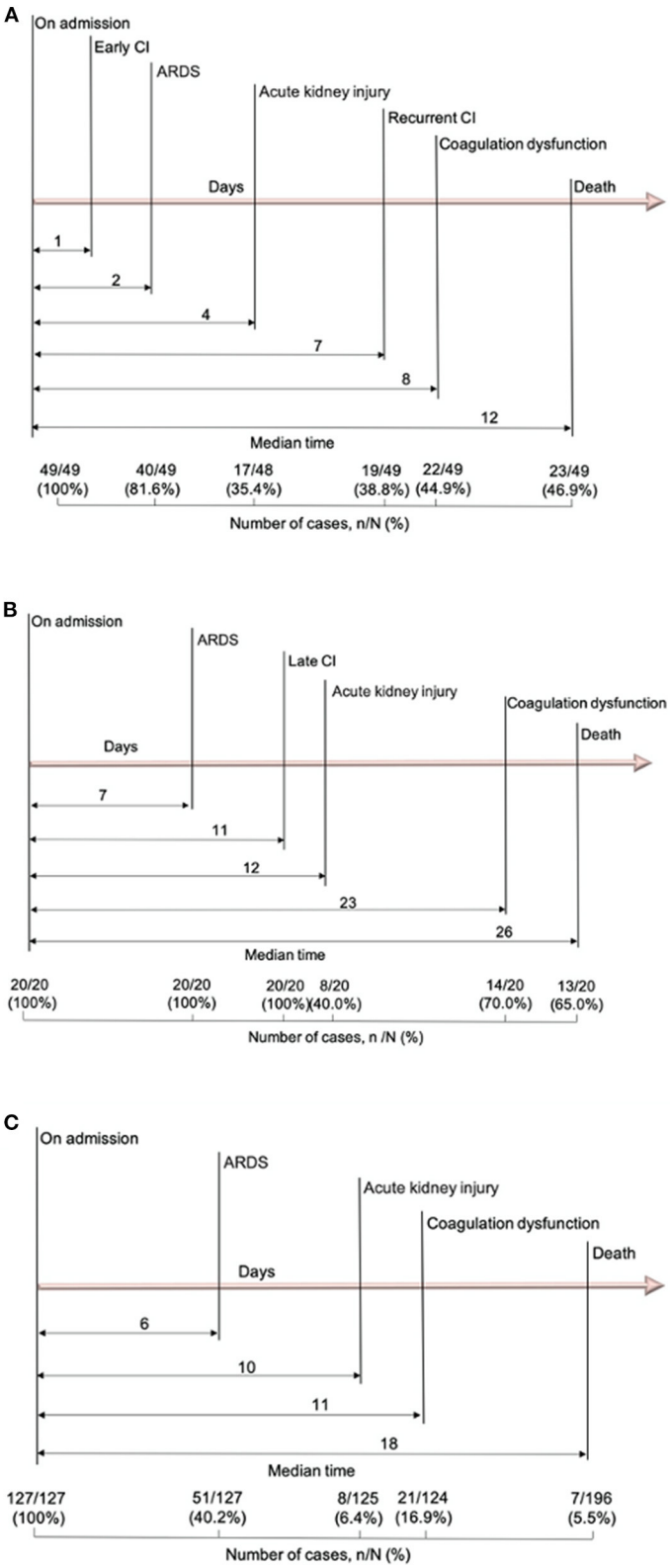


FIGURE 1 | Timeline of COVID-19 patients after admission. **(A)** Timeline of COVID-19 patients with early CI; **(B)** Timeline of COVID-19 patients with late CI; **(C)** Timeline of COVID-19 patients without CI. COVID-19, coronavirus disease 2019; CI, cardiac injury.

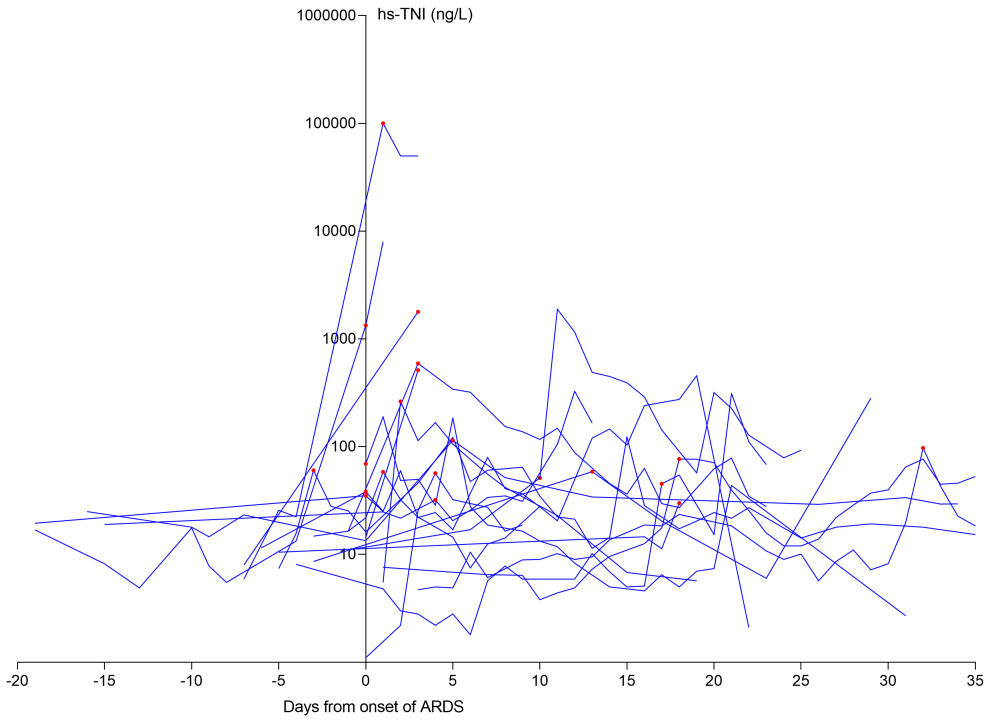


FIGURE 2 | The dynamic profile of hs-TNI levels in late CI patients in relation to ARDS onset. 15/20 (75.0%) patients suffering from late CI after the onset of ARDS. The levels of hs-TN were log transformed. ARDS, acute respiratory distress syndrome; CI, cardiac injury; hs-TNI, high-sensitivity troponin I.

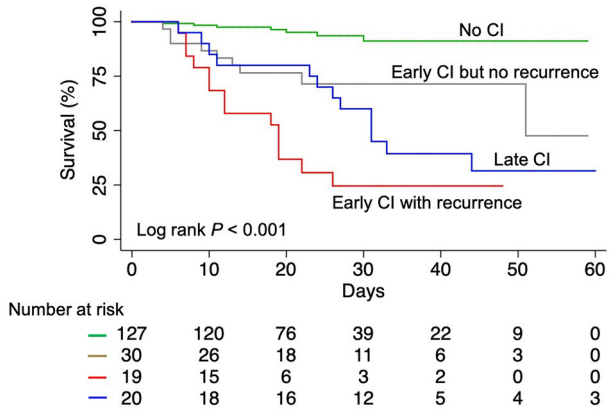


FIGURE 3 | Kaplan-Meier survival curves for COVID-19 patients. COVID-19, coronavirus disease 2019; CI, cardiac injury.

being out of danger despite absence of CI in the first 72 h since it can occur late. Similarly, a fall after the early rise in Trop I should not translate into a lower risk status since recurrence of CI can occur. Conversely, we also found that CI is closely related to the occurrence of ARDS as opposed to a primary cardiac event. Accordingly, our study suggested the need for a more systematic assessment of cardiac troponins for risk stratification of COVID-19 patients on admission, with repeat measures for those who already have initial CI or who are at risk for clinical deterioration. With the advent of multiple therapies now to reduce the morbidity and mortality of COVID-19 (35–37), early identification of higher risk patients with cardiac biomarkers may be helpful to balance the cost vs. the effect of therapy.

LIMITATIONS

Our study has some limitations. First, this was a relatively small sample size and single-center retrospective observational study for which residual confounding cannot be excluded and we did not include all the potential factors associated with mortality in our study. Second, only 196 patients both had the data of hs-TNI within and after 72 h of admission, which may have underestimated the true incidence of CI in our study. Therefore, future studies should be multi-centered, with larger sample size and systematic data collection, in order to promote a more comprehensive understanding of the association between cardiac injury and mortality in hospitalized patients with COVID-19.

CONCLUSIONS

CI is a common condition that can occur early on or late after, the initial 72 h of admission and is associated with ARDS and an increased risk of in-hospital mortality. Both late CI

TABLE 3 | Univariate and multivariate logistic regression analysis of factors associated with in-hospital mortality of COVID-19 patients.

Factors	Univariate		Multivariate	
	Unadjusted OR (95% confidence interval)	*P*-value	Adjusted OR (95% confidence interval)	*P*-value
Age group (years)				
<45	1 (ref)			
45–54	0.680 (0.040, 11.632)	0.79		
55–64	4.675 (0.559, 39.116)	0.155		
65–74	7.650 (0.944, 61.980)	0.057		
>74	6.581 (0.787, 55.045)	0.082		
Sex				
Female	1 (ref)		1 (ref)	
Male	3.632 (1.632, 8.086)	0.002	8.828 (2.463, 31.643)	0.001
Respiratory rate (breaths/min)				
12–24	1 (ref)		1 (ref)	
>24	3.667 (1.804, 7.451)	<0.001	3.773 (1.188, 11.983)	0.024
Heart rate (bpm)				
70–109	1 (ref)			
40–69	2.321 (0.547, 9.845)	0.253		
110–139	3.095 (1.261, 7.600)	0.014		
140–179	4.643 (0.627, 34.377)	0.133		
SpO_2 (%)				
>89	1 (ref)		1 (ref)	
<75	24.833 (2.661, 231.712)	0.005	8.264 (0.694, 98.332)	0.095
75–85	31.042 (6.405, 150.435)	<0.001	11.129 (1.341, 92.388)	0.026
86–89	31.042 (3.474, 277.332)	0.002	74.421(3.121, 1774.444)	0.008
Temperature (°C)				
<37.2	1 (ref)			
37.2–38.9	1.991 (0.855, 4.633)	0.11		
>38.9	2.031 (0.741, 5.564)	0.168		
Mean arterial pressure (mmHg)				
70–109	1 (ref)			
50–69	8.065 (0.708, 91.829)	0.094		
110–129	1.578 (0.664, 3.748)	0.302		
130–159	1.008 (0.109, 9.340)	0.994		
Coma (yes vs. no) (9 vs. 320)	8.108 (1.937, 33.944)	0.004		
Hypertension (yes vs. no) (139 vs. 190)	2.974 (1.467, 6.029)	0.003		
Lymphocytes (%)				
≥20	1 (ref)			
<20	10.551 (3.594, 30.980)	<0.001		
CRP (mg/L)				
≤8	1 (ref)			
>8	17.500 (4.086, 74.956)	<0.001		
LDH (U/L)				
≤245	1 (ref)			
>245	8.129 (3.239, 20.398)	<0.001		
Type of CI				
Early CI but no recurrence	1 (ref)		1 (ref)	
Early CI with recurrence	6.533 (1.807, 23.627)	0.004	7.184 (1.472, 35.071)	0.015
Late CI	4.333 (1.298, 14.471)	0.017	5.019 (1.125, 22.388)	0.034
No CI	0.136 (0.046, 0.405)	<0.001	0.119 (0.030, 0.475)	0.003

OR, odds ratio; CI, cardiac injury; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; LDH, lactate dehydrogenase.

and recurrent CI after the initial episode were associated with worse outcomes than patients with early CI alone. This study highlights the importance of early examination and periodical monitoring of cardiac biomarkers to identify predictors and markers of clinical deterioration in COVID-19 patients to guide intervention.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology. Written informed consent was waived for all participants with emerging infectious diseases. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MX, W-CW, PH, and LZ: conception and design of study. WS, YaZ, CW, SW, DZ, HY, YoZ, LC, YL, JW, YY, and QL: acquisition of data. YaZ, WS, SW, ML, and YiZ: analysis and/or interpretation of data. LZ, WS, YaZ, and CW: drafting the manuscript. MX, W-CW, PH, and LZ: revising the manuscript critically for important intellectual content. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China (Grant No. 81922033 to LZ; Grant No. 81727805 to MX).

ACKNOWLEDGMENTS

The authors would like to express their appreciation for all of the emergency services, nurses, doctors, and other hospital staff for their efforts to combat the COVID-19 outbreak.

REFERENCES

1. World Health Organization. *Novel Coronavirus (2019-nCoV): Situation Report-88*. Available online at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200417-sitrep-88-covid-191b6cccd94f8b4f219377bff55719a6ed.pdf (accessed December 22, 2020).
2. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
3. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. (2020) 382:1708–20. doi: 10.1056/NEJMoa2002032
4. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. (2020) 323:1061–9. doi: 10.1001/jama.2020.1585
5. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3
6. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. (2020) 8:475–81. doi: 10.1016/S2213-2600(20)30079-5
7. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol*. (2020) 5:802–10. doi: 10.1001/jamacardio.2020.0950
8. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol*. (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017
9. Shi S, Qin M, Cai Y, Liu T, Shen B, Yang F, et al. Characteristics and clinical significance of myocardial injury in patients with severe coronavirus disease 2019. *Eur Heart J*. (2020) 41:2070–9. doi: 10.1093/eurheartj/ehaa408
10. Ferrante G, Fazzari F, Cozzi O, Maurina M, Bragato R, D'Orazio F, et al. Risk factors for myocardial injury and death in patients with COVID-19: insights from a cohort study with chest computed tomography. *Cardiovasc Res*. (2020) 116:2239–46. doi: 10.1093/cvr/cvaa193
11. Deng Q, Hu B, Zhang Y, Wang H, Zhou X, Hu W, et al. Suspected myocardial injury in patients with COVID-19: evidence from front-line clinical observation in Wuhan, China. *Int J Cardiol*. (2020) 311:116–21. doi: 10.1016/j.ijcard.2020.03.087
12. World Health Organization. *Clinical Management of Severe Acute Respiratory Infection When Novel Coronavirus (nCoV) Infection Is Suspected: Interim Guidance, 25 January 2020*. World Health Organization (2020). Available online at: https://apps.who.int/iris/handle/10665/330854 (accessed December 22, 2020).
13. World Health Organization. *COVID-19 Clinical Management: Living Guidance* (2020). Available online at: https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-1 (accessed February 12, 2021).
14. Kidney Disease Improving Global Outcomes. Acute Kidney Injury Work Group: KDIGO clinical practice guideline for acute kidney injury. *Kidney Int*. (2012) 2:1–138. doi: 10.1038/kisup.2012.1
15. World Health Organization. *Nutritional Anaemias*. Report of a WHO Scientific Group. World Health Organization technical report series no. 405. Geneva: World Health Organization (1968). p. 5–37.
16. Hu H, Yao N, Qiu Y. Comparing rapid scoring systems in mortality prediction of critically ill patients with novel coronavirus disease. *Acad Emerg Med*. (2020) 27:461–8. doi: 10.1111/acem.13992
17. Yan L, Zhang H, Goncalves J, Xiao Y, Wang M, Guo Y, et al. An interpretable mortality prediction model for COVID-19 patients. *Nat Mach Intell*. (2020) 2:283–8. doi: 10.1038/s42256-020-0180-7
18. Shi Y, Yu X, Zhao H, Wang H, Zhao R, Sheng J. Host susceptibility to severe COVID-19 and establishment of a host risk score: findings of 487 cases outside Wuhan. *Crit Care*. (2020) 24:108. doi: 10.1186/s13054-020-2833-7
19. Turner AJ, Hiscox JA, Hooper NM. ACE2: from vasopeptidase to SARS virus receptor. *Trends Pharmacol Sci*. (2004) 25:291–4. doi: 10.1016/j.tips.2004.04.001
20. Gallagher PE, Ferrario CM, Tallant EA. Regulation of ACE2 in cardiac myocytes and fibroblasts. *Am J Physiol Heart Circ Physiol*. (2008) 295:H2373–9. doi: 10.1152/ajpheart.00426.2008
21. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med*. (2020) 8:420–2. doi: 10.1016/S2213-2600(20)30076-X

22. Tavazzi G, Pellegrini C, Maurelli M, Belliato M, Sciutti F, Bottazzi A, et al. Myocardial localization of coronavirus in COVID-19 cardiogenic shock. *Eur J Heart Fail*. (2020) 22:911–5. doi: 10.1002/ejhf.1828
23. Wichmann D, Sperhake JP, Lütgehetmann M, Steurer S, Edler C, Heinemann A, et al. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort Study. *Ann Intern Med*. (2020) 173:268–77. doi: 10.7326/L20-1206
24. Lindner D, Fitzek A, Bräuninger H, Aleshcheva G, Edler C, Meissner K, et al. Association of cardiac infection with SARS-CoV-2 in confirmed COVID-19 autopsy cases. *JAMA Cardiol*. (2020) 5:1281–5. doi: 10.1001/jamacardio.2020.3551
25. Schaller T, Hirschbühl K, Burkhardt K, Braun G, Trepel M, Märkl B, et al. Postmortem examination of patients with COVID-19. *JAMA*. (2020) 323:2518–20. doi: 10.1001/jama.2020.8907
26. Tang Y, Liu J, Zhang D, Xu Z, Ji J, Wen C. Cytokine storm in COVID-19: the current evidence and treatment strategies. *Front Immunol*. (2020) 11:1708. doi: 10.3389/fimmu.2020.01708
27. Mahmudpour M, Roozbeh J, Keshavarz M, Farrokhi S, Nabipour I. COVID-19 cytokine storm: the anger of inflammation. *Cytokine*. (2020) 133:155151. doi: 10.1016/j.cyto.2020.155151
28. Zhang L, Feng X, Zhang D, Jiang C, Mei H, Wang J, et al. Deep vein thrombosis in hospitalized patients with COVID-19 in Wuhan, China: prevalence, risk factors, and outcome. *Circulation*. (2020) 142:114–28. doi: 10.1161/CIR.0000000000000887
29. Han Q, Li G, Ip MS, Zhang Y, Zhen Z, Mak JC, et al. Haemin attenuates intermittent hypoxia-induced cardiac injury via inhibiting mitochondrial fission. *J Cell Mol Med*. (2018) 22:2717–26. doi: 10.1111/jcmm.13560
30. Eltzschig HK, Carmeliet P. Hypoxia and inflammation. *N Engl J Med*. (2011) 364:656–65. doi: 10.1056/NEJMra0910283
31. Ni C, Ma P, Wang R, Lou X, Liu X, Qin Y, et al. Doxorubicin-induced cardiotoxicity involves IFNγ-mediated metabolic reprogramming in cardiomyocytes. *J Pathol*. (2019) 247:320–32. doi: 10.1002/path.5192
32. Violi F, Calvieri C, Cangemi R. Effect of corticosteroids on myocardial injury among patients hospitalized for community-acquired pneumonia: rationale and study design. The colosseum trial. *Intern Emerg Med*. (2020) 15:79-86. doi: 10.1007/s11739-019-02117-0
33. Mumoli N, Cei M, Mazzone A, Conte G. Cardiac injury as prognostic value in COVID-19: more remains to be clarified. *Intern Emerg Med*. (2021) 16:267–8. doi: 10.1007/s11739-020-02540-8
34. Olsson T, Terent A, Lind L. Rapid emergency medicine score: a new prognostic tool for in-hospital mortality in nonsurgical emergency department patients. *J Intern Med*. (2004) 255:579–87. doi: 10.1111/j.1365-2796.2004.01321.x
35. Grein J, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, et al. Compassionate use of remdesivir for patients with severe COVID-19. *N Engl J Med*. (2020) 382:2327–36. doi: 10.1056/NEJMc2015312
36. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. *JAMA*. (2020) 324:782–93. doi: 10.1001/jama.2020.12839
37. Robbiani DF, Gaebler C, Muecksch F, Lorenzi JCC, Wang Z, Cho A, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature*. (2020) 584:437–42. doi: 10.1038/s41586-020-2456-9

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 31 May 2021
doi: 10.3389/fcvm.2021.577398

Association of ACEi/ARB Use and Clinical Outcomes of COVID-19 Patients With Hypertension

Jing Ma[1,2,3†], *Xiaowei Shi*[1,2†], *Jiong Yu*[1,2†], *Feifei Lv*[3], *Jian Wu*[1,4], *Xinyu Sheng*[1,2], *Qiaoling Pan*[1,2], *Jinfeng Yang*[1,2], *Hongcui Cao*[1,2*] *and Lanjuan Li*[1,2]

[1] State Key Laboratory for the Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, [2] Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Hangzhou, China, [3] Department of Laboratory Medicine, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, [4] Department of Laboratory Medicine, The First People's Hospital of Yancheng City, Yancheng, China

OPEN ACCESS

Edited by:
Shuyang Zhang,
Peking Union Medical College Hospital, China

Reviewed by:
Piyali Chatterjee,
Central Texas Veterans Health Care System, United States
Raffaella Lombardi,
University of Naples Federico II, Italy

***Correspondence:**
Hongcui Cao
hccao@zju.edu.cn

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Hypertension, a section of the journal Frontiers in Cardiovascular Medicine

Received: *22 January 2021*
Accepted: *04 May 2021*
Published: *31 May 2021*

Citation:
Ma J, Shi X, Yu J, Lv F, Wu J, Sheng X, Pan Q, Yang J, Cao H and Li L (2021) Association of ACEi/ARB Use and Clinical Outcomes of COVID-19 Patients With Hypertension. Front. Cardiovasc. Med. 8:577398. doi: 10.3389/fcvm.2021.577398

Objectives: Evidence has shown that angiotensin-converting enzyme 2 (ACE2), which can be upregulated after angiotensin-converting enzyme inhibitor (ACEi) and angiotensin receptor blocker (ARB) treatment, may play a dual role in the pathogenesis and progression of coronavirus disease 2019 (COVID-19). We aimed to assess the association between the use of ACEi/ARB and the outcome of COVID-19 patients with preexisting hypertension in non-endemic areas.

Methods: From January 17, 2020, to February 19, 2020, 286 patients with hypertension were enrolled in this retrospective study out of 1,437 COVID-19 patients from 47 centers in Zhejiang and Jiangsu Province. The composite endpoints consisted of mechanical ventilation, intensive care unit (ICU) admission, or death. Cox proportional hazards analysis was performed to assess the association between ACEi/ARB and clinical outcomes of COVID-19 patients with hypertension.

Results: In the main analysis, 103 patients receiving ACEi/ARB were compared with 173 patients receiving other regimens. Overall, 44 patients (15.94%) had an endpoint event. The risk probability of crude endpoints in the ACEi/ARB group (12.62%) was lower than that in the non-ACEi/ARB group (17.92%). After adjusting for confounding factors by inverse probability weighting, the results showed that the use of ACEi/ARB reduced the occurrence of end events by 47% [hazard ratio (HR) = 0.53; 95% CI, 0.34–0.83]. Similar results were obtained in multiple sensitivity analyses.

Conclusions: In this retrospective study, among COVID-19 patients with hypertension, the use of ACEi/ARB is not associated with an increased risk of disease severity compared with patients without ACEi/ARB. The trends of beneficial effects of ACEi/ARB need to be further evaluated in randomized clinical trials.

Keywords: angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, COVID-19, hypertension, SARS-CoV-2

INTRODUCTION

The novel coronavirus disease 2019 (COVID-19) is spreading worldwide, with an increasing number of confirmed cases and deaths, and has received widespread attention from the World Health Organization. It is currently known that COVID-19 patients with hypertension are prone to have poor clinical outcomes (1). Angiotensin-converting enzyme inhibitors (ACEis) and angiotensin receptor blockers (ARBs) are widely used in the treatment of hypertension. In animal studies, the expression of angiotensin-converting enzyme 2 (ACE2) is upregulated after ACEi and ARB treatment (2). Intriguingly, ACE2 plays a dual role in COVID-19 progression. On one hand, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds with ACE2 to enter the host cell during invasion (3), resulting in a decrease in ACE2 and subsequently causing vasoconstriction. Based on this, patients with a medical history of ACEi/ARB may be more likely to suffer from SARS-COV-2 infection and severe progression due to elevated ACE2 expression, and it has proposed that alternative treatments be sought for those with a high risk of infection (4). On the other hand, evidence from various acute respiratory distress syndrome (ARDS) animal models showed that exogenous ACE2 supplementation can reduce inflammation and increase oxygenation (2). The absence of the protective role of ACE2 may lead to renin–angiotensin system (RAS) dysregulation and potentially give rise to extensive endothelial dysfunction and acute lung injury (5). Thus, ACEi/ARB may, in turn, be beneficial as it prevents RAS overactivation by increasing ACE2 expression, reducing the risk of acute lung injury and acute respiratory distress syndrome.

Several studies have indicated that ACEi/ARB use was associated with decreased mortality in patients with COVID-19 (6–8), but most studies supported that ACEi/ARB use was not related to disease severity (1, 8–12). A recent meta-review of ours also concluded that ACEi/ARB therapy was associated with a lower risk of mortality compared to those who have non-ACEi/ARB antihypertensive drugs but not associated with a higher risk of COVID-19 severity (13). Indeed, the use of ACEi/ARB in patients with COVID-19 remains controversial. And very few large-sample studies are conducted outside the pandemic area in China (14, 15). Therefore, the present study aimed to assess the association between ACEi/ARB use and its impact on the risk of severity in COVID-19 patients with hypertension in non-endemic areas by inverse probability of treatment weighting (IPTW) analysis.

METHODS

Patients

Patients diagnosed with COVID-19 were recruited for this multicenter retrospective study from 47 centers in Zhejiang and Jiangsu Province between January 17, 2020, and February 19, 2020. All patients enrolled in this study were diagnosed with hypertension and COVID-19 according to the diagnostic criteria of the National Health Commission. This study was approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University (No. IIT20200005C), and complied with the ethical guidelines of the Declaration of Helsinki. Written informed consent was waived, as this study was conducted on an emerging infectious disease and the researchers analyzed only anonymous data.

Data Collection

Epidemiological, demographic, comorbidities, clinical, laboratory, time from illness onset to hospital admission, time to the first dose of antiviral delivery, chest radiological findings at admission, and outcome data were collected from patients' electronic medical records, with verification by independent doctors. The COVID-19 cases were all confirmed by throat swab specimens from the upper respiratory tract using sequencing or RT-PCR assay. Clinical outcomes were followed up to March 15, 2020.

Definition

The patients were classified into four types: mild, moderate, severe, and critical type according to the guidelines on the Diagnosis and Treatment of COVID-19 by the National Health Commission (16). All patients taking ACEi and ARB antihypertensive drugs, whether combined or not, were classified in the ACEi/ARB group based on their main complaint at admission. In principle, the antihypertensive regimens remained the same as the drugs used by patients before admission. Hypertension grades were defined as Grade 1, Grade 2, and Grade 3 according to 2018 guidelines of the European Society of Hypertension (ESH). The onset of COVID-19 was defined as the time when symptoms were first noticed. The endpoint of this study was defined as a composite measure consisting of mechanical ventilation, intensive care unit (ICU) admission, or death. Briefly, the endpoint represented at least one criterion: respiratory failure occurs and mechanical ventilation is required, develops other organ failures and needs ICU monitoring and treatment, or death (17). If the patient met several criteria for the event, the calculation will be based on the time of the first criterion appearance and follow-up until the patient was discharged.

Statistical Analysis

Continuous variables were expressed as medians and interquartile range (IQR) 25–75% and were compared by *t*-test or Mann–Whitney U-test. Categorical variables were expressed as percentages and tested with chi-square test or Fisher's exact test. To assess the association between ACEi/ARB use and clinical outcomes of COVID-19 patients, our main analysis compared the 103 participants who received ACEi/ARB with the 173 who received other regimens. Cox proportional hazards regression models were used to assess the association between ACEi/ARB use and the composite endpoint of intubation, ICU admission, or death. The primary analyses adjusted for benchmark covariates, including sex, age, body mass index (BMI), smoking status, duration from onset to admission, C-reactive protein (CRP), treatment of antivirus drugs, clinical type on admission, grade of hypertension, and comorbidities. The main analysis was performed by IPTW to minimize

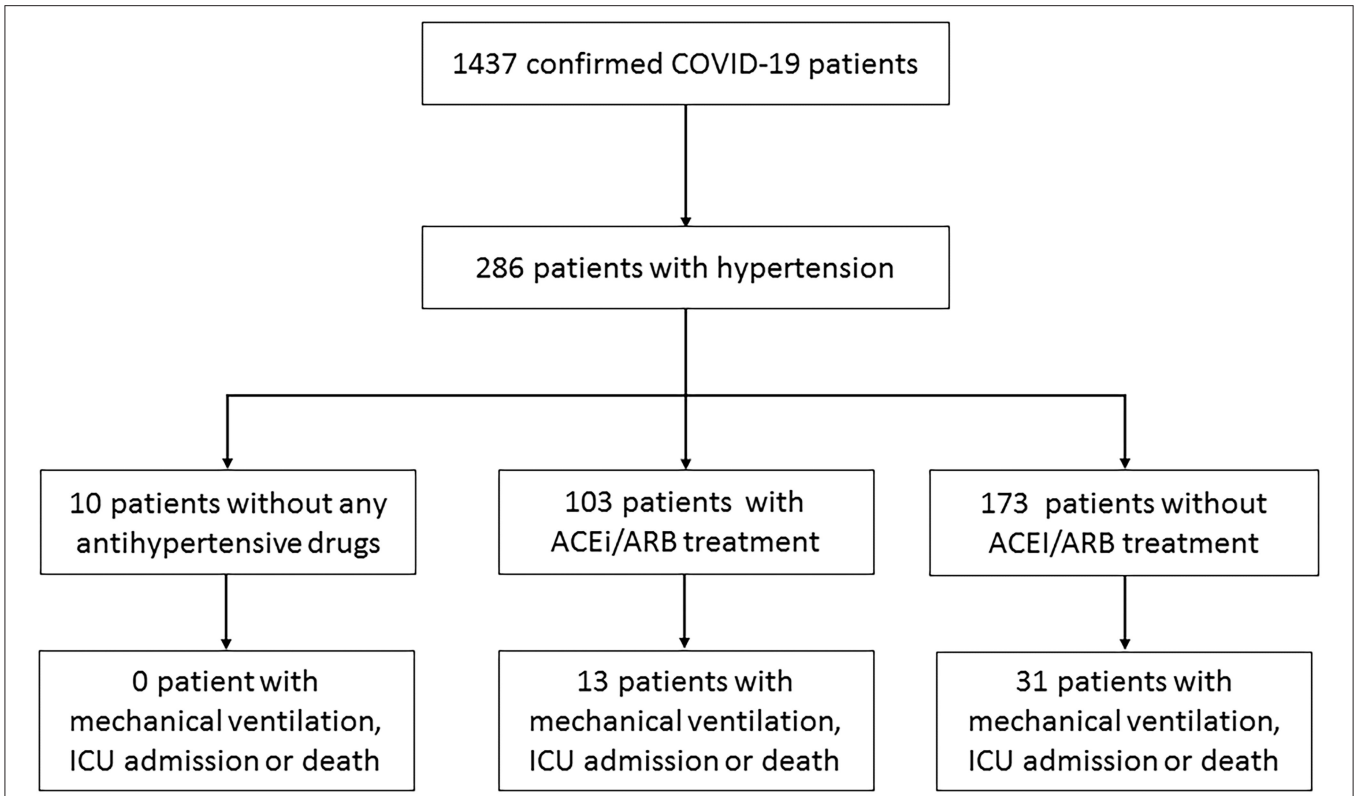


FIGURE 1 | Flowchart of patient selection.

the effect of ACEi/ARB use selection bias and to control for potential confounding factors (18), which included the same covariates as the Cox regression model (19). The estimated propensity score was obtained as the predicted probability of each subject treated with ACEi/ARB. The standardized differences were examined to assess the covariates included in estimating propensity scores before and after weighting, with a statistic <10% indicating a clinically meaningful balance between the two groups (19). Missing data were performed through multiple imputations by chained equations using the other variables available (20). All statistical analyses were performed by Statistical Package for the Social Sciences version 19.0 (International Business Machines Corporation, Armonk, NY) and R version 3.4 (R Foundation, Vienna, Austria). All tests were two–tailed, and $p < 0.05$ was considered to indicate statistical significance.

Other Sensitivity Analyses

In addition, we conducted eight prespecified subgroups and sensitivity analyses to evaluate the robustness of the composite endpoint: (1) age (age <60 vs. ≥60 years), (2) sex (male vs. female), (3) median value of onset to admission (<4 vs. ≥4 days), (4) CRP (<8 vs. ≥8 mg/L), (5) BMI (<25 vs. ≥25 kg/m^2), (6) presence of diabetes (yes vs. no), (7) clinical type on admission (mild/moderate vs. severe), (8) grade of hypertension (1 vs. 2 vs. 3).

Second, all patients eligible for the study were analyzed, and those without any antihypertensive drugs were analyzed in the control group.

RESULTS

Clinical Characteristics and Symptoms on Admission

From January 17, 2020, to February 19, 2020, 286 patients with hypertension were enrolled in this study out of 1,437 COVID-19 patients in 47 centers of Zhejiang and Jiangsu Province (**Figure 1**). Among the patients, 103 patients received ACEi/ARB therapy, including 12 with ACEi, 91 with ARB, and 46 combined with other types of drugs. Besides, 173 patients were treated with other regimens, including 143 (82.66%) with calcium channel blockers, 20 (11.56%) with beta-blockers, 40 (22.73%) with diuretics, and three (1.73%) with centrally acting agents (**Table 2**) and 10 without any antihypertensive drugs.

Clinical characteristics of patients from the ACEi/ARB group and other regimens group are shown in **Table 1**. There were no significant differences in either age or sex between the two groups ($p > 0.05$). Fever and cough were the main symptoms in the ACEi/ARB group and other regimens group, and the proportion in the two groups had no significant differences. In addition to hypertension, 97 (35.14%) patients had at least one comorbidity other than hypertension. The ACEi/ARB group included 22 cases

TABLE 1 | Characteristics of COVID-19 patients with hypertension with or without ACEi/ARB therapy.

Characteristic	Non-ACEi/ARB ($n = 173$)	ACEi/ARB ($n = 103$)	*p*-value
Age (years)	62 (52–68)	59 (52–67)	0.450
BMI (kg/m^2)*	25.53 (23.52–27.28)	24.84 (22.58–27.30)	0.190
Duration from onset to admission (days)	4 (2–7)	5 (3–7)	0.928
Temperatures (°C)	38.00 (37.50–38.50)	38.00 (37.40–38.50)	0.211
Female (%)	76 (43.93%)	55 (53.40%)	0.128
Current smoker (%)	15 (8.67%)	9 (8.74%)	1
Exposure history			
Contact with patients (%)	91 (52.60%)	54 (52.43%)	0.978
Cluster (%)	31 (17.92%)	19 (18.45%)	0.912
From Wuhan (%)	50 (28.90%)	34 (33.01%)	0.473
Symptoms			
Fever (%)	139 (80.35%)	79 (76.70%)	0.472
Cough (%)	107 (61.85%)	69 (66.99%)	0.390
Expectoration (%)	52 (30.06%)	25 (24.27%)	0.300
Sore throat (%)	15 (8.67%)	13 (12.62%)	0.293
Muscle ache (%)	19 (10.98%)	10 (9.71%)	0.739
Fatigue (%)	43 (24.86%)	26 (25.24%)	0.943
Shortness of breath (%)	19 (10.98%)	12 (11.65%)	0.865
Diarrhea (%)	15 (8.67%)	6 (5.83%)	0.485
Sick (%)	6 (3.47%)	3 (2.91%)	1
Headache (%)	6 (3.47%)	4 (3.88%)	1
Coexisting comorbidity			
Cardiovascular diseases (%)	21 (12.14%)	5 (4.85%)	0.055
Diabetes (%)	32 (18.50%)	22 (21.36%)	0.562
COPD (%)	2 (1.16%)	0 (0.00%)	0.530
Asthma (%)	2 (1.16%)	0 (0.00%)	0.530
Cancer (%)	6 (3.47%)	1 (0.97%)	0.263
Chronic liver disease (%)	13 (7.51%)	9 (8.74%)	0.819
Chronic renal disease (%)	3 (1.73%)	3 (2.91%)	0.674
Chest x-ray/CT findings			0.520
Normal (%)	12 (6.94%)	3 (2.97%)	
Unilateral pneumonia (%)	20 (11.56%)	10 (9.90%)	
Bilateral pneumonia (%)	96 (55.49%)	59 (58.42%)	
Multiple mottling and ground-glass opacity (%)	45 (26.01%)	29 (28.71%)	
Grade of hypertension			0.003
Grade 1 (%)	109 (63.01%)	54 (52.43%)	
Grade 2 (%)	33 (19.08%)	38 (36.89%)	
Grade 3 (%)	31 (17.92%)	11 (10.68%)	
Severe/critical type on admission (%)	14 (8.09%)	10 (9.71%)	0.663
C-reactive protein (mg/L)**	15.53 (4.54–43.11)	15.80 (5.42–34.16)	0.164

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019.

**21 patients with missing data of BMI.*

***2 patients with missing data of C-reactive protein.*

of diabetes, five cases of cardiovascular diseases, and nine cases of chronic liver disease. And there were 32 with diabetes, 21 with cardiovascular disease, and 13 with chronic liver disease in the non-ACEi/ARB group. There are significant differences in the grade of hypertension: the proportion of grade 1 hypertension was 54 (52.43%) in the ACEi/ARB group vs. 109 (63.01%) in the non-ACEi/ARB group; grade 2, 38 (36.89%) vs. 33 (19.08%); and grade 3, 11 (10.68%) vs. 31 (17.92%), respectively ($p = 0.003$) (**Table 1**). The results of the remaining laboratory tests were shown in **Supplementary Table 1**.

TABLE 2 | In-hospital management and outcomes of ACEi/ARB and non-ACEI/ARB groups.

Variable	Non-ACEi/ARB ($n = 173$)	ACEi/ARB ($n = 103$)	*p*-value
Interferon-α	110 (63.58%)	64 (63.37%)	0.971
Oseltamivir	3 (1.73%)	5 (4.95%)	0.149
Fapiravir	6 (3.47%)	5 (5.00%)	0.535
Arbidol	127 (73.41%)	71 (70.30%)	0.579
Lopinavir/ritonavir	96 (55.49%)	61 (60.40%)	0.428
Darunavir	3 (1.84%)	2 (2.27%)	1
Chloroquine phosphate	2 (1.23%)	1 (1.14%)	0.95
Glucocorticoids	60 (34.68%)	33 (32.04%)	0.653
IVIGt	47 (27.17%)	25 (24.27%)	0.596
Antibiotics drug	80 (46.24%)	45 (43.69%)	0.68
Antihypertensive agents			
Calcium channel blockers	137 (82.66%)	47 (45.63%)	<0.001
ACEi	0 (0.00%)	12 (11.65%)	<0.001
ARB	0 (0.00%)	91 (88.35%)	<0.001
Beta-blockers	20 (11.56%)	7 (6.80%)	0.198
Diuretics	34 (19.65%)	12 (11.65%)	0.084
Centrally antihypertensive agents	3 (1.73%)	0 (0.00%)	0.296
Shock	1 (0.55%)	1 (0.97%)	1
Admission to ICU	24 (13.87%)	10 (9.71%)	0.309
Mechanical Ventilation	22 (12.72%)	9 (8.74%)	0.311
Venovenous hemofiltration	3 (1.73%)	1 (0.97%)	1
ECMO	6 (3.47%)	2 (1.94%)	0.714
Lung transplantation	1 (0.97%)	0 (0.00%)	1
Composite endpoint	31 (17.92%)	13 (12.62%)	0.245

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ICU, intensive care unit; ECMO, extracorporeal membrane oxygenation; IVIGt, intravenous immunoglobulin treatment.

The Association of Angiotensin-Converting Enzyme Inhibitor/Angiotensin Receptor Blocker Use With the Composite Endpoints

With a median time of 9 days, 44 patients had disease progression or death in the entire cohort. In detail, two had septic shock and were given vasoactive medications, 34 (12.32%) were admitted to the ICU, 31 (11.23%) received mechanical ventilation, one patient died after intubation, one had lung transplantation, and eight (2.90%) received extracorporeal membrane oxygenation (ECMO) (**Table 2**). Until March 15, 2020, nine patients had not been discharged, and one of them was in the ACEi/ARB group. The composite endpoints were documented in 13 of 103 (12.62%) patients who received ACEi/ARB therapy compared with 31 of 173 (17.92%) patients in the non-ACEi/ARB group. The rate of events was numerically lower in the ACEi/ARB group than in

the non-ACEi/ARB group, but the difference was not significant. The median progression event time was significantly different in the ACEi/ARB group compared with the non-ACEi/ARB group (12 vs. 9 days, $p = 0.003$). In the crude unadjusted analysis, Kaplan–Meier curves for events-free survival showed a hazard ratio (HR) of 0.65 (95% CI, 0.34–1.25; $p = 0.2002$); after adjusting the benchmark covariate, the HR was 0.41 (95% CI, 0.19–0.88; $p = 0.0211$) in the primary multivariable analysis (**Figure 2A**).

In the IPTW analysis, baseline characteristics were balanced in the two groups (**Supplementary Figure 1**, **Supplementary Table 2**). Among the 276 patients in the two groups, the events-free survival was 89.48% in the ACEi/ARB group and 81.85% in the non-ACEi/ARB group; the weighted HR was 0.53 (95% CI, 0.34–0.83; $p = 0.006$; **Figure 2B**).

Other Sensitivity Analyses

To further confirm whether the observed findings were robust to potential confounders, we performed stratified analyses by prespecified subgroups; all analyses were adjusted for all variables as the Cox regression model except for the stratification variable itself. Compared with the non-ACEi/ARB group, the risk of composite endpoint events probability did not increase in the ACEi/ARB group, with HRs ranging from 0.07 to 0.80 (**Figure 3**), and no statistically significant interaction was found. In addition, adding the 10 patients who were not taking any antihypertensive drugs in the control group did not change the result; the weighted HR was 0.48 (95% CI, 0.30–0.77; $p = 0.0022$; **Supplementary Figure 2**). The results of the sensitivity analyses support our main findings.

DISCUSSION

In this multicenter retrospective study, our results suggest that chronic treatment with ACEi/ARB is not associated with an increased severity of clinical outcome in COVID-19 patients with hypertension. The values of HRs were below 1 in all subgroups considered and after careful adjustments, including an IPTW analysis. In addition, the median progression event time of the ACEi/ARB group was significantly longer than that of the non-ACEi/ARB group (12 vs. 9 days, $p < 0.001$). This finding supported the continued use of RAS inhibitors in COVID-19 patients with hypertension, which provides clinical evidence for the recommendations of international societies.

RAS plays an important role in the pathogenesis and development of hypertension. ACEis and ARBs are commonly used in hypertensive patients as two targeted RAS system inhibitors. There is evidence demonstrating that activation of RAS is associated with acute lung injury in the SARS-CoV-infected model with downregulated ACE2 expression in the lungs, but the lung failure in this setting could be attenuated by treatment with ACEi/ARB (21–23). Furthermore, a systematic meta-study showed that ACEi/ARB can reduce the incidence of community-acquired pneumonia and pneumonia-related mortality (24). A recent study found that angiotensin II was significantly elevated in COVID-19 patients and was in a positive linear correlation with viral load and lung injury (25). Another study also supported the use of ACEi/ARB in improving clinical outcomes of COVID-19 patients with hypertension, as they found that using ACEi/ARB could significantly reduce the level of interleukin 6 while increasing the level of peripheral blood T cells (26).

To assess the potential effects of ACEi/ARB use on these in-hospital patients with COVID-19, we limited our analysis to a cohort of patients with coexisting hypertension and excluded those without hypertension. Several previous studies included the patients without hypertension in the non-ACEi/ARB group and concluded that the use of ACEi/ARB was not related to the severity of the disease (27–30), which may underestimate the effect of ACEi/ARB on patients with COVID-19, since hypertension itself was a risk factor for disease progression (31).

To the best of our knowledge, several observational studies have evaluated the impact of ACEi/ARB use on clinical outcomes in patients with COVID-19 (1, 6–11) and have offered different perspectives. Observational studies may be prone to bias and cannot provide robust results because interventions are not randomly assigned. Despite such shortcomings, observational data represent current clinical practice and apply modern methods to minimize selection bias to assess the effectiveness of clinical interventions and may help guide clinical decision-making. Two recent systematic reviews and meta-analyses concluded that the use of ACEi/ARB is significantly associated with decreased mortality in COVID-19 patients with hypertension but not associated with disease severity (13, 32). These systematic reviews recognized similar limitations, such as research heterogeneity, all studies included were observational, and most studies only adjusted for age and gender without considering other potential confounders and selection bias. Therefore, it is impossible to determine whether ACEi/ARB use is actually effective in SARS-COV-2-infected patients.

Feng et al. (33) first reported from Wuhan that there was a significant difference in ACEi/ARB usage among patients of different severities; the number of severe or critical patients was significantly lower in the ACEi/ARB group than in the non-ACEi/ARB group, but this research did not consider confounding factors, as other studies have done (8, 10). Another multicenter retrospective study performed an analysis among 1,128 COVID-19 patients with preexisting hypertension, which included 188 patients on treatment with ACEi/ARB (7). The effect of ACEi/ARB treatment was analyzed using a multivariate adjustment for confounding variables and propensity score (PS) matching. And results stated that ACEi/ARB was associated with a lower rate of severe outcomes with SARS-CoV-2 infection. These data are in concordance with our results, but the mortality rate in our patients was substantially lower. This discrepancy might result from several factors, i.e., delayed hospitalization after symptom onset in Hubei may lead to disease progression (34). In addition, in every five death cases of COVID-19, only one received invasive mechanical ventilation or further active respiratory support, suggesting that ventilation equipment was limited and intubation was delayed for many patients (35). But the authors did not provide many details about the duration between the onset of symptoms to admission (36) and the grade of hypertension like another study from Korea (9), which were

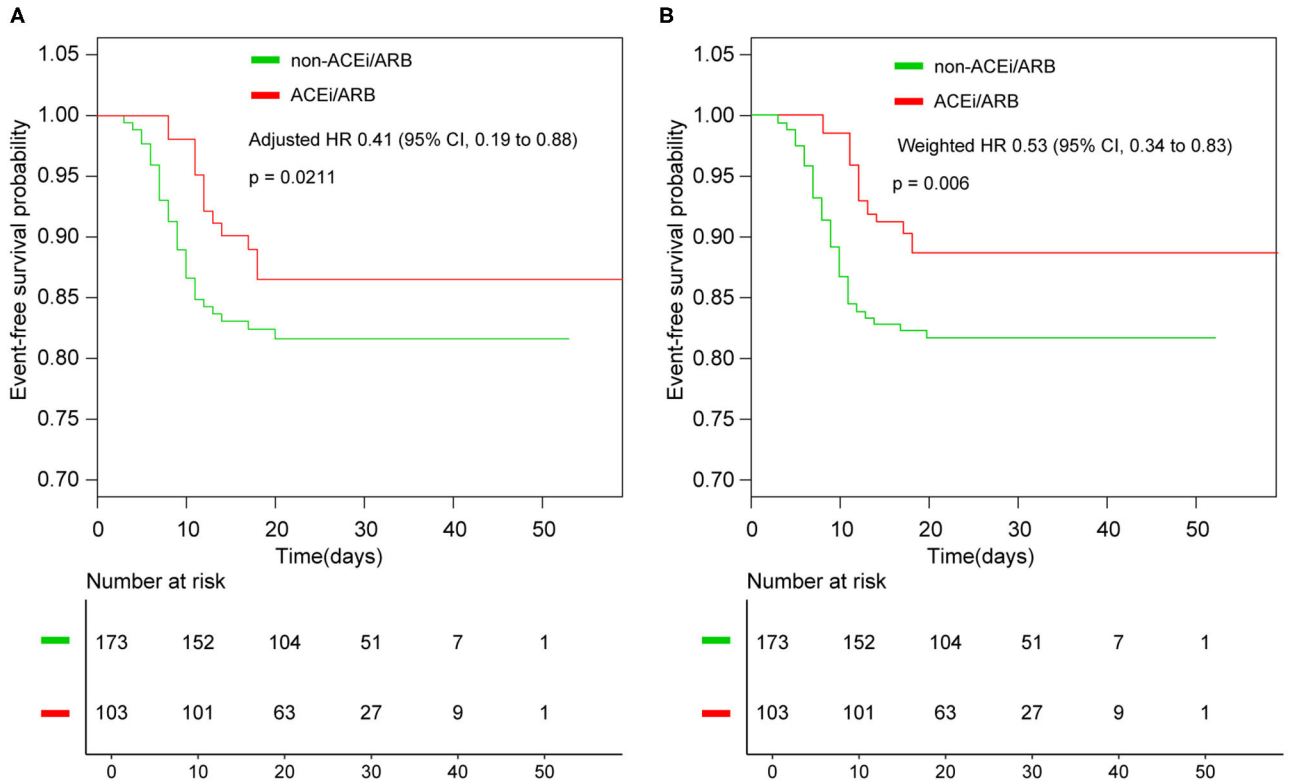


FIGURE 2 | Kaplan–Meier curves for survival without events. **(A)** Kaplan–Meier curves for event-free survival without weighted; **(B)** Kaplan–Meier curves for event-free survival after inverse probability of treatment weighting. ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; HR, hazard ratio.

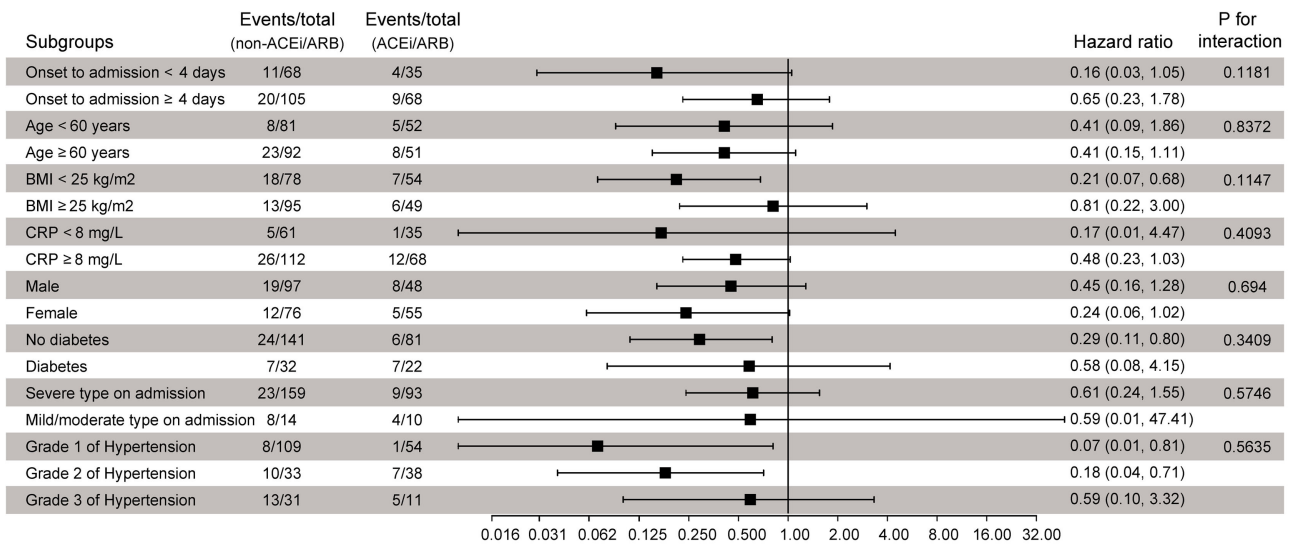


FIGURE 3 | Subgroup analysis of component endpoints according to ACEi/ARB treatment. ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CRP, C-reactive protein; HR, hazard ratio.

found to be significantly associated with the severity of COVID-19 in our study (**Supplementary Table 3**). After controlling important confounding factors through multivariate adjustment and IPTW analysis, the results suggested a favorable association of using ACEi/ARB and less severity in COVID-19 patients. Furthermore, sensitivity analyses supported our main finding.

The main advantage of this study is exploring the association between chronic treatment with ACEi/ARB and COVID-19

progression after adjusting the major confounding factors such as the interval between symptom onset to admission and grade of hypertension, and our sample size is relatively larger compared with studies conducted in non-endemic areas in China (14, 15). However, we recognize some limitations. First, due to the relatively lower mortality rate, we could not assess the association between ACEi/ARB use and mortality in COVID-19 patients with hypertension. Since the study was conducted in a non-epidemic pandemic area and there were sufficient medical resources to support the treatment of patients with COVID-19, this study can better reduce confounding factors caused by a shortage of medical resources. Second, the sample size in this study is not big enough. This study included 103 patients receiving ACEi/ARB therapy; only 12 of whom received ACEi. Therefore, subgroup analysis of the differences between the two drugs could not be performed. Third, since patients were not randomly allocated to ACEi/ARB therapy or other regimens, the results may be affected by selection/collider bias. IPTW analysis was used to minimize selection bias, which is a powerful and flexible approach to adjust for collider bias and reduce observational bias and is the best evidence available in observational studies. But IPTW analysis may also have limitations, as this approach may not reflect possible biases in observational studies, and some residual confounding may persist. Fourth, our results were obtained from patients with COVID-19 in non-endemic areas of China. Due to policy reasons, the impact of using ACEi/ARB in other countries/regions on SARS-CoV-2-infected patients needs further study. Whether the current results are applicable to other global populations, long-term prospective studies and randomized clinical trials are still needed to investigate the effects of these treatments.

CONCLUSION

In a group of hospitalized COVID-19 patients with preexisting hypertension, chronic treatment with ACEi/ARB does not seem to increase the risk of disease severity after adequate adjustment by IPTW. ACEi/ARB could be continued as antihypertensive therapy for COVID-19 patients with hypertension according to the recommendations of international societies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This study was approved by the First Affiliated Hospital, College of Medicine, Zhejiang University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HC and LL: concept and study design. JYu, XShi, JM, FL, JW, XShe, QP, and JYa: data acquisition. JYu and XShi: data analyses. JYu and JM: statistics. JYu: manuscript preparation. JYu, XShi, JM, FL, JW, QP, JYa, HC, and LL: review of the manuscript. All authors approved the final version of the manuscript.

FUNDING

This work was supported by the National Key Research and Development Program of China (No. 2016YFA0101001).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.577398/full#supplementary-material

REFERENCES

1. Li J, Wang X, Chen J, Zhang H, Deng A. Association of renin-angiotensin system inhibitors with severity or risk of death in patients with hypertension hospitalized for coronavirus disease 2019 (COVID-19) infection in Wuhan, China. *JAMA Cardiol*. (2020) 5:825–30. doi: 10.1001/jamacardio.2020.1624
2. Zhang H, Baker A. Recombinant human ACE2: acing out angiotensin II in ARDS therapy. *Crit Care*. (2017) 21:305. doi: 10.1186/s13054-017-1882-z
3. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. (2020) 579:270–3. doi: 10.1038/s41586-020-2012-7
4. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir Med*. (2020) 8:e21. doi: 10.1016/S2213-2600(20)30116-8
5. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. Penninger: a crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med*. (2005) 11:875–9. doi: 10.1038/nm1267
6. Zhou F, Liu YM, Xie J, Li H, Lei F, Yang H, et al. Comparative impacts of angiotensin converting enzyme inhibitors versus angiotensin II receptor blockers on the risk of COVID-19 mortality. *Hypertension*. (2020) 76:e15–7. doi: 10.1161/HYPERTENSIONAHA.120.15622
7. Zhang P, Zhu L, Cai J, Lei F, Qin J, Xie J, et al. Association of inpatient use of angiotensin converting enzyme inhibitors and angiotensin ii receptor blockers with mortality among patients with hypertension hospitalized with COVID-19. *Circ Res*. (2020) 126:e142–3. doi: 10.1161/CIRCRESAHA.120.317242
8. Tan ND, Qiu Y, Xing XB, Ghosh S, Chen MH, Mao R. Associations between angiotensin converting enzyme inhibitors and angiotensin ii receptor blocker use, gastrointestinal symptoms, and mortality among patients with COVID-19. *Gastroenterology*. (2020) 159:1170–2.e1. doi: 10.1053/j.gastro.2020.05.034
9. Jung SY, Choi JC, You SH, Kim Y. Association of renin-angiotensin-aldosterone system inhibitors with COVID-19-related outcomes in Korea: a nationwide population-based cohort study. *Clin Infect Dis*. (2020) 71:2121–8. doi: 10.1093/cid/ciaa624
10. Yang G, Tan Z, Zhou L, Yang M, Peng L, Liu J, et al. Effects of ARBs and ACEIs on virus infection, inflammatory status and clinical outcomes in COVID-19 patients with hypertension: a single center retrospective study. *Hypertension*. (2020) 76:51–8. doi: 10.1161/HYPERTENSIONAHA.120.15143
11. Mehta N, Kalra A, Nowacki AS, Anjewierden S, Han Z, Bhat P, et al. Association of use of angiotensin-converting enzyme

inhibitors and angiotensin II receptor blockers with testing positive for coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:1020–6. doi: 10.1001/jamacardio.2020.1855
12. Ren L, Yu S, Xu W, Overton JL, Chiamvimonvat N, Thai N. Lack of association of antihypertensive drugs with the risk and severity of COVID-19: a meta-analysis. *J Cardiol.* (2020) 77:482–91. doi: 10.1016/j.jjcc.2020.10.015
13. Zhang X, Yu J, Pan LY, Jiang HY. ACEI/ARB use and risk of infection or severity or mortality of COVID-19: a systematic review and meta-analysis. *Pharmacol Res.* (2020) 158:104927. doi: 10.1016/j.phrs.2020.104927
14. Hu J, Zhang X, Zhang X, Zhao H, Lian J, Hao S, et al. Sheng: COVID-19 is more severe in patients with hypertension; ACEI/ARB treatment does not influence clinical severity and outcome. *J Infect.* (2020) 81:979–97. doi: 10.1016/j.jinf.2020.05.056
15. Liu X, Liu Y, Chen K, Yan S, Bai X, Li J, et al. Efficacy of ACEIs/ARBs vs CCBs on the progression of COVID-19 patients with hypertension in Wuhan: a hospital-based retrospective cohort study. *J Med Virol.* (2021) 93:854–62. doi: 10.1002/jmv.26315
16. Wu J, Liu J, Zhao X, Liu C, Wang W, Wang D, et al. Clinical characteristics of imported cases of COVID-19 in Jiangsu Province: a multicenter descriptive study. *Clin Infect Dis.* (2020) 71:706–12. doi: 10.1093/cid/ciaa199
17. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. China medical treatment expert group for: clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med.* (2020) 382:1708–20. doi: 10.1101/2020.02.06.20020974
18. Griffith GJ, Morris TT, Tudball MJ, Sterne J, Palmer TM, Davey Smith G, et al. Collider bias undermines our understanding of COVID-19 disease risk and severity. *Nat Commun.* (2020) 11:5749. doi: 10.1038/s41467-020-19478-2
19. McCaffrey DF, Griffin BA, Almirall D, Slaughter M, Ramchand R, Burgette LF. A tutorial on propensity score estimation for multiple treatments using generalized boosted models. *Stat Med.* (2013) 32:3388–414. doi: 10.1002/sim.5753
20. Su YS, Gelman A, Hill J, Yajima M. Multiple imputation with diagnostics (mi) in R: opening windows into the black box. *J Stat Softw.* (2011) 45:1–31. doi: 10.1007/s10822-011-9505-2
21. Imai Y, Kuba K, Penninger JM. The discovery of angiotensin-converting enzyme 2 and its role in acute lung injury in mice. *Exp Physiol.* (2008) 93:543–8. doi: 10.1113/expphysiol.2007.040048
22. Guo J, Huang Z, Lin L, Lv J. Coronavirus disease 2019 (COVID-19) and cardiovascular disease: a viewpoint on the potential influence of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers on onset and severity of severe acute respiratory syndrome coronavirus 2 infection. *J Am Heart Assoc.* (2020) 9:e016219. doi: 10.1161/JAHA.120.016219
23. Li Y, Zeng Z, Li Y, Huang W, Zhou M, Zhang X, et al. Angiotensin-converting enzyme inhibition attenuates lipopolysaccharide-induced lung injury by regulating the balance between angiotensin-converting enzyme and angiotensin-converting enzyme 2 and inhibiting mitogen-activated protein kinase activation. *Shock.* (2015) 43:395–404. doi: 10.1097/SHK.0000000000000302
24. Caldeira D, Alarcao J, Vaz-Carneiro A, Costa J. Risk of pneumonia associated with use of angiotensin converting enzyme inhibitors and angiotensin receptor blockers: systematic review and meta-analysis. *BMJ.* (2012) 345:e4260. doi: 10.1136/bmj.e4260
25. Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci.* (2020) 63:364–74. doi: 10.1007/s11427-020-1643-8
26. Meng J, Xiao G, Zhang J, He X, Ou M, Bi J, et al. Renin-angiotensin system inhibitors improve the clinical outcomes of COVID-19 patients with hypertension. *Emerg Microbes Infect.* (2020) 9:757–60. doi: 10.1080/22221751.2020.1746200
27. Tedeschi S, Giannella M, Bartoletti M, Trapani F, Tadolini M, Borghi C, et al. Clinical impact of renin-angiotensin system inhibitors on in-hospital mortality of patients with hypertension hospitalized for COVID-19. *Clin Infect Dis.* (2020) 155:473–81. doi: 10.1093/cid/ciaa492
28. Reynolds HR, Adhikari S, Pulgarin C, Troxel AB, Hausvater A, Newman JD, et al. Renin-angiotensin-aldosterone system inhibitors and risk of covid-19. *N Engl J Med.* (2020) 382:2441–8. doi: 10.1056/NEJMoa2008975
29. de Abajo FJ, Rodriguez-Martin S, Lerma V, Mejia-Abril G, Aguilar M, Angeles Galvez M, et al. Use of renin-angiotensin-aldosterone system inhibitors and risk of COVID-19 requiring admission to hospital: a case-population study. *Lancet.* (2020) 395:1705–14. doi: 10.1016/S0140-6736(20)31030-8
30. Mancia G, Rea F, Ludergnani M, Apolone G, Corrao G. Renin-angiotensin-aldosterone system blockers and the risk of Covid-19. *N Engl J Med.* (2020) 382:2431–40. doi: 10.1056/NEJMoa2006923
31. Guan WJ, Liang WH, Zhao Y, Liang H, Chen ZS, Li YM, et al. China medical treatment expert group for: comorbidity and its impact on 1590 patients with Covid-19 in China: a nationwide analysis. *Eur Respir J.* (2020) 55:2000547. doi: 10.1183/13993003.00547-2020
32. Guo X, Zhu Y, Hong Y. Decreased mortality of COVID-19 with renin-angiotensin-aldosterone system inhibitors therapy in patients with hypertension: a meta-analysis. *Hypertension.* (2020) 76:e13–4. doi: 10.1161/HYPERTENSIONAHA.120.15572
33. Feng Y, Ling Y, Bai T, Xie Y, Huang J, Li J, et al. COVID-19 with different severity: a multi-center study of clinical features. *Am J Respir Crit Care Med.* (2020) 201:1380–8. doi: 10.1164/rccm.202002-0445OC
34. Liang WH, Guan WJ, Li CC, Liang HR, Zhao Y, Liu XQ, et al. Clinical characteristics and outcomes of hospitalised patients with COVID-19 treated in Hubei (epicenter) and outside Hubei (non-epicenter): a nationwide analysis of China. *Eur Respir J.* (2020) 55:2000562. doi: 10.1183/13993003.00562-2020
35. Xie J, Tong Z, Guan X, Du B, Qiu H. Clinical characteristics of patients who died of coronavirus disease 2019 in China. *JAMA Netw Open.* (2020) 3:e205619. doi: 10.1001/jamanetworkopen.2020.5619
36. Wu J, Li W, Shi X, Chen Z, Jiang B, Liu J, et al. Early antiviral treatment contributes to alleviate the severity and improve the prognosis of patients with novel coronavirus disease (COVID-19). *J Intern Med.* (2020) 288:128–38. doi: 10.1111/joim.13063

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 04 June 2021
doi: 10.3389/fcvm.2021.584108

Impaired Myocardial Function Is Prognostic for Severe Respiratory Failure in the Course of COVID-19 Infection

Alvaro Petersen-Uribe[1†], Alban Avdiu[1†], Peter Martus[2], Katja Witzel[1], Philippa Jaeger[1], Monika Zdanyte[1], David Heinzmann[1], Elli Tavlaki[1], Verena Warm[1], Tobias Geisler[1], Karin Müller[1], Meinrad Gawaz[1] and Dominik Rath[1]*

[1] Department of Cardiology and Angiology, University Hospital Tübingen, Eberhard Karls Universität Tübingen, Tübingen, Germany, [2] Institute for Clinical Epidemiology and Applied Biostatistics, University Hospital Tübingen, Eberhard Karls Universität Tübingen, Tübingen, Germany

OPEN ACCESS

Edited by:
Mingxing Xie,
Huazhong University of Science and Technology, China

Reviewed by:
Hack-Lyoung Kim,
Seoul Metropolitan Government - Seoul National University Boramae Medical Center, South Korea
Andrew F. James,
University of Bristol, United Kingdom

***Correspondence:**
Dominik Rath
dominik.rath@med.uni-tuebingen.de

[†]These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Cardiovascular Epidemiology and Prevention, a section of the journal Frontiers in Cardiovascular Medicine

Received: 16 July 2020
Accepted: 30 April 2021
Published: 04 June 2021

Citation:
Petersen-Uribe A, Avdiu A, Martus P, Witzel K, Jaeger P, Zdanyte M, Heinzmann D, Tavlaki E, Warm V, Geisler T, Müller K, Gawaz M and Rath D (2021) Impaired Myocardial Function Is Prognostic for Severe Respiratory Failure in the Course of COVID-19 Infection. Front. Cardiovasc. Med. 8:584108. doi: 10.3389/fcvm.2021.584108

COVID-19 may lead to severe acute respiratory distress syndrome (ARDS) resulting in increased morbidity and mortality. Heart failure and/or pre-existing cardiovascular disease may correlate with poor outcomes and thus require special attention from treating physicians. The present study sought to investigate a possible impact of impaired myocardial function as well as myocardial distress markers on mortality or ARDS with need for mechanical ventilation in 157 consecutive patients with confirmed SARS-CoV-2 infection. All patients were admitted and treated at the University Hospital of Tübingen, Germany, during the first wave of the pandemic. Electrocardiography, echocardiography, and routine blood sampling were performed at hospital admission. Impaired left-ventricular and right-ventricular function, tricuspid regurgitation > grade 1, and elevated RV-pressure as well as thrombotic and myocardial distress markers (D-dimers, NT-pro-BNP, and troponin-I) were associated with mechanical ventilation and/or all-cause mortality. Impaired cardiac function is more frequent amidst ARDS, leading to subsequent need for mechanical ventilation, and thus denotes a poor outcome in COVID-19. Since a causal treatment for SARS-CoV-2 infection is still lacking, guideline-compliant cardiovascular evaluation and treatment remains the best approach to improve outcomes in COVID-19 patients with cardiovascular comorbidities.

Keywords: COVID-19, mechanical ventilation, mortality, myocardial function, prognosis

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an emerging cause of acute respiratory distress syndrome (ARDS) (1). Depending on the severity of ARDS, mechanical ventilation is the cornerstone for treatment of these critically ill patients (2). Patients in need for mechanical ventilation endure prolonged intra-hospital stay, neurological dysfunctions associated with concomitant anesthesia, and increased incidence of thrombosis and thromboembolism due to pro-thrombotic effects of SARS-CoV-2 and immobilization (3). Most importantly, severe respiratory failure is strongly associated with increased mortality in COVID-19 patients (4).

COVID-19 may cause severe acute myocardial injury or exacerbate an underlying chronic cardiovascular disease. Elevated levels of myocardial distress markers NT-pro-BNP and troponin are common findings in these patients (5). Moreover, pre-existing cardiovascular disease and compromised myocardial function have been associated with worse outcomes (6). Electrocardiography (ECG), echocardiography, and blood sampling for specific myocardial distress markers, e.g., troponin I and NT-pro-BNP, are essential for identifying COVID-19 patients with cardiovascular risk in order to improve management and consequently course of the disease. Since we currently lack a specific treatment for COVID-19, management of pre-existing or developing cardiac impairment is critical for improving outcomes in severely affected patients. Effects of impaired myocardial function on development of progressive respiratory failure and subsequent need for mechanical ventilation are unknown so far. Here, we report that markers of myocardial distress and impaired myocardial function are associated with progressive respiratory failure and increased mortality.

MATERIALS AND METHODS

Study Design and Participants

In March and April 2020, this prospective study enrolled 157 consecutive patients diagnosed with severe COVID-19-associated respiratory failure, including the first wave of COVID-19 infections at the University Hospital of Tübingen, Germany. The aim of the current study was to enroll all COVID-19-positive patients requiring hospital admission. Hence, a confirmed SARS-CoV-2 infection requiring hospital admission represented the only selection criterion. According to our official hospital database, 187 patients with confirmed SARS-CoV-2 infection were treated in our university hospital in March and April 2020. We managed to include 84.0% of these COVID-19 patients into the current study. Within 24 h after hospital admission, an extensive cardiovascular assessment including ECG, transthoracic echocardiography (TTE), and testing for myocardial distress biomarkers (e.g., pro-NT-BNP and troponin I) was performed. Written informed consent was obtained wherever possible ($n = 128$, 81.5%). We strongly assume that the remaining patients would not have refused to participate in the study since the cardiologic assessment was performed routinely and not purely study associated. In mechanically ventilated patients, the patient consent was obtained once invasive ventilation was discontinued or after discharge. In these patients, no study-associated measurements were performed but already existing clinical data was analyzed in accordance with the local ethics committee. The study was approved by the institutional ethics committee (238/2018BO2) and complies with the Declaration of Helsinki and good clinical practice guidelines (7–9).

Diagnosis of SARS-CoV-2 Infection and ARDS

SARS-CoV-2 was detected from nasopharyngeal secretions using real-time reverse transcriptase polymerase chain reaction. Severe

TABLE 1 | Baseline characteristics of the overall cohort ($n = 157$).

	All ($n = 157$)
Age, years (mean ± SD)	68 (±15)
Male, *n* (%)	99 (63.1)
Body mass index (mean ± SD)	29 (±5)
Cardiovascular risk factors, *n* (%)	
Arterial hypertension	110 (70.1)
Dyslipidemia	55 (36.2)
Diabetes mellitus	36 (23.1)
Current smokers	7 (4.6)
Obesity	39 (25.8)
Atrial fibrillation	36 (23.1)
Known CAD	34 (22.4)
Chronic kidney disease	20 (12.7)
Echocardiography	
Left ventricular function, % (mean ± SD)	57 (±7)
Left ventricular hypertrophy, *n* (%)	94 (69.1)
Visually estimated normal right ventricular function, *n* (%)	112 (82.4)
Visually estimated impaired right ventricular function, *n* (%)	17 (12.5)
Right ventricular dilatation, *n* (%)	51 (37.5)
TAPSE, mm (mean ± SD)	22 (±5)
RV pressure, mmHg (mean ± SD)	29 (±11)
Aortic stenosis >1, *n* (%)	5 (3.7)
Aortic regurgitation >1, *n* (%)	12 (8.8)
Mitral regurgitation >1, *n* (%)	31 (22.8)
Tricuspid regurgitation >1, *n* (%)	34 (25.0)
Pericardial effusion, *n* (%)	64 (47.1)
Electrocardiography	
Rate, bpm (mean ± SD)	84 (±22)
Sinus rhythm, *n* (%)	108 (81.2)
QRS, ms (mean ± SD)	93 (±20)
Regular R progression, *n* (%)	78 (58.6)
Right bundle branch block, *n* (%)	4 (3.0)
Left bundle branch block, *n* (%)	2 (1.5)
PQ segment, ms (mean ± SD)	170 (±87)
QTc, ms (mean ± SD)	437 (±65)
Negative T wave, *n* (%)	14 (10.5)
ST segment depression, *n* (%)	2 (1.5)
ST segment elevation, *n* (%)	0 (0.0)
Admission laboratory, median (25th percentile–75th percentile)	
Leucocytes, 1,000/μL	6.6 (4.8–9.5)
Lymphocytes, 1,000/μL	0.8 (0.6–1.1)
Creatinine, mg/dL	0.9 (0.7–1.3)
GFR, mL/m²	74 (48–92)
D-Dimer, μg/dL	1.3 (0.7–2.8)
C-reactive protein, mg/dL	8.2 (2.6–16.0)
Procalcitonin, ng/mL	0.14 (0.07–0.74)
Troponin I, ng/dL	17 (6–56)
NT pro-BNP, ng/L	458 (139–2827)
CK, U/L	149 (74–346)
AST, U/L	43 (27–70)
ALT, U/L	32 (21–47)
LDH, U/L	337 (232–446)
Medication at admission, *n* (%)	
Oral anticoagulation	21 (14.8)
ACEi/ARB	78 (54.9)
Aldosterone inhibitors	17 (12.0)
Diuretics	52 (36.6)
Calcium channel blockers	32 (22.5)
Beta blockers	58 (40.8)
Statins	51 (35.9)
ASA	36 (25.4)
P2Y12 blockers	3 (2.1)

TABLE 2 | Baseline characteristics stratified according to the combined endpoint.

	Combined endpoint		
	No ($n = 85$)	**Yes ($n = 72$)**	***p* value**
Age, years (mean ± SD)	67 (±14)	68 (±16)	0.575
Male, *n* (%)	48 (56.5)	51 (70.8)	0.063
Body mass index (mean ± SD)	29 (±6)	29 (±5)	0.709
Cardiovascular risk factors, *n* (%)			
Arterial hypertension	53 (62.4)	57 (79.2)	**0.022**
Dyslipidemia	34 (40.0)	21 (29.2)	0.179
Diabetes mellitus	19 (22.4)	17 (23.6)	0.546
Current smokers	5 (5.9)	2 (2.8)	0.368
Obesity	21 (24.7)	18 (25.0)	0.870
Atrial fibrillation	15 (17.6)	21 (29.2)	0.095
Known CAD	14 (16.5)	20 (27.8)	0.311
Chronic kidney disease	9 (10.6)	11 (15.3)	0.380
Echocardiography			
Left ventricular function, % (mean ± SD)	59 (±4)	54 (±10)	**0.002**
Left ventricular hypertrophy, *n* (%)	57 (78.1)	37 (62.7)	0.514
Visually estimated normal right ventricular function, *n* (%)	71 (93.4)	41 (69.5)	**0.008**
Visually estimated impaired right ventricular function, *n* (%)	5 (6.6)	12 (20.3)	**0.008**
Right ventricular dilatation, *n* (%)	29 (39.7)	22 (37.3)	0.164
TAPSE, mm (mean ± SD)	22 (±5)	21 (±6)	0.441
RV pressure, mmHg (mean ± SD)	27 (±9)	32 (±12)	**0.045**
Aortic stenosis >1, *n* (%)	2 (2.7)	3 (5.1)	0.478
Aortic regurgitation >1, *n* (%)	7 (9.6)	5 (8.5)	0.989
Mitral regurgitation >1, *n* (%)	15 (20.5)	16 (27.1)	0.185
Tricuspid regurgitation >1, *n* (%)	13 (17.8)	21 (35.6)	**0.004**
Pericardial effusion, *n* (%)	32 (43.8)	30 (50.8)	0.180
Electrocardiography			
Rate, bpm (mean ± SD)	80 (±18)	88 (±26)	**0.029**
Sinus rhythm, *n* (%)	64 (84.2)	44 (77.2)	0.566
QRS, ms (mean ± SD)	93 (±23)	93 (±16)	0.931
Regular R progression, *n* (%)	47 (61.8)	31 (54.4)	0.385
Right bundle branch block, *n* (%)	2 (2.6)	2 (3.5)	0.877
Left bundle branch block, *n* (%)	2 (2.6)	0 (0.0)	0.243
PQ segment, ms (mean ± SD)	167 (±83)	173 (±93)	0.722
QTc, ms (mean ± SD)	427 (±81)	451 (±31)	**0.041**
Negative T wave, *n* (%)	4 (5.3)	10 (17.5)	**0.036**
ST segment depression, *n* (%)	1 (1.3)	1 (1.7)	0.821
ST segment elevation, *n* (%)	0 (0.0)	0 (0.0)	0.557
Admission laboratory, median (25th percentile−75th percentile)			
Leucocytes, 1,000/μL	5.7 (4.2–7.5)	7.7 (5-9–11.9)	**<0.001**
Lymphocytes, 1,000/μL	0.9 (0.7–1.2)	0.7 (0.5–1.0)	**0.005**
Creatinine, mg/dL	0.9 (0.7–1.2)	1.0 (0.8–1.6)	**0.027**
GFR, mL/m²	79.0 (58.9–97.2)	68.3 (37.5–91.6)	0.071
D-Dimer, μg/dL	0.8 (0.5–1.5)	2.4 (1.2–5.9)	**<0.001**
C-reactive protein, mg/dL	3.5 (1.3–8.7)	16.3 (9.2–27.4)	**<0.001**
Procalcitonin, ng/mL	0.08 (0.05–0.17)	0.58 (0.13–2.01)	**<0.001**
Troponin I, ng/dL	9 (4–18)	33 (18–124)	**<0.001**
NT pro-BNP, ng/L	310 (93–839)	1815 (401–6026)	**<0.001**
CK, U/L	121 (67–240)	273 (91–727)	**0.001**
AST, U/L	34 (20–47)	61 (39–102)	**<0.001**
ALT, U/L	28 (19–38)	41 (26–66)	**<0.001**
LDH, U/L	265 (207–361)	429 (337–494)	**<0.001**
Medication at admission, *n* (%)			
Oral anticoagulation	12 (14.1)	9 (12.5)	0.919
ACEi/ARB	44 (51.8)	34 (47.7)	0.640
Aldosterone inhibitors	9 (10.6)	8 (11.1)	0.642
Diuretics	29 (34.1)	23 (31.9)	0.701
Calcium channel blockers	19 (22.4)	13 (18.1)	0.843
Beta blockers	31 (36.5)	27 (37.6)	0.343
Statins	29 (34.1)	22 (370.6)	0.815
ASA	21 (24.7)	15 (20.8)	0.973
P2Y12 blockers	1 (1.2)	2 (2.8)	0.370

Bold values indicate statistical significance.

respiratory failure was defined according to the Berlin Definition of Acute Respiratory Distress Syndrome (10).

Twelve-Channel ECG and Laboratory Parameters

Twelve-channel ECG was registered according to standard procedure. Peripheral venous blood was drawn for routine laboratory parameters.

Transthoracic Echocardiography

TTE was performed by our Cardio-COVID-19 team. Left ventricular ejection fraction (LVEF) was assessed visually and measured using Simpson's method (11). Impaired LVEF was defined as an EF <50% (12). Impaired right ventricular function (RV-function) was evaluated combining visual assessment and measuring of tricuspid annular plane systolic excursion (TAPSE). TAPSE was assessed by placing an M-mode cursor through the lateral tricuspid valve annulus in the apical four-chamber view. Then, the total systolic excursion distance of the tricuspid annulus was measured. Impaired RV-function was defined by TAPSE < 20 mm (13). Mitral regurgitation was assessed based on regurgitant orifice area and width of vena contracta (14). Severity of aortic stenosis was defined based on valve area measured by continuity equation and planimetry (15). Jet/left ventricular outflow tract width ratio, pressure half time, as well as diastolic flow reversal in proximal descending aorta were used to quantify severity of aortic regurgitation (16). Central jet area and width of vena contracta were applied to determine tricuspid regurgitation (14). Right ventricular pressure was estimated using the simplified Bernoulli equitation [$RVPsys = 4 \times (Vmax)^2$] (17) when tricuspid

regurgitation was present (18). High probability of pulmonary hypertension was defined as RV-pressure > 35 mmHg (19). Finally, the presence of pericardial effusion (PE) was visually assessed (20).

Clinical Follow-Up

All patients were followed up for 30 days after study inclusion for the primary combined endpoint (poor outcome): mechanical ventilation and/or mortality. Secondary endpoint included all-cause mortality and mechanical ventilation.

Statistical Analysis

SPSS version 26.0 (SPSS Inc., Chicago, IL) and GraphPad Prism8.4.0 (GraphPad Software, San Diego, CA) were used for all statistical analyses. Student's *t*-test was applied for normally distributed data, whereas Mann–Whitney *U*-test served for analysis of non-normally distributed data. Accordingly, mean values are presented as mean ± standard deviation and median values are presented as median and 25th/75th percentiles. Categorical endpoints were analyzed *via* cross-tabulations and Chi-square tests. Correlations of non-normally distributed were assessed using Spearman's rank correlation coefficient (rho). Kaplan–Meier curves with log rank tests were applied to compare survival between groups, whereas multiple Cox regression analyses were used to analyse independent associations between myocardial distress markers and the combined endpoint after adjustment for epidemiological factors. Regarding Cox regression analyses, LVEF, RV-pressure, and age were included as continuous variables, whereas RV-function (normal vs. impaired), significant TR, arterial hypertension, coronary artery disease, as well as diabetes mellitus (no vs. yes) were coded as binary variables. Discriminatory performance of myocardial distress markers and other clinical factors was evaluated using receiver operator curves (ROC) and expressed as c-statistics with 95% CI. Depending on the area under the curve (AUC), ROC 0.5 suggests no discrimination, ≥0.7–<0.8 acceptable,

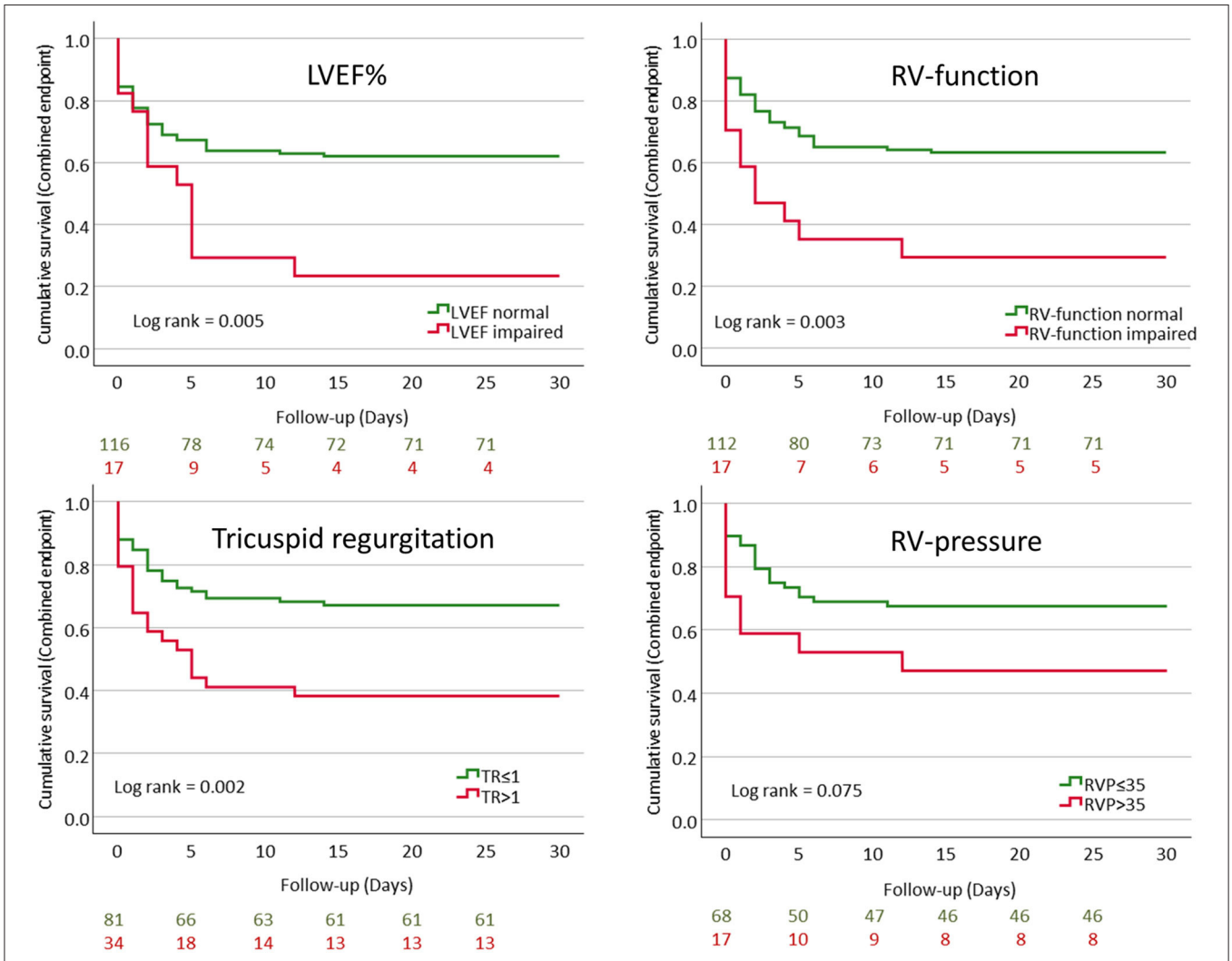


FIGURE 1 | Kaplan–Meier curves showing cumulative event-free survival for the combined endpoint (mechanical ventilation and/or mortality) stratified according to LVEF%, RV-function, tricuspid regurgitation, and RV-pressure.

TABLE 3 | Cox regression with markers of myocardial function as well as epidemiological factors as independent variables and the combined endpoint as dependent variables.

	p-value	HR	95% CI
Age	0.167	0.985	(0.964–1.006)
Arterial hypertension	0.321	1.456	(0.693–3.061)
Coronary artery disease	0.873	1.047	(0.594–1.845)
Diabetes mellitus	0.409	1.283	(0.709–2.321)
LVEF	**0.002**	0.955	(0.926–0.984)
Age	0.332	0.989	(0.969–1.011)
Arterial hypertension	0.292	1.496	(0.707–3.166)
Coronary artery disease	0.798	1.086	(0.579–2.037)
Diabetes mellitus	0.901	1.040	(0.563–1.922)
RV-Function	**0.010**	2.463	(1.239–4.895)
Age	0.062	0.977	(0.954–1.001)
Arterial hypertension	0.357	1.463	(0.651–3.288)
Coronary artery disease	0.335	1.312	(0.756–2.276)
Diabetes mellitus	0.505	1.233	(0.666–2.283)
Significant TR	**0.002**	2.851	(1.480–5.490)
Age	0.070	0.970	(0.939–1.002)
Arterial hypertension	0.074	3.085	(0.898–10.596)
Coronary artery disease	0.426	1.348	(0.647–2.808)
Diabetes mellitus	0.979	0.979	(0.451–2.170)
RV-pressure	**0.025**	1.040	(1.005–1.076)

Bold values indicate statistical significance.

≥0.8–<0.9 excellent, and ≥0.9 outstanding discrimination (21). ROC analyses using combinations of predictors were based on multiple logistic regression analysis with leaving one out correction. Ninety-five percent CIs of these areas are included in the figures. Course of biomarkers and respective associations with poor outcome were analyzed *via* linear mixed-models with random intercept.

RESULTS

A total of 157 patients were included, and their baseline characteristics are shown in **Table 1**. Stratification according to incidence of the combined endpoint is presented in **Table 2**. Routine blood sampling was performed in the whole collective; ECG and echocardiography were performed in 136 (86.6%) and 133 (84.7%) patients, respectively. Rate of mechanical ventilation within 30 days after hospital admission was 44.6% ($n = 70$). Twenty (12.7%) patients developed severe ARDS in the course of the hospital stay. Twenty-two (14.0%) patients were already mechanically ventilated at admission. Twenty-eight (17.8%) patients were intubated due to rapidly increasing respiratory failure and for airway protection. A total of 25 patients died (15.9%); two patients died without being mechanically ventilated (1.3%).

Patients with poor outcome displayed a significantly lower LVEF, worse RV-function, more severe tricuspid regurgitation, and increased RV pressure when compared to those with milder course of COVID-19 (**Table 2** and **Figure 1**).

Multivariable Cox-regression analysis revealed that impaired LVEF and RV-function as well as tricuspid regurgitation >1 and increased RV-pressure were independently associated with poor outcome (**Table 3**).

Amidst patients with poor outcome, leucocyte count, D-dimers, C-reactive protein, procalcitonin, troponin I, NT-pro-BNP, CK, AST, and LDH levels were significantly higher when compared to COVID-19 patients with a more favorable course of disease (**Table 2**).

Increased QTc interval and a higher heart rate, just as a larger proportion of T wave inversion, were more frequently observed in patients requiring ventilation in the course of disease (**Table 2**). The locations of inverted T waves were distributed as follows: Lead I: $n = 6$ (42.9%), lead II: $n = 2$ (14.3%), lead III: $n = 5$ (35.7%), lead aVR: $n = 10$ (71.4%), lead aVL: $n = 6$ (42.9%), lead aVF: $n = 5$ (35.7%), lead V_1: $n = 6$ (42.9%), lead V_2: $n = 5$ (35.7%), lead V_3: $n = 7$ (50.0%), lead V_4: $n = 6$ (42.9%), lead V_5: $n = 4$ (28.6%), and lead V_6: $n = 3$ (21.4%), respectively.

Mechanically ventilated patients showed significantly progressive D-dimer levels when compared to the remaining subjects ($p = 0.043$). Furthermore, non-survivors showed significantly progressive NT-pro-BNP and troponin-I levels when compared to survivors ($p = 0.002$ and $p < 0.001$, respectively) (**Table 4**, **Figure 2**).

LVEF correlated significantly with troponin I and NT-pro-BNP at admission (rho $= -0.310$, $p < 0.001$ and rho $= -0.456$, $p < 0.001$, respectively). TAPSE correlated significantly with troponin I (rho $= -0.293$, $p = 0.003$). Finally, RV-pressure was significantly associated with troponin I and NT-pro-BNP (rho $= 0.310$, $p = 0.005$ and rho $= 0.511$, $p < 0.001$, respectively) (**Figure 3**).

ROC analyses (combined endpoint) revealed an AUC of 0.588 for a multivariable model containing age, arterial hypertension, coronary artery disease, diabetes mellitus type II, and LVEF, 0.475 for a combination of age, arterial hypertension, coronary artery disease, diabetes mellitus type II, and RV-function, 0.520 for age, arterial hypertension, coronary artery disease, diabetes mellitus type II, and significant TR, and 0.590 for age, arterial hypertension, coronary artery disease, diabetes mellitus type II, and elevated RV-pressure. Cardiac biomarkers and D-dimer showed significantly better predictive performance (AUC 0.737 for D-dimers, 0.764 for NT-pro-BNP, and 0.735 for troponin-I). The best discrimination performance was achieved by a model including D-dimers, NT-pro-BNP, and troponin-I (AUC 0.788), whereas a combined model including age, arterial hypertension, coronary artery disease, diabetes mellitus type II, LVEF, RV-function, significant TR, and elevated RV-pressure performed poorly in predicting the combined endpoint (AUC 0.603) (**Figure 4**).

DISCUSSION

The major findings of this study are as follows: (1) Early impaired left and right ventricular systolic function, higher degree tricuspid regurgitation, and higher RV-pressure are more prevalent among COVID-19-positive patients with poor

TABLE 4 | D-dimer, troponin-I, and NT-pro-BNP levels at admission (1st sample), median of hospital stay (interval sample), and discharge/death (close-up sample) stratified according to mechanical ventilation, all-cause mortality, and the combined endpoint.

		1st sample	Interval sample	Close-up sample	*p*-value (int)	*p*-value (time)	*p*-value (group)
Mechanical ventilation							
D-dimers (±SD)	Ventilated	10.3 (±18.1) 0.5 (±0.6)	7.3 (±9.0) 0.6 (±0.4)	7.1 (±8.8) 0.6 (±0.4)	**0.043**	0.760	**<0.001**
	Non-ventilated	4.1 (±11.6) 0.1 (±0.5)	1.7 (±2.6) 0.0 (±0.4)	1.7 (±2.9) −0.2 (±0.4)			
Troponin-I (±SD)	Ventilated	167 (±412) 1.7 (±0.6)	351 (±1061) 1.8 (±0.7)	202 (±586) 1.6 (±0.7)	0.345	**0.023**	**<0.001**
	Non-ventilated	38 (±66) 1.1 (±0.6)	40 (±73) 1.2 (±0.6)	32 (±48) 1.1 (±0.6)			
NT pro-BNP (±SD)	Ventilated	6894 (±9695) 3.4 (±0.8)	9114 (±8691) 3.6 (±0.8)	11623 (±12862) 3.7 (±0.8)	0.985	**<0.001**	**<0.001**
	Non-ventilated	7818 (±39032) 2.6 (±0.9)	7352 (±36658) 2.8 (±0.8)	6550 (±29070) 2.9 (±0.7)			
All-cause mortality							
D-dimers (±SD)	Non-survivors	9.6 (±14.3) 0.5 (±0.7)	5.6 (±5.4) 0.6 (±0.3)	7.4 (±7.1) 0.7 (±0.4)	0.258	0.360	**<0.001**
	Survivors	6.7 (±15.7) 0.2 (±0.6)	4.5 (±7.7) 0.3 (±0.5)	3.8 (±6.9) 0.2 (±0.5)			
Troponin-I (±SD)	Non-survivors	244 (±535) 1.8 (±0.7)	141 (±253) 1.8 (±0.5)	352 (±816) 2.0 (±0.6)	**<0.001**	0.860	**0.002**
	Survivors	84 (±248) 1.4 (±0.6)	264 (±949) 1.5 (±0.8)	82 (±311) 1.2 (±0.7)			
NT pro-BNP (±SD)	Non-survivors	5064 (±6750) 3.3 (±0.7)	8931 (±5697) 3.8 (±0.4)	16023 (±15442) 4.1 (±0.3)	**0.002**	**<0.001**	**0.002**
	Survivors	7854 (±34447) 2.8 (±0.9)	7819 (±31940) 3.0 (±0.9)	7137 (±25882) 3.0 (±0.8)			
Combined endpoint (CE)							
D-dimers (±SD)	CE yes	10.3 (±18.0) 0.5 (±0.6)	7.2 (±9.0) 0.6 (±0.4)	7.1 (±8.7) 0.6 (±0.4)	0.070	0.779	**<0.001**
	CE no	4.0 (±11.7) 0.1 (±0.5)	1.7 (±2.6) 0.0 (±0.4)	1.7 (±3.0) −0.0 (±0.4)			
Troponin-I (±SD)	CE yes	167 (±412) 1.7 (±0.6)	352 (±1062) 1.8 (±0.7)	202 (±586) 1.6 (±0.7)	0.345	**0.023**	**<0.001**
	CE no	38 (±66) 1.2 (±0.6)	40 (±73) 1.2 (±0.6)	32 (±48) 1.1 (±0.6)			
NT pro-BNP (±SD)	CE yes	6894 (±9695) 3.4 (±0.8)	9114 (±8691) 3.6 (±0.8)	11623 (±12862) 3.7 (±0.8)	0.985	**<0.001**	**<0.001**
	CE no	7818 (±39032) 2.6 (±0.9)	7352 (±36658) 2.8 (±0.8)	6550 (±29070) 2.9 (±0.7)			

Both raw and logarithmic values are shown. P-values were calculated for logarithmic data. Int, interaction. Bold values indicate statistical significance.

outcome. (2) The course of the myocardial distress markers NT-pro-BNP and troponin-I may predict outcome in COVID-19 patients. (3) Troponin I and NT-pro-BNP correlate with LVEF, RV-function, and RV pressure at admission. (4) A combined model including D-dimers, troponin-I, and NT-pro-BNP may facilitate risk assessment in COVID-19 patients.

The current findings provide further evidence that an extensive cardiologic assessment of patients suffering from COVID-19 is required at the earliest time point before severe respiratory symptoms are evident.

Our current data and previous reports emphasize that myocardial injury represents a prevalent finding in COVID-19 patients with respiratory insufficiency. Severe respiratory failure and ARDS are currently considered as the main cause of COVID-19-associated morbidity and mortality (22). Recently, Richardson and collaborators reported that 12.2% of hospitalized COVID-19 patients require mechanical ventilation (23). Among those, up to 20% developed cardiac injury, defined as an increase in troponin I (24). Interestingly, in our consecutive collective, ∼45% of patients required mechanical ventilation, with 24% showing significant troponin I elevation. Susceptibility to SARS-CoV-2 infection seems to be higher in patients with pre-existing cardiovascular disease (25). Furthermore, these patients suffer from increased morbidity and mortality (26).

As the precise mechanisms leading to myocardial damage in COVID-19 await a thorough investigation, current research

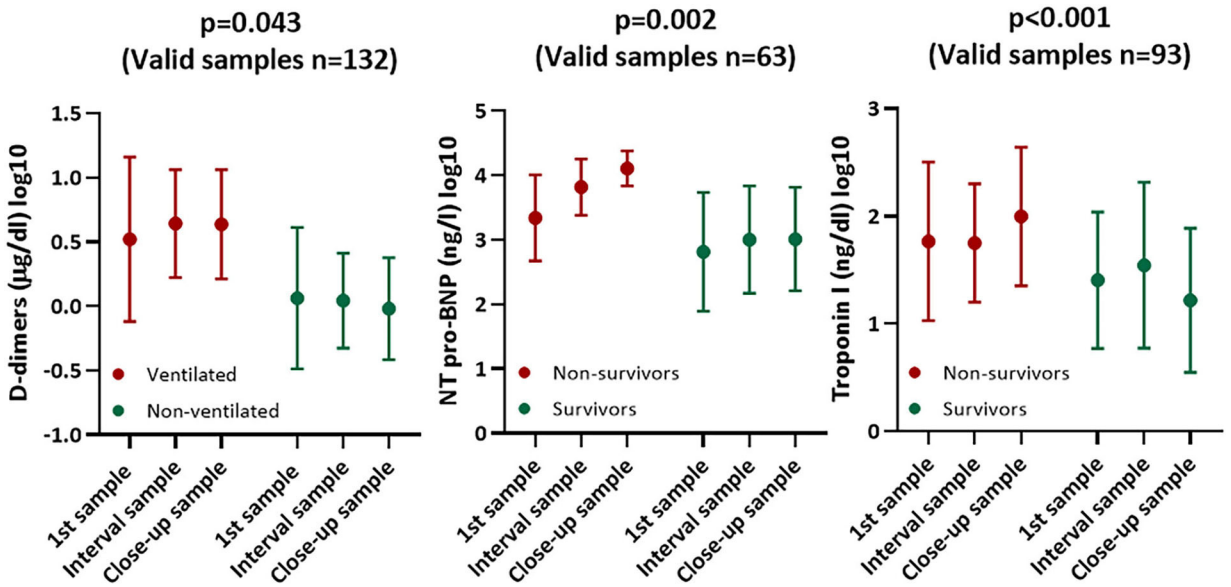


FIGURE 2 | Diagrams (mean ± SD) showing course of cardiac and thrombotic biomarkers stratified according to survival and mechanical ventilation.

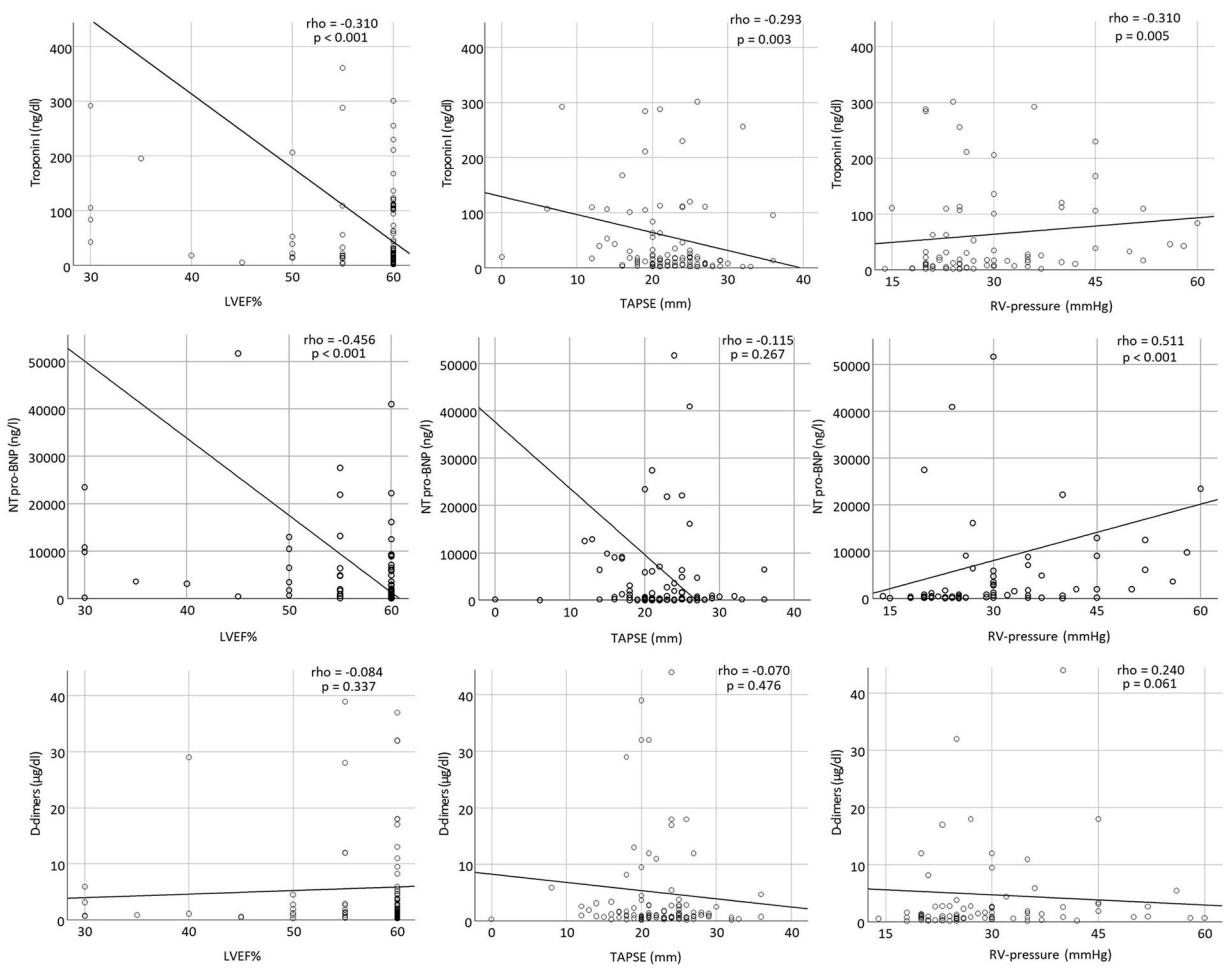


FIGURE 3 | Scatter plots showing correlations between troponin I, NT-pro-BNP, and D-dimers with LVEF%, TAPSE, and RV-pressure at admission.

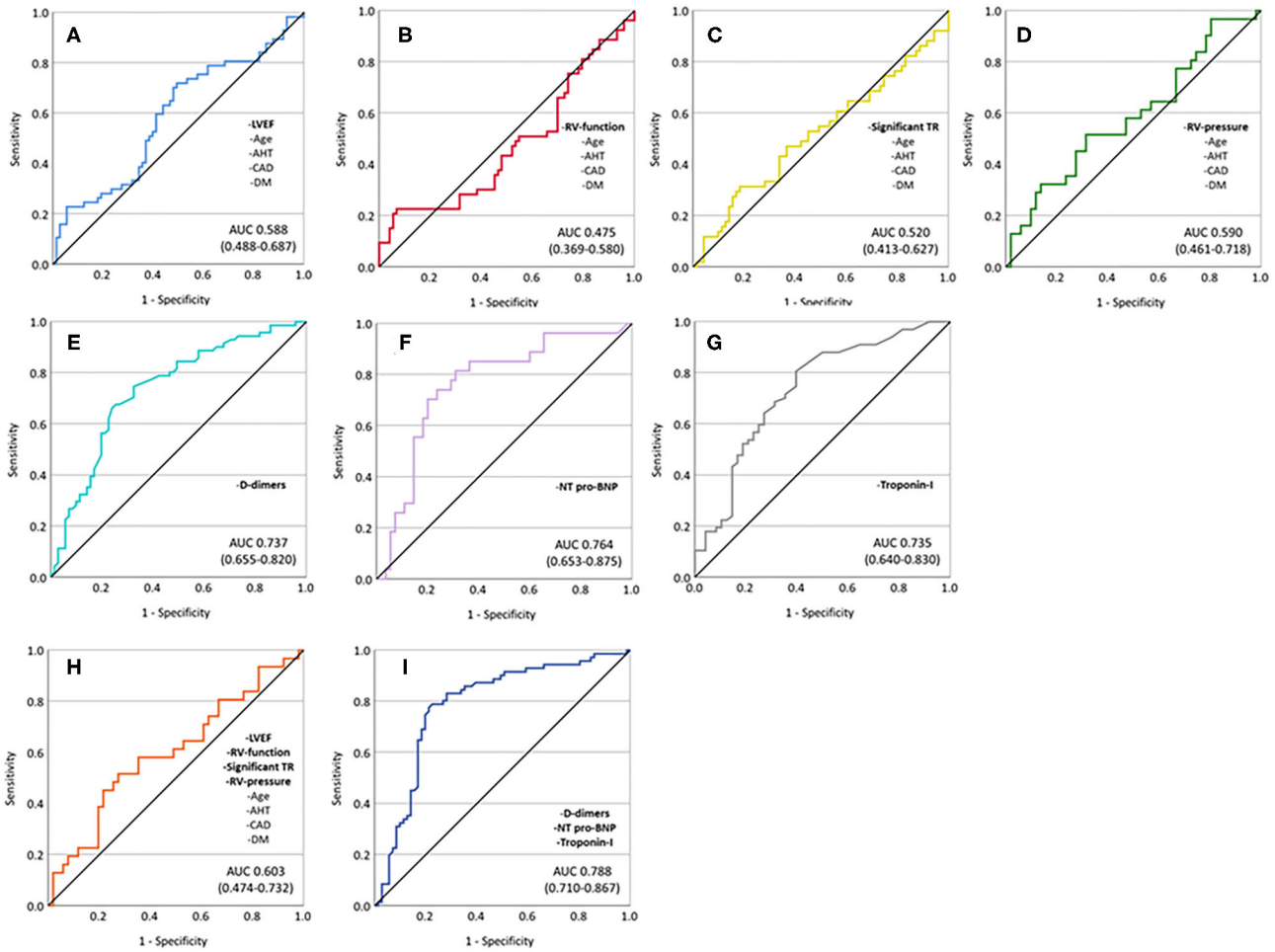


FIGURE 4 | ROC analyses showing predictive performance of different univariate and multivariable models. **(A)** Age, arterial hypertension, coronary artery disease, diabetes mellitus, and impaired LVEF; **(B)** age, arterial hypertension, coronary artery disease, diabetes mellitus, and impaired RV-function; **(C)** age, arterial hypertension, coronary artery disease, diabetes mellitus, and significant TR; **(D)** age, arterial hypertension, coronary artery disease, diabetes mellitus, and elevated RV-pressure; **(E)** D-dimers; **(F)** NT-pro-BNP; **(G)** troponin-I; **(H)** age, arterial hypertension, coronary artery disease, diabetes mellitus, impaired LVEF, impaired RV-function, significant TR, and elevated RV-pressure; and **(I)** D-dimers, NT-pro-BNP, and troponin-I.

suggests that myocardial damage may result from direct viral or inflammatory myocardial injury and may be augmented by systemic inflammatory response, which further promotes microcirculatory impairment or arrest (22). Diagnosing COVID-19-induced direct myocardial damage is a challenging process requiring myocardial biopsy as a gold standard, although SARS-CoV-2 genome could not be identified within the myocardium in biopsy and autopsy findings so far (27). Furthermore, cardiac MRI may help identify myocarditis as a cause of impaired LV-function. These two diagnostic modalities were, however, not applied in our department during the first COVID-19 wave due to patient overload and protection of clinic personnel.

According to available echocardiography findings prior to the diagnosis of SARS-CoV-2 infection, impaired systolic LV-function was commonly a chronic condition, whereas impaired RV-function tended to be a new finding in the current patient cohort. This suggests that elevated RV-pressure and RV-dysfunction is an acute process caused by COVID-19-induced ARDS. Significantly elevated BNP and troponin-I levels found in mechanically ventilated COVID-19 patients in our cohort support the development of acute right ventricular failure, which is consistent with recent findings (28, 29). Furthermore, pulmonary distress could fittingly account for QRS prolongation and higher amount of abnormal T waves seen in our collective (30). Impaired LV-function at hospital admission may fasten this process by congestion caused by elevated LVEDP and thus raising pulmonary artery pressure. Our hypothesis of COVID-19-induced right ventricular failure as a result of major hemodynamic stress is presented in **Figure 5**.

CONCLUSION

As cardiovascular comorbidities and myocardial injury significantly contribute to mortality in COVID-19, early

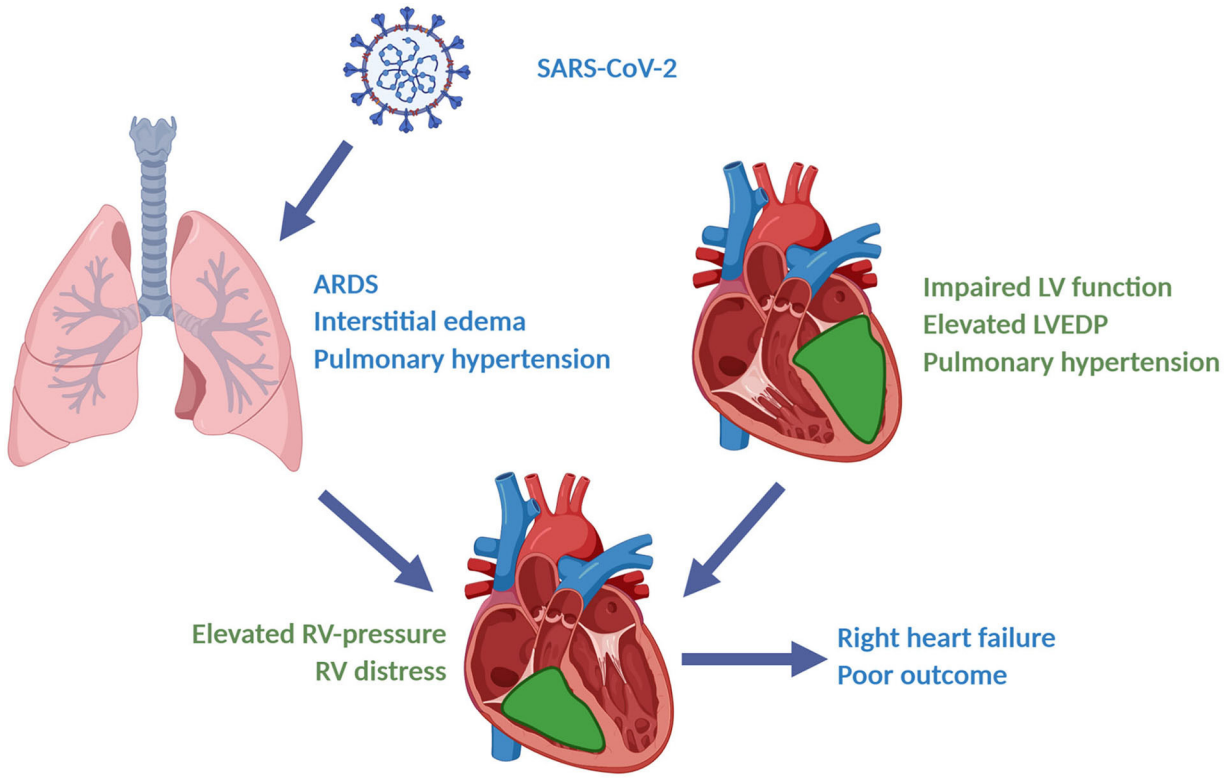


FIGURE 5 | Right heart failure caused by COVID-19-induced severe ARDS and preexisting left heart failure: A hypothesis. (Figure created with BioRender®).

cardiologic assessment and identification of high-risk patients is of critical importance to optimize the management and improve prognosis of COVID-19 patients.

Limitations

The current study offers several major limitations. First, we could not differentiate between COVID-19-induced and non-COVID-19-induced impairment of myocardial function, which may have affected outcome to an unknown degree. Second, the number of patients enrolled was low, rendering generation of risk prediction models difficult. Third, we were not able to include all COVID-19-positive patients admitted to our hospital during the first wave of the disease. Finally, biomarker levels were not available for all patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This study was approved by the institutional ethics committee (238/2018BO2) and complies with the declaration of Helsinki and good clinical practice guidelines. The patients/participants provided their written informed consent to participate in this study.

AUTHOR'S NOTE

We submitted this manuscript at the end of the first COVID-19 wave at the University Hospital Hospital of Tübingen. We have previously submitted an interimanalysis of COVID-19 positive patients to a different journal, which has been accepted for publication onMay 28th (Rath et al., Clin Res Cardiol 2020 Jun 14:1-9). The endpoint differed however and the number of events was significantly lower. Fewer patients were enrolled. Finally, additional analyses were performed in the current manuscript.

AUTHOR CONTRIBUTIONS

AP-U and AA: data collection, data analysis, and drafting of the manuscript. PM: expert data analysis. KW, PJ, MZ, DH, ET, VW, TG, and KM: data collection and critical revision. MG: study concept and drafting of the manuscript. DR: data

collection, and data analysis, drafting of the manuscript, and study concept. All authors contributed to the article and approved the submitted version.

FUNDING

This project was supported by the German Research Foundation (DFG) KFO-274–Project number 190538538, by the German Research Foundation (DFG)–Project number 374031971–TRR 240, and funded by the German Research Foundation (DFG)–Project number 335549539–GRK2381.

ACKNOWLEDGMENTS

We thankfully acknowledge the work of Lydia Laptev for assisting us to isolate and process the blood samples. We acknowledge support by Open Access Publishing Fund of University of Tübingen.

REFERENCES

1. Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): the epidemic and the challenges. *Int J Antimicrob Agents*. (2020) 55:105924. doi: 10.1016/j.ijantimicag.2020.105924
2. Aoyama H, Uchida K, Aoyama K, Pechlivanoglou P, Englesakis M, Yamada Y, et al. Assessment of therapeutic interventions and lung protective ventilation in patients with moderate to severe acute respiratory distress syndrome: a systematic review and network meta-analysis. *JAMA Netw Open*. (2019) 2:e198116. doi: 10.1001/jamanetworkopen.2019.8116
3. Fogarty H, Townsend L, Ni Cheallaigh C, Bergin C, Martin-Loeches I, Browne P, et al. More on COVID-19 coagulopathy in Caucasian patients. *Br J Haematol*. (2020) 189:1044–9. doi: 10.1111/bjh.16791
4. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. *JAMA*. (2020) 323:1574–81. doi: 10.1001/jama.2020.5394
5. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol*. (2020) 17:259–60. doi: 10.1038/s41569-020-0360-5
6. Bansal M. Cardiovascular disease and COVID-19. *Diabetes Metab Syndr*. (2020) 14:247–50. doi: 10.1016/j.dsx.2020.03.013
7. World Medical Association Declaration of Helsinki. Recommendations guiding physicians in biomedical research involving human subjects. *Cardiovasc Res*. (1997) 35:2–3.
8. ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice. *J Postgrad Med*. (2001) 47:199–203.
9. Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the member states relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. *Med Etika Bioet*. (2002) 9:12–9.
10. Force ADT, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, et al. Acute respiratory distress syndrome: the Berlin Definition. *JAMA*. (2012) 307:2526–33. doi: 10.1001/jama.2012.5669
11. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr*. (1989) 2:358–67. doi: 10.1016/S0894-7317(89)80014-8
12. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*. (2016) 37:2129–200. doi: 10.1093/eurheartj/ehw128
13. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr*. (2015) 28:1–39.e14. doi: 10.1016/j.echo.2014.10.003
14. Lancellotti P, Moura L, Pierard LA, Agricola E, Popescu BA, Tribouilloy C, et al. European Association of Echocardiography recommendations for the assessment of valvular regurgitation. Part 2: mitral and tricuspid regurgitation (native valve disease). *Eur J Echocardiogr*. (2010) 11:307–32. doi: 10.1093/ejechocard/jeq031
15. Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP III, Guyton RA, et al. 2014 AHA/ACC guideline for the management of patients with valvular heart disease: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. (2014) 63:2438–88. doi: 10.1161/CIR.0000000000000029
16. Lancellotti P, Tribouilloy C, Hagendorff A, Moura L, Popescu BA, Agricola E, et al. European Association of Echocardiography recommendations for the assessment of valvular regurgitation. Part 1: aortic and pulmonary regurgitation (native valve disease). *Eur J Echocardiogr*. (2010) 11:223–44. doi: 10.1093/ejechocard/jeq030
17. Yock PG, Popp RL. Noninvasive estimation of right ventricular systolic pressure by Doppler ultrasound in patients with tricuspid regurgitation. *Circulation*. (1984) 70:657–62. doi: 10.1161/01.CIR.70.4.657
18. Parasuraman S, Walker S, Loudon BL, Gollop ND, Wilson AM, Lowery C, et al. Assessment of pulmonary artery pressure by echocardiography-a comprehensive review. *Int J Cardiol Heart Vasc*. (2016) 12:45–51. doi: 10.1016/j.ijcha.2016.05.011
19. Galie N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Heart J*. (2016) 37:67–119. doi: 10.1093/eurheartj/ehv317
20. Pepi M, Muratori M. Echocardiography in the diagnosis and management of pericardial disease. *J Cardiovasc Med*. (2006) 7:533–44. doi: 10.2459/01.JCM.0000234772.73454.57
21. Hosmer David W. Lemeshow S. *Model-Building Strategies and Methods for Logistic Regression. Applied Logistic Regression.* (2000). p. 91–142. doi: 10.1002/0471722146
22. Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: a review. *Clin Immunol*. (2020) 215:108427. doi: 10.1016/j.clim.2020.108427
23. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City Area. *JAMA*. (2020) 323:2052–9. doi: 10.1001/jama.2020.6775
24. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol*. (2020) 5:802–10. doi: 10.1001/jamacardio.2020.0950
25. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, et al. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. *Int J Infect Dis*. (2020) 94:91–5. doi: 10.1016/j.ijid.2020.03.017
26. Santoso A, Pranata R, Wibowo A, Al-Farabi MJ, Huang I, Antariksa B. Cardiac injury is associated with mortality and critically ill pneumonia in COVID-19: a meta-analysis. *Am J Emerg Med*. (2020). doi: 10.1016/j.ajem.2020.04.052. [Epub ahead of print].
27. Ho JS, Sia CH, Chan MY, Lin W, Wong RC. Coronavirus-induced myocarditis: a meta-summary of cases. *Heart Lung*. (2020) 49:681–5. doi: 10.1016/j.hrtlng.2020.08.013

28. Sandoval Y, Januzzi JL, Jr., Jaffe AS. Cardiac troponin for assessment of myocardial injury in COVID-19: JACC review topic of the week. *J Am Coll Cardiol*. (2020) 76:1244–58. doi: 10.1016/j.jacc.2020.06.068
29. Deng Q, Hu B, Zhang Y, Wang H, Zhou X, Hu W, et al. Suspected myocardial injury in patients with COVID-19: evidence from front-line clinical observation in Wuhan, China. *Int J Cardiol*. (2020) 311:116–21. doi: 10.1016/j.ijcard.2020.03.087
30. Stein PD, Matta F, Ekkah M, Saleh T, Janjua M, Patel YR, et al. Electrocardiogram in pneumonia. *Am J Cardiol*. (2012) 110:1836–40. doi: 10.1016/j.amjcard.2012.08.019

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 07 June 2021
doi: 10.3389/fcvm.2021.680604

Elderly Male With Cardiovascular-Related Comorbidities Has a Higher Rate of Fatal Outcomes: A Retrospective Study in 602 Patients With Coronavirus Disease 2019

Xiao-Yong Zhan[1†], Liang Li[1†], Yuhai Hu[2†], Qiang Li[3†], Huimin Kong[1], Margaret H. L. Ng[4], Chun Chen[1], Yulong He[1], Bihui Huang[1*] and Mo Yang[1*]*

[1] The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen, China, [2] Department of Clinical Laboratory, Hankou Hospital, Wuhan, China, [3] Department of Clinical Laboratory, Nanfang Hospital, Southern Medical University, Guangzhou, China, [4] Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Hong Kong, China

Elderly with comorbidities have shown a higher rate of fatal outcomes when suffering coronavirus disease 2019 (COVID-19). However, a delineation of clinical significances of hematologic indices and underlying comorbidities in the progression and outcome of COVID-19 remains undefined. Six hundred two COVID-19 patients with established clinical outcomes (discharged or deceased) from Hankou Hospital of Wuhan, China between January 14, 2020 and February 29, 2020 were retrospectively analyzed. Of the 602 patients with COVID-19, 539 were discharged and 63 died in the hospital. The deceased group showed higher leukocyte and neutrophil counts but lower lymphocyte and platelet counts. Longer activated partial thromboplastin time (APTT) and prothrombin time (PT), as well as higher D-dimer and C-reactive protein levels, were found in non-survivors. Our observations suggest that these parameters could serve as potential predictors for the fatal outcome and in the discharged group. A higher neutrophil count and D-dimer level but lower lymphocyte were associated with a longer duration of hospitalization. A multivariable Cox regression analysis showed that higher neutrophil count, prolonged PT, and low lymphocyte count were risk factors for patients with COVID-19. Also, we found an association of lower lymphocyte count and higher C-reactive protein levels with the elderly group and those with cardiovascular-related comorbidities. The significantly different hematologic profiles between survivors and non-survivors support that distinct hematologic signatures in COVID-19 patients will dictate different outcomes as a prognostic marker for recovery or fatality. Lymphopenia and aggressive inflammatory response might be major causes for fatal outcomes in the elderly male and especially those with cardiovascular-related comorbidities.

Keywords: COVID-19, cardiovascular-related comorbidities, aggressive inflammatory response, lymphopenia, elderly male

OPEN ACCESS

Edited by:
Shuyang Zhang,
Peking Union Medical College Hospital, China

Reviewed by:
Kun Wang,
Qingdao University, China
Owais Bhat,
Virginia Commonwealth University, United States

***Correspondence:**
Mo Yang
yangm1091@126.com
Yulong He
heyulong@mail.sysu.edu.cn
Bihui Huang
huangbh7@mail.sysu.edu.cn

† These authors have contributed equally to this work

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *15 March 2021*
Accepted: *19 April 2021*
Published: *07 June 2021*

Citation:
Zhan X-Y, Li L, Hu Y, Li Q, Kong H, Ng MHL, Chen C, He Y, Huang B and Yang M (2021) Elderly Male With Cardiovascular-Related Comorbidities Has a Higher Rate of Fatal Outcomes: A Retrospective Study in 602 Patients With Coronavirus Disease 2019. Front. Cardiovasc. Med. 8:680604. doi: 10.3389/fcvm.2021.680604

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged and is now a worldwide health threat (1). Up to December 2020, tens of thousands of patients are still diagnosed with the coronavirus disease 2019 (COVID-19) every day all over the world. Typically, affected individuals display a variable extent of dyspnea and radiological signs (2–4). Through the unremitting efforts of researchers, we have a deeper understanding of COVID-19. Clinical studies have detected a cytokine storm in critical patients with COVID-19 (5), which is considered to be one of the major causes of acute respiratory distress syndrome (ARDS) and multiple-organ failure at the beginning of the SARS-COV-2 outbreak (6). Thrombotic complications in patients with COVID-19 are common and contribute to organ failure and mortality (7, 8), which suggests that platelet hyperreactivity is associated with SARS-CoV-2 infection and participating in COVID-19 pathophysiology (9). Several reports have described significant procoagulant events, including life-threatening pulmonary embolism (PE) (10–12). According to the last available sex-related study from Italy, lethality is 17.7% in men and 10.8% in women, suggesting gender might also be a risk factor for COVID-19 patients (13, 14). Although many clinical studies have been done on COVID-19, laboratory indices to predict disease progression and prognosis are not well-established yet (15).

Hematological findings and thrombocytopenia with SARS and COVID-19 have been reported in our previous publications (15, 16). In the present study, we present a retrospective analysis to describe clinical outcomes, underlying comorbidities, and hematological indices in 602 laboratory-confirmed hospitalized COVID-19 patients. We aimed to explore the potential factors that predict the prognosis and survival outcome of COVID-19 inpatients. With multiple analyses of bio-indices among patients with different underlying comorbidities or age and gender, we sought to delineate how underlying comorbidities, age, and gender influence the disease outcome.

METHODS

Ethical Statement

The study was approved by the Ethics Committee of the Seventh Affiliated Hospital, Sun Yat-sen University. No informed consent of patients was required.

Data Sources

We obtained the medical records of 602 hospitalized patients with a laboratory-confirmed diagnosis of COVID-19 from Wuhan Hospital between January 14, 2020, and February 29, 2020. Admission criteria are as follows: the patient has clinical symptoms, a positive nucleic acid test, and CT suggests viral pneumonia. Demographic information, medical history, comorbidities, signs and symptoms, and laboratory findings on admission were collected from electronic medical records.

A laboratory-confirmed case of COVID-19 was defined as a positive real-time reverse transcriptase–polymerase chain reaction (RT-qPCR) test result obtained through oral pharyngeal swab specimens. Investigators collected demographic information, exposure history, medical history, comorbidities, signs and symptoms, chest computed tomography, laboratory findings on admission, and clinical outcomes from electronic medical records. Laboratory results (blood count, biochemical analysis, and coagulation testing) were included in laboratory profile testing. The dates of disease onset, SARS-CoV-2 laboratory confirmation, hospital admission, discharge, and death were also recorded.

Statistical Analysis

Continuous variables are presented as medians with interquartile ranges (IQRs). For categorical variables, we calculated the frequency rates and percentages of patients in each category. Continuous variables were compared using the Mann–Whitney *U*-test. Proportions for categorical variables were compared using the chi-square test, and Fisher's exact test was used when the data were limited. $P < 0.05$ was considered statistically significant. Spearman's correlation analysis was used to analyze the relationship between different indices. Survival curves were estimated by the Kaplan–Meier method and compared by the log-rank test. Multivariate Cox regression was performed to investigate the hazard ratio by the Cox proportional hazards model. GraphPad Prism 8 (GraphPad Software) was used for graphing. Statistical analyses were performed using SPSS 25.0 (IBM software). A principal component analysis (PCA) for hematologic indices was performed using Origin (OriginLab).

RESULTS

Baseline Clinical Characteristics

A total of 602 patients (383 males and 389 females) with a laboratory-confirmed diagnosis of COVID-19 were included, in which 539 were discharged and 63 died in hospital. The baseline characteristics of these patients are shown in **Table 1**. The median age for all patients was 62 years, with significantly older age for deceased than for discharged (71.0 vs. 61.0 years; $P < 0.0001$). Among all deceased patients, 65.08% were males, which was significantly more than females ($P = 0.039$). There were 386 patients (386/580, 66.55%) (due to some data missing, only 580 patients had underlying comorbidity records) who had at least one underlying comorbidity, of which 24.66% had only cardiovascular-related underlying comorbidities (CRUC) including hypertension, diabetes, coronary heart disease, and cerebrovascular disease; 18.62% had other underlying comorbidities including thyroid nodules, fracture, chronic renal failure, lymphoma, hepatitis B, gallstone, etc.; and 23.28% had at least two types of underlying comorbidities, in which one was CRUC. The incidence of underlying comorbidities was significantly higher in the deceased than in the discharged ($P < 0.001$).

Laboratory Findings Among Hospitalized Patients With Different Outcomes

The hematologic profile among patients with different outcomes is shown in **Table 1**. Compared with the discharged, higher leukocyte and neutrophil counts (**Figures 1A,B**), but lower

TABLE 1 | Baseline characteristics of the recovered patients and patients who died of COVID-19.

Characteristics	All patients (*n* = 602)	Discharged (*n* = 539)	Deceased (*n* = 63)	*P*-value
Demographic				
Survival or death rate (%)		89.53	10.47	N/A
Age, years	62.0 (51.0–70.0)	61.0 (50.0–69.0)	71.0 (64.0–79.0)	<0.0001
Gender				
Female, *n* (%)	289 (48.01)	267 (49.54)	22 (34.92)	0.039*
Male, *n* (%)	313 (51.99)	272 (50.46)	41 (65.08)	
Hospitalization, days	18 (12–26)	19 (12–27)	7 (3–13)	N/A
Comorbidity				
No comorbidity, *n* (%)	194 (33.44)	186 (35.84)	8 (13.11)	<0.001*&
Generalized vascular disease, *n* (%)	143 (24.66)	121 (32.31)	22 (36.07)	
Other comorbidity, *n* (%)	108 (18.62)	102 (19.65)	6 (9.84)	
Two and more comorbidities, *n* (%)	135 (23.28)	110 (21.20)	25 (40.98)	
Laboratory findings				
Hematologic				
Leukocyte count,10^9/l	5.70 (4.30–7.88)	5.40 (4.20–7.20)	11.70 (8.50–15.50)	<0.0001
<4 × 10^9/l, *n* (%)	95 (17.24)	94 (18.91)	1 (1.85)	<0.001*#
4–10 × 10^9/l, *n* (%)	383 (69.51)	363 (73.04)	20 (3.70)	
>10 × 10^9/l, *n* (%)	73 (13.25)	40 (8.05)	33 (61.11)	
Neutrophil count, 10^9/l	4.00 (2.70–6.20)	3.80 (2.60–5.40)	10.50 (7.80–13.48)	<0.0001
<1.8 × 10^9/l, *n* (%)	33 (6.00)	33 (6.65)	0 (0)	<0.001*#
1.8–6.3 × 10^9/l, *n* (%)	384 (69.82)	375 (75.60)	9 (16.67)	
>6.3 × 10^9/l, *n* (%)	133 (24.18)	88 (17.74)	45 (83.33)	
Lymphocyte count, 10^9/l	1.00 (0.70–1.50)	1.10 (0.70–1.50)	0.5 (0.30–0.70)	<0.0001
<0.8 × 10^9/l, *n* (%)	169 (30.73)	127 (25.60)	42 (77.78)	<0.001*
0.8–4.0 × 10^9/l, *n* (%)	381 (69.27)	369 (74.40)	12 (22.22)	
Hemoglobin, g/l	126.00 (115.00–135.00)	126.00 (115.00–135.00)	131.00 (110.00–139.00)	0.2559
Platelet count, 10^9/l	221.00 (169.00–290.00)	230.00 (174.00–294.00)	170.00 (78.00–231.30)	<0.0001
<100 × 10^9/l, *n* (%)	25 (6.19)	14 (3.88)	11 (25.58)	<0.001*$, 0.117^
100–300 × 10^9/l, *n* (%)	295 (73.02)	268 (74.24)	27 (62.79)	
>300 × 10^9/l, *n* (%)	84 (20.79)	79 (21.88)	5 (11.63)	
Other indices				
APTT, s	34.40 (31.30–37.90)	34.20 (31.30–37.20)	37.00 (31.30–42.70)	0.0152
≤47 s, *n* (%)	386 (94.84)	352 (96.97)	34 (72.27)	<0.001*
>47 s, *n* (%)	21 (5.16)	11 (3.03)	10 (22.73)	
Prothrombin time (PT), s	13.85 (12.93–15.00)	13.70 (12.80–14.70)	17.10 (14.60–19.95)	<0.0001
≤17 s, *n* (%)	380 (93.37)	349 (96.14)	21 (47.73)	<0.001*
>17 s, *n* (%)	37 (6.63)	14 (3.86)	23 (52.27)	
Thrombin time (TT), s	15.70 (15.00–16.60)	15.70 (15.00–16.40)	16.30 (14.85–17.90)	0.0664
≤19 s, *n* (%)	393 (96.56)	357 (98.35)	36 (81.82)	<0.001*
>19 s, *n* (%)	14 (3.44)	6 (1.65)	8 (18.18)	
D-dimer, mg/l	0.65 (0.18–3.02)	0.54 (0.15–2.02)	6.96 (0.95–8.00)	<0.0001
<0.5 mg/l, *n* (%)	181 (44.47)	177 (48.76)	4 (9.09)	<0.001*
≥0.5 mg/l, *n* (%)	226 (55.53)	186 (51.24)	40 (90.91)	
Fibrinogen (FIB), g/l	3.29 (2.58–4.18)	3.29 (2.59–4.18)	3.06 (1.46–4.41)	0.6432
<2, *n* (%)	35 (8.60)	23 (6.34)	12 (27.27)	<0.001*$, 0.412*^
2–4, *n* (%)	246 (60.44)	230 (63.36)	16 (36.36)	
>4, *n* (%)	126 (30.96)	110 (30.30)	16 (36.36)	
International normalized ratio (INR)				
≤1.5, *n* (%)	385 (94.59)	355 (97.80)	30 (68.18)	<0.001*
>1.5, *n* (%)	22 (5.41)	8 (2.20)	14 (31.82)	

(Continued)

TABLE 1 | Continued

Characteristics	All patients (n = 602)	Discharged (n = 539)	Deceased (n = 63)	P-value
C-reactive protein (CRP), mg/l	10.30 (2.08–49.15)	7.97 (1.79–42.20)	115.00 (42.88–199.30)	<0.0001
≤10 mg/l, *n* (%)	152 (49.51)	150 (53.38)	2 (7.69)	<0.001*
>10 mg/l, *n* (%)	155 (50.49)	131 (46.62)	24 (92.31)	

*Chi-square tests or Fisher's exact test was used to compare the COVID-19 mortality between the patients with different indices. & Patients with or without comorbidities were compared. # COVID-19 mortality of patients with high neutrophil count (>6.3 × 10^9/l) or leukocyte count (>6.3 × 10^9/l) was compared with the other two groups. $ COVID-19 mortality of low-platelet-count group (<100 × 10^9/L) and low-level FIB group (<2.0 g/l) was compared with the other two groups. ^ COVID-19 mortality of high-platelet-count group (>100 × 10^9/l) and high-level FIB group (>4.0 g/l) was compared with the other two groups. N/A, not available.

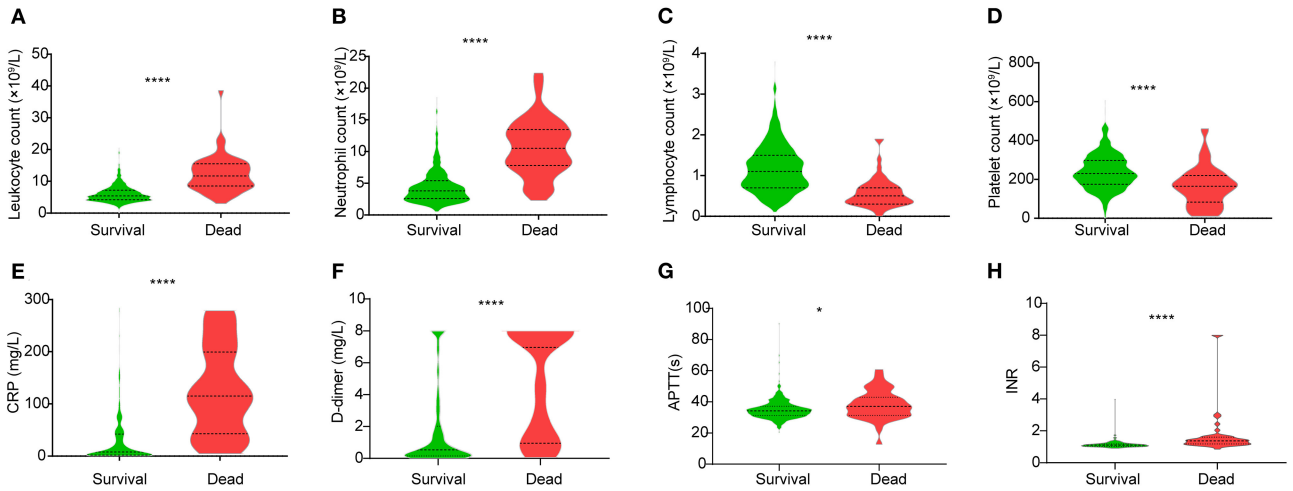


FIGURE 1 | Different levels of hematologic indices **(A)** leukocyte count, **(B)** neutrophil count, **(C)** lymphocyte count, **(D)** platelet count, **(E)** CRP level, **(F)** D-dimer level, **(G)** APTT, and **(H)** INR between the discharged and deceased patients. Data are shown as a violin plot with median and 25 and 75% percentile lines. *P < 0.05, ****P < 0.0001. CRP, C-reactive protein; APTT, activated partial thromboplastin time; INR, international normalized ratio.

lymphocyte and platelet counts (**Figures 1C,D**), were found in deceased patients. Lymphopenia (<1 × 10^9/l) was more common in non-survivors than survivors (**Table 1**).

Compared with discharged patients, the deceased also showed significantly increased levels of C-reactive protein (CRP) and D-dimer (**Figures 1E,F**). Regarding the coagulation indicators, activated partial thromboplastin time (APTT), thrombin time (TT), prothrombin time (PT), and international normalized ratio (INR) were all increased in deceased patients (**Figures 1G,H**, **Table 1**).

Association of Patient Characteristics and Laboratory Indices With the Survival Rate of COVID-19 Patients

The Kaplan–Meier analysis with log-rank test for the hematological indicators showed a significant difference in survival curve in COVID-19 patients categorized by the levels of leukocyte (**Figure 2A**), neutrophil (**Figure 2B**), lymphocyte (**Figure 2C**), CRP (**Figure 2D**), and those indices related to coagulation function, including platelets (**Figure 2E**), APTT (**Figure 2F**), PT (**Figure 2G**), TT (**Figure 2H**), fibrinogen (FIB; **Figure 2I**), INR (**Figure 2J**), and D-dimer (**Figure 2K**), respectively. COVID-19 patients with a higher leukocyte count (>10 × 10^9/l) had a worse prognosis. No significant difference in prognosis was observed in COVID-19 patients with lower leukocyte count (<4 × 10^9/l) compared with those with normal leukocyte levels (**Figure 2A**). Similar results were found on neutrophil and FIB, that a low level of neutrophil count (<1.8 × 10^9/l) and a high level of FIB (>4 g/l) did not significantly contribute to the worse prognosis than the normal-level group (**Figures 2B,I**). Our Kaplan–Meier analysis also showed that patients with high levels of CRP (>10 μg/l), D-dimer (>0.5 mg/l), extended APTT (>47 s), PT (>17 s), TT (>19 s), and high INR were associated with worse prognosis (**Figures 2D,F–K**). In contrast, normal levels of lymphocyte (>0.8 × 10^9/l) and platelet (>100 × 10^9/l) were associated with better prognosis (**Figures 2A,E**). Collectively, these results suggested that these hematological parameters and patients' characteristics could be a potential prognostic marker for COVID-19.

Furthermore, based on the multivariate Cox regression analysis, we found that among the 11 laboratory indices that could predict the prognosis of COVID-19 mentioned above, lymphocyte count <0.8 × 10^9/l [hazard ratio (HR), 2.911; 95%

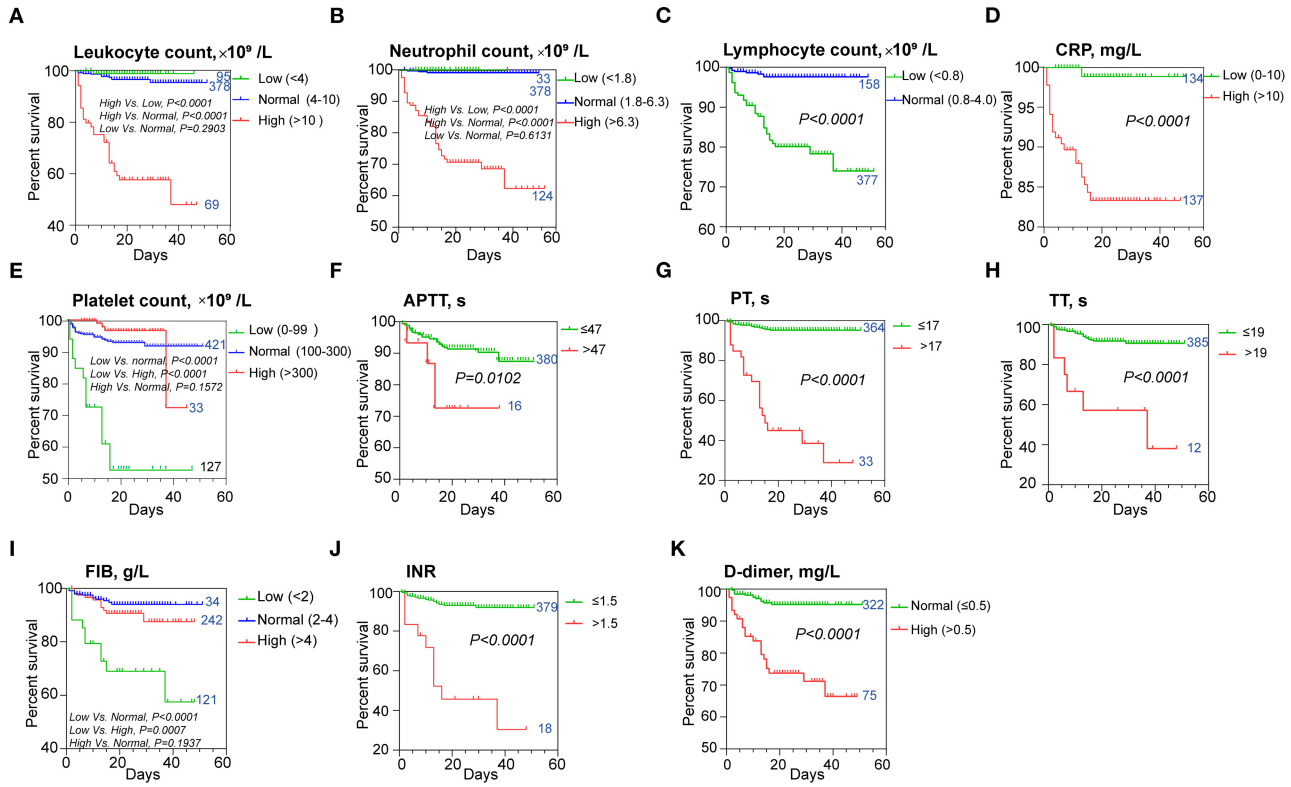


FIGURE 2 | Kaplan–Meier survival curves for different prognostic factors. The curves according to **(A)** leukocytes, **(B)** neutrophils, **(C)** lymphocytes, **(D)** CRP levels, **(E)** platelets, **(F)** APTT, **(G)** PT, **(H)** TT, **(I)** FIB levels, **(J)** INR, and **(K)** D-dimer levels. The patient number of each group was indicated next to the curve. CRP, C-reactive protein; APTT, activated partial thromboplastin time; INR, international normalized ratio; PT, prothrombin time; TT, thrombin time; FIB, fibrinogen.

TABLE 2 | Risk factors of fatal outcome in the multivariate cox proportional hazards regression model.

Variables	Level	HR	95% CI	*P*-value
Lymphocyte count ($\times 10^9$/l)	<0.8 vs. ≥0.8	2.911	1.172–7.229	0.021
Neutrophil count ($\times 10^9$/l)	>6.3 vs. ≤6.3	15.679	4.643–52.945	<0.001
PT (s)	>17 vs. ≤17	6.864	3.389–13.901	<0.001

confidence interval (CI), 1.172–7.229], neutrophil count >10 × 10^9/l (HR, 15.679; 95% CI, 4.643–52.945), and PT > 17 s (HR, 6.864; 95% CI, 3.389–13.901) on admission were the risk factors for a fatal outcome (**Table 2**).

Correlation of Characteristics and Hospitalization Days Within Discharged Patients

Here, we defined the correlations of characteristics and hospitalization days of the discharged patients by using Spearman's correlation analysis. Due to the limit of sample size, $|r| > 0.2$ and $P < 0.05$ were set as cutoff values for correlation. We observed that hospitalization days of discharged patients were negatively correlated with lymphocyte count and blood saturation levels on admission ($P < 0.0001$ and $P = 0.0002$, $r < -0.2$) (**Figures 3A,B**), while they were positively correlated with neutrophil count and D-dimer levels ($P < 0.0001$, $r > 0.2$; **Figures 3C,D**).

Correlation Networks and Principal Component Analysis for Hematologic Indices

Both survivors and non-survivors showed strong positive correlations between leukocytes and neutrophils ($r = 0.94$ and $r = 0.99$, respectively), and between INR and PT (both $r = 1.00$). Similarly, a moderate negative correlation between FIB and TT was found on both survivors and non-survivors ($r = -0.31$ and $r = -0.59$, respectively) (**Figure 4**).

We observed a negative correlation between lymphocytes and D-dimer in the survivors ($r = -0.3338$). However, this correlation was positive in the non-survivors ($r = 0.4323$). Lymphocyte counts and CRP levels had four and three more

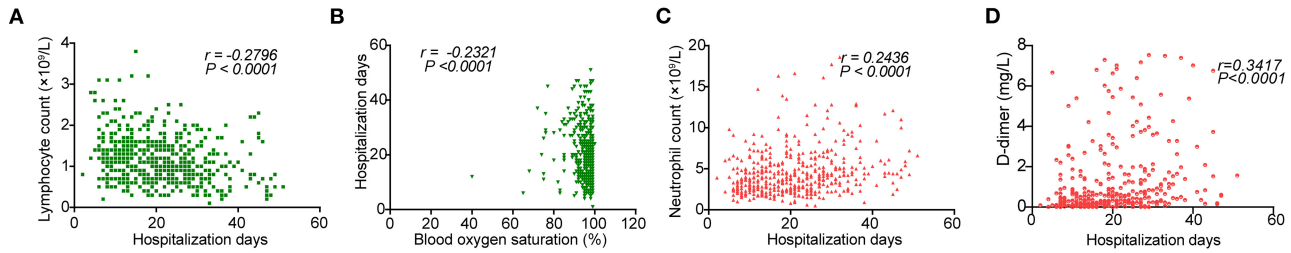


FIGURE 3 | Correlations between hospitalization days and on admission levels of **(A)** leukocytes, **(B)** blood oxygen saturation, **(C)** neutrophils, and **(D)** D-dimers.

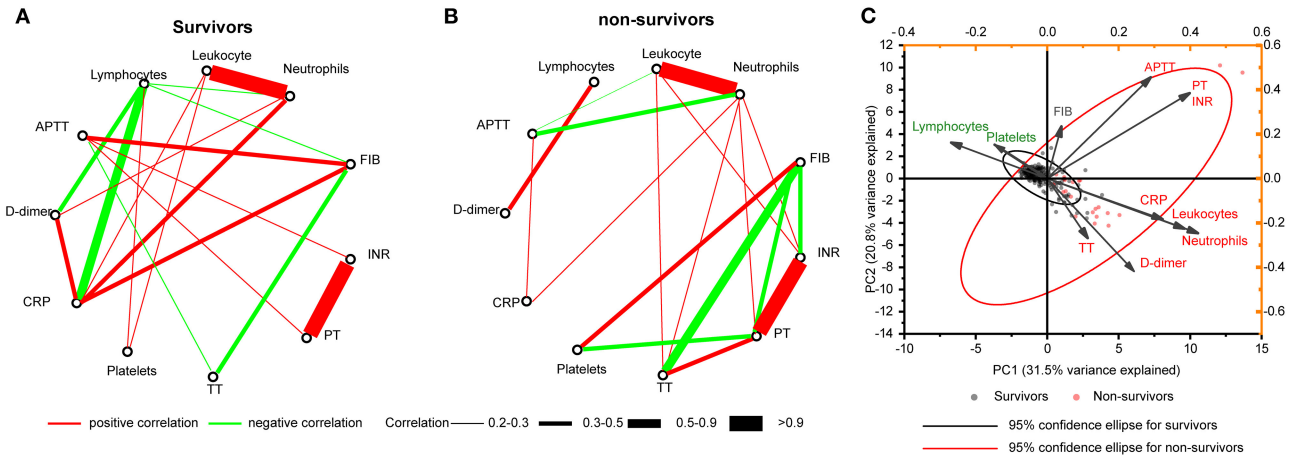


FIGURE 4 | Correlation networks for hematologic indices. Networks showed different profiles of correlations in COVID-19 survivors **(A)** and non-survivors **(B)**, on admission. **(C)** PCA biplot of hematologic indices on admission. Individuals are shown as dots and colored by outcomes (survivors and non-survivors). Indices showed as lines with arrows and colored by positive or negative contribution to PC1. The configuration of indices in biplot represented the relationship between variables and principal components. PCA, principal component analysis.

highly connected hub nodes in the survivors than in the non-survivors (six and five edges, respectively) (**Figures 4A,B**). In contrast, neutrophil counts and PT had two and three more connected hub nodes in the non-survivors (six and five edges, respectively) (**Figures 4A,B**). We did not observe a correlation between D-dimer and other coagulation indicators including platelets, APTT, TT, PT, INR, and FIB in both survivors and non-survivors (**Figures 4A,B**). We found that APTT lost correlation with other coagulation indicators including PT, TT, FIB, and INR in non-survivors (**Figures 4A,B**). A biplot via PCA indicated the configuration of hematologic indices on admission, which is shown in **Figure 4C**. The first principal component (PC1) could roughly separate non-survivors from survivors, with neutrophils (42.20%), leukocytes (38.42%), and CRP (32.32%) having the biggest positive contribution. In contrast, lymphocytes (26.91%) and platelets (14.71%) had a negative contribution to PC1.

Cardiovascular-Related Underlying Comorbidities Were Associated With Poor Prognosis of COVID-19

Based on the underlying comorbidity description, we categorized the patients into four groups including the no underlying comorbidity, CRUC, other-comorbidity group, more than two comorbidities, and at least one was CRUC. The Kaplan–Meier analysis with log-rank test showed a significantly different survival curve among the four groups (**Figure 5**). Except for the cardiovascular-related comorbidity (hypertension, diabetes, coronary heart disease, cerebrovascular disease, etc.), other comorbidities did not significantly affect the survival rate of the patients as compared to those without. Although a relatively low survival rate was observed in patients with more than two types of comorbidities as compared to those without, it had no significant difference as compared with those with only CRUC (**Figure 5A**). These results together indicated that CRUC might be the main factor that decreased the survival rate of COVID-19 patients.

To explore why the four groups of patients had such significantly different outcomes, we analyzed the hematologic indices that were associated with mortality in these patients. As shown in **Supplementary Figure 1** and **Supplementary Table 1**, we did not find a significant difference in coagulation indices such as platelet count, PT, TT, D-dimer, INR, and FIB between patients without any comorbidity and those only with CRUC. Combined with the above results, the role of coagulation dysfunction in decreasing the survival rate of patients with CRUC was excluded.

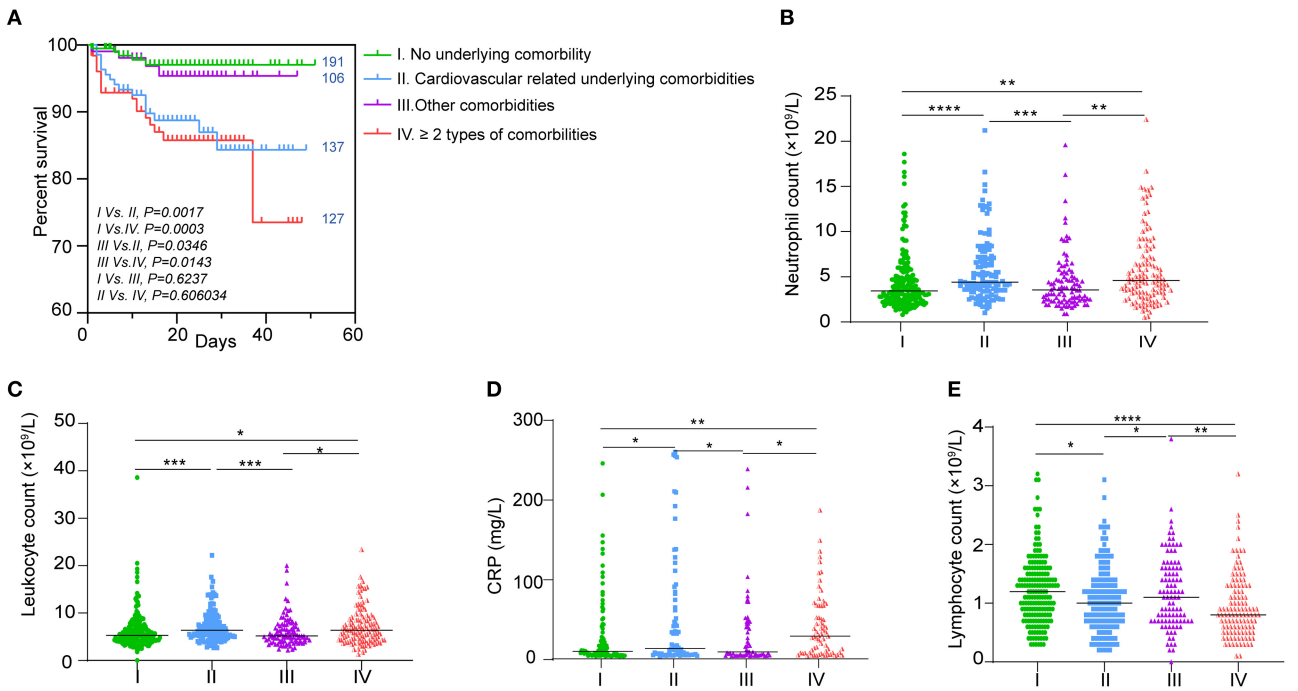


FIGURE 5 | Association of underlying comorbidities and hematologic indices. **(A)** Kaplan–Meier survival curves for different underlying comorbidities. The number of patients in each group is indicated next to the curve. Hematologic indices in the four groups that showed to be better fit the survival curves including **(B)** neutrophils, **(C)** lymphocytes, **(D)** leukocytes, and **(E)** CRP levels. Data are shown as dots with median lines. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$.

In contrast, we found that neutrophil, lymphocyte, leukocyte, and CRP levels in the four groups all fit the trend of survival curves better (**Figures 5B–D**). Significantly high levels of neutrophil, leukocyte, and CRP were found in patients with CRUC as compared with those without any comorbidity. Comparable levels of neutrophil, leukocyte, and CRP were found in patients with an underlying comorbidity other than CRUC as compared with those without any comorbidity (**Figures 5B–D**). Similarly, we also found comparable levels of neutrophil, leukocyte, and CRP between patients with more than two types of comorbidities and those with only CRUC. Lymphocyte levels in the four groups showed the opposite trend (**Figure 5E**).

The Age-Related Poor Prognosis of COVID-19 Patients Was Associated With CRUC

The Kaplan–Meier analysis with log-rank tests showed that elderly patients (>60 years of age) had a poorer outcome than those who are younger (≤60) (**Figure 6A**), that at day 47 after admission, 95.58% of young patients survived, while only 83.24% of elderly patients did. We also found significantly different survival curves between patients with or without underlying comorbidities (**Figure 6B**). We hypothesized that age-related poor prognosis might be related to a higher frequency of underlying comorbidities that happened in the older age group, which was observed in our dataset (**Figure 6C**). To validate this hypothesis, we analyzed the survival curves of the young and the old with each category of underlying comorbidities. We found no significant difference in survival curves between the young and the old who had no underlying comorbidities (**Figure 6D**). Similar results were also observed in the patients without CRUC and patients with only CRUC (**Figures 6E,F**). However, we found a significant difference in survival curves between the young and the old who had CRUC (**Figure 6G**). Altogether, these results highlighted the contribution of CRUC in the age-related poor prognosis. Then, we analyzed the hematologic index differences between the old and the young. We found a significantly lower level of lymphocyte and a higher level of neutrophil and CRP in the old (**Figures 6H–J**). Besides, significant correlations ($P < 0.05$, $|r| > 0.2$) between the age and these hematologic indices (lymphocyte count, neutrophil count, and CRP level) were found in the patients (**Figures 6K–M**). However, most other hematologic indices were found not to be significantly different between the young and old or have a correlation with age (**Supplementary Figures 2A–O**, **Supplementary Table 2**), except the D-dimer level (**Supplementary Figure 2P**).

Male COVID-19 Patients Had a Poorer Outcome

As shown in **Figure 7A**, a significant difference in survival curves was observed between male and female patients, suggesting that male patients had a poorer outcome than female. We did not observe different occurrences of underlying comorbidities between the male and female (**Figure 7B**, **Supplementary Table 3**). To explore a possible explanation, we

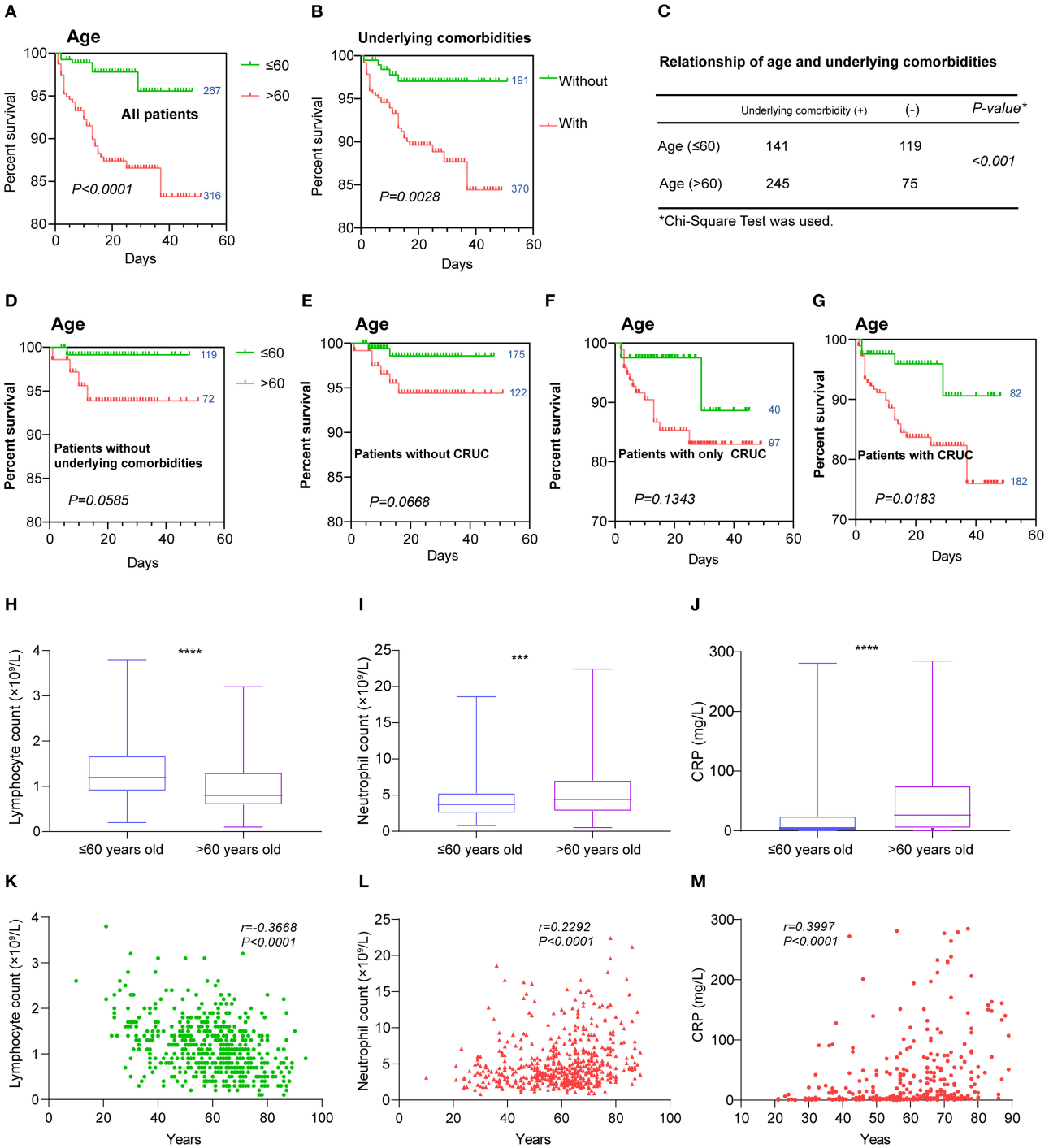


FIGURE 6 | Hematologic variations between the young and old. Kaplan–Meier survival curves for **(A)** age and **(B)** underlying comorbidities are shown. **(C)** The relationship of age and underlying comorbidities was analyzed by chi-square test. Kaplan–Meier survival curves for age in those patients **(D)** without any underlying comorbidities, **(E)** those without cardiovascular-related comorbidities, **(F)** those with only cardiovascular-related underlying comorbidities, and **(G)** those with cardiovascular-related underlying comorbidities. Hematologic indices that were found to have different levels between the young and old including **(H)** lymphocytes, **(I)** neutrophils, and **(J)** CRP and were also correlated with age **(K–M)**. Data are shown as boxes and whiskers. Correlations are colored by positive (red) or negative (green). The numbers next to the survival curves indicate quantities of patients in such a group. ***$P < 0.001$ and ****$P < 0.0001$.

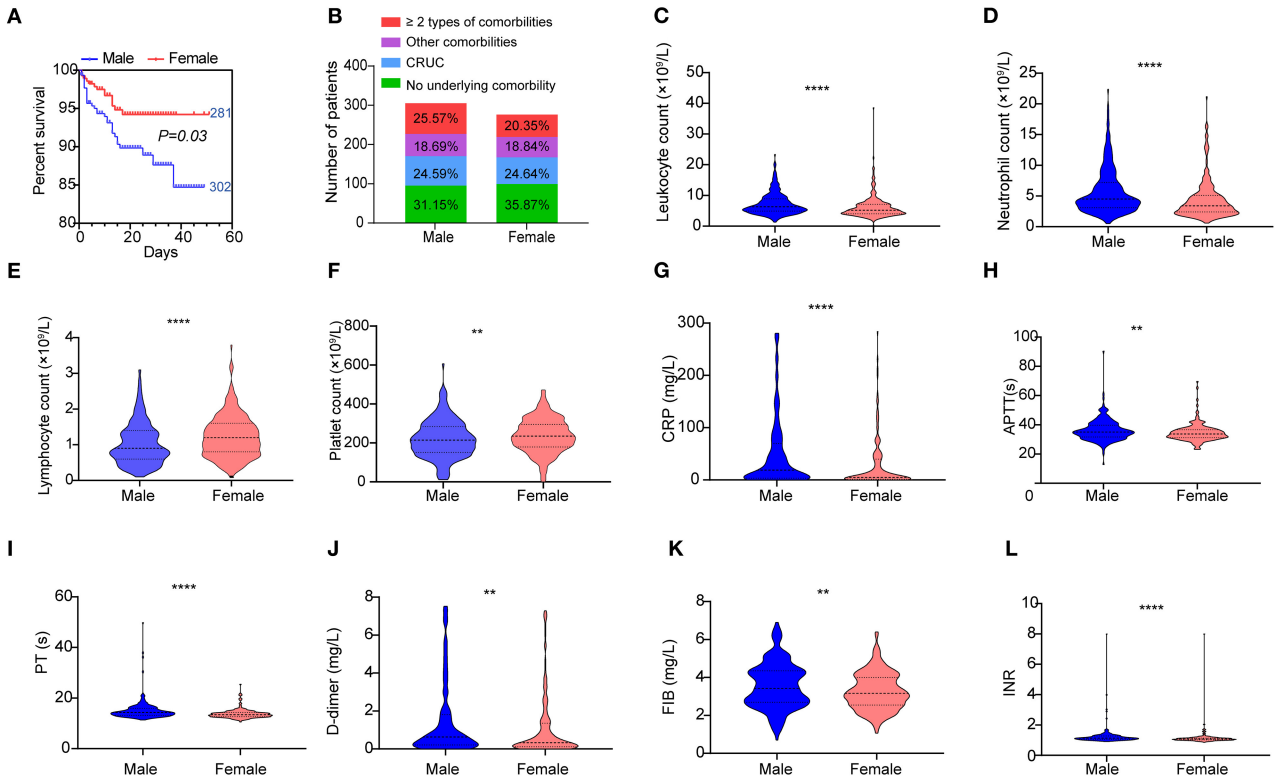


FIGURE 7 | Hematologic variations between the male and female. **(A)** Kaplan–Meier survival curves for genders are shown. **(B)** Composition of different underlying comorbidities in the male and female. Different levels of hematologic indices **(C)** leukocytes, **(D)** neutrophils, **(E)** lymphocytes, **(F)** platelets, **(G)** CRP level, **(H)** APTT, **(I)** PT, **(J)** D-dimer level, **(K)** FIB, and **(L)** INR between the male and female. Data are shown as a violin plot with median and 25 and 75% percentile lines. $^{**}P < 0.01$, $^{****}P < 0.0001$. The numbers next to the survival curves indicate quantities of patients in such a group.

analyzed hematologic indices between the male and female. By using the Mann–Whitney *U*-test, we found that male patients had significantly higher levels of leukocytes, neutrophils, CRP, D-dimer, FIB, and INR; extended APTT and PT; and lower levels of platelets and lymphocytes (**Figures 7C–L**). However, when counting the frequencies of normal and abnormal levels of these indices, we found that most coagulation indices including platelet, APTT, TT, and INR were not significantly different between the male and female (**Supplementary Table 3**).

Taken together, these results indicated that higher inflammatory conditions that manifested as higher levels of leukocytes, neutrophils, CRP, and D-dimer and lower lymphocyte count were the main factors associated with the poorer outcome of male patients. In contrast, coagulation disorders might have a limited contribution to the poorer outcome of male patients.

DISCUSSION

In this study, hematologic biomarkers associated with the progression of COVID-19 were investigated, and some novel findings were documented. First, patient characteristics including the hematologic indices that could predict the fatal outcome of COVID-19 or be associated with the patient's duration of hospitalization in discharged people were detailed and documented in the present study. Second, lymphopenia, hyper inflammation status, and coagulation derangements were shown to be associated with fatal outcome of COVID-19 patients, and their contribution to the fatal outcome of different types of patients (patients with different types of underlying comorbidities, young or old, and male or female) was elucidated.

In our study, results were also found with the incidence of 30.73% (lymphopenia), 24.18% (neutrophilia) (17), and 6.19% (thrombocytopenia) (18) among all the patients, respectively. Higher incidences of these hematological changes were found in deceased patients as compared to the discharged patients (**Table 1**). A higher neutrophil level on admission was found in deceased patients and could predict poor outcomes in our cohort (**Figure 2B**). The involvement of elements of the hematopoietic system is prominent in severe cases and associated with poor outcomes and mortality (19). Blood counts and coagulation parameters are also frequently dysregulated in severe COVID-19 (20, 21). A severe disease is commonly complicated by lymphopenia (22), thrombocytopenia, and coagulopathy, often progressing to disseminated intravascular coagulation (DIC) (23).

Our study also indicated that decreased platelet count might be able to serve as a potential clinical indicator of

mortality during hospitalization (**Figure 2E**). This result was also consistent with our previous studies (15, 16). The mechanism of the reduction of platelet counts in COVID-19 patient may include (1) the inhibition of hematopoiesis in the bone marrow through certain receptors causes decreased primary platelet formation (24, 25); (2) the hyperreactivity of platelets increases the consumption of platelets/megakaryocytes; and (3) the lung functions as one of the hematopoietic organs (26), and SARS-CoV-2 may disrupt its function like SARS. An abnormal coagulation status is an important phase for COVID-19 patients (27). Many coagulation indices, including APTT, PT, TT, FIB, and INR, and some other hematologic indices, including leukocyte, CRP, and D-dimer on admission, were shown to be different between the survivors and non-survivors and could be used as prognostic indicators for a fatal outcome of COVID-19 (**Table 1, Figure 1**). However, the multivariate Cox regression model suggested that lymphopenia, neutrophilia, and prolonged PT serve as the predictors of fatal outcome (**Table 2**). This was partially consistent with the results obtained by the correlation networks and PCA, that these three indices had more connected hub nodes in the survivors or non-survivors or had the biggest positive/negative contribution for PC1 (**Figure 4**). Furthermore, findings on the correlation of hematologic characteristics and hospitalization days confirmed the role of these biomarkers for predicting the prognosis and might help us to build a model to predict the length of hospitalization (**Figures 2**, **3**).

Patients with underlying comorbidities may have a worse outcome than those without (28, 29). Our present study provides further evidence to substantiate this notion (**Table 1**, **Figure 5A**). We observed that CRUC, but not other comorbidities, might contribute to higher mortality for COVID-19 patients. We did not categorize the comorbidities into smaller types, but into four major categories mentioned above (**Table 1**). Then, we researched the survival curves of the patients with each category of comorbidity. It was shown that only CRUC, but not other comorbidities, was associated with poorer clinical outcomes (**Figure 5A**).

Hematological indicators fitted this result perfectly including lymphocyte, leukocyte, and neutrophil counts and CRP levels (**Figures 5B–E**). These indicators were found to have identical trends among the survival curves of the four comorbidity categories. Thus, these four comorbidity clinical indicators might carry the implications of specific hematological changes and their associated poor outcomes in patients with such comorbidities. First, the decrease in lymphocyte, especially in the T cells, might be frequently found in those patients with CRUC (30, 31). These might represent the defects of these cells, which might in turn cause T cells to unable to efficiently combat viral infections (31). Second, patients with CRUC also showed higher inflammation levels in our study, which was manifested as elevated neutrophil count and CRP levels (**Figures 5D,E**). Inflammatory processes and systematic inflammation play a central role in CRUC (32, 33). Hyperinflammation that drives lung or multiorgan injury was often found on COVID-19 patients with worse outcomes (34). Therefore, we could conclude that CRUC contributing to worse outcomes might be related to lymphocyte dysfunction and high background inflammatory state.

Our results confirmed that older age was associated with increased death (**Figure 6A**). This may be associated with age-related underlying comorbidities, particularly the CRUC (**Figures 6D–G**) and age-dependent defects in T and B-cell function (**Figures 6H,K**). As markers of inflammatory reactions, neutrophil and CRP levels were higher in deceased patients and associated with fatal outcome (**Figures 1B,E**, **2B,D**, **Table 1**). They were found to be positively correlated with COVID-19 patients' age ($r = 0.2292$ and 0.3997, $P < 0.0001$) in our study (**Figures 6L,M**). This result further confirmed that irresistible and overexuberant inflammatory response was a potential risk factor that caused the death in SARS-CoV-2 infection given that viral load might not be correlated with the worsening of symptoms, which highlighted the rationality of combining antiviral and anti-inflammatory treatments for COVID-19 (35, 36). Another evidence of a higher inflammatory status of the old was found in the D-dimer level, which was higher and positively correlated with age (**Supplementary Figure 2P**). In COVID-19 patients, D-dimer was found to be related to markers of inflammation (37, 38). Thus, strengthening cellular immunity and anti-inflammation could be an option for COVID-19 therapy, especially for the old with CRUC (39). In contrast, coagulation disorders were shown not to be the main factors that contributed to the different outcomes between the old and young (**Supplementary Figure 2**). Furthermore, the reverse correlation of lymphocyte and D-dimer between the survivors and non-survivors was interesting, and we proposed that it was caused by an incongruent decrease of lymphocyte and an increase of D-dimer happened in non-survivors.

We also found that male patients had a worse outcome than female or the young (**Figure 7A**). No association of underlying comorbidities and gender was found in the cohort, indicating underlying comorbidities may not contribute to such difference. An explanation showed that the female patients mounted significantly more robust T-cell activation than male patients during SARS-CoV-2 infection, which was sustained in the old (40). As we knew, a large proportion (>70%) of lymphocytes were T-cells. We also found that lymphocyte level in female COVID-19 patients was higher than that in the male. However, this was not observed in the deceased patient (**Supplementary Figure 3**). Taken together, we speculated that the lymphocyte level of COVID-19 patients might reflect the level of activated T-cells targeting virus-infected cells (41). Additionally, we also found levels of many hematologic indices to be different between the male and female, which indicated that they might contribute to different outcomes (42).

However, the main factors that contributed to the worse outcome of male patients were lymphocyte dysfunction and hyperinflammation, while coagulation disorders might have partly contributed as most of the coagulation indices were not significantly different between the male and female. The finding of higher inflammatory conditions in the male than in the female patients may be associated with sex hormone differences. Differing in their immunological reactions to foreign and self-antigens, males and females are distinctively different in innate and adaptive immune responses. Importantly, these sex-based immunological differences contribute to variations

in the incidence of autoimmune diseases and malignancies, susceptibility to infectious diseases, and responses to vaccines in males and females (43). Besides, X-chromosome mosaicism in the female is associated with varied genes involved in inflammation. This biased response from X chromosome also promotes differential immunological responses observed in women and men (44). Taken together, these results might indicate different treatment strategies for different types of patients. For example, for patients with CRUC, immune-supportive treatment and anti-inflammatory therapy were of ultimate importance, while for the old and male patients, besides the two above treatment strategies, coagulation support treatment could not be ignored.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Seventh Affiliated Hospital, Sun Yat-sen University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

X-YZ, LL, BH, YHe, and MY had the idea and designed the study. X-YZ, LL, and HK contributed to the writing of the manuscript. BH, MN, MY, CC, YHe, YHu, and QL contributed to the critical revision of the manuscript. X-YZ and LL contributed to the statistical analysis. X-YZ, BH, LL, and MY have verified the underlying data. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81770116 to MY and 31870001 to X-YZ); the Science, Technology and Innovation Commission of Shenzhen; the China's Postdoctoral Science Foundation (Grant No. 2019TQ0383); and the Sanming Project of Medicine in Shenzhen (Grant No. SZSM202011004).

ACKNOWLEDGMENTS

We thank Hankou Hospital for providing the data of these patients.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.680604/full#supplementary-material

Supplementary Figure 1 | Hematologic indices in four categories of underlying comorbidities that showed to not fit the survival curves including **(A)** platelets, **(B)** APTT, **(C)** PT, **(D)** TT, **(E)** D-dimer, **(F)** FIB, and **(G)** INR. Data are shown as dots with median lines. $^{**}P < 0.01$, $^{****}P < 0.0001$.

Supplementary Figure 2 | Hematologic indices that were shown to be not different between the young and old or the levels of which were not significantly correlated with age ($P > 0.05$ or $|r| < 0.2$), including **(A,I)** leukocytes, **(B,J)** platelets, **(C,K)** APTT, **(D,L)** PT, **(E,M)** TT, **(F,N)** FIB, **(G,O)** INR. **(H,P)** D-dimer levels between the young and old and its correlation with age. Data are shown as boxes and whiskers. Correlations are colored by positive (red) or negative (green) or no correlation ($P > 0.05$) (gray). $^{*}P < 0.05$, $^{****}P < 0.0001$.

Supplementary Figure 3 | Lymphocyte levels in the male and female survivors and non-survivors. $^{****}P < 0.0001$; n.s., not significant.

Supplementary Table 1 | Baseline characteristics of patients with or without cardiovascular-related underlying comorbidities (CRUC). #COVID-19 mortality of patients with high neutrophil counts (>6.3 × 10^9/l) or leukocyte counts (>10 × 10^9/l) was compared with other two groups. $COVID-19 mortality of low-platelet-count group (<100 × 10^9/l) and low FIB (<2 g/l) was compared with the other two groups. ^COVID-19 mortality of high-platelet-count group (>300 × 10^9/l) and low FIB (>4 g/l) was compared with the other two groups.

Supplementary Table 2 | Baseline characteristics of old and young patients. *Fisher's exact test was used to compare the COVID-19 mortality between the patients with different indices. &Patients with or without comorbidities were compared. @Patients with or without CRUC were compared. #COVID-19 mortality of patients with high neutrophil counts (>6.3 × 10^9/l) or leukocyte counts (>10 × 10^9/l) was compared with the other two groups. $COVID-19 mortality of low-platelet-count group (<100 × 10^9/l) and low FIB (<2 g/l) was compared with the other two groups. ^COVID-19 mortality of high-platelet-count group (>300 × 10^9/l) and low FIB (>4 g/l) was compared with the other two groups.

Supplementary Table 3 | Baseline characteristics of male and female patients. *Chi-square tests or Fisher's exact test were used to compare the COVID-19 mortality between the patients with different indices. &Patients with or without underlying comorbidities were compared. @Patients with or without CRUC were compared. #COVID-19 mortality of patients with high neutrophil counts (>6.3 × 10^9/l) or leukocyte counts (>10 × 10^9/l) was compared with the other two groups. $COVID-19 mortality of low-platelet-count group (<100 × 10^9/l) and low FIB (<2 g/l) was compared with the other two groups. ^COVID-19 mortality of high-platelet-count group (>300 × 10^9/l) and low FIB (>4 g/l) was compared with the other two groups.

REFERENCES

1. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of Coronavirus disease 2019 (COVID-19): a review. *JAMA*. (2020) 324:782–93. doi: 10.1001/jama.2020.12839
2. Lake MA. What we know so far: COVID-19 current clinical knowledge and research. *Clin Med*. (2020) 20:124–7. doi: 10.7861/clinmed.2019-coron
3. Pascarella G, Strumia A, Piliego C, Bruno F, Del Buono R, Costa F, et al. COVID-19 diagnosis and management: a comprehensive review. *J Intern Med*. (2020) 288:192–206. doi: 10.1111/joim.13091
4. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. (2020) 382:1708–20. doi: 10.1056/NEJMoa2002032
5. Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M. The cytokine storm in COVID-19: an overview of the involvement of the chemokine/

chemokine-receptor system. *Cytokine Growth Factor Rev.* (2020) 53:25–32. doi: 10.1016/j.cytogfr.2020.05.003
6. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the 'cytokine storm' in COVID-19. *J Infect.* (2020) 80:607–13. doi: 10.1016/j.jinf.2020.03.037
7. Shah A, Donovan K, McHugh A, Pandey M, Aaron L, Bradbury CA, et al. Thrombotic and haemorrhagic complications in critically ill patients with COVID-19: a multicentre observational study. *Crit Care.* (2020) 24:561. doi: 10.1186/s13054-020-03260-3
8. Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. *J Am Coll Cardiol.* (2020) 75:2950–73. doi: 10.1016/j.jacc.2020.04.031
9. Manne BK, Denorme F, Middleton EA, Portier I, Rowley JW, Stubben CJ, et al. Platelet gene expression and function in COVID-19 patients. *Blood.* (2020) 136:1317–29. doi: 10.1182/blood.2020007214
10. Sakr Y, Giovini M, Leone M, Pizzilli G, Kortgen A, Bauer M, et al. Pulmonary embolism in patients with coronavirus disease-2019 (COVID-19) pneumonia: a narrative review. *Ann Intensive Care.* (2020) 10:124. doi: 10.1186/s13613-020-00741-0
11. Ranucci M, Ballotta A, Di Dedda U, Bayshnikova E, Dei Poli M, Resta M, et al. The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. *J Thromb Haemost.* (2020) 18:1747–51. doi: 10.1111/jth.14854
12. Denorme F, Manne BK, Portier I, Petrey AC, Middleton EA, Kile BT, et al. COVID-19 patients exhibit reduced procoagulant platelet responses. *J Thromb Haemost.* (2020) 18:3067–73. doi: 10.1111/jth.15107
13. Wenham C, Smith J, Morgan R, Gender, Group C-W. COVID-19: the gendered impacts of the outbreak. *Lancet.* (2020) 395:846–8. doi: 10.1016/S0140-6736(20)30526-2
14. Foresta C, Rocca MS, Di Nisio A. Gender susceptibility to COVID-19: a review of the putative role of sex hormones and X chromosome. *J Endocrinol Invest.* (2020) 44:951–6. doi: 10.1007/s40618-020-01383-6
15. Zhang Y, Zeng X, Jiao Y, Li Z, Liu Q, Ye J, et al. Mechanisms involved in the development of thrombocytopenia in patients with COVID-19. *Thromb Res.* (2020) 193:110–5. doi: 10.1016/j.thromres.2020.06.008
16. Yang M, Li CK, Li K, Hon KL, Ng MH, Chan PK, et al. Hematological findings in SARS patients and possible mechanisms (review). *Int J Mol Med.* (2004) 14:311–5. doi: 10.3892/ijmm.14.2.311
17. Yang L, Liu S, Liu J, Zhang Z, Wan X, Huang B, et al. COVID-19: immunopathogenesis and immunotherapeutics. *Signal Transduct Target Ther.* (2020) 5:128. doi: 10.1038/s41392-020-00243-2
18. Yang X, Yang Q, Wang Y, Wu Y, Xu J, Yu Y, et al. Thrombocytopenia and its association with mortality in patients with COVID-19. *J Thromb Haemost.* (2020) 18:1469–72. doi: 10.1111/jth.14848
19. Mina A, van Besien K, Platanias LC. Hematological manifestations of COVID-19. *Leuk Lymphoma.* (2020) 61:2790–8. doi: 10.1080/10428194.2020.1788017
20. Connors JM, Levy JH. COVID-19 and its implications for thrombosis and anticoagulation. *Blood.* (2020) 135:2033–40. doi: 10.1182/blood.2020006000
21. Ye J, Jiao Y, Zhang Y, Li Z, Zeng X, Deng H, et al. Hematological changes in patients with COVID-19 (review). *Mol Med Rep.* (2020) 22:4485–91. doi: 10.3892/mmr.2020.11581
22. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, Kastritis E, Sergentanis TN, Politou M, et al. Hematological findings and complications of COVID-19. *Am J Hematol.* (2020) 95:834–47. doi: 10.1002/ajh.25829
23. Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost.* (2020) 18:1094–9. doi: 10.1111/jth.14817
24. Xu P, Zhou Q, Xu J. Mechanism of thrombocytopenia in COVID-19 patients. *Ann Hematol.* (2020) 99:1205–8. doi: 10.1007/s00277-020-04019-0
25. Zini G, Bellesi S, Ramundo F, d'Onofrio G. Morphological anomalies of circulating blood cells in COVID-19. *Am J Hematol.* (2020) 95:870–2. doi: 10.1002/ajh.25824
26. Lefrançais E, Ortiz-Muñoz G, Caudrillier A, Mallavia B, Liu F, Sayah DM, et al. The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. *Nature.* (2017) 544:105–9. doi: 10.1038/nature21706
27. Li T, Lu H, Zhang W. Clinical observation and management of COVID-19 patients. *Emerg Microbes Infect.* (2020) 9:687–90. doi: 10.1080/22221751.2020.1741327
28. Guan WJ, Liang WH, Zhao Y, Liang HR, Chen ZS, Li YM, et al. Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. *Euro Respir J.* (2020) 55:2000547. doi: 10.1183/13993003.01227-2020
29. Ji W, Huh K, Kang M, Hong J, Bae GH, Lee R, et al. Effect of underlying comorbidities on the infection and severity of COVID-19 in Korea: a nationwide case-control study. *J Korean Med Sci.* (2020) 35:e237. doi: 10.3346/jkms.2020.35.e237
30. Siedlinski M, Jozefczuk E, Xu X, Teumer A, Evangelou E, Schnabel RB, et al. White blood cells and blood pressure: a mendelian randomization study. *Circulation.* (2020) 141:1307–17. doi: 10.1161/CIRCULATIONAHA.119.045102
31. Youn JC, Yu HT, Lim BJ, Koh MJ, Lee J, Chang DY, et al. Immunosenescent CD8+ T cells and C-X-C chemokine receptor type 3 chemokines are increased in human hypertension. *Hypertension.* (2013) 62:126–33. doi: 10.1161/HYPERTENSIONAHA.113.00689
32. Ruparelia N, Chai JT, Fisher EA, Choudhury RP. Inflammatory processes in cardiovascular disease: a route to targeted therapies. *Nat Rev Cardiol.* (2017) 14:314. doi: 10.1038/nrcardio.2017.33
33. Boukhris M, Hillani A, Moroni F, Annabi MS, Addad F, Ribeiro MH, et al. Cardiovascular implications of the COVID-19 pandemic: a global perspective. *Can J Cardiol.* (2020) 36:1068–80. doi: 10.1016/j.cjca.2020.05.018
34. Didangelos A. COVID-19 hyperinflammation: what about neutrophils? *mSphere.* (2020) 5:e00367–20. doi: 10.1128/mSphere.00367-20
35. Stebbing J, Phelan A, Griffin I, Tucker C, Oechsle O, Smith D, et al. COVID-19: combining antiviral and anti-inflammatory treatments. *Lancet Infect Dis.* (2020) 20:400–2. doi: 10.1016/S1473-3099(20)30132-8
36. Asif M, Saleem M, Saadullah M, Yaseen HS, Al Zarzour R. COVID-19 and therapy with essential oils having antiviral, anti-inflammatory, and immunomodulatory properties. *Inflammopharmacology.* (2020) 28:1153–61. doi: 10.1007/s10787-020-00744-0
37. Yu B, Li X, Chen J, Ouyang M, Zhang H, Zhao X, et al. Evaluation of variation in D-dimer levels among COVID-19 and bacterial pneumonia: a retrospective analysis. *J Thromb Thrombolysis.* (2020) 50:548–57. doi: 10.1007/s11239-020-02171-y
38. Cao W, Li T. COVID-19: towards understanding of pathogenesis. *Cell Res.* (2020) 30:367–9. doi: 10.1038/s41422-020-0327-4
39. Wang L, Zhang Y, Zhang S. Cardiovascular impairment in COVID-19: learning from current options for cardiovascular anti-inflammatory therapy. *Front Cardiovasc Med.* (2020) 7:78. doi: 10.3389/fcvm.2020.00078
40. Takahashi T, Ellingson MK, Wong P, Israelow B, Lucas C, Klein J, et al. Sex differences in immune responses that underlie COVID-19 disease outcomes. *Nature.* (2020) 588:315–20. doi: 10.1101/2020.06.06.20123414
41. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol.* (2020) 20:355–62. doi: 10.1038/s41577-020-0331-4
42. Marquez EJ, Trowbridge J, Kuchel GA, Banchereau J, Ucar D. The lethal sex gap: COVID-19. *Immun Ageing.* (2020) 17:13. doi: 10.1186/s12979-020-00183-z
43. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol.* (2016) 16:626–38. doi: 10.1038/nri.2016.90
44. Fischer J, Jung N, Robinson N, Lehmann C. Sex differences in immune responses to infectious diseases. *Infection.* (2015) 43:399–403. doi: 10.1007/s15010-015-0791-9

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 14 June 2021
doi: 10.3389/fcvm.2021.667721

Myocardial Work Efficiency, A Novel Measure of Myocardial Dysfunction, Is Reduced in COVID-19 Patients and Associated With In-Hospital Mortality

Anum S. Minhas[1†], Nisha A. Gilotra[1†], Erin Goerlich[1], Thomas Metkus[1], Brian T. Garibaldi[2], Garima Sharma[1], Nicole Bavaro[1], Susan Phillip[1], Erin D. Michos[1] and Allison G. Hays[1]*

[1] Division of Cardiology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2] Division of Pulmonary and Critical Care Medicine, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States

OPEN ACCESS

Edited by:
Mingxing Xie, Huazhong University of Science and Technology, China

Reviewed by:
Lígia Mendes, Hospital da Luz Setúbal, Portugal
Alessandro Faragli, Deutsches Herzzentrum Berlin, Germany

***Correspondence:**
Allison G. Hays
ahays2@jhmi.edu

[†] *These authors have contributed equally to this work*

Specialty section:
This article was submitted to Cardiovascular Imaging, a section of the journal Frontiers in Cardiovascular Medicine

Received: *14 February 2021*
Accepted: *22 April 2021*
Published: *14 June 2021*

Citation:
Minhas AS, Gilotra NA, Goerlich E, Metkus T, Garibaldi BT, Sharma G, Bavaro N, Phillip S, Michos ED and Hays AG (2021) Myocardial Work Efficiency, A Novel Measure of Myocardial Dysfunction, Is Reduced in COVID-19 Patients and Associated With In-Hospital Mortality. Front. Cardiovasc. Med. 8:667721. doi: 10.3389/fcvm.2021.667721

Background: Although troponin elevation is common in COVID-19, the extent of myocardial dysfunction and its contributors to dysfunction are less well-characterized. We aimed to determine the prevalence of subclinical myocardial dysfunction and its association with mortality using speckle tracking echocardiography (STE), specifically global longitudinal strain (GLS) and myocardial work efficiency (MWE). We also tested the hypothesis that reduced myocardial function was associated with increased systemic inflammation in COVID-19.

Methods and Results: We conducted a retrospective study of hospitalized COVID-19 patients undergoing echocardiography ($n = 136$), of whom 83 and 75 had GLS (abnormal $>-16\%$) and MWE (abnormal $<95\%$) assessed, respectively. We performed adjusted logistic regression to examine associations of GLS and MWE with in-hospital mortality. Patients were mean 62 ± 14 years old (58% men). While 81% had normal left ventricular ejection fraction (LVEF), prevalence of myocardial dysfunction was high by STE; [39/83 (47%) had abnormal GLS; 59/75 (79%) had abnormal MWE]. Higher MWE was associated with lower in-hospital mortality in unadjusted [OR 0.92 (95% CI 0.85–0.99); $p = 0.048$] and adjusted models [aOR 0.87 (95% CI 0.78–0.97); $p = 0.009$]. In addition, increased systemic inflammation measured by interleukin-6 level was associated with reduced MWE.

Conclusions: Subclinical myocardial dysfunction is common in COVID-19 patients with clinical echocardiograms, even in those with normal LVEF. Reduced MWE is associated with higher interleukin-6 levels and increased in-hospital mortality. Non-invasive STE represents a readily available method to rapidly evaluate myocardial dysfunction in COVID-19 patients and can play an important role in risk stratification.

Keywords: echo, strain, COVID-19, non-invasive, ultrasoud diagnosis

INTRODUCTION

COVID-19, the disease caused by the novel coronavirus SARS-CoV2, carries high acute cardiovascular morbidity and mortality (1, 2). The mechanisms for cardiac injury are not fully understood, with hypotheses ranging from systemic inflammation due to cytokine release syndrome, angiotensin converting enzyme-2 mediated direct viral myocardial toxicity, autoimmune myocarditis, and sympathetic stress response (1, 3). Over 25% of hospitalized COVID-19 patients have acute cardiac injury as detected by elevated cardiac troponin, associated with greater in-hospital mortality (1, 3, 4). However, troponin alone has limited specificity and sensitivity in myocarditis and can also rise in acute respiratory distress syndrome (ARDS), another recognized complication of COVID-19 (5–7). Additionally, although multiple inflammatory pathways, such as interleukin-6 (IL-6), are implicated in myocardial injury in COVID-19, their effect on indices of cardiac function is unknown and a better understanding of the degree and determinants of myocardial function may improve risk stratification and lead to new therapeutic approaches (8–10).

Studies examining the degree of myocardial dysfunction in COVID-19 are limited, and assessment with cardiac imaging has been challenging due to exposure concerns to echocardiography staff. Thus, it is likely that the true prevalence of cardiac dysfunction is underreported (11). Speckle tracking echocardiography (STE) can rapidly quantify myocardial dysfunction (e.g., using global longitudinal strain [GLS]) with increased sensitivity compared with standard echocardiographic measures (12–14). More recently, a novel technique to measure LV function based on STE, global myocardial work (MW), was developed (15, 16). The advantage of MW [assessed by myocardial work index (MWI) and myocardial work efficiency (MWE)], is that it provides a more load independent measure of LV function by accounting for afterload; MW is also highly reproducible and adds incremental value to GLS in predicting adverse events (17).

Given the high mortality and severity of complications with COVID-19, we conducted a clinical cardiac imaging study in hospitalized COVID-19 patients with echocardiograms performed with the following aims: (1) to determine the prevalence and extent of myocardial dysfunction using STE (GLS and MWE), (2) to examine the association of myocardial dysfunction with in-hospital mortality, and (3) to investigate clinical and inflammatory biomarker risk factors associated with worsened subclinical myocardial dysfunction.

Abbreviations: COVID-19, SARS-CoV2; ARDS, acute respiratory distress syndrome; GLS, global longitudinal strain; MW, myocardial work; MWI, myocardial work index; MWE, myocardial work efficiency; LV, left ventricular; RV, right ventricular; EF, ejection fraction; LVEDD, left ventricular end diastolic diameter; RVEDD, right ventricular end diastolic diameter; TAPSE, tricuspid annular plane systolic excursion; TR, tricuspid regurgitation; STE, speckle tracking echocardiography; ASE, American Society of Echocardiography; BMI, body mass index; CRP, C-reactive protein; IL-6, interleukin 6; Pro-BNP, N-terminal pro-hormone B-type natriuretic peptide.

METHODS

Study Design

This retrospective, single-center cohort study included 136 consecutive patients with confirmed COVID-19 who were hospitalized at Johns Hopkins Hospital and underwent clinically indicated transthoracic echocardiogram between March 25, 2020 and May 19, 2020, with follow-up completed by June 22, 2020. All echocardiograms were ordered by the patient's clinical care team. The study was approved by the Johns Hopkins Institutional Review Board and informed consent was waived per IRB guidelines.

Clinical Data

Patient characteristics, including demographics, medical history, clinical presentation, laboratory testing, and clinical outcomes were extracted from the electronic medical record. Initial values after admission for the following serum biomarkers were collected: cardiac troponin I, IL-6, C-reactive protein (CRP), ferritin, fibrinogen, and d-dimer. In- hospital all-cause mortality during index hospitalization was ascertained from electronic medical records through the end of follow-up. Two separate investigators independently reviewed the data.

Transthoracic Echocardiography

Conventional 2D Echocardiographic Analysis

Bedside transthoracic echocardiographic (TTE) examinations were performed by experienced sonographers using Vivid^{TM} E95 ultrasound system (GE Vingmed Ultrasound; Horten, Norway). Both standard 2D and Doppler echocardiography were acquired. Measurements including LV, right ventricular (RV) parameters and diastology were performed by a dedicated research sonographer based on the American Society of Echocardiography (ASE) guidelines (18, 19). To limit exposure to patients and staff, measurements that were not essential, including STE analyses, were performed offline, removed from the patient's room, and limited studies were performed according to COVID-19 specific imaging guidelines (20).

Speckle Tracking Echocardiography Analysis

STE analyses were conducted according to ASE recommendations in a subset of TTEs that were (1) deemed to be of fair quality or greater for subendocardial image visualization by two independent readers and (2) in a patient free of atrial or ventricular arrhythmias at the time of exam ($n = 83$) (18). Two-dimensional images from the apical four-chamber, two-chamber, and long-axis views were acquired with frame rates between 50 and 80 frames/s to enable GLS. GLS was quantified using semiautomated analysis software (EchoPAC version 202; GE Vingmed Ultrasound). The automated algorithm traces and tracks the LV myocardium, with manual adjustments made when appropriate, and the software calculates GLS from the weighted average of the peak systolic longitudinal strain of all segments using the 17 segment model. GLS is quantified as a negative number with cutoff as −18%, and more negative as normal for this system, but based on prior literature supporting use of a cutoff of −16% as the threshold

for normal, analyses were conducted with > −16% as the cutoff for normal (21–25). Tracking quality was assessed by the operator and over-ridden in segments with two or fewer rejected regions where the operator deemed tracking quality to be acceptable. Images were analyzed by two independent observers blinded to clinical data on a dedicated offline research workstation. Intraobserver and interobserver variability of STE measures, specifically MWE, were assessed by intra- class correlation coefficient (individual ICC of 0.994 and average ICC of 0.997 for intraobserver and 0.992 and 0.995 for interobserver, respectively), and Bland-Altman analysis (all differences in measurements within ±1 SD). The time between intraobserver measurements was 1 day.

Myocardial Work

Myocardial work (MW) was determined from non-invasive LV pressure-strain analysis, which has previously been described and validated (26, 27). MW is calculated as the area of the pressure-strain loop, similar in concept to deriving LV stroke work using pressure volume loops invasively. In this technique, pressures are assessed using brachial systolic pressure and valvular event timing and strain measured with STE (15, 16). MW indices were calculated with the same software as above to evaluate LV performance by incorporating afterload determination using blood pressure; this provides a more load-independent measure compared with GLS (27). Blood pressure was measured by sphygmomanometry at the time of the

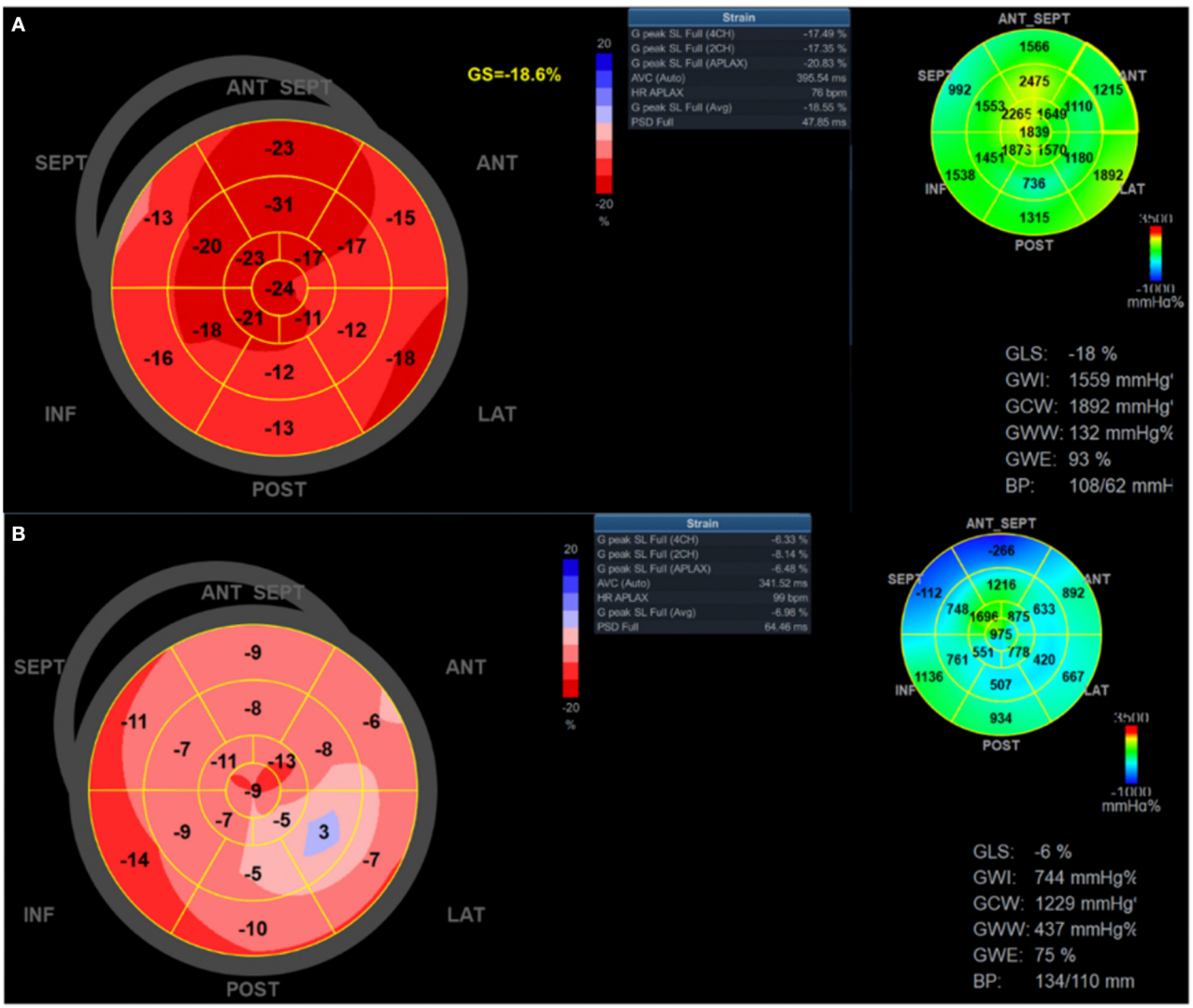


FIGURE 1 | Global longitudinal strain and myocardial work efficiency measurement in patients with COVID-19. Global longitudinal strain and myocardial work index bull's eye mapping for two patients with COVID-19. **(A)** representative patient with relatively normal strain and myocardial work; **(B)** representative patient with severely reduced global longitudinal strain (apical predominant), myocardial work index, and work efficiency. ANT, anterior; ANT SEPT, anterospetal; APLAX, apical long axis; AVC, aortic valve closure; CH, chamber; GS, global strain; HR, heart rate; INF, inferior; LAT, lateral; POST, posterior; PSD, peak systolic dispersion; SEPT, septal; SL, strain length.

echocardiogram immediately before acquiring images for STE. The MW software then constructs a non-invasive LV pressure curve adjusted according to the duration of isovolumic and ejection phases defined by the timing of aortic and mitral valve opening and closing events (28). Global MW was quantified by calculating the rate of regional shortening by differentiation of the strain tracing and multiplying by instantaneous LV pressure (estimated) integrated over time. During LV ejection time, segments were analyzed for wasted work and constructive work, with global values determined as the averages of all segmental values (see example **Figure 1**). The following parameters were acquired using EchoPAC software: Global MW index (MWI, mmHg%) defined as the area within the global LV pressure-strain loop and global MW efficiency (MWE, %), defined as constructive MW divided by the sum of constructed work and wasted work, expressed as a percentage. Abnormal MWE was defined as <95%, consistent with other studies (16). For myocardial work, MWE was chosen as the primary variable of interest as it provides a comprehensive assessment of the ratio between constructive work performed by the LV and the sum of both wasted and constructive work, and has previously shown to have prognostic value in other populations (29–31).

Statistical Methods

Descriptive statistical analyses were performed for clinical and echocardiographic parameters. Continuous variables are presented as mean ± standard deviation (normally distributed variables) or median (IQR) (non-normally distributed variables). Differences between groups were compared using parametric two-sample Student's *t*-test or non-parametric Mann–Whitney *U*-test. Categorical variables are presented as number (%) and groups compared using Chi-squared test. For relevant analyses, normal LVEF was defined as >50%.

We then performed unadjusted and adjusted logistic regression to estimate the odds of mortality with either GLS or MWE as the primary independent variable of interest, analyzed continuously. Covariates included were clinical characteristics (age, sex, diabetes, and hypertension) and echocardiographic measurements, selected one at a time for addition to the model as the primary covariate of interest (LVEF, GLS, MWE, TAPSE, RVSP, TR peak velocity, and E/E′). Clinical covariates selected for inclusion in the adjusted models were chosen based on prior literature suggesting possible confounding, and included age, sex, history of hypertension, and diabetes (32–36). Model 1 included the echocardiographic covariate of interest, adjusted for age and sex. Model 2 included the echocardiographic covariate of interest, adjusted for age, sex, diabetes, and hypertension. All variables for logistic regression were analyzed as continuous variables.

To further understand the incremental value of STE analysis over standard echocardiographic LVEF assessment for mortality prediction, we performed subgroup analyses in patients with normal (>50%) or abnormal (<50%) LV EF. We also performed subgroup analyses in patients with the presence or absence of acute respiratory distress syndrome (ARDS). A $p \leq 0.05$ was considered significant.

Last, for a subset of the cohort, linear regression was then performed to evaluate inflammatory markers (divided into tertiles given non-normal distribution) as predictors of MWE. Values within each tertile are included in the supplement. These markers included IL6, troponin, ferritin, C-reactive protein (CRP), d-dimer, and fibrinogen. Missing data were considered to occur at random, and patients with missing inflammatory data were not included in this analysis.

RESULTS

Clinical Characteristics of Patients Undergoing Echocardiogram

Median time of symptom duration prior to admission was 6 days (3–8 days). Median time to echocardiogram after admission was 4 days (2–8 days) and median overall time of admission was 16.5 days (9–31 days).

Clinical characteristics of hospitalized patients with COVID-19 who had echocardiogram performed are shown in **Table 1** ($n = 136$). The mean age was 62 years, 79 (58%) were men and 63 (47%) African American. Approximately 63% of patients required mechanical ventilation, 57% were diagnosed with ARDS and 53% had shock (septic, distributive, cardiogenic or otherwise) (**Table 1**).

The cohort of patients with echocardiograms performed was comparable to the subset of patients with GLS and MWE measured (**Table 1**). The majority of patients (81%) undergoing echocardiogram had normal LV systolic function by LVEF measurement. Follow-up (discharged as alive or deceased) was complete for 131/136 patients, while 5/136 (3.7%) were administratively censored (still admitted at the time of analysis).

Clinical and Echocardiographic Characteristics for Patients With Global Longitudinal Strain Assessed

Among the patients with GLS performed ($n = 83$), 44 patients had normal GLS and 39 (47%) had abnormal GLS (**Table 1**). There were no significant differences in age, sex, race, or history of hypertension or CAD between patients with and without abnormal GLS. There was higher prevalence of diabetes mellitus in the abnormal compared with normal GLS group (51 vs. 27%, $p = 0.025$). Body mass index (BMI) was significantly higher in patients with abnormal compared with normal GLS (median 31.4 vs. 27.8 kg/m^2, $p = 0.017$). Patients with abnormal GLS had lower LVEF (55 vs. 62%, $p < 0.001$), and lower TAPSE (1.7 vs. 2.0 cm, $p = 0.005$) when compared with those with normal GLS.

Among the inflammatory markers, interleukin-6 was higher among patients with abnormal GLS [median 164 (69–815)] compared with normal GLS [median 86 (32–167)], $p = 0.034$. All other inflammatory markers were not significantly different (**Table 1**). The value ranges of each inflammatory marker per tertile are presented in **Supplementary Table 1**.

Clinical and Echocardiographic Characteristics for Patients With Myocardial Work Efficiency Assessed

Among the subgroup of patients with myocardial work imaging performed ($N = 75$), abnormal MWE (defined as <95%) was

TABLE 1 | Comparison of clinical characteristics and echocardiographic parameters in the cohort of hospitalized patients with COVID-19 and subgroups with normal vs. abnormal global longitudinal strain (GLS) and myocardial work efficiency (MWE).

Variables	Overall cohort *N* = 136	Normal GLS *N* = 44	Abnormal GLS *N* = 39	*p*-value	Normal MWE *N* = 16	Abnormal MWE *N* = 59	*p*-value
Age, years	62.4 ± 13.9	61.9 ± 13.4	63.4 ± 14.4	0.614	55.2 ± 16.5	64.3 ± 13.1	**0.023**
Male	79 (58%)	27 (61%)	22 (56%)	0.647	13 (81%)	32 (53%)	**0.039**
Race				0.347			0.082
White	34 (25%)	10 (23%)	5 (13%)		3 (19%)	12 (21%)	
African American	63 (47%)	20 (45%)	23 (61%)		5 (31%)	33 (57%)	
Other	37 (27%)	14 (32%)	10 (26%)		8 (50%)	13 (22%)	
Body mass index, kg/m^2	30.0 (26.4–35.8)	27.8 (25.6–31.3)	31.4 (26.5–38.4)	**0.017**	27.7 (25.7–31.8)	28.7 (25.7–34.5)	0.544
Comorbidities							
Hypertension	97 (72%)	29 (66%)	30 (77%)	0.269	7 (44%)	46 (78%)	**0.008**
Diabetes mellitus	55 (41%)	12 (27%)	20 (51%)	**0.025**	1 (6%)	29 (49%)	**0.002**
Coronary artery disease	20 (15%)	4 (9%)	8 (21%)	0.140	0 (0%)	10 (17%)	0.077
Heart failure	20 (15%)	2 (5%)	12 (31%)	**0.001**	0 (0%)	12 (20%)	**0.049**
Clinical presentation							
Heart rate, beats per min	99 ± 20	97 ± 17	103 ± 21	0.151	95 ± 18	100 ± 20	0.392
Systolic blood pressure, mmHg	129 ± 25	129 ± 24	134 ± 24	0.368	126 ± 27	132 ± 23	0.343
Diastolic blood pressure, mmHg	71 ± 16	71 ± 16	74 ± 15	0.389	74 ± 16	71 ± 16	0.546
Laboratory measurements							
White blood cell count, K/cu mm	6.7 (5.0–9.3)	6.4 (4.6–8.7)	6.0 (4.8–8.3)	0.773	6.4 (4.8–9.0)	6.4 (4.8–9.1)	0.946
Absolute lymphocyte count, K/cu mm	0.6 (0.1–1.1)	0.6 (0.1–1.0)	0.5 (0.0–1.3)	0.794	0.7 (0.0–1.2)	0.7 (0.03–1.2)	0.992
D-dimer, mg/L	2.0 (0.8–5.3)	2.0 (0.8–4.6)	2.2 (0.9–7.3)	0.433	2.0 (0.4–4.7)	2.2 (0.9–4.5)	0.213
Interleukin-6, pg/ml	130 (51–409)	86 (32–167)	164 (69–815)	**0.034**	114 (47–422)	125 (45–406)	0.695
CRP, mg/dl	15.3 (4.9–34.7)	11.7 (3.3–20.5)	13.7 (5.1–37.7)	0.410	4.9 (2.3–15.3)	15 (6.6–34.3)	**0.009**
Ferritin, ng/ml	735 (395–1,424)	737 (427–1,130)	800 (402–2,898)	0.525	830 (289–1,677)	719 (412–1,125)	0.897
Fibrinogen, mg/dl	596 (445–703)	737 (427–1,130)	800 (402–2,898)	0.695	568 (463–729)	597 (457–722)	0.694
Pro-BNP, pg/ml	422 (157–1,956)	242 (99–589)	564 (164–3,992)	**0.044**	176 (70–385)	392 (164–2,611)	**0.032**
Troponin I, ng/ml	0.03 (0.03–0.05)	0.03 (0.03–0.03)	0.03 (0.03–0.08)	0.454	0.03 (0.03–0.03)	0.03 (0.03–0.05)	0.305
Clinical events							
Shock	72 (53%)	17 (39%)	23 (59%)	0.064	4 (25%)	30 (51%)	0.065
Mechanical ventilation	86 (63%)	22 (50%)	26 (67%)	0.125	5 (31%)	38 (64%)	**0.017**
ARDS	78 (57%)	19 (43%)	25 (64%)	0.057	5 (31%)	32 (54%)	0.103
DVT or PE	31 (23%)	8 (18%)	8 (21%)	0.788	3 (19%)	12 (20%)	0.888
Death	25 (19%)	7 (16%)	8 (21%)	0.620	2 (12%)	9 (16%)	0.764
Echocardiographic parameters							
LA volume, ml	44 (35–71)	41 (29–45)	48 (39–95)	**0.046**	39.5 (28–42)	47 (39–55)	0.222
LVEDD, cm	4.2 (3.7–4.8)	4.1 (3.8–4.6)	4.3 (3.4–4.9)	0.378	4.4 (3.8–4.9)	4.1 (3.5–4.7)	0.276
LVEF, %	62 (52–62)	62 (57–64)	55 (40–62)	**<0.001**	62 (62–64)	57 (50–62)	**0.011**
Normal LVEF (>50%)	109 (81%)	43 (64%)	24 (36%)	**<0.001**	16 (100%)	45 (74%)	**0.031**
RVEDD, cm	3.6 ± 0.7	3.4 ± 0.6	3.6 ± 0.7	0.224	3.4 ± 0.7	3.6 ± 0.6	0.225
Normal RV function	63 (81%)	22 (85%)	18 (72%)	0.274	12 (92%)	24 (73%)	0.147
TAPSE, cm	1.8 ± 0.4	2.0 ± 0.4	1.7 ± 0.4	**0.005**	2.1 ± 0.3	1.8 ± 0.4	**0.003**
RVSP, mmHg	37 (30–50)	37 (29–48)	34 (32–53)	0.742	31 (30–33)	37 (29–49)	0.288
Mean PAP, mmHg	34 ± 12	35 ± 9	34 ± 11	0.754	27 ± 12	35 ± 9	0.087
Peak TR gradient, mmHg	31 (25–42)	32 (25–42)	31 (25–40)	0.899	29 (25–38)	31 (24–43)	0.832
PCWP, mmHg	14 (10–18)	13 (9–17)	12 (9–16)	0.820	12 (10–16)	15 (12–21)	0.422
E/E'	10 (8–13)	10 (7–12)	9 (7–13)	0.665	9 (7–11)	9 (7–13)	0.561
GLS, %	−16.1 ± 4.3	−19.2 ± 2.4	−12.6 ± 3.0	**<0.001**	−19.7 ± 3.1	−15.5 ± 4.1	**<0.001**
MWI, mmHg%	1,412 ± 425	1,579 ± 362	1,227 ± 417	**<0.001**	1,723 ± 399	1,331 ± 396	**<0.001**
MWE, %	92 (87–94)	94 (91–95)	89 (82–92)	**<0.001**	96 (95–96)	91 (86–93)	**<0.001**

Categorical variables are presented as number (%) and continuous variables are presented as mean ± standard deviation or median (interquartile range).

CRP, C-reactive protein; Pro-BNP, N-terminal pro-hormone B-type natriuretic peptide; ARDS, acute respiratory distress syndrome; DVT, deep venous thrombosis; PE, pulmonary embolism; LA, left atrium; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; GLS, global longitudinal strain; RVEDD, right ventricular end diastolic diameter; TAPSE, tricuspid annular plane systolic excursion; RVSP, right ventricular systolic pressure; PAP, pulmonary artery pressure; TR, tricuspid regurgitation; GLS, global longitudinal strain; MWI, myocardial work index; MWE, myocardial work efficiency.

The bold values represent significant p-values, with significant defined as <0.05.

present in the majority (59/75, 79%). There were no significant differences in demographics or clinical presentation between patients with normal vs. abnormal MWE (**Table 1**). A history of hypertension was more common among patients with abnormal MWE compared with normal MWE (78 vs. 44%, $p = 0.008$), as was a prior history of diabetes (29 vs. 1%, $p = 0.002$). Patients with abnormal MWE compared with those with normal MWE had lower LVEF (57 vs. 62%, $p = 0.011$), and lower TAPSE (1.8 vs. 2.1 cm, $p = 0.003$).

Among patients with normal LVEF ($n = 67$), a high percentage had evidence of subclinical myocardial dysfunction using STE: 36% had abnormal GLS (GLS>−16%) and 74% had abnormal MWE (MWE <95%) (**Figure 2**).

Association of Clinical Characteristics and Speckle Tracking Echocardiography Measurements With Mortality

During hospital admission, 25 (19%) of patients experienced in-hospital death. No clinical characteristics were independently associated with mortality in univariate analysis. MWE was the only echocardiographic parameter independently associated with mortality [unadjusted OR 0.92 (95% CI 0.85–0.999), $p = 0.048$]. In adjusted Models 1–2, MWE remained associated with mortality, with the strongest association in Model 2 [OR 0.87 (95% CI 0.78–0.97), $p = 0.009$] (**Table 2**), suggesting that a 1% increase in MWE was associated with 13% lower odds of death.

Additional subgroup analyses performed to confirm the relationship of MWE and mortality showed similar findings. Among patients with normal LVEF, higher MWE was again independently inversely associated with death [unadjusted OR 0.89 (95% CI 0.78–1.00), $p = 0.050$]. MWE was also associated with in-hospital death after adjusting for age and sex [aOR 0.85 (95% CI 0.74–0.99), $p = 0.038$]. GLS was not associated with death in adjusted or unadjusted analysis. No echocardiographic parameter (LVEF, GLS, or MWE) was associated with mortality in subgroup analyses of patients with and without ARDS (**Supplementary Table 2**).

As MWE was the only echocardiographic parameter associated with mortality, we then evaluated systemic inflammatory markers as predictors of abnormal MWE in a subset of patients with available inflammatory marker data. We observed that MWE was 2.04% lower per higher tertile of IL-6 level ($p = 0.021$), indicating that greater degree of inflammation reflected by IL-6 levels were associated with worse myocardial function as measured using MWE. All other inflammatory markers tested were associated with no difference in MWE (**Figure 3**).

DISCUSSION

We report that subclinical cardiac dysfunction measured by GLS and MWE on STE is common in hospitalized COVID-19 patients with clinically indicated echocardiograms performed. To our knowledge, this is one of the first reports characterizing novel echocardiographic indices of myocardial dysfunction (GLS and the newer imaging parameter MWE) in hospitalized patients with COVID-19. We report several unique findings in our population: (1) Subclinical myocardial dysfunction is prevalent among COVID-19 patients even in the setting of normal LVEF, especially in those with traditional cardiovascular risk factors, (2) lower, more abnormal MWE, which is a sensitive measure of load independent myocardial dysfunction, is associated with greater in-hospital mortality, and (3) higher level of the inflammatory marker, IL-6, is predictive of lower MWE. Importantly, the finding of the association of MWE with mortality held true even after analyzing patients with normal LVEF, suggesting the prognostic benefit of MWE over LVEF and supporting use of MWE in addition to LVEF for hospitalized patients with COVID-19.

Speckle Tracking Echocardiography for the Detection of Subclinical Myocardial Dysfunction in COVID-19 Patients

Both GLS and MWE are sensitive measures of LV function and cardiac injury, and the current study is among the first to characterize these indices in the setting of acute COVID-19 (37, 38). Compared with LVEF, GLS improves risk stratification, enhances disease classification, and may guide the treatment approach in asymptomatic patients with subclinical LV dysfunction (14, 38). Both GLS and MWE measurements are validated, reproducible, and do not require additional imaging beyond standard TTE, reducing potential additional provider exposure during image acquisition. Prior studies have consistently demonstrated reduced GLS despite a preserved LVEF among patients at increased risk for cardiac injury and dysfunction (39). MWE is a newer load-independent measure that permits both global and regional ventricular mechanics to be analyzed through the relationship between myocardial contractility and LV pressure (15). A previous study showed that non-invasive indices of myocardial work are more sensitive than GLS for the detection of significant CAD in patients with normal regional wall motion and preserved LVEF (17). The present study supports these prior findings, as abnormal MWE was even more prevalent than abnormal GLS (79 vs. 46% of patients). Additionally, patients with abnormal STE indices were more likely to have cardiovascular risk factors than those with normal indices, even among those with normal LVEF.

Myocardial Work Efficiency and Mortality

While recent data has suggested high incidence of acute cardiac injury by troponin levels in COVID-19, investigations into the extent and implications of myocardial dysfunction on adverse outcomes such as death are limited (1, 7, 13, 40–42). In the present study, in a cohort with comparable in-hospital mortality to prior studies in COVID-19, we demonstrate the ability of MWE to predict mortality while GLS and LVEF did not. Prior studies suggest that the amount of myocardial work is related to uptake of fluro-deoxy-glucose at myocardial positron emission tomography scan, suggesting a relationship between myocardial work efficiency and metabolism (27). It is possible that impaired

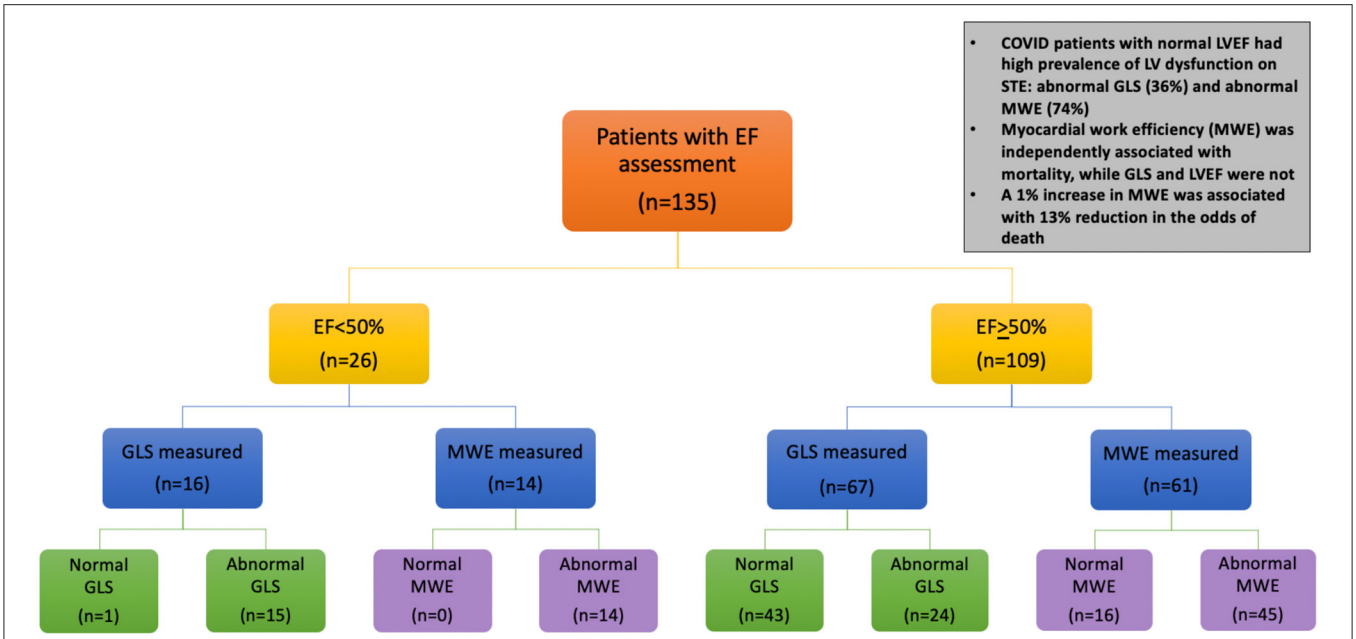


FIGURE 2 | Echocardiogram evaluation and main findings in hospitalized patients with COVID-19. Flow diagram of the study shows number of patients undergoing echocardiogram, including with speckle tracking technique for strain measures. GLS, global longitudinal strain; LVEF, left ventricular ejection fraction; MWE, myocardial work efficiency. Abnormal GLS is defined as ≤16% (the absolute value of −16%). Additional abbreviations in **Figure 1**.

TABLE 2 | Association of each echocardiographic parameter with mortality in hospitalized patients with COVID-19.

	Unadjusted	Model 1 (age and sex) odds ratio (95% CI)	Model 2 (age, sex, diabetes, hypertension) odds ratio (95% CI)
LVEF	1.00 (0.96–1.03) $P = 0.248$	1.00 (0.96–1.04) $P = 0.934$	1.00 (0.96–1.04) $P = 0.918$
GLS	1.07 (0.94–1.22) $P = 0.287$	1.08 (0.94–1.23) $P = 0.287$	1.15 (0.98–1.35) $P = 0.089$
MWE	0.92 (0.85–0.999) ***P* = 0.048**	0.90 (0.81–0.98) ***P* = 0.021**	0.87 (0.78–0.97) ***P* = 0.009**
TAPSE	0.43 (0.11–1.71) $P = 0.230$	0.41 (0.10–1.74) $P = 0.228$	0.30 (0.06–1.45) $P = 0.135$
RVSP	1.04 (1.00–1.09) $P = 0.051$	1.04 (1.00–1.09) $P = 0.073$	1.04 (1.0–1.09) $P = 0.081$
TR peak velocity	1.03 (0.99–1.07) $P = 0.182$	1.03 (0.98–1.07) $P = 0.219$	1.03 (0.98–1.07) $P = 0.235$
E/E'	0.97 (0.91–1.05) $P = 0.459$	0.96 (0.87–1.06) $P = 0.392$	0.97 (0.90–1.05) $P = 0.498$

The bold values represent significant p-values, with significant defined as <0.05.

MWE may be related to derangements in myocardial metabolism that can occur in the setting of increased systemic inflammation.

Based on these findings, it is possible that STE measures of subclinical LV dysfunction may provide incremental value to standard echo measures in patients with COVID-19. Given the acuity of presentation and cardiovascular complications of COVID-19, a better understanding of the extent of myocardial injury and dysfunction early in the disease course may help triage at risk patients and implement early interventions aimed at reducing mortality.

Systemic Inflammation and Cardiac Dysfunction

Although recent studies have aimed to describe pathophysiologic processes leading to RV strain and dilation in acute COVID-19 (43, 44), LV dysfunction and particularly subclinical dysfunction on STE, have not been as well-investigated. Studies suggest that increased systemic inflammation and impaired immune function may play a role (6, 7). Potential causes of myocardial dysfunction include myocarditis, ischemic injury (caused by microvascular dysfunction or epicardial CAD), stress cardiomyopathy or

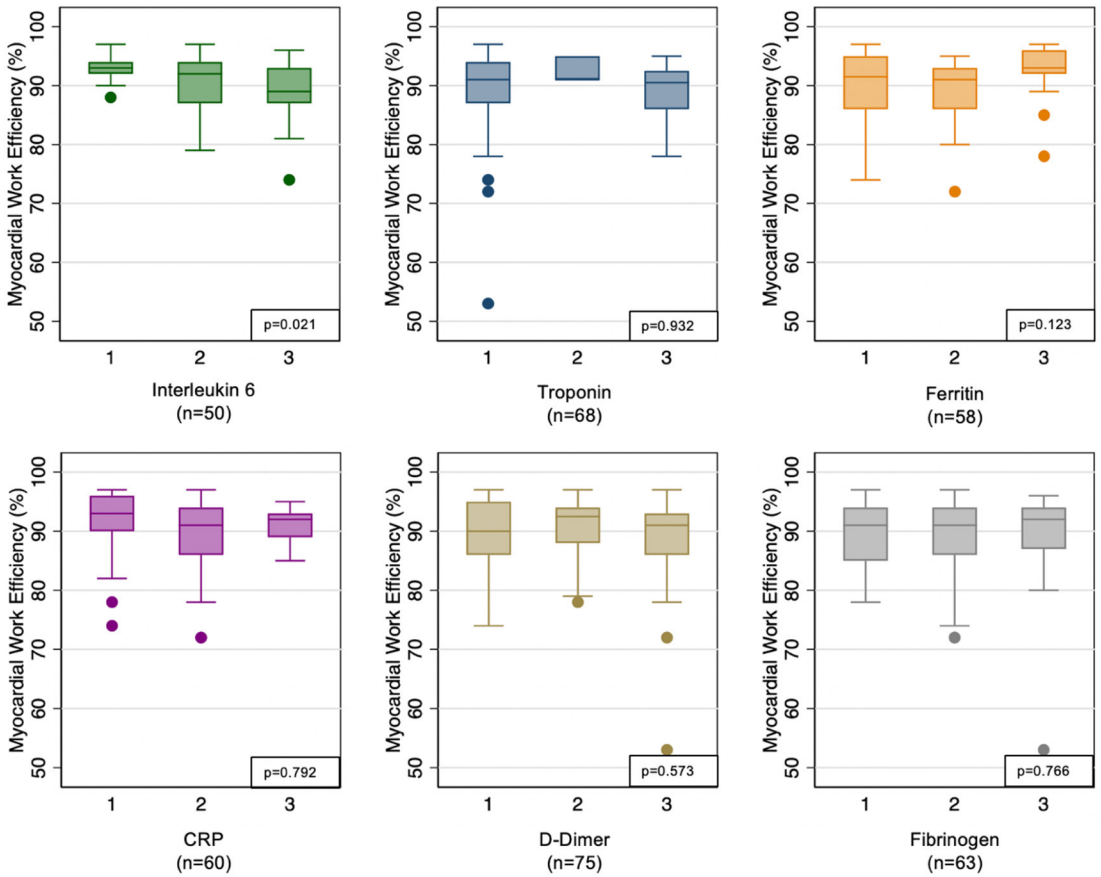


FIGURE 3 | Association of myocardial work efficiency with inflammatory markers. Inflammatory markers are analyzed by tertile of each marker given non-normal distribution.

cytokine release syndrome (45). Autopsy studies of severe COVID-19 disease suggest there can also be direct viral-induced injury of multiple organs, including the heart (46). However, the relative contribution and determinants of myocardial dysfunction have not been well-characterized, partially due to limited ability to obtain widespread cardiac testing in these patients. Given these limitations, the true prevalence of cardiac dysfunction has likely been underreported thus far, and is mainly limited to case reports (6, 7).

In our study, patients underwent echocardiography at a median 4 days after hospital admission and 6 days of symptom onset, suggesting that impaired GLS and MWE occur early in COVID-19 during the systemic inflammatory response, and cannot entirely be explained by a more chronic myocardial process such as fibrosis. In addition, COVID-19 patients with LV dysfunction on STE had more obesity, which is a pro-inflammatory state that initiates oxidative stress and adversely affects immune function, leading to cardiac injury (40, 47, 48). Finally, although inflammatory pathways have been implicated in myocardial injury related to COVID-19, their effect on important indices of cardiac function has not been well-characterized. In the present study, we show that subclinical myocardial dysfunction is related to the degree of systemic inflammation measured by IL-6. IL-6 has previously been shown to act as a key cytokine in producing downstream effects resulting in organ damage, including reduced myocardial contractility (49–51).

Additionally, IL-6 levels in the setting of COVID-19 have been reported to be elevated in several studies and have been shown to correlate with mortality (52–54). Our study, along with these prior studies, supports a potential role of IL-6 and heightened inflammation in mediating myocardial dysfunction, thereby increasing risk of death. Of note, we did not find a similar relationship with troponin and myocardial dysfunction, likely related to the primarily normal-range troponin values for the majority of patients.

By characterizing subclinical myocardial dysfunction using STE, the present study provides incremental knowledge, linking increased systemic inflammation (by IL-6 levels) to the pathophysiology of myocardial injury and dysfunction in COVID-19.

Limitations

The main limitation of this study is the relatively small sample size and retrospective cohort study design. Larger prospective studies are needed to further explore these novel echocardiographic parameters (GLS and MWE) with regard to cardiovascular mortality and other clinically meaningful outcomes in COVID-19 disease. Also, not all hospitalized

COVID-19 patients underwent echocardiogram and STE, which could result in selection bias and inability to detect true prevalence of abnormal GLS or MWE among COVID-19 patients. Lastly, a minority of patients with GLS and MWE performed did not have all inflammatory markers tested clinically, thus limiting the analyses.

CONCLUSIONS

In summary, sensitive indices of LV dysfunction, GLS and MWE, measured with STE are abnormal in a substantial portion of hospitalized COVID-19 patients who underwent echocardiograms, even in those with normal LVEF. Impaired MWE is independently associated with in-hospital mortality in COVID-19 patients. Higher IL-6 levels are associated with reduced MWE, providing a possible pathophysiologic link between increased inflammation and adverse outcomes in COVID-19. Based on these findings, it is possible that STE measures of subclinical LV dysfunction may provide incremental value to standard echocardiographic measures in patients with COVID-19. Given the acuity of presentation and cardiovascular complications of COVID-19, a better understanding of the extent of myocardial injury and dysfunction early in the disease course may help triage at risk patients and implement early interventions aimed at reducing mortality. Further longitudinal studies are needed to investigate persistence of impaired cardiac function in the setting of COVID-19.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Johns Hopkins IRB. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AM, NG, EG, and AH drafted the manuscript. BG, TM, GS, SP, and NB edited the manuscript. AM, NG, EG, and NB collected the data. AM analyzed the data and performed statistical analysis. All authors contributed to the article and approved the submitted version.

FUNDING

AM was supported by the National Heart, Lung, and Blood Institute training Grant T32HL007024. AH was supported by the National Heart, Lung, and Blood Institute Grant 1R01HL147660. This study received device and funding support from GE Healthcare. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.667721/full#supplementary-material

REFERENCES

1. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017
2. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* (2020) 5:802–10. doi: 10.1001/jamacardio.2020.0950
3. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell.* (2020) 181:271–80.e8. doi: 10.1016/j.cell.2020.02.052
4. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. *JAMA.* (2020) 323:2052–9. doi: 10.1001/jama.2020.6775
5. Gilotra NA, Minkove N, Bennett MK, Tedford RJ, Steenbergen C, Judge DP, et al. Lack of relationship between serum cardiac troponin I level and giant cell myocarditis diagnosis and outcomes. *J Cardiac Fail.* (2016) 22:583–5. doi: 10.1016/j.cardfail.2015.12.022
6. Hu H, Ma F, Wei X, Fang Y. Coronavirus fulminant myocarditis treated with glucocorticoid and human immunoglobulin. *Eur Heart J.* (2020) 4:260. doi: 10.1093/eurheartj/ehaa190
7. Inciardi RM, Lupi L, Zaccone G, Italia L, Raffo M, Tomasoni D, et al. Cardiac involvement in a patient with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:819–24. doi: 10.1001/jamacardio.2020.1096
8. Zeng F, Huang Y, Guo Y, Yin M, Chen X, Xiao L, et al. Association of inflammatory markers with the severity of COVID-19: A meta-analysis. *Int J Infect Dis.* (2020) 96:467–74. doi: 10.1016/j.ijid.2020.05.055
9. Rali AS, Ranka S, Shah Z, Sauer AJ. Mechanisms of myocardial injury in coronavirus disease 2019. *Card Fail Rev.* (2020) 6:e15. doi: 10.15420/cfr.2020.10
10. Chen C, Zhou Y, Wang DW. SARS-CoV-2: a potential novel etiology of fulminant myocarditis. *Herz.* (2020) 45:230–2. doi: 10.1007/s00059-020-04909-z
11. Cooper LT, Baughman KL, Feldman AM, Frustaci A, Jessup M, Kuhl U, et al. The role of endomyocardial biopsy in the management of cardiovascular disease. *J Am Coll Cardiol.* (2007) 50:1914–31. doi: 10.1016/j.jacc.2007.09.008
12. Kostakou PM, Kostopoulos VS, Tryfou ES, Giannaris VD, Rodis IE, Olympios CD, et al. Subclinical left ventricular dysfunction and correlation with regional strain analysis in myocarditis with normal ejection fraction. A new diagnostic criterion. *Int J Cardiol.* (2018) 259:116–21. doi: 10.1016/j.ijcard.2018.01.058
13. Han J, Mou Y, Yan D, Zhang Y-T, Jiang T-A, Zhang Y-Y, et al. Transient cardiac injury during H7N9 infection. *Eur J Clin Invest.* (2015) 45:117–25. doi: 10.1111/eci.12386
14. Awadalla M, Mahmood SS, Groarke JD, Hassan MZO, Nohria A, Rokicki A, et al. Global longitudinal strain and cardiac events in patients with immune checkpoint inhibitor-related myocarditis. *J Am College Cardiol.* (2020) 75:467–78. doi: 10.1016/j.jacc.2019.11.049

15. Sörensen J, Harms HJ, Aalen JM, Baron T, Smiseth OA, Flachskampf FA. Myocardial efficiency. *JACC: Cardiovasc Imag.* (2019) 13:1564–76. doi: 10.1016/j.jcmg.2019.08.030
16. Chan J, Edwards NFA, Khandheria BK, Shiino K, Sabapathy S, Anderson B, et al. A new approach to assess myocardial work by non-invasive left ventricular pressure–strain relations in hypertension and dilated cardiomyopathy. *Eur Heart J Cardiovasc Imaging.* (2019) 20:31–9. doi: 10.1093/ehjci/jey131
17. Edwards NFA, Scalia GM, Shiino K, Sabapathy S, Anderson B, Chamberlain R, et al. Global myocardial work is superior to global longitudinal strain to predict significant coronary artery disease in patients with normal left ventricular function and wall motion. *J Am Soc Echocardiogr.* (2019) 32:947–57. doi: 10.1016/j.echo.2019.02.014
18. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American society of echocardiography and the European association of cardiovascular imaging. *J Am Soc Echocardiogr.* (2015) 28:1–39.e14. doi: 10.1016/j.echo.2014.10.003
19. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF, Dokainish H, Edvardsen T, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American society of echocardiography and the European association of cardiovascular imaging. *J Am Soc Echocardiograp.* (2016) 29:277–314. doi: 10.1016/j.echo.2016.01.011
20. Kirkpatrick JN, Mitchell C, Taub C, Kort S, Hung J, Swaminathan M. ASE statement on protection of patients and echocardiography service providers during the 2019 novel coronavirus outbreak. *J Am College Cardiol.* (2020) 75:3078–84. doi: 10.1016/j.jacc.2020.04.002
21. Farsalinos KE, Daraban AM, Ünlü S, Thomas JD, Badano LP, Voigt J-U. Head-to-head comparison of global longitudinal strain measurements among nine different vendors. *J Am Soc Echocardiograp.* (2015) 28:1171–81.e2. doi: 10.1016/j.echo.2015.06.011
22. Haji K, Marwick TH. Clinical utility of echocardiographic strain and strain rate measurements. *Curr Cardiol Rep.* (2021) 23:18. doi: 10.1007/s11886-021-01444-z
23. D'Elia N, Caselli S, Kosmala W, Lancellotti P, Morris D, Muraru D, et al. Normal global longitudinal strain. *JACC: Cardiovasc Imaging.* (2020) 13:167–9. doi: 10.1016/j.jcmg.2019.07.020
24. Pieske B, Tschöpe C, de Boer RA, Fraser AG, Anker SD, Donal E, et al. How to diagnose heart failure with preserved ejection fraction: the HFA–PEFF diagnostic algorithm: a consensus recommendation from the heart failure association (HFA) of the European Society of Cardiology (ESC). *Eur Heart J.* (2019) 40:3297–317. doi: 10.1093/eurheartj/ehz641
25. Potter E, Marwick TH. Assessment of left ventricular function by echocardiography. *JACC: Cardiovascu Imaging.* (2018) 11:260–74. doi: 10.1016/j.jcmg.2017.11.017
26. Russell K, Eriksen M, Aaberge L, Wilhelmsen N, Skulstad H, Gjesdal O, et al. Assessment of wasted myocardial work: a novel method to quantify energy loss due to uncoordinated left ventricular contractions. *Am J Physiol Heart Circul Physiol.* (2013) 305:H996–1003. doi: 10.1152/ajpheart.00191.2013
27. Russell K, Eriksen M, Aaberge L, Wilhelmsen N, Skulstad H, Remme EW, et al. A novel clinical method for quantification of regional left ventricular pressure-strain loop area: a non-invasive index of myocardial work. *Eur Heart J.* (2012) 33:724–33. doi: 10.1093/eurheartj/ehs016
28. Hubert A, Le Rolle V, Leclercq C, Galli E, Samset E, Casset C, et al. Estimation of myocardial work from pressure-strain loops analysis: an experimental evaluation. *Eur Heart J Cardiovasc Imaging.* (2018) 19:1372–9. doi: 10.1093/ehjci/jey024
29. Manganaro R, Marchetta S, Dulgheru R, Ilardi F, Sugimoto T, Robinet S, et al. Echocardiographic reference ranges for normal non-invasive myocardial work indices: results from the EACVI NORRE study. *Eur Heart J Cardiovasc Imaging.* (2019) 20:582–90. doi: 10.1093/ehjci/jey188
30. El Mahdiui M, van der Bijl P, Abou R, Ajmone Marsan N, Delgado V, Bax JJ. Global left ventricular myocardial work efficiency in healthy individuals and patients with cardiovascular disease. *J Am Soc Echocardiograp.* (2019) 32:1120–7. doi: 10.1016/j.echo.2019.05.002
31. Bouali Y, Donal E, Gallard A, Laurin C, Hubert A, Bidaut A, et al. Prognostic usefulness of myocardial work in patients with heart failure and reduced ejection fraction treated by sacubitril/valsartan. *Am J Cardiol.* (2020) 125:1856–62. doi: 10.1016/j.amjcard.2020.03.031
32. Abou R, Leung M, Khidir MJH, Wolterbeek R, Schalij MJ, Ajmone Marsan N, et al. Influence of aging on level and layer-specific left ventricular longitudinal strain in subjects without structural heart disease. *Am J Cardiol.* (2017) 120:2065–72. doi: 10.1016/j.amjcard.2017.08.027
33. Liu J-H, Chen Y, Yuen M, Zhen Z, Chan CW-S, Lam KS-L, et al. Incremental prognostic value of global longitudinal strain in patients with type 2 diabetes mellitus. *Cardiovasc Diabetol.* (2016) 15:22. doi: 10.1186/s12933-016-0333-5
34. Wierzbowska-Drabik K, Trzos E, Kurpesa M, Rechciński T, Miśkowiec D, Cieślik-Guerra U, et al. Diabetes as an independent predictor of left ventricular longitudinal strain reduction at rest and during dobutamine stress test in patients with significant coronary artery disease. *Eur Heart J Cardiovasc Imaging.* (2018) 19:1276–86. doi: 10.1093/ehjci/jex315
35. Vrettos A, Dawson D, Grigoratos C, Nihoyannopoulos P. Correlation between global longitudinal peak systolic strain and coronary artery disease severity as assessed by the angiographically derived SYNTAX score. *Echo Res Pract.* (2016) 3:29–34. doi: 10.1530/ERP-16-0005
36. Liou K, Negishi K, Ho S, Russell EA, Cranney G, Ooi S-Y. Detection of obstructive coronary artery disease using peak systolic global longitudinal strain derived by two-dimensional speckle-tracking: a systematic review and meta-analysis. *J Am Soc Echocardiograp.* (2016) 29:724–35.e4. doi: 10.1016/j.echo.2016.03.002
37. Smiseth OA, Torp H, Opdahl A, Haugaa KH, Urheim S. Myocardial strain imaging: how useful is it in clinical decision making? *Eur Heart J.* (2016) 37:1196–207. doi: 10.1093/eurheartj/ehv529
38. Thavendiranathan P, Poulin F, Lim K-D, Plana JC, Woo A, Marwick TH. Use of myocardial strain imaging by echocardiography for the early detection of cardiotoxicity in patients during and after cancer chemotherapy. *J Am College Cardiol.* (2014) 63:2751–68. doi: 10.1016/j.jacc.2014.01.073
39. Stokke TM, Hasselberg NE, Smedsrud MK, Sarvari SI, Haugaa KH, Smiseth OA, et al. Geometry as a confounder when assessing ventricular systolic function. *J Am College Cardiol.* (2017) 70:942–54. doi: 10.1016/j.jacc.2017.06.046
40. Honce R, Schultz-Cherry S. Impact of obesity on influenza A virus pathogenesis, immune response, and evolution. *Front Immunol.* (2019) 10:1071. doi: 10.3389/fimmu.2019.01071
41. Estabragh ZR, Mamas MA. The cardiovascular manifestations of influenza: A systematic review. *Int J Cardiol.* (2013) 167:2397–403. doi: 10.1016/j.ijcard.2013.01.274
42. Cummings MJ, Baldwin MR, Abrams D, Jacobson SD, Meyer BJ, Balough EM, et al. Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York City: a prospective cohort study. *Lancet.* (2020) 395:1763–70. doi: 10.1016/S0140-6736(20)31189-2
43. Li Y, Li H, Zhu S, Xie Y, Wang B, He L, et al. Prognostic value of right ventricular longitudinal strain in patients with COVID-19. *JACC: Cardiovasc Imaging.* (2020) 13:2287–99. doi: 10.1016/j.jcmg.2020.04.014
44. Argulian E, Sud K, Vogel B, Bohra C, Garg VP, Talebi S, et al. Right ventricular dilation in hospitalized patients with COVID-19 infection. *JACC: Cardiovasc Imaging.* (2020) 13:2459–61. doi: 10.1016/j.jcmg.2020.05.010
45. Atri D, Siddiqi HK, Lang J, Nauffal V, Morrow DA, Bohula EA. COVID-19 for the cardiologist: a current review of the virology, clinical epidemiology, cardiac and other clinical manifestations and potential therapeutic strategies. *JACC: Basic Transl Sci.* (2020) 5:518–36. doi: 10.1016/j.jacbts.2020.04.002
46. Buja LM, Wolf DA, Zhao B, Akkanti B, McDonald M, Lelenwa L, et al. The emerging spectrum of cardiopulmonary pathology of the coronavirus disease 2019 (COVID-19): report of 3 autopsies from Houston, Texas, and review of autopsy findings from other United States cities. *Cardiovasc Pathol.* (2020) 48:107233. doi: 10.1016/j.carpath.2020.107233
47. The GBD 2015 Obesity Collaborators. Health effects of overweight and obesity in 195 countries over 25 years. *N Engl J Med.* (2017) 377:13–27. doi: 10.1056/NEJMoa1614362
48. Kass DA, Duggal P, Cingolani O. Obesity could shift severe COVID-19 disease to younger ages. *Lancet.* (2020) 395:1544–5. doi: 10.1016/S0140-6736(20)31024-2
49. Pathan N, Hemingway CA, Alizadeh AA, Stephens AC, Boldrick JC, Oragui EE, et al. Role of interleukin 6 in myocardial dysfunction of meningococcal septic shock. *Lancet.* (2004) 363:203–9. doi: 10.1016/S0140-6736(03)15326-3

50. Johnson DE, O'Keefe RA, Grandis JR. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. *Nat Rev Clin Oncol.* (2018) 15:234–8. doi: 10.1038/nrclinonc.2018.8
51. Liu B, Li M, Zhou Z, Guan X, Xiang Y. Can we use interleukin-6 (IL-6) blockade for coronavirus disease 2019 (COVID-19)-induced cytokine release syndrome (CRS)? *J Autoimmun.* (2020) 111:102452. doi: 10.1016/j.jaut.2020.102452
52. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
53. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* (2020) 395:507–13. doi: 10.1016/S0140-6736(20)30211-7
54. Gao Y, Li T, Han M, Li X, Wu D, Xu Y, et al. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. *J Med Virol.* (2020) 92:791–6. doi: 10.1002/jmv.25770

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

SYSTEMATIC REVIEW
published: 17 June 2021
doi: 10.3389/fcvm.2021.695206

COVID-19 and Cardiomyopathy: A Systematic Review

Fatemeh Omidi[1], Bahareh Hajikhani[2]*, Seyyedeh Neda Kazemi[3], Ardeshir Tajbakhsh[4], Sajedeh Riazi[5], Mehdi Mirsaeidi[6], Ali Ansari[7], Masoud Ghanbari Boroujeni[7], Farima Khalili[7], Sara Hadadi[6] and Mohammad Javad Nasiri[2]**

[1] Department of Cardiology, Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [2] Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [3] Preventative Gynecology Research Center, Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [4] Anesthesiology Research Center, Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [5] Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [6] Department of Pulmonary and Critical Care, University of Miami Miller School of Medicine, Miami, FL, United States, [7] School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

OPEN ACCESS

Edited by:
Reza Beigverdi, Tehran University of Medical Sciences, Iran

Reviewed by:
Roshan Dinparastisaleh, The Johns Hopkins Hospital, United States
Yu-Che Lee, University at Buffalo, United States

***Correspondence:**
Bahareh Hajikhani
b.hajikhani@gmail.com
Fatemeh Omidi
dr.fateme.omidi@gmail.com
Mohammad Javad Nasiri
mj.nasiri@hotmail.com

Specialty section:
This article was submitted to Cardiovascular Epidemiology and Prevention, a section of the journal Frontiers in Cardiovascular Medicine

Received: *14 April 2021*
Accepted: *21 May 2021*
Published: *17 June 2021*

Citation:
Omidi F, Hajikhani B, Kazemi SN, Tajbakhsh A, Riazi S, Mirsaeidi M, Ansari A, Ghanbari Boroujeni M, Khalili F, Hadadi S and Nasiri MJ (2021) COVID-19 and Cardiomyopathy: A Systematic Review. Front. Cardiovasc. Med. 8:695206. doi: 10.3389/fcvm.2021.695206

Background: Cardiomyopathies (CMPs) due to myocytes involvement are among the leading causes of sudden adolescent death and heart failure. During the COVID-19 pandemic, there are limited data available on cardiac complications in patients with COVID-19, leading to severe outcomes.

Methods: We conducted a systematic search in Pubmed/Medline, Web of Science, and Embase databases up to August 2020, for all relevant studies about COVID-19 and CMPs.

Results: A total of 29 articles with a total number of 1460 patients were included. Hypertension, diabetes, obesity, hyperlipidemia, and ischemic heart disease were the most reported comorbidities among patients with COVID-19 and cardiomyopathy. In the laboratory findings, 21.47% of patients had increased levels of troponin. Raised D-dimer levels were also reported in all of the patients. Echocardiographic results revealed mild, moderate, and severe Left Ventricular (LV) dysfunction present in 17.13, 11.87, and 10% of patients, respectively.

Conclusions: Cardiac injury and CMPs were common conditions in patients with COVID-19. Therefore, it is suggested that cardiac damage be considered in managing patients with COVID-19.

Keywords: COVID-19, cardiomyopathy, cardiac injury and regeneration, systematic review, SARS-CoV-2

INTRODUCTION

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was first reported on 31 December 2019 from Wuhan, China, resulted in an unprecedented outbreak of Coronavirus disease 2019 (COVID-19). The most common manifestation of COVID-19 is pulmonary complications. However, this novel disease's presentations have a broad spectrum of signs and symptoms from asymptomatic infection or mild flu-like symptoms to multiorgan failure resulting in death (1, 2). Cardiovascular disease (CVD) has been reported in patients infected with COVID-19 (3). Based on the literature, 20–30% of hospitalized patients showed cardiovascular manifestations associated with worse outcomes (4, 5). Cardiovascular complications of COVID-19

are thought to be a combination of direct viral injury and the host's immune response resulting in vascular inflammation, plaque instability, and myocardial inflammation (6–9). Cardiomyopathies (CMPs) which resulted from heart muscle involvement, are among the main causes of adolescent sudden death and heart failure (10). SARS-CoV-2 infection in patients suffering from CMPs represents an actual risk of exacerbating patient clinical status (11).

Although many authors have reported various aspects of respiratory-related symptoms of COVID-19, the increasing prevalence of cardiac complications in COVID-19 patients should be taken into considerations (12–17). Thus, this study was aimed to systematically review the current published literature to evaluate clinical and paraclinical characteristics of CMPs in patients infected with SARS-CoV-2.

METHODS

Search Strategy

In the following bibliographic databases, we carried out a comprehensive systematic search of literature: PubMed/Medline, Embase, and Web of Science. We searched for any relevant articles published in English up to August 2020. The search included keywords including COVID-19, severe acute respiratory syndrome coronavirus 2, SARS-CoV-2, in combinations with cardiomyopathy, or CMP, cardiomyopathies, myocardiopathy, cardiac injury, or myocarditis.

Additionally, all references of selected papers were searched manually for additional related articles. The present systematic review conforms to the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) statement (18).

Study Selection

Studies reported any data about CMPs in patients with confirmed COVID-19 were included. Abstracts, commentary, letter to editor, guidelines, and review articles were excluded.

All retrieved publications were screened for eligibility in two phases. First, two reviewers independently screened the titles and abstracts of potentially relevant articles identified in the primary search. Subsequently, a review of the full texts of all remaining articles was done by the same authors. Any discrepancy in the article selection or technical uncertainties were discussed and resolved between review authors.

Data Extraction

The following variables were extracted from all included studies: first author, year of publication, type of study, country where the research was conducted, study population, COVID-19 diagnosis technique, laboratory findings, treatment protocols, and type of CMPs. Two authors independently extracted the data from the selected studies. The data was jointly reconciled, and disagreements were discussed and resolved between review authors.

RESULTS

As shown in **Figure 1**, a total of 186 studies were identified from databases. After removing 45 duplicates, 141 non-duplicate studies remained for further assessments. After applying the inclusion/exclusion criteria, 29 articles (22 case reports and 7 case series) were included with a total number of 1460 unique cases of COVID-19 with a mean age of 58 years. The characteristics of the included studies are described in **Table 1**.

Table 2 shows the outcomes and prognosis of CMPs in patients with COVID-19. 98 out of 1,212 evaluated patients developed cardiogenic shock (8.08%). Six studies reported mortality rates, showing 48 out of 192 (25%) of patients deceased.

As presented in **Table 3**, hypertension, diabetes, obesity, hyperlipidemia, ischemic disease, and obstructive sleep apnea were the most reported comorbidities among them.

As shown in **Table 3**, cough and fever were reported as the most prevalent symptoms in 14 out of 29 studies. Dyspnea was reported in 9 studies. According to these studies, 90% of the evaluated patients had this complication. Evaluation of laboratory findings showed elevated troponin levels in 18 studies with 308 out of 1,412 patients (21.47%). Increased D-dimer levels were reported in 5 case reports, of which six patients showed this elevated marker.

CMPs evidence in patients with COVID-19 indicates in **Table 4**. Common ECG findings were: tachycardia, premature beats, ST-segment elevation, blocks, and inverted T wave. Inverted T waves were seen in EKG findings of 9 studies (91.66% of evaluated patients). Left ventricular (LV) involvement is a hallmark of primary CMPs. Echocardiographic findings revealed mild (17.1%), moderate (11.85%), and severe (9.98%) LV dysfunction, which was discussed in 6, 4, and 11 studies, respectively. Aneurysm formation, a sign of stress-induced cardiomyopathy followed COVID-19, was found in all 11 evaluated patients (100%). Regional wall motion abnormalities (RWMA) as another sign were found in 46/1,217 (1.15%) patients. Right ventricular (RV) involvement and high pulmonary artery pressure (PAP) are signs of the destruction of the right heart. RV enlargement was presented in 14.88% of tested patients (181/1,216). RV dysfunction was also found in 26.01% (315/1,211) of patients' echocardiograms. Findings of Chest X-Ray (CXR) and Chest CT scan showed ground-glass opacification (GGO) patterns (26 of 34 patients) and consolidation (7 of 7 patients) as the most common findings (**Table 4**). Among the type of CMPs, COVID CMPs, and hypertrophic cardiomyopathy were among the most reported type in 39.13 and 18.75% cases, respectively.

In terms of treatment, 10 out of 14 patients (71.42%) reported in 11 studies received β-Blocker as part of their treatment regimen. The use of Diuretic agents was reported in 7 studies which included 7 out of 9 (77.77%) patients (**Table 5**).

DISCUSSION

COVID-19 has resulted in other organ involvement, and CMPs are among the most significant complications of this rapidly

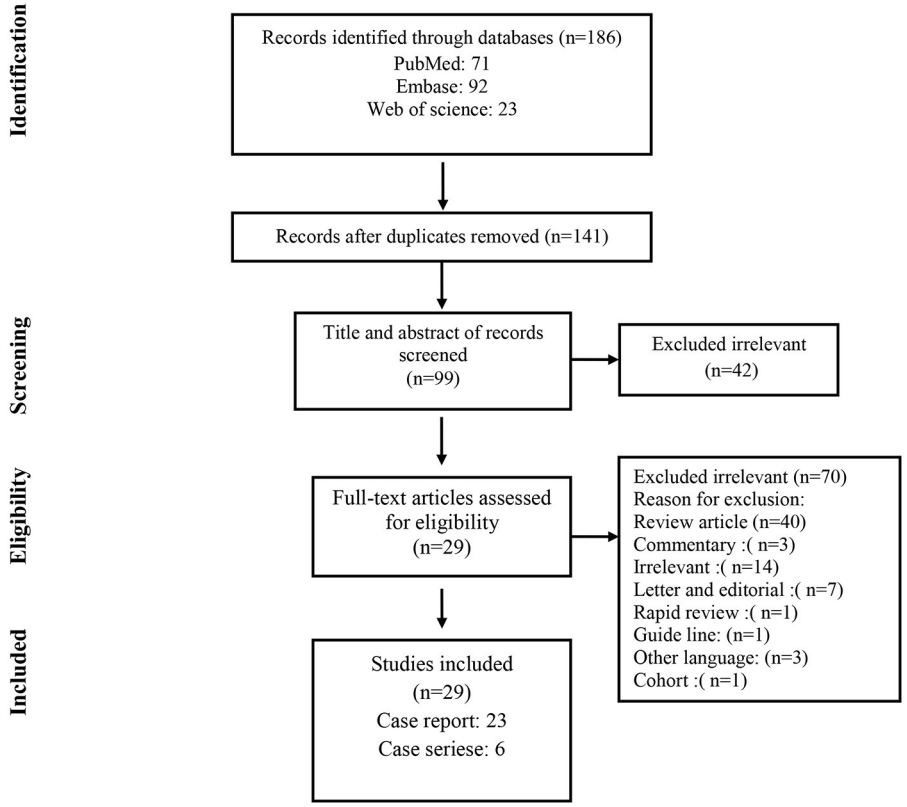


FIGURE 1 | Flow chart of study selection for inclusion in the systematic review.

emerging disease, causing more severe disease and increased mortality rates (48, 49). In this systematic review, we studied the cardiac injuries in patients with SARS-CoV-2 infection that resulted in CMPs. Echocardiographic results showed a range of mild to severe left ventricular dysfunction in 10% to 17.13% of the studied patients.

The patients' recovery and death rates were assessed in 20 studies that showed that 28.7% of patients with one type of CMPs died following SARS-CoV-2 infection. Patients with cardiovascular comorbidities had a higher risk of developing cardiac injury (50).

In a study on twenty-one critically ill patients admitted in intensive care units (ICU), one-third developed CMPs (51). Yang et al. showed 52 critically ill COVID-19 patients 12 (23%) presented with cardiac injury (52).

The results of a cohort study showed that 23% of patients experienced new heart failure or exacerbation of chronic heart failure, of which 28 survived, and 16 died (50).

Based on the included studies that examined patients' mortality rate with CMPs and COVID-19, 25% of these patients were deceased. As a result, it can be inferred that cardiac injury is a significant predisposing factor for increasing the mortality rate of COVID-19.

Huang et al. demonstrated a "Cytokine storm" model that results in a pro-inflammatory markers surge that may lead to myocardial injury (53). Similar effects have been observed with MERS-CoV and SARS-CoV infections previously (54). Furthermore, the virus may be involved in a primary myocardial injury by entering the myocytes through the ACE-2 receptor (55).

Overall, SARS-CoV-2 can cause cardiac complications through the following pathways: (1) Indirect cardiac injury due to increased release of cytokines and inflammatory pathways. (2) Direct invasion of the SARS-COV-2 in cardiac myocytes. (3) Respiratory damage can cause hypoxia, myocardial supply-demand mismatch, followed by oxidative stress and damage to cardiomyocytes (56, 57).

There are different manifestations of cardiac involvement in COVID-19, including acute myocardial infarction, acute heart failure, cardiogenic shock, myocarditis, and fatal arrhythmias (58). Myocardial injury is a common condition in COVID-19 hospitalized, which is characterized by increased troponin levels (59). Another definition of cardiac injury is reported as abnormality in cardiac biomarkers, electrocardiography, or echocardiography relative to the patient's previous condition. In a cohort study of 416 patients, 19.7% of hospitalized patients had a cardiac injury (34).

Cardiomyopathy was defined as evidence of new left ventricular systolic dysfunction on trans-thoracic echocardiography with one of the following criteria: 1. Clinical signs of cardiogenic shock, 2. Increase in creatine kinase or troponin level, and 3. Reduction in oxygen saturation of the central vein below 70% (43).

TABLE 1 | Characteristics of the included studies.

References	Country	Type of study	No. of patients	Male/female	Mean age
Doyen et al. (19)	Italy	Case report	1	1 M	69
Paul et al. (20)	France	Case report	1	1 M	35
Huyut (21)	Turkey	Case report	1	1 F	59
Pasqualetto et al. (22)	Italy	Case series	3	2 M-1 F	83.33
Deng et al. (23)	China	Case series	14	10 M-4 F	74
Taza et al. (24)	USA	Case report	1	1 M	52
Roca et al. (25)	Italy	Case report	1	1 F	87
Minhas et al. (26)	USA	Case report	1	1 F	52
Juusela et al. (27)	USA	Case series	2	2 F	35.5
Meyer et al. (28)	Switzerland	Case report	1	1 F	83
Khalid et al. (29)	Italy	Case report	1	1 F	76
Nguyen et al. (30)	Belgium	Case report	1	1 F	71
Bonnet et al. (31)	France	Case report	1	1 M	27
Zhang et al. (32)	Multicenter	Case series	2	1 M-1 F	59
Dabbagh et al. (33)	USA	Case report	1	1 F	67
Guo et al. (34)	China	Case series	187	91 M-96 F	58.5
Tavazzi et al. (35)	Italy	Case report	1	1 M	69
Hua et al. (36)	UK	Case report	1	1 M	47
Villanueva et al. (37)	USA	Case report	1	1 M	68
Kir et al. (38)	USA	Case report	1	1 M	49
Dweck et al. (39)	Multicenter	Case series	1209	844 M-365 F	62
Irabien-Ortiz et al. (40)	Spain	Case report	1	1 M	59
Craver et al. (41)	USA	Case report	1	1 M	17
Bobeck et al. (42)	USA	case report	1	1 M	80
Arentz et al. (43)	USA	Case series	21	10 M-11 F	70
Yildirim and Karaagac (44)	Turkey	Case report	1	1 F	7
Chadha (45)	USA	Case report	1	1 F	85
Kim et al. (46)	Korea	Case report	1	1 F	21
Luetkens et al. (47)	Germany	Case report	1	1 M	79

TABLE 2 | The outcomes and prognosis of CMPs.

Outcomes	No of study	*n/N*	Percentage (%)
Deceased	6	48/192	25
Cured	13	14/16	87.5
Prognosis			
Cardiogenic shock	4	98/1,212	8.08
MOD[a]	3	81/191	42.40
ARDS[b]	8	67/215	31.16

n, number of patients with any variables; N, the total number of studied patients.
[a]MOD,Multi organ disease; [b]ARDS, Adult respiratory distress syndrome.

Our results showed that ARDS was present in 31.45% of patients following COVID-19 and cardiomyopathy. The cardiogenic shock occurred in 8% of patients. Reported data from Germany and the United States (56, 60) showed that cardiogenic shock is a significant complication of COVID-19. According to the evaluated studies in our systematic review, ∼8% of patients developed heart failure/cardiogenic shock as a manifestation of COVID-19.

We showed that common symptoms of COVID-19 in patients with cardiac injury include fever, cough, headache, and fatigue. These findings are broadly consistent with other studies examining clinical signs in patients with COVID-19 (13, 14).

Our review of published studies showed the most common abnormal laboratory findings in patients with cardiomyopathy were increased IL-6 level, elevated ferritin, and High D-dimer. Some studies were reported that Serum concentrations of IL-6 were higher in severe cases of COVID-19 compared with moderate cases. Moreover, in deceased patients, levels of this cytokine were substantially higher than in recovered ones. So, continuous measurement of IL-6 level for early prediction of severity of infection has been suggested (61, 62).

The elevated level of fibrin degradation products, especially D-dimers ($>2590/ng{\cdot}mL^{-1}$), was shown to be an indicator of pulmonary embolism in hospitalized COVID-19 patients. It also contributed to poor prognosis and high mortality in patients with a more severe form of COVID-19 (63).

Our systematic review showed that 21.7% of patients presented with high troponin levels that were investigated in 14 studies. Gue et al. indicated the importance of monitoring

TABLE 3 | Clinical and laboratories findings in patients with COVID-19.

	Variable	No of study	*n/N*	%
Clinical manifestations	Chest pain	9	128/1,232	10.38
	Dyspnea	9	9/10	90
	Shortness of breath	11	39/45	88.66
	Cough	14	38/49	77.55
	Fever	14	40/51	78.43
	Fatigue	5	5/5	5
	Tachypnea	7	8/21	30.09
	Crackles	7	7/8	87.5
	Diarrhea	3	3/3	100
	Nausea & vomiting	4	5/6	83.33
Signs	Elevated pulse rate	12	195/1,413	13.8
	Elevated temperature	12	32/33	96.96
Comorbidities	Hypertension	15	526/1,424	36.93
	Diabetes	10	279/1,440	19.37
	Obesity	3	11/17	64.7
	Hyperlipidemia	5	5/5	100
	Ischemic disease	4	200–1,431	13.97
	Obstructive sleep apnea	2	10/35	25.57
	COPD[a]	3	12/222	5.4
	CKD[b]	3	17/209	8.13
	CA[c]	3	15/189	7.93
Laboratory findings	elevated NTproBNP	11	26/214	12.2
	High IL-6	5	5/5	100
	High D-dimer	5	6/6	100
	High ferritin	6	6/6	100
	High CRP[d]	14	14/201	6.96
	High Troponin	18	307/1412	21.74

n, number of patients with any variables; N, the total number of studied patients.
[a]COPD, Chronic obstructive pulmonary disease; [b]CKD, Chronic kidney disease; [c]CA, Copd/Asthma; [d]CRP, C-reactive protein.

TABLE 4 | Cardiomyopathy evidence in patients with COVID-19.

	Variable	No of study	*n/N*	%
EKG	Sinus tachycardia	7	8/8	100
	Bradycardia	2	2/2	100
	Premature beats	2	4/4	100
	ST elevation	5	5/5	100
	ST depression	2	2/2	100
	Blocks	2	4/4	100
	Inverted T wave	9	11/12	91.66
	VT[a]	2	49/1,403	3.49
Echocardiography	LVE (LV[b] enlargement)	3	68/1,219	5.57
	Mild LV dysfunction	6	208/1,216	17.10
	Moderate LV dysfunction	4	144/1,215	11.85
	Severe LV dysfunction	11	122/1,222	9.98
	RVE (RV[c] enlargement)	1	181/1,216	14.88
	RV dysfunction	3	315/1,211	26.01
	High PAP[d]	1	99/1,216	8.14
	Aneurysm formation	10	11/11	100
	RWMA[e]	10	46/1,217	1.15
	Pericardial effusion	3	3/3	100
	LVH[f]	4	4/4	100
	Pericardial effusion	3	3/3	100
	Endocarditis	1	14/1,216	1.15
	Tamponade	3	13/1,218	1.06
	Echo MI[g]	2	37/1,230	3
	Echo Myocarditis	1	35/1,216	2.87
	D shap LV	1	49/1,216	4.02
CXR	Diffuse involvement	6	7/7	100
	Cardiomegaly	2	2/3	66.66
CT scan	Ground-glass opacities	11	26/34	76.47
	Consolidation	5	7/7	100
Angiogram	Abnormal angiogram	5	6/7	85.71
	Normal angiogram	1	1/1	100
Type of cardiomyopathy	DCM[h]	3	71/1,225	5.79
	HCM[i]	3	3/16	18.75
	Myocarditis	8	55/1,229	4.47
	Myocardial injury	13	303/1,408	2.3
	Takotsubo	14	32/1,222	2.61
	Ischemic after COVID	1	36/1,216	2.96
	COVID cardiomyopathy	3	9/23	39.13

n, number of patients with any variables; N, the total number of studied patients.
[a]VT, Ventricular tachycardia; [b]LV, Left ventricular; [c]RV, Right ventricular; [d]PAP, Pulmonary artery pressure; [e]RWMA, Regional wall motion abnormalities; [f]LVH, Left ventricular hypertrophy; [g]M, myocardial infarction; [h]DCM, Dilated cardiomyopathy; [i]HCM, Hyper trophic cardiomyopathy.

troponin levels to predict the likelihood of cardiovascular events. Patients with high troponin levels had higher levels of other cardiac biomarkers and more fatal arrhythmias (34).

The results of our analysis revealed that hypertension, obesity, and hyperlipidemia were the most common comorbidities among patients with COVID-19. The association between hypertension and inflammation is well-known; inflammatory responses increase the disease's severity and complications in patients (64, 65). In a systematic review study, hypertension was the most common underlying condition in CMPs following COVID-19, reported in 33% of patients (66). Moreover, the presence of hyperinflammatory conditions in the airways interferes with the virus's clearance (67). It is inferred that the potential synergistic effect of inflammation due to hypertension and COVID-19 can aggravate this effect on the heart and result in CMPs.

Studies have shown that obesity is a risk factor for developing ARDS in COVID-19 (68). Moreover, hyperlipidemia has been more prevalent among hospitalized and more severe cases of COVID-19 compared to non-hospitalized ones (69, 70).

One of the common diagnostic modalities for COVID-19 is CT-scan. Bilateral and peripheral predominant ground-glass

TABLE 5 | Treatment agents used in the included studies.

		Variable	No of study	*n/N*	%
Non-pharmacologic treatment		O2 nasal	8	10/11	90.9
		Intubation	14	72/223	32.28
		Pericardiocentesis	3	3/3	100
Pharmacologic treatment	Antimicrobial agents	Antibacterial drugs	6	188/193	97.4
		Azithromycin	6	6/7	85.71
		Antiviral drugs	4	171/192	89.06
	Immunomodulators	Hydroxychloroquine	9	10/12	83.33
		IVIG[a]	3	23/189	12.16
		steroid	8	113/194	58.24
		Tocilizumab	4	4/5	80
	Anticoagulant	Fondaparinux	3	4/5	80
		Anti-platelet	3	4/5	80
		Heparin/LMWH[b]	6	6/7	85.71
	Others	ACE/ARB[c]	4	4/4	100
		β-Blocker	10	10/14	71.42
		NEP[d]	5	5/6	83.33
		Diuretic	7	7/9	77.77
		Vasopressor	5	5/5	100

[a]IVIG, Intravenous immune globulin; [b]LMWH, Low molecular weight heparin; [c]ACE/ARB, angiotensin converting enzyme inhibitors/angiotensin-receptor blockers; [d]NEP, Norepinephrine.

opacity, multifocal patchy consolidation, and interstitial changes with the peripheral distribution are among these features (71).

According to the included articles in our study, 76.47% of evaluated patients demonstrated Ground-glass opacities in their chest CT scan examination.

Different pharmacological and non-pharmacological treatments have been studied and applied for COVID-19. The included studies showed that nasal oxygen and intubation were among the most common non-pharmacological treatments for patients. Hydroxychloroquine, azithromycin, antiviral drugs, and β-Blockers were the most common pharmacological treatments. Due to the wide range of disease symptoms and complications, further studies related to each organ involvement are required to manage the disease better and prevent the complications.

In the end, it is necessary to point out the limitations of the present study. Since only case reports and case series studies have been selected for this review, this increases the potential risk of bias. Another issue is the small number of patients enrolled in the study. Due to the scarcity of randomized controlled trial (RCT)/quasi-randomized studies, we could not include them in the present study. We have not adopted the publications as abstracts or letters as data presented in this format is not high quality. Further investigations are required to include a broader range of studies, including clinical trials in patients with COVID-19 and CMPs.

In conclusion, cardiac injury and CMPs, including exacerbation of an underlying CMPs or the emergence of new CMPs, are common in COVID-19 patients. Moreover, they are associated with higher mortality and morbidity in these patients. Common fatal conditions in patients with COVID-19 CMPs include multiorgan damage, ARDS, and cardiogenic shock. Therefore, diagnostic measures of COVID-19 should consist of underlying cardiovascular comorbidities. History, signs, and symptoms of cardiac injury should be considered in evaluating these patients early in the course of this novel disease, and prompt therapeutic measures for the prevention of exacerbating cardiac condition should be sought.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

FO, MN, and BH: designed the study. FO, SK, AT, SR, AA, SH, MG, and FK: performed the search, study selection, and data synthesis. BH, FO, and MN: wrote the first draft of the manuscript. MN, BH, and MM: revised the article. All authors contributed to the paper and approved the submitted version.

REFERENCES

1. Dadashi M, Hajikhani B, Yaslianifard S, Goudarzi M, Owlia P, Nasiri MJ, et al. COVID-19 and Skin manifestations: An overview of case reports/case series and meta-analysis of the prevalence studies. *Front Med.* (2020) 7:573188. doi: 10.3389/fmed.2020.573188
2. Hajikhani B, Calcagno T, Nasiri MJ, Jamshidi P, Dadashi M, Goudarzi M, et al. Olfactory and gustatory dysfunction in COVID-19 patients: A meta-analysis study. *Physiol Rep.* (2020) 8:e14578. doi: 10.14814/phy2.14578
3. Inciardi RM, Lupi L, Zaccone G, Italia L, Raffo M, Tomasoni D, et al. Cardiac involvement in a patient with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:819–24. doi: 10.1001/jamacardio.2020.1096

4. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* (2020) 5:802–10. doi: 10.1001/jamacardio.2020.0950
5. Cizgici AY, Agus HZ, Yildiz M. COVID-19 myopericarditis: it should be kept in mind in today's conditions. *Am J Emerg Med.* (2020) 38:1547.e5-1547.e6. doi: 10.1016/j.ajem.2020.04.080
6. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med.* (2020) 46:846–8. doi: 10.1007/s00134-020-05991-x
7. Pérez-Bermejo JA, Kang S, Rockwood SJ, Simoneau CR, Joy DA, Ramadoss GN, et al. SARS-CoV-2 infection of human iPSC-derived cardiac cells predicts novel cytopathic features in hearts of COVID-19 patients. *BioRxiv [Preprint].* (2020). doi: 10.1101/2020.08.25.265561
8. Siripanthong B, Nazarian S, Muser D, Deo R, Santangeli P, Khanji MY, et al. Recognizing COVID-19–related myocarditis: The possible pathophysiology and proposed guideline for diagnosis and management. *Heart Rhythm.* (2020) 17:1463–71. doi: 10.1016/j.hrthm.2020.05.001
9. Shafi AM, Shaikh SA, Shirke MM, Iddawela S, Harky A. Cardiac manifestations in COVID-19 patients—A systematic review. *J Cardiac Surg.* (2020) 35:1988–2008. doi: 10.1111/jocs.14808
10. Limongelli G, Crotti L. COVID-19 pandemia and inherited cardiomyopathies and channelopathies: a short term and long term perspective. *Orphanet J Rare Dis.* (2020) 15:1–7. doi: 10.1186/s13023-020-01444-2
11. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of coronaviruses on the cardiovascular system: a review. *JAMA Cardiol.* (2020) 5:831–40. doi: 10.1001/jamacardio.2020.1286
12. Nasiri MJ, Haddadi S, Tahvildari A, Farsi Y, Arbabi M, Hasanzadeh S, et al. COVID-19 clinical characteristics, and sex-specific risk of mortality: systematic review and meta-analysis. *Front Med.* (2020) 7:459. doi: 10.3389/fmed.2020.00459
13. Tahvildari A, Arbabi M, Farsi Y, Jamshidi P, Hasanzadeh S, Calcagno TM, et al. Clinical features, diagnosis, and treatment of COVID-19 in hospitalized patients: a systematic review of case reports and case series. *Front Med.* (2020) 7:231. doi: 10.3389/fmed.2020.00231
14. Vetter P, Vu DL, L'Huillier AG, Schibler M, Kaiser L, Jacquerioz F. Clinical features of covid-19. *Br Med J Publishing Group.* (2020) 369:m1470. doi: 10.1136/bmj.m1470
15. Marini JJ, Gattinoni L. Management of COVID-19 respiratory distress. *JAMA.* (2020) 323:2329–30. doi: 10.1001/jama.2020.6825
16. Ziehr DR, Alladina J, Petri CR, Maley JH, Moskowitz A, Medoff BD, et al. Respiratory pathophysiology of mechanically ventilated patients with COVID-19: a cohort study. *Am J Respirat Crit Care Med.* (2020) 201:1560–4. doi: 10.1164/rccm.202004-1163LE
17. Gattinoni L, Chiumello D, Caironi P, Busana M, Romitti F, Brazzi L, et al. COVID-19 pneumonia: different respiratory treatments for different phenotypes?. *Intensive Care Med.* (2020) 46:1099–102. doi: 10.1007/s00134-020-06033-2
18. Knobloch K, Yoon U, Vogt PM. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement and publication bias. *J Cranio-Maxillofacial Surg.* (2011) 39:91–2. doi: 10.1016/j.jcms.2010.11.001
19. Doyen D, Moceri P, Ducreux D, Dellamonica J. Myocarditis in a patient with COVID-19: a cause of raised troponin and ECG changes. *Lancet.* (2020) 395:1516. doi: 10.1016/S0140-6736(20)30912-0
20. Paul J-F, Charles P, Richaud C, Caussin C, Diakov C. Myocarditis revealing COVID-19 infection in a young patient. *Eur Heart J Cardiovasc Imaging.* (2020) 21:776. doi: 10.1093/ehjci/jeaa107
21. Huyut MA. Novel coronavirus pneumonia and cardiomyopathy: a case report. *Arq Bras Cardiol.* (2020) 114:843–5. doi: 10.36660/abc.20200268
22. Pasqualetto MC, Secco E, Nizzetto M, Scevola M, Altafini L, Cester A, et al. Stress cardiomyopathy in COVID-19 Disease. *Eur J Case Rep Internal Med.* (2020) 7:001718. doi: 10.12890/2020_001718
23. Deng Q, Hu B, Zhang Y, Wang H, Zhou X, Hu W, et al. Suspected myocardial injury in patients with COVID-19: Evidence from front-line clinical observation in Wuhan, China. *Int J Cardiol.* (2020) 311:116–21. doi: 10.1016/j.ijcard.2020.03.087
24. Taza F, Zulty M, Kanwal A, Grove D. Takotsubo cardiomyopathy triggered by SARS-CoV-2 infection in a critically ill patient. *BMJ Case Rep CP.* (2020) 13:e236561. doi: 10.1136/bcr-2020-236561
25. Roca E, Lombardi C, Campana M, Vivaldi O, Bigni B, Bertozzi B, et al. Takotsubo syndrome associated with COVID-19. *Eur J Case Rep Internal Med.* (2020) 7:1665. doi: 10.12890/2020_001665
26. Minhas AS, Scheel P, Garibaldi B, Liu G, Horton M, Jennings M, et al. Takotsubo syndrome in the setting of COVID-19 infection. *JACC: Case Rep.* (2020) 2:1321–5. doi: 10.1016/j.jaccas.2020.04.023
27. Juusela A, Nazir M, Gimovsky M. Two cases of COVID-19 related cardiomyopathy in pregnancy. *Am J Obstetri Gynecol MFM.* (2020) 2:100113. doi: 10.1016/j.ajogmf.2020.100113
28. Meyer P, Degrauwe S, Van Delden C, Ghadri J-R, Templin C. Typical takotsubo syndrome triggered by SARS-CoV-2 infection. *Euro Heart J.* (2020) 41:1860. doi: 10.1093/eurheartj/ehaa306
29. Khalid Y, Dasu N, Dasu K. A case of novel coronavirus (COVID-19)-induced viral myocarditis mimicking a Takotsubo cardiomyopathy. *HeartRhythm Case Rep.* (2020) 6:473. doi: 10.1016/j.hrcr.2020.05.020
30. Nguyen D, Nguyen T, De Bels D, Rodriguez JC. A case of Takotsubo cardiomyopathy with COVID 19. *Euro Heart J Cardiovasc Imaging.* (2020) 21:1052. doi: 10.1093/ehjci/jeaa152
31. Bonnet M, Craighero F, Harbaoui B. Acute myocarditis with ventricular non compaction in a COVID-19 patient. *Jacc Heart Failure.* (2020) 8:599–600. doi: 10.1016/j.jchf.2020.05.004
32. Zhang L, Wang B, Zhou J, Kirkpatrick J, Xie M, Johri AM. Bedside focused cardiac ultrasound in COVID-19 from the Wuhan epicenter: the role of cardiac point-of-care ultrasound, limited transthoracic echocardiography, and critical care echocardiography. *J Am Soc Echocardiography.* (2020) 33:676–82. doi: 10.1016/j.echo.2020.04.004
33. Dabbagh MF, Aurora L, D'Souza P, Weinmann AJ, Bhargava P, Basir MB. Cardiac tamponade secondary to COVID-19. *JACC: Case Rep.* (2020) 2:1326–30. doi: 10.1016/j.jaccas.2020.04.009
34. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017
35. Tavazzi G, Pellegrini C, Maurelli M, Belliato M, Sciutti F, Bottazzi A, et al. Myocardial localization of coronavirus in COVID-19 cardiogenic shock. *Eur J Heart Failure.* (2020) 22:911–5. doi: 10.1002/ejhf.1828
36. Hua A, O'Gallagher K, Sado D, Byrne J. Life-threatening cardiac tamponade complicating myo-pericarditis in COVID-19. *Eur Heart J.* (2020) 41:2130. doi: 10.1093/eurheartj/ehaa253
37. Villanueva D-DH, Lusby HP, Islam SP, Gupte AA, Beatty NL. Heart failure exacerbation as only presenting sign of COVID-19. *IDCases.* (2020) 21:e00870. doi: 10.1016/j.idcr.2020.e00870
38. Kir D, Mohan C, Sancassani R. Heart brake: an unusual cardiac manifestation of COVID-19. *JACC: Case Rep.* (2020) 2:1252–5. doi: 10.1016/j.jaccas.2020.04.026
39. Dweck MR, Bularga A, Hahn RT, Bing R, Lee KK, Chapman AR, et al. Global evaluation of echocardiography in patients with COVID-19. *Eur Heart J Cardiovasc Imaging.* (2020) 21:949–58. doi: 10.1093/ehjci/jeaa178
40. Irabien-Ortiz Á, Carreras-Mora J, Sionis A, Pàmies J, Montiel J, Tauron M. Fulminant myocarditis due to COVID-19. *Rev Española Cardiol.* (2020) 73:503–4. doi: 10.1016/j.rec.2020.04.005
41. Craver R, Huber S, Sandomirsky M, McKenna D, Schieffelin J, Finger L. Fatal eosinophilic myocarditis in a healthy 17-year-old male with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2c). *Fetal Pediatr Pathol.* (2020) 39:26–8. doi: 10.1080/15513815.2020.1761491
42. Bobeck KA, Holtzclaw AW, Brown TE, Clark PA. Effective use of angiotensin ii in coronavirus disease 19–associated mixed shock state: a case report. *A&a Practice.* (2020) 14:e01221. doi: 10.1213/XAA.0000000000001221
43. Arentz M, Yim E, Klaff L, Lokhandwala S, Riedo FX, Chong M, et al. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington State. *JAMA.* (2020) 323:1612–4. doi: 10.1001/jama.2020.4326
44. Yildirim AI, Karaagac AT. COVID-19 in a Young Girl with Restrictive Cardiomyopathy and Chronic Lung Disease. *Indian Pediatri.* (2020) 57:577. doi: 10.1007/s13312-020-1863-1
45. Chadha S. 'COVID-19 Pandemic'Anxiety induced Tako-tsubo Cardiomyopathy. *QJM.* (2020) 113:488–90. doi: 10.1093/qjmed/hcaa135

46. Kim I-C, Kim JY, Kim HA, Han S. COVID-19-related myocarditis in a 21-year-old female patient. *Eur Heart J.* (2020) 41:1859. doi: 10.1093/eurheartj/ehaa288
47. Luetkens JA, Isaak A, Zimmer S, Nattermann J, Sprinkart AM, Boesecke C, et al. Diffuse myocardial inflammation in COVID-19 associated myocarditis detected by multiparametric cardiac magnetic resonance imaging. *Circulation: Cardiovasc Imaging.* (2020) 13:e010897. doi: 10.1161/CIRCIMAGING.120.010897
48. Bonow RO, Fonarow GC, O'Gara PT, Yancy CW. Association of coronavirus disease 2019 (COVID-19) with myocardial injury and mortality. *JAMA Cardiol.* (2020) 5:751–3. doi: 10.1001/jamacardio.2020.1105
49. Zheng Y-Y, Ma Y-T, Zhang J-Y, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol.* (2020) 17:259–60. doi: 10.1038/s41569-020-0360-5
50. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3
51. Xu X-W, Wu X-X, Jiang X-G, Xu K-J, Ying L-J, Ma C-L, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. *Bmj.* (2020) 368:m606. doi: 10.1136/bmj.m606
52. Yang X, Yu Y, Xu J, Shu H, Liu H, Wu Y, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respirat Med.* (2020) 8:475–81. doi: 10.1016/S2213-2600(20)30079-5
53. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
54. Mahallawi WH, Khabour OF, Zhang Q, Makhdoum HM, Suliman BA. MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile. *Cytokine.* (2018) 104:8–13. doi: 10.1016/j.cyto.2018.01.025
55. Lang JP, Wang X, Moura FA, Siddiqi HK, Morrow DA, Bohula EA. A current review of COVID-19 for the cardiovascular specialist. *Am Heart J.* (2020) 226:29–44. doi: 10.1016/j.ahj.2020.04.025
56. Bemtgen X, Krüger K, Supady A, Duerschmied D, Schibilsky D, Bamberg F, et al. First successful treatment of coronavirus disease 2019 induced refractory cardiogenic plus vasoplegic shock by combination of percutaneous ventricular assist device and extracorporeal membrane oxygenation: a case report. *Asaio J.* (2020) 66:607–9. doi: 10.1097/MAT.0000000000001178
57. Tan W, Aboulhosn J. The cardiovascular burden of coronavirus disease 2019 (COVID-19) with a focus on congenital heart disease. *Int J Cardiol.* (2020) 309:70–7. doi: 10.1016/j.ijcard.2020.03.063
58. Fried JA, Ramasubbu K, Bhatt R, Topkara VK, Clerkin KJ, Horn E, et al. The variety of cardiovascular presentations of COVID-19. *Circulation.* (2020) 141:1930–6. doi: 10.1161/CIRCULATIONAHA.120.047164
59. Driggin E, Madhavan MV, Bikdeli B, Chuich T, Laracy J, Biondi-Zoccai G, et al. Cardiovascular considerations for patients, health care workers, and health systems during the COVID-19 pandemic. *J Am College Cardiol.* (2020) 75:2352–71. doi: 10.1016/j.jacc.2020.03.031
60. Harari R, Bangalore S, Chang E, Shah B. COVID-19 complicated by acute myocardial infarction with extensive thrombus burden and cardiogenic shock. *Catheterization Cardiovasc Interven.* (2020) 97:E661–6. doi: 10.1002/ccd.28992
61. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunologic features in severe and moderate forms of coronavirus disease. *J Clin Invest.* (2019) 130:2620–9. doi: 10.1172/JCI137244
62. Gao Y, Li T, Han M, Li X, Wu D, Xu Y, et al. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. *J Med Virol.* (2020) 92:791–6. doi: 10.1002/jmv.25770
63. Mouhat B, Besutti M, Bouiller K, Grillet F, Monnin C, Ecarnot F, et al. Elevated D-dimers and lack of anticoagulation predict PE in severe COVID-19 patients. *Eur Respirat J.* (2020) 56:2001811. doi: 10.1183/13993003.01811-2020
64. Jayedi A, Rahimi K, Bautista LE, Nazarzadeh M, Zargar MS, Shab-Bidar S. Inflammation markers and risk of developing hypertension: a meta-analysis of cohort studies. *Heart.* (2019) 105:686–92. doi: 10.1136/heartjnl-2018-314216
65. McMaster WG, Kirabo A, Madhur MS, Harrison DG. Inflammation, immunity, and hypertensive end-organ damage. *Circulat Res.* (2015) 116:1022–33. doi: 10.1161/CIRCRESAHA.116.303697
66. Sawalha K, Abozenah M, Kadado AJ, Battisha A, Al-Akchar M, Salerno C, et al. Systematic review of COVID-19 related myocarditis: Insights on management and outcome. *Cardiovasc Revasculariz Med.* (2020) 23:107–13. doi: 10.1016/j.carrev.2020.08.028
67. Trump S, Lukassen S, Anker MS, Chua RL, Liebig J, Thürmann L, et al. Hypertension delays viral clearance and exacerbates airway hyperinflammation in patients with COVID-19. *Nat Biotechnol.* (2020). doi: 10.1038/s41587-020-00796-1. [Epub ahead of print].
68. Gong MN, Bajwa EK, Thompson BT, Christiani DC. Body mass index is associated with the development of acute respiratory distress syndrome. *Thorax.* (2010) 65:44–50. doi: 10.1136/thx.2009.117572
69. Iqbal Z, Ho JH, Adam S, France M, Syed A, Neely D, et al. Managing hyperlipidaemia in patients with COVID-19 and during its pandemic: An expert panel position statement from HEART UK. *Atherosclerosis.* (2020) 313:126–36. doi: 10.1016/j.atherosclerosis.2020.09.008
70. Wei X, Zeng W, Su J, Wan H, Yu X, Cao X, et al. Hypolipidemia is associated with the severity of COVID-19. *J Clin Lipidol.* (2020) 14:297–304. doi: 10.1016/j.jacl.2020.04.008
71. Nouri E, Delashoub O, Shahabi-Rabori MA, Afzalipour R, Jafari S. Interpretation of CT scan findings during the COVID-19 pandemic. *Hormozgan Med J.* (2020) 24:107909. doi: 10.5812/hmj.107909

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

SYSTEMATIC REVIEW
published: 24 June 2021
doi: 10.3389/fcvm.2021.690790

B-Type Natriuretic Peptide Concentrations, COVID-19 Severity, and Mortality: A Systematic Review and Meta-Analysis With Meta-Regression

Angelo Zinellu[1], *Salvatore Sotgia*[1], *Ciriaco Carru*[1,2] *and Arduino A. Mangoni*[3,4]*

[1] Department of Biomedical Sciences, University of Sassari, Sassari, Italy, [2] Quality Control Unit, University Hospital of Sassari, Sassari, Italy, [3] Discipline of Clinical Pharmacology, College of Medicine and Public Health, Flinders University, Adelaide, SA, Australia, [4] Department of Clinical Pharmacology, Flinders Medical Centre, Southern Adelaide Local Health Network, Adelaide, SA, Australia

Alterations in cardiac biomarkers have been reported in patients with coronavirus disease 2019 (COVID-19) in relation to disease severity and mortality. We conducted a systematic review and meta-analysis with meta-regression of studies reporting B-type natriuretic peptide (BNP) or N-terminal proBNP (NT-proBNP) plasma concentrations in COVID-19. We searched PubMed, Web of Science, and Scopus, between January 2020 and 2021, for studies reporting BNP/NT-proBNP concentrations, measures of COVID-19 severity, and survival status (PROSPERO registration number: CRD42021239190). Forty-four studies in 18,856 COVID-19 patients were included in the meta-analysis and meta-regression. In pooled results, BNP/NT-proBNP concentrations were significantly higher in patients with high severity or non-survivor status when compared to patients with low severity or survivor status during follow up (SMD = 1.07, 95% CI: 0.89–1.24, and $p < 0.001$). We observed extreme between-study heterogeneity ($I^2 = 93.9\%$, $p < 0.001$). In sensitivity analysis, the magnitude and the direction of the effect size were not substantially modified after sequentially removing individual studies and re-assessing the pooled estimates, (effect size range, 0.99 – 1.10). No publication bias was observed with the Begg's ($p = 0.26$) and Egger's ($p = 0.40$) t-tests. In meta-regression analysis, the SMD was significantly and positively associated with D-dimer ($t = 2.22$, $p = 0.03$), myoglobin ($t = 2.40$, $p = 0.04$), LDH ($t = 2.38$, $p = 0.02$), and procalcitonin ($t = 2.56$, $p = 0.01$) concentrations. Therefore, higher BNP/NT-proBNP plasma concentrations were significantly associated with severe disease and mortality in COVID-19 patients.

Keywords: B-type natriuretic peptide, COVID-19, disease severity, mortality, biomarkers

OPEN ACCESS

Edited by:
Shuyang Zhang,
Peking Union Medical College Hospital, China

Reviewed by:
John David Horowitz,
University of Adelaide, Australia
Carmine Morisco,
University of Naples Federico II, Italy

***Correspondence:**
Arduino A. Mangoni
arduino.mangoni@flinders.edu.au

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *04 April 2021*
Accepted: *03 June 2021*
Published: *24 June 2021*

Citation:
Zinellu A, Sotgia S, Carru C and Mangoni AA (2021) B-Type Natriuretic Peptide Concentrations, COVID-19 Severity, and Mortality: A Systematic Review and Meta-Analysis With Meta-Regression. Front. Cardiovasc. Med. 8:690790. doi: 10.3389/fcvm.2021.690790

INTRODUCTION

A significant number of clinical and demographic factors have been studied in patients with coronavirus disease 19 (COVID-19) in regard to their association with specific clinical presentations and measures of clinical severity (1, 2). The evidence of an excessive activation of inflammatory and immunomodulating pathways in patients with the more severe forms of

the disease, typically characterized by the development of respiratory failure with or without multi-organ dysfunction, have prompted the search for specific biomarkers of inflammation and immuno-activation in order to develop better predictive models to assist with management (3). The increasing evidence of significant alterations of different organs and/or systems in patients with COVID-19 has also led to the investigation of the predictive capacity of additional, organ-specific, biomarkers. For example, the presence of myocardial injury, associated with several cardiac manifestations, including myocarditis, acute coronary syndrome, and arrhythmias, has been well-documented in COVID-19 patients with or without pre-existing cardiovascular history (4). Notably, cardiac abnormalities in this group are independently associated with an increased risk of mortality (5). While the exact mechanisms involved in the onset and progression of COVID-19 related myocardial injury remain to be elucidated, several circulating markers of myocardial damage, particularly creatine kinase (CK), and troponin, are being increasingly studied in terms of their predictive capacity (6). Another cardiac complication, heart failure, has been observed in about a quarter of patients with COVID-19 and has been associated with an increased risk of adverse outcomes (7, 8). The active peptide B-type natriuretic peptide (BNP) and the inactive peptide N-terminal proBNP (NT-proBNP) are both derived from the human BNP precursor proBNP in the ventricular myocytes. The increased secretion of BNP and NT-proBNP from the heart, in response to high ventricular filling pressures, is routinely used as a diagnostic and prognostic marker in heart failure and, by some, as a marker of the size or severity of ischaemic insults (9–11). However, its biological and clinical role in patients with COVID-19 is not well-established. We addressed this issue by conducting a systematic review and meta-analysis with meta-regression of studies reporting plasma BNP or NT-proBNP concentrations in COVID-19 patients with different disease severity, based on clinical guidelines or need for hospitalization, mechanical ventilation, or transfer to the intensive care unit (ICU), and clinical outcomes, particularly survival status during follow up.

MATERIALS AND METHODS

Search Strategy, Eligibility Criteria, and Study Selection

We conducted a systematic literature search, using the terms "brain natriuretic peptide" or "BNP" or "NT-proBNP" or "N-terminal pro-brain natriuretic peptide" and "coronavirus disease 19" or "COVID-19," in PubMed, Web of Science and Scopus, from January 2020 to January 2021, to identify peer-reviewed studies reporting BNP/NT-proBNP concentrations in COVID-19 patients according to disease severity and/or mortality. We accessed the references of the retrieved articles to identify additional studies. Eligibility criteria were (a) reporting continuous data on plasma BNP/NT-proBNP concentrations in COVID-19 patients, (b) investigating COVID-19 patients with different disease severity or survival status during follow up, (c) adult patients, (d) English language, (e) >10 patients, and (f) full-text available. Two investigators independently screened individual abstracts. If relevant, they independently reviewed the full articles (PROSPERO registration number: CRD42021239190). We used the Newcastle-Ottawa scale to assess study quality, with a score ≥6 indicating high quality (12).

Statistical Analysis

We calculated standardized mean differences (SMD) and 95% confidence intervals (CIs) in BNP/NT-proBNP concentrations between COVID-19 patients with low vs. high severity or survivor vs. non-survivor status. A $p < 0.05$ was considered statistically significant. When studies reported medians and interquartile ranges (IQR) the corresponding means and standard deviations were estimated (13). We assessed between-study heterogeneity in SMD values using the Q-statistic (significance level at $p < 0.10$). Inconsistency across studies

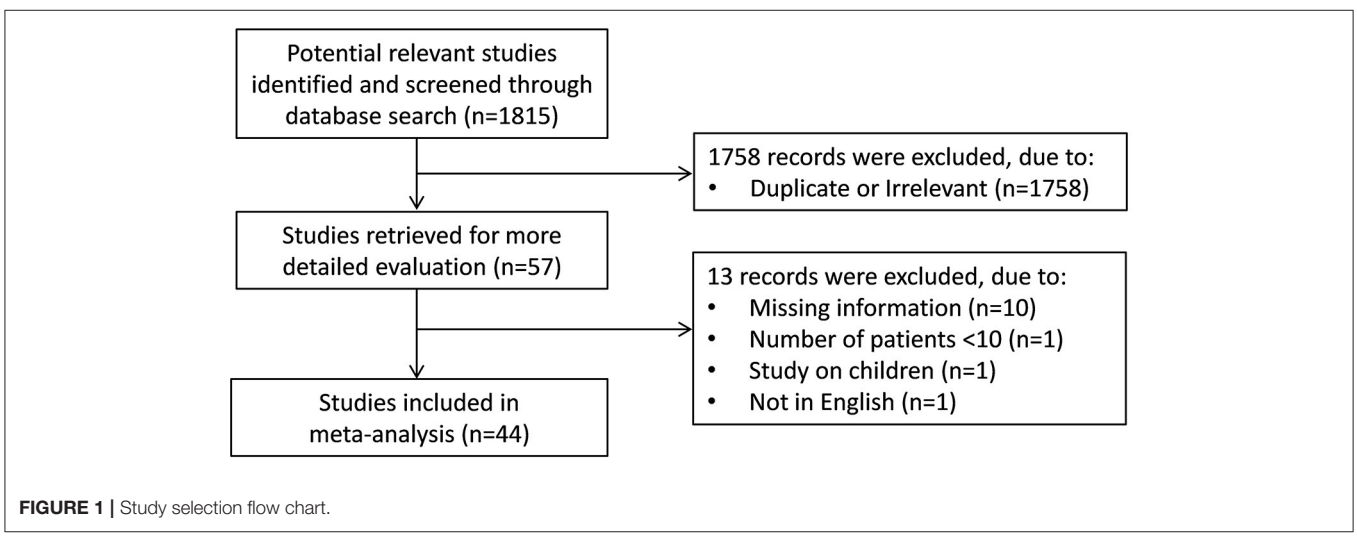


FIGURE 1 | Study selection flow chart.

TABLE 1 | Characteristics of the selected studies.

References	Country	Study design	Endpoint	NOS (stars)	Low severity or survivor				High severity or non-survivor			
					n	Age (Years)	Gender (M/F)	BNP pg/mL (Mean ± SD)	*n*	Age (Years)	Gender (M/F)	BNP pg/mL (Mean ± SD)
Abdeladim et al. (21)	Morocco	R	Disease severity	6	39	50	11/28	81 ± 50*	34	61	12/22	2,982 ± 3,172*
Aladag et al. (22)	Turkey	R	Survival status	7	35	68	22/13	3,318 ± 5,054*	15	68	6/9	15,511 ± 13,638*
Almeida Junior et al. (23)	Brazil	R	Survival status or MV	7	139	64	86/53	73 ± 90	44	76	34/10	287 ± 485
Bao et al. (24)	China	P	Disease severity	5	129	NR	NR	11 ± 25	49	NR	NR	53 ± 85
Belarte-Tornero et al. (25)	Spain	NR	Survival status	7	82	77	45/37	518 ± 528*	47	86	18/29	5,192 ± 6,673*
Chen et al. (26)	China	NR	Survival status	8	1,651	57	781/870	67 ± 93	208	70	153/55	685 ± 987
Chen et al. (7)	China	R	Survival status	6	161	51	88/73	92 ± 122*	113	68	83/30	1,002 ± 1,058*
Chen et al. (27)	China	R	Survival status	8	53	64	27/26	336 ± 298*	20	69	15/5	840 ± 898*
Ciceri et al. (28)	Italy	NR	Survival status	8	291	62	207/84	206 ± 259*	95	76	70/25	1,583 ± 2,176*
Cui et al. (29)	China	R	Survival status	8	699	61	353/346	153 ± 158*	137	70	86/51	1,244 ± 1,649*
D'Alto et al. (30)	Italy	P	Survival status	8	69	62	53/16	686 ± 1,224*	25	68	17/8	3,375 ± 3,891*
Deng et al. (31)	China	R	Survival status	8	212	63	97/115	227 ± 293*	52	75	33/19	1,248 ± 1,478*
Du et al. (32)	China	R	Transfer to ICU	6	58	73	31/27	852 ± 620*	51	68	40/11	564 ± 654*
Feng et al. (33)	China	R	Disease severity	6	352	51	190/162	41 ± 64	124	60	81/43	65 ± 67
Ferrari et al. (34)	Italy	R	Survival status	6	40	60	27/13	690 ± 1, 075*	42	74	30/12	6,296 ± 17,528*
Gan et al. (35)	China	R	Survival status	8	56	62	30/26	1,653 ± 289	39	70	28/11	1,848 ± 784
Gavin et al. (36)	USA	R	Survival status	6	118	57	58/60	160 ± 51	18	73	11/7	587 ± 184
Gottlieb et al. (37)	USA	R	Hospitalization	8	7,190	38	3,935/3,255	33 ± 35	1,483	58	792/691	73 ± 74
Guo et al. (38)	China	R	Survival status	8	28	59	NR	1,741 ± 2,363*	46	72	NR	3,544 ± 7,998*
Han et al. (39)	China	R	Disease severity	6	198	59	127/71	145 ± 169*	75	59	26/49	624 ± 1,027*
He et al. (40)	China	NR	Disease severity	6	32	42	15/17	42 ± 66*	21	57	13/8	822 ± 1,100*
He et al. (41)	China	R	Disease severity	8	530	60	241/289	83 ± 95*	501	66	297/204	381 ± 498*
Hui et al. (42)	China	R	Survival status	8	65	55	42/23	176 ± 178*	47	66	29/18	1,631 ± 2,453*
Koc et al. (43)	Turkey	R	Disease severity	6	60	65	37/23	53 ± 35*	30	61	20/10	267 ± 339*
Li et al. (44)	China	R	Transfer to ICU	8	312	49	131/181	100 ± 129*	211	62	119/92	103 ± 132*
Li et al. (45)	China	R	Survival status	6	60	62	33/27	327 ± 455	14	71	11/3	854 ± 849
Liu et al. (46)	China	P	Survival status	8	21	64	15/6	1,859 ± 2,599*	22	65	7/15	7,530 ± 8,820*
Lorente et al. (47)	Soain	P	Survival status	7	118	64	53/65	538 ± 789*	25	71	7/18	3,370 ± 4,218*
Ma et al. (48)	China	R	Disease severity	6	429	42	230/199	180 ± 273	94	50	59/35	663 ± 641
Myhre et al. (49)	Norway	P	Survival status or transfer to ICU	8	88	58	46/42	126 ± 170*	35	64	25/10	186 ± 181*
Pan et al. (50)	China	R	Survival status	8	35	65	18/17	63 ± 73	89	69	67/22	107 ± 113
Qin et al. (51)	China	R	Survival status	8	239	63	113/126	93 ± 102	23	69	10/13	499 ± 468
Rath et al. (52)	Germany	P	Survival status	7	107	67	65/42	808 ± 1,320*	16	73	12/4	3,376 ± 5,410*

(Continued)

TABLE 1 | Continued

References	Country	Study design	Endpoint	NOS (stars)	Low severity or survivor				High severity or non-survivor			
					n	Age (Years)	Gender (M/F)	BNP pg/mL (Mean ± SD)	*n*	Age (Years)	Gender (M/F)	BNP pg/mL (Mean ± SD)
Sun et al. (53)	China	R	Survival status	8	123	67	51/72	2 ± 2*	121	72	82/39	13 ± 16*
Sun et al. (54)	China	P	Disease severity	7	49	52	26/23	9 ± 6	50	71	34/16	163 ± 232
Tao et al. (55)	China	R	Disease severity	7	202	54	72/130	198 ± 352*	20	65	8/12	811 ± 1,367*
Vrillon et al. (56)	France	P	Survival status	8	54	90	19/35	184 ± 242	22	90	15/7	367 ± 371
Wang et al. (57)	China	R	Disease severity	6	72	NR	24/48	48 ± 46	38	NR	24/14	206 ± 228
Xie et al. (58)	China	R	Disease severity	7	38	61	26/12	29 ± 29	24	72	12/12	97 ± 106
Yang et al. (59)	China	R	Disease severity	6	99	44	49/50	1,705 ± 2,326*	15	60	7/8	243 ± 165*
Yu et al. (60)	China	R	Survival status	8	123	80	46/77	299 ± 287*	18	84	11/7	2 ± 349,941*
Zhang et al. (61)	China	R	Survival status	6	62	60	35/27	310 ± 441*	36	71	23/13	3,200 ± 5,144*
Zhao et al. (62)	China	R	Disease severity	8	19	49	7/12	97 ± 115	31	60	23/8	703 ± 641
Zheng et al. (63)	China	R	Disease severity	6	32	44	NR	67 ± 91*	67	64	NR	1,086 ± 3,217*

*ICU, intensive care unit; MV, mechanical ventilation; NOS, Newcastle-Ottawa quality assessment scale for case-control studies; NR, not reported; P, prospective; R, retrospective; *, NT-proBNP.*

was evaluated using the I^2 statistic, where $I^2 < 25\%$ indicated no heterogeneity, between 25 and 50% moderate heterogeneity, between 50 and 75% large heterogeneity, and >75% extreme heterogeneity (14, 15). Random-effect models were used to calculate the pooled SMD and 95% CIs if significant heterogeneity was present. In sensitivity analyses, the influence of individual studies on the overall effect size was assessed using the leave-one-out method (16). The presence of publication bias was assessed using the Begg's and the Egger's test, at the $p < 0.05$ level of significance (17, 18), and the Duval and Tweedie "trim and fill" procedure (19). To identify factors contributing to the between-study variance, we investigated the effects of several biologically and/or clinically plausible factors on the SMD by univariate meta-regression analysis. These factors included age, gender, clinical endpoint, study design (retrospective or prospective), geographical area where the study was conducted, aspartate aminotransferase (AST), alanine aminotransferase (ALT), D-dimer, serum creatinine, myoglobin, troponin, CK, albumin, ferritin, lactate dehydrogenase (LDH), procalcitonin, C-reactive protein (CRP), white blood cell count (WBC), diabetes, hypertension and cardiovascular disease. Statistical analyses were performed using Stata 14 (STATA Corp., College Station, TX, USA). The study was fully compliant with the PRISMA statement (20).

RESULTS

Literature Search and Study Selection

We initially identified 1,815 studies. A total of 1,758 studies were excluded after the first screening because they were duplicates or irrelevant. Following full-text revision of the remaining 57 articles, 13 were further excluded because they did not meet the inclusion criteria. Thus, 44 studies in 18,856 COVID-19 patients, 14,569 (53% males, mean age 48 years) with low severity or survivor status and 4,287 (59% males, mean age 61 years) with high severity or non-survivor status, were included in the final analysis (**Figure 1** and **Table 1**) (7, 21–63). Thirty-two studies were conducted in Asia (7, 22, 24, 26, 27, 29, 31–33, 35, 38–46, 48, 50, 51, 53–55, 57–63), eight in Europe (25, 28, 30, 34, 47, 49, 52, 56), three in America (23, 36, 37), and one in Africa (21). Thirty-two studies were retrospective (7, 21–23, 27, 29, 31–39, 41–45, 48, 50, 51, 53, 55, 57–63), eight prospective (24, 30, 46, 47, 49, 52, 54, 56), whereas the remaining four did not report the study design (25, 26, 28, 40). Clinical endpoints included disease severity based on current clinical guidelines in 15 studies (21, 24, 33, 39–41, 43, 48, 54, 55, 57–59, 62, 63), hospitalization in one (37), ICU transfer in three (32, 44, 49), or need for mechanical ventilation in one (23), and survival status in 24 studies (7, 22, 25–31, 34–36, 38, 42, 45–47, 50–53, 56, 60, 61). Sixteen studies reported plasma BNP concentrations (23, 24, 26, 33, 35–37, 45, 48, 50, 51, 54, 56–58, 62), whereas the remaining 28 reported plasma NT-proBNP concentrations (7, 21, 22, 25, 27–32, 34, 38–44, 46, 47, 49, 52, 53, 55, 59–61, 63). Only one study reported cumulative 7-day mean plasma NT-proBNP concentrations (34), whereas another reported BNP concentrations on initial presentation to the emergency department (37). The remaining 42 studies

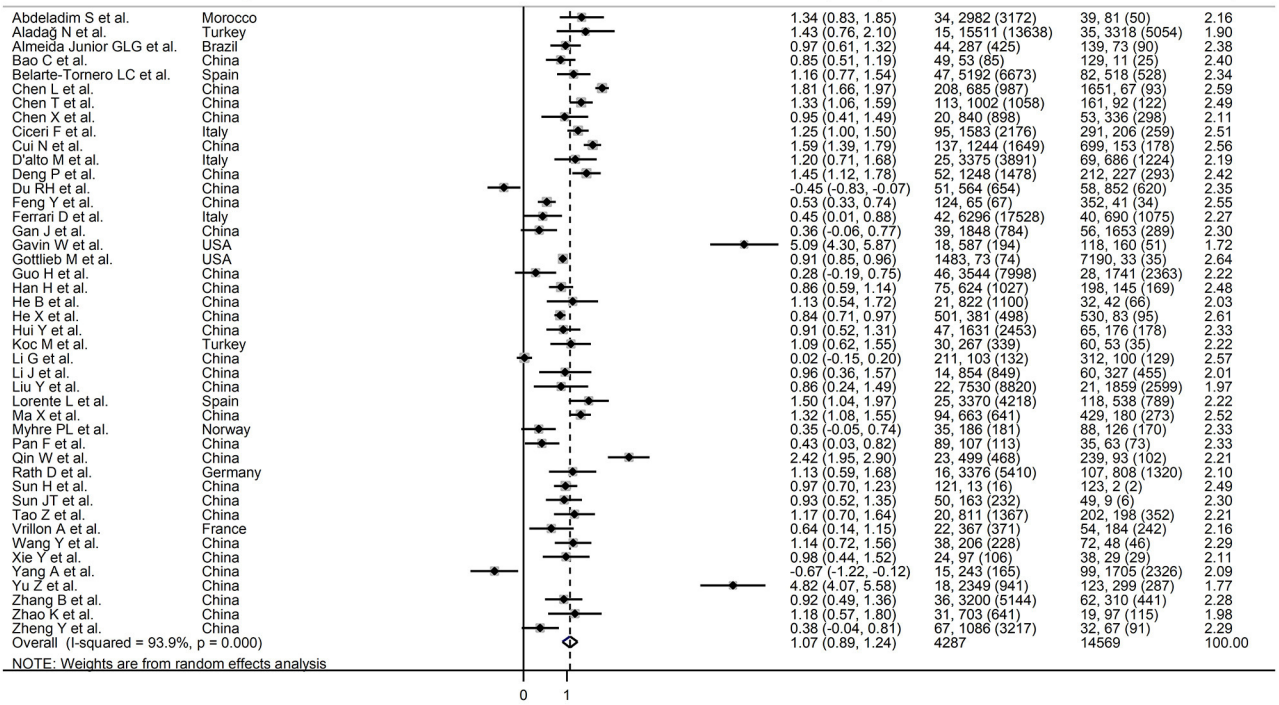


FIGURE 2 | Forest plot of studies reporting BNP concentrations in patients with COVID-19.

reported BNP or NT-proBNP concentrations within the first 24–48 h from admission.

Meta-Analysis

The overall SMD in BNP/NT-proBNP concentrations between COVID-19 patients with low vs. high severity or survivor vs. non-survivor status is shown in **Figure 2**. In two studies, patients with high severity or non-survivor status had significantly lower BNP/NT-proBNP concentrations when compared to those with low severity or survivor status (mean difference range, −0.45 to −0.67) (31, 59). By contrast, in the remaining studies BNP/NT-proBNP concentrations were lower in patients with low severity or survivor status (mean difference range, 0.02 – 5.09), with a non-significant difference in five studies (35, 38, 44, 50, 63). Pooled results confirmed that BNP/NT-proBNP concentrations were significantly higher in patients with severe disease or non-survivor status (SMD = 1.07, 95% CI: 0.89 – 1.24, and $p < 0.001$; **Figure 2**). There was extreme between-study heterogeneity ($I^2 = 93.9\%$, $p < 0.001$). BNP/NT-proBNP concentrations remained significantly higher (SMD = 1.06, 95% CI: 0.86 – 1.26, and $p < 0.001$; $I^2 = 93.0\%$, $p < 0.001$) in patients with high severity or non-survivor status after excluding two relatively large studies, accounting for nearly 56% of the overall sample size (26, 37).

In sensitivity analysis, the magnitude and the direction of the effect size were not substantially modified after sequentially removing each study and re-assessing the pooled estimates (effect size range, 0.99 – 1.10; **Figure 3**). No publication bias was observed with the Begg's ($p = 0.26$) and Egger's ($p = 0.40$) t-tests. However, using the trim-and-fill method, we identified three potential missing studies to be added to the left side of the funnel plot to ensure symmetry (**Figure 4**). The adjusted SMD, albeit attenuated, remained significant (SMD = 0.90, 95% CI: 0.70 – 1.09, and $p < 0.001$).

Sub-group analysis of the 42 studies reporting BNP/NT-proBNP concentrations on admission showed that the SMD remained significantly higher in patients with high severity or non-survivor status (SMD = 1.10, 95% CI: 0.88 – 1.31, and $p < 0.001$) with an extreme between-study variance ($I^2 = 94.1\%$, $p < 0.001$). Additionally, the pooled SMD value in studies assessing disease severity (SMD = 0.87, 95% CI: 0.68 – 1.07, and $p < 0.001$; $I^2 = 79.5$, $p < 0.001$) was non-significantly lower than those investigating survivor status (SMD = 1.37, 95% CI: 1.08 – 1.66, $p < 0.001$; $I^2 = 92.3$, $p < 0.001$; $t = 1.63$, $p = 0.11$; **Figure 5**). Similarly, non-significantly higher SMD values were observed in retrospective (SMD = 1.06, 95% CI: 0.86 – 1.27, $p < 0.001$; $I^2 = 94.5$, $p < 0.001$) vs. prospective studies (SMD = 0.92, 95% CI: 0.67 – 1.18, $p < 0.001$; $I^2 = 59.4$, $p = 0.016$; $t = -0.41$, $p = 0.69$; **Figure 6**). The pooled SMD value in European studies (SMD = 0.96, 95% CI: 0.67 – 1.26, $p < 0.002$; $I^2 = 75.5\%$, $p < 0.001$) was non-significantly lower than that observed in Asian (SMD = 1.01, 95% CI: 0.77 – 1.24, $p < 0.001$; $I^2 = 94.5\%$, $p < 0.001$) and American studies (SMD = 2.24, 95% CI: 0.83 – 3.64, $p < 0.001$;

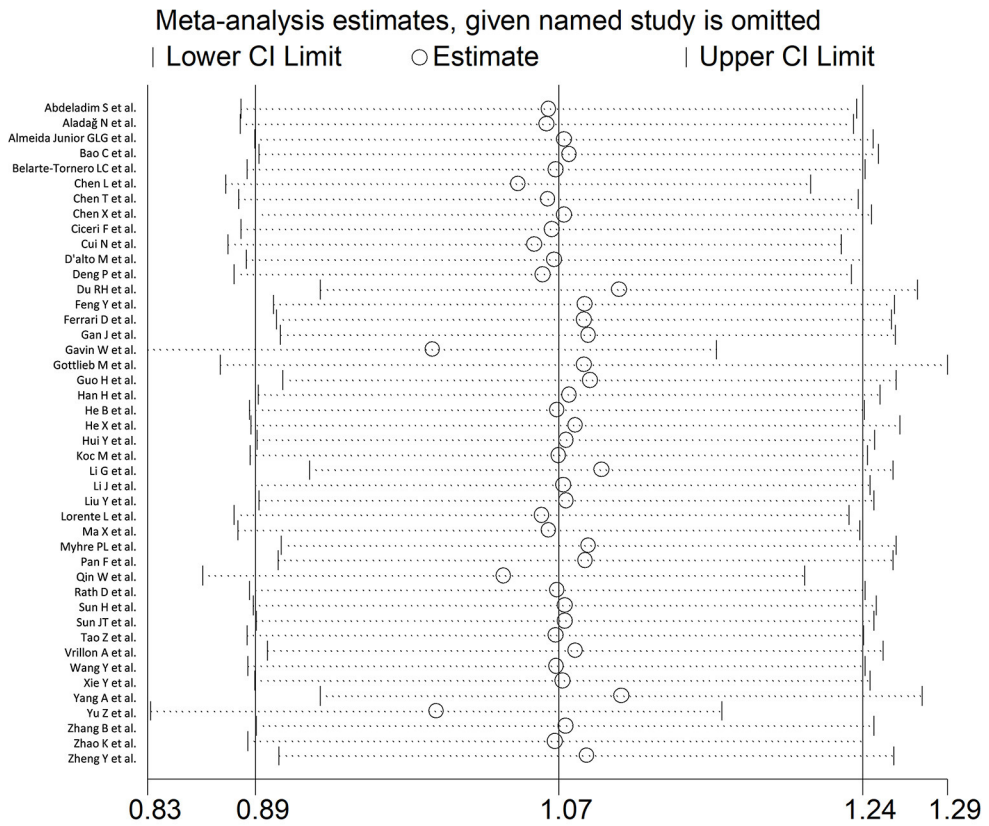


FIGURE 3 | Sensitivity analysis of the association between BNP and COVID-19 disease, assessed by investigating the influence of individual studies on the overall standardized mean difference (SMD). The SMD and the 95% confidence intervals (CIs) are indicated by the middle and the lateral vertical axes, respectively. The pooled SMD and the 95% CIs are indicated by the hollow circles and the two ends of each broken line, respectively.

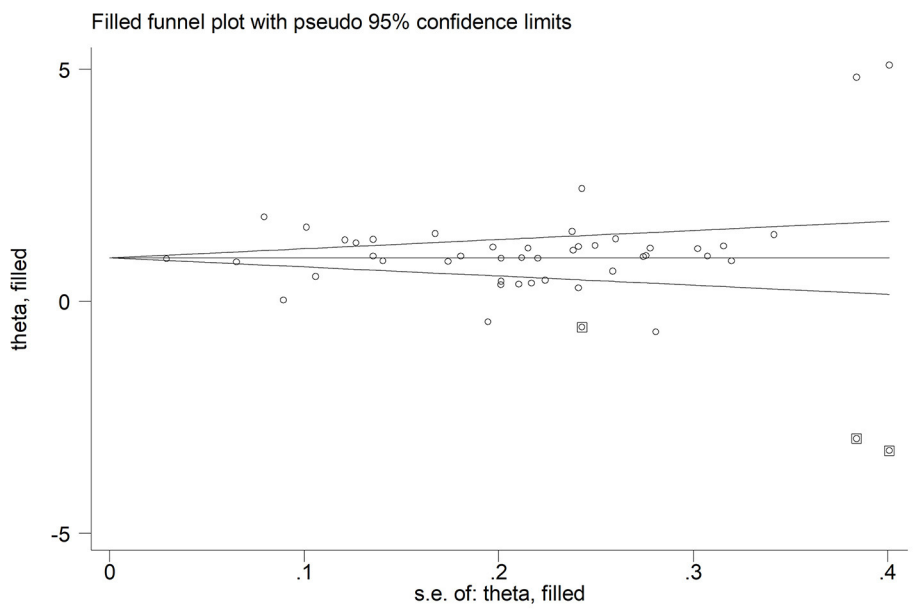


FIGURE 4 | Funnel plot of studies investigating disease severity or survival status after trimming and filling. Enclosed and free circles indicate dummy and genuine studies, respectively.

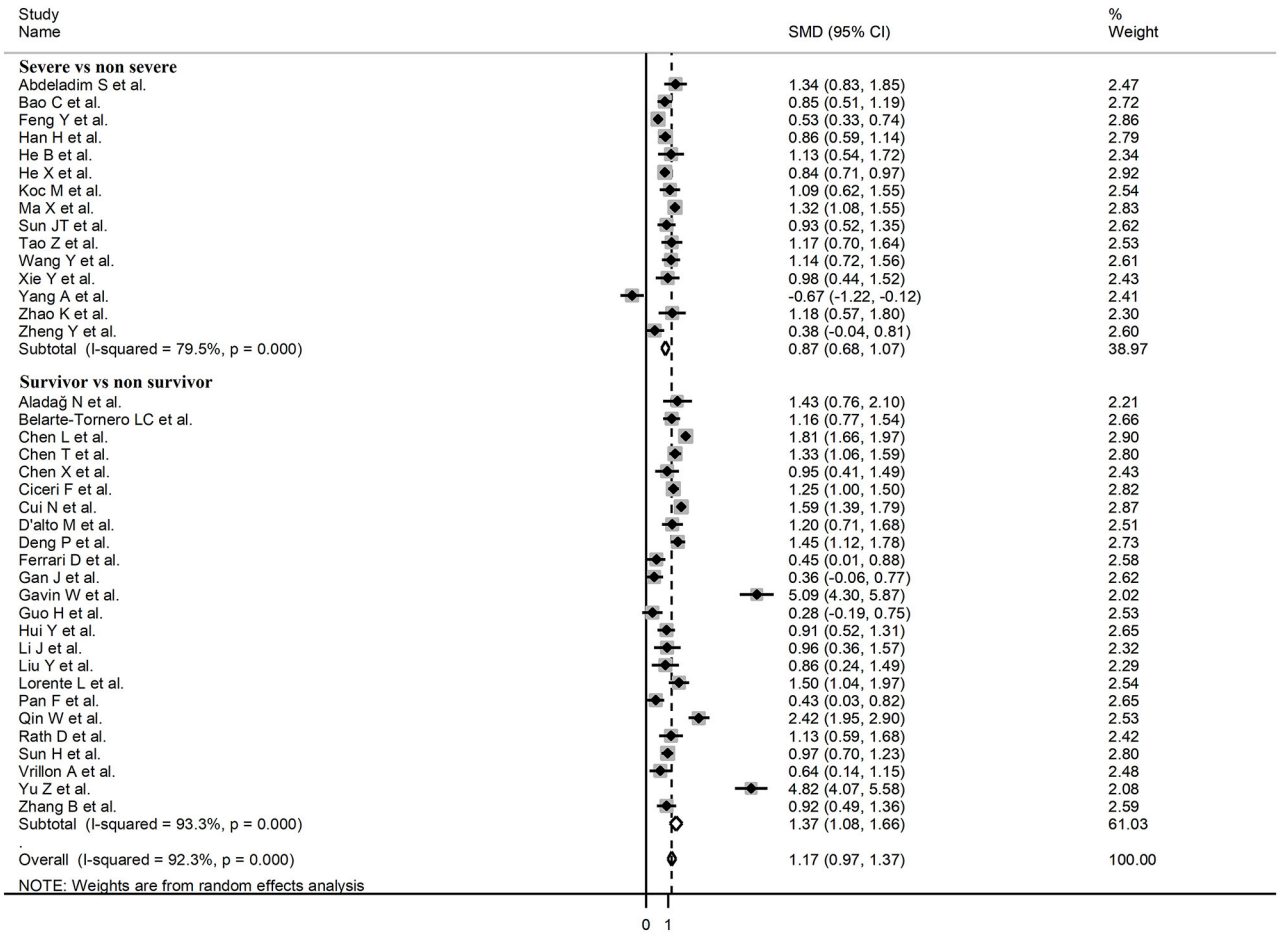


FIGURE 5 | Forest plot of studies reporting BNP concentrations in patients with COVID-19 according to disease severity or survival status.

$I^2 = 98.2\%$, $p < 0.001$; $t = 1.36$, $p = 0.18$; **Figure 7**). Finally, the pooled SMD value in studies reporting plasma NT-proBNP concentrations (SMD = 0.98, 95% CI: 0.74 – 1.23, $p < 0.001$; $I^2 = 93.2\%$, $p < 0.001$) was non-significantly lower than that observed in studies reporting plasma BNP concentrations (SMD = 1.22, 95% CI: 0.93 – 1.52, $p < 0.001$; $I^2 = 95.0\%$, $p < 0.001$; $t = -0.85$, $p = 0.40$; **Figure 8**). A relatively lower heterogeneity was observed in prospective ($I^2 = 59.4\%$) and European studies ($I^2 = 75.5\%$), and in those investigating disease severity ($I^2 = 79.5\%$).

Meta-Regression

The D-dimer ($t = 2.22$, $p = 0.03$), myoglobin ($t = 2.40$, $p = 0.04$), LDH ($t = 2.38$, $p = 0.02$), and procalcitonin ($t = 2.56$, $p = 0.01$) concentrations were significantly and positively associated with the pooled SMD. By contrast, no significant correlations were observed between the SMD and age ($t = -0.30$, $p = 0.76$), gender ($t = 0.26$, $p = 0.80$), AST ($t = 0.25$, $p = 0.81$), ALT ($t = -0.89$, $p = 0.38$), creatinine ($t = 0.93$, $p = 0.36$), troponin ($t = 0.18$, $p = 0.86$), CK ($t = 0.85$, $p = 0.41$), albumin ($t = 0.70$, $p = 0.49$), ferritin ($t = -1.29$, $p = 0.22$), CRP ($t = 0.96$, $p = 0.34$), WBC ($t = 0.08$, $p = 0.94$), diabetes ($t = -0.59$, $p = 0.56$), hypertension ($t = -0.01$, $p = 0.99$), and cardiovascular disease ($t = -0.53$, $p = 0.60$).

DISCUSSION

In our study, plasma concentrations of BNP and NT-proBNP, generally measured within the first 24–48 h from admission, were significantly higher in COVID-19 patients with severe disease, based on clinical assessment or the need for hospitalization, mechanical ventilation, or ICU transfer, and in those who did not survive when compared to patients with mild disease or who survived during follow up. The observed SMD values for combined natriuretic peptide concentrations or BNP and NT-proBNP separately, 1.07, 1.22, and 0.98, respectively, suggest a biologically and clinically significant effect size (64). Although between-study heterogeneity was extreme, in sensitivity analysis the effect size was not influenced when individual studies were sequentially removed. The Begg's and Egger's *t*-tests did not show any evidence of publication bias. In meta-regression analysis, significant associations were observed between the SMD value and D-dimer, myoglobin, LDH, and procalcitonin,

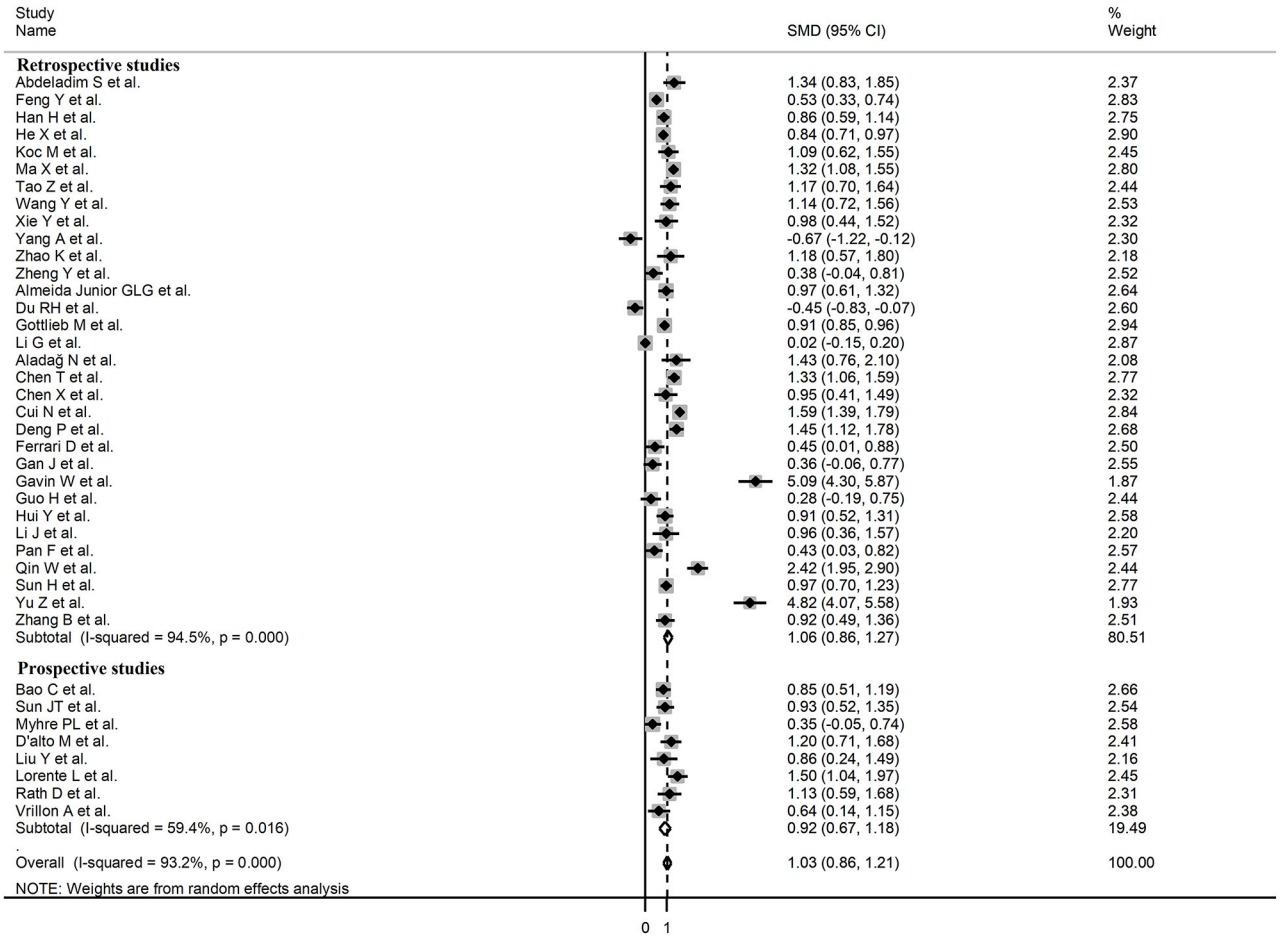


FIGURE 6 | Forest plot of studies reporting BNP concentrations in patients with COVID-19 according to study design (retrospective or prospective).

but not with age, gender, AST, ALT, creatinine, troponin, CK, albumin, ferritin, CRP, WBC, diabetes, hypertension, or cardiovascular disease.

Differently from the inactive NT-proBNP, the BNP exerts significant biological effects through its binding to the guanylyl cyclase-coupled natriuretic receptors A and B. The consequent increase in cyclic guanosine monophosphate causes vasodilatation, diuresis, natriuresis, inhibition of the renin-angiotensin-aldosterone system, inhibition of fibrosis, hypertrophy, cell apoptosis and inflammation, including suppression of superoxide generation by neutrophils, and improvement in myocardial relaxation (10). Notably, there is no evidence of significant associations between BNP and cyclic guanosine monophosphate concentrations in human studies. Furthermore, specific BNP-mediated protective effects, particularly the suppression of neutrophil-mediated generation of superoxide via nicotinamide adenine dinucleotide phosphate oxidase, are impaired in the context of acute heart failure, even in the presence of increased BNP concentrations (65). Whilst such effects are partially restored with pharmacological treatment, the failure of BNP-related suppression of superoxide release might lead to sustained tissue inflammation in heart failure, with or without concomitant COVID-19. There are other differences between the BNP and the NT-proBNP, with the latter being characterized by a higher molecular mass, a longer half-life (>60 vs. 15–20 min), a higher degree of *in vivo* glycosylation, and a lower degree of intra-individual biological variation (66). The better analytical characteristics of the available immunoassay methods for the measurement of NT-proBNP concentrations, when compared to those for the assessment of the BNP, have prompted some experts to advocate the measurement and monitoring of NT-proBNP concentrations as the best strategy for the management of patients with heart failure (66). These issues notwithstanding, in our meta-analysis the studies reporting BNP vs. NT-proBNP plasma concentrations had similar SMD values and degrees of heterogeneity.

The significant association observed between plasma BNP/NT-proBNP concentrations, disease severity and mortality in patients with COVID-19 is likely to reflect the presence of heart failure and its adverse sequelae in this group. In this context, these routine and relatively inexpensive biomarkers might assist the clinician with the early diagnosis of cardiac dysfunction

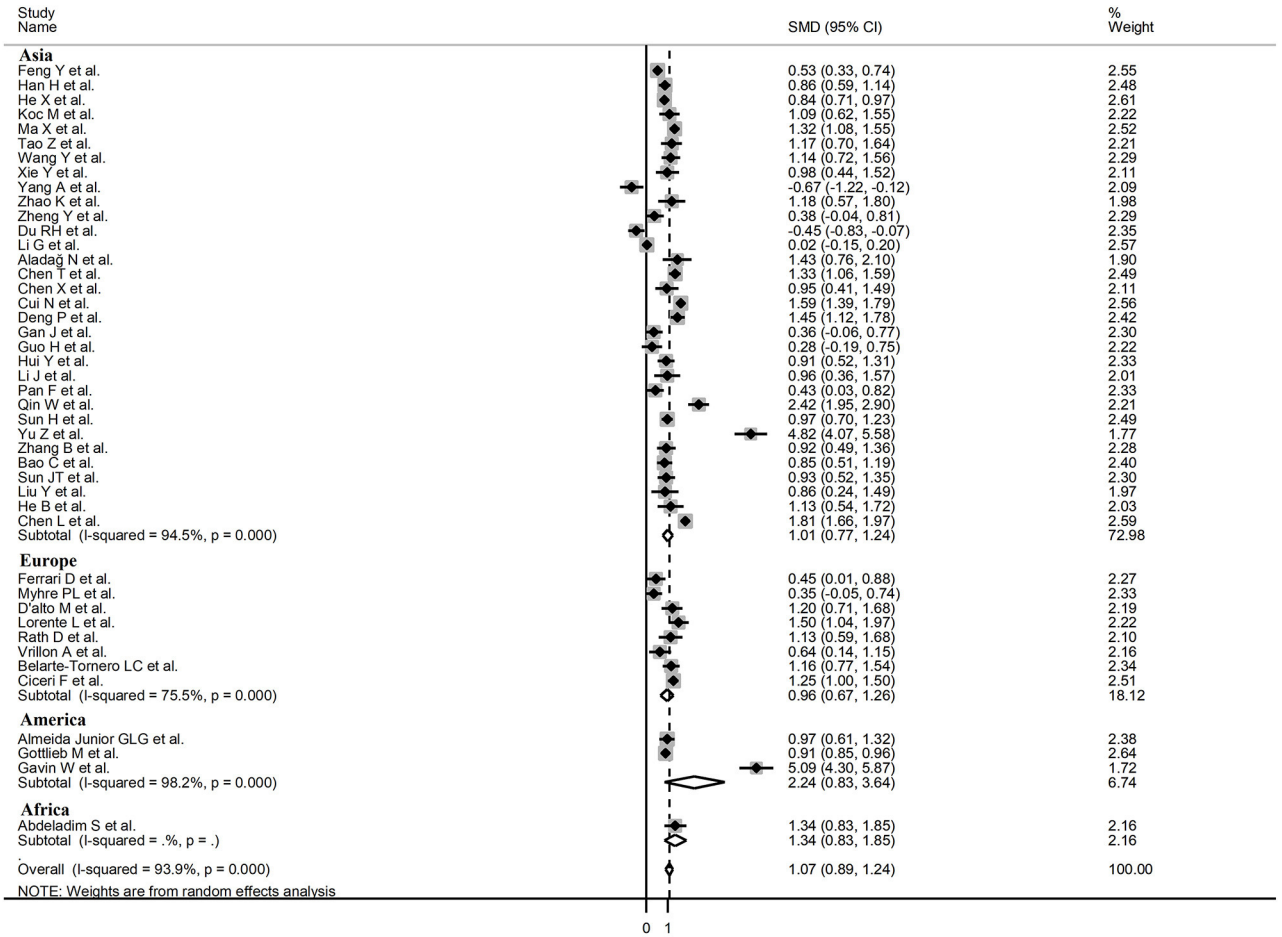


FIGURE 7 | Forest plot of studies examining BNP concentrations in patients with COVID-19 according to the geographic area where the study was conducted.

and the prompt initiation of appropriate pharmacological and non-pharmacological therapies (67). Further research is warranted to determine whether the assessment of BNP/NT-proBNP might also be incorporated into predictive tools that are specifically developed and validated in COVID-19 patients. The reported associations, in meta-regression analysis, between the SMD of BNP/NT-proBNP and D-dimer, myoglobin, LDH, and pro-calcitonin suggests that the effect size is particularly correlated with markers of pro-coagulant activity, skeletal muscle and other tissue damage, and severe sepsis, respectively. Notably, these markers have, in turn, been shown to have significant associations with COVID-19 severity and outcomes (68–70). By contrast, the lack of associations observed with other cardiac biomarkers, e.g., troponin and CK, suggests that the measurement of BNP/NT-proBNP may provide complementary, rather than redundant, information regarding the presence of cardiac abnormalities in patients with COVID-19.

The extreme between-study heterogeneity represents a limitation of our study. However, we did not observe significant publication bias and the overall effect size was not substantially affected in sensitivity analyses. The lack of significant associations between the SMD and several patient and study characteristics, except for D-dimer, myoglobin, LDH, and procalcitonin concentrations, suggests that other unreported factors might have contributed to the observed heterogeneity. Such factors may include the relationship between the SMD values and the presence of new onset vs. acute on chronic heart failure and the information regarding the specific analytical methods used for the determination of BNP and NT-proBNP plasma concentrations (66). In this context, the lack of available information on indexes of left ventricular function prevented the conduct of further meta-regression analyses of the association between such indexes and the SMD values. Furthermore, virtually all selected studies reported isolated, rather than serial, measurements of natriuretic peptide shortly after hospital admission. This issue is particularly important as the routine monitoring of BNP/NT-proBNP concentrations has been shown to be beneficial in heart failure (71). Further studies should investigate the prognostic value of single vs. serial BNP/NT-proBNP measurements also in patients with COVID-19.

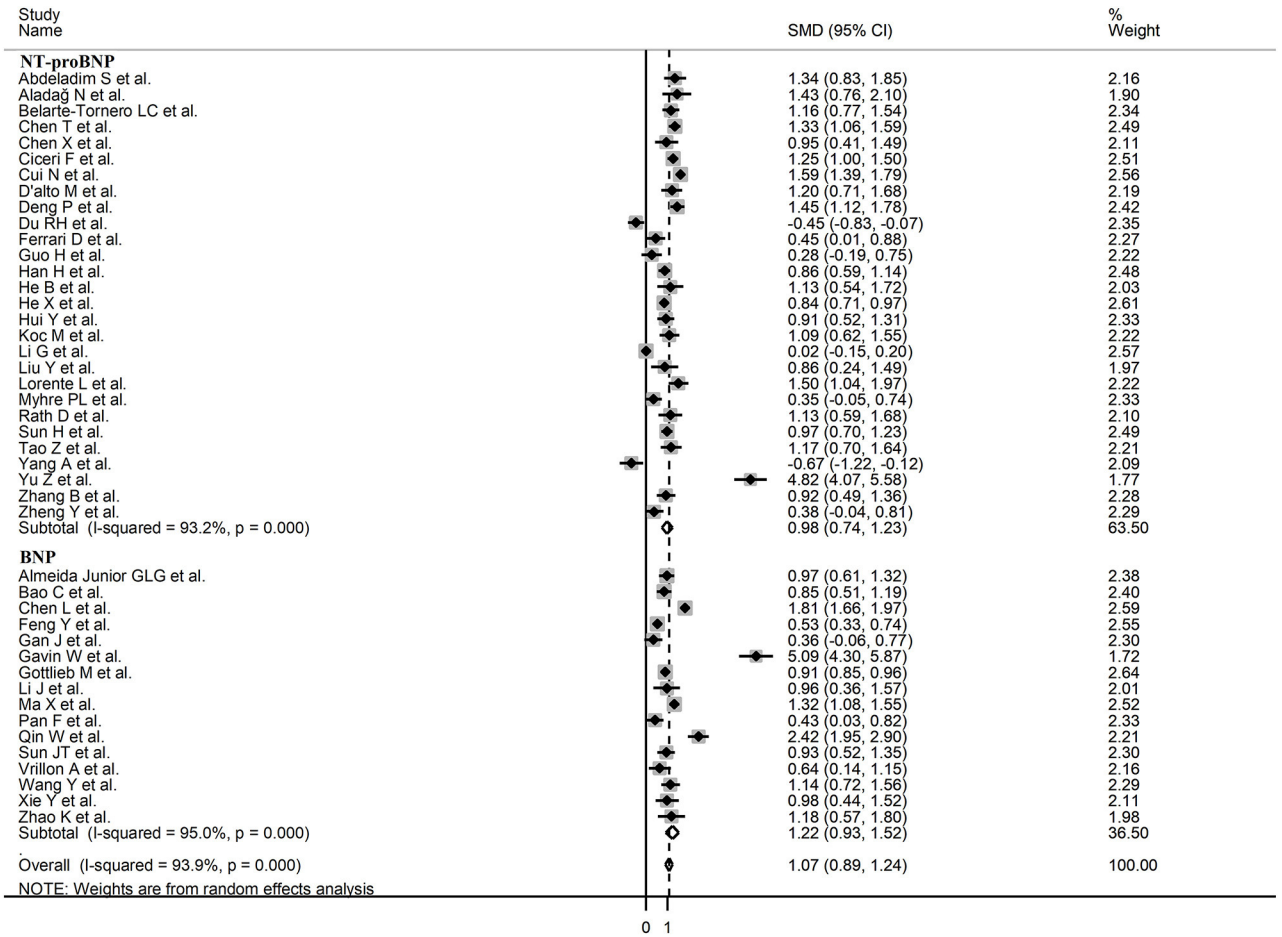


FIGURE 8 | Forest plot of studies examining BNP concentrations in patients with COVID-19 according to whether plasma NT-proBNP or BNP was measured.

In conclusion, higher plasma concentrations of BNP or NT-proBNP are significantly associated with higher disease severity and increased mortality in COVID-19. Additional studies are required to determine whether these cardiac biomarkers can be incorporated into robust predictive tools that further assist with early management and monitoring in this patient group.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

AZ: initial idea. AZ and SS: data collection and analysis. AZ, SS, CC, and AM: data interpretation and writing—review and editing. AM: writing—first draft. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.690790/full#supplementary-material

REFERENCES

1. Huang C, Soleimani J, Herasevich S, Pinevich Y, Pennington KM, Dong Y, et al. Clinical characteristics, treatment, and outcomes of critically ill patients with COVID-19: a scoping review. *Mayo Clin Proc.* (2021) 96:183–202. doi: 10.1016/j.mayocp.2020.10.022
2. Pijls BG, Jolani S, Atherley A, Derckx RT, Dijkstra JIR, Franssen GHL, et al. Demographic risk factors for COVID-19 infection, severity, ICU admission and death: a meta-analysis of 59 studies. *BMJ Open.* (2021) 11:e044640. doi: 10.1136/bmjopen-2020-044640
3. Fajgenbaum DC, June CH. Cytokine Storm. *N Engl J Med.* (2020) 383:2255–73. doi: 10.1056/NEJMra2026131

4. Nishiga M, Wang DW, Han Y, Lewis DB, Wu JC. COVID-19 and cardiovascular disease: from basic mechanisms to clinical perspectives. *Nat Rev Cardiol.* (2020) 17:543–58. doi: 10.1038/s41569-020-0413-9
5. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* (2020) 5:802–10. doi: 10.1001/jamacardio.2020.0950
6. Yang J, Liao X, Yin W, Wang B, Yue J, Bai L, et al. Elevated cardiac biomarkers may be effective prognostic predictors for patients with COVID-19: a multicenter, observational study. *Am J Emerg Med.* (2021) 39:34–41. doi: 10.1016/j.ajem.2020.10.013
7. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ.* (2020) 368:m1091. doi: 10.1136/bmj.m1091
8. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3
9. Maisel AS, Duran JM, Wettersten N. Natriuretic peptides in heart failure: atrial and B-type natriuretic peptides. *Heart Fail Clin.* (2018) 14:13–25. doi: 10.1016/j.hfc.2017.08.002
10. Potter LR, Yoder AR, Flora DR, Antos LK, Dickey DM. Natriuretic peptides: their structures, receptors, physiologic functions and therapeutic applications. *Handb Exp Pharmacol.* (2009) 191:341–66. doi: 10.1007/978-3-540-68964-5_15
11. Omland T, Persson A, Ng L, O'Brien R, Karlsson T, Herlitz J, et al. N-terminal pro-B-type natriuretic peptide and long-term mortality in acute coronary syndromes. *Circulation.* (2002) 106:2913–8. doi: 10.1161/01.CIR.0000041661.63285.AE
12. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses.* The Ottawa Hospital Research Institute (2013). Available online at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (accessed June 11, 2021).
13. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol.* (2014) 14:135. doi: 10.1186/1471-2288-14-135
14. Bowden J, Tierney JF, Copas AJ, Burdett S. Quantifying, displaying and accounting for heterogeneity in the meta-analysis of RCTs using standard and generalised Q statistics. *BMC Med Res Methodol.* (2011) 11:41. doi: 10.1186/1471-2288-11-41
15. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med.* (2002) 21:1539–58. doi: 10.1002/sim.1186
16. Tobias A. Assessing the influence of a single study in the meta-analysis estimate. *Stata Tech Bull.* (1999) 47:15–7.
17. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics.* (1994) 50:1088–101. doi: 10.2307/2533446
18. Sterne JA, Egger M. Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. *J Clin Epidemiol.* (2001) 54:1046–55. doi: 10.1016/S0895-4356(01)00377-8
19. Duval S, Tweedie R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics.* (2000) 56:455–63. doi: 10.1111/j.0006-341X.2000.00455.x
20. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ.* (2009) 339:b2700. doi: 10.1136/bmj.b2700
21. Abdeladim S, Oualim S, Elouarradi A, Bensahi I, Aniq Filali R, El Harras M, et al. Analysis of cardiac injury biomarkers in COVID-19 patients. *Arch Clin Infect Dis.* (2020) 15:e105515. doi: 10.5812/archcid.105515
22. Aladag N, Atabey RD. The role of concomitant cardiovascular diseases and cardiac biomarkers for predicting mortality in critical COVID-19 patients. *Acta Cardiol.* (2021) 76:132–9. doi: 10.1080/00015385.2020.1810914
23. Almeida Junior GLG, Braga F, Jorge JK, Nobre GF, Kalichsztein M, Faria PMP, et al. Prognostic value of troponin-T and B-type natriuretic peptide in patients hospitalized for COVID-19. *Arq Bras Cardiol.* (2020) 115:660–6. doi: 10.36660/abc.20200385
24. Bao C, Tao X, Cui W, Yi B, Pan T, Young KH, et al. SARS-CoV-2 induced thrombocytopenia as an important biomarker significantly correlated with abnormal coagulation function, increased intravascular blood clot risk and mortality in COVID-19 patients. *Exp Hematol Oncol.* (2020) 9:16. doi: 10.1186/s40164-020-00172-4
25. Belarte-Tornero LC, Valdivielso-More S, Vicente Elcano M, Sole-Gonzalez E, Ruiz-Bustillo S, Calvo-Fernandez A, et al. Prognostic implications of chronic heart failure and utility of NT-proBNP levels in heart failure patients with SARS-CoV-2 infection. *J Clin Med.* (2021) 10:323. doi: 10.3390/jcm10020323
26. Chen L, Yu J, He W, Chen L, Yuan G, Dong F, et al. Risk factors for death in 1859 subjects with COVID-19. *Leukemia.* (2020) 34:2173–83. doi: 10.1038/s41375-020-0911-0
27. Chen X, Yan L, Fei Y, Zhang C. Laboratory abnormalities and risk factors associated with in-hospital death in patients with severe COVID-19. *J Clin Lab Anal.* (2020) 34:e23467. doi: 10.1002/jcla.23467
28. Ciceri F, Castagna A, Rovere-Querini P, De Cobelli F, Ruggeri A, Galli L, et al. Early predictors of clinical outcomes of COVID-19 outbreak in Milan, Italy. *Clin Immunol.* (2020) 217:108509. doi: 10.1016/j.clim.2020.108509
29. Cui N, Yan R, Qin C, Zhao J. Clinical characteristics and immune responses of 137 deceased patients with COVID-19: a retrospective study. *Front Cell Infect Microbiol.* (2020) 10:595333. doi: 10.3389/fcimb.2020.595333
30. D'Alto M, Marra AM, Severino S, Salzano A, Romeo E, De Rosa R, et al. Right ventricular-arterial uncoupling independently predicts survival in COVID-19 ARDS. *Crit Care.* (2020) 24:670. doi: 10.1186/s13054-020-03385-5
31. Deng P, Ke Z, Ying B, Qiao B, Yuan L. The diagnostic and prognostic role of myocardial injury biomarkers in hospitalized patients with COVID-19. *Clin Chim Acta.* (2020) 510:186–90. doi: 10.1016/j.cca.2020.07.018
32. Du RH, Liu LM, Yin W, Wang W, Guan LL, Yuan ML, et al. Hospitalization and critical care of 109 decedents with COVID-19 pneumonia in Wuhan, China. *Ann Am Thorac Soc.* (2020) 17:839–46. doi: 10.1513/AnnalsATS.202003-225OC
33. Feng Y, Ling Y, Bai T, Xie Y, Huang J, Li J, et al. COVID-19 with different severities: a multicenter study of clinical features. *Am J Respir Crit Care Med.* (2020) 201:1380–8. doi: 10.1164/rccm.202002-0445OC
34. Ferrari D, Seveso A, Sabetta E, Ceriotti D, Carobene A, Banfi G, et al. Role of time-normalized laboratory findings in predicting COVID-19 outcome. *Diagnosis (Berl).* (2020) 7:387–94. doi: 10.1515/dx-2020-0095
35. Gan J, Li J, Li S, Yang C. Leucocyte subsets effectively predict the clinical outcome of patients with COVID-19 pneumonia: a retrospective case-control study. *Front Public Health.* (2020) 8:299. doi: 10.3389/fpubh.2020.00299
36. Gavin W, Campbell E, Zaidi SA, Gavin N, Dbeibo L, Beeler C, et al. Clinical characteristics, outcomes and prognosticators in adult patients hospitalized with COVID-19. *Am J Infect Control.* (2021) 49:158–65. doi: 10.1016/j.ajic.2020.07.005
37. Gottlieb M, Sansom S, Frankenberger C, Ward E, Hota B. Clinical course and factors associated with hospitalization and critical illness among COVID-19 patients in Chicago, Illinois. *Acad Emerg Med.* (2020) 27:963–73. doi: 10.1111/acem.14104
38. Guo H, Shen Y, Wu N, Sun X. Myocardial injury in severe and critical coronavirus disease 2019 patients. *J Card Surg.* (2021) 36:82–8. doi: 10.1111/jocs.15164
39. Han H, Xie L, Liu R, Yang J, Liu F, Wu K, et al. Analysis of heart injury laboratory parameters in 273 COVID-19 patients in one hospital in Wuhan, China. *J Med Virol.* (2020) 92:819–23. doi: 10.1002/jmv.25809
40. He B, Wang J, Wang Y, Zhao J, Huang J, Tian Y, et al. The metabolic changes and immune profiles in patients with COVID-19. *Front Immunol.* (2020) 11:2075. doi: 10.3389/fimmu.2020.02075
41. He X, Wang L, Wang H, Xie Y, Yu Y, Sun J, et al. Factors associated with acute cardiac injury and their effects on mortality in patients with COVID-19. *Sci Rep.* (2020) 10:20452. doi: 10.1038/s41598-020-77172-1
42. Hui Y, Li Y, Tong X, Wang Z, Mao X, Huang L, et al. The risk factors for mortality of diabetic patients with severe COVID-19: a retrospective study of 167 severe COVID-19 cases in Wuhan. *PLoS One.* (2020) 15:e0243602. doi: 10.1371/journal.pone.0243602
43. Koc M, Sumbul HE, Gulumsek E, Koca H, Bulut Y, Karakoc E, et al. Disease severity affects ventricular repolarization parameters in patients with COVID-19. *Arq Bras Cardiol.* (2020) 115:907–13. doi: 10.36660/abc.20200482
44. Li G, Zhou CL, Ba YM, Wang YM, Song B, Cheng XB, et al. Nutritional risk and therapy for severe and critical COVID-19 patients: a multicenter retrospective observational study. *Clin Nutr.* (2021) 40:2154–61. doi: 10.1016/j.clnu.2020.09.040

45. Li J, Xu G, Yu H, Peng X, Luo Y, Cao C. Clinical characteristics and outcomes of 74 patients with severe or critical COVID-19. *Am J Med Sci.* (2020) 360:229–35. doi: 10.1016/j.amjms.2020.05.040
46. Liu Y, Xie J, Gao P, Tian R, Qian H, Guo F, et al. Swollen heart in COVID-19 patients who progress to critical illness: a perspective from echo-cardiologists. *ESC Heart Fail.* (2020) 7:3621–32. doi: 10.1002/ehf2.12873
47. Lorente L, Martin MM, Argueso M, Sole-Violan J, Perez A, Marcos YRJA, et al. Association between red blood cell distribution width and mortality of COVID-19 patients. *Anaesth Crit Care Pain Med.* (2020) 40:100777. doi: 10.1016/j.accpm.2020.10.013
48. Ma X, Li A, Jiao M, Shi Q, An X, Feng Y, et al. Characteristic of 523 COVID-19 in henan province and a death prediction model. *Front Public Health.* (2020) 8:475. doi: 10.3389/fpubh.2020.00475
49. Myhre PL, Prebensen C, Strand H, Roysland R, Jonassen CM, Rangberg A, et al. Growth differentiation factor 15 provides prognostic information superior to established cardiovascular and inflammatory biomarkers in unselected patients hospitalized with COVID-19. *Circulation.* (2020) 142:2128–37. doi: 10.1161/CIRCULATIONAHA.120.050360
50. Pan F, Yang L, Li Y, Liang B, Li L, Ye T, et al. Factors associated with death outcome in patients with severe coronavirus disease-19 (COVID-19): a case-control study. *Int J Med Sci.* (2020) 17:1281–92. doi: 10.7150/ijms.46614
51. Qin WD, Bai W, Liu K, Liu Y, Meng X, Zhang K, et al. Clinical course and risk factors of disease deterioration in critically ill patients with COVID-19. *Hum Gene Ther.* (2021) 32:310–5. doi: 10.1089/hum.2020.255
52. Rath D, Petersen-Uribe A, Avdiu A, Witzel K, Jaeger P, Zdanyte M, et al. Impaired cardiac function is associated with mortality in patients with acute COVID-19 infection. *Clin Res Cardiol.* (2020) 109:1491–9. doi: 10.1007/s00392-020-01683-0
53. Sun H, Ning R, Tao Y, Yu C, Deng X, Zhao C, et al. Risk factors for mortality in 244 older adults with COVID-19 in Wuhan, China: a retrospective study. *J Am Geriatr Soc.* (2020) 68:E19–E23. doi: 10.1111/jgs.16533
54. Sun JT, Chen Z, Nie P, Ge H, Shen L, Yang F, et al. Lipid profile features and their associations with disease severity and mortality in patients with COVID-19. *Front Cardiovasc Med.* (2020) 7:584987. doi: 10.3389/fcvm.2020.584987
55. Tao Z, Xu J, Chen W, Yang Z, Xu X, Liu L, et al. Anemia is associated with severe illness in COVID-19: a retrospective cohort study. *J Med Virol.* (2021) 93:1478–88. doi: 10.21203/rs.3.rs-39184/v1
56. Vrillon A, Hourregue C, Azuar J, Grosset L, Boutelier A, Tan S, et al. COVID-19 in older adults: a series of 76 patients aged 85 years and older with COVID-19. *J Am Geriatr Soc.* (2020) 68:2735–43. doi: 10.1111/jgs.16894
57. Wang Y, Zhou Y, Yang Z, Xia D, Hu Y, Geng S. Clinical characteristics of patients with severe pneumonia caused by the SARS-CoV-2 in Wuhan, China. *Respiration.* (2020) 99:649–57. doi: 10.1159/000507940
58. Xie Y, You Q, Wu C, Cao S, Qu G, Yan X, et al. Impact of cardiovascular disease on clinical characteristics and outcomes of coronavirus disease 2019 (COVID-19). *Circ J.* (2020) 84:1277–83. doi: 10.1253/circj.CJ-20-0348
59. Yang A, Qiu Q, Kong X, Sun Y, Chen T, Zuo Y, et al. Clinical and Epidemiological Characteristics of COVID-19 Patients in Chongqing China. *Front Public Health.* (2020) 8:244. doi: 10.3389/fpubh.2020.00244
60. Yu Z, Ke Y, Xie J, Yu H, Zhu W, He L, et al. Clinical characteristics on admission predict in-hospital fatal outcome in patients aged >/=75 years with novel coronavirus disease (COVID-19): a retrospective cohort study. *BMC Geriatr.* (2020) 20:514. doi: 10.1186/s12877-020-01921-0
61. Zhang B, Dong C, Li S, Song X, Wei W, Liu L. Triglyceride to high-density lipoprotein cholesterol ratio is an important determinant of cardiovascular risk and poor prognosis in coronavirus disease-19: a retrospective case series study. *Diabetes Metab Syndr Obes.* (2020) 13:3925–36. doi: 10.2147/DMSO.S268992
62. Zhao K, Huang J, Dai D, Feng Y, Liu L, Nie S. Serum iron level as a potential predictor of coronavirus disease 2019 severity and mortality: a retrospective study. *Open Forum Infect Dis.* (2020) 7:ofaa250. doi: 10.1093/ofid/ofaa250
63. Zheng Y, Xu H, Yang M, Zeng Y, Chen H, Liu R, et al. Epidemiological characteristics and clinical features of 32 critical and 67 noncritical cases of COVID-19 in Chengdu. *J Clin Virol.* (2020) 127:104366. doi: 10.1016/j.jcv.2020.104366
64. Cohen J. *Statistical Power Analysis for the Behavioral Sciences, 2nd Edn.* Hillsdale, NJ: Erlbaum (1988).
65. Liu S, Ngo DT, Chong CR, Amarasekera AT, Procter NE, Licari G, et al. Suppression of neutrophil superoxide generation by BNP is attenuated in acute heart failure: a case for 'BNP resistance'. *Eur J Heart Fail.* (2015) 17:475–83. doi: 10.1002/ejhf.242
66. Clerico A, Zaninotto M, Passino C, Plebani M. New issues on measurement of B-type natriuretic peptides. *Clin Chem Lab Med.* (2017) 56:32–9. doi: 10.1515/cclm-2017-0433
67. Zhang Y, Coats AJS, Zheng Z, Adamo M, Ambrosio G, Anker SD, et al. Management of heart failure patients with COVID-19: a joint position paper of the Chinese Heart Failure Association & National Heart Failure Committee and the Heart Failure Association of the European Society of Cardiology. *Eur J Heart Fail.* (2020) 22:941–56. doi: 10.1002/ejhf.1915
68. Paliogiannis P, Mangoni AA, Dettori P, Nasrallah GK, Pintus G, Zinellu A. D-Dimer Concentrations and COVID-19 severity: a systematic review and meta-analysis. *Front Public Health.* (2020) 8:432. doi: 10.3389/fpubh.2020.00432
69. Ghahramani S, Tabrizi R, Lankarani KB, Kashani SMA, Rezaei S, Zeidi N, et al. Laboratory features of severe vs. non-severe COVID-19 patients in Asian populations: a systematic review and meta-analysis. *Eur J Med Res.* (2020) 25:30. doi: 10.1186/s40001-020-00432-3
70. Qin JJ, Cheng X, Zhou F, Lei F, Akolkar G, Cai J, et al. Redefining cardiac biomarkers in predicting mortality of inpatients with COVID-19. *Hypertension.* (2020) 76:1104–12. doi: 10.1161/HYPERTENSIONAHA.120.15528
71. Januzzi JL, Troughton R. Are serial BNP measurements useful in heart failure management? Serial natriuretic peptide measurements are useful in heart failure management. *Circulation.* (2013) 127:500–7; discussion 8. doi: 10.1161/CIRCULATIONAHA.112.120485

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 09 July 2021
doi: 10.3389/fcvm.2021.684528

Prognostic Value of Coronary Artery Calcium Score in Hospitalized COVID-19 Patients

Maria-Luiza Luchian[1†], Stijn Lochy[1†], Andreea Motoc[1], Dries Belsack[2], Julien Magne[3,4], Bram Roosens[1], Johan de Mey[2], Kaoru Tanaka[2], Esther Scheirlynck[1], Sven Boeckstaens[1], Karen Van den Bussche[1], Tom De Potter[5], Berlinde von Kemp[1], Xavier Galloo[1], Clara François[1], Caroline Weytjens[1], Steven Droogmans[1] and Bernard Cosyns[1]*

[1] Department of Cardiology, University Hospital of Brussels (Centrum voor Hart-en Vaat ziekten, Universitair Ziekenhuis Brussel), Brussels, Belgium, [2] Department of Radiology, University Hospital of Brussels, Brussels, Belgium, [3] CHU Limoges, Hôpital Dupuytren, Service Cardiologie, Limoges, France, [4] INSERM 1094, Faculté de médecine de Limoges, 2, rue Marcland, Limoges, France, [5] Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel, Brussels, Belgium

OPEN ACCESS

Edited by:
Ranil De Silva,
Imperial College London,
United Kingdom

Reviewed by:
Tarun Mittal,
Harefield Hospital, United Kingdom
Richard Nethononda,
Chris Hani Baragwanath Hospital,
South Africa

***Correspondence:**
Andreea Motoc
andreea.motoc@gmail.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Cardiovascular Imaging, a section of the journal Frontiers in Cardiovascular Medicine

Received: 23 March 2021
Accepted: 10 June 2021
Published: 09 July 2021

Citation:
Luchian M-L, Lochy S, Motoc A, Belsack D, Magne J, Roosens B, de Mey J, Tanaka K, Scheirlynck E, Boeckstaens S, Van den Bussche K, De Potter T, von Kemp B, Galloo X, François C, Weytjens C, Droogmans S and Cosyns B (2021) Prognostic Value of Coronary Artery Calcium Score in Hospitalized COVID-19 Patients. Front. Cardiovasc. Med. 8:684528. doi: 10.3389/fcvm.2021.684528

Background: The association of known cardiovascular risk factors with poor prognosis of coronavirus disease 2019 (COVID-19) has been recently emphasized. Coronary artery calcium (CAC) score is considered a risk modifier in the primary prevention of cardiovascular disease. We hypothesized that the absence of CAC might have an additional predictive value for an improved cardiovascular outcome of hospitalized COVID-19 patients.

Materials and methods: We prospectively included 310 consecutive hospitalized patients with COVID-19. Thirty patients with history of coronary artery disease were excluded. Chest computed tomography (CT) was performed in all patients. Demographics, medical history, clinical characteristics, laboratory findings, imaging data, in-hospital treatment, and outcomes were retrospectively analyzed. A composite endpoint of major adverse cardiovascular events (MACE) was defined.

Results: Two hundred eighty patients (63.2 ± 16.7 years old, 57.5% male) were included in the analysis. 46.7% patients had a CAC score of 0. MACE rate was 21.8% (61 patients). The absence of CAC was inversely associated with MACE (OR 0.209, 95% CI 0.052–0.833, $p = 0.027$), with a negative predictive value of 84.5%.

Conclusion: The absence of CAC had a high negative predictive value for MACE in patients hospitalized with COVID-19, even in the presence of cardiac risk factors. A semi-qualitative assessment of CAC is a simple, reproducible, and non-invasive measure that may be useful to identify COVID-19 patients at a low risk for developing cardiovascular complications.

Keywords: Corona virus, coronary artery calcium score, major adverse cardiac and cerebral event, chest computed tomography, risk stratification

INTRODUCTION

Coronavirus disease 2019 (COVID-19) has significantly impacted the healthcare system, due to the rapid spread of infection and unpredictable disease course. Studies have shown that advanced age and comorbidities including hypertension, diabetes mellitus, cardiovascular diseases, and cerebrovascular diseases are predictors of an unfavorable prognosis and mortality in COVID-19 infection (1–4). Coronary artery calcium (CAC) score assessed by computed tomography (CT) is considered a risk modifier in primary prevention of cardiovascular disease (5, 6).

The CAC score offers two main assets: (1) it has an independent additional value in the prediction of all-cause mortality and mortality due to coronary artery disease in asymptomatic individuals; (2) it may reclassify patients considered as being at low or intermediate risk according to the clinical risk scores at high risk of atherosclerotic coronary events (6–9).

However, data regarding the role of CAC score in the prediction of cardiovascular events and outcome in COVID-19 patients are still scarce.

We hypothesized that the absence of CAC might have an additional predictive value for an improved cardiovascular outcome of hospitalized COVID-19 patients.

MATERIALS AND METHODS

We prospectively included 310 consecutive hospitalized patients with confirmed COVID-19 by real-time reverse transcription polymerase chain reaction (RT-PCR) test, between March 2020 and April 2020. Thirty patients with a history of coronary artery disease (stable angina, unstable angina, history of acute coronary syndrome) were excluded from the analysis. Demographics, medical history, clinical characteristics, laboratory findings, imaging data, in-hospital treatment, and outcomes were retrospectively analyzed. A composite endpoint [major adverse cardiovascular events (MACE)] was defined as all-cause mortality, heart failure, acute coronary syndrome, atrial fibrillation, and stroke.

In the absence of widely available RT-PCR at the beginning of the pandemic, chest CT had been systematically performed in all suspected COVID-19 patients. All patients were scanned on an Apex Revolution CT (GE Healthcare, Milwaukee, WI, USA). The low-dose non-contrast CT thorax scan protocol consisted of a 128 × 0.625 mm spiral acquisition with pitch 1, rotation time 0.35 s, automated kVp selection and automated mA modulation. Images with 1.25 mm slice thickness were reconstructed with deep learning image reconstruction (DLIR) set at medium level. The average volume CT dose index (CTDIvol) and dose-length product (DLP) were 4.4 mGy (95% CI: 4.3–4.5) and 159 mGy·cm (95% CI: 157–162), respectively. Visual assessment of CAC was performed using ordinal scoring: each of the four main coronary arteries was identified (left main, left anterior descending, left circumflex, and right coronary artery). Calcium was scored as 0, 1, 2, or 3 for every artery, corresponding to absent, mild, moderate, or severe CAC. Mild CAC was defined as involvement of less than one third of the vessel length, moderate as involvement of one to two thirds of the vessel length and severe CAC as involvement of more than two thirds of the vessel length. A total score was calculated by summing the score of each vessel. The total score was then categorized as 0 (undetectable), 1–3 (mild), 4–5 (moderate), and ≥ 6 (severe) (10) (**Figure 1**).

Intraobserver and interobserver reproducibility analyses of CAC score were performed by repeating the measurements in 20 random patients by the same primary investigator 2 weeks after the first assessment and by an additional investigator, respectively. During the repeated analysis, the investigators were blinded to any previous results.

The study was approved by the local Ethical Committee of the University Hospital of Brussels and was carried out in accordance with the ethical principles for medical research involving human subjects established by the Declaration of Helsinki, protecting the privacy of all participants as well as the confidentiality of their personal information. All data were fully anonymized. The need for consent in this study was waived by the ethical committee.

Statistical Analysis

Continuous variables were presented as means with standard deviations (SD) or median [interquartile range (IQR)] for skewed variables. Categorical variables were expressed as percentages. Normality of data was tested using Kolmogorov–Smirnov test. Comparisons of continuous variables were done using Student *t*-test or Mann–Whitney *U*-test and of binominal variables using chi-square or Fisher exact test, respectively. Intraobserver and interobserver variability for CAC score assessment was tested by Cohen's Kappa coefficient. The following criteria for Kappa coefficient were used to interpret the results: <0.00 = poor, 0.00–0.20 = light, 0.21–0.40 = fair, 0.41–0.60 = moderate, 0.61–0.80 = substantial, and 0.81–0.99 = almost perfect (11). Univariate and multivariate logistic regression models were used to evaluate potential predictors of MACE. Variables included in the multivariate analysis were chosen based on their statistical significance in the univariate analysis ($p < 0.05$) and on their clinical significance. Specificity, sensitivity, and negative predictive value of CAC score = 0 were calculated using a cross-tabulation table. Specificity was defined as the probability that a test result will be negative when the disease is not present (true negative rate). Sensitivity was defined as the probability that a test result will be positive when the disease is present (true positive rate). Negative predictive value was defined as the probability that the disease is not present when the test is negative (12, 13). Statistical significance was considered for a $p < 0.05$. Statistical analyses were performed using IBM SPSS Statistic for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA).

RESULTS

A total of 280 patients (63.2 ± 16.7 years old, 57.5% male) were included in the analysis.

Mean length of hospitalization was 13.6 ± 13.2 days. Sixty-one patients (21.8%) had at least one MACE: 16 (5.7%) patients presented acute heart failure, 15 (5.3%) patients had atrial

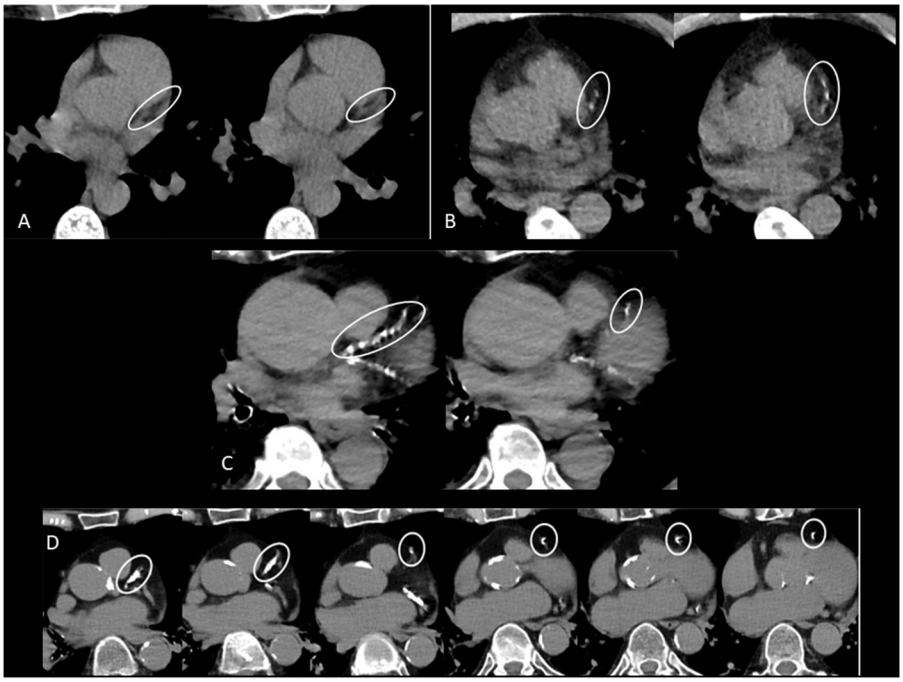


FIGURE 1 | (A) CAC score zero in left anterior descending (LAD) coronary artery. **(B)** Mild CAC in LAD. **(C)** Moderate CAC in LAD. **(D)** Severe CAC in LAD.

fibrillation, 4 (1.4%) patients presented acute coronary syndrome [2 (0.7%) patients had a non-ST elevation myocardial infarction and 2 (0.7%) patients had unstable angina], and 3 (1.0%) patients presented a stroke, respectively. In-hospital mortality rate was 16.1% (45 patients). CAC score = 0 was found in 46.7% (131) patients, vs. 53.2% (149) patients with CAC score ≥1. The baseline characteristics of the study population and the comparison between patients with a CAC score = 0 and CAC score ≥ 1 are shown in **Table 1**.

Univariate analysis for the prediction of MACE is shown in **Supplementary Table 1**.

Multivariate analysis (**Table 2**) showed that a CAC score of 0 was inversely associated with the occurrence of MACE [p = 0.027, odds ratio (OR) = 0.209, 95% confidence interval (CI) 0.052–0.833]. The negative predictive value of CAC score for MACE was 84.5% (sensitivity 72%, specificity 55%).

Reproducibility of CAC score assessment using Cohen's k showed substantial intraobserver and interobserver agreement for the total CAC score assessment (k = 0.859, 95% CI 0.678–1.000, p < 0.001 and k = 0.795, 95% CI 0.581–1.000, p < 0.001, respectively).

DISCUSSION

The main findings of this study were the following: (1) MACE rate in COVID-19 hospitalized patients was 21.8%; (2) the absence of CAC was independently associated with a lower rate of MACE in COVID-19 hospitalized patients.

COVID-19 promotes a rapid systemic inflammation and cytokine storm, which can cause vascular dysfunction, destabilization of atherosclerotic plaques, or myocardial infiltration, which are potential pathways for cardiovascular complications (14). The most commonly reported MACE in COVID-19 hospitalized patients include heart failure, arrhythmia, and acute coronary syndrome, similar to results from the present study (14–16). Moreover, patients with pre-existing cardiac disease are more predisposed to develop cardiac complications during hospitalization for COVID-19 (14, 15).

Similar to previous reports, in the present study, older age was independently associated with worse outcome of COVID-19 patients (17, 18). Moreover, an increased cardiac troponin independently predicted MACE, which is in line with recent studies showing evidence of myocardial injury in hospitalized COVID-19 patients and subsequently increased disease severity (2, 19).

Current guidelines consider CAC score to be a risk modifier in primary prevention of cardiovascular disease (5, 6). Moreover, CAC score has been shown to improve cardiovascular risk prediction in addition to classical risk factors (5, 6, 20) and to be a potential tool for risk reclassification (21–24). The Multi-Ethnic Study of Atherosclerosis (MESA) showed that CAC improved risk prediction at 10-year follow-up compared with traditional risk factors alone (25).

Interestingly, multiple studies have focused on the role of the absence of CAC as a potential downward cardiovascular risk reclassification (26–29). In the present study, the absence of CAC score independently predicted lower MACE rate in COVID-19 hospitalized patients.

TABLE 1 | Comparison between patients with CAC score 0 and those with CAC score ≥1.

	Total ($n = 280$)	CAC score = 0 ($n = 131$)	CAC score ≥ 1 ($n = 149$)	p-value
Age (years)	63.2 ± 16.7	53.7 ± 13.1	72.7 ± 13.2	<0.001
Weight (kg)	80.5 ± 16.7	84.4 ± 16.3	76.6 ± 15.8	<0.001
BMI (kg/m^2)	27.8 ± 5.2	28.9 ± 5.2	26.8 ± 4.9	0.001
Male gender (*n*, %)	161 (57.5)	76 (58.0)	76 (60.3)	0.707
History				
Heart failure (*n*, %)	6 (2.1)	1 (0.8)	5 (4.0)	0.089
Valve disease (*n*, %)	6 (2.1)	1 (0.8)	5 (4.0)	0.089
Atrial fibrillation (*n*, %)	14 (5.0)	3 (2.2)	11 (7.3)	0.023
CKD (*n*, %)	32 (11.4)	9 (6.9)	23 (18.3)	0.006
Chronic pulmonary disease (*n*, %)	43 (15.3)	20 (15.2)	23 (15.4)	0.718
Cancer (*n*, %)	28 (10)	7 (5.3)	21 (16.7)	0.004
Risk factors				
Hypertension (*n*, %)	128 (45.7)	40 (30.5)	78 (61.9)	<0.001
DM (*n*, %)	64 (22.8)	33 (25.2)	31 (24.6)	0.913
Dyslipidemia (*n*, %)	89 (31.8)	39 (29.8)	50 (39.7)	0.095
Smoking (*n*, %)	30 (10.7)	13 (9.9)	17 (13.5)	0.373
Laboratory values				
Hemoglobin (g/dl)	13.5 ± 1.8	13.7 ± 1.7	13.4 ± 2.0	0.205
Platelets (10^3/mm^3)	218.4 ± 86.8	218.3 ± 84.1	212.2 ± 86.5	0.572
WBC (10^3/mm^3)	7.8 ± 4.4	7.2 ± 3.0	8.1 ± 5.0	0.072
CRP (mg/L)	135.7 ± 96.5	128.0 ± 97.9	140.1. 94.1	0.312
D-dimers (ng/ml)	1,638.6 ± 2,720.7	1,048.5 ± 1,442.4	1,830.9 ± 2,613.0	0.042
LDH (U/L)	968.4 ± 1,183.8	988.6 ± 608.8	1,000.3 ± 595.8	0.877
Lactate (mmol/L)	1.0 ± 0.6	1.0 ± 0.5	1.1 ± 0.6	0.659
cTnT (μg/L)	0.02 ± 0.04	0.01 ± 0.01	0.02 ± 0.01	<0.001
Creatinine (mg/dl)	1.2 ± 1.2	1.0 ± 0.9	1.3 ± 1.4	0.012
Chest CT				
Ground-glass opacity (*n*, %)	226 (80.7)	115 (87.7)	111 (74.4)	0.854
Interlobular septal thickening (*n*, %)	25 (8.9)	9 (6.8)	17 (11.4)	0.076
Pulmonary consolidation (*n*, %)	94 (33.5)	44 (33.5)	50 (33.5)	0.267
Pleural effusion (*n*, %)	14 (5.0)	4 (3.0)	10 (6.7)	0.068
ICU admission (*n*, %)	71 (18.2)	33 (25.1)	39 (26.1)	0.500

BMI, body mass index; CKD, chronic kidney disease; DM, diabetes mellitus; WBC, white blood cells; NLR, neutrophil to lymphocyte ratio; CRP, C-reactive protein; LDH, lactate dehydrogenase; cTnT, cardiac troponin T; CT, computed tomography; ICU, intensive care unit; CAC, coronary artery calcium.

Dillinger et al. (30) evaluated the role of CAC in COVID-19 patients hospitalized at the intensive care unit (ICU) and showed that the presence of CAC score was associated with the occurrence of mechanical ventilation, extracorporeal membrane oxygenation, or death. Compared to the present study and other previous series, mortality rate in the study of Dillinger et al. (30) was significantly lower, even if the authors reported only the mortality among ICU patients (2, 19, 31, 32). In our cohort, 71 (25.7%) patients were transferred to ICU, among whom 19 (26.7%) died. Surprisingly, the proportion of elevated CAC score in patients younger than 61 years old was higher in the study of Dillinger et al. (30) compared to the results from our cohort. For the same group of ethnicity, the MESA study showed that CAC score increased with age, which is comparable to data from the present study (33). In contrast to MESA, there was no significant difference in CAC score between genders in this study.

In another recent report by Nai Fovino et al. the presence of CAC in COVID-19 patients was associated with ICU admission and in-hospital mortality (34). However, this study had a small sample population in whom CAC score was evaluated as high or low-intermediate, and potential confounders were not included; therefore, the results cannot be compared to our cohort. Zimmerman et al. also evaluated the role of CAC in the prediction of ICU admission and death in COVID-19 patients (35). Nevertheless, in this study, patients with a history of coronary artery disease were not excluded from the analysis, and the potential relationship between CAC and inflammatory markers was not assessed.

Although recent studies focused on the power of CAC score 0 to predict an improved cardiovascular outcome, data regarding the role of CAC in COVID-19 patients with classical cardiac risk factors are still limited (27, 36). In this study, the absence of CAC translated into a low risk for MACE in COVID-19

TABLE 2 | Predictors of MACE.

	OR	95% CI	*p*
Age	1.067	1.009–1.129	0.024
Male gender	0.702	0.221–2.228	0.548
Atrial fibrillation	1.175	0.182–7.595	0.865
Creatinine	1.018	0.49–2.090	0.962
CRP	1.009	1.004–1.015	0.001
cTnT	1.072	1.026–1.120	0.002
CAC score = 0	0.209	0.052–0.833	0.027

CRP, C-reactive protein; cTnT, cardiac troponin T; CAC, coronary artery calcium; OR, odds ratio; CI, confidence interval; MACE, major adverse cardiovascular events.

patients, independent of age and the presence of risk factors or inflammation, reinforcing the idea that the assessment of CAC score in hospitalized COVID-19 patients could be a useful marker for patients' risk stratification and management.

At the beginning of the pandemic, RT-PCR tests were not widely available; therefore, a systematic chest CT was performed in almost all COVID-19 patients. The ability to assess CAC score on non-gated chest CT allows the application of CAC to the risk evaluation of COVID-19 patients with no additional cost or time consumption. Moreover, most studies report a low-dose radiation for chest CT in COVID-19 patients (37). The semi-qualitative assessment of CAC on routine chest CT has proved to be accurate and reproducible when compared to Agatston scoring (10). Similarly, in our study intraobserver and interobserver reproducibility of CAC score was very good.

Evidence that viral infections represent a trigger for cardiovascular events is increasing, but data regarding long-term follow-up of patients admitted with respiratory viral diseases are still scarce (38, 39). Future directions should focus on the implementation of CAC score into mid-term and long-term follow-up of this particular population, to provide a more precise and earlier estimation of cardiovascular risk.

STUDY LIMITATIONS

This was a single-center study, the sample size was relatively small, and no comparison with a control group was performed; therefore, the extrapolation of these results is limited. The method used to assess CAC is a semi-qualitative scoring system using a non-gated chest CT. The absence of triggering, the lower temporal resolution, and larger field of view which alters the voxel size might modify CAC score assessment. However, this method has been previously validated against quantitative CAC assessment, and its accuracy to predict Agatston score was demonstrated (10).

CONCLUSION

In this study, the absence of CAC had a high negative predictive value for MACE in patients hospitalized with COVID-19, independent of the presence of cardiac risk factors. A semi-qualitative assessment of CAC is a simple, reproducible, and non-invasive measure that may be useful for the risk stratification of COVID-19 patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethics Committee, University Hospital of Brussels. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

M-LL, SL, AM, and BC contributed to the conception and design of the study. M-LL, AM, and SL wrote the first draft of the manuscript. AM and JMa performed the statistical analysis. DB, JMe, SB, and KT performed CT analysis. ES, BR, KV, TD, BK, XG, and CF participated to the investigation, clinical assessment and database. CW, SD, and BC contributed to project administration, supervision and validation. All authors contributed to manuscript revision, read, and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.684528/full#supplementary-material

REFERENCES

1. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. potential effects of coronaviruses on the cardiovascular system: a review. *JAMA Cardiol.* (2020) 5:831–40. doi: 10.1001/jamacardio.2020.1286
2. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* (2020) 5:802–10. doi: 10.1001/jamacardio.2020.0950
3. Wang Y, Lu X, Li Y, Chen H, Chen T, Su N, et al. Clinical course and outcomes of 344 intensive care patients with COVID-19. *Am J Respir Crit Care Med.* (2020) 201:1430–4. doi: 10.1164/rccm.202003-0736LE
4. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3
5. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. 2019 ACC/AHA guideline on the primary

prevention of cardiovascular disease: executive summary: a report of the American college of cardiology/American heart association task force on clinical practice guidelines. *J Am Coll Cardiol.* (2019). 74:1376–414. doi: 10.1161/CIR.0000000000000677

6. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European guidelines on cardiovascular disease prevention in clinical practice: the sixth joint task force of the european society of cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of 10 societies and by invited experts)developed with the special contribution of the European association for cardiovascular prevention & rehabilitation (EACPR). *Eur Heart J.* (2016). 37:2315–81. doi: 10.1016/j.atherosclerosis.2016.05.037
7. Akram K, O'Donnell RE, King S, Superko HR, Agatston A, Voros S. Influence of symptomatic status on the prevalence of obstructive coronary artery disease in patients with zero calcium score. *Atherosclerosis.* (2009) 203:533–7. doi: 10.1016/j.atherosclerosis.2008.07.008
8. Hussain A, Ballantyne CM, Nambi V. Zero coronary artery calcium score: desirable, but enough? *Circulation.* (2020) 142:917–9. doi: 10.1161/CIRCULATIONAHA.119.045026
9. Knez A, Becker A, Leber A, White C, Becker CR, Reiser MF, et al. Relation of coronary calcium scores by electron beam tomography to obstructive disease in 2,115 symptomatic patients. *Am J Cardiol.* (2004) 93:1150–2. doi: 10.1016/j.amjcard.2004.01.044
10. Azour L, Kadoch MA, Ward TJ, Eber CD, Jacobi AH. Estimation of cardiovascular risk on routine chest CT: ordinal coronary artery calcium scoring as an accurate predictor of agatston score ranges. *J Cardiovasc Comput Tomogr.* (2017) 11:8–15. doi: 10.1016/j.jcct.2016.10.001
11. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics.* (1977) 33:159–74. doi: 10.2307/2529310
12. Antonelli P, Chiumello D, Cesana BM. Statistical methods for evidence-based medicine: the diagnostic test. Part II. *Minerva Anestesiol.* (2008) 74:481–8.
13. Cesana BM, Antonelli P, Chiumello D. Statistical methods for evidence-based medicine: the diagnostic test. Part I. *Minerva Anestesiol.* (2008) 74:431–7.
14. Guzik TJ, Mohiddin SA, Dimarco A, Patel V, Savvatis K, Marelli-Berg FM, et al. COVID-19 and the cardiovascular system: implications for risk assessment, diagnosis, and treatment options. *Cardiovasc Res.* (2020) 116:1666–87. doi: 10.1093/cvr/cvaa106
15. Linschoten M, Peters S, van Smeden M, Jewbali LS, Schaap J, Siebelink HM, et al. Cardiac complications in patients hospitalised with COVID-19. *Eur Heart J Acute Cardiovasc Care.* (2020) 9:817–23. doi: 10.1177/2048872620974605
16. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
17. Guan WJ, Liang WH, Zhao Y, Liang HR, Chen ZS, Li YM, et al. Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. *Eur Respir J.* (2020) 55:2001227. doi: 10.1183/13993003.01227-2020
18. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, et al. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. *Int J Infect Dis.* (2020) 94:91–5. doi: 10.1016/j.ijid.2020.03.017
19. Lala A, Johnson KW, Januzzi JL, Russak AJ, Paranjpe I, Richter F, et al. Prevalence and impact of myocardial injury in patients hospitalized with COVID-19 infection. *J Am Coll Cardiol.* (2020) 76:533–46. doi: 10.1101/2020.04.20.20072702
20. Hadamitzky M, Freissmuth B, Meyer T, Hein F, Kastrati A, Martinoff S, et al. Prognostic value of coronary computed tomographic angiography for prediction of cardiac events in patients with suspected coronary artery disease. *JACC Cardiovasc Imaging.* (2009) 2:404–11. doi: 10.1016/j.jcmg.2008.11.015
21. Budoff MJ, Achenbach S, Blumenthal RS, Carr JJ, Goldin JG, Greenland P, et al. Assessment of coronary artery disease by cardiac computed tomography: a scientific statement from the American heart association committee on cardiovascular imaging and intervention, council on cardiovascular radiology and intervention, and committee on cardiac imaging, council on clinical cardiology. *Circulation.* (2006) 114:1761–91. doi: 10.1161/CIRCULATIONAHA.106.178458
22. Greenland P, Bonow RO, Brundage BH, Budoff MJ, Eisenberg MJ, Grundy SM, et al. ACCF/AHA 2007 clinical expert consensus document on coronary artery calcium scoring by computed tomography in global cardiovascular risk assessment and in evaluation of patients with chest pain: a report of the American college of cardiology foundation clinical expert consensus task force (accf/aha writing committee to update the 2000 expert consensus document on electron beam computed tomography) developed in collaboration with the society of atherosclerosis imaging and prevention and the society of cardiovascular computed tomography. *J Am Coll Cardiol.* (2007) 49:378–402. doi: 10.1016/j.jacc.2006.10.001
23. Peters SAE, den Ruijter HM, Bots ML, Moons KGM. Improvements in risk stratification for the occurrence of cardiovascular disease by imaging subclinical atherosclerosis: a systematic review. *Heart.* (2012) 98:177–84. doi: 10.1136/heartjnl-2011-300747
24. Radford NB, DeFina LF, Barlow CE, Lakoski SG, Leonard D, Paixao AR, et al. Progression of CAC score and risk of incident CVD. *JACC Cardiovasc Imaging.* (2016) 9:1420–9. doi: 10.1016/j.jcmg.2016.03.010
25. Budoff MJ, Young R, Burke G, Jeffrey Carr J, Detrano RC, Folsom AR, et al. Ten-year association of coronary artery calcium with atherosclerotic cardiovascular disease (ASCVD) events: the multi-ethnic study of atherosclerosis (MESA). *Eur Heart J.* (2018) 39:2401–8. doi: 10.1093/eurheartj/ehy217
26. Piepoli MF, Abreu A, Albus C, Ambrosetti M, Brotons C, Catapano AL, et al. Update on cardiovascular prevention in clinical practice: a position paper of the European association of preventive cardiology of the European society of cardiology. *Eur J Prev Cardiol.* (2020) 27:181–205. doi: 10.1177/2047487319893035
27. Blaha MJ, Cainzos-Achirica M, Greenland P, McEvoy JW, Blankstein R, Budoff MJ, et al. Role of coronary artery calcium score of zero and other negative risk markers for cardiovascular disease: the multi-ethnic study of atherosclerosis (MESA). *Circulation.* (2016) 133:849–58. doi: 10.1161/CIRCULATIONAHA.115.018524
28. Mortensen MB, Fuster V, Muntendam P, Mehran R, Baber U, Sartori S, et al. Negative risk markers for cardiovascular events in the elderly. *J Am Coll Cardiol.* (2019) 74:1–11. doi: 10.1016/j.jacc.2019.04.049
29. Sarwar A, Shaw LJ, Shapiro MD, Blankstein R, Hoffmann U, Cury RC, et al. Diagnostic and prognostic value of absence of coronary artery calcification. *JACC Cardiovasc Imaging.* (2009) 2:675–88. doi: 10.1016/j.jcmg.2008.12.031
30. Dillinger JG, Benmessaoud FA, Pezel T, Voicu S, Sideris G, Chergui N, et al. Coronary artery calcification and complications in patients with COVID-19. *JACC Cardiovasc Imaging.* (2020) 13:2468–70. doi: 10.1016/j.jcmg.2020.07.004
31. Chen FF, Zhong M, Liu Y, Zhang Y, Zhang K, Su DZ, et al. The characteristics and outcomes of 681 severe cases with COVID-19 in China. *J Crit Care.* (2020) 60:32–7. doi: 10.1016/j.jcrc.2020.07.003
32. Du RH, Liang LR, Yang CQ, Wang W, Cao TZ, Li M, et al. Predictors of mortality for patients with COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study. *Eur Respir J.* (2020) 55:2000524. doi: 10.1183/13993003.00524-2020
33. McClelland RL, Chung H, Detrano R, Post W, Kronmal RA. Distribution of coronary artery calcium by race, gender, and age: results from the multi-ethnic study of atherosclerosis (MESA). *Circulation.* (2006) 113:30–7. doi: 10.1161/CIRCULATIONAHA.105.580696
34. Nai Fovino L, Cademartiri F, Tarantini G. Subclinical coronary artery disease in COVID-19 patients. *Eur Heart J Cardiovasc Imaging.* (2020) 21:1055–6. doi: 10.1093/ehjci/jeaa202
35. Zimmermann GS, Fingerle AA, Muller-Leisse C, Gassert F, von Schacky CE, Ibrahim T, et al. Coronary calcium scoring assessed on native screening chest CT imaging as predictor for outcome in COVID-19: an analysis of a hospitalized German cohort. *PLoS ONE.* (2020) 15:e0244707. doi: 10.1371/journal.pone.0244707
36. Arbas Redondo E, Tebar Marquez D, Poveda Pinedo ID, Dalmau Gonzalez-Gallarza R, Valbuena Lopez SC, Guzman Martinez G, et al. Diagnostic and prognostic value of coronary artery calcium score of zero: is it

time for guidelines to change? *European Heart Journal.* (2020) 41 (Suppl. 2):1383. doi: 10.1093/ehjci/ehaa946.1383
37. Kalra MK, Homayounieh F, Arru C, Holmberg O, Vassileva J. Chest CT practice and protocols for COVID-19 from radiation dose management perspective. *Eur Radiol.* (2020) 30:1–7. doi: 10.1007/s00330-020-07034-x
38. Siriwardena AN. Increasing evidence that influenza is a trigger for cardiovascular disease. *J Infect Dis.* (2012) 206:1636–8. doi: 10.1093/infdis/jis598
39. Xiong TY, Redwood S, Prendergast B, Chen M. Coronaviruses and the cardiovascular system: acute and long-term implications. *Eur Heart J.* (2020) 41:1798–800. doi: 10.1093/eurheartj/ehaa231

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

MINI REVIEW
published: 14 July 2021
doi: 10.3389/fcvm.2021.684780

Review of COVID-19 Myocarditis in Competitive Athletes: Legitimate Concern or Fake News?

Zulqarnain Khan[1]*, *Jonathan S. Na*[1] *and Scott Jerome*[2]

[1] *Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, United States,* [2] *Division of Cardiovascular Medicine, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, United States*

Since the first reported case of COVID-19 in December 2019, the global landscape has shifted toward an unrecognizable paradigm. The sports world has not been immune to these ramifications; all major sports leagues have had abbreviated seasons, fan attendance has been eradicated, and athletes have opted out of entire seasons. For these athletes, cardiovascular complications of COVID-19 are particularly concerning, as myocarditis has been implicated in a significant portion of sudden cardiac death (SCD) in athletes (up to 22%). Multiple studies have attempted to evaluate post-COVID myocarditis and develop consensus return-to-play (RTP) guidelines, which has led to conflicting information for internists and primary care doctors advising these athletes. We aim to review the pathophysiology and diagnosis of viral myocarditis, discuss the heterogeneity regarding incidence of COVID myocarditis among athletes, and summarize the current expert recommendations for RTP. The goal is to provide guidance for practitioners who will be managing and advising athletes in the COVID era.

Keywords: COVID myocarditis, COVID-19, cardiac complications of COVID, COVID athletes, return to play, sports after COVID, pre-participation physicals, sudden cardiac death athletes

OPEN ACCESS

Edited by:
Andrew F. James,
University of Bristol, United Kingdom

Reviewed by:
Bernhard Maisch,
University of Marburg, Germany
Andrew D'Silva,
King's College London,
United Kingdom

***Correspondence:**
Zulqarnain Khan
zulqarnain.khan@som.umaryland.edu

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *24 March 2021*
Accepted: *09 June 2021*
Published: *14 July 2021*

Citation:
Khan Z, Na JS and Jerome S (2021) Review of COVID-19 Myocarditis in Competitive Athletes: Legitimate Concern or Fake News? Front. Cardiovasc. Med. 8:684780. doi: 10.3389/fcvm.2021.684780

INTRODUCTION

In December 2019, the first case of COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was reported in Wuhan, China. As the global landscape has shifted to reflect the pandemic, the sports world has not been immune to these ramifications. Professional and college athletic seasons were abbreviated, fan attendance eliminated, and estimated losses of $92.6K per minute for sports occupations, along with 1.3 million jobs lost (1). While COVID infections have affected competitive athletes in similar rates to the general population, the cardiovascular implications and their ability to resume athletic participation remains unclear. Of particular concern is viral myocarditis, cardiovascular inflammation associated with a significant portion of sudden cardiac death (SCD) in athletes (ranging from 5 to 22% pre-COVID) (2). In 2020, multiple athletes opted to forgo the season due to uncertainty about returning to play following the diagnosis of COVID myocarditis, including Boston Red Sox pitcher, Eduardo Rodriguez. We will briefly review the pathophysiology and diagnosis of viral myocarditis, discuss the incidence of COVID myocarditis among athletes, and reconcile the current recommendations for return-to-play (RTP).

PATHOPHYSIOLOGY

Myocarditis is a nonischemic inflammatory process affecting the myocardium and inducing myocardial injury of varying clinical severity. The etiology of myocarditis may be infectious (viral, bacterial) or noninfectious (toxins, hypersensitivity, autoimmune disorders, and radiation). In viral myocarditis, which may or may not directly translate to COVID, injury to the cardiac muscle is attributable to direct virus-induced damage, as well as subsequent autoimmune inflammation. The acute phase (within hours) of viral myocarditis is comprised of viral entry into myocytes mediated by cell surface receptors (3). Once intracellular, the viral genome is translated into viral proteins, which may disrupt key dystrophin-glycoprotein interactions to impair cardiac function and injure myocyte cytoskeleton to cause myocyte death (4). During the second phase, there is an innate immune response to the viral antigen mediated by humoral (B-cell) and cell-mediated (T-cell) mechanisms. In the third phase, the host immune system may recognize intracellular components released as a result of virus-induced injury as foreign antigens, which may induce an immunologic response and autoantibodies against the myocyte (*via* CD4+ cells stimulating B-cells, cytotoxic CD8+ cells, and cytokines). Over time, these autoantigens may cause chronic myocardial inflammation, further myocyte necrosis, and progression of structural heart disease (dilated cardiomyopathy).

Per Siripanthong et al. (5), the pathophysiology of COVID myocarditis is postulated to be similar with SARS-CoV-2 entering the cell by binding to angiotensin-converting enzyme 2 (ACE2) receptors on cardiomyocyte surfaces, inducing viral replication, and setting off the lymphocytic inflammatory cascade augmented by interleukin 6 (IL-6) mediated cytokine release (**Figure 1**). Based on this animal model, the severity of COVID associated myocarditis may reflect the immune response generated by the host, so young, otherwise healthy, athletes may generate a more robust immunologic reaction to viral infection and experience greater lymphocytic proliferation and cytokine storm.

DIAGNOSIS

Clinical Presentation

The initial presentation of myocarditis is often nonspecific, so a high index of suspicion is required by the clinician. A viral prodrome (congestion, rhinorrhea, cough, and/or fever) may precede viral myocarditis. Young patients, particularly athletes, without coronary artery disease (CAD) risk factors may present with severe chest pain and ST-segment elevations on an electrocardiogram (ECG), described as an "infarct-like" pattern associated with viral myocarditis (6). Alternatively, patients may report various degrees of exertional dyspnea, atypical chest pain, palpitations, and/or generalized fatigue. In extreme cases, previously healthy patients may present with decompensated heart failure or cardiogenic shock (volume overload, depressed cardiac index, and cool extremities). The most morbid presentation is one of a patient with life-threatening arrhythmia or SCD, as a result of the nonischemic ventricular scarring induced by myocarditis, which is a nidus for re-entrant circuits (7).

Exam

The physical exam may demonstrate subtle positional or reproducible chest pain. There may be signs of congestive heart failure, including jugular venous distension (JVD), ascites, abdominal pain, peripheral extremity edema, or crackles on a lung exam. Given the propensity for dysrhythmia, examiners should keenly evaluate for rhythm irregularities, ectopic beats, or rate discrepancies (bradyarrhythmia and tachyarrhythmia). Rarely, patients may present in fulminant cardiogenic shock as a result of COVID myocarditis with hypotension, narrow arterial pulse pressure, cool extremities, and altered mental status (8).

Biomarkers

If viral myocarditis is suspected, clinicians should obtain markers of myocardial injury, including elevated troponin (I or T) and creatinine kinase. Elevated brain natriuretic peptide (BNP) may indicate ventricular dilation or strain from myocardial injury (9). Viral serology testing, although low sensitivity, may be reasonable if evaluating for viral myocarditis [including full respiratory viral panel, as well as SARS-CoV-2 polymerase chain reaction (PCR) testing or antibodies]. Particularly in athletes, alternative etiologies of cardiomyopathy should be excluded, such as substances (i.e., cocaine) and metabolic derangements (thyroid) with urine toxicology, and serum thyroid stimulating hormone (TSH) (10). Inflammatory markers [e.g., C-reactive protein (CRP)] can be obtained and trended with treatment.

Electrocardiogram

In myocarditis, a 12-lead ECG may demonstrate changes such as diffuse ST-segment elevations, T-wave inversions, low-voltage QRS complexes, or even q-waves. As noted above, the infiltrative nature of viral myocarditis may ultimately result in scarring, which can impair the electrophysiological components of the heart. Even transient myocardial inflammation may induce intraventricular conduction delay, AV-block, supraventricular tachycardia (SVT), ventricular tachycardia (VT), ventricular fibrillation (VF), atrial fibrillation, or nonspecific ectopy. If inflammation extends to the pericardium, the ECG may also demonstrate PR-interval depressions (11).

Transthoracic Echocardiography

The diagnostic workup for myocarditis should include a TTE, which can be useful in evaluating for myocarditis as well as excluding alternative etiologies of cardiomyopathy, such as valvular pathology or other structural heart disease (11). In the acute phase of viral infection, myocardial inflammation may be characterized by impaired ventricular function, abnormal ventricular dimensions (i.e., dilation or increased myocardial wall thickness), and/or pericardial effusion. Specifically in this scenario, increased wall thickness in the setting of low voltage on the ECG is suggestive of myocardial edema or infiltrative disease. Chronic myocardial inflammation may cause ventricular dilation, as well as hypokinesis, which may be global or regional (12). Although TTE findings in myocarditis can be nonspecific, specialized modalities that attempt to quantify motion of specific myocardial segments [such as strain rate imaging (SRI)] are

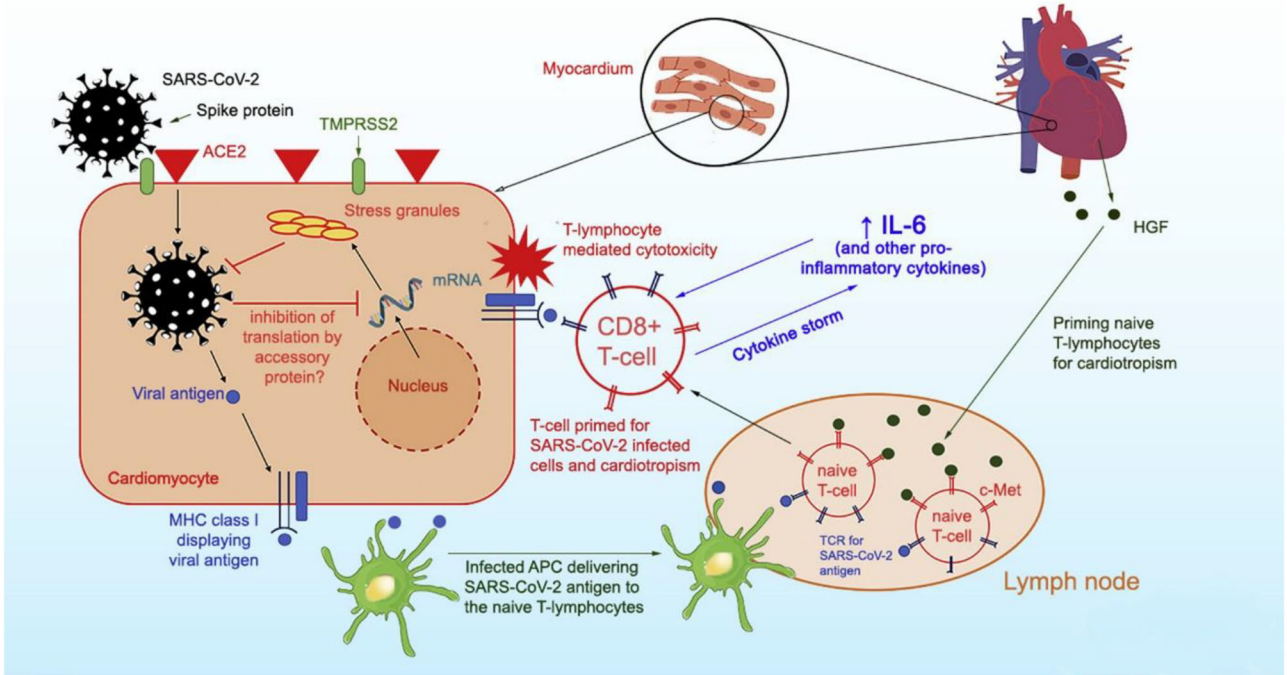


FIGURE 1 | Proposed mechanism of SARS-CoV-2 entry into myocytes and inflammatory pathways causing viral myocarditis. Reprinted with permission (5).

nonstandardized and have only been utilized in case reports (13–15).

Cardiac MRI

Given the nonspecific nature of biomarkers, symptoms, ECG, and TTE in myocarditis, CMR has been heralded as the noninvasive gold standard to evaluate myocardial inflammation, including segments not ideal for biopsy (i.e., epicardium, pericardium) (16). In 2018, the American College of Cardiology (ACC) updated the CMR diagnostic criteria for myocarditis, known as Lake Louise Criteria (LLC), to increase specificity (see **Supplementary Figure 1**) (12, 17). On CMR, there are three proposed diagnostic targets indicative of myocardial inflammation: myocardial edema (mediated by inflammation), hyperemia (due to increased permeability of vascular beds), and myocardial necrosis/scar (reflective of myocyte death).

According to Ferreira et al., these changes are reflected in signal intensity of various modalities within CMR imaging. Myocardial edema leads to prolonged myocardial relaxation time, which can be measured on T1 or T2 weighted images, as well as hyperintensity on T2-weighted images. An expanded extracellular space within myocardium is visualized by increased extracellular volume (ECV) or by administration of gadolinium-based contrast (GBCA), which localizes to inflamed myocardium when measured in T1 weighted imaging, known as early gadolinium enhancement (EGE). Finally, myocardial necrosis leads to scarring, which allows delayed GBCA accumulation known as late gadolinium enhancement (LGE) in T1-weighted imaging. To fulfill the updated LLC for acute myocardial inflammation (**Supplementary Figure 1**), CMR must identify at least one criterion of both myocardial edema (T2-based) AND nonischemic myocardial injury (T1-based). Moreover, the LLC boasts particularly high sensitivity and specificity in acute viral myocarditis, which is characterized by a CMR pattern of subepicardial edema and patchy necrosis [often at the basal inferolateral or lateral wall of the left ventricle (LV)], which may extend to mid-myocardial regions (12).

In addition to diagnostic utility, CMR also has prognostication value, per Gräni et al. In their 2017 CMR evaluation (prior to revision of LLC in 2018) of 670 suspected myocarditis patients, a 2–3 fold increase in hazard ratio was observed in development of major adverse cardiovascular events (MACE) in patients who had LGE on CMR (18). In a prognostic study more relevant for COVID myocarditis, which can present with "infarct-like" findings (positive biomarkers, ST elevations on ECG, and LGE on CMR), Chopra et al. found a greater risk of MACE compared to noninfarct-like presentations (6).

While CMR-based LLC is very accurate for diagnosis of acute inflammation, its sensitivity is reduced as myocardial inflammation becomes more diffuse. In a cost-conscious world, CMR and trained radiologists also remain cost-prohibitive for nonacademic centers.

Endomyocardial Biopsy

The gold standard for identifying myocarditis remains endomyocardial biopsy (EMB) because it allows for

histopathological, immunohistochemically, and molecular biology analysis with few complications (12, 19, 20). Given the patchy distribution of myocarditis, five or six EMB samples are recommended to reduce false negative results, but fewer may be obtained in practice (21). Histological analysis of viral myocarditis demonstrates lymphocytic infiltration of myocardium. Suspected myocardial samples can also be analyzed *via* viral nucleic acid stains and quantitative PCR or RT-PCR to evaluate for the presence of a viral genome. However, given the inherent risks of EMB (albeit cited as <1% by experienced interventionalists) and low sensitivity of obtaining affected samples, centers are more inclined to evaluate for myocarditis noninvasively with CMR and biomarkers.

INCIDENCE

Despite the prowess of diagnostic modalities, reported cases of COVID myocarditis have varied considerably from study to study. An in-depth evaluation reveals that earlier studies were reporting higher incidence of COVID-related myocarditis compared to those published more recently. In July 2020, Puntmann et al. (22) evaluated 100 German patients (nonathletes) recovered from COVID-19 with CMR at a median of 71 days from initial diagnosis and reported that 78% of patients had "abnormal CMR" indicative of cardiac involvement, while 60% had evidence of ongoing myocardial inflammation. These abnormal CMR findings are described as "at least one of the following" from increased myocardial T1 or T2 time, myocardial LGE, or pericardial enhancement. Interestingly, the authors do not directly reconcile their CMR findings with updated or original LLC parameters. Additionally, CMR imaging should ideally be performed in temporal proximity to the acute phase of infection but was done at a median of 71 days after COVID-19 diagnosis in the study, which makes it difficult to interpret clinical significance of the CMR changes. In September 2020, Rajpal et al. (23) published the first major study regarding COVID myocarditis in athletes from The Ohio State University. Twenty-six athletes (football, soccer, lacrosse, basketball, and track), who had PCR-confirmed COVID infection, underwent CMR, TTE, ECG, and troponin measurements following recommended quarantine (11–53 days). The published results indicate that four of these athletes (about 15%) fulfilled 2018 LLC for myocarditis with two out of those four reporting mild dyspnea, while eight others had evidence of LGE without T2 changes (23). While more expeditious than the Puntmann study, there was still latency to perform CMR in Rajpal et al., which may have failed to capture the acute inflammatory period of myocarditis in some cases. Additionally, while the incidence of myocarditis was 15%, the presence of LGE in eight athletes (which represents myocardial scarring) is certainly concerning.

In early 2021, another significant COVID myocarditis study including 145 student athletes was published by Starekova et al. (24), from the University of Wisconsin, who were recovering from COVID asymptomatically or with mild to moderate symptoms. In this elegantly designed study, these athletes underwent CMR, a median of 15 days after diagnosis, as well as measurement of biomarkers, ECG, and TTE. Of the 145 athletes, only two (1.4%) had CMR evidence of myocarditis per updated LLC, as reviewed by two experienced radiologists (24). Notably, one athlete was largely asymptomatic with mild elevation of biomarkers (troponin-I peaked at 0.09 ng/mL), while the other had mild to moderate symptoms for 3 days in the setting of normal biomarkers, and both had normal LV function. As such, the authors questioned the use of CMR as a screening tool for myocarditis in athletes without significant symptoms or abnormal ECG/biomarkers. With similar skepticism, Kawakami et al. (25) published a January 2021 pathological review with autopsy evaluation of 16 hearts (obtained from patients who had died from SARS-CoV-2) and found that only two hearts had PCR-detectable SARS-CoV-2 in the myocardium, but without pathological evidence of myocarditis. Senior author, Dr. Aloke V. Finn, noted that incidence of myocarditis with SARS-CoV-2 is lower than initially reported and cautioned that EMB be reserved for severe cases but admits that these pathological findings are mostly from older patients with co-morbidities, which do not directly translate to a younger population (i.e., athletes). Most recently, two large studies have further elucidated the prevalence of myocardial inflammation in athletes following COVID infection. In March 2021, Martinez et al. (26) evaluated 789 professional North American league athletes following COVID infection, ultimately finding that just five (0.6%) of the 789 had CMR evidence of myocarditis/pericarditis. Subsequently, Moulson et al. (27) released their findings in April 2021 that among 3,018 collegiate athletes who tested positive for COVID, 21 (0.7%) had cardiac involvement per updated LLC.

EXPERT RECOMMENDATIONS

Based on the known risk of SCD in athletes with myocarditis and aforementioned data on COVID myocarditis, various cardiology societies have attempted to generate a RTP consensus. The most up-to-date RTP recommendations for adult athletes from American, European, and Canadian societies are summarized in **Figure 2**. It too is worth mentioning that each society has a slight variation with respect to the isolation or convalescence period in their recommendations (e.g., 7 days to 2 weeks). While there is data extrapolated from animal models suggesting that viral replication and subsequent myocardial injury can be worsened by vigorous activity, there is no guiding data specific to the SARS-CoV-2 virus. As such, each society is basing their recommendations on epidemiologic data, which suggests that SARS-CoV-2 concentration and transmission peaks within the first week of infection, incubation lasts from 2 to 12 days, and cultivable virus is absent after 8 days (28). Taking the data into account, isolation periods ranging from 7 to 14 days seem reasonable to encompass the incubation period and allow athletes to resume their respective RTP workup.

According to the AHA/ACC, adult athletes should abstain from exercise for 10 days (or symptom resolution/no fever for 24 h) following an asymptomatic diagnosis of COVID-19 and gradually return to their previous level of activity with athletic trainer supervision. Meanwhile, per these recommendations,

	Asymptomatic	Mild Symptoms	Moderate Symptoms	Severe Symptoms and/or Hospitalization
American Heart Association (AHA)/American College of Cardiology (ACC) [29]	1. Self-isolation and abstinence from exercise for 10 days from positive test. 2. No cardiovascular risk stratification necessary. 3. Gradual RTP with athletic trainer supervision. 4. If any cardiovascular symptoms, see mild symptoms section.	1. Self-isolation and abstinence from exercise for 10 days from symptom onset. 2. No cardiovascular testing necessary after FULL resolution of symptoms. 3. Gradual RTP with supervision. 4. If protracted, not self-resolving illness, consider using moderate symptom section.	1. Self-isolation and complete rest ≥ 10 days from symptom onset and after symptom resolution. 2. Clinical evaluation, including ECG and troponin and TTE. 3. If normal, gradual RTP with supervision. 4. If abnormal, repeat troponin and consider CMR.	1. If hospitalized, perform troponin and ECG and TTE. 2. If normal, not performed, or non-hospitalized, rest for 2 more weeks after symptoms resolution. 3. Clinical evaluation, ECG, troponin, and TTE (if not done prior). 4. If normal, gradual RTP. 5. If abnormal or new symptoms, repeat workup +/- CMR.
European Association of Preventative Cardiology (EAPC) [33]	1. Basic pre-participation evaluation by team doctor. 2. Gradual RTP with supervision.	1. ECG and TTE 2. Consider CMR if symptoms suggestive of myocarditis. 3. If ECG/TTE normal, perform exercise stress test. 4. RTP if normal stress test. 5. If abnormal, further workup per sports cardiology.	Same as mild symptoms.	1. ECG and TTE and troponin +/- CRP and CMR 2. If above normal, exercise stress test and ambulatory ECG monitoring. 3. If stress test and monitoring normal, gradual RTP with supervision. 4. If any of above abnormal, further workup per sports cardiology.
European Society of Cardiology (ESC) [32]	1. Rest for 2 weeks 2. Symptom evaluation 3. Resting ECG 4. Eligible for RTP if normal. 5. If abnormal, follow mild symptoms recommendations.	1. Rest for at least 2 to 4 weeks 2. Physical Exam and 3. Resting + Exercise ECG and TTE 4. RTP if normal 5. If abnormal, treat as myocarditis.	Same as mild symptoms.	Same as mild symptoms.
Canadian Cardiovascular Society (CCS) [30]	1. Refrain from moderate exercise for ≥ 7 days after resolution of viral symptoms. 2. Focused cardiac symptom history (loss of consciousness, chest pain, dyspnea, increased resting heart rate, palpitations, or reduced fitness). 3. If none of above, gradual RTP. 4. If present, proceed to mild symptoms section.	1. Continued restriction of exercise until after resolution of viral symptoms. 2. Detailed cardiac history and physical exam. 3. Consider ECG and Troponin. 4. If abnormal findings, referral to cardiology and advanced imaging (TTE and/or CMR).	Do not recommend stratifying based on severity of COVID-19 viral symptoms. Follow mild symptoms section.	Do not recommend stratifying based on severity of COVID-19 viral symptoms. Follow mild symptoms section.

FIGURE 2 | Summary of return to play (RTP) recommendations from major cardiology societies. ECG, electrocardiogram; TTE, transthoracic echocardiogram; CMR, cardiac magnetic resonance imaging; CRP, C-reactive protein.

mildly symptomatic athletes recovering from COVID-19 do not require extensive risk stratification beyond history and physical exam if their mild symptoms were self-limited. However, in athletes with moderate to severe or not self-resolving symptoms, extensive cardiovascular risk stratification is needed, including ECG, biomarkers, and TTE (29). If testing is normal, then athletes may RTP gradually with supervision of athletic trainers, while abnormal testing or development of new cardiovascular symptoms warrants repeat biomarkers and CMR.

In contrast to the American recommendations, the European and Canadian societies are more pragmatic with RTP screening, while acknowledging the inability to offer universal cardiovascular testing in all COVID-infected athletes. Yet there are key differences between the Canadian Cardiovascular Society (CCS) and European Association of Preventative Cardiology (EAPC)/European Society of Cardiology (ESC) recommendations particularly when it pertains to COVID-symptom based stratification. According to McKinney et al., athletes should not be risk stratified based on their viral COVID illness symptoms, rather with the reporting or development of cardiovascular symptoms following recovery from acute viral illness. The CCS recommendation is based on the lack of association between severity of COVID illness and development of myocarditis, which is consistent with recent studies that have mostly identified myocarditis in asymptomatic or mildly symptomatic athletes. At that point, a cardiac symptom questionnaire should be administered; if no cardiac symptoms are reported, athletes may gradually RTP following at least 7 days of viral symptom resolution. COVID-infected athletes who report having the aforementioned cardiac symptoms require a focused history and physical exam, consideration of ECG/troponin, and referral to cardiology (for TTE and/or CMR) if any abnormal findings noted (30). Meanwhile, the EAPC and ESC advocate for use of exercise stress testing in symptomatic

athletes more than the Canadian or American societies; the EAPC recommends athletes with mild to moderate symptoms should undergo ECG and TTE, then exercise stress testing for eligibility to RTP if normal, while the ESC recommends exercise ECG in tandem with TTE. However, while the ESC maintains the same recommendations for severe/hospitalization symptoms as mild to moderate cases (akin to CCS), the EAPC is more in line with the AHA/ACC in recommending a more rigorous cardiovascular evaluation consisting of imaging, biomarkers, and stress testing.

In athletes diagnosed with COVID myocarditis, the 2015 recommendations for sports eligibility by "Task Force 3" (comprised of AHA and ACC) (31) should be adapted (see **Supplementary Figure 2**).

CONCLUSION

Early pandemic studies in nonathletes reported higher incidence of COVID-related cardiac involvement, while recent publications indicate that incidence of COVID myocarditis in adult athletes is not robust as initially feared. While the recommendations by various cardiology societies are an excellent resource, there remain limitations with regards to stratifying athletes by symptoms of viral illness. In the cited cases of athletes with CMR-proven COVID myocarditis, the affected athletes had mild to no symptoms, which means they could have been eligible for RTP without further workup per AHA/ACC and CCS guidelines (23, 24). Furthermore, at least in the Starekova et al. study, both athletes with COVID myocarditis had normal LV function, so they may have also evaded the EAPC/ESC recommendations for further workup. Nonetheless, as suggested by Moulson et al. (27), primary screening *via* CMR is also low yield unless prompted by ECG, TTE, or biomarkers. Mitigating the low prevalence of cardiac involvement in athletes with COVID with the risk of SCD, moving forward with a symptom-based approach, suggested by most societies, to guide RTP seems most appropriate.

AUTHOR CONTRIBUTIONS

JN and SJ contributed to the development and writing of the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors would like to acknowledge the University of Maryland School of Medicine, Department of Internal Medicine and Division of Cardiology.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.684780/full#supplementary-material

REFERENCES

1. Burrow G. *The Economic Impact of COVID-19 on US Sports*. Economic Modeling. Available online at: https://www.economicmodeling.com/2020/05/28/the-economic-impact-of-covid-19-on-us-sports-up-to-92-6k-lost-every-minute/ (accessed January 02, 2021).
2. Shah N, Phelan DMJ. *Myocarditis in the Athlete*. American College of Cardiology (2018). Available online at: https://www.acc.org/latest-in-cardiology/articles/2018/01/18/15/00/myocarditis-in-the-athlete (accessed January 2, 2021).
3. Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, et al. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. *Science.* (1997) 275:1320–3. doi: 10.1126/science.275.5304.1320
4. Badorff C, Lee GH, Lamphear BJ, Martone ME, Campbell KP, Rhoads RE, et al. Enteroviral protease 2A cleaves dystrophin: evidence of cytoskeletal disruption in an acquired cardiomyopathy. *Nat Med.* (1999) 5:320–6. doi: 10.1038/6543
5. Siripanthong B, Nazarian S, Muser D, Deo R, Santangeli P, Khanji MY, et al. Recognizing COVID-19 related myocarditis: the possible pathophysiology and proposed guideline for diagnosis and management. *Heart Rhythm.* (2020) 17:1463–71. doi: 10.1016/j.hrthm.2020.05.001
6. Chopra H, Arangalage D, Bouleti C, Zarka S, Fayard F, Chillon S, et al. Prognostic value of the infarct- and non-infarct like patterns and cardiovascular magnetic resonance parameters on long-term outcome of patients after acute myocarditis. *Int J Cardiol.* (2016) 212:63–9. doi: 10.1016/j.ijcard.2016.03.004
7. Peretto G, Sala S, Rizzo S, De Luca G, Campochiaro C, Sartorelli S, et al. Arrhythmias in myocarditis: state of the art. *Heart Rhythm.* (2019) 16:793–801. doi: 10.1016/j.hrthm.2018.11.024
8. Kociol RD, Cooper LT, Fang JC, Moslehi JJ, Pang PS, Sabe MA, et al. Recognition and initial management of fulminant myocarditis: a scientific statement from the American Heart Association. *Circulation.* (2020) 141:e69–e92. doi: 10.1161/CIR.0000000000000745
9. Inciardi RM, Lupi L, Zaccone G, Italia L, Raffo M, Tomasoni D, et al. Cardiac involvement in a patient with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:819–24. doi: 10.1001/jamacardio.2020.1096
10. Trachtenberg BH, Hare JM. Inflammatory cardiomyopathic syndromes. *Circ Res.* (2017) 121:803–18. doi: 10.1161/CIRCRESAHA.117.310221
11. Caforio AL, Pankuweit S, Arbustini E, Basso C, Gimeno-Blanes J, Felix SB, et al. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J.* (2013) 34:2636–48. 2648a–d. doi: 10.1093/eurheartj/eht210
12. Ferreira VM, Schulz-Menger J, Holmvang G, Kramer CM, Carbone I, Sechtem U, et al. Cardiovascular magnetic resonance in nonischemic myocardial inflammation: expert recommendations. *J Am Coll Cardiol.* (2018) 72:3158–76. doi: 10.1016/j.jacc.2018.09.072
13. Kaluzynski K, Chen X, Emelianov SY, Skovoroda AR, O'Donnell M. Strain rate imaging using two-dimensional speckle tracking. *IEEE Trans Ultrason Ferroelectr Freq Control.* (2001) 48:1111–23. doi: 10.1109/58.935730
14. Uziebło-Zyczkowska B, Mielniczuk M, Ryczek R, Krzesiński P. Myocarditis successfully diagnosed and controlled with speckle tracking echocardiography. *Cardiovasc Ultrasound.* (2020) 18:19. doi: 10.1186/s12947-020-00203-4
15. Kostakou PM, Kostopoulos VS, Tryfou ES, Giannaris VD, Rodis IE, Olympios CD, et al. Subclinical left ventricular dysfunction and correlation with regional strain analysis in myocarditis with normal ejection fraction. A new diagnostic criterion. *Int J Cardiol.* (2018) 259:116–21. doi: 10.1016/j.ijcard.2018.01.058

16. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC guidelines for the diagnosis treatment of acute chronic heart failure: the Task Force for the Diagnosis Treatment of Acute Chronic Heart Failure of the European Society of Cardiology (ESC) developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*. (2016) 37:2129–200. doi: 10.1093/eurheartj/ehw128
17. Luetkens JA, Faron A, Isaak A, Dabir D, Kuetting D, Feisst A, et al. Comparison of original and 2018 Lake Louise criteria for diagnosis of acute myocarditis: results of a validation cohort. *Radiol Cardiothorac Imaging*. (2019) 1:e190010. doi: 10.1148/ryct.2019190010
18. Gräni C, Eichhorn C, Bière L, Murthy VL, Agarwal V, Kaneko K, et al. Prognostic value of cardiac magnetic resonance tissue characterization in risk stratifying patients with suspected myocarditis. *J Am Coll Cardiol*. (2017) 70:1964–76. doi: 10.1016/j.jacc.2017.08.050
19. Cooper LT, Baughman KL, Feldman AM, Frustaci A, Jessup M, Kuhl U, et al. The role of endomyocardial biopsy in the management of cardiovascular disease: a scientific statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology. Endorsed by the Heart Failure Society of America and the Heart Failure Association of the European Society of Cardiology. *J Am Coll Cardiol*. (2007) 50:1914–31. doi: 10.1093/eurheartj/ehm456
20. Yilmaz A, Kindermann I, Kindermann M, Mahfoud F, Ukena C, Athanasiadis A, et al. Comparative evaluation of left and right ventricular endomyocardial biopsy: differences in complication rate and diagnostic performance. *Circulation*. (2010) 122:900–9. doi: 10.1161/CIRCULATIONAHA.109.924167
21. Tschöpe C, Ammirati E, Bozkurt B, Caforio ALP, Cooper LT, Felix SB, et al. Myocarditis and inflammatory cardiomyopathy: current evidence and future directions. *Nat Rev Cardiol*. (2020) 18:169–93. doi: 10.1038/s41569-020-00435-x
22. Puntmann VO, Carerj ML, Wieters I, Fahim M, Arendt C, Hoffmann J, et al. Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19). *JAMA Cardiol*. (2020) 5:1265–73. doi: 10.1001/jamacardio.2020.3557
23. Rajpal S, Tong MS, Borchers J, Zareba KM, Obarski TP, Simonetti OP, et al. Cardiovascular magnetic resonance findings in competitive athletes recovering from COVID-19 infection. *JAMA Cardiol*. (2020) 6:116–8. doi: 10.1001/jamacardio.2020.4916
24. Starekova J, Bluemke DA, Bradham WS, Eckhardt LL, Grist TM, Kusmirek JE, et al. Evaluation for myocarditis in competitive student athletes recovering from coronavirus disease 2019 with cardiac magnetic resonance imaging. *JAMA Cardiol*. (2021). doi: 10.1001/jamacardio.2020.7444. [Epub ahead of print].
25. Kawakami R, Sakamoto A, Kawai K, Gianatti A, Pellegrini D, Nasr A, et al. Pathological evidence for SARS-CoV-2 as a cause of myocarditis: JACC review topic of the week. *J Am Coll Cardiol*. (2021) 77:314–25. doi: 10.1016/j.jacc.2020.11.031
26. Martinez MW, Tucker AM, Bloom OJ, Green G, DiFiori JP, Solomon G, et al. Prevalence of inflammatory heart disease among professional athletes with prior COVID-19 infection who received systematic return-to-play cardiac screening. *JAMA Cardiol*. (2021). doi: 10.1001/jamacardio.2021.0565. [Epub ahead of print].
27. Moulson N, Petek BJ, Drezner JA, Harmon KG, Kliethermes SA, Patel MR, et al. SARS-CoV-2 cardiac involvement in young competitive athletes. *Circulation*. (2021). doi: 10.1161/CIRCULATIONAHA.121.054824. [Epub ahead of print].
28. Atlani-Duault L, Lina B, Malvy D, Yazdanpanah Y, Chauvin F, Delfraissy JF. COVID-19: France grapples with the pragmatics of isolation. *Lancet Public Health*. (2020) 5:e573–e4. doi: 10.1016/S2468-2667(20)30235-8
29. Kim JH, Levine BD, Phelan D, Emery MS, Martinez MW, Chung EH, et al. Coronavirus disease 2019 and the athletic heart: emerging perspectives on pathology, risks, and return to play. *JAMA Cardiol*. 6:219–27. (2020). doi: 10.1001/jamacardio.2020.5890
30. McKinney J, Connelly KA, Dorian P, Fournier A, Goodman JM, Grubic N, et al. COVID-19-myocarditis and return to play: reflections and recommendations from a Canadian Working Group. *Can J Cardiol*. (in press). doi: 10.1016/j.cjca.2020.11.007
31. Maron BJ, Udelson JE, Bonow RO, Nishimura RA, Ackerman MJ, Estes NA 3rd, et al. Eligibility and disqualification recommendations for competitive athletes with cardiovascular abnormalities: Task Force 3: hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy and other cardiomyopathies, and myocarditis: a scientific statement from the American Heart Association and American College of Cardiology. *Circulation*. (2015) 132:e273–80. doi: 10.1161/CIR.0000000000000239
32. Schellhorn P, Klingel K, Burgstahler C. Return to sports after COVID-19 infection. *Eur Heart J*. (2020) 41:4382–4. doi: 10.1093/eurheartj/ehaa448
33. Bhatia RT, Marwaha S, Malhotra A, Iqbal Z, Hughes C, Börjesson M, et al. Exercise in the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) era: a question and answer session with the experts endorsed by the section of sports cardiology & exercise of the European Association of Preventive Cardiology (EAPC). *Eur J Prev Cardiol*. (2020) 27:1242–51. doi: 10.1177/2047487320930596

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 20 July 2021
doi: 10.3389/fcvm.2021.691609

Pulmonary Rehabilitation Accelerates the Recovery of Pulmonary Function in Patients With COVID-19

Pengfei Zhu[1†], *Zhengchao Wang*[2†], *Xiaomi Guo*[3†], *Zhiyong Feng*[4], *Chaochao Chen*[4], *Ai Zheng*[4], *Haotian Gu*[5*‡] and *Yu Cai*[4*‡]

[1] Department of Cardiology, Wuhan Fourth Hospital, Puai Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [2] Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [3] Department of Ultrasound, Wuhan Asia General Hospital, Wuhan, China, [4] Department of Rehabilitation, Wuhan Fourth Hospital, Puai Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [5] British Heart Foundation Centre of Research Excellence, King's College London, London, United Kingdom

OPEN ACCESS

Edited by:
Sebastian Kelle,
Deutsches Herzzentrum
Berlin, Germany

Reviewed by:
Wen-Yi Yang,
Shanghai Jiao Tong University, China
Jing Wang,
Wuhan University, China

***Correspondence:**
Yu Cai
caiy_kf@163.com
Haotian Gu
haotian.gu@kcl.ac.uk

†These authors share first authorship

‡These authors share senior authorship

Specialty section:
This article was submitted to Cardiovascular Imaging, a section of the journal Frontiers in Cardiovascular Medicine

Received: 06 April 2021
Accepted: 21 June 2021
Published: 20 July 2021

Citation:
Zhu P, Wang Z, Guo X, Feng Z, Chen C, Zheng A, Gu H and Cai Y (2021) Pulmonary Rehabilitation Accelerates the Recovery of Pulmonary Function in Patients With COVID-19. Front. Cardiovasc. Med. 8:691609. doi: 10.3389/fcvm.2021.691609

Objectives: To evaluate the effect of in-hospital pulmonary rehabilitation (PR) on short-term pulmonary functional recovery in patients with COVID-19.

Methods: Patients with COVID-19 ($n = 123$) were divided into two groups (PR group or Control group) according to recipient of pulmonary rehabilitation. Six-min walk distance (6MW), heart rate (HR), forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), diffusing capacity of the lung for carbon monoxide (DL_{CO}), and CT scanning were measured at the time of discharge, 1, 4, 12, and 24 weeks.

Results: At week one, both PR group and Control group showed no significant changes in pulmonary function. At 4 and 12 weeks, 6MW, HR, FVC, FEV_1, and DL_{CO} improved significantly in both groups. However, the improvement in the PR group was greater than the Control group. Pulmonary function in the PR group returned to normal at 4 weeks [FVC (% predicted, PR vs. Control): 86.27 ± 9.14 vs. 78.87 ± 7.55; FEV1 (% predicted, PR vs. Control) 88.76 ± 6.22 vs. 78.96 ± 6.91; DLCO (% predicted, PR vs. Control): 87.27 ± 6.20 vs. 77.78 ± 5.85] compared to 12 weeks in the control group [FVC (% predicted, PR vs. Control): 90.61 ± 6.05 vs. 89.96 ± 4.05; FEV1 (% predicted, PR vs. Control) 94.06 ± 0.43 vs. 93.85 ± 5.61; DLCO (% predicted, PR vs. Control): 91.99 ± 8.73 vs. 88.57 ± 5.37]. Residual lesions on CT disappeared at week 4 in 49 patients in PR group and in 28 patients in control group ($p = 0.0004$).

Conclusion: Pulmonary rehabilitation could accelerate the recovery of pulmonary function in patients with COVID-19.

Keywords: pulmonary training, corona virus disease 2019, pulmonary function, pulmonary rehabilitation, 2019-nCoV

INTRODUCTION

Corona Virus Disease (COVID-19) caused by a novel coronavirus named as Severe Acute Respiratory Syndrome (SARS)-CoV (Corona Virus)-2 has been rapidly occurring the world and is not completely controlled till now (1, 2). Transmissions through fecal-oral route and ocular are also considered to be possible while evidences are not sufficient till now (3, 4). All age groups are susceptible to SARS-CoV-2, while the elderlies and people with underlying diseases are more likely to develop severe conditions such as severe pneumonia and respiratory failure in a short period of time (2). The therapeutic principles of COVID-19 include general treatment (vital sign monitoring, mechanical ventilation, etc.), drug therapy (anti-infection drugs, traditional Chinese medicine, etc.), pulmonary rehabilitation (PR), nutrition management and mental support.

Pulmonary rehabilitation, as a comprehensive intervention including exercise training, education and behavioral changes that aims to improve the physical and psychological condition in patients with respiratory disease and to promote high long-term quality of life. It has also been confirmed to be an important part of the integrated care strategy for chronic obstructive pulmonary disease (COPD) (5, 6). Its positive effects in preoperative pulmonary rehabilitation were also discovered including reducing the sensation of dyspnea, reducing muscle strength loss associated with dyspnea, and improving psychologic states (7). As for infectious disease of respiratory system, Hsieh et al. (8) found that survivors of acute respiratory distress syndrome (ARDS) caused by influenza A (H1N1) who received pulmonary rehabilitation for 2 months had improved pulmonary function, exercise capacity, and quality of life.

Therefore, the aim of present study was to evaluate the effect of in-hospital pulmonary rehabilitation on short-term pulmonary functional recovery in patients with COVID-19.

METHODS

Patients and Data Collection

We conducted a perspective observational study in patients with COVID-19.

Participants were recruited from Puai Hospital, Wuhan Forth Hospital and Huazhong University of Science and Technology, and were divided into two groups according to whether patients received in-hospital pulmonary rehabilitation. Patients who underwent in-hospital pulmonary rehabilitation were based on the clinical judgements by attending physicians. No patients were directly involved in the design, planning and conception of this study. Inclusion criteria were: (1) patients with COVID-19; (2) able to receive pulmonary rehabilitation; (3) no co-infection of other pathogene; (4) sign the informed consent. Exclusion criteria include: (1) suffering from high blood pressure, diabetes, or other chronic or basic diseases; (2) COVID-19 recurrence during the follow-up period. (3) infection of other pathogene during the follow-up period. (4) pregnancy before or during the follow-up period. Data were collected at the time of discharge and 1, 4, 12, 24 weeks after discharge. The study was approved by Chinese Clinical Trial Registry (ChiCTR2000031751).

Pulmonary Rehabilitation

In the PR group, all patients underwent a standardized rehabilitation scheme (ref) when their clinical condition was stable and capable of PR. Detailed PR protocol as follow: (1) allow patients to maintain regular movement, such as chest expansion and ambulation, in the isolation ward for at least 1 h per day while monitoring heart rate and respiratory rate during movement to avoid overexertion in terms of heart and lung function; (2) provide respiratory control training: Help the patients sit in an upright position to avoid orthopnea. If the patients could not sit upright, lift the head of bed by 60 degrees. Let the patients relax their shoulder muscles by placing one hand on the chest and the other on the abdomen, instruct the patients to deeply breathe in through their nose and breathe out through their mouth to expand the lower chest. (3) pursed lip breathing: Keep the same patient position as with respiratory control. Let the patients breathe in through their nose, hold their breath for 2 s, then deeply breathe out using their abdomen for 3–5 s with their mouth pursed as if they are whistling; this increases the expiratory resistance and prolongs the expiratory time. For (2) and (3) above, the patients were trained repeatedly for 10–15 min each and 4 times per day. The patients could train along with light music if possible. If any discomfort occurred, the training should be stopped immediately.

Outcome Measures

Six-min walk distance (6MW), Heart rate (HR), forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), and Diffusing capacity of the lung for carbon monoxide (DL_{CO}) were measured. CT scanning was conducted at discharge, 4, and 24 weeks. FVC and FEV_1 were measure using spirometry. Spirometry was performed using the Medical Graphics CPXD (Minneapolis, MN, US). Diffusing capacity of the lung for carbon monoxide (DL_{CO}) were assessed using the rebreathe technique and a mass spectrometer (Perkin Elmer, St. Louis, MO, USA) as previously described (9, 10). CT scan was conducted using a 64-slice spiral CT machine (NeuSoft, NeuViz64). The CT images was evaluated by two experienced imaging clinicians. If their opinions were different, a third clinician was invited to make the final decision.

Statistical Analysis

Statistical analysis was completed using SPSS 21.0. Baseline differences between groups were analyzed by Student's *t*-test for continuous data and by the χ^2 test for categorical data. Continuous data are expressed as the means ± SDs, and the normality of distribution was tested by a QQ plot. The data were analyzed using Student's *t*-test and repeated measures analysis of variance (ANOVA). As for repeated measures ANOVA, *post-hoc* test of *p*-value was adopted by Bonferroni correction and effect size was expressed as eta-square. A value of $p < 0.05$ was considered statistically significant. Because of a small sample size, *p*-valued between 0.05 and 0.1 was marked with specific value.

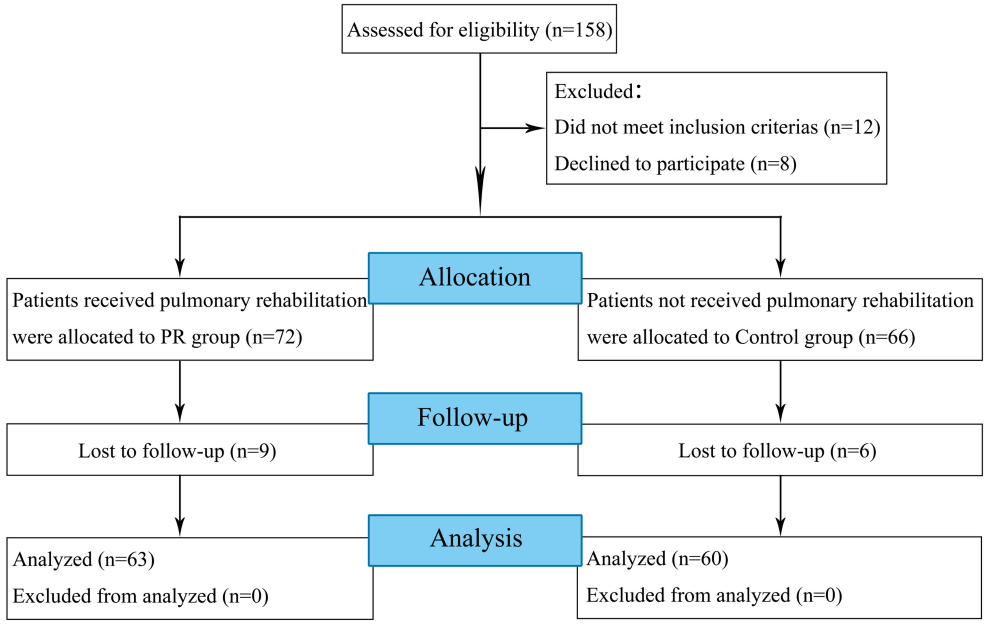


FIGURE 1 | Flow diagram of the study design.

RESULTS

A total of 158 participants were screened between February 1st 2020 to March 31st 2020, out of whom 20 patients were excluded because of not meeting the inclusion criteria or declined to participate in this study (**Figure 1**). Fifteen participants were lost to follow-up before 4 weeks follow-up. Baseline demographics were shown in **Tables 1**–**3**.

6MW and HR were shown in **Table 4**. At the time of discharge, 6MW distance in PR group was longer than the Control group and the HR was lower than the Control group, but did not reach significant. At the time of week 1 and 4, there were significant improvements of 6MW and HR in PR group compared to those at the time of discharge (week 1: 495.88 ± 34.67 vs. 470.83 ± 35.70 $p < 0.05$ and 83.24 ± 8.46 vs. 97.05 ± 14.24 $p < 0.001$; week 4: 557.94 ± 38.44 vs. 514.22 ± 43.47 $p < 0.01$ and 78.59 ± 6.73 vs. 88.61 ± 9.37 $p < 0.001$). However, in the Control group, only an improvement of 6MW was found at 4 weeks and was smaller than the PR group. At 12 and 24 weeks, 6MW and HR were similar in two groups.

The measurements of FVC and FEV_1 is shown in **Table 5**. At the time of discharge and week 1, FEV_1 in the PR group was significantly larger than that in the Control group. There was no significant difference in FVC between the two groups at week 1 (2.05 ± 0.26 vs. 1.91 ± 0.21, $p = 0.096$). Although FVC and FEV1 improved significantly in both groups, there was greater improvement in the PR groups than the Control group at week 4. FEV_1 and FVC in the PR group exceeded 80% of predicted values at 4 weeks [FVC (% predicted): 86.27 ± 9.14 vs. 78.87 ± 7.55, $p < 0.05$; FEV1 (% predicted) 88.76 ± 6.22 vs. 78.96 ± 6.91, $p < 0.001$]. At 12 and 24 weeks, there were no significant difference in FEV_1 and FVC between two groups and FEV1 and FVC reached 90% of predicted values. There was no significant change in FEV_1 to FVC ratio during the entire follow-up period.

TABLE 1 | General characteristics in PR and control groups.

	No. (%)		*p*-value
	PR Group (*n* = 63)	**Control Group (*n* = 60)**	
Age (years)	36.59 ± 7.01	35.47 ± 7.58	0.40
Gender			0.53
Male	34	29	
Female	29	31	
Blood pressure (mmHg, at discharge)			
Systolic pressure	116.3 ± 4.4	115.8 ± 5.2	0.57
Diastolic pressure	78.7 ± 3.2	78.1 ± 3.5	0.32
Weight (kg, at discharge)	62.4 ± 11.3	64.0 ± 10.9	0.43
Height (cm, at discharge)	167.6 ± 13.2	167.8 ± 12.9	0.93
Personal habits			
Smoking	7 (11.1)	9 (15.0)	0.52
Drinking	4 (6.3)	3 (5.0)	0.75
Education			0.66
Junior high school or below	12	9	
High school or vocational school	19	24	
College degree	16	17	
Bachelor degree	12	8	
Postgraduate degree or above	4	2	

PR, pulmonary rehabilitation.

TABLE 2 | Clinical characteristics in PR and control groups.

	No. (%)		*p*-value
	PR Group (*n* = 63)	**Control Group (*n* = 60)**	
Clinical Presentation			
Fever	62 (98.8)	59 (98.3)	1.00
Dry cough	45 (71.4)	41 (68.3)	0.71
Headache	5 (7.9)	4(6.7)	1.00
Sore throat	7 (11.1)	5 (8.3)	0.40
Myalgia	21(33.3)	18 (30.0)	0.69
Fatigue	24 (38.1)	19 (31.7)	0.46
Dyspnoea	28 (44.4)	21 (35.0)	0.29
Rhinorrhoea	13 (20.6)	11 (18.3)	0.75
Nausea & vomiting	18 (28.6)	19 (31.7)	0.71
Diarrhea	12 (19.0)	10 (16.7)	0.73
Length of Hospital stay (days)	21.18 ± 4.98	21.94 ± 3.24	0.32

PR, pulmonary rehabilitation.

TABLE 3 | Results of laboratory examination at discharge.

	PR Group (*n* = 63)	**Control Group (*n* = 60)**	***p*-value**
Blood Count			
WBC ($\times 10^9$/L)	7.14 ± 3.41	6.86 ± 2.99	0.63
Lymphocyte count ($\times 10^9$/L)	0.62 ± 0.08	0.66 ± 0.09	0.20
PLT at discharge ($\times 10^9$/L)	243 ± 99	216 ± 71	0.09
Hemoglobin (g/dL)	118 ± 23	125 ± 17	0.10
Coagulation Function			
PT (s)	14.1 ± 3.3	13.2 ± 1.4	0.08
APTT (s)	37.6 ± 9.0	37.2 ± 6.2	0.82
D-dimer (mg/L)	1.7 ± 2.4	1.3 ± 1.9	0.24
Blood Biochemistry			
TP (g/L)	64.5 ± 10.3	66.5 ± 7.2	0.21
Albumin (g/L)	34.6 ± 5.8	37.5 ± 6.4	0.10
ALT (U/L)	35 ± 19	40 ± 22	0.14
AST (U/L)	30 ± 15	34 ± 19	0.20
TB (μmol/L)	11.8 ± 5.5	12.5 ± 6.2	0.51
Sodium (mmol/L)	137.7 ± 5.3	138.5 ± 3.3	0.30
Potassium (mmol/L)	4.1 ± 0.5	3.9 ± 0.4	0.09
Creatinine (μmol/L)	71.2 ± 27.5	69.1 ± 20.4	0.62
BUN (mmol/L)	5.2 ± 2.1	5.4 ± 2.3	0.66
LDH (U/L)	239 ± 133	213 ± 127	0.27
CK-MB (U/L)	10.9 ± 8.5	11.8 ± 7.7	0.53
Infection-Related Biomarkers			
CRP (mg/L)	23 ± 34	18 ± 25	0.34
PCT (ng/ml)	0.18 ± 0.45	0.11 ± 0.18	0.21

PR, pulmonary rehabilitation; RBC, red blood cell, WBC, white blood cell; PT, prothrombin time; PLT, platelet; APTT, activated partial thromboplastin time; FBG, fasting blood glucose; TP, total protein; ALT, alanine transaminase; AST, aspertate aminotransfera; TB, total bilirubin; BUN, blood urea nitrogen; LDH, lactate dehydrogenase; CK-MB, creatine kinase–MB; CRP, hypersensitive C-reactive protein; PCT, procalcitonin.

DL_{CO} was shown in **Table 6**. At the first week after discharge, no improvements were discovered in DL_{CO}. Meanwhile, the DL_{CO} of PR group was higher than Control group (19.65 ± 2.12 vs. 17.03 ± 1.94, $p < 0.01$). Significant improvements were discovered at 4 weeks, while level of DL_{CO} in the PR group was higher than the Control group [DLCO (% predicted): 87.27 ± 6.20 vs. 77.78 ± 5.85, $p < 0.001$]. At 12 and 24 weeks, DL_{CO} reached normal level and had no significantly differences between two groups.

As shown in **Figure 2**, in the PR group, little parenchymal bands with group-glass opacity were observed at the time of discharge in all patients. The lesions of 49 patients (77.8%) in PR group basically disappeared at 4 weeks follow-up and no changes were discovered at 24 weeks. The CT images of 60 patients (95.2%) in PR group were basically normal at 24 weeks. In the control group, little parenchymal bands with more group-glass opacities were observed at the time of discharge in all patients. At 4 weeks follow-up, some group-glass opacities still existed in CT images of 32 patients (53.3%). The lesions of only 28 patients (46.7%, $p = 0.0004$ vs. PR group) in control group basically disappeared at 4 weeks follow-up and no changes were discovered at 24 weeks. The CT images of 56 patients (93.3%, $p = 0.65$ vs. PR group) in control group were basically normal at 24 weeks.

DISCUSSION

The main physiological change in patient recovery from COVID-19 is poorer cardio-pulmonary function, and lower FVC, FEV_1, and DL_{CO}. Meanwhile most of the values of FEV_1/FVC were still abnormal. The main imaging changes from CT scanning were little parenchymal bands with residual group-glass opacity. As a result, the pathologic changes in the lung of patients after discharge might be: (1) residual unabsorbed exudative lesion; (2) mild lung fibrosis. These changes result in the functional disorders include: (1) decreasing in lung capacity; (2) decreasing in lung compliance; (3) decreasing in diffusion function. However, all impairments disappeared within 12 weeks, which means the pathological and functional changes are reversible.

The residual lesions of lung function are not rarely in viral pneumonia. Studies have discovered that survivors from SARS had significantly impaired pulmonary function, limited physical and psychology function, and reduced life quality (11, 12). Regarding influenza A virus H1N1, a study found that over half of these patients had signs of more severe abnormal pulmonary function, including diffusion disorders and small airway dysfunction, 1 year after discharge (13). From our results, we found that the residual lesions of lung function caused by SARS-CoV-2 is relatively short-term and reversible. It might attribute to the relatively lower virulence of the virus or the participants we included were not severe and critical.

Pulmonary rehabilitation is a comprehensive intervention that includes but is not limited to exercise training, education and behavioral changes with the aim to improve the physical and psychological conditions of people with respiratory disease

TABLE 4 | Six-min walk distance and heart rate.

	Discharge	1 week	4 weeks	12 weeks	24 weeks
6MW (m)					
PR group	462.12 ± 31.61	495.88 ± 34.67*	557.94 ± 38.44*†	584.41 ± 20.12*†	598.71 ± 22.35*†‡
Control group	448.56 ± 31.10	470.83 ± 35.70	514.22 ± 43.47*†	573.11 ± 29.20*†‡	590.33 ± 19.88*†‡
PR vs. control	$p > 0.05$	$p < 0.05$	$p < 0.01$	$p > 0.05$	$p > 0.05$
p and η^2 for ANOVA	$p_{time} < 0.001$, $\eta^2_{time} = 0.932$, $p_{group} < 0.05$, $\eta^2_{group} = 0.124$, $p_{time*group} < 0.001$, $\eta^2_{time*group} = 0.168$				
HR (beats/min)					
PR group	90.71 ± 9.30	83.24 ± 8.46*	78.59 ± 6.73*	76.06 ± 6.09*†	76.06 ± 6.09*†
Control group	97.44 ± 10.39	97.05 ± 14.24	88.61 ± 9.37	78.61 ± 9.37*†‡	77.00 ± 6.16*†‡
PR vs. control	$p = 0.052$	$p < 0.001$	$p < 0.001$	$p > 0.05$	$p > 0.05$
p and η^2 for ANOVA	$p_{time} < 0.001$, $\eta^2_{time} = 0.778$, $p_{group} < 0.05$, $\eta^2_{group} = 0.143$, $p_{time*group} < 0.001$, $\eta^2_{time*group} = 0.332$				

PR, pulmonary rehabilitation; 6MW, 6-min walk distance; HR, heart rate.
P-value of PR. vs. Control was from Student's t-test between two groups.
*P-value of the comparison between different times was from post-hoc test with Bonferroni correction for multiple comparisons [C5(2)]: *p <0.05/10 vs. discharge; †p < 0.05/10 vs. 1 week; ‡p < 0.05/10 vs. 4 weeks.*

TABLE 5 | Forced vital capacity and forced expiratory volume in 1 s.

	Discharge	1 week	4 weeks	12 weeks	24 weeks
FVC (L)					
PR group	2.05 ± 0.26	2.11 ± 0.29	2.75 ± 0.30*†	2.89 ± 0.22*†	2.95 ± 0.15*†
Control group	1.91 ± 0.21	2.02 ± 0.19	2.51 ± 0.20*†	2.86 ± 0.12*†‡	2.91 ± 0.10*†‡
PR vs. control	$p = 0.096$	$p > 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$
p and η^2 for ANOVA	$p_{time} < 0.001$, $\eta^2_{time} = 0.947$, $p_{group} = 0.053$, $\eta^2_{group} = 0.109$, $p_{time*group} < 0.05$, $\eta^2_{time*group} = 0.288$				
FVC (% predicted)					
PR group	64.25 ± 7.94	66.29 ± 9.14	86.27 ± 9.14*†	90.61 ± 6.05*†	92.64 ± 3.27*†
Control group	60.04 ± 6.28	63.46 ± 6.32	78.87 ± 7.55*†	89.96 ± 4.05*†‡	91.51 ± 2.62*†‡
PR vs. control	$p = 0.090$	$p > 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$
p and η^2 for ANOVA	$p_{time} < 0.001$, $\eta^2_{time} = 0.946$, $p_{group} = 0.061$, $\eta^2_{group} = 0.102$, $p_{time*group} < 0.05$, $\eta^2_{time*group} = 0.295$				
FEV_1 (L)					
PR group	1.52 ± 0.12	1.54 ± 0.14	2.12 ± 0.11*†	2.25 ± 0.10*†‡	2.29 ± 0.14*†‡
Control group	1.43 ± 0.11	1.48 ± 0.09	1.88 ± 0.12*†	2.24 ± 0.10*†‡	2.31 ± 0.13*†‡
PR vs. control	$p < 0.05$	$p < 0.05$	$p < 0.001$	$p > 0.05$	$p > 0.05$
p and η^2 for ANOVA	$p_{time} < 0.001$, $\eta^2_{time} = 0.980$, $p_{group} < 0.01$, $\eta^2_{group} = 0.302$, $p_{time*group} < 0.001$, $\eta^2_{time*group} = 0.499$				
FEV_1 (% predicted)					
PR group	63.62 ± 5.82	64.54 ± 7.11	88.76 ± 6.22*†	94.06 ± 0.43*†	95.83 ± 5.29*†‡
Control group	59.81 ± 4.94	61.58 ± 5.29	78.96 ± 6.91*†	93.85 ± 5.61*†‡	97.01 ± 5.79*†‡
PR vs. control	$p < 0.05$	$p < 0.05$	$p < 0.001$	$p > 0.05$	$p > 0.05$
p and η^2 for ANOVA	$p_{time} < 0.001$, $\eta^2_{time} = 0.938$, $p_{group} < 0.05$, $\eta^2_{group} = 0.152$, $p_{time*group} < 0.001$, $\eta^2_{time*group} = 0.198$				
FEV_1/FVC (%)					
PR group	74.73 ± 5.89	73.67 ± 8.08	77.70 ± 6.70	78.15 ± 5.96	77.62 ± 4.25
Control group	74.99 ± 5.55	74.39 ± 6.63	75.32 ± 5.43	78.30 ± 4.37	78.55 ± 5.35
PR vs. control	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
p and η^2 for ANOVA	$p_{time} < 0.001$, $\eta^2_{time} = 0.565$, $p_{group} > 0.05$, $\eta^2_{group} = 0.004$, $p_{time*group} > 0.05$, $\eta^2_{time*group} = 0.210$				

PR, pulmonary rehabilitation; FVC, forced vital capacity; FEV1, forced expiratory volume in 1s.
P-value of PR. vs. Control was from Student's t-test between two groups.
*P-value of the comparison between different times was from post-hoc test with Bonferroni correction for multiple comparisons [C5(2)]: *p < 0.05/10 vs. discharge; †p < 0.05/10 vs. 1 week; ‡p < 0.05/10 vs. 4 weeks.*

TABLE 6 | Diffusing capacity of the lung for carbon monoxide.

	Discharge	1 week	4 weeks	12 weeks	24 weeks
DL_{CO} [ml/(min·mmHg)]					
PR group	18.53 ± 2.03	19.65 ± 2.12	21.76 ± 2.19*	22.88 ± 2.12*†	22.94 ± 2.33*†
Control group	16.00 ± 1.46	17.03 ± 1.94*	18.83 ± 1.86*	21.50 ± 2.38*†‡	22.72 ± 2.16*†‡
PR vs. control	$p < 0.001$	$p < 0.01$	$p < 0.001$	$p = 0.079$	$p > 0.05$
p and η^2 for ANOVA	$p_{time} < 0.001$, $\eta^2_{time} = 0.753$, $p_{group} < 0.01$, $\eta^2_{group} = 0.271$, $p_{time*group} < 0.01$, $\eta^2_{time*group} = 0.145$				
DL_{CO} (% predicted)					
PR group	74.36 ± 6.59	78.81 ± 6.57	87.27 ± 6.20*†	91.99 ± 8.73*†	92.12 ± 8.32*†
Control group	66.24 ± 6.20	70.32 ± 7.46	77.78 ± 5.85*†	88.57 ± 5.037*†‡	93.94 ± 8.29*†‡
PR vs. control	$p < 0.01$	$p < 0.01$	$p < 0.001$	$p > 0.05$	$p > 0.05$
p and η^2 for ANOVA	$p_{time} < 0.001$, $\eta^2_{time} = 0.740$, $p_{group} < 0.01$, $\eta^2_{group} = 0.283$, $p_{time*group} < 0.01$, $\eta^2_{time*group} = 0.143$				

PR, pulmonary rehabilitation; FVC, forced vital capacity; DL_{CO}, diffusing capacity of the lung for carbon monoxide.
P-value of PR. vs. Control was from Student's t-test between two groups.
*P-value of the comparison between different times was from post-hoc test with Bonferroni correction for multiple comparisons [C5(2)]: *$p < 0.05/10$ vs. discharge; †$p < 0.05/10$ vs. 1 week; ‡$p < 0.05/10$ vs. 4 weeks.*

and promote long-term quality of life (6). Previous studies have confirmed the positive effects of pulmonary rehabilitation on pulmonary diseases such as COPD and H1N1 pneumonia (8, 12, 14). Besides, pulmonary rehabilitation has been proved to benefit the lung function and life quality in interstitial lung diseases such as idiopathic pulmonary fibrosis and interstitial pneumonias (15–17). Based on clinical practice, the program of pulmonary mainly contained three aspects: (1) physical training, (2) respiratory training, and (3) psychological regulation. Therefore, there are three main benefits of PR: (1) improve the patients' exercise capacity, (2) improve the patients' pulmonary function, and (3) improve the patients' psychological state. During the whole follow-up from the time of discharge to 24 weeks later, we can find that the pulmonary function of PR group was basically normal at 4 weeks, while Control group was basically normal at 12 weeks. As a result, the pulmonary rehabilitation could accelerate the recovery of pulmonary lesions and cardio-pulmonary function. According to the changes in CT imaging, we suspected that the effects of pulmonary rehabilitation may attribute to the promotion in absorption of exudation and fibrosis lesions, result in improvement of lung capacity, compliance, and diffusion function.

Because of the flexibility, feasibility and low cost, pulmonary rehabilitation could be a relatively practical way to improve patient condition. Most of patients suffered from COVID-19 are mild and common type, which makes it easy to carry out pulmonary rehabilitation. As for critical patient, whether, when, and how to carry out pulmonary rehabilitation should be further considered. Moreover, the intensity of training relies on the patients' condition; hence, the therapists should pay more attention each patient's vital signs and subjective feelings to not only maximize the effectiveness of the training but also avoid adverse events.

The main limitation of our study is that we only reported the results of 24 weeks follow-up, whether COVID-19 have sequela in respiratory system or other systems should be further studied.

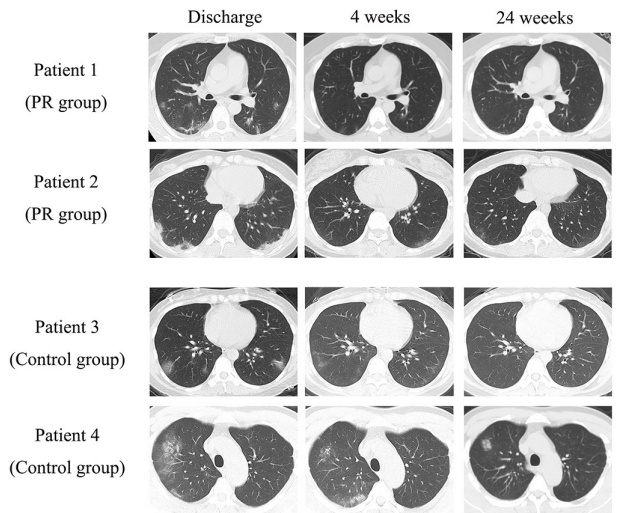


FIGURE 2 | Typical CT imaging of each group.

On the other hand, the characteristics of socio-economic of patients might affect patients' choice for accepting pulmonary rehabilitation, which might also lead to a better recovery. However, the socio-economic data were not available, which could be another limitation for this research.

In conclusion, pulmonary rehabilitation could accelerate the recovery of pulmonary function for COVID-19 patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Wuhan Fourth Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PZ, HG, and YC conceived and designed the study. YC and PZ contributed to the literature search. CC, ZF, and AZ contributed to data collection. AZ, ZF, and XG contributed to data analysis. PZ and XG contributed to data interpretation. ZW contributed to the figures. ZW, HG, and PZ drafted the article. All authors contributed to the article and approved the submitted version.

FUNDING

This study was funded by National Natural Science Foundation of Wuhan (Grant Nos. WX18Q21 and WX19Q24); Health commission of Hubei Province scientific research project (Grant No. WJ2019H371); and The National Natural Science Foundationof Hubei (Grant No. 2019CFB806). We acknowledge the financial support from the National Institute for Health Research, UK (ICA-CL-2018-04-ST2-012) to HG and by British Heart Foundation, UK (PG/19/23/34259) to HG.

ACKNOWLEDGMENTS

We are grateful for all our colleagues for their support of the present study. We are also grateful to the many front-line medical staffs for their dedication in the face of this outbreak, despite the potential threat to their own lives and the lives of their families.

REFERENCES

1. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet*. (2020) 395:514–23. doi: 10.1016/S0140-6736(20)30154-9
2. Chen ZM, Fu JF, Shu Q, Chen YH, Hua CZ, Li FB, et al. Diagnosis and treatment recommendations for pediatric respiratory infection caused by the 2019 novel coronavirus. *World J Pediatrics*. (2020) 16:240–6. doi: 10.1007/s12519-020-00345-5
3. Zhang H, Kang Z, Gong H, Xu D, Wang J, Li Z, et al. The digestive system is a potential route of 2019-nCov infection: a bioinformatics analysis based on single-cell transcriptomes. *bioRxiv*. 2020.2001.2030.927806 (2020). doi: 10.1101/2020.01.30.927806
4. Lu CW, Liu XF, Jia ZF. 2019-nCoV transmission through the ocular surface must not be ignored. *Lancet*. (2020) 395:e39. doi: 10.1016/S0140-6736(20)30313-5
5. Spruit MA, Pulmonary rehabilitation. *Eur Respir Rev*. (2014) 23:55–63. doi: 10.1183/09059180.00008013
6. Spruit MA, Singh SJ, Garvey C, ZuWallack R, Nici L, Rochester C, et al. An official American thoracic society/european respiratory society statement: key concepts and advances in pulmonary rehabilitation. *Am J Respir Crit Care Med*. (2013) 188:e13–64. doi: 10.1164/rccm.201309-1634ST
7. Pehlivan E, Balci A, Kilic L, Kadakal F. Preoperative pulmonary rehabilitation for lung transplant: effects on pulmonary function, exercise capacity, and quality of life; first results in Turkey. *Exp Clin Transp*. (2018) 16:455–60. doi: 10.6002/ect.2017.0042
8. Hsieh MJ, Lee WC, Cho HY, Wu MF, Hu HC, Kao KC, et al. Recovery of pulmonary functions, exercise capacity, and quality of life after pulmonary rehabilitation in survivors of ARDS due to severe influenza A (H1N1) pneumonitis. *Influenza Respir Viruses*. (2018) 12:643–8. doi: 10.1111/irv.12566
9. Snyder EM, Johnson BD, Beck KC. An open-circuit method for determining lung diffusing capacity during exercise: comparison to rebreathe. *J Appl Physiol*. (2005) 99:1985–91. doi: 10.1152/japplphysiol.00348.2005
10. Wheatley CM, Baker SE, Daines CM, Phan H, Martinez MG, Morgan WJ, et al. Influence of the Vibralung Acoustical Percussor on pulmonary function and sputum expectoration in individuals with cystic fibrosis. *Ther Adv Respir Dis*. (2018) 12:1753466618770997. doi: 10.1177/1753466618770997
11. Ngai JC, Ko FW, Ng SS, To KW, Tong M, Hui DS. The long-term impact of severe acute respiratory syndrome on pulmonary function, exercise capacity and health status. *Respirology*. (2010) 15:543–50. doi: 10.1111/j.1440-1843.2010.01720.x
12. Herridge MS, Tansey CM, Matte A, Tomlinson G, Diaz-Granados N, Cooper A, et al. Functional disability 5 years after acute respiratory distress syndrome. *N Engl J Med*. (2011) 364:1293–304. doi: 10.1056/NEJMoa1011802
13. Liu W, Peng L, Liu H, Hua S. Pulmonary function and clinical manifestations of patients infected with mild Influenza A virus subtype H1N1: a one-year follow-up. *PloS ONE*. (2015) 10:e0133698. doi: 10.1371/journal.pone.0133698
14. Carreiro A, Santos J, Rodrigues F. Impact of comorbidities in pulmonary rehabilitation outcomes in patients with chronic obstructive pulmonary disease. *Rev Port Pneumol*. (2013) 19:106–113. doi: 10.1016/j.rppnen.2012.12.001
15. Dowman L, Hill CJ, Holland AE. Pulmonary rehabilitation for interstitial lung disease. *Cochrane Database Syst Rev*. (2014) Cd006322. doi: 10.1002/14651858.CD006322.pub3
16. Vainshelboim B, Oliveira J, Yehoshua L, Weiss I, Fox BD, Fruchter O, et al. Exercise training-based pulmonary rehabilitation program is clinically beneficial for idiopathic pulmonary fibrosis. *Respir*. (2014) 88:378–88. doi: 10.1159/000367899
17. Yu X, Li X, Wang L, Liu R, Xie Y, Li S, et al. Pulmonary rehabilitation for exercise tolerance and quality of life in IPF patients: a systematic review and meta-analysis. *BioMed Res Int*. (2019) 2019:8498603. doi: 10.1155/2019/8498603

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 22 July 2021
doi: 10.3389/fcvm.2021.698923

Impact of COVID-19 Pandemic on Mechanical Reperfusion in ST-Segment-Elevation Myocardial Infarction Undergoing Primary Percutaneous Coronary Intervention: A Multicenter Retrospective Study From a Non-epicenter Region

Qi Mao[1], Jianhua Zhao[1], Youmei Li[1], Li Xie[1], Han Xiao[1], Ke Wang[1], Youzhu Qiu[1], Jianfei Chen[2], Qiang Xu[3], Zhonglin Xu[4], Yang Yu[5], Ying Zhang[6], Qiang Li[7], Xiaohua Pang[8], Zhenggong Li[9], Boli Ran[10], Zhihui Zhang[11], Zhifeng Li[12], Chunyu Zeng[13], Shifei Tong[14], Jun Jin[1], Lan Huang[1] and Xiaohui Zhao[1]**

[1] Department of Cardiology, Institute of Cardiovascular Research, Xinqiao Hospital, Army Medical University, Chongqing, China, [2] Department of Cardiology, People's Hospital of Banan District, Chongqing, China, [3] Department of Cardiology, The Fifth People's Hospital, Chongqing, China, [4] Department of Cardiology, The Ninth People's Hospital, Chongqing, China, [5] Department of Cardiology, People's Hospital of Dianjiang District, Chongqing, China, [6] Department of Cardiology, Emergency Medical Center, Chongqing, China, [7] Department of Cardiovascular Medicine, People's Hospital of Nanchuan District, Chongqing, China, [8] Department of Cardiovascular Medicine, The Three Gorges Central Hospital, Chongqing, China, [9] Department of Cardiac Intervention Therapy, Zhongshan Hospital District, Chongqing General Hospital, University of Chinese Academy of Sciences, Chongqing, China, [10] Department of Cardiology, The Third Hospital District, Chongqing General Hospital, University of Chinese Academy of Sciences, Chongqing, China, [11] Department of Cardiology, Southwest Hospital, Army Medical University, Chongqing, China, [12] Department of Cardiology, Yongchuan Hospital, Chongqing Medical University, Chongqing, China, [13] Department of Cardiology, Daping Hospital, Army Medical University, Chongqing, China, [14] Department of Cardiology, The Third Affiliated Hospital of Chongqing Medical University, Chongqing, China

Objective: The COVID-19 pandemic placed heavy burdens on emergency care and posed severe challenges to ST-segment-elevation myocardial infarction (STEMI) treatment. This study aimed to investigate the impact of COVID-19 pandemic on mechanical reperfusion characteristics in STEMI undergoing primary percutaneous coronary intervention (PPCI) in a non-epicenter region.

Methods: STEMI cases undergoing PPCI from January 23 to March 29 between 2019 and 2020 were retrospectively compared. PPCI parameters mainly included total ischemic time (TIT), the period from symptom onset to first medical contact (S-to-FMC), the period from FMC to wire (FMC-to-W) and the period from door to wire (D-to-W). Furthermore, the association of COVID-19 pandemic with delayed PPCI risk was further analyzed.

Results: A total of 14 PPCI centers were included, with 100 and 220 STEMI cases undergoing PPCI in 2020 and 2019, respectively. As compared to 2019, significant prolongations occurred in reperfusion procedures ($P < 0.001$) including TIT (420 vs. 264 min), S-to-FMC (5 vs. 3 h), FMC-to-W (113 vs. 95 min) and D-to-W (83 vs. 65 min).

OPEN ACCESS

Edited by:
Hendrik Tevaearai Stahel,
Bern University Hospital, Switzerland

Reviewed by:
Mehmet Tolga Dogru,
Kirikkale University, Turkey
Gianluca Campo,
University Hospital of Ferrara, Italy

***Correspondence:**
Lan Huang
huanglan260@126.com
Xiaohui Zhao
doctorzhaoxiaohui@yahoo.com

Specialty section:
This article was submitted to Coronary Artery Disease, a section of the journal Frontiers in Cardiovascular Medicine

Received: 22 April 2021
Accepted: 28 June 2021
Published: 22 July 2021

Citation:
Mao Q, Zhao J, Li Y, Xie L, Xiao H, Wang K, Qiu Y, Chen J, Xu Q, Xu Z, Yu Y, Zhang Y, Li Q, Pang X, Li Z, Ran B, Zhang Z, Li Z, Zeng C, Tong S, Jin J, Huang L and Zhao X (2021) Impact of COVID-19 Pandemic on Mechanical Reperfusion in ST-Segment-Elevation Myocardial Infarction Undergoing Primary Percutaneous Coronary Intervention: A Multicenter Retrospective Study From a Non-epicenter Region. Front. Cardiovasc. Med. 8:698923. doi: 10.3389/fcvm.2021.698923

Consistently, delayed reperfusion surged including TIT ≥ 12 h (22.0 vs.3.6%), FMC-to-W ≥ 120 min (34.0 vs. 6.8%) and D-to-W ≥ 90 min (19.0 vs. 4.1%). During the pandemic, the patients with FMC-to-W ≥ 120 min had longer durations in FMC to ECG completed (6 vs. 5 min, $P = 0.007$), FMC to DAPT (24 vs. 21 min, $P = 0.001$), catheter arrival to wire (54 vs. 43 min, $P < 0.001$) and D-to-W (91 vs. 78 min, $P < 0.001$). The pandemic was significantly associated with high risk of delayed PPCI (OR = 7.040, 95% CI 3.610–13.729, $P < 0.001$).

Conclusions: Even in a non-epicenter region, the risk of delayed STEMI reperfusion significantly increased due to cumulative impact of multiple procedures prolongation.

Keywords: COVID-19, ST-segment-elevation myocardial infarction, primary percutaneous coronary intervention, mechanical reperfusion, non-epicenter region

INTRODUCTION

ST-segment-elevation myocardial infarction (STEMI) is a major cardiovascular emergency requiring early diagnosis and timely reperfusion (1). Mechanical reperfusion is mainly based on rapid and standardized emergency procedures for chest pain (2). Since the outbreak in December 2019, over 110 million coronavirus-2019 disease (COVID-19) infected cases have been diagnosed and 2.4 million confirmed deaths (3). The continuing pandemic placed heavy burdens on emergency care and posed severe challenges to STEMI treatment (4).

On the one hand, protective measures against COVID-19 cause delays in primary percutaneous coronary intervention (PPCI) and prolonged ischemia time thus may lead to poor prognosis. On the other hand, emergency process without protection greatly increases the risk of virus spread, especially serious infection in hospital (5, 6). Therefore, how to balance prevention and treatment is a great ordeal for medical institutions. Considering the pandemic may last for a long time, as a core issue in health governance, it will profoundly affect the public health system and chest pain practice. In previous studies, decline of admitted STEMI was reported both in Europe, US etc, and increased delays in PPCI were also observed in COVID-19 epicenters (7–9). However, in non-epicenters, few studies on detailed mechanical reperfusion characteristics were reported.

METHODS

Study Population

This multicenter retrospective study included 14 PPCI centers, which were certified by the China Chest Pain Center (CCPC) with standardized catheterization lab. In light of changes in epidemic and adjustments in public health response, the COVID-19 pandemic was defined as the period from January 23 (the day on which Wuhan City entered into a state of full-scale wartime through the lockdown, and then other regions including Chongqing City also upgraded their public health response to prevent the spread of the epidemic) to March 29 in 2020 (the day on which Chongqing City downgraded local public health response due to the absolute clearance of COVID-19 cases). Also, similar patients at the same period last year were included to reduce the biases of seasonal variation and festive events on the incidence. The patients with confirmed or suspected COVID-19 were excluded. Our study protocol complied with the Declaration of Helsinki and was approved by Xinqiao Hospital Ethics Committee, Army Medical University.

Treatment Procedure During the Pandemic

Although Chongqing City was a non-epicenter during the pandemic, local public health response was still upgraded on January 23 to minimize the spread of virus. Except for the lockdown, social restrictive measures were implemented to reduce external input and local transmission. For medical institutions, all admitted patients were screened for SARS-COV-2 according to Clinical Guideline of COVID-19 Diagnosis and Treatment (7 th edition) (10). In brief, the patients with confirmed or suspected COVID-19 would be transferred to the designated hospitals as soon as possible; the patients without exclusion of COVID-19 temporarily would be first transferred to the special clinics for isolation and treatment, if further tests were positive, they would be immediately transferred to the designated hospitals; while non-COVID-19 patients underwent conventional treatment procedures (11). Reperfusion therapy was determined based on benefit/risk assessment and consensus recommendation (12). Compared to the epicenters, PPCI remained the preferred option for local reperfusion therapy rather than thrombolysis-first. The flowchart of emergency procedure was shown in **Figure 1**.

Definition and Data Collection

Acute myocardial infarction refers to the fourth universal definition, when troponin value exceeds the 99 th percentile upper reference limit and combines at least one of following characteristics: (1) symptoms of myocardial ischemia; (2) new changes in ischemic electrocardiogram or emerging pathological

Abbreviations: COVID-19, coronavirus-2019 disease; STEMI, ST-segment-elevation myocardial infarction; PPCI, primary percutaneous coronary intervention; SBP, systolic blood pressure; DBP, diastolic blood pressure; Scr, serum creatinine; EMS, emergency medical service; CCPC, China Chest Pain Center; FMC, first medical contact; S-to-FMC, symptom onset to FMC; DAPT, dual antiplatelet therapy; D-to-W, door to wire; FMC-to-W, FMC to wire; TIT, total ischemic time.

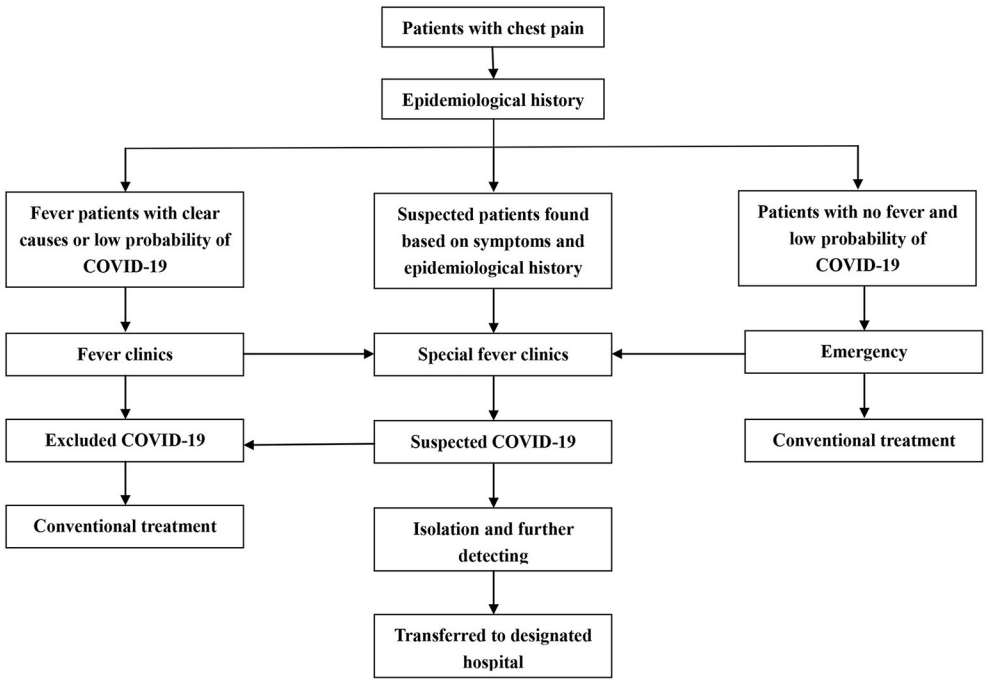


FIGURE 1 | Flowchart of emergency procedure.

Q waves; (3) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality (13). Global Registry of Acute Coronary Events (GRACE) risk score is applied to stratification and prediction of risk in patients with ACS and is calculated based on the clinical data, electrocardiogram (ECG), and laboratory parameters at admission (14).

Arrival patterns included walk-in, in-hospital onset, emergency medical services (EMS) and inter-facility transports; walk-in and in-hospital onset were defined as non-transferred pattern, while EMS and inter-facility transports were regarded as transferred pattern. PPCI parameters mainly included the period from symptom onset to first medical contact (S-to-FMC), the period from FMC to wire through culprit (FMC-to-W), and the period from door to wire through culprit (D-to-W) (15). Total ischemic time (TIT) was composed of S-to-FMC and FMC-to-W. D-to-W $\geq$ 90 min, FMC-to-W $\geq$ 120 min and TIT $\geq$ 12 h were deemed as pivotal timelines for delayed mechanical reperfusion (16). Clinical data and mechanical reperfusion characteristics were obtained from medical records.

Statistical Analysis

Continuous variables are presented as mean $\pm$ SD for symmetric distributions and median (interquartile range, IQR) for skewed distributions. Categorical variables are expressed as frequency (percentage). In comparisons between groups, the *t*-test was performed for symmetric distributed variables, and nonparametric Mann-Whitney U test was applied for skewed distributed variables. Differences in categorical variables were compared by the Chi-squared test or Fisher exact test. Taking the dichotomous delay PPCI indicators as the dependent variables, we conducted logistic regression analysis to explore the association of COVID-19 pandemic with delayed mechanical reperfusion, and sub-group analysis was utilized to further assess this correlation. Two-tailed *P*-values < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS software version 24.0 (SPSS, Inc, Chicago, Illinois).

RESULTS

Composition and Grouping

STEMI collaboration network from 14 PPCI centers implemented a unified procedure in accordance with CCPC specification in chest pain emergency (17). During the pandemic from 23 th January 2020 to 29 th March 2020 in China, a total of 145 consecutive patients admitted to chest pain emergency were diagnosed with STEMI, and 100 patients (69.0%) met the inclusion criteria after exclusion of non-mechanical reperfusion cases among these cases. During the same period in 2019, a total of 278 consecutive STEMI patients arrived in chest pain emergency after symptom onset, and 220 cases (79.1%) were included after screening (**Figure 2**).

Comparison of Study Population Before and During the Pandemic

Overall, we identified 320 non-COVID-19 patients with STEMI undergoing PPCI as the study population (**Table 1**). As compared to 2019, the cases of STEMI (decreased by 47.8%) and PPCI (decreased by 54.5%) had a significant reduction. In terms of

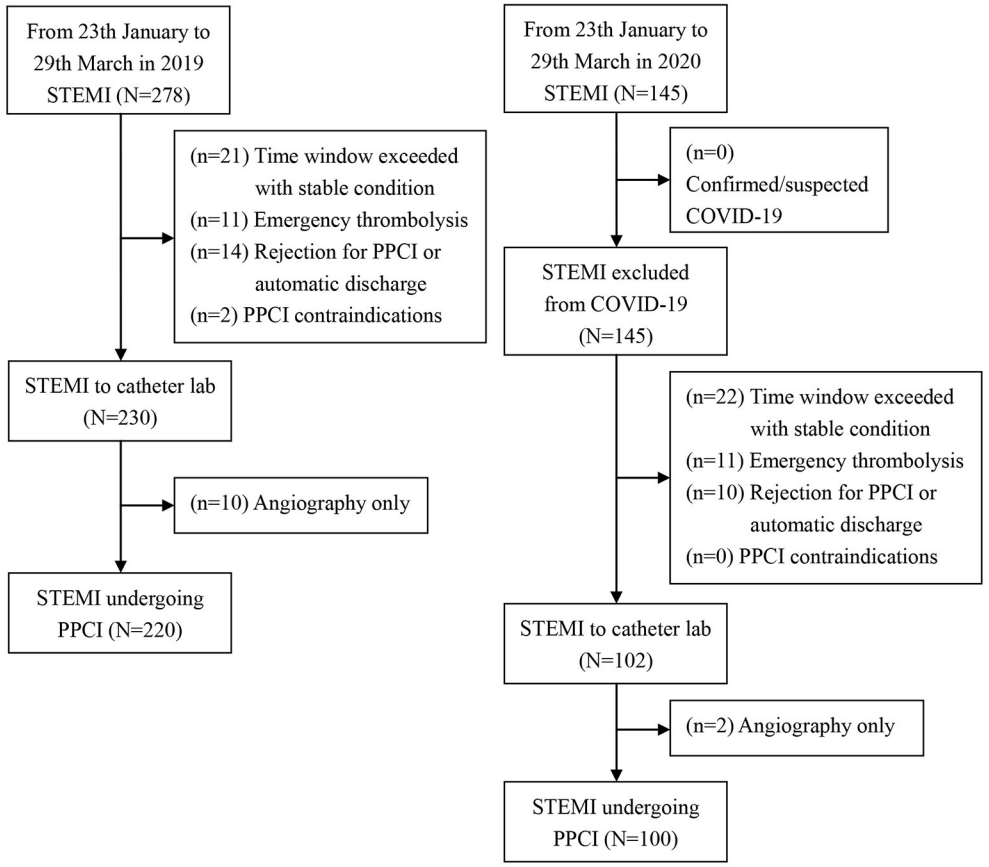


FIGURE 2 | Flowchart of study population.

clinical characteristics, there were no differences in age, gender, heart rate, Killip class, serum creatinine and GRACE scores between the two groups ($P > 0.05$). Although arrival during non-offices hours did not differ significantly between the two groups ($P > 0.05$), more non-transferred patients with less inter-facility transports (24.0 vs. 41.4%) and more walk-in (61.0 vs. 48.6%) appeared during the pandemic ($P < 0.05$). In terms of mechanical reperfusion characteristics, significant prolongations occurred in PPCI parameters ($P < 0.001$) including TIT (420 vs. 264 min), S-to-FMC (5 vs. 3 h), FMC-to-W (113 vs. 95 min) and D-to-W (83 vs. 65 min). Further analysis revealed that median time of TIT increased by 156 min during the pandemic; COVID-19 outbreak delayed the median time of FMC-to-W for 18 min. Consistently, delayed reperfusion surged including TIT $\geq$ 12 h (22.0 vs.3.6%), FMC-to-W $\geq$ 120 min (34.0 vs. 6.8%) and D-to-W $\geq$ 90 min (19.0 vs. 4.1%) significantly ($P < 0.001$). Of note, the ratio of S-to-FMC to TIT increased significantly during the pandemic (72.8 vs. 63.7%, $P < 0.001$).

PPCI Parameters Between Different Groups During the Pandemic

No differences occurred in PPCI parameters between office periods and non-office periods during the pandemic ($P > 0.05$) (**Table 2**). Compared to the transferred patients, the periods of FMC to ECG completed and FMC to DAPT were decreased by 2 and 3 min, respectively, in non-transferred patients ($P = 0.002$); whereas the periods of telephone to catheter activated (15 vs. 9 min, $P < 0.001$) and catheter arrival to wire (47 vs. 44 min, $P < 0.042$) significantly extended for non-transferred patients; non-transferred pattern increased the proportion of patients with TIT $\geq$ 12 h ($P = 0.045$) (**Table 3**).

In **Table 4**, the patients with FMC-to-W $\geq$ 120 min had longer durations in FMC to ECG completed (6 vs. 5 min, $P = 0.007$), FMC to DAPT (24 vs. 21 min, $P = 0.001$), catheter arrival to wire (54 vs. 43 min, $P < 0.001$) and D-to-W (91 vs. 78 min, $P < 0.001$) than the patients with FMC-to-W < 120 min; while S-to-FMC and TIT showed no differences between the two groups ($P > 0.05$).

Association of the Pandemic With the Risk of Delayed PPCI

Logistic regression analysis was used to explore the association between the pandemic and delayed PPCI. The binary delayed PPCI indicators and COVID-19 pandemic status were included as dependent and independent variables in the model, respectively. The results indicated the pandemic was

TABLE 1 | Characteristics of study population before and during COVID-19 pandemic.

Characteristics	From 23rd January to 29th March in 2019 (*N* = 220)	From 23 rd January to 29th March in 2020 (*N* = 100)	*P*-value
Male, *n* (%)	177 (80.5)	77 (77.0)	0.479
Age (years)	63 (54–73)	64 (55–75)	0.768
Heart rate (min)	78 (70–89)	75 (65–88)	0.082
SBP (mmHg)	122 (110–142)	125 (110–150)	0.790
DBP (mmHg)	77 (68–88)	78 (68–92)	0.667
Killip class			
Killip class I, *n* (%)	138 (62.7)	62 (62.0)	
Killip class II, *n* (%)	57 (25.9)	22 (22.0)	
Killip class III, *n* (%)	9 (4.1)	2 (2.0)	
Killip class IV, *n* (%)	15 (6.8)	15 (15.0)	
Killip class ≥ II, *n* (%)	82 (37.3)	39 (39.0)	0.768
Scr (μmol/L)	74.0 (61.9–90.4)	71.3 (60.3–91.4)	0.624
GRACE scores in hospital	143 (121–163)	139 (119–166)	0.804
Arrival During non-office hours, *n* (%)	99 (45.0)	51 (51.0)	0.319
Pattern of patients arrival			
Walk-in	107 (48.6)	61 (61.0)	
In-hospital onset	2 (0.9)	1 (1.0)	
EMS	20 (9.1)	14 (14.0)	
Inter-facility transports	91 (41.4)	24 (24.0)	
Non-transferred patients, *n* (%)	109 (49.5)	62 (62.0)	0.038
S-to-FMC (hours)	3.0 (2.0–4.5)	5.0 (3.0–10.0)	<0.001
FMC to ECG completed (min)	3 (2–6)	5 (3–7)	<0.001
Door to Troponin completed (min)	12 (11–15)	13 (12–14)	0.475
FMC to DAPT (min)	19 (17–22)	22 (19–25)	<0.001
Telephone to catheter activated (min)	9 (6–12)	13 (9–17)	<0.001
Catheter arrival to wire (min)	36 (31–42)	45 (41–53)	<0.001
D-to-W (min)	65 (57–76)	83 (75–89)	<0.001
D-to-W ≥ 90 min, *n* (%)	9 (4.1)	19 (19.0)	<0.001
FMC-to-W (min)	95 (87–108)	113 (106–124)	<0.001
FMC-to-W ≥120 min, *n* (%)	15 (6.8)	34 (34.0)	<0.001
TIT (min)	264 (204–367)	420 (295–688)	<0.001
TIT ≥ 12 h, *n* (%)	8 (3.6)	22 (22.0)	<0.001
S-to-FMC/TIT ratio (%)	63.7 (50.4–74.5)	72.8 (62.6–83.8)	<0.001
FMC-to-W/TIT ratio (%)	36.3 (25.5–49.6)	27.2 (16.2–37.4)	

Data are expressed as median (interquartile range) or number (percentage) as appropriate.

TABLE 2 | Comparison of PPCI parameters between different arrival periods during COVID-19 pandemic.

Parameters	During office hours (*N* = 49)	During non-office hours (*N* = 51)	*P*-value
Killip class≥II, *n* (%)	18 (36.7)	21 (41.2)	0.649
GRACE scores in hospital	137 (120–161)	147 (118–172)	0.546
Non-transferred patients, *n* (%)	32 (65.3)	30 (58.8)	0.504
S-to-FMC (hours)	6.0 (3.0–11.0)	4.0 (3.0–9.0)	0.076
FMC to ECG completed (min)	5 (3–7)	6 (4–7)	0.113
Door to Troponin completed (min)	13 (11–14)	13 (12–15)	0.186
FMC to DAPT (min)	22 (19–25)	22 (19–27)	0.836
Telephone to catheter activated (min)	14 (10–17)	13 (9–18)	0.751
Catheter arrival to wire (min)	45 (41–53)	46 (42–54)	0.394
D-to-W (min)	82 (75–89)	83 (75–87)	0.753
D-to-W ≥ 90 min, *n* (%)	10 (20.4)	9 (17.6)	0.725
FMC-to-W (min)	113 (106–121)	114 (107–127)	0.574
FMC-to-W ≥ 120 min, *n* (%)	14 (28.6)	20 (39.2)	0.261
TIT (min)	462 (315–750)	374 (289–639)	0.116
TIT ≥ 12 h, *n* (%)	14 (28.6)	8 (15.7)	0.120
S-to-FMC/TIT ratio (%)	77.3 (64.3–84.0)	67.3 (58.4–82.9)	0.073
FMC-to-W/TIT ratio (%)	22.7 (16.0–35.7)	32.7 (17.1–41.6)	

Data are expressed as median (interquartile range) or number (percentage) as appropriate.

significantly associated with high risk of delayed TIT (OR = 7.474, 95% CI 3.195–17.484, $P < 0.001$), delayed FMC-to-W (OR = 7.040, 95% CI 3.610–13.729, $P < 0.001$) and delayed D-to-W (OR = 5.499, 95% CI 2.390–12.655, $P < 0.001$). Sub-group analysis stratified by clinical characteristics further examined this association (**Table 5**). Meanwhile, constituent ratios of TIT and PPCI were shown in **Figure 3**.

DISCUSSION

In this study, we found that risk of delayed STEMI reperfusion significantly increased due to cumulative impact of multiple procedures in a non-epicenter region.

Evidence from Europe indicated that compared with the same period in 2019, PPCI cases decreased by 19.3%, and the median time of TIT and D-to-W were delayed by 9 and 2 min, respectively (18). The data from North America showed an estimated 38% reduction in U.S. cardiac catheterization activation after the outbreak (19). Consistently, the analysis from China's epicenter (Hubei Province) also revealed a 62.3% decline in STEMI cases during the pandemic, while the proportion of non-transferred patients characterized by walk-in increased significantly (8). In a non-epicenter, our results also revealed that a significant reduction occurred in cases of admitted STEMI and PPCI during the pandemic. Common reasons had been formulated to explain the reduction in cases including fear of infection, social distancing, and medical care avoidance. However, the decline in cases could not be simply ascribed to

TABLE 3 | Comparison of PPCI parameters between different transferred methods during COVID-19 pandemic.

Parameters	Non-transferred Patients (*N* = 62)	Transferred Patients (*N* = 38)	*P*-value
Killip class≥II, *n* (%)	21 (33.9)	18 (47.4)	0.179
GRACE scores in hospital	134 (115–160)	143 (133–177)	0.053
Arrival during non-office hours, *n* (%)	30 (48.4)	21 (55.3)	0.504
S-to-FMC (hours)	5.5 (3.0–11.0)	5.0 (3.0–9.0)	0.322
FMC to ECG completed (min)	5 (3–6)	7 (4–10)	0.002
Door to Troponin completed (min)	13 (11–14)	14 (12–15)	0.377
FMC to DAPT (min)	21 (18–24)	24 (20–30)	0.002
Telephone to catheter activated (min)	15 (12–18)	9 (6–14)	<0.001
Catheter arrival to wire (min)	47 (42–54)	44 (36–50)	0.042
D–to-W (min)	83 (77–89)	83 (74–88)	0.511
D-to-W ≥ 90 min, *n* (%)	12 (19.4)	7 (18.4)	0.908
FMC-to-W (min)	113 (106–122)	115 (107–126)	0.649
FMC-to-W ≥ 120 min, *n* (%)	17 (27.4)	17 (44.7)	0.076
TIT (min)	443 (294–774)	400 (303–641)	0.347
TIT ≥ 12 h, *n* (%)	18 (29.0)	4 (10.5)	0.045
S-to-FMC/TIT ratio (%)	76.0 (63.1–84.5)	68.1 (62.2–82.6)	0.248
FMC-to-W/TIT ratio (%)	24.0 (15.5–36.9)	31.9 (17.4–37.8)	

Data are expressed as median (interquartile range) or number (percentage) as appropriate.

TABLE 4 | Comparison of parameters between timely PPCI and delayed PPCI during COVID-19 pandemic.

Parameters	FMC-to-W <120 min (*N* = 66)	FMC-to-W ≥ 120 min (*N* = 34)	*P*-value
Killip class ≥ II, *n* (%)	26 (39.4)	13 (38.2)	0.910
GRACE scores in hospital	137 (115–162)	142 (123–177)	0.142
Arrival during non-office hours, *n* (%)	31 (47.0)	20 (58.8)	0.261
Non-transferred patients, *n* (%)	45 (68.2)	17 (50.0)	0.076
S-to-FMC (hours)	6.0 (3.0–9.0)	4.0 (3.0–10.0)	0.818
FMC to ECG completed (min)	5 (3–6)	6 (4–9)	0.007
Door to Troponin completed (min)	13 (11–14)	14 (12–15)	0.181
FMC to DAPT (min)	21 (18–23)	24 (20–29)	0.001
Telephone to catheter activated (min)	12 (9–16)	14 (11–20)	0.050
Catheter arrival to wire (min)	43 (40–48)	54 (47–57)	<0.001
D-to-W (min)	78 (69–83)	91 (85–106)	<0.001
D-to-W ≥ 90 min, *n* (%)	0	19 (55.9)	<0.001
FMC-to-W (min)	108 (101–113)	128 (124–138)	<0.001
TIT (min)	462 (289–654)	396 (320–724)	0.702
TIT ≥ 12 h, *n* (%)	12 (18.2)	10 (29.4)	0.199
S-to-FMC/TIT ratio (%)	77.1 (63.3–84.1)	66.3 (58.3–83.0)	0.137
FMC-to-W/TIT ratio (%)	22.9 (15.9–36.7)	33.7 (17.0–41.7)	

Data are expressed as median (interquartile range) or number (percentage) as appropriate.

individual behaviors, and we should also pay attention to the comprehensive impact of the pandemic on chest pain procedure. STEMI rescue includes pre-hospital and in-hospital segments. Both S-to-FMC and D-to-W were apparently prolonged, which led to cumulative delays in reperfusion procedure.

Mechanical reperfusion for STEMI is a competition with time. The 1-year mortality of STEMI increases by 15% with every 1 h extension in time to reperfusion (20). Quality control of PPCI based on standardized procedure can help shorten TIT, reduce infarction sizes and mortality (21). Although COVID-19 has been shown to directly cause myocardial injury and induce thrombosis, heart failure, arrhythmia and even cardiac arrest; for non-COVID-19 patients, delayed PPCI affected by the pandemic might be the determinant for the poor prognosis in STEMI (22). In previous studies, Tam et al. (23) showed longer median time in all components of PPCI parameters compared with historical data from prior year in Hong Kong, yet limited by very small sample size (7 cases) and non-contemporaneous data comparison. Siudak et al. (24) reported that time from FMC to inflation significantly increased compared with analogous time period last year in Poland, but the impact of the virus infection on delayed PPCI had not been ruled out. An observational study from Canada revealed that significant delay appeared in reperfusion procedure and predominantly ascribed to patient-level and transfer-level during the pandemic (25). Of note, our study found that delays in mechanical reperfusion should be attributed to the cumulative effect of multiple processes. In addition to pre-hospital level, in-hospital delays should also not be ignored. In a non-hot spot region from America, Hammad et al. found that although no difference occurred in total D-to-B between pre-COVID-19 and post-COVID-19, a higher proportion of patients in the post-COVID-19 period presented with >12-h delay compared with the pre-COVID-19 period, and those patients with >12-h delay also had a longer average D-to-B time (26). Similarly, we also observed the adverse effect of COVID-19 pandemic on reperfusion procedure in another non-epicenter region. However, our results revealed the apparent prolongations in S-to-FMC and FMC-to-W after the outbreak through detailed parameter analysis. We speculated that this might be associated with stricter social restrictions and upgraded public health response after the first wave pandemic in China. In epicenter region (Hubei Province) from China, although differences of median time in S-to-FMC and FMC-to-W seemed to be not significant, delays in timelines was still apparent due to the highly fluctuated time and limited sample size (8). Compared with our study, reperfusion strategy of this epicenter had been adjusted to meet the needs of high-intensity epidemic control. A

TABLE 5 | Logistic analyses for the association of COVID-19 pandemic with delayed PPCI.

	Delayed PPCI								
	TIT ≥ 12 h			FMC-to-W ≥ 120 min			D-to-W ≥ 90 min		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Overall	7.474	(3.195–17.484)	<0.001	7.040	(3.610–13.729)	<0.001	5.499	(2.390–12.655)	<0.001
Age									
≥65 years	4.694	(1.733–12.717)	0.002	7.759	(3.090–19.479)	<0.001	6.803	(2.039–22.694)	0.002
<65 years	25.929	(3.189–210.805)	0.002	6.216	(2.333–16.563)	<0.001	4.353	(1.350–14.037)	0.014
Gender									
Male	7.475	(2.798–19.973)	<0.001	8.514	(3.849–18.832)	<0.001	5.541	(2.258–13.598)	<0.001
Female	7.235	(1.325–39.497)	0.022	4.053	(1.142–14.392)	0.030	6.300	(0.616–64.426)	0.121
Killip class									
Killip class ≥ II	5.850	(1.675–20.435)	0.006	6.333	(2.183–18.370)	0.001	2.868	(0.725–11.343)	0.133
Killip class < II	9.073	(2.821–29.179)	<0.001	7.525	(3.192–17.741)	<0.001	7.923	(2.707–23.190)	<0.001
GRACE score									
GRACE > 140	5.294	(1.930–14.524)	0.001	8.053	(3.277–19.793)	<0.001	6.568	(1.909–22.594)	0.003
GRACE ≤ 140	24.318	(3.022–195.704)	0.003	6.538	(2.367–18.062)	<0.001	4.682	(1.512–14.494)	0.007
Office hours or not									
Non–office hours	18.233	(2.211–150.318)	0.007	5.742	(2.425–13.598)	<0.001	3.321	(1.111–9.932)	0.032
Office hours	6.514	(2.437–17.412)	<0.001	9.280	(3.124–27.569)	<0.001	10.085	(2.641–38.518)	0.001
Transferred or not									
Transferred	6.412	(1.125–36.548)	0.036	9.175	(3.603–23.359)	<0.001	8.129	(1.984–33.304)	0.004
Non-transferred	7.023	(2.612–18.882)	<0.001	6.485	(2.399–17.530)	<0.001	4.120	(1.461–11.617)	0.007

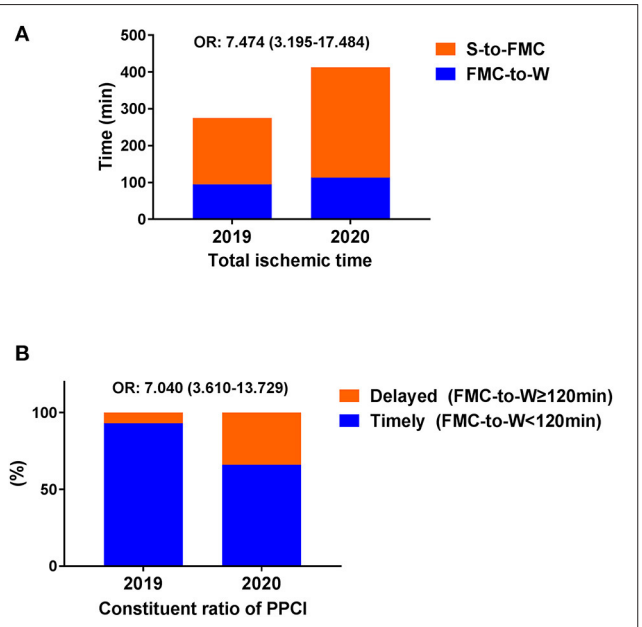


FIGURE 3 | Constituent ratios of TIT and PPCI.

large number of patients from epicenter received thrombolytic therapy at the first time, given that thrombolysis could be considered as the recommended reperfusion option during the pandemic (12).

Compared to other regions, we discovered that delays in mechanical reperfusion were still rather serious in non-COVID-19 STEMI patients from a non-epicenter implying severe condition might be not the only driving factor for admission. Medical responses affected by the pandemic might be also important for seeking assistance at symptom onset. Interestingly, an observational study from Italy found that although myocardial infarction hospitalizations significantly decreased, FMC-balloon time remained unchanged after the outbreak (27). The result might be firstly attributed to the excellent reorganization for local hospital activities. Secondly, compared with our study, Italian patients were younger and had fewer cardiovascular risk factors, and were more likely to seek medical assistance timely due to striking symptoms and maintain high medical compliance in rescue procedure. FITT-STEMI study from Germany showed high-standard treatment and management for STEMI, reperfusion parameters were almost unaffected during the pandemic (16). This achievement was due to quick public response, very high proportion of EMS transport, high-level routine procedure and pre-existing care network. Based on our findings, we noticed that the pandemic might magnify the shortcomings of the pre-existing treatment pathway, thus still caused a significant delay even in a non-epicenter region. This also meant that only a high-level treatment pathway maintained for a long time could effectively deal with medical burden caused by the pandemic. In the present study, we further provided new evidence for cumulative delays in reperfusion procedure; S-to-FMC was the determinant for prolonged TIT, while slow activation in hospital was pivotal to delayed PPCI. Furthermore,

our findings showed the significant correlation between the pandemic and high risk of delayed PPCI. In our opinion, longer FMC-to-W might be interpreted by institutional delays due to protective protocols for screening patients, preparing for equipment and activating personnel in catheter lab. Meanwhile, emergency care overload and staff fatigue should also be taken into consideration certainly. Hence, we proposed the insight as optimizing mechanical reperfusion by controlling cumulative delays.

LIMITATIONS

Our study had several limitations. First, this study was subject to the biases inherent to its retrospective design. Second, clinical characteristics and PPCI parameters were evaluated by trained investigators in each center, without central reconfirmation, potentially resulting biases and errors. Third, our study had a small sample size and no follow-up data for *post-hoc* analysis.

CONCLUSION

The COVID-19 pandemic significantly increased the risk of delayed STEMI reperfusion in a non-epicenter region, probably due to cumulative impact of multiple procedures prolongation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Xinqiao Hospital Ethics Committee, Army Medical University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

XZ was responsible for the design. QM, JZ, JC, QX, ZX, YY, YZ, QL, XP, ZheL, BR, ZZ, ZhiL, CZ, and ST contributed to collect and clean the data. QM, JZ, YL, LX, HX, KW, and YQ performed the data analysis. QM wrote the draft of this manuscript. JJ, LH, and XZ contributed to the writing and revision of the paper. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the National Natural Science Foundation of China (Grant no. 81670428).

ACKNOWLEDGMENTS

We are grateful for all the participants and investigators for their support.

REFERENCES

1. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. *Eur Heart J.* (2018) 39:119–77. doi: 10.1093/eurheartj/ehx393
2. Danchin N, Popovic B, Puymirat E, Goldstein P, Belle L, Cayla G, et al. Five-year outcomes following timely primary percutaneous intervention, late primary percutaneous intervention, or a pharmaco-invasive strategy in ST-segment elevation myocardial infarction: the FAST-MI programme. *Eur Heart J.* (2020) 41:858–66. doi: 10.1093/eurheartj/ehz665
3. World Health Organization. *Coronavirus Disease 2019 Situation Report*. Available online at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/ (accessed February 24, 2021).
4. Boukhris M, Hillani A, Moroni F, Annabi MS, Addad F, Ribeiro MH, et al. Cardiovascular implications of the COVID-19 pandemic: a global perspective. *Can J Cardiol.* (2020) 36:1068–80. doi: 10.1016/j.cjca.2020.05.018
5. Jacobs AK, Ali M, Best PJ, Bieniarz M, Cohen MG, French WJ, et al. Temporary emergency guidance to STEMI systems of care during the COVID-19 pandemic. *Circulation.* (2020) 142:199–202. doi: 10.1161/CIRCULATIONAHA.120.048180
6. Roffi M, Guagliumi G, Ibanez B. The obstacle course of reperfusion for ST-segment-elevation myocardial infarction in the COVID-19 pandemic. *Circulation.* (2020) 141:1951–3. doi: 10.1161/CIRCULATIONAHA.120.047523
7. Rodríguez-Leor O, Cid-Álvarez B, Pérez De Prado A, Rossello X, Ojeda S, Serrador A, et al. Impact of COVID-19 on ST-segment elevation myocardial infarction care. The Spanish experience. *Rev Esp Cardiol.* (2020) 73:994–1002. doi: 10.1016/j.recesp.2020.07.033
8. Xiang D, Xiang X, Zhang W, Yi S, Zhang J, Gu X, et al. Management and outcomes of patients with STEMI during the COVID-19 pandemic in China. *J Am Coll Cardiol.* (2020) 76:1318–24. doi: 10.1016/j.jacc.2020.06.039
9. Garcia S, Albaghdadi MS, Meraj PM, Schmidt C, Garberich R, Jaffer FA, et al. Reduction in ST-segment elevation cardiac catheterization laboratory activations in the United States during COVID-19 pandemic. *J Am Coll Cardiol.* (2020) 75:2871–2. doi: 10.1016/j.jacc.2020.04.011
10. NHCotPsRo, China. *Chinese Clinical Guideline for COVID-19 Diagnosis and Treatment.* 7th ed. National Health Committee (2020). Available online at: http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml (accessed March 4, 2020).
11. Xiang D, Huo Y, Ge J. Expert consensus on operating procedures at chest pain centers in China during the coronavirus infectious disease-19 epidemic. *Cardiol Plus.* (2020) 5:21–32. doi: 10.4103/cp.cp_5_20
12. Han Y, Zeng H, Jiang H, Yang Y, Yuan Z, Cheng X, et al. CSC expert consensus on principles of clinical management of patients with severe emergent cardiovascular diseases during the COVID-19 epidemic. *Circulation.* (2020) 141:e810–6. doi: 10.1161/CIRCULATIONAHA.120.047011
13. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth universal definition of myocardial infarction (2018). *Circulation.* (2018) 138:e618–51. doi: 10.1161/CIR.0000000000000617
14. Fox KA, Dabbous OH, Goldberg RJ, Pieper KS, Eagle KA, Van de Werf F, et al. Prediction of risk of death and myocardial infarction in the six months after presentation with acute coronary syndrome: prospective multinational observational study (GRACE). *BMJ.* (2006) 333:1091. doi: 10.1136/bmj.38985.646481.55
15. Mahmud E, Dauerman HL, Welt FGP, Messenger JC, Rao SV, Grines C, et al. Management of acute myocardial infarction during the COVID-19 pandemic: a position statement from the society for cardiovascular angiography and interventions (SCAI), the American college of cardiology (ACC), and the

American college of emergency physicians (ACEP). *J Am Coll Cardiol.* (2020) 76:1375–84. doi: 10.1016/j.jacc.2020.04.039
16. Scholz KH, Lengenfelder B, Thilo C, Jeron A, Stefanow S, Janssens U, et al. Impact of COVID-19 outbreak on regional STEMI care in Germany. *Clin Res Cardiol.* (2020) 109:1511–21. doi: 10.1007/s00392-020-01703-z
17. Zhang Y, Yu B, Han Y, Wang J, Yang L, Wan Z, et al. Protocol of the China ST-segment elevation myocardial infarction (STEMI) care project (CSCAP): a 10-year project to improve quality of care by building up a regional STEMI care network. *BMJ Open.* (2019) 9:e26362. doi: 10.1136/bmjopen-2018-026362
18. De Luca G, Verdoia M, Cercek M, Jensen LO, Vavlukis M, Calmac L, et al. Impact of COVID-19 pandemic on mechanical reperfusion for patients with STEMI. *J Am Coll Cardiol.* (2020) 76:2321–30. doi: 10.1016/j.jacc.2020.09.546
19. Welt F, Shah PB, Aronow HD, Bortnick AE, Henry TD, Sherwood MW, et al. Catheterization laboratory considerations during the coronavirus (COVID-19) pandemic: from the ACC's interventional council and SCAI. *J Am Coll Cardiol.* (2020) 75:2372–5. doi: 10.1016/j.jacc.2020.03.021
20. Hannan EL, Zhong Y, Jacobs AK, Holmes DR, Walford G, Venditti FJ, et al. Effect of onset-to-door time and door-to-balloon time on mortality in patients undergoing percutaneous coronary interventions for ST-segment elevation myocardial infarction. *Am J Cardiol.* (2010) 106:143–7. doi: 10.1016/j.amjcard.2010.02.029
21. Vogel B, Claessen BE, Arnold SV, Chan D, Cohen DJ, Giannitsis E, et al. ST-segment elevation myocardial infarction. *Nat Rev Dis Primers.* (2019) 5:39. doi: 10.1038/s41572-019-0090-3
22. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol.* (2020) 17:259–60. doi: 10.1038/s41569-020-0360-5
23. Tam CF, Cheung K, Lam S, Wong A, Yung A, Sze M, et al. Impact of coronavirus disease 2019 (COVID-19) outbreak on ST-segment-elevation myocardial infarction care in Hong Kong, China. *Circ Cardiovasc Qual Outcomes.* (2020) 13:e6631. doi: 10.1161/CIRCOUTCOMES.120.006631
24. Siudak Z, Grygier M, Wojakowski W, Malinowski KP, Witkowski A, Gasior M, et al. Clinical and procedural characteristics of COVID-19 patients treated with percutaneous coronary interventions. *Catheter Cardiovasc Interv.* (2020) 96:E568–75. doi: 10.1002/ccd.29134
25. Clifford C R, Le May M, Chow A, Boudreau R, Fu A, Barry Q, et al. Delays in ST-elevation myocardial infarction care during the COVID-19 lockdown: an observational study. *CJC Open.* (2020) 3:565–73. doi: 10.1016/j.cjco.2020.12.009
26. Hammad T A, Parikh M, Tashtish N, Lowry CM, Gorbey D, Forouzandeh F, et al. Impact of COVID-19 pandemic on ST-elevation myocardial infarction in a non-COVID-19 epicenter. *Catheter Cardiovasc Interv.* (2021) 97:208–14. doi: 10.1002/ccd.28997
27. Campo G, Fortuna D, Berti E, De Palma R, Pasquale GD, Galvani M, et al. In- and out-of-hospital mortality for myocardial infarction during the first wave of the COVID-19 pandemic in Emilia-Romagna, Italy: a population-based observational study. *Lancet Reg Health Eur.* (2021) 3:100055. doi: 10.1016/j.lanepe.2021.100055

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 27 July 2021
doi: 10.3389/fcvm.2021.702507

IL-10 and IL-12 (P70) Levels Predict the Risk of Covid-19 Progression in Hypertensive Patients: Insights From the BRACE-CORONA Trial

Renata Moll-Bernardes[1], Andrea Silvestre de Sousa[1,2], Ariane V. S. Macedo[1,3,4], Renato D. Lopes[1,5,6], Narendra Vera[7], Luciana C. R. Maia[7], André Feldman[1,8], Guilherme D. A. S. Arruda[9], Mauro J. C. Castro[10], Pedro M. Pimentel-Coelho[7], Denílson C. de Albuquerque[1,11], Thiago Ceccatto de Paula[3], Thyago A. B. Furquim[12], Vitor A. Loures[8], Karla G. D. Giusti[13], Nathália M. de Oliveira[13], Fábio A. De Luca[14], Marisol D. M. Kotsugai[14], Rafael A. M. Domiciano[8], Mayara Fraga Santos[1], Olga Ferreira de Souza[1,15], Fernando A. Bozza[1,2], Ronir Raggio Luiz[1,16†] and Emiliano Medei[1,7,17†]*

[1] D'Or Institute for Research and Education, Rio de Janeiro, Brazil, [2] Evandro Chagas National Institute of Infectious Disease, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, [3] Hospital São Luiz Jabaquara, São Paulo, Brazil, [4] Santa Casa de São Paulo, São Paulo, Brazil, [5] Duke Clinical Research Institute, Duke University Medical Center, Durham, NC, United States, [6] Brazilian Clinical Research Institute, São Paulo, Brazil, [7] Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [8] Hospital São Luiz Anália Franco, São Paulo, Brazil, [9] Hospital São Luiz São Caetano, São Caetano do Sul, Brazil, [10] Instituto de Microbiologia Paulo de Góes, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [11] Cardiology Department, Rio de Janeiro State University, Rio de Janeiro, Brazil, [12] Hospital Sino Brasileiro, Osasco, Brazil, [13] Hospital Villa Lobos, São Paulo, Brazil, [14] Hospital São Luiz Morumbi, São Paulo, Brazil, [15] Hospital Copa Star, Rio de Janeiro, Brazil, [16] Institute for Studies in Public Health—IESC, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [17] National Center for Structural Biology and Bioimaging, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

OPEN ACCESS

Edited by:
Masanori Aikawa,
Brigham and Women's Hospital and Harvard Medical School, United States

Reviewed by:
Angelo Silverio,
University of Salerno, Italy
Valeria Visco,
University of Salerno, Italy

***Correspondence:**
Emiliano Medei
emedei70@biof.ufrj.br

†These authors have contributed equally to this work and share last authorship

Specialty section:
This article was submitted to Hypertension, a section of the journal Frontiers in Cardiovascular Medicine

Received: 29 April 2021
Accepted: 02 July 2021
Published: 27 July 2021

Citation:
Moll-Bernardes R, Sousa AS, Macedo AVS, Lopes RD, Vera N, Maia LCR, Feldman A, Arruda GDAS, Castro MJC, Pimentel-Coelho PM, Albuquerque DC, de Paula TC, Furquim TAB, Loures VA, Giusti KGD, Oliveira NM, De Luca FA, Kotsugai MDM, Domiciano RAM, Santos MF, Souza OF, Bozza FA, Luiz RR and Medei E (2021) IL-10 and IL-12 (P70) Levels Predict the Risk of Covid-19 Progression in Hypertensive Patients: Insights From the BRACE-CORONA Trial. Front. Cardiovasc. Med. 8:702507. doi: 10.3389/fcvm.2021.702507

Background: Cardiovascular comorbidities such as hypertension and inflammatory response dysregulation are associated with worse COVID-19 prognoses. Different cytokines have been proposed to play vital pathophysiological roles in COVID-19 progression, but appropriate prognostic biomarkers remain lacking. We hypothesized that the combination of immunological and clinical variables at admission could predict the clinical progression of COVID-19 in hypertensive patients.

Methods: The levels of biomarkers, including C-reactive protein, lymphocytes, monocytes, and a panel of 29 cytokines, were measured in blood samples from 167 hypertensive patients included in the BRACE-CORONA trial. The primary outcome was the highest score during hospitalization on the modified WHO Ordinal Scale for Clinical Improvement. The probability of progression to severe disease was estimated using a logistic regression model that included clinical variables and biomarkers associated significantly with the primary outcome.

Results: During hospitalization, 13 (7.8%) patients showed progression to more severe forms of COVID-19, including three deaths. Obesity, diabetes, oxygen saturation, lung involvement on computed tomography examination, the C-reactive protein level, levels of 15 cytokines, and lymphopenia on admission were associated with progression to severe COVID-19. Elevated levels of interleukin-10 and interleukin-12 (p70) combined

with two or three of the abovementioned clinical comorbidities were associated strongly with progression to severe COVID-19. The risk of progression to severe disease reached 97.5% in the presence of the five variables included in our model.

Conclusions: This study demonstrated that interleukin-10 and interleukin-12 (p70) levels, in combination with clinical variables, at hospital admission are key biomarkers associated with an increased risk of disease progression in hypertensive patients with COVID-19.

Keywords: hypertension, cytokine, COVID-19, biomarker, inflammation, prognosis

INTRODUCTION

COVID-19 may evolve to severe viral pneumonia and acute respiratory distress syndrome with a high mortality rate. Importantly, patients with cardiac comorbidities have been found in various studies to be at greater risk of severe disease (1–6). In addition, patients with cardiovascular disease are more prone to myocardial injury development after SARS-CoV-2 infection (7–9).

The pathophysiological mechanisms related to these increased risks in patients with cardiac comorbidities are not completely understood. Concern has been raised about the use of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) in hypertensive patients, as preclinical studies have suggested that renin-angiotensin-aldosterone system inhibitors increase the expression of angiotensin-converting enzyme 2, the functional SARS-CoV-2 receptor (10–12). A recent randomized trial from our group (the BRACE-CORONA trial), in which 659 hypertensive patients were included, demonstrated that the discontinuation of ACEIs and ARBs for 30 days does not impact the number of days over a 30-day follow-up period that patients hospitalized with mild to moderate COVID-19 remain alive and out of the hospital (13, 14).

In addition to cardiac risk factors, several studies have suggested the occurrence of a dysregulated inflammatory response, characterized by the simultaneous release of pro- and anti-inflammatory mediators, known as a cytokine storm and established as a key factor in the physiopathology and clinical progression of COVID-19 in a subset of patients (15, 16). An exacerbated immune response is well-accepted to potentially strongly impair cardiac function (17–20). Several cytokines have been proposed to be potential biomarkers of COVID-19 severity (21–24); interferon gamma–induced protein 10 (IP-10), interleukin (IL)-6, and IL-10 have been associated consistently with greater severity of this disease (25–28).

The uncertainty and variability of the innate immune response, associated with an unpredictable disease course ranging from mild to fatal, highlights the need to identify prognostic factors related to a greater risk of progression to severe disease, particularly in more susceptible patients with comorbidities such as hypertension. To our knowledge, however, no data have been provided about biomarkers that could allow clinicians to identify, in the first 48 h after hospital admission, hypertensive patients at increased risk of disease progression, thereby helping them to choose the best therapeutic option.

This study was conducted to test the hypothesis that the immunological profiles of hypertensive patients upon admission to hospital with COVID-19 provide additional information about disease severity and progression. The analysis of cellular components, such as lymphocytes and monocytes, and the quantification of cytokine concentrations were performed to identify potential biomarkers.

MATERIALS AND METHODS

Population and Design

Patients included in this study were from the BRACE-CORONA trial (14), an academically led, investigator-initiated phase IV multicenter open-label registry-based randomized trial involving 659 patients on ACEIs/ARBs with confirmed COVID-19 diagnoses at 29 centers in Brazil. The present study was conducted with blood samples from 167 hospitalized hypertensive patients enrolled consecutively in the trial at six centers in the state of São Paulo, Brazil. The samples were collected within 24 h of COVID-19 diagnosis confirmation between 21 May and 27 June 2020. The trial protocol (13) was approved by the Brazilian Ministry of Health National Commission for Research Ethics and by institutional review boards or ethics committees at participating sites. All patients provided informed consent before enrollment.

Patients eligible for the BRACE-CORONA trial were aged ≥18 years and chronic ACEI/ARB users. Patients with clinical indications for ACEI/ARB treatment termination, such as hypotension, acute kidney injury, and/or shock, were excluded. Patients on mechanical ventilation and those with hemodynamic instability, acute renal failure, or shock also were excluded (14). The inclusion and exclusion criteria are provided in full in the **Supplementary Data**.

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; AUC, area under the receiver operating characteristic curve; CI, confidence interval; CRP, C-reactive protein; CT, computed tomography; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICU, intensive care unit; IFN, interferon; IL, interleukin; IP-10, interferon gamma–induced protein 10; LOS, length of stay; MCP-1, monocyte chemoattractant protein-1; MIP, macrophage inflammatory protein; OR, odds ratio; TNF, tumor necrosis factor; WHO, World Health Organization.

Outcomes

The primary outcome was defined as the highest score during hospitalization on the modified WHO Ordinal Scale for Clinical Improvement [range, 0 (no evidence of infection) to 8 (death)]. COVID-19 was classified as non-severe (mild to moderate, scores of 3–5), ranging from the lack of need for oxygen therapy to conditions requiring noninvasive ventilation, and severe (scores of 6–8), including disease necessitating the use of mechanical ventilation, inotropic support, and/or renal replacement therapy, and that causing death (**Supplementary Table 1**) (29). Secondary outcomes were the lengths of stay (LOSs) in the hospital and intensive care unit (ICU), acute myocardial infarction, new or worsening heart failure, hypertensive crisis, transient ischemic attack, stroke, myocarditis, pericarditis, arrhythmias requiring treatment, and thromboembolic events.

Biomarker Quantification

Blood samples were collected in tubes containing ethylenediaminetetraacetic acid as an anticoagulant and centrifuged immediately for plasma separation. Plasma samples were then frozen and stored at −20°C until analysis. Levels of epidermal growth factor, eotaxin, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-α2, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2–8, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1α, MIP-1β, tumor necrosis factor (TNF)-α, TNF-β, and vascular endothelial growth factor in undiluted samples were measured using the MILLIPLEX MAP human cytokine/chemokine magnetic bead panel (HCYTMAG-60K-PX29; Merck Millipore, Billerica, MA, USA) according to the manufacturer's instructions. The assay plates were read immediately and analyzed in a MAGPIX® system (Merck Millipore). All samples and standards were measured in duplicate. Lymphocyte and monocyte quantification was performed in an automized Horiba ABX Micros 60 system (Horiba Medical, Montpellier, France) using photometry. C-reactive protein (CRP) was measured by latex-enhanced immunoturbidimetric assay. Cytokines not detected in >50% of the patient samples were excluded from further analyses.

TABLE 1 | Baseline patient characteristics by primary outcome*.

Clinical Conditions	Total	Score 3–5 (*n* = 154/167)	Score 6–8 (*n*= 13/167)	Fisher's exact test *P*-value
	n	*n* (%)	*n* (%)	
Sex				
Male	110	99 (90.0)	11 (10.0)	0.22
Female	57	55 (96.5)	2 (3.5)	
Age				
<60 years old	114	105 (92.1)	9 (7.9)	1.00
60 and older	53	49 (92.5)	4 (7.5)	
Signs of pulmonary involvement				
O_2 sat > 93% and CT ≤ 50%†	131	126 (96.2)	5 (3.8)	0.001
O_2 sat ≤ 93% or CT > 50%	36	28 (77.8)	8 (22.2)	
Obesity				
No (BMI < 30 kg/m²) †	79	78 (98.7)	1 (1.3)	0.003
Yes (BMI ≥ 30 kg/m²)	88	76 (86.4)	12 (13.6)	
Diabetes				
No	126	123 (97.6)	3 (2.4)	< 0.001
Yes	41	31 (75.6)	10 (24.4)	
Asthma/COPD				
No	164	151 (92.1)	13 (7.9)	1.00
Yes	3	3 (100.0)	0 (0.0)	
Dyslipidemia				
No	138	128 (92.8)	10 (7.2)	0.70
Yes	29	26 (89.7)	3 (10.3)	
Coronary disease				
No	163	151 (92.6)	12 (7.4)	0.28
Yes	4	3 (75.0)	1 (25.0)	

**Highest modified World Health Organization WHO Ordinal Scale for Clinical Improvement.*

†Extent of lung involvement on CT examination.

BMI, body mass index; O_2 sat, oxygen saturation; COPD, chronic obstructive pulmonary disease; CT, computed tomography.

Statistical Analysis

Continuous variables were described as medians, means, and standard deviations; categorical variables were characterized by proportions. For the primary outcome, 95% confidence intervals (CIs) were calculated. Fisher's exact test was used to detect statistical associations between the outcome and categorical clinical variables. For continuous variables, receiver operating characteristic (ROC) curves were used to discriminate between severe and non-severe cases, and those associated statistically with the primary outcome were dichotomized using cutoff points of 90% sensitivity. $P \leq 0.05$ was used to define significance and for automatic forward stepwise selection of clinical variables for inclusion in a binary logistic regression model. The significance levels for entry and removal of variables selected by the automatic regression model were defined at 5 and 10%, respectively. The beta coefficients and odd ratios were calculated for all variables in each step of the model to quantify the association with the outcome. The goodness of fit for the final model was evaluated by the Hosmer–Lemeshow test and by ROC curve. Predicted probabilities for the primary outcome were estimated using variables showing significant associations in the final model. All analysis were performed using SPSS software (version 24.0; IBM Corporation, Armonk, NY, USA).

RESULTS

Of the 167 hypertensive patients, 13.8% were using ACEIs and 86.2% were using ARBs. The mean patient age was 54.1 ± 12.3 years; 57 (34.1%) patients were female, 88 (52.7%) were obese, 41 (24.6%) had diabetes, and 29 (17.4%) had dyslipidemia. Coronary artery disease and chronic pulmonary disease were present in 2.4% of the cases each, and 2.4% of the patients were smokers. Data on all comorbidities are provided in **Supplementary Figure 1**.

Cough (62.3%), fever (57.5%), myalgia (46.7%), shortness of breath (44.9%), and fatigue (44.9%) were the most common symptoms at presentation (**Supplementary Figure 2**). The mean interval from symptom onset to hospital presentation was 5 ± 3.1 days, and 20.4% of patients had ≤93% baseline oxygen saturation. All patients included in this study had non-severe COVID-19 (WHO scores of 3–5) on admission. On chest computed tomography (CT) examinations, 59.9% of patients showed ≤25% lung involvement, 35.3% showed 26–50% involvement, and 4.8% showed >50% lung involvement. Thirty-six (21.6%) cases presented criteria for significant pulmonary involvement (oxygen saturation ≤ 93% and/or >50% lung involvement on CT) at admission (**Supplementary Table 2**).

Primary Outcome

Worst WHO clinical improvement scores during hospitalization were 3 (mild disease) in 81 (48.5%; 95% CI, 41.0–56.1%) cases, 4 or 5 (moderate disease) in 73 (43.7%; 95% CI, 36.3–51.3%) cases, and 6–8 (severe disease) in 13 (7.8%; 95% CI, 4.4–12.6) cases (**Supplementary Table 3**). Progression to severe disease was associated with obesity ($p = 0.003$), diabetes ($p < 0.001$), and oxygen saturation ($p = 0.001$) and lung involvement ($p = 0.001$) on admission, but not with age or sex (**Table 1**).

Secondary Outcomes

The mean hospital LOS was 9.1 ± 6.9 days. In total, 119 patients were admitted to the ICU; the mean ICU LOS was 7.6 ± 6.8 days (**Supplementary Table 4**). According to the report on the

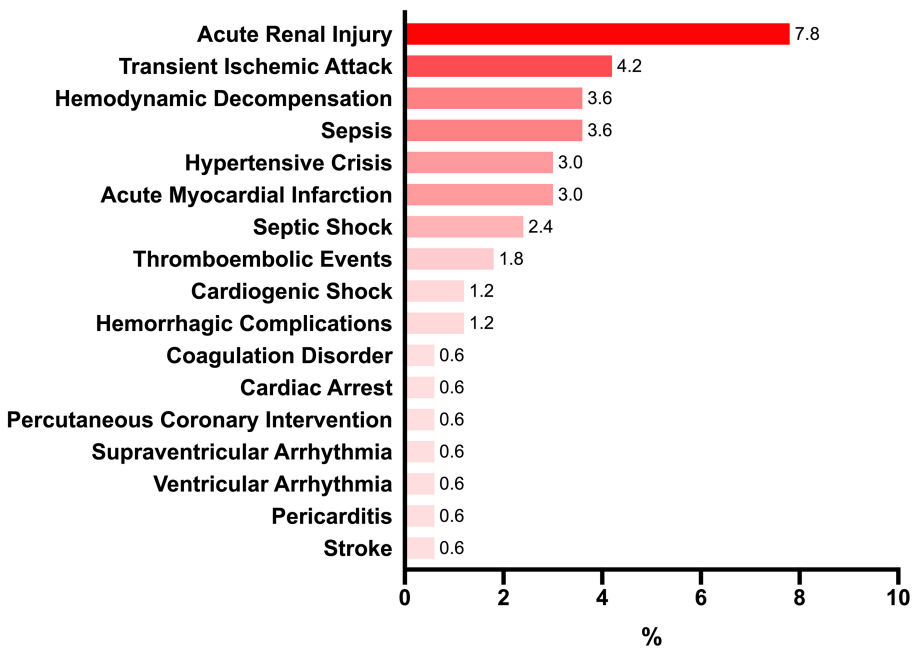


FIGURE 1 | Main complications occurring during hospitalization (%).

TABLE 2 | Biomarker levels according to WHO Ordinal Scale for Clinical Improvement.

	All			Non-severe (score 3–5)			Severe (score 6–8)		
	Median	**Mean**	**SD**	**Median**	**Mean**	**SD**	**Median**	**Mean**	**SD**
Lymphocytes*	1.40	1.51	0.74	1.44	1.53	0.65	0.73	1.30	1.45
Monocytes*	0.23	0.36	0.38	0.23	0.35	0.32	0.10	0.53	0.82
CRP†	2.23	4.53	5.28	2.08	4.05	4.95	10.70	10.20	6.01
MIP-1β	21.8	22.5	11.1	22.0	22.9	11.0	13.8	17.6	10.5
VEGF	57.2	80.5	83.9	55.0	81.1	86.8	67.4	72.8	34.6
TNF-β	0.9	27.1	102.1	0.8	28.9	106.1	2.6	6.4	11.1
TNF-α	15.8	16.0	7.4	15.6	15.8	7.4	18.5	18.1	6.6
MIP-1α	5.1	7.3	11.0	5.1	7.3	11.4	7.6	7.4	5.1
MCP-1	466	584	428	435	573	435	792	721	316
IP-10	2,357	2,765	2,482	1,842	2,509	2,155	5,434	5,798	3,904
IL-17A	0.4	2.7	5.9	0.2	2.6	6.0	2.0	4.3	5.2
IL-15	5.1	6.1	4.8	5.1	5.9	4.8	9.2	9.2	3.9
IL-13	1.7	13.1	43.0	1.3	13.8	44.6	1.7	4.7	11.4
IL-12 (p70)	0.8	1.4	2.1	0.6	1.4	2.0	1.6	2.5	2.3
IL-12 (p40)	1.3	4.2	6.6	1.2	4.1	6.6	4.3	6.3	6.2
IL-10	15.5	26.3	29.4	13.4	23.2	25.2	43.8	62.0	49.2
IL-8	8.9	14.2	18.5	8.6	13.8	18.5	13.9	18.8	19.2
IL-7	7.7	10.0	12.7	7.0	10.1	13.2	9.1	9.1	5.0
IL-6	5.5	28.4	76.8	5.1	28.1	78.8	12.6	32.1	49.3
IL-5	1.5	6.4	19.1	1.4	6.6	19.8	2.0	4.3	6.8
IL-4	0.0	239	916	0.0	254	951	10	62	185
IL-3	0.0	0.06	0.11	0.0	0.05	0.11	0.13	0.12	0.08
IL-2	0.48	1.11	2.01	0.29	1.06	2.03	1.15	1.72	1.71
IL-1ra	52	112	196	48	91	138	231	358	464
IL-1β	0.58	1.25	2.08	0.58	1.26	2.15	0.83	1.12	0.93
IL-1α	25.4	59.6	137.8	23.6	59.9	143.1	48.7	55.9	39.0
IFN-γ	5.5	12.6	22.5	5.4	12.5	23.2	11.9	13.4	11.2
IFN-α2	28.2	40.8	54.2	26.4	37.8	54.1	74.1	75.7	43.2
GM-CSF	2.68	4.01	5.00	1.92	3.84	5.09	5.37	5.97	3.37
G-CSF	57.8	65.7	55.4	53.4	61.3	52.7	118.6	118.3	61.2
eotaxin	177	195	96	177	196	99	182	186	51
EGF	208	247	205	204	251	211	214	197	111

CRP, C-reactive protein; EGF, epidermal growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IP-10, interferon gamma-induced protein 10; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage inflammatory protein; SD, standard deviation; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; WHO, World Health Organization.
$*10^9$ cells/L, †mg/L; all other biomarker units are pg/mL.

BRACE-CORONA trial (13), the mean numbers of days alive and out of hospital did not differ among patients hospitalized with mild to moderate COVID-19 according to ACEI/ARB discontinuation or continuation.

At least one complication occurred during hospitalization in 29 (17.4%) patients. The number of complications per patient ranged from one to nine. The most common complication was acute renal injury [$n = 13$ (7.8%); **Figure 1**]. The criteria used for the identification of these complications have been provided in the BRACE-CORONA trial (13). The most commonly administered treatments were antibiotics (98.2%), anticoagulants (68.3%), and corticosteroids (59.9%; **Supplementary Figure 3**).

Biomarkers

Blood samples were collected a mean of 2.8 days after hospitalization. Levels of IL-10, IP-10, G-CSF, IFN- α2, IL-1ra, IL-15, IL-1α, IL-12 (p70), IL-2, IL-17A, GM-CSF, IL-8, IL-6, MCP-1, and CRP were higher in patients with severe than in those with non-severe disease. In contrast, levels of lymphocytes and MIP-1β were lower in patients with severe than in those with non-severe disease (**Table 2**). IL-3 and IL-4 were not detected in >50% of patients and were excluded from further analyses.

Fifteen cytokines were found to be useful for the prediction of progression to severe COVID-19 [areas under the ROC curve (AUCs), 0.667–0.836]. Increased levels of 14 cytokines

TABLE 3 | Distinction of severe (modified WHO score 6–8) and non-severe (modified WHO score 3–5) cases by areas under ROC curves.

Biomarkers	Area under curve	*P*-value	Cut-off for 90% sensitivity
IL-10	**0.836**	**<0.001**	**26.0**
CRP†	**0.825**	**<0.001**	**2.70**
IP-10	**0.812**	**<0.001**	**2400**
G-CSF	**0.788**	**0.001**	**54.0**
IFN-α2	**0.775**	**0.001**	**19.4**
IL-1ra	**0.759**	**0.002**	**29.5**
IL-15	**0.750**	**0.003**	**5.1**
Lymphocytes*§	**0.742**	**0.004**	**2.11**
IL-1α	**0.731**	**0.006**	**26.1**
IL-12 (p70)	**0.730**	**0.006**	**0.91**
IL-2	**0.715**	**0.010**	**0.35**
IL-17A	**0.711**	**0.012**	**0.21**
GMCSF	**0.710**	**0.012**	**2.69**
MIP-1β§	**0.686**	**0.026**	**32.6**
IL-8	**0.682**	**0.030**	**6.2**
IL-6	**0.678**	**0.033**	**2.6**
MCP-1	**0.667**	**0.045**	**406**
IL-12 (p40)	0.664	0.051	#
TNF-β	0.644	0.085	#
TNF-α	0.607	0.202	#
IFN-γ	0.607	0.200	#
IL-1β	0.601	0.225	#
MIP-1α	0.584	0.313	#
IL-5	0.581	0.330	#
IL-7	0.576	0.366	#
VEGF	0.564	0.441	#
IL-13	0.539	0.637	#
Eotaxin	0.503	0.971	#
EGF	0.473	0.743	#
Monocytes*	0.378	0.143	#

CRP, C-reactive protein; EGF, epidermal growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IP-10, interferon gamma-induced protein 10; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage inflammatory protein; ROC, receiver operating characteristic; SD, standard deviation; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; WHO, World Health Organization.

**10^9 cells/L, †mg/L; all other biomarker units are pg/mL; §In contrast to other biomarkers, reduced values are predictors of disease progression; Bold values indicate significance at $p < 0.05$.*

#Not calculated due to lack of statistical association at established value.

and decreased levels of MIP-1β were associated with COVID-19 severity. AUCs for IL-10, IP-10, G-CSF, IFN- α2, IL-1ra, and IL-15 were ≥0.75 (**Table 3**). Increased CRP levels and reduced lymphocyte counts were also associated with disease severity (AUCs, 0.825 and 0.742, respectively; **Supplementary Figure 4**).

Predictive Model

The initial model for the prediction of the risk of progression of COVID-19 included diabetes, obesity, hypoxemia, lung involvement on CT, the CRP level, the lymphocyte count, and levels of 15 cytokines. Five variables were selected automatically in a forward stepwise manner: the IL-10 level (>26.0 pg/mL), diabetes, the IL-12 (p70) level (>0.91 pg/mL), obesity, and significant lung involvement on admission (oxygen saturation ≤ 93% or >50% lung involvement on CT). The IL-10 level was associated strongly with disease severity [odds ratio (OR) = 32]. The ORs for the other four variables also showed associations with progression to severe disease (**Table 4**), and the predictive value of the model increased strongly with the addition of these variables (OR = 78.3). The ROC curve for the predictive model showed a very high discriminatory power between the two groups with an AUC of 0.981 (**Supplementary Figure 5**).

TABLE 4 | Forward stepwise logistic regression results for COVID-19 severity.

Variables in the equation		β	*P*-value	Odds ratio
Step 1	IL-10 > 26	3.47	0.001	32.0
Step 2	Diabetes	2.82	< 0,001	16.8
	IL-10 > 26	3.68	0.001	39.6
Step 3	IL-12 (p70) > 0.91	3.30	0.009	27.1
	Diabetes	3.54	0.000	34.3
	IL-10 > 26	3.89	0.002	49.1
Step 4	Obesity	2.86	0.046	17.5
	IL-12 (p70) > 0.91	3.33	0.024	28.0
	Diabetes	3.89	0.001	49.0
	IL-10 > 26	4.35	0.002	77.2
Step 5 (final model)*	Lung involvement†	2.16	0.045	8.7
	Obesity	3.81	0.032	45.2
	IL-12p70 > 0.91	3.89	0.026	49.0
	Diabetes	3.58	0.004	35.9
	IL-10 > 26	4.36	0.005	78.3

IL, interleukin.

**Constant = −14.15 and Hosmer–Lemeshow test p = 1.000.*

†Significant lung involvement on admission (oxygen saturation ≤ 93% or >50% lung involvement on computed tomography examination).

Biomarker values are presented in pg/mL.

In the presence of two or three clinical comorbidities, the predictive capability of these biomarkers increased markedly (**Figure 2**). In patients with diabetes and obesity, for example, the likelihood of disease progression increased from 0.1% with low IL-10 and IL-12 (p70) levels to >80% with levels of these cytokines exceeding the 90% sensitivity thresholds. Similarly, the risk of progression to severe disease in the presence of three clinical comorbidities was 1.0% with IL-10 and IL-12 (p70) levels below the thresholds and 97.5% with levels exceeding the thresholds (**Table 5**).

DISCUSSION

In this study, we analyzed immune response patterns, including levels of 29 cytokines, CRP, monocytes, and lymphocytes, in a large sample (n = 167) of hospitalized hypertensive patients from the BRACE-CORONA trial (13). In univariate analysis, progression to severe COVID-19 was associated with clinical factors (diabetes, obesity, and lung involvement on admission) and levels of biomarkers, including 15 cytokines, CRP, and

lymphocytes. We propose a logistic regression model that includes clinical variables (diabetes, obesity, and significant lung involvement) and critical biomarkers [IL-10 and IL-12 (p70)]. This combined use of clinical risk factors and biomarkers for the prediction of COVID-19 severity at admission is novel.

Clinical comorbidities, particularly diabetes, hypertension, and other cardiovascular diseases, have been associated with COVID-19 severity, as they are more prevalent in non-survivors and patients requiring ICU care (1, 30–32). However, the mechanisms involved in the increased risk of COVID-19 in these patients are not understood completely. Infections are more prevalent and have more complicated courses in patients with diabetes, possibly due to disturbances in humoral and cellular immunity and exaggerated pro-inflammatory cytokine responses (33, 34). In addition, obesity has been associated with ICU admission and mortality in patients with COVID-19, which may be related to the presence of angiotensin-converting enzyme 2 receptors in adipose tissue, elevated pro-inflammatory cytokine levels, increased susceptibility to infection by various pathogens (35), and pro-coagulant profiles (36). Moreover, the extent of CT lung involvement has been correlated with COVID-19 severity, and severity scores for chest CT findings have been proposed to enable the differentiation of clinical forms and prediction of clinical outcomes (37, 38). The lack of association between age and the outcome in the present study may be related to the relative young mean age of our sample, due to the exclusion of patients with severe disease in the first 24 h after admission.

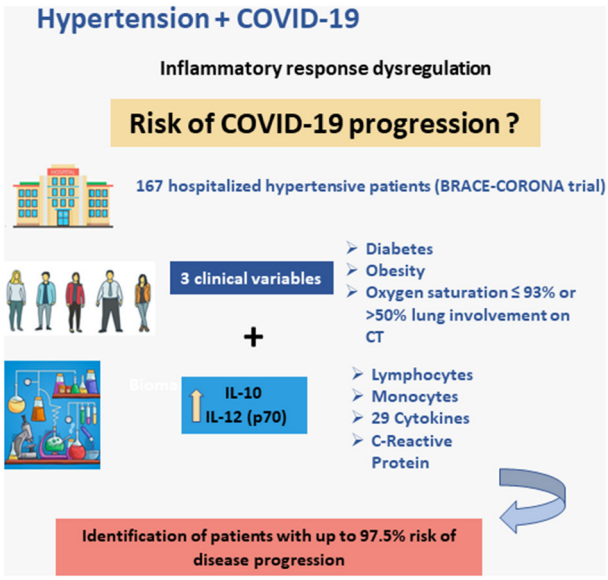


FIGURE 2 | Schematic illustration of the main findings: model for the prediction of the risk of progression of COVID-19 including three clinical variables and two biomarkers: IL-10 and IL-12 (p70).

In this study, admission levels of 17 biomarkers (increased levels of CRP and 14 cytokines and reduced levels of MIP-1β and lymphocytes) were associated significantly with progression to severe disease. Our biomarker findings are similar to previously reported associations of the levels of several cytokines (e.g., IL-1ra, IL-2, IL-6, IL-8, IL-10, and IP-10) with COVID-19 severity and mortality (25, 27, 39–41). The association of the IL-10 level with COVID-19 progression to severity has been reported in a considerable number of publications (40–42). IL-10 is an immunoregulatory cytokine with the main functions of limiting inflammatory responses and regulating immune cell differentiation and proliferation (43). Information about the role of IL-12 (p70) in COVID-19 is more limited. Consistent with our findings, higher levels of IL-12 (p70) have been associated with severe COVID-19 (44, 45). IL-12 is a heterodimeric cytokine composed of p35 and p40 subunits that enhances connections between the innate and adaptive immune responses; its expression is induced via a pathogen-associated molecular response when a virus enters a cell (46).

Although the ability of clinical and laboratory variables to independently predict COVID-19 severity has been assessed

TABLE 5 | Probability of COVID-19 progression according to the final logistic model.

	IL-10 ≤ 26 and IL12p70 ≤ 0.91	IL10 ≤ 26 and IL12p70 > 0.91	IL10 > 26 and IL12p70 ≤ 0.91	IL10 > 26 and IL12p70 > 0.91
No clinical risk factors[†]	<0.1%	<0.1%	<0.1%	0.3%
Only diabetes	<0.1%	0.1%	0.2%	8.9%
Only obesity	<0.1%	0.2%	0.3%	10.9%
Only significant lung involvement[‡]	<0.1%	<0.1%	<0.1%	2.3%
Diabetes + obesity	0.1%	5.3%	8.3%	81.5%
Diabetes + significant lung involvement	< 0.1%	1.1%	1.7%	45.9%
Obesity + significant lung involvement	< 0.1%	1.3%	2.1%	51.6%
Diabetes + obesity + significant lung involvement	1.0%	32.8%	43.9%	97.5%

IL, interleukin.
Biomarker values are presented in pg/mL.
[†]*Clinical risk factors in the model are diabetes, obesity, and significant lung involvement on admission.*
[‡]*Oxygen saturation ≤ 93% or >50% lung involvement on computed tomography examination.*

extensively and predictive models have been proposed, no definitive prognostic biomarker or effective predictive model for the identification, at the time of hospital admission, of patients who will require ICU care, mechanical ventilation, or inotropic support has emerged (47, 48). According to the model we propose, the probability of progression to severe disease in hypertensive patients with obesity and diabetes is 0.1% in the absence of increased IL-10 and IL-12 (p70) levels, but 81.5% with levels of these two cytokines exceeding the 90% sensitivity thresholds. Similarly, in the presence of the three clinical comorbidities (obesity, diabetes, and oxygen saturation $\leq$93% or $>$50% lung involvement on CT), the probability of progression is 1% with lower IL-10 and IL-12 (p70) levels, but 97.5% with elevated levels of these biomarkers. A practical approach to model application for the estimation of the risk of progression to severe COVID-19 would be to measure IL-10 and IL-12 (p70) levels on admission in hypertensive patients with two or three of the relevant clinical comorbidities.

Limitations

This study has some limitations. Blood samples were collected a mean of 2.8 days after hospitalization (usually within 24 h after confirmation of SARS-CoV-2 infection); with a median 6-day interval between symptom onset and hospital admission, and our population included only hypertensive patients who were taking ACEi or ARBs, which might limit the generalizability of our results. Nevertheless, we believe that the widespread use of these drugs in the hypertensive population associated with the multicentric nature of the study might help to ensure a good external validity. Besides, we were not able to validate our model with a different patient sample. Additional studies are needed to validate the results obtained here in more heterogeneous populations of hypertensive patients and also to evaluate the applicability of the proposed model in non-hypertensive COVID-19 populations.

CONCLUSION

The measurement of IL-10 and IL-12 (p70) levels on admission may be useful for the identification of hypertensive patients at greater risk of COVID-19 progression, particularly in the presence of classical clinical comorbidities (obesity, diabetes, and extensive lung involvement). We propose a new biomarker-based approach to improve the prediction of COVID-19 progression in hypertensive patients, which may help physicians identify patients at high risk who would benefit from more intensive surveillance and treatment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Brazilian Ministry of Health's National Commission for Research Ethics (CAAE # 30432020.2.0000.5249). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RM-B, EM, AS, FB, and RRL: study design. OS, AM, RDL, AF, GA, DA, and MS: patient recruitment, data, and sample collection organization. RM-B and EM: application for the funding and writing-original draft preparation. AM, AF, GA, TP, TF, VL, KG, NO, FD, MK, and RD: patient recruitment and sample collection. NV, LM, MC, and PP-C: biomarker processing and analyses. RDL, RM-B, AM, and RRL: data curation. RRL: statistical analyses. AS, FB, and RDL writing-review and editing. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by intramural grants from the D'Or Institute of Research and Education, the Brazilian Research Institute, the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (nos. E-26/210.155/2020, E-26/203.169/2017, E-26/210.191/2020, and E-26/210.253/2020), the CNPq (no. 310681/2018-9), CAPES, FINEP, and the Serrapilheira Institute.

ACKNOWLEDGMENTS

We thank all staff and research assistants from: D'Or Institute of Research and Education (IDOR), Brazilian Clinical Research Institute (BCRI) and Rede D'Or Hospitals which took part in this study. In addition, the authors thank all patients for accepting participating in this project.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.702507/full#supplementary-material

REFERENCES

1. Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A, et al. COVID-19 and cardiovascular disease. *Circulation.* (2020) 141:1648–55. doi: 10.1161/CIRCULATIONAHA.120.046941
2. Mehra MR, Desai SS, Kuy S, Henry TD, Patel AN. Cardiovascular disease, drug therapy, and mortality in Covid-19. *N Engl J Med.* (2020) 382:e102. doi: 10.1056/NEJMoa2007621
3. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA.* (2020) 323:1061–69. doi: 10.1001/jama.2020.1585

4. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol.* (2020) 17:259–60. doi: 10.1038/s41569-020-0360-5
5. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3
6. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med.* (2020) 180:934–43. doi: 10.1001/jamainternmed.2020.0994
7. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017
8. Liu PP, Blet A, Smyth D, Li H. The science underlying COVID-19: implications for the cardiovascular system. *Circulation.* (2020) 142:68–78. doi: 10.1161/CIRCULATIONAHA.120.047549
9. Nguyen LS, Dolladille C, Drici M-D, Fenioux C, Alexandre J, Mira J-P, et al. Cardiovascular toxicities associated with hydroxychloroquine and azithromycin: an analysis of the World Health Organization Pharmacovigilance Database. *Circulation.* (2020) 142:303–5. doi: 10.1161/CIRCULATIONAHA.120.048238
10. Vaduganathan M, Vardeny O, Michel T, McMurray JJ, Pfeffer MA, Solomon SD. Renin-angiotensin-aldosterone system inhibitors in patients with Covid-19. *N Engl J Med.* (2020) 382:1653–9. doi: 10.1056/NEJMsr2005760
11. Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong J-C, Turner AJ, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. *Circ Res.* (2020) 126:1456–74. doi: 10.1161/CIRCRESAHA.120.317015
12. Reynolds HR, Adhikari S, Pulgarin C, Troxel AB, Iturrate E, Johnson SB, et al. Renin-angiotensin-aldosterone system inhibitors and risk of Covid-19. *N Engl J Med.* (2020) 382:2441–8. doi: 10.1056/NEJMoa2008975
13. Lopes RD, Macedo AVS, de Barros ESPGM, Moll-Bernardes RJ, Dos Santos TM, Mazza L, et al. Effect of discontinuing vs continuing angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers on days alive and out of the hospital in patients admitted with COVID-19: a randomized clinical trial. *JAMA.* (2021) 325:254–64. doi: 10.1001/jama.2020.25864
14. Lopes RD, Macedo AVS, de Barros ESPGM, Moll-Bernardes RJ, Feldman A, D'Andrea Saba Arruda G, et al. Continuing versus suspending angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: Impact on adverse outcomes in hospitalized patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–The BRACE CORONA Trial. *Am Heart J.* (2020) 226:49–59. doi: 10.1016/j.ahj.2020.05.002
15. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet.* (2020) 395:1033–4. doi: 10.1016/S0140-6736(20)30628-0
16. Lowenstein CJ, Solomon SD. Severe COVID-19 is a microvascular disease. *Circulation.* (2020) 142:1609–11. doi: 10.1161/CIRCULATIONAHA.120.050354
17. Monnerat G, Alarcón ML, Vasconcellos LR, Hochman-Mendez C, Brasil G, Bassani RA, et al. Macrophage-dependent IL-1β production induces cardiac arrhythmias in diabetic mice. *Nat Commun.* (2016) 7:1–15. doi: 10.1038/ncomms13344
18. Alarcon MML, Trentin-Sonoda M, Panico K, Schleier Y, Duque T, Moreno-Loaiza O, et al. Cardiac arrhythmias after renal I/R depend on IL-1β. *J Mol Cell Cardiol.* (2019) 131:101–11. doi: 10.1016/j.yjmcc.2019.04.025
19. Belhadjer Z, Méot M, Bajolle F, Khraiche D, Legendre A, Abakka S, et al. Acute heart failure in multisystem inflammatory syndrome in children in the context of global SARS-CoV-2 pandemic. *Circulation.* (2020) 142:429–36. doi: 10.1161/CIRCULATIONAHA.120.048360
20. Lazzerini PE, Boutjdir M, Capecchi PL. COVID-19, arrhythmic risk, and inflammation: mind the gap! *Circulation.* (2020) 142:7–9. doi: 10.1161/CIRCULATIONAHA.120.047293
21. Wan S, Yi Q, Fan S, Lv J, Zhang X, Guo L, et al. Relationships among lymphocyte subsets, cytokines, and the pulmonary inflammation index in coronavirus (COVID-19) infected patients. *Br J Haematol.* (2020) 189:428–37. doi: 10.1111/bjh.16659
22. Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, Ruiz C, Melguizo-Rodriguez L. SARS-CoV-2 infection: the role of cytokines in COVID-19 disease. *Cytokine Growth Factor Rev.* (2020) 54:62–75. doi: 10.1016/j.cytogfr.2020.06.001
23. Conti P, Ronconi G, Caraffa A, Gallenga CE, Ross R, Frydas I, Kritas SK. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. *J Biol Regul Homeost Agents.* (2020) 34:327–31. doi: 10.23812/CONTI-E
24. Han H, Ma Q, Li C, Liu R, Zhao L, Wang W, et al. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. *Emerg Microbes Infect.* (2020) 9:1123–30. doi: 10.1080/22221751.2020.1770129
25. Yang Y, Shen C, Li J, Yuan J, Wei J, Huang F, et al. Plasma IP-10 and MCP-3 levels are highly associated with disease severity and predict the progression of COVID-19. *J Allergy Clin Immunol.* (2020) 146:119–27.e114. doi: 10.1016/j.jaci.2020.04.027
26. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest.* (2020) 130:2620–9. doi: 10.1172/JCI137244
27. Sun HB, Zhang YM, Huang LG, Lai QN, Mo Q, Ye XZ, et al. The changes of the peripheral CD4+ lymphocytes and inflammatory cytokines in Patients with COVID-19. *PLoS One.* (2020) 15:e0239532. doi: 10.1371/journal.pone.0239532
28. Dhar SK, K V, Damodar S, Gujar S, Das M. IL-6 and IL-10 as predictors of disease severity in COVID-19 patients: results from meta-analysis and regression. *Heliyon.* (2021) 7:e06155. doi: 10.1016/j.heliyon.2021.e06155
29. World Health Organization. *WHO R&D Blueprint: COVID-19 Therapeutic Trial Synopsis*. (2020). Available online at: https://www.who.int/publications/i/item/covid-19-therapeutic-trial-synopsis (accessed October 20, 2020).
30. Iaccarino G, Grassi G, Borghi C, Ferri C, Salvetti M, Volpe M. Age and multimorbidity predict death among COVID-19 patients: results of the SARS-RAS study of the Italian Society of Hypertension. *Hypertension.* (2020) 76:366–72. doi: 10.1161/HYPERTENSIONAHA.120.15324
31. Semenzato L, Botton J, Drouin J, Baricault B, Vabre C, Cuenot F, et al. Antihypertensive drugs and COVID-19 risk: a cohort study of 2 million hypertensive patients. *Hypertension.* (2021) 77:833–42. doi: 10.1161/HYPERTENSIONAHA.120.16314
32. Silverio A, Di Maio M, Citro R, Esposito L, Iuliano G, Bellino M, et al. Cardiovascular risk factors and mortality in hospitalized patients with COVID-19: systematic review and meta-analysis of 45 studies and 18,300 patients. *BMC Cardiovasc Disord.* (2021) 21:23. doi: 10.1186/s12872-020-01816-3
33. Geerlings SE, Hoepelman AI. Immune dysfunction in patients with diabetes mellitus (DM). *FEMS Immunol Med Microbiol.* (1999) 26:259–65. doi: 10.1111/j.1574-695X.1999.tb01397.x
34. Pal R, Bhansali A. COVID-19, diabetes mellitus and ACE2: the conundrum. *Diabetes Res Clin Pract.* (2020) 162:108132. doi: 10.1016/j.diabres.2020.108132
35. Dicker D, Bettini S, Farpour-Lambert N, Frühbeck G, Golan R, Goossens G, et al. Obesity and COVID-19: the two sides of the coin. *Obes Facts.* (2020) 13:430–8. doi: 10.1159/000510005
36. Sattar N, McInnes IB, McMurray JJ. Obesity is a risk factor for severe COVID-19 infection: multiple potential mechanisms. *Circulation.* (2020) 142:4–6. doi: 10.1161/CIRCULATIONAHA.120.047659
37. Yang R, Li X, Liu H, Zhen Y, Zhang X, Xiong Q, et al. Chest CT severity score: an imaging tool for assessing severe COVID-19. *Radiology.* (2020) 2:e200047. doi: 10.1148/ryct.2020200047
38. Francone M, Iafrate F, Masci GM, Coco S, Cilia F, Manganaro L, et al. Chest CT score in COVID-19 patients: correlation with disease severity and short-term prognosis. *Eur Radiol.* (2020) 30:6808–17. doi: 10.1007/s00330-020-07033-y

39. Jin M, Shi N, Wang M, Shi C, Lu S, Chang Q, et al. CD45: a critical regulator in immune cells to predict severe and non-severe COVID-19 patients. *Aging (Albany NY).* (2020) 12:19867. doi: 10.18632/aging.103941
40. Varchetta S, Mele D, Oliviero B, Mantovani S, Ludovisi S, Cerino A, et al. Unique immunological profile in patients with COVID-19. *Cell Mol Immunol.* (2020) 18:1–11. doi: 10.21203/rs.3.rs-23953/v1
41. Li Q, Xu W, Li W, Huang C, Chen L. Dynamics of cytokines and lymphocyte subsets associated with the poor prognosis of severe COVID-19. *Eur Rev Med Pharmacol Sci.* (2020) 24:12536–44. doi: 10.26355/eurrev_202012_24051
42. Liu QQ, Cheng A, Wang Y, Li H, Hu L, Zhao X, et al. Cytokines and their relationship with the severity and prognosis of coronavirus disease 2019 (COVID-19): a retrospective cohort study. *BMJ Open.* (2020) 10:e041471. doi: 10.1136/bmjopen-2020-041471
43. Asadullah K, Sterry W, Volk H. Interleukin-10 therapy-review of a new approach. *Pharmacol Rev.* (2003) 55:241–69. doi: 10.1124/pr.55.2.4
44. Young BE, Ong SWX, Ng LFP, Anderson DE, Chia WN, Chia PY, et al. Viral dynamics and immune correlates of COVID-19 disease severity. *Clin Infect Dis.* (2020). doi: 10.1093/cid/ciaa1280. [Epub ahead of print].
45. Liu Y, Chen D, Hou J, Li H, Cao D, Guo M, et al. An inter-correlated cytokine network identified at the center of cytokine storm predicted COVID-19 prognosis. *Cytokine.* (2021) 138:155365. doi: 10.1016/j.cyto.2020.155365
46. Guo Y, Cao W, Zhu Y. Immunoregulatory functions of the IL-12 family of cytokines in antiviral systems. *Viruses.* (2019) 11:772. doi: 10.3390/v11090772
47. Wynants L, Van Calster B, Collins GS, Riley RD, Heinze G, Schuit E, et al. Prediction models for diagnosis and prognosis of covid-19: systematic review and critical appraisal. *BMJ.* (2020) 369:m1328. doi: 10.1136/bmj.m1328
48. Collins GS, van Smeden M Riley RD. COVID-19 prediction models should adhere to methodological and reporting standards. *Eur Respir J.* (2020) 56:2002643. doi: 10.1183/13993003.02643-2020

Conflict of Interest: RDL reports receiving grant support from Bristol-Myers Squibb, GlaxoSmithKline, Medtronic, Sanofi, and Pfizer, and consulting fees from Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi-Sankyo, GlaxoSmithKline, Medtronic, Merck, Pfizer, Sanofi, and Portola. AM reports receiving consulting fees from Pfizer, Bayer, AstraZeneca, Novartis, Daiichi-Sankyo, Zodiac, Roche, and Janssen. AF reports receiving consulting fees from Pfizer, Bayer, Daiichi-Sankyo, Boehringer, and Servier. GA reports receiving consulting fees from Bayer, Pfizer, Servier, AstraZeneca, and Daichii Sankyo. DA reports receiving consulting fees from Boehringer Ingelheim, AstraZeneca, Bayer, and Servier. OS reports receiving grant support from Boehringer Ingelheim and consulting fees from Pfizer, Bayer, Daiichi-Sankyo, and Boehringer Ingelheim.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

CASE REPORT
published: 29 July 2021
doi: 10.3389/fcvm.2021.695010

Case Report: Co-occurrence of Myocarditis and Thrombotic Microangiopathy Limited to the Heart in a COVID-19 Patient

Thomas Menter[1], *Nadine Cueni*[2], *Eva Caroline Gebhard*[2] *and Alexandar Tzankov*[1]*

[1] Department of Pathology, Institute of Medical Genetics and Pathology, University Hospital Basel, University of Basel, Basel, Switzerland, [2] Intensive Care Unit, University Hospital Basel, Basel, Switzerland

We report on an impressive case of a previously healthy 47-year-old female Caucasian SARS-CoV-2 positive patient who died within 48 h after initial cardiac symptoms. Autopsy revealed necrotizing myocarditis and extensive microthrombosis as the cause of death. The interesting feature of this case is the combination of both myocarditis and extensive localized microthrombosis of cardiac capillaries. Microthrombosis was not present in other organs, and the patient did not show typical features of diffuse alveolar damage in the lungs. Taken together, our morphologic findings illustrate the angiocentric, microangiopathic, thromboinflammatory disease with significant thrombotic diathesis prevalent in COVID-19, which has been previously described in the literature, likely warranting thromboprophylaxis even in oligosymptomatic circumstances. This case also delineates several potential etiologies for microthrombosis, i.e., inflammatory reactions and primary hypercoagulative states. Further systematic analyses on risk stratification for receipt of prophylactic anticoagulation in COVID-19 are urgently required.

Keywords: COVID-19, heart, myocarditis acute and fulminant, thrombus, thromboinflammation

OPEN ACCESS

Edited by:
Marijana Tadic, Universität Ulm, Germany

Reviewed by:
Evgeny Belyavskiy, Charité – Universitätsmedizin Berlin, Germany
Jelena Suzic Lazic, University Hospital Center Dr Dragiša Mišović, Serbia

***Correspondence:**
Alexandar Tzankov
alexandar.tzankov@usb.ch

Specialty section:
This article was submitted to Heart Failure and Transplantation, a section of the journal Frontiers in Cardiovascular Medicine

Received: *14 April 2021*
Accepted: *30 June 2021*
Published: *29 July 2021*

Citation:
Menter T, Cueni N, Gebhard EC and Tzankov A (2021) Case Report: Co-occurrence of Myocarditis and Thrombotic Microangiopathy Limited to the Heart in a COVID-19 Patient. Front. Cardiovasc. Med. 8:695010. doi: 10.3389/fcvm.2021.695010

INTRODUCTION

Based on early empiric evidence and autopsy observations (1–3), anticoagulation has emerged as an important topic in treatment of COVID-19 (4). It is now well-established that thromboses significantly contribute to disease burden in COVID-19 and thus warrant immediate attention by treating physicians regardless of disease severity (5, 6). Myocarditis is also rare but nevertheless an acknowledged comorbidity in COVID-19 (7). Few cases have been examined by histopathology so far, and they showed divergent features ranging from subtle inflammatory infiltrates not fulfilling diagnostic criteria for borderline myocarditis to overt necrotizing inflammation (8–10).

Here, we present a case of a previously healthy patient positive for SARS-CoV-2 who died of cardiac complications consisting of necrotizing myocarditis and extensive microthrombosis within 48 h after initial cardiac symptoms.

CASE

A 47-year-old female suffered from oligosymptomatic flu-like disease for a week before she was found unconscious and apneic at home. Advanced cardiac life support was promptly administered by paramedics. Upon hospital admission, electrocardiography (ECG) showed ST-segment

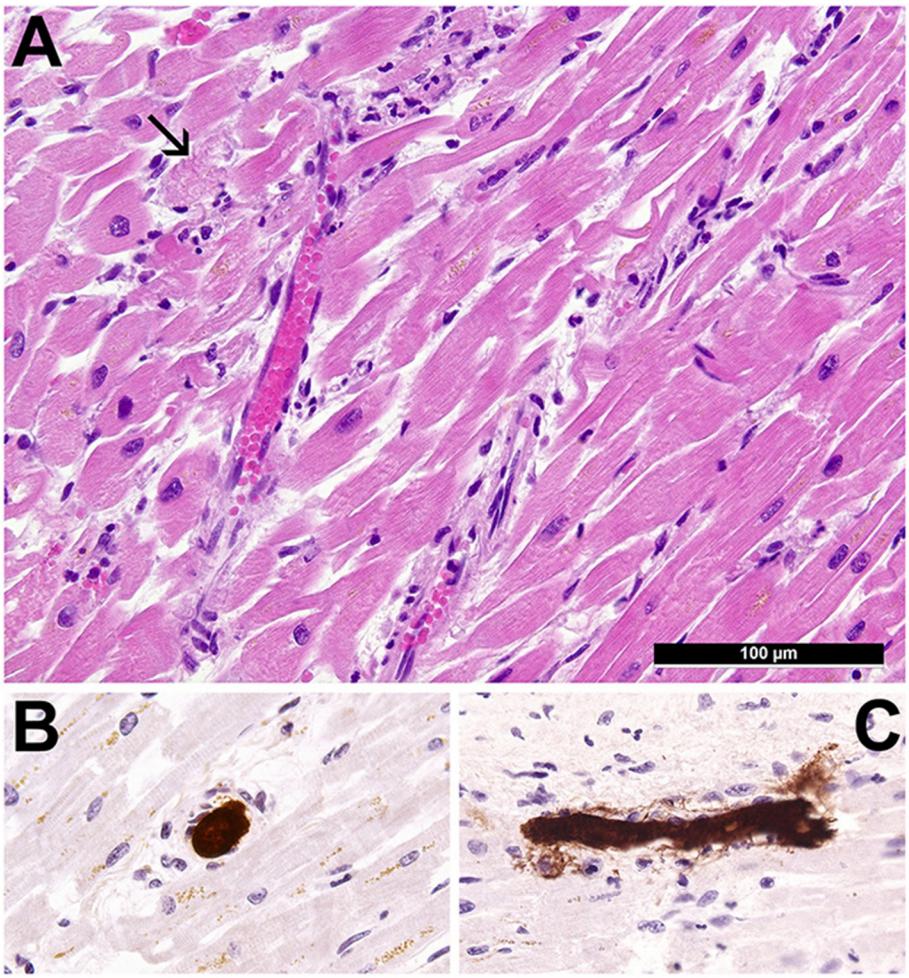


FIGURE 1 | Cardiopathological findings. **(A)** Morphology of the heart showing multifocal inflammatory infiltrates consisting of neutrophilic granulocytes, lymphocytes and histiocytes, capillarostasis, and perifocal single-cell necroses of cardiomyocytes (arrow) (H&E, ×400). **(B,C)** Immunohistochemical staining for fibrin demonstrating cross section and longitudinal section of capillaries with prominent microthrombi occluding the lumens (immunohistochemistry for fibrin, ×400).

depression in all the leads and elevations in augmented vector right (aVR). Subsequently performed coronary angiography revealed no relevant coronary stenosis. Echocardiography detected moderately reduced left ventricular function (left ventricular ejection fraction (LVEF) 30%) and normal right ventricular size and function. There was no evidence of left ventricular hypertrophy or dilatation. Computerized tomography excluded pulmonary thromboembolism but showed bilateral lower-lobe consolidations. High-sensitivity troponin T (hsTropT) was elevated (272 ng/l at admission; peak of 507 ng/l 10 h after admission), as were brain natriuretic peptide (>70,000 ng/l) and C-reactive protein (282 mg/l). The leucocyte count was within normal range. A nasopharyngeal swab was positive for SARS-CoV-2. Apart from mild thrombocytopenia (140 G/L) and mildly prolonged activated partial thromboplastin time (aPTT) (39 s) at the time of admission, all other coagulation parameters were within normal ranges. Despite exhaustive invasive intensive care interventions including continuous adrenalin/noradrenaline infusion, prophylactic antibiotic therapy (piperacillin/tazobactam), and two further attempts of cardiac resuscitation, the patient died of cardio-respiratory failure within 48 h of admission. Her detailed clinical course is shown in the timeline section. Previous clinical history included episodes of depression, which had been treated with venlafaxine, and a cholecystectomy.

An autopsy was performed. No relevant comorbidities apart from obesity [body mass index (BMI) 31.6] were noticed. Major findings included moderate bilateral suppurative pneumonia with COVID-19-characteristic capillary stasis, yet without diffuse alveolar damage. Most notably, the heart presented as normotrophic and irregularly perfused with mild diffuse necrotizing myocarditis (**Figure 1A**) accompanied by extensive thrombotic microangiopathy of cardiac capillaries (**Figures 1B,C**; microthrombi in cardiac capillaries immunohistochemically stained for fibrin), which was determined as the cause of death. RT-qPCR of

heart tissue was positive for SARS-CoV-2-*N*-gene (Ct 35.7). Immunohistochemistry for adenovirus was negative.

TIMELINE

Date	Event
1 week before admission to hospital	Flu-like symptoms with symptomatic treatment (SARS-CoV-2 test negative)
Day 1	Advanced cardiac life support due to asystole (after at least 10 min without basic life support measurements) with return of spontaneous circulation after 10 min
	Referral to the emergency department of a tertiary care center (SARS-CoV-2 test positive)
	Diagnostics: moderate left ventricular ejection dysfunction (LVEF 30%), lower pulmonary lobe opacities, no evidence of coronary artery disease or thrombosis, nor pulmonary embolisms or pneumothorax, normal electrolyte values
Day 2	Admission to intensive care unit requiring mechanical ventilation and with multiorgan failure
	One episode of ventricular fibrillation treated with defibrillation
	Extracorporeal membrane oxygenation not performed, as the time period between first reanimation attempts was prolonged, inducing severe hypoxic encephalopathy with extensively elevated neuron-specific enolase in the absence of hemolysis
Day 3	Death after renewed unsuccessful reanimation for 20 min

DISCUSSION

The interesting feature of this COVID-19 case study is the combination of both myocarditis and extensive microthrombosis of cardiac capillaries.

Our group, amongst others, has previously presented comprehensive autopsy cohorts of patients succumbing to COVID-19 describing microthrombosis predominantly in the lungs and further organs (2, 11, 12). Microthrombi in the pulmonary capillary bed and subsequently increased intravascular pressure in the pulmonary circulation have been attributed to heart failure in several studies. A recent report focusing on heart pathology described microthrombi in 12/15 COVID-19 cases (13); thrombi were also found in some control cases with influenza infection, metastatic carcinoma, or advanced severe bacterial pneumonia. In line with this case, we have previously investigated cardiopathological characteristics of patients succumbing to COVID-19 associated respiratory failure, similarly demonstrating a high incidence of capillary dilatation, stasis, and microthrombosis, especially in cases with detectable SARS-CoV-2 cardiac viral load (14).

It is well-acknowledged that COVID-19 predisposes to a procoagulatory state. The underlying pathophysiology for thromboinflammation is likely multifaceted, involving direct endothelial damage by SARS-CoV-2, secondary inflammatory endothelial damage, an overexpression of procoagulatory genes (e.g., *SERPINE* genes) in target organs, and generation of neutrophilic extracellular traps, and immunological, particularly antiphospholipid-mediated processes [rev. in (15)].

Myocarditis, in particular borderline myocarditis, in COVID-19 patients has been described (7). In a systematic review of 41 studies compiling 316 cases of COVID-19 autopsies, Roshdy et al. identified five cases (i.e., 1.5%) with inflammatory infiltrates fulfilling the Dallas criteria of myocarditis (8). In ≈10% of cases, mild focal inflammatory infiltrates in the myocardial interstitium had been noticed. In one study on endomyocardial biopsies taken for elucidating the cause of acute heart failure or in suspicion of myocarditis (16), isolated cases showed both presence of SARS-CoV-2 genomes and inflammatory infiltrates also affecting small vessels, while thrombi had not been described in this series.

In our case, the patient exclusively presented with localized cardiac microthrombi; other organs were not affected (also confirmed by immunohistochemistry). Microthrombi were partially associated with inflammatory infiltrates and single-cell cardiomyocyte necrosis, which we interpret as a sequela of the thrombotic microangiopathy. Acute heart failure, which is the non-disputable cause of death, can be attributed to both features—myocarditis and microthrombi—and our findings strongly support that both morphological features can be collectively interpreted as a rare but severe COVID-19-related thromboinflammatory cardiac complication. There was no evidence of a preexisting heart condition based on imaging and autopsy findings as well as the clinical history of the patient. Admittedly, it has to be considered that her intake of antidepressant (venlafaxine) might have contributed to cardiac pathology, yet the features described in single case reports and a review article of individuals treated with venlafaxine and cardiac problems were not evident in this case (17–19). Furthermore, there was no evidence of serotonin syndrome, mydriasis, or seizures.

Taken together, our morphologic findings and the preexisting literature illustrate that COVID-19 is an angiocentric, particularly microangiopathic, thromboinflammatory disease with significant thrombotic diathesis, in all likelihood warranting thromboprophylaxis even in oligosymptomatic circumstances. This case also delineates several potential etiologies for microthrombi: inflammatory reaction and primary hypercoagulative state. Further systematic analyses on risk stratification for receipt of prophylactic anticoagulation in COVID-19 are urgently required.

PATIENT PERSPECTIVE

Heart failure is a severe complication of COVID-19. As illustrated in this case, it can also arise in previously healthy patients and might develop independently of pulmonary findings. Myocarditis and microthrombosis of the cardiac capillaries are potentially treatable etiologies of heart failure in such instances, yet their diagnosis may be difficult without histological examination. Thorough investigation of both coagulation parameters and the myocardium might be therefore required in patients with unexplained or rapidly

deteriorating heart failure in the setting of COVID-19. It remains to be determined if prophylactic anticoagulation even in oligosymptomatic COVID-19 patients is feasible.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AT performed the autopsy. AT and TM designed the study and wrote the manuscript. NC and EG took care of the patient and provided clinical data. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Botnar Research Centre for Child Health, BRCCH.

ACKNOWLEDGMENTS

We thank Dr. med. J. D. Haslbauer for critically proofreading the manuscript.

REFERENCES

1. Wichmann D, Sperhake JP, Lutgehetmann M, Steurer S, Edler C, Heinemann A, et al. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. *Ann Intern Med.* (2020) 173:268–77. doi: 10.7326/M20-2003
2. Menter T, Haslbauer JD, Nienhold R, Savic S, Hopfer H, Deigendesch N, et al. Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. *Histopathology.* (2020) 77:198–209. doi: 10.1111/his.14134
3. Lax SF, Skok K, Zechner P, Kessler HH, Kaufmann N, Koelblinger C, et al. Pulmonary arterial thrombosis in COVID-19 with fatal outcome : results from a prospective, single-center, clinicopathologic case series. *Ann Intern Med.* (2020) 173:350–61. doi: 10.7326/M20-2566
4. Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. *J Am Coll Cardiol.* (2020) 75:2950–73. doi: 10.1016/j.jacc.2020.04.031
5. Rentsch CT, Beckman JA, Tomlinson L, Gellad WF, Alcorn C, Kidwai-Khan F, et al. Early initiation of prophylactic anticoagulation for prevention of coronavirus disease 2019 mortality in patients admitted to hospital in the United States: cohort study. *BMJ.* (2021) 372:n311. doi: 10.1136/bmj.n311
6. Giannis D, Allen S, Tsang J, Flint S, Pinhasov T, Williams S, et al. Post-Discharge thromboembolic outcomes and mortality of hospitalized COVID-19 patients: the CORE-19 registry. *Blood.* (2021) 137:2838–47. doi: 10.1182/blood.2020010529
7. Mele D, Flamigni F, Rapezzi C, Ferrari R. Myocarditis in COVID-19 patients: current problems. *Intern Emerg Med.* (2021) 23:1–7. doi: 10.1007/s11739-021-02635-w
8. Roshdy A, Zaher S, Fayed H, Coghlan JG. COVID-19 and the heart: a systematic review of cardiac autopsies. *Front Cardiovasc Med.* (2020) 7:626975. doi: 10.3389/fcvm.2020.626975
9. Craver R, Huber S, Sandomirsky M, McKenna D, Schieffelin J, Finger L. Fatal eosinophilic myocarditis in a healthy 17-year-old male with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2c). *Fetal Pediatr Pathol.* (2020) 39:263–8. doi: 10.1080/15513815.2020.1761491
10. Basso C, Leone O, Rizzo S, De Gaspari M, van der Wal AC, Aubry MC, et al. Pathological features of COVID-19-associated myocardial injury: a multicentre cardiovascular pathology study. *Eur Heart J.* (2020) 41:3827–35. doi: 10.1093/eurheartj/ehaa664
11. Duarte-Neto AN, Monteiro RAA, da Silva LFF, Malheiros D, de Oliveira EP, Theodoro-Filho J, et al. Pulmonary and systemic involvement in COVID-19 patients assessed with ultrasound-guided minimally invasive autopsy. *Histopathology.* (2020) 77:186–97. doi: 10.1111/his.14160
12. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet.* (2020) 395:1417–8. doi: 10.1016/S0140-6736(20)30937-5
13. Bois MC, Boire NA, Layman AJ, Aubry MC, Alexander MP, Roden AC, et al. COVID-19-associated nonocclusive fibrin microthrombi in the heart. *Circulation.* (2021) 143:230–43. doi: 10.1161/CIRCULATIONAHA.120.050754
14. Haslbauer JD, Tzankov A, Mertz KD, Schwab N, Nienhold R, Twerenbold R, et al. Characterisation of cardiac pathology in 23 autopsies of lethal COVID-19. *J Pathol Clin Res.* (2021) 7:326–37. doi: 10.1002/cjp2.212
15. Bosmuller H, Matter M, Fend F, Tzankov A. The pulmonary pathology of COVID-19. *Virchows Arch.* (2021) 478:137–50. doi: 10.1007/s00428-021-03053-1
16. Escher F, Pietsch H, Aleshcheva G, Bock T, Baumeier C, Elsaesser A, et al. Detection of viral SARS-CoV-2 genomes and histopathological changes in endomyocardial biopsies. *ESC Heart Fail.* (2020) 7:2440–7. doi: 10.1002/ehf2.12805
17. Vinetti M, Haufroid V, Capron A, Classen JF, Marchandise S, Hantson P. Severe acute cardiomyopathy associated with venlafaxine overdose and possible role of CYP2D6 and CYP2C19 polymorphisms. *Clin Toxicol.* (2011) 49:865–9. doi: 10.3109/15563650.2011.626421
18. Elikowski W, Malek-Elikowska M, Ganowicz-Kaatz T, Fertala N, Zawodna M, Baszko A, et al. Transient left ventricular hypertrophy in a 30-year-old female with chronic emotional stress and depression treated with venlafaxine. *Pol Merkur Lekarski.* (2019) 47:144–9.
19. Howell C, Wilson AD, Waring WS. Cardiovascular toxicity due to venlafaxine poisoning in adults: a review of 235 consecutive cases. *Br J Clin Pharmacol.* (2007) 64:192–7. doi: 10.1111/j.1365-2125.2007.02849.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

CASE REPORT
published: 29 July 2021
doi: 10.3389/fcvm.2021.694542

Case Report: An Unusual Case of Biventricular Thrombosis in a COVID-19 Patient With Ischemic Dilated Cardiomyopathy: Assessment of Mass Mobility and Embolic Risk by Tissue Doppler Imaging

Andrea Sonaglioni[1], Adriana Albini[2], Gian Luigi Nicolosi[3], Elisabetta Rigamonti[1], Douglas M. Noonan[2,4] and Michele Lombardo[1]*

[1] Department of Cardiology, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Multi Medica, Milan, Italy, [2] Scientific and Technological Pole, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Multi Medica, Milan, Italy, [3] Department of Cardiology, Policlinico San Giorgio, Pordenone, Italy, [4] Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy

OPEN ACCESS

Edited by:
Marijana Tadic,
Universität Ulm, Germany

Reviewed by:
Daniel Morris,
Charité Universitätsmedizin Berlin, Germany
Branislava Ivanovic,
Institute for Cardiovascular Diseases, Serbia

***Correspondence:**
Adriana Albini
albini.adriana@gmail.com

Specialty section:
This article was submitted to Cardiovascular Imaging, a section of the journal Frontiers in Cardiovascular Medicine

Received: 13 April 2021
Accepted: 21 June 2021
Published: 29 July 2021

Citation:
Sonaglioni A, Albini A, Nicolosi GL, Rigamonti E, Noonan DM and Lombardo M (2021) Case Report: An Unusual Case of Biventricular Thrombosis in a COVID-19 Patient With Ischemic Dilated Cardiomyopathy: Assessment of Mass Mobility and Embolic Risk by Tissue Doppler Imaging. Front. Cardiovasc. Med. 8:694542. doi: 10.3389/fcvm.2021.694542

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein binds to angiotensin-converting enzyme 2 (ACE2) receptor on vascular cells. As a consequence, patients with COVID-19 have an increased incidence of thromboembolic complications of the SARS-CoV-2 infection and subsequent endothelial cell damage with consequence of development of systemic vasculitis and diffuse intravascular coagulation. The present case describes a COVID-19 female patient with ischemic dilated cardiomyopathy, who presented with congestive heart failure and echocardiographic evidence of biventricular apical thrombi. The peak antegrade longitudinal velocity (Va) of each thrombotic mass was measured by pulsed wave tissue Doppler imaging (PW-TDI). Both left ventricular and right ventricular apical thrombi were found with a TDI-derived mass peak Va < 10 cm/s. There was no clinical evidence of neither systemic nor pulmonary embolization, probably due to the hypomobility of both left and right ventricular masses.

Keywords: COVID-19, biventricular thrombosis, pulsed wave tissue Doppler imaging, ACE2, dilated cardiomyopathy

INTRODUCTION

Left ventricular (LV) thrombosis can complicate both ischemic and non-ischemic cardiomyopathies and can lead to arterial embolic complications such as stroke (1, 2). However, the occurrence of biventricular thrombi is very rare and only few cases have been previously described in literature (3–9). Here, we report the case of an 80-year-old woman infected by Coronavirus 2019 (COVID-19), presenting with congestive heart failure (CHF) due to ischemic dilated cardiomyopathy (DCM), who was diagnosed with biventricular apical thrombi by transthoracic echocardiography (TTE).

CLINICAL COURSE

An 80-year-old woman, BSA 1.62 m^2, body mass index (BMI) 22.6 Kg/m^2 with history of coronary artery disease presented to the Emergency Department (ED) with worsening dyspnea, non-productive cough, fatigue, and bilateral leg swelling. She had prior anterior myocardial infarction treated with percutaneous transluminal coronary angioplasty of the proximal left anterior descending coronary artery in 2019 and subsequent unfavorable evolution in DCM with severe systolic dysfunction (estimated left ventricular ejection fraction of 20%) and chronic renal failure (estimated glomerular filtration rate of 30 ml/min/1.73 m^2). She was in home-therapy with acetyl salicylic acid 75 mg/die, furosemide 50 mg/die, spironolactone 25 mg/die, and rosuvastatin 5 mg/die.

Parameters recorded at the admission were the following: body temperature 36.5°C, heart rate 82 beats per minute, blood pressure 150/90 mmHg, respiratory rate 28 times per minute, and oxygen saturation 90% on ambient air.

Blood tests showed a white blood cell count of 8,720/mmc (88.0% neutrophils and 8.0% lymphocytes), hemoglobin 16.5 g/dl, C-reactive protein at the level of 11.1 mg/dl (reference range 0.05–0.50 mg/dl), estimated glomerular filtration rate 25 ml/min/1.73 m^2, B-type natriuretic peptide level >20,000 pg/ml, D-dimer at the level of 17,108 ng/ml (reference range 1–500 ng/ml), and troponin I at the level of 0.08 ng/ml (reference range 0.00–0.04 ng/ml).

The electrocardiogram showed sinus rhythm, poor R wave progression in precordials, suggestive of old large anterior myocardial infarction and QRS voltage <5 mm in all limb leads.

Chest x-ray showed cardiomegaly, bilateral interstitial infiltrates and bilateral pleural effusions (**Figure 1**). A positive rapid antigen test for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) was confirmed by the molecular test at hospital admission. Therefore, the patient was transferred from the ED to the semi-intensive care unit for COVID-19 patients.

A bedside TTE revealed the presence of large and roundish masses in the apex of both ventricles associated with severe biventricular dilatation (LV end-diastolic short axis diameter of 65 mm, right ventricular inflow tract of 45 mm) and dysfunction. Left ventricular ejection fraction (LVEF) estimated by modified Simpson's method was 15%, with dyskinesia/aneurism of the apex, and interventricular septum and marked hypokinesis of the other segments of the left ventricle. Right ventricular (RV) systolic function, measured by the tricuspid annular plane systolic excursion (TAPSE), was also severely impaired (TAPSE = 9 mm).

Both masses were acoustically distinct from underlying myocardium, with hypoechoic central space and hyperechoic border, well-circumscribed and sessile, attached to the apex of the left ventricle and of the right ventricle. The LV mass measured 23 mm × 21 mm, while the RV mass measured 18 mm × 15 mm. Both the masses were hypomobile and prominent in the apical 4-chamber view. Bilateral ventricular thrombi were diagnosed.

To precisely assess the mobility of the intracardiac thrombi, we employed pulsed-wave tissue Doppler imaging (PW-TDI) placing the sample volume at the level of the body of each mass. The peak antegrade longitudinal velocity (Va) of both LV and RV apical thrombi was measured.

Figure 2 illustrates the LV apical thrombus (**Figure 2A**) and the corresponding TDI-derived peak mass Va (**Figure 2B**), while **Figure 3** depicts the RV apical thrombus (**Figure 3A**) and the relative TDI-derived peak mass Va (**Figure 3B**). Moderate mitral and tricuspid regurgitation, dilatation of the inferior vena cava and moderate pulmonary hypertension (the estimated systolic pulmonary artery pressure was 60 mmHg) were also detected.

The above-mentioned echocardiographic examination was compared with a previous TTE performed in October 2020 that showed severe biventricular dilatation and dysfunction (LV ejection fraction of 20%, TAPSE of 13 mm), with no evidence of ventricular thrombi.

The diagnosis of CHF due to ischemic DCM complicated with biventricular thrombi in a COVID-19 patient was made. Thrombosis at other sites was excluded; no deep vein thrombosis of the abdomen and lower extremities was found by ultrasonography.

Conventional treatment for CHF with loop diuretics (intravenous furosemide 120 mg/die and canrenone 100 mg/die) and beta-blockers (bisoprolol 2.5 mg/die) was started. Moreover, the patient received antibiotic treatment (intravenous piperacillin and tazobactam three times daily) and oxygen therapy via nasal cannula (2 l/min). Low molecular weight heparin (enoxaparin sodium) was administered for the treatment of biventricular thrombosis (4,000 IU twice daily by subcutaneous injection).

During the hospitalization, the patient underwent diagnostic thrombophilia testing. Results showed that serum levels of protein C, protein S, antithrombin III, factor V Leiden, and antiphospholipid antibody were normal.

A subsequent TTE, performed after 10 days of anticoagulant treatment, showed the complete dissolution of both left and right ventricular thrombi (**Figure 4**). There was no clinical evidence of neither systemic nor pulmonary embolization, probably due to the hypomobility of both left, and right ventricular masses.

However, severe biventricular dysfunction persisted, and refractory CHF occurred, despite intensive diuretic therapy. The patient underwent serial chest x-ray which showed progressive increase of pulmonary congestion and interstitial infiltrates. Computed tomography scan was not performed due to the critical condition of the patient and the absence of clinical signs of pulmonary/systemic embolization. Finally, the patient's clinical conditions worsened, and she died 15 days after hospitalization.

DISCUSSION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein binds to angiotensin-converting enzyme 2 (ACE2) receptor on vascular cells (10, 11). On endothelial cells from arterial and venous vessels there is ACE2 expression and there is evidence that endothelial cells are prone to SARS-CoV-2 infection which causes subsequent endothelial cell damage with development of systemic vasculitis and disseminated intravascular coagulation (DIC) (10). Since the

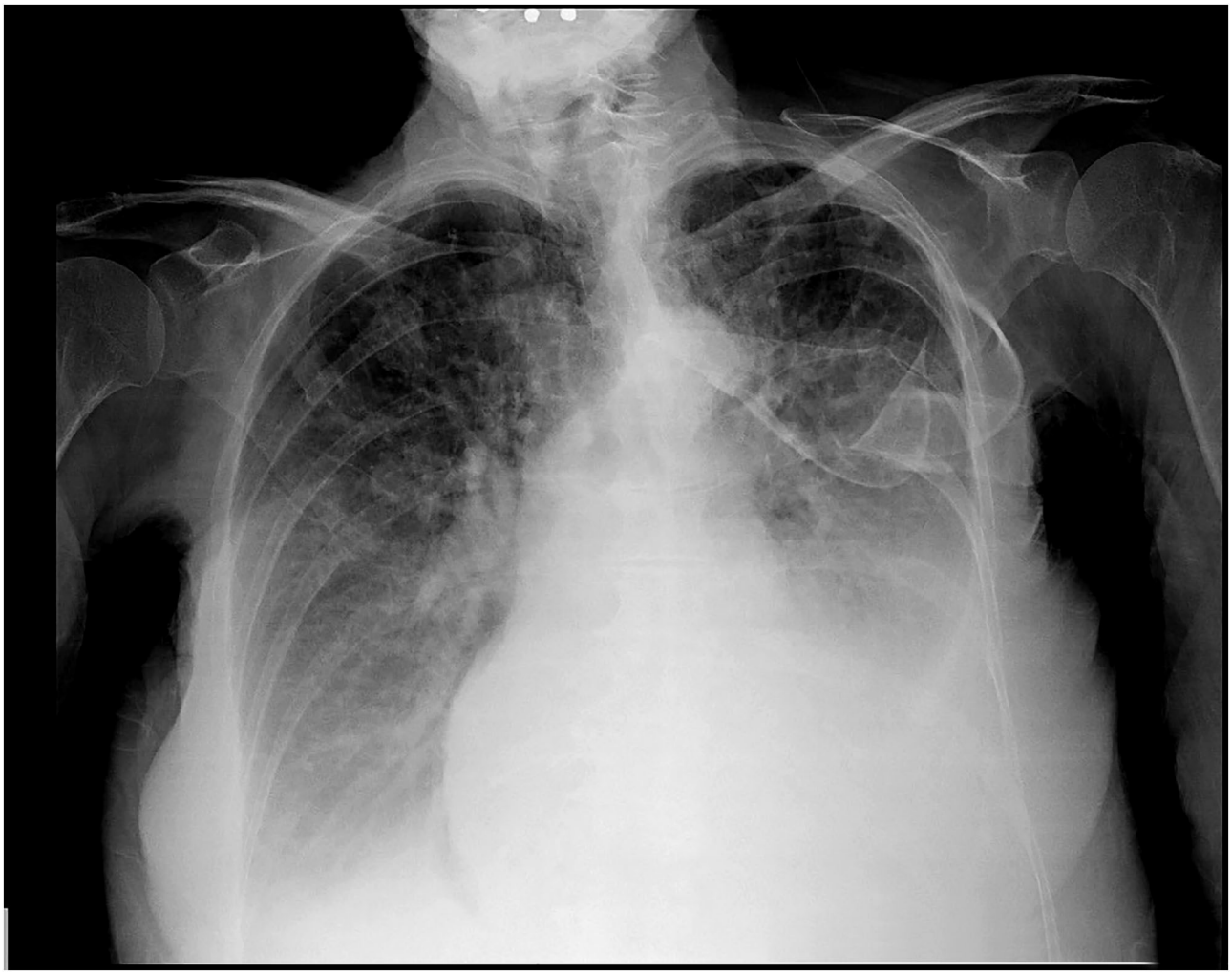


FIGURE 1 | Posteroanterior chest x-ray view revealing cardiomegaly, bilateral interstitial infiltrates and bilateral pleural effusions.

beginning of the COVID-19 pandemic, severe hypercoagulability and serious thrombotic complications have been reported in infected patients, especially in those patients who are admitted to intensive care unit (12–16). The most common thromboembolic complications detected in COVID-19 patients were deep vein thrombosis, acute pulmonary embolism, coronary and cerebral thrombosis, systemic arterial embolism, and placental thrombosis (17–21).

The occurrence of biventricular thrombi is a rare, but serious condition which may increase the risk of both systemic and pulmonary embolization. Previous cases of biventricular thrombosis have been described in patients with severe ventricular dysfunction, autoimmune disease, HIV infection, nephrotic syndrome, hypereosinophilic syndrome, heparin-induced thrombocytopenia, and antiphospholipid syndrome (3–9).

To date, there are only three case reports in literature who described biventricular thrombi in a COVID-19 patient (22–24).

One patient, a 63-year-old woman, with a known medical history of emphysema and active smoker, 2-week history of worsening dyspnea, nonproductive cough, and chills, she was positive for SARS-CoV-2 as detected by PCR (22). After, few hours from admission she had a cardiac arrest with successful cardiorespiratory resuscitation. The patient had 93% oxygen saturation, elevated troponin I, creatinine kinase, and lactate, with normal platelet count, coagulation parameters, and fibrinogen (22). Cardiac tomography revealed a right ventricular thrombus, measuring 4 mm by 10 mm, a left ventricular thrombus 12-mm in thickness extending over a 6-cm perimeter (22). The patient died of cardiogenic and pulmonary septic shock.

Another patient, a 58-year-old man, had obesity (BMI of 31 kg/m^2 on admission) and hypertension, he presented with intermittent fever and worsening shortness of breath on exertion he was positive for PCR SARS-CoV-2 (23). The patient initial clinical examination was normal except for an

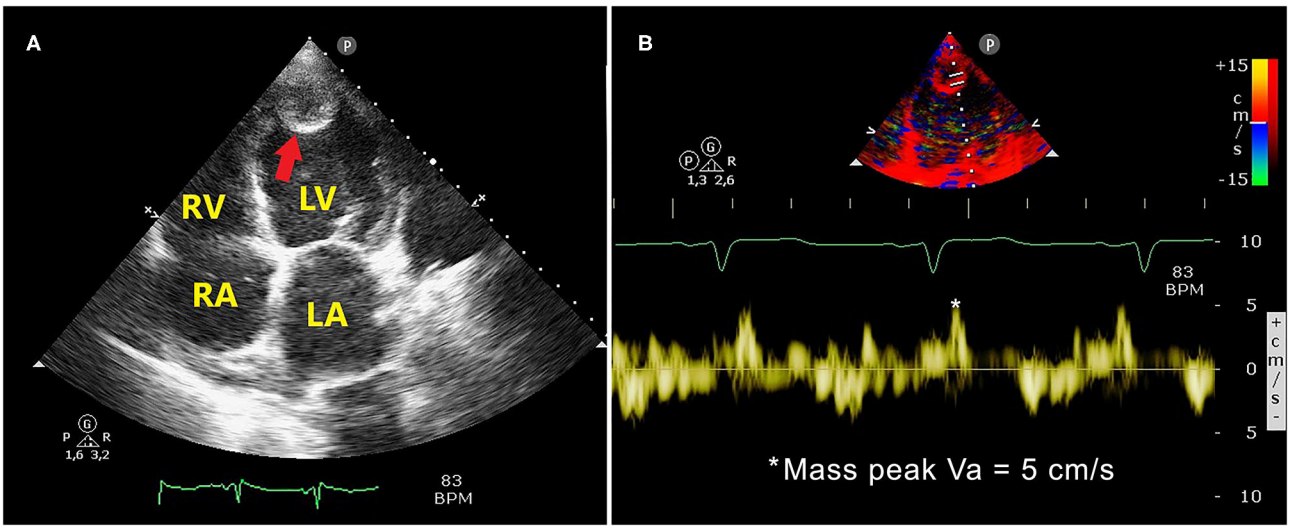


FIGURE 2 | Apical 4-chamber echocardiographic view showing a large and roundish LV apical thrombus with hypoechoic central space and hyperechoic border (**A**, red arrow). The LV thrombotic mass measured 23 mm × 21 mm. The TDI-derived LV mass peak Va was 5 cm/s, as depicted in panel **(B)**. LA, left atrium; LV, left ventricular/left ventricle; TDI, tissue Doppler imaging; RA, right atrium; RV, right ventricle; Va, antegrade velocity.

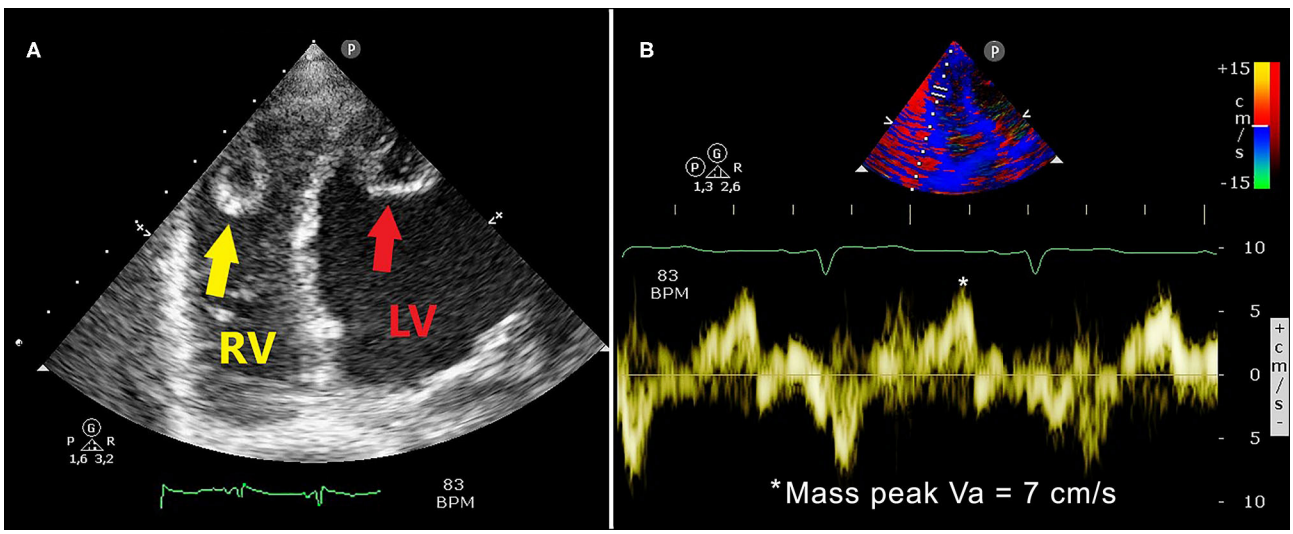


FIGURE 3 | Right ventricular focused 4-chamber echocardiographic view demonstrating RV (**A**, yellow arrow) and LV (**A**, red arrow) apical thrombi: both masses were found with hypoechoic central space and hyperechoic border. The RV thrombotic mass measured 18 mm × 15 mm. The TDI-derived RV mass peak Va was 7 cm/s, as depicted in panel **(B)**. LV, left ventricular/left ventricle; TDI, tissue Doppler imaging; RV, right ventricular/right ventricle; Va, antegrade velocity.

outstanding oxygen requirement. The patient had high C-reactive protein (CRP) D-dimer was significantly elevated, with normal prothrombin time and activated partial thromboplastin time, no pulmonary thromboembolism appeared on CT pulmonary angiography (23). On day 4 the D-dimer levels were noted to have risen, and on day 9 they were steadily rising. On day 9, CT pulmonary angiography revealed simultaneous bilateral pulmonary thromboembolism, biventricular cardiac thrombi (23). The patient with multiple thromboses had appropriate prophylactic and therapeutic LMWH is this case (23). The patient was successfully discharged on day 19.

A 58-year-old African-American male, with a history of hypertension and diabetes mellitus, was brought to emergency room (24). He had 60% oxygen saturation the clinical laboratory test showed a hypercoagulable state (Fibrinogen was low and high D-dimer levels, PT 36 s, INR 3.6, and aPTT of 100 s) normal troponin I, CK, CK-MB at normal levels (24). He was taking care of parents, confirmed positive with COVID-19. In this patient transthoracic echocardiography has seen left ventricle extensive mural thrombus and highly mobile thrombus the in right atrium with extensive biventricular thrombi (24). The patient died of ventricular fibrillation within 24 h.

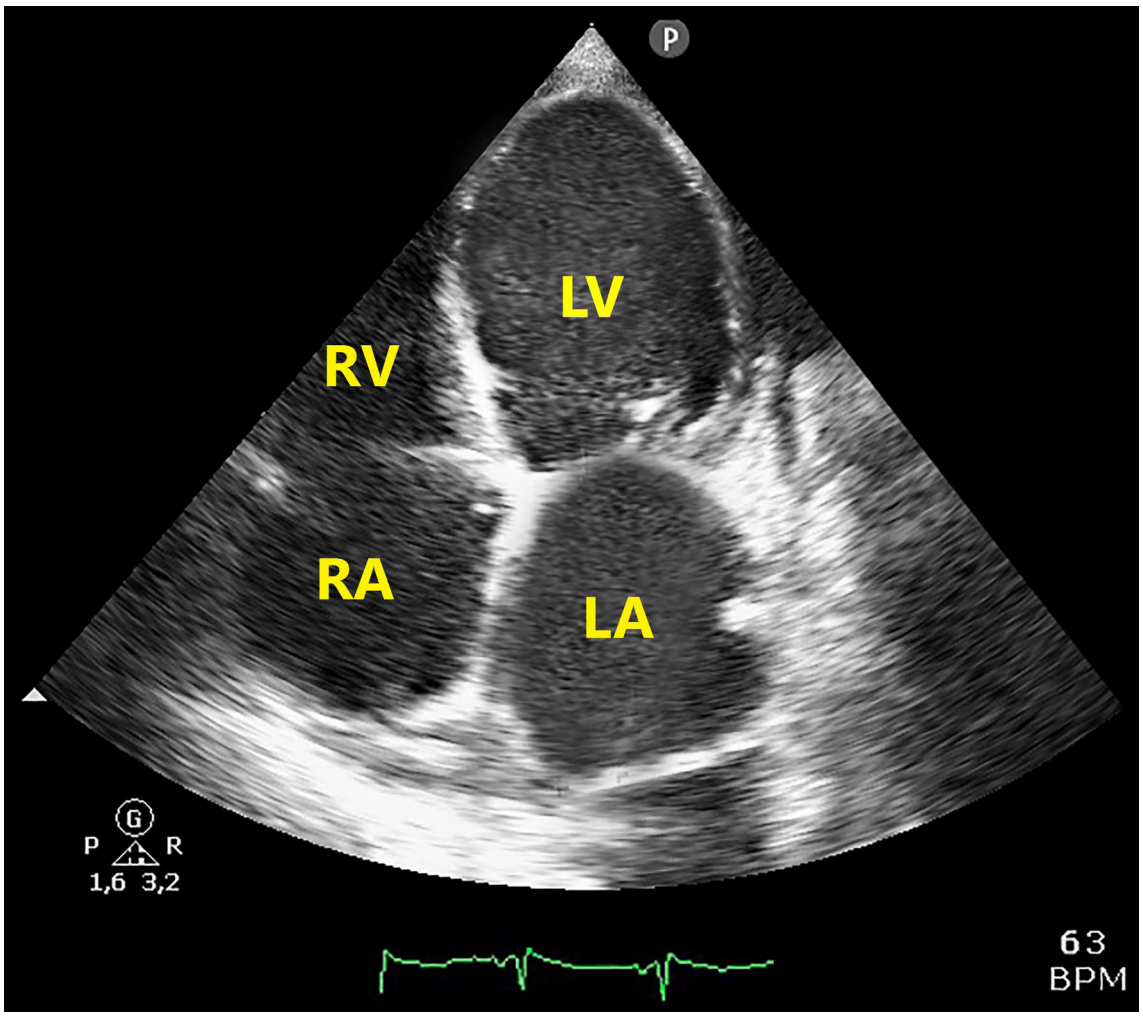


FIGURE 4 | Apical 4-chamber echocardiographic view showing disappearance of both RV and LV thrombotic masses after 10 days of anticoagulant treatment. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle.

Obesity is one of the complications of the COVID-19 patients (25). The BMI of the African-American male, with a history of hypertension and diabetes mellitus, and 63-year-old woman active smoker and emphysema was not mentioned in these case reports (22, 24). In all the case reports of biventricular thrombi and COVID-19 the patients were ∼60 years.

In this case report, a 80 years COVID-19 patient with CHF due to ischemic DCM was diagnosed with LV and RV apical thrombi. Our patient had severely depressed systolic biventricular function, suggesting a high risk of ventricular thrombi. However, she was never diagnosed with ventricular thrombi prior to admission and was tested negative for thrombophilia screening during hospitalization. As far as we know, this is the first case of biventricular thrombi described in a COVID-19 patient with ischemic dilated cardiomyopathy.

As proposed by Mehta et al. (26), the combination of a progressive dysregulated coagulative response to SARS-CoV-2 with consequent activation of a state of hypercoagulability and the blood stasis due to severe biventricular dilatation and contractile dysfunction might have contributed to the formation of LV and RV apical thrombi.

In the present case, the D-dimer level of the patient was 17,108 ng/ml, well-above reference values, and the patient was treated with low-molecular-weight heparin, while the surgical thrombectomy was not considered due to the patient's advanced age and the severe CHF. The 63 years woman patient coagulation parameters were normal, but the authors mention the D-dimer (22). In the two male cases D-dimers were highly elevated (23, 24).

In the present case, contrast echocardiography, which is particularly advantageous for detection of small or mural thrombi (27), was not performed to confirm the diagnosis of LV and RV apical thrombi, because both thrombotic masses were large in size and protuberant in shape. On the

other hand, PW-TDI was useful to precisely assessing the mobility of the intracardiac thrombi. Therefore, the diagnosis of biventricular thrombosis was performed and confirmed by TTE, whereas PW-TDI provided a rapid characterization of mass-mobility. Differently from previous case reports which described biventricular thrombi detected by TTE in different clinical settings (3–9), our case is the only one that employed a TTE implemented with PW-TDI assessment of the thrombotic mass mobility in a COVID-19 patient.

Our previous prospective analysis performed on 72 patients with echocardiographically detected LV thrombi revealed that a TDI-derived mass peak Va $\geq$ 10 cm/s, was the most important and independent predictor of outcome at mid-term follow-up (28). Therefore, we demonstrated that the TDI-derived mass peak Va might represent a new objective marker of thrombotic mass motility and that a mass peak Va $\geq$ 10 cm/s might stratify the hospitalized patients with increased probability of embolic events in a mid-term follow-up, regardless of the mass dimension.

In this present case, both LV and RV apical thrombi were found with a mass peak Va $<$ 10 cm/sec, as assessed by PW-TDI, and the clinical course was not clinically complicated by systemic nor pulmonary embolization. However, the patient's clinical conditions quickly worsened due to SARS-CoV-2 severe pneumonia.

CONCLUSION

This is a rare case of ischemic dilated cardiomyopathy complicated with biventricular apical thrombi early detected by TTE in a patient that was infected by COVID-19. The present case demonstrates the clinical usefulness of TTE implemented with PW-TDI for detecting ventricular thrombi and for measuring the thrombotic mass mobility. Although a biventricular thrombosis is a rare COVID-19 complication, performing appropriate diagnostic tests could decrease COVID-19 mortality in patients with dilated cardiomyopathy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was obtained for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

AS, ER, and ML performed the provided clinical procedures and collected the clinical data. AS, GN, ER, and ML analyzed the data and prepared the figures. AS, AA, DN, and ML discussed data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This research was funded by a grant of the Ministero della Salute COVID-2020-12371849 to DN and work was also supported by the Italian Ministry of Health Ricerca Corrente—IRCCS MultiMedica.

REFERENCES

1. Habash F, Vallurupalli S. Challenges in management of left ventricular thrombus. *Ther Adv Cardiovasc Dis.* (2017) 11:203–13. doi: 10.1177/1753944717711139
2. Lee JM, Park JJ, Jung HW, Cho YS, Oh IY, Yoon CH, et al. Left ventricular thrombus and subsequent thromboembolism, comparison of anticoagulation, surgical removal, antiplatelet agents. *J Atheroscler Thromb.* (2013) 20:73–93. doi: 10.5551/jat.13540
3. Missault L, Koch A, Colardyn F, Clement D. Biventricular thrombi in dilated cardiomyopathy: massive simultaneous pulmonary and systemic embolisation. *Eur Heart J.* (1994) 15:713–4. doi: 10.1093/oxfordjournals.eurheartj.a060574
4. Nishi I, Ishimitsu T, Ishizu T, Ueno Y, Suzuki A, Seo Y, et al. Peripartum cardiomyopathy and biventricular thrombi. *Circ J.* (2002) 66:863–5. doi: 10.1253/circj.66.863
5. Goncalves LF, Souto FM, Faro FN, Oliveira JL, Barreto-Filho JA, Sousa AC. Biventricular thrombus and endomyocardial fibrosis in antiphospholipid syndrome. *Arq Bras Cardiol.* (2012) 99:e162–5. doi: 10.1590/S0066-782X2012001400017
6. Hatanaka N, Ueda T. [Removal of biventricular thrombi for dilated cardiomyopathy in a patient with acute heart failure]. *Kyobu Geka.* (2014) 67:895–8.
7. Nkoke C, Kuate LM, Luchuo EB, Edie SD, Boombhi J, Menanga A. Biventricular thrombi in dilated cardiomyopathy in a patient with human immunodeficiency virus infection: a case report. *BMC Res Notes.* (2015) 8:168. doi: 10.1186/s13104-015-1140-x
8. Iwano T, Yunoki K, Tokunaga N, Shigetoshi M, Sugiyama H, Yamamoto H, et al. A case of biventricular thrombi in a patient with dilated cardiomyopathy: utility of multimodality imaging for diagnosis and management of treatment strategy. *J Cardiol Cases.* (2017) 15:91–4. doi: 10.1016/j.jccase.2016.10.013
9. Kammari CB, Rallabandi S, Rallabandi H, Daggubati SR, Adapa S, Naramala S, et al. Case report: dilated cardiomyopathy with biventricular thrombus secondary to impaired coagulation in a patient with HIV. *F1000Res.* (2020) 9:610. doi: 10.12688/f1000research.24016.2
10. Albini A, Di Guardo G, Noonan DM, Lombardo M. The SARS-CoV-2 receptor, ACE-2, is expressed on many different cell types: implications for ACE-inhibitor- and angiotensin II receptor blocker-based cardiovascular therapies. *Intern Emerg Med.* (2020) 15:759–66. doi: 10.1007/s11739-020-02364-6
11. Yang J, Petitjean SJL, Koehler M, Zhang Q, Dumitru AC, Chen W, et al. Molecular interaction and inhibition of SARS-CoV-2 binding to the ACE2 receptor. *Nat Commun.* (2020) 11:4541. doi: 10.1038/s41467-020-18319-6
12. Akel T, Qaqa F, Abuarqoub A, Shamoon F. Pulmonary embolism: a complication of COVID 19 infection. *Thromb Res.* (2020) 193:79–82. doi: 10.1016/j.thromres.2020.05.033
13. Klok FA, Kruip M, van der Meer NJM, Arbous MS, Gommers D, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res.* (2020) 191:145–7. doi: 10.1016/j.thromres.2020.04.013

14. Middeldorp S, Coppens M, van Haaps TF, Foppen M, Vlaar AP, Muller MCA, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. *J Thromb Haemost.* (2020) 18:1995–2002. doi: 10.1111/jth.14888
15. Paterson RW, Brown RL, Benjamin L, Nortley R, Wiethoff S, Bharucha T, et al. The emerging spectrum of COVID-19 neurology: clinical, radiological and laboratory findings. *Brain.* (2020) 143:3104–20. doi: 10.1093/brain/awaa240
16. Poissy J, Goutay J, Caplan M, Parmentier E, Duburcq T, Lassalle F, et al. Pulmonary embolism in patients with COVID-19: awareness of an increased prevalence. *Circulation.* (2020) 142:184–6. doi: 10.1161/CIRCULATIONAHA.120.047430
17. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med.* (2020) 46:1089–98. doi: 10.1007/s00134-020-06062-x
18. Kollias A, Kyriakoulis KG, Stergiou GS, Syrigos K. Heterogeneity in reporting venous thromboembolic phenotypes in COVID-19: methodological issues and clinical implications. *Br J Haematol.* (2020) 190:529–32. doi: 10.1111/bjh.16993
19. Marietta M, Ageno W, Artoni A, De Candia E, Gresele P, Marchetti M, et al. COVID-19 and haemostasis: a position paper from Italian Society on Thrombosis and Haemostasis (SISET). *Blood Transfus.* (2020) 18:167–9. doi: 10.2450/2020.0083-20
20. Oxley TJ, Mocco J, Majidi S, Kellner CP, Shoirah H, Singh IP, et al. Large-vessel stroke as a presenting feature of Covid-19 in the young. *N Engl J Med.* (2020) 382:e60. doi: 10.1056/NEJMc2009787
21. Wise J. Covid-19 and thrombosis: what do we know about the risks and treatment? *BMJ.* (2020) 369:m2058. doi: 10.1136/bmj.m2058
22. Soltani M, Mansour S. Biventricular thrombi associated with myocardial infarction in a patient with COVID-19. *Can J Cardiol.* (2020) 36:1326.e9–1326.e11. doi: 10.1016/j.cjca.2020.06.016
23. Ferguson K, Quail N, Kewin P, Blyth KG. COVID-19 associated with extensive pulmonary arterial, intracardiac and peripheral arterial thrombosis. *BMJ Case Rep.* (2020) 13:e237460. doi: 10.1136/bcr-2020-237460
24. Ozer M, Abbasi F, Mahdi M, Goksu SY, Struble E. Massive biventricular thrombi complicating new-onset heart failure in a patient with suspected COVID-19. *J Cardiol Cases.* (2021). doi: 10.1016/j.jccase.2021.02.016. [Epub ahead of print].
25. Caci G, Albini A, Malerba M, Noonan DM, Pochetti P, Polosa R. COVID-19 and obesity: dangerous liaisons. *J Clin Med.* (2020) 9:2511. doi: 10.3390/jcm9082511
26. Mehta JL, Calcaterra G, Bassareo PP. COVID-19, thromboembolic risk, and Virchow's triad: lesson from the past. *Clin Cardiol.* (2020) 43:1362–7. doi: 10.1002/clc.23460
27. Aggeli C, Dimitroglou Y, Raftopoulos L, Sarri G, Mavrogeni S, Wong J, et al. Cardiac masses: the role of cardiovascular imaging in the differential diagnosis. *Diagnostics (Basel).* (2020) 10:1088. doi: 10.3390/diagnostics10121088
28. Sonaglioni A, Nicolosi GL, Lombardo M, Anza C, Ambrosio G. Prognostic relevance of left ventricular thrombus motility: assessment by pulsed wave tissue doppler imaging. *Angiology.* (2021) 72:355–63. doi: 10.1177/0003319720974882

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

REVIEW
published: 10 August 2021
doi: 10.3389/fcvm.2021.713560

COVID-19 and Heart Failure: From Epidemiology During the Pandemic to Myocardial Injury, Myocarditis, and Heart Failure Sequelae

*Leonardo Italia, Daniela Tomasoni, Stefano Bisegna, Edoardo Pancaldi, Lorenzo Stretti, Marianna Adamo and Marco Metra**

Cardiology, ASST Spedali Civili and Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, Brescia, Italy

A close and intriguing relationship has been suggested between heart failure (HF) and coronavirus disease 2019 (COVID-19). First, COVID-19 pandemic represented a global public health emergency in the last year and had a catastrophic impact on health systems worldwide. Several studies showed a reduction in HF hospitalizations, ranging from 30 to 66% in different countries and leading to a subsequent increase in HF mortality. Second, pre-existing HF is a risk factor for a more severe clinical course of COVID-19 and an independent predictor of in-hospital mortality. Third, patients hospitalized for COVID-19 may develop both an acute decompensation of chronic HF and *de-novo* HF as a consequence of myocardial injury and cardiovascular (CV) complications. Myocardial injury occurred in at least 10% of unselected COVID-19 cases and up to 41% in critically ill patients or in those with concomitant CV comorbidities. Few cases of COVID-19-related acute myocarditis, presenting with severe reduction in the left ventricular (LV) ejection fraction and peculiar histopathological findings, were described. However, recent data suggested that COVID-19 may be associated with both systolic and diastolic LV dysfunction, with LV diastolic impairment, pulmonary hypertension, and right ventricular dysfunction representing the most frequent findings in echocardiographic studies. An overview of available data and the potential mechanisms behind myocardial injury, possibly leading to HF, will be presented in this review. Beyond the acute phase, HF as a possible long-term consequence of cardiac involvement in COVID-19 patients has been supposed and need to be investigated yet.

Keywords: COVID-19, SARS-CoV-2 infection, heart failure, myocardial injury, epidemiology, myocarditis, pathophysiology

OPEN ACCESS

Edited by:
Hendrik Tevaearai Stahel, Bern University Hospital, Switzerland

Reviewed by:
Kumaravelu Jagavelu, Central Drug Research Institute (CSIR), India
Alessandro Iadanza, University of Siena, Italy

****Correspondence:***
Marco Metra
metramarco@libero.it

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *23 May 2021*
Accepted: *12 July 2021*
Published: *10 August 2021*

Citation:
Italia L, Tomasoni D, Bisegna S, Pancaldi E, Stretti L, Adamo M and Metra M (2021) COVID-19 and Heart Failure: From Epidemiology During the Pandemic to Myocardial Injury, Myocarditis, and Heart Failure Sequelae. Front. Cardiovasc. Med. 8:713560. doi: 10.3389/fcvm.2021.713560

INTRODUCTION

Coronavirus disease 2019 (COVID-19) rapidly spread around the world becoming a global public health emergency. It is caused by a novel enveloped, positively stranded RNA beta coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1). So far, more than one hundred million of confirmed COVID-19 cases can be counted worldwide, with a total of more than three million deaths, as of June 1, 2021, according to the World Health Organization (2).

Although COVID-19 was initially considered a respiratory disease, it has rapidly become clear that a multiorgan involvement was common. In particular, the heart often represents a target organ and patients may develop heart failure (HF) (3–6).

Of note, the link between COVID-19 and HF is more complex. First, COVID-19 pandemic has an impact on HF management and a reduction of hospitalizations due to HF has been shown during the pandemic period, possibly leading to an increase in HF mortality. Second, history of HF is a risk factor for a more severe clinical course of COVID-19. Third, HF can be a consequence of COVID-19-related myocardial damage.

The aim of this review is to describe the epidemiology of HF during the pandemic, the role of cardiac injury and HF in COVID-19, and its pathogenetic mechanisms.

HEART FAILURE EPIDEMIOLOGY DURING COVID-19

COVID-19 pandemic upsets the epidemiology and the management of acute HF. Urgent cardiovascular (CV) hospital admission showed a general decline during the pandemic period, with also a delay in urgent care and an increased risk of complications (7, 8). Similarly, several studies reported a reduction in HF hospitalizations ranging from 30 to 66% (**Table 1**) (9–13). An analysis from a tertiary Heart Failure Unit in London showed that the number of HF hospitalizations had a significant decline by 66% during the COVID-19 pandemic, compared both with a pre-COVID period in the same year and the corresponding time periods from 2017 to 2019 (9). Patients hospitalized for HF during the pandemic were sicker, with higher rates of NYHA class III or IV symptoms and severe peripheral oedema, which are known predictors of poor outcomes in acute HF. The authors speculated that patients with less severe acute HF might have avoided presenting to hospital during the pandemic, due to the fear of acquiring infection (9). Further studies aimed to compare not only the rates of HF hospitalizations but also in-hospital outcomes. Despite similar demographic characteristics, patients admitted with HF in two referral centers in South London in 2020 experienced worse outcomes compared with those admitted in the previous year (12). Hospitalization for HF in 2020 was independently associated with increased mortality risk (12). Similarly, in Germany, there was a decrease by 30% in urgent HF hospitalizations during the pandemic ($p < 0.01$) with a concomitant higher in-hospital mortality compared with both same-year and previous-year control groups (13).

COVID-19 AND CARDIOVASCULAR COMORBIDITIES

The clinical presentation and the course of COVID-19 is extremely variable, ranging from an asymptomatic or pauci-symptomatic illness, presenting with mild symptoms (e.g., fever, dry cough, and fatigue), to a severe disease [e.g., severe pneumonia and acute respiratory distress syndrome (ARDS)] with possibly fatal outcome (14–18). The earliest reports from China and Italy showed a high prevalence of comorbidities and their association with the severity of COVID-19 and increased mortality (19–21). In a report of 72,314 cases, the overall case-fatality rate of COVID-19 was 2.3%, with higher rates in patients with pre-existing comorbidities [10.5, 7.3, 6.3, and 6.0% in patients with cardiovascular disease (CVD), diabetes, chronic respiratory disease, and hypertension, respectively] (22). A more recent meta-analysis suggested that CVD and cardiovascular risk factors (hypertension and diabetes) were closely related to fatal outcomes in COVID-19 patients, across and independently from all ages (23).

IMPACT OF PRE-EXISTING HEART FAILURE ON COVID-19 CLINICAL COURSE

Further studies showed that the prevalence of HF as a comorbid condition ranged from 3.3 to 21% among SARS-CoV-2-infected patients (**Table 2**) (19–21, 24–28). In a multicenter retrospective study from New York City area, including nearly 3,000 patients with laboratory-confirmed SARS-CoV-2 infection, the prevalence of HF was 10.1% (25). HF patients were more prone to develop myocardial injury, defined as increased troponin levels. HF history was also found to be associated with an increased risk of hospitalization and a severe clinical course in COVID-19 patients. In a prospective cohort study, among 5,279 people with laboratory confirmed SARS-CoV-2 infection, more than a half were admitted to hospital, of whom 1,904 (69.5%) were discharged alive (19). Besides age, HF was one of the strongest predictor for in-hospital admission [odds ratio (OR), 4.43; 95% confidence interval (CI), 2.59–8.04; $p < 0.001$] and critical illness (OR, 1.9; 95% CI, 1.4–2.5; $p < 0.001$) (19). A retrospective analysis conducted in Spain showed that HF was associated with higher risk of mechanical ventilation and mortality among patients hospitalized for COVID-19, regardless of left ventricle ejection fraction (LVEF) (28). Similar results were found in an Italian multicenter study, with HF resulting as an independent predictor of mortality and a risk factor for in-hospital complications, namely, acute HF, acute renal failure, and multiorgan failure (27).

THE PATHOPHYSIOLOGY OF COVID-19 MYOCYTE INJURY

Indirect Mechanisms

The pathogenesis of myocardial injury in COVID-19 is still not completely clear and likely involves multiple pathways. Overall, myocardial damage can be summarized distinguishing two different mechanisms of injury: the first, "indirect" or "aspecific," common with other severe infections, and the second, "direct" or "specific," related to the peculiar effects mediated by SARS-CoV-2 (3). The mechanisms of myocardial damage are highlighted in **Figure 1**.

First, COVID-19 has general deleterious effects on the cardiovascular system, which were already described in other infections (i.e., influenza and community-acquired pneumoniae).

TABLE 1 | Reduction in hospitalizations due to HF during COVID-19 pandemic period, compared with same period in the previous year or a different period in the same year (before COVID-19).

Study (year)	Number of patients	Country	Study and control periods	Reduction in HF hospitalizations
Bromage et al. (9)	104	England and Wales	2 March−19 April 2020 vs. control period in 2020 (pre-COVID) and the same periods in 2017–2019	−66%, $p < 0.01$
Cox et al. (10)	–	Vanderbilt University Medical Center, Nashville, Tennessee	22 March−20 April 2020 vs. same period in 2019	$-62 \pm 7\%$, $p < 0.01$
Hall et al. (11)	–	USA	Mean weekly hospitalization from January 2020 to 11 April. The significant and progressive decline described in 2020 was not observed in 2019, excluding potential confounding based on seasonal trends.	−50% (after the first case of COVID-19)
Cannatà et al. (12)	1,372	South London	7 January−14 June 2020 vs. same period in 2019	−40%, $p < 0.001$
Konig et al. (13)	13,484	Germany	13 March−21 May 2020 vs. control periods in 2020 (1 January−12 March) and 2019 (13 March−21 May)	−30%, $p < 0.01$

COVID-19, Coronavirus Disease 2019; HF, heart failure.

Fever and sympathetic activation cause tachycardia with a consequent increase in myocardial oxygen consumption (29–31). Moreover, prolonged bed rest and systemic inflammation favor coagulation disorders. Both venous and unusual arterial thromboembolic events were observed in COVID-19 patients (32, 33). Hypoxemia, another hallmark of COVID-19, is associated with enhanced oxidative stress with reactive oxygen species production and subsequent intracellular acidosis, mitochondrial damage, and cell death (29, 34).

A second series of indirect mechanisms are those related with the peculiar abnormal inflammatory response that COVID-19 may elicit: the presence of a proinflammatory surge, the so-called cytokine storm, may happen in a week after the infection and is thought to be central in the pathogenesis of the acute lung injury/ARDS spectrum, as it is reported in severely ill patients (35, 36). Indeed, during the acute phase of the infection, an imbalanced response of types 1 and 2 T helper cells may lead to a hyperinflammatory response (35, 36), resulting in an excessive release of cytokines: in particular, higher levels of interleukin-1β (IL-1β), interleukin-6, interferon-γ, tumor necrosis factor (TNF), macrophage inflammatory protein, and vascular endothelial growth factor (VEGF) have been described in patients affected by severe COVID-19 (16–18), and are independently associated with a severe course of the infection and eventually death (16, 37). In addition, the hyperinflammation syndrome seems to be pivotal in the development of cardiac injury, since a positive correlation has been described between the increase in inflammatory markers and myocardial damage in COVID-19 (38–41). Consistently, previous *in-vitro* studies have shown that the release of proinflammatory cytokines such as TNF and IL-1β, in other septic conditions, were responsible for myocardial cells depression (42–44), through modulation of calcium channel activity and nitric oxide production (43, 44).

Cytokine storm may be as well the cause of acute HF: the inflammatory activation and oxidative stress are similarly present in HF and may predispose, combined with COVID-19, to a more severe clinical course (45–47). Finally, the marked inflammatory response takes place also in the endothelium, as demonstrated by post-mortem histological findings showing lymphocytic endotheliitis with apoptotic bodies and viral inclusion structures in multiple organs (48, 49). Endotheliitis can lead to disseminated intravascular coagulation with small or large vessels thrombosis and infarction and significant new vessel growth through a mechanism of intussusceptive angiogenesis (49, 50).

Consequently, anti-inflammatory therapies and thromboprophylaxis have been the mainly studied drugs for COVID-19 (51–54). Dexamethasone was found to be associated with lower 28-day mortality in the controlled, open-label Randomized Evaluation of COVID-19 Therapy (RECOVERY) trial (51). Beneficial effects were limited to those patients receiving ventilatory support (either invasive or non-invasive), while neutral effects were reported among patients not requiring oxygen therapy. The efficacy of steroids was confirmed in further retrospective series and in one meta-analysis of seven randomized trials, including 1,703 patients (53).

Finally, drugs that have been used as COVID-19 therapy may cause themselves myocardial injury. At the beginning of the pandemic, many drugs were proposed in an expedited manner (55). Hydroxychloroquine was initially proposed as an effective drug for the therapy of COVID-19. It is known that hydroxychloroquine has cardiovascular toxicity, as it may cause arrhythmias and heart failure (56). A recent meta-analysis, including a total of 5,652 COVID-19 patients, showed that treatment with hydroxychloroquine or chloroquine was associated with risk of drug-induced QT prolongation and higher incidence of torsades de pointes, ventricular tachycardia, or cardiac arrest (57), while no efficacy was found in the treatment of hospitalized patients with COVID-19 for hydroxychloroquine in the RECOVERY trial (58). Similarly, azithromycin was initially recommended in patients with COVID-19, but it may increase the risk of adverse CV events (high risk of QTc

TABLE 2 | Prevalence, incidence, and mortality of pre-existing and acute HF in COVID-19.

Study (year)	Number of patients	Number of patients with history of HF	Prevalence of HF history (%)	Main outcome of patients with history of HF	Incidence of acute HF during COVID-19 (%)	Outcome in acute HF patients
Inciardi et al. (21)	99	21	21	Higher mortality in HF vs. non-HF patients (57 vs. 18%, $p = 0.009$)	–	–
Shi et al. (24)	671	22	3.3	History of HF was more prevalent in dead patients vs. survivors (21 vs. 1.5%, $p < 0.001$)	–	Acute HF was the cause of death in 19.4% of cases.
Petrilli et al. (19)	5,279	367	7	Adjusted HR for death 1.77 (95% CI, 1.43–2.2, $p < 0.001$)	–	–
Lala et al. (25)	2,736	276	10.1	HR for death 1.03 (95% CI, 0.77–1.37, $p = 0.867$)	–	–
Richardson et al. (20)	5,700	371	6.9	–	–	–
Rey et al. (26)	3,080	152	4.9	Higher mortality in HF vs. non-HF patients (48.7 vs. 19%, $p < 0.001$)	2.5	Acute HF patients had higher mortality (46.8 vs. 19.7%, $p < 0.001$
Tomasoni et al. (27)	692	90	13	Adjusted HR for death 2.25 (95% CI, 1.26–4.02, $p = 0.006$)	9.1	Acute HF patients had higher mortality (40.0 vs. 21.8%, $p = 0.004$)
Zhou et al. (17)	191	–	–	–	23	Acute HF was more prevalent in dead patients vs. survivors (52 vs. 12%, $p < 0.0001$)
Alvarez-Garcia et al. (28)	6,439	422	6.6	Adjusted OR for death 1.88 (1.27–2.78, $p = 0.002$)	0.6 (*de-novo* HF)	*De-novo* HF patients had increased risk of ICU (HR, 2.2; 95% CI, 1.2–3.8) and intubation (HR, 2.2; 95% CI, 1.2–4.3), but not mortality (HR, 1.1, 95% CI, 0.6–2.0)

CI, confidence interval; COVID-1, Coronavirus Disease 2019; HF, heart failure; HR, hazard ratio; OR, odds ratio.

prolongation, especially when administered concomitantly with hydroxychloroquine (59). Several antiviral drugs are known to cause mitochondrial dysfunction and cardiotoxicity (60, 61).

Direct Mechanisms: the Role of ACE 2

Angiotensin-converting enzyme (ACE)2 is the key to understand the consequences of SARS-CoV-2 infection on the CV system. ACE2 is a membrane protein, that is highly expressed in different organs, including heart, lungs, gut, and kidneys. It mediates SARS-CoV-2 entry into the host cells (62). Thus, ACE2 may facilitate organ damage by direct virus entry, with different clinical implications, according to the target organ. The virus, once inside the cell, uses host's ribonucleic acid (RNA)-dependent RNA polymerase to replicate its own structural proteins and, when assembled, new virus is released from the cells; as a consequence, host cell can be damaged/destroyed in this process (5). Consistently, SARS-CoV-2 positivity in cardiac tissues could be documented in autoptic studies in consecutive patients who died as a consequence of COVID-19 (63). Supportively, in engineered heart tissue model of COVID-19 myocardial pathology, SARS-CoV-2 was found capable to directly infect cardiomyocytes through ACE2, resulting in contractile deficits, cytokine production, sarcomere disassembly, and cell death (64).

In addition, ACE2 may be not only a simple bystander in the pathophysiology of myocardial injury: indeed, besides being the receptor of SARS-CoV-2, is an enzyme involved in the renin–angiotensin–aldosterone system (RAAS). Once binding is complete, the virus attaches ACE2 throughout membrane fusion and invagination, causing a downregulation in the activity of ACE2 (65). Particularly, ACE2 cleaves angiotensin II into angiotensin 1–7, which has vasodilating and anti-inflammatory effects. ACE2 has also a weak affinity for angiotensin I and can convert it into the non-apeptide angiotensin 1–9, limiting angiotensin II synthesis by ACE, and with vasodilatatory effects through angiotensin type 2 (AT2) receptor stimulation. Thus, ACE2 can counteract the untoward effects of angiotensin II with vasodilatatory, antioxidant, and antifibrotic effects (66). Interestingly, ACE2 has also immunomodulatory properties both direct, through its interaction with macrophages, and indirect, reducing angiotensin II which stimulates inflammation (67). ACE2 downregulation by SARS-CoV-2 infection may

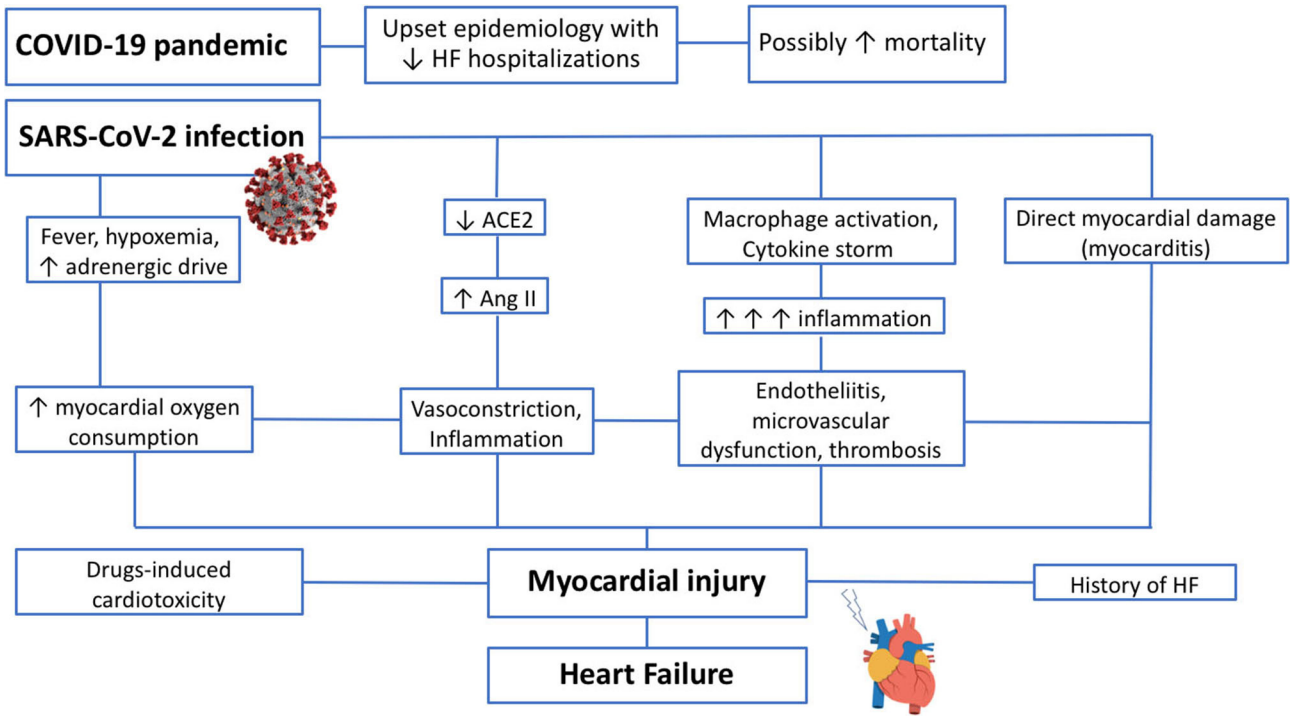


FIGURE 1 | COVID-19 and Heart Failure: mechanisms of myocardial damage in COVID-19. ACE2, angiotensin-converting enzyme2; Ang II, angiotensin II; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; HF, heart failure.

increase angiotensin II levels, favoring AT1 receptor activity, with a subsequent vasoconstriction, fibrotic, proliferative, and proinflammatory effects (3).

CLINICAL PRESENTATIONS OF MYOCARDIAL INJURY IN COVID-19

COVID-19 often affects the heart. The clinical manifestations of cardiac involvement could range from an absolute lack of symptoms in the presence of increased troponin levels, with or without ECG or imaging abnormalities, to arrhythmia and sudden cardiac death, pulmonary embolism, acute coronary syndromes, myocarditis, acute HF, and cardiogenic shock (3, 68, 69). The majority of patients with cardiac injury, as assessed by serum troponin elevation, do not have clear cardiac symptoms, whereas a minority is diagnosed with myocarditis or acute myocardial infarction. In more than a half of cases, ECG abnormalities compatible with myocardial ischemia (T-wave depression and inversion, ST segment depression, Q-waves) were described (38).

Recently, it has been shown that patients with cardiac injury have a greater prevalence of left ventricle (LV), right ventricle (RV), and pericardial abnormalities (69). Diastolic dysfunction was more frequent in patients with myocardial injury, possibly reflecting the higher prevalence of hypertension, diabetes, and chronic kidney disease among those patients, known risk factors for HF with preserved ejection fraction (HFpEF) (70). Transthoracic echocardiography (TTE) abnormalities and concomitant cardiac injury were correlated with an increased risk of death; thus, TTE evaluation might be useful to characterize the underlying cardiac substrate, for risk stratification, and to guide clinical decisions (69).

SUBCLINICAL ACUTE MYOCARDIAL INJURY IN COVID-19

Since the first Chinese reports, a high incidence of cardiac injury, defined as the presence of elevated troponin (Tn) levels above the 99th percentile of the reference interval, was found in COVID-19 patients. The prevalence of cardiac injury ranges from 12% in unselected COVID-19 cases up to 41% in critically ill patients and patients with pre-existing cardiovascular diseases with a further rise to 75.8% in non-survivors (**Table 3**) (14, 17, 18, 24, 25, 38, 39, 71–82). The presence of elevated Tn levels is associated with abnormal laboratory findings (including white blood cells count, neutrophil and lymphocyte count, C-reactive protein, procalcitonin, N-terminal pro-B-type natriuretic peptide, D-dimer, transaminases, lactate dehydrogenase, total bilirubin, albumin, prothrombin time, and cytokines) (18) and a higher grade of pulmonary involvement in radiographic findings, suggesting an important multiorgan involvement (21, 38, 73). This may be a consequence of the derangement in the innate and adaptive immune response, with a cytokine storm, similar to that previously observed in

TABLE 3 | Cardiac injury prevalence and risk of in-hospital death in different geographical settings.

Study (date of publication)	Country	Number of patients	Severity	Patients with cardiac injury (*n* (%))	In-hospital deaths among cardiac injury patients (*n* (%))	HR (95%CI) for death in cardiac injury group	OR (95% CI) for death in cardiac injury group
Huang et al. (18)	China	41	Mixed (inpatients)	5 (12%)	–	–	–
Shi et al. (38)	China	416	Mixed (inpatients)	82 (19.7%)	42 (51.2%)	3.41 (1.62–7.16)	–
Guo et al. (39)	China	187	Mixed (inpatients)	52 (27.8%)	31 (59.6%)	–	–
Zhou et al. (17)	China	191	Mixed (inpatients)	– (17%)	–	–	80 (10.3–620.4) (univariate)
Chen et al. (14)	China	274	Moderate to severe/critical	– (41%)	–	–	–
Deng et al. (71)	China	112	Mixed (inpatients)	42 (37.5%)	14 (33.3%)	8.9 (1.9–40.6) (peak troponin T)	–
Wei et al. (72)	China	101	Mixed (inpatients)	16 (15.8%)	3 (18.8%)	–	6.63 (2.24–19.65) (univariate) (progression to severe disease)
Shi et al. (24)	China	671	Severe and critical	– (15.8%)		4.56 (1.28–16.28) (multivariate)	–
Lala et al. (25)	USA	2,736	Mixed (inpatients)	Troponin I >0.03 to 0.09 ng/ml: $n = 455$ (16.6%); troponin I > 0.09 ng/dl: $n = 530$ (19.4%)	–	1.75 (1.37–2.24); 3.03 (2.42–3.80)	
Lombardi et al. (73)	Italy	614	Mixed (inpatients)	278 (45.3%)	104 (37.4%)	1.71 (1.13–2.59) (multivariate)	–
Calvo-Fernandez et al. (74)	Spain	872	Mixed (inpatients)	(34.6%)	(29.3%)	2.91 (1.21–7.04) (multivariate)	–
Fan et al. (75)	China	353	Mixed (inpatients)	79 (22.4%)	45 (57%)	1.65 (1.17–2.34) (multivariate)	–
Giustino et al. (69)	International (USA, Italy)	305	Mixed (inpatients with TTE and ECG)	190 (62.3%)	51 (26.8%)	–	6.67 (2.76–16.11) (univariate)
He et al. (76)	China	1,031	Mixed (inpatients)	215 (20.7%)	131 (60.9%)	–	2.34 (1.23–4.45) (multivariate) (among severe patients)
De Almeida et al. (77)	Brazil	183	Mixed (inpatients)	–	–	1.12 (1.03–1.47) (multivariate)	–
Manocha et al. (78)	USA	446	Mixed (inpatients)	112 (25.1%)	51 (45%)	–	4.38 (2.32–8.28) (multivariate) (30-day in-hospital mortality)
Bardaji et al. (79)	Spain	186	Mixed (inpatients)	41 (22%)	–	3.54 (1.70–7.34) (multivariate)	–
Efros et al. (80)	Israel	320	Mixed (inpatients)	91 (28.4%)	33 (36%)	4.32 (2.8–8.99) (multivariate)	–
Ali et al. (81)	Pakistan	466	Mixed (inpatients)	168 (36%)	130 (77.4%)	3.61 (0.70–1.86) (multivariate)	–
Tanboga et al. (82)	Turkey	14,855	Mixed (inpatients)	1,027 (6,9%)	–	1.89 (1.62–2.21) (multivariate) (30-day in-hospital mortality)	–

CI, confidence interval; ECG, electrocardiogram; HF, heart failure; HR, hazard ratio; OR, odds ratio; TTE, transthoracic echocardiogram; USA, United States of America.

SARS and Middle East Respiratory Syndrome (MERS) (3). The presence of cardiac injury in COVID-19 is associated with more severe manifestations, complications, and adverse prognosis (14, 17, 18, 24, 25, 38, 39, 71–82). Data about the association between cardiac injury and mortality are summarized in **Table 3**.

Chinese reports firstly described the impact of comorbidities and underlying CVD on the development of myocardial injury and subsequent fatal outcomes (15, 39). In-hospital mortality was 7.6% for patients without underlying CVD and normal Tn levels, 13.3% for those with underlying CVD and normal Tn levels, 37.5% for those without underlying CVD but elevated Tn levels, and 69.4% for those with underlying CVD and elevated Tn (39). Moreover, the mortality rate increases with the magnitude of the troponin elevation: the mortality rate is higher among

patients with vs. without cardiac injury [42 (51.2%) vs. 15 (4.5%); $p < 0.001$] (39). After adjusting for multiple variables (e.g., age, pre-existing cardiovascular diseases, cerebrovascular diseases, diabetes mellitus, chronic obstructive pulmonary disease, renal failure, cancer, ARDS, creatinine levels, and NT-proBNP levels), the hazard ratio (HR) for death among patients with cardiac injury was 4.26 (95% CI, 1.92–9.49), $p < 0.01$ (39). The prognostic role of cardiac injury was confirmed in further and larger studies from different countries (including European, American, and Asiatic nations), with mortality HR ranging from 1.12 to 8.9 depending on the regression model used and OR up to 80 in a single univariate model (17, 71–82) (**Table 3**). Data from a recent meta-analysis including 12,262 patients from 13 studies summarized that elevated Tn is associated with increased mortality (OR, 4.75; 95% CI, 4.07–5.53; $p < 0.001$; $I^2 = 19.9\%$), with 55% sensitivity and 80% specificity (83). Therefore, Tn test offers an important prognostic tool for the stratification of the risk of mortality in patients affected by COVID-19.

THE DILEMMA OF MYOCARDITIS IN COVID-19

Sometimes, cardiac involvement is clinically evident, and besides elevation of serum Tn, patients complain of chest pain, palpitation, or symptoms of HF (14, 17, 84). They may develop LV or biventricular dysfunction, in the absence of obstructive epicardial coronary disease, raising the clinical concern for myocarditis. Plenty of clinical reports described cases of acute myocarditis, presenting with cardiogenic shock, as a possible manifestation of COVID-19 (85–90).

However, myocarditis diagnosis can be controversial. Most of those cases were diagnosed based on cardiac magnetic resonance (CMR) findings that may show diffuse ventricular wall thickening and edema with wall pseudohypertrophy, with or without late gadolinium enhancement. Tissue diagnosis criteria were met only in few cases with endomyocardial biopsy (EMB) showing different degrees of aspecific myocardial inflammation and limited or absent myocardial necrosis (85, 86, 88, 90–92). Tissue findings in COVID-19 related to supposed myocarditis are enlisted in **Table 4** (5, 48, 85, 86, 88, 92–106).

SARS-CoV-2 was shown within macrophages, but not in cardiomyocytes, in an earliest case report of clinically suspected acute myocarditis (88). Further studies documented viral invasion and necrosis of myocytes (5, 107). In a series of 104 EMB, performed in COVID-19 patients with suspected myocarditis or unexplained HF, SARS-CoV-2 was identified through RT-PCR only in five samples (102). In a multicentric post-mortem study, Basso et al. found that the most common cardiac autoptic evidence in patients dying for COVID-19 was aspecific interstitial macrophage infiltration (in 86% of cases), whereas 14% of the patients presented a multifocal lymphocytic myocarditis (92) (**Table 4**). A literature review, identifying 277 cardiac autopsies from 22 studies, showed that the prevalence of myocarditis in SARS-CoV-2-infected patients was 1.4% (108). The most common histopathologic findings were myocardial hypertrophy (from 70 to 100% of cases) and myocardial fibrosis (80–100% of cases). Lymphocytic infiltrate and pericarditis were present in <25% of cases, while common findings were endothelitis, macro- or microvascular thrombi, and macrophage infiltrate (86% of cases) (108). Macrophage infiltration is an aspecific inflammatory histological finding, which can be found also in normal human hearts or in patients dying from bacterial sepsis and may be due to systemic hyperinflammatory response or other underlying disease rather than COVID-19 itself (109, 110).

Although a few cases of direct virus-related myocarditis may exist, the most common cardiac findings were non-myocarditis inflammatory infiltrate and single cell ischemia, showing how multiple and complex mechanisms are responsible for myocardial injury in COVID-19 patients, as stated above (67, 111–113).

CARDIOVASCULAR INVOLVEMENT AFTER COVID-19 VACCINATION

COVID-19 vaccines are a critical tool for controlling the ongoing global pandemic. In large, randomized controlled trials, COVID-19 vaccines were found to be safe and efficacious in preventing symptomatic, laboratory-confirmed, COVID-19. However, many adverse effects, namely, CV complications, have been described. Myocarditis may be a complication after mRNA COVID-19 vaccination (i.e., Pfizer-BioNTech and Moderna). Up to June 2021, more than a thousand cases of possible myocarditis and pericarditis have been signaled to the Vaccine Adverse Event Reporting System (VAERS) in the USA[1]. The cases are rare (hundreds of millions of doses have been administrated up to now) and were reported to be more common in young and adolescent males and after the second dose of the vaccine[1]. Similarly, the European Medicines Agency (EMA) reports less than a thousand cases of myopericarditis up to June 2021[2]. The cases described in literature usually present with fever, chest pain, and dyspnea, together with changes on the electrocardiogram and cardiac magnetic resonance findings consistent with myocarditis; the symptoms usually resolved rapidly (114–120). The patients were treated with non-steroidal anti-inflammatory drugs (NSAIDs) only, but, in some cases, required intravenous immune globulin (IVIG) and corticosteroids (114–120). However, up-to-date, no causal relationship between vaccine administration and myocarditis has been established.

The Centers for Disease Control (CDC) continues to recommend COVID-19 vaccination for everyone 12 years of age and older[1]. Another possible but life-threatening complication of COVID-19 vaccination is vaccine-induced thrombotic thrombocytopenia (VITT) (also referred to as vaccine-induced prothrombotic immune thrombocytopenia or thrombosis with thrombocytopenia syndrome); VITT is characterized by thrombosis, often in unusual sites (specifically, cerebral venous sinus thrombosis or thrombosis in the portal, splanchnic, or

[1]https://www.cdc.gov/coronavirus/2019-ncov/vaccines/safety/myocarditis.html.
[2]https://www.ema.europa.eu/en/news/covid-19-vaccines-update-ongoing-evaluation-myocarditis-pericarditis.

TABLE 4 | Studies reporting cardiac tissue findings in COVID-19 patients.

Study (year)	Number of patients	Design	Findings
Basso et al. (92)	21	Multicenter pathology study, post-mortem	Increased interstitial macrophage infiltration was present in 86% of the cases, whereas lymphocytic myocarditis was present in 14% of the cases
Varga et al. (48)	3	Case reports, post-mortem	Lymphocytic endotheliitis in lung, heart, kidney, and liver but no sign of lymphocytic myocarditis.
Menter et al. (93)	21	Multicenter, post-mortem	Myocardial hypertrophy (71% of cases), senile amyloidosis (29% of cases), peracute myocardial necrosis (14% cases), acute myocardial infarction (5% cases)
Lax et al. (94)	11	Single-center, prospective study, post-mortem	Myocardial hypertrophy (100%), coronary small vessel disease (54%), myocardial fibrosis (91%), focal lymphocytic infiltrate (9%)
Buja et al. (95)	3	Multicenter, post-mortem	Lymphocytic myocarditis was reported in 1 case.
Duarte-Neto et al. (96)	10	Single-center, case series, post-mortem	Cardiomyocyte hypertrophy (90%), myocardial fibrosis (90%), previous myocardial infarction (40%), interstitial oedema (90%) myocarditis (20%), and fibrin thrombi (20%)
Bradley et al. (97)	14	Multicenter, case series, post-mortem	Cardiac findings were mostly non-specific: fibrosis (100%) and myocyte hypertrophy (93%). Myocarditis was present with aggregates of lymphocytes surrounding necrotic myocytes in 7%
Rapkiewicz et al. (98)	7	Single-center, case series, post-mortem	1 case had focal acute lymphocyte-predominant inflammation in the myocardium. Otherwise, cardiac histopathological changes were limited to minimal epicardial inflammation ($n = 1$), early ischemic injury ($n = 3$), and mural fibrin thrombi ($n = 2$)
Grosse et al. (99)	14	Single-center, case series, post-mortem	Myocardial hypertrophy (92.9%), acute myocardial infarction (21.4%), focal myocardial fibrosis (42.9%), amyloidosis (7.1%), mononuclear inflammatory cells in the myocardial interstitium (100%)
Hanley et al. (100)	10	Multicenter, case series, post-mortem	Acute coronary thrombosis (10%), thrombi in the microcirculation (56%), aright atrial thrombus (11%). Pericarditis (22%); marantic endocarditis in 11%
Oprinca et al. (101)	3	Single-center, case series, post-mortem	Mild to moderate perivascular edema, vascular congestion, small number of scattered lymphocytes between the myocardial fibers
Sala et al. (86)	1	Case report with EMB	Diffuse T-lymphocytic inflammatory infiltrates with huge interstitial oedema and limited foci of necrosis. No replacement fibrosis
Tavazzi et al. (88)	1	Case report with EMB	Low-grade interstitial and endocardial inflammation, with macrophages containing virions of coronaviruses. Cardiac myocytes showed non-specific features consisting of focal myofibrillar lysis and lipid droplets.
Escher et al. (102)	104	Multicenter, EMB study	5 EMBs were positive for SARS-CoV-2 E-gene-specific sequences. Other findings were active myocarditis (13.4%), inflammatory cardiomyopathy (32.6%), borderline myocarditis (2.9 %); dilated cardiomyopathy (41.3%), and amyloidosis (9.6%)
Lindner et al. (63)	39	Cohort study, post-mortem	Viral presence within the myocardium could be documented in 41% but was not associated with an influx of inflammatory cells
Kawakami et al. (103)	15	Literature review, post-mortem	None of the cases met the criteria of myocarditis, although in 3 cases microvascular infarction was described. In 2 cases, the virus was detected by RT-PCR in the atria, but no inflammation was described.
Haslbauer et al. (104)	23	Multicenter, post-mortem	60% of cases had myocardial RT-PCR positivity by SARS-CoV-2 PCR. Significantly higher levels of capillary fibrin deposition, capillary dilatation, and parenchymal microhemorrhages (consistent with microvascular dysfunction) compared with 10 autopsies without SARS-CoV-2. Five cases presented with increased cardioinflammatory infiltrate presented but without cardiomyocyte necrosis. Only while 1 case presented with active lymphohistiocytic myocarditis.
Bearse et al. (105)	41	Single-center, consecutive cases, post-mortem	Cardiac infection by SARS-CoV-2 (assessed by RT-PCR) was present in 30/41 cases. Cardiac infection by SARS-CoV-2 is associated with more cardiac inflammation (monocytes and macrophages). Four cases met criteria for myocarditis.
Fox et al. (106)	10	Single-center, case series, post-mortem	No evidence of lymphocytic myocarditis. In the COVID-19-affected cases, diffuse number of infiltrative cells of monocytes/macrophage lineage was noticed, with upper quantiles as compared to both matched control hearts.

EMB, endomyocardial biopsy; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RT-PCR, reverse transcriptase-polymerase chain reaction.

hepatic veins), and concomitant thrombocytopenia (121). The cases reported were noticed after adenovirus-based vaccination (i.e., AstraZeneca or Johnson & Johnson/Janssen) in patients without prior exposure to heparin (122–124). The majority of patients in these cases were women younger than 50 years of age, some of whom were receiving estrogen-replacement therapy or oral contraceptives (122–125). The pathogenesis is similar to heparin-induced thrombocytopenia (HIT): the enzyme-linked immunosorbent assay test in these cases identified antibodies directed against the platelet factor 4 (PF4)-heparin complex which activate platelets, similar to HIT antibodies (121–125). Patients usually presented with a median platelet counts at diagnosis of 20,000–30,000/mm^3 with concomitant high levels of D-dimer and low levels of fibrinogen; almost 40% of the

patients died, some from ischemic brain injury or superimposed hemorrhage (122–125). In the case of cerebral venous sinus thrombosis (CVST), patients usually presented with headache and progressive lethargy. The consensus treatment is based on the administration of intravenous immunoglobulin or corticosteroids (126).

The estimated incidence of CVST is 3.6 per million people after the AstraZeneca COVID-19 vaccine and 0.9 per million people after Johnson and Johnson vaccine (which is much lower than the rate of CVST in COVID-19, estimated at 207 per million) (127). According to the EMA, the risk of death and serious outcomes of COVID-19 (including thrombosis) outweighs the risk of VITT[3].

HEART FAILURE AS A CONSEQUENCE OF COVID-19

Acute HF was found to be a possible consequence of COVID-19, with a dramatic impact on mortality (26). During COVID-19 hospitalization, about one-third of patients with previous HF had an acute decompensation of HF (27); however, acute HF can be developed not only as a decompensation of chronic HF but also as a new-onset HF (128) (**Table 2**). In an Italian multicenter study, acute HF occurred in 9.1% of patients during hospitalization for COVID-19, and almost half of them were "*de-novo*" HF in patients with no HF history (27). Among 3,080 consecutive patients with confirmed COVID-19 infection hospitalized in a tertiary center in Madrid (Spain), 2.5% of patients were diagnosed with acute HF and suffered from significantly higher mortality as compared with patients without HF(46.8 vs. 19.7%; $p < 0.001$) (128). Arrhythmias during hospital admission and chronic HF were the main predictors of acute HF; however, 77.9% of acute HF did not have a previous history of HF (128).

In COVID-19 patients presenting acute HF, LV systolic function is not usually compromised; on the contrary, impairment of right ventricular (RV) systolic function and LV diastolic function can be found (129). Out of 100 patients hospitalized for COVID-19, 32% were reported to have normal echocardiography, whereas 39% presented RV dilatation and dysfunction and 16% LV diastolic dysfunction, whereas reduced LV EF was reported only in <10% (130). Similar results are described in other small series (131, 132) and in a large international cohort study (69). Accordingly, LV diastolic impairment with elevated LV filling pressures (E/e' ratio) could be observed in a quarter of patients admitted for COVID-19 (132). Consistently, patients hospitalized with COVID-19 showed high likelihood of presence of HF with preserved ejection fraction (HFpEF) as compared with patients without COVID-19 according to the score of the Heart Failure Association (HFA) of the European Society of Cardiology (ESC), and HFpEF was found associated with cardiac structural and functional alterations and myocardial injury (133). Moreover, the longitudinal function could be impaired earlier than LVEF: in a Danish prospective multicenter cohort study, no differences were found between cases and controls from the general population regarding LVEF; on the contrary, LV global longitudinal strain (GLS) was significantly reduced (134). Speckle tracking was found to be able to identify a reduced basal LV longitudinal strain in more than a half of hospitalized COVID-19 patients (135, 136). Moreover, RV systolic function [assessed by RV longitudinal strain and tricuspid annular plane systolic excursion (TAPSE)] can be impaired in COVID-19 patients (137). A more pronounced reduction of mean values of LV-GLS and RV longitudinal strain could be found in severe COVID-19 patients, and speckle tracking analysis could predict mortality even after adjusting for multiple confounders (130, 137, 138).

LONG TERM CONSEQUENCES OF COVID-19 ON THE HEART

Concerning data are emerging regarding the possibility of long-term subacute myocarditis following the recovery from SARS-CoV-2 infection and the development of HF as a long-term consequence of COVID-19 inflammatory cardiomyopathy. Follow-up clinical studies are starting to report the long-term COVID-19 consequences with many people still suffering from fatigue, dyspnea, and palpitations 3–6 months after the recovery from acute infection (139–142). In this context, imaging tests taken months after recovery from COVID-19 have shown ongoing signs of damage to the heart, even in people who experienced only mild COVID-19 symptoms. A German study suggested that 2 months after SARS-CoV-2 positivity, 78% of survivors had persistent heart involvement, of which 60% presented ongoing signs of myocarditis, revealed with cardiac magnetic resonance (CMR) (142). In a study including competitive athletes, referred to the sports medicine clinic after testing positive for COVID-19, 15% of patients had CMR findings suggestive of ongoing myocarditis and 30.8% suggestive of prior myocardial injury (143). In another CMR multicenter study evaluating 148 patients during convalescence, 2 months after severe COVID-19 infection with troponin elevation, myocarditis-like injury can be encountered in almost a half of cases (144). A large CMR cohort study among 1,597 US competitive athletes from the Big Ten Universities recently affected by SARS-CoV-2, reported 37 athletes with clinical and subclinical myocarditis (145). Echocardiographic assessment of patients with recent COVID-19 may, as well, show abnormalities in terms of higher degrees of diastolic dysfunction, lower men values of LV GLS, and presence of pericardial effusion, consistent with CMR findings, up to 2 months after COVID-19 recovery (146–148).

The meaning of those imaging findings are currently unknown; however, persistent myocardial damage and fibrosis in the subacute and chronic phases after recovery suggest that COVID-19 may be an independent risk factor for the development of HF (70). The early identification of patients with cardiac abnormalities is of pivotal importance as they may benefit from cardioprotective therapy and need different follow-up strategies.

[3]https://www.ema.europa.eu/en/news/covid-19-vaccine-astrazeneca-benefits-still-outweigh-risks-despite-possible-link-rare-blood-clots.

CONCLUSIONS

COVID-19 and HF have a strong connection that go beyond pathophysiology. First of all, COVID-19 pandemic had an impact on HF hospitalization: a reduction on hospital admission for HF has been extensively described, and this may have an impact on HF mortality. Second, history of HF is a frequent comorbidity in patients hospitalized for COVID-19. It is associated with a higher mortality and more complications during the clinical course, and this association is independent from other variables related with HF and COVID-19 severity.

Third, we have shown the high prevalence of cardiac injury following COVID-19 which is often diagnosed only through biomarker measurements. However, besides subclinical myocardial damage, SARS-CoV-2 infection can cause myocarditis with a severe reduction of LVEF, or diastolic dysfunction in a larger number of patients. Finally, HF may be a short- or long-term consequence of COVID-19 inflammatory cardiomyopathy with a dramatic consequence on the prognosis.

AUTHOR CONTRIBUTIONS

LI contributed to the design and conception of the manuscript and wrote the first draft. SB, LS, and EP wrote sections of the manuscript. DT reviewed and edited the manuscript. MM and MA supervised and edited the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Coronaviridae study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol.* (2020) 5:536–44. doi: 10.1038/s41564-020-0695-z
2. WHO. *Coronavirus Disease (COVID-19) Dashboard*. Available online at: https://covid19.who.int (accessed June 1, 2021).
3. Tomasoni D, Italia L, Adamo M, Inciardi RM, Lombardi CM, Solomon SD, et al. COVID-19 and heart failure: from infection to inflammation and angiotensin II stimulation. Searching for evidence from a new disease. *Eur J Heart Fail.* (2020) 22:957–66. doi: 10.1002/ejhf.1871
4. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol.* (2020) 17:259–60. doi: 10.1038/s41569-020-0360-5
5. Liu PP, Blet A, Smyth D, Li H. The science underlying COVID-19: implications for the cardiovascular system. *Circulation.* (2020) 142:68–78. doi: 10.1161/CIRCULATIONAHA.120.047549
6. Xiong TY, Redwood S, Prendergast B, Chen M. Coronaviruses and the cardiovascular system: acute and long-term implications. *Eur Heart J.* (2020) 41:1798–800. doi: 10.1093/eurheartj/ehaa231
7. Solomon MD, McNulty EJ, Rana JS, Leong TK, Lee C, Sung SH, et al. The Covid-19 pandemic and the incidence of acute myocardial infarction. *N Engl J Med.* (2020) 383:691–3. doi: 10.1056/NEJMc2015630
8. Tomasoni D, Adamo M, Italia L, Branca L, Chizzola G, Fiorina C, et al. Impact of COVID-2019 outbreak on prevalence, clinical presentation and outcomes of ST-elevation myocardial infarction. *J Cardiovasc Med.* (2020) 21:874–81. doi: 10.2459/JCM.0000000000001098
9. Bromage DI, Cannatà A, Rind IA, Gregorio C, Piper S, Shah AM, et al. The impact of COVID-19 on heart failure hospitalization and management: report from a Heart Failure Unit in London during the peak of the pandemic. *Eur J Heart Fail.* (2020) 22:978–84. doi: 10.1002/ejhf.1925
10. Cox ZL, Lai P, Lindenfeld J. Decreases in acute heart failure hospitalizations during COVID-19. *Eur J Heart Fail.* (2020) 22:1045–6. doi: 10.1002/ejhf.1921
11. Hall ME, Vaduganathan M, Khan MS, Papadimitriou L, Long RC, Hernandez GA, et al. Reductions in heart failure hospitalizations during the COVID-19 pandemic. *J Card Fail.* (2020) 26:462–3. doi: 10.1016/j.cardfail.2020.05.005
12. Cannatà A, Bromage DI, Rind IA, Gregorio C, Bannister C, Albarjas M, et al. Temporal trends in decompensated heart failure and outcomes during COVID-19: a multisite report from heart failure referral centres in London. *Eur J Heart Fail.* (2020) 22:2219–24. doi: 10.1002/ejhf.1986
13. König S, Hohenstein S, Meier-Hellmann A, Kuhlen R, Hindricks G, Bollmann A; Helios Hospitals, Germany. In-hospital care in acute heart failure during the COVID-19 pandemic: insights from the German-wide Helios hospital network. *Eur J Heart Fail.* (2020) 22:2190–201. doi: 10.1002/ejhf.2044
14. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ.* (2020) 368:m1091. doi: 10.1136/bmj.m1091
15. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA.* (2020) 323:1061–69. doi: 10.1001/jama.2020.1585
16. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med.* (2020) 8:475–81. doi: 10.1016/S2213-2600(20)30079-5
17. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3
18. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
19. Petrilli CM, Jones SA, Yang J, Rajagopalan H, O'Donnell L, Chernyak Y, et al. Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: prospective cohort study. *BMJ.* (2020) 369:m1966. doi: 10.1136/bmj.m1966
20. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City Area. *JAMA.* (2020) 323:2052–9. doi: 10.1001/jama.2020.6775
21. Inciardi RM, Adamo M, Lupi L, Cani DS, Di Pasquale M, Tomasoni D, et al. Characteristics and outcomes of patients hospitalized for COVID-19 and cardiac disease in Northern Italy. *Eur Heart J.* (2020) 41:1821–9. doi: 10.1093/eurheartj/ehaa388
22. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese center for disease control and prevention. *JAMA.* (2020) 323:1239–42. doi: 10.1001/jama.2020.2648
23. Bae S, Kim SR, Kim M, Shim WJ, Park SM. Impact of cardiovascular disease and risk factors on fatal outcomes in patients with COVID-19 according to age: a systematic review and meta-analysis. *Heart.* (2021) 107:373–80. doi: 10.1136/heartjnl-2020-317901
24. Shi S, Qin M, Cai Y, Liu T, Shen B, Yang F, et al. Characteristics and clinical significance of myocardial injury in patients with severe coronavirus disease 2019. *Eur Heart J.* (2020) 41:2070–9. doi: 10.1093/eurheartj/ehaa408
25. Lala A, Johnson KW, Januzzi JL, Russak AJ, Paranjpe I, Richter F, et al. Prevalence and impact of myocardial injury in patients hospitalized with COVID-19 infection. *J Am Coll Cardiol.* (2020) 76:533–46. doi: 10.1101/2020.04.20.20072702
26. Rey JR, Caro-Codón J, Rosillo SO, Iniesta ÁM, Castrejón-Castrejón S, Marco-Clement I et al. Heart failure in COVID-19 patients: prevalence, incidence and prognostic implications. *Eur J Heart Fail.* (2020) 22:2205–15. doi: 10.1002/ejhf.1990

27. Tomasoni D, Inciardi RM, Lombardi CM, Tedino C, Agostoni P, Ameri P, et al. Impact of heart failure on the clinical course and outcomes of patients hospitalized for COVID-19. Results of the Cardio-COVID-Italy multicentre study. *Eur J Heart Fail.* (2020) 22:2238–47. doi: 10.1002/ejhf.2052
28. Alvarez-Garcia J, Lee S, Gupta A, Cagliostro M, Joshi AA, Rivas-Lasarte M, et al. Prognostic impact of prior heart failure in patients hospitalized with COVID-19. *J Am Coll Cardiol.* (2020) 76:2334–48. doi: 10.1016/j.jacc.2020.09.549
29. Violi F, Cangemi R, Falcone M, Taliani G, Pieralli F, Vannucchi V, et al. Cardiovascular complications and short-term mortality risk in community-acquired pneumonia. *Clin Infect Dis.* (2017) 64:1486–93. doi: 10.1093/cid/cix164
30. Madjid M, Miller CC, Zarubaev VV, Marinich IG, Kiselev OI, Lobzin YV, et al. Influenza epidemics and acute respiratory disease activity are associated with a surge in autopsy-confirmed coronary heart disease death: results from 8 years of autopsies in 34,892 subjects. *Eur Heart J.* (2007) 28:1205–0. doi: 10.1093/eurheartj/ehm035
31. Corrales-Medina VF, Alvarez KN, Weissfeld LA, Angus DC, Chirinos JA, Chang CC, et al. Association between hospitalization for pneumonia and subsequent risk of cardiovascular disease. *JAMA.* (2015) 313:264–74. doi: 10.1001/jama.2014.18229
32. Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res.* (2020) 191:145–7. doi: 10.1016/j.thromres.2020.04.013
33. de Roquetaillade C, Chousterman BG, Tomasoni D, Zeitouni M, Houdart E, Guedon A, et al. Unusual arterial thrombotic events in Covid-19 patients. *Int J Cardiol.* (2021) 323:281–4. doi: 10.1016/j.ijcard.2020.08.103
34. Takasu O, Gaut JP, Watanabe E, To K, Fagley RE, Sato B, et al. Mechanisms of cardiac and renal dysfunction in patients dying of sepsis. *Am J Respir Crit Care Med.* (2013) 187:509–17. doi: 10.1164/rccm.201211-1983OC
35. Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 cytokine storm; what we know so far. *Front Immunol.* (2020) 11:1446. doi: 10.3389/fimmu.2020.01446
36. Fajgenbaum DC, June CH. Cytokine storm. *N Engl J Med*. (2020) 383:2255–73. doi: 10.1056/NEJMra2026131
37. Del Valle DM, Kim-Schulze S, Huang H-H, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. *Nat Med*. (2020) 26:1636–43. doi: 10.1038/s41591-020-1051-9
38. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* (2020) 5:802–10. doi: 10.1001/jamacardio.2020.0950
39. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017
40. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. et al. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet.* (2020) 395:1033–4. doi: 10.1016/S0140-6736(20)30628-0
41. Arévalos V, Ortega-Paz L, Rodríguez-Arias JJ, Calvo M, Castrillo L, Salazar A, et al. Myocardial injury in COVID-19 patients: association with inflammation, coagulopathy and in-hospital prognosis. *J Clin Med*. (2021) 10:2096. doi: 10.3390/jcm10102096
42. Kumar A, Thota V, Dee L, Olson J, Uretz E, Parrillo JE. Tumor necrosis factor alpha and interleukin 1beta are responsible for *in vitro* myocardial cell depression induced by human septic shock serum. *J Exp Med*. (1996) 183:949–58. doi: 10.1084/jem.183.3.949
43. Kumar A, Brar R, Wang P, Dee L, Skorupa G, Khadour F, et al. Role of nitric oxide and cGMP in human septic serum-induced depression of cardiac myocyte contractility. *Am J Physiol.* (1999) 276:R265–76. doi: 10.1152/ajpregu.1999.276.1.R265
44. Joulin O, Petillot P, Labalette M, Lancel S, Neviere R. Cytokine profile of human septic shock serum inducing cardiomyocyte contractile dysfunction. *Physiol Res.* (2007) 56:291–7.
45. Unudurthi SD, Luthra P, Bose RJC, McCarthy JR, Kontaridis MI. Cardiac inflammation in COVID-19: lessons from heart failure. *Life Sci.* (2020) 260:118482. doi: 10.1016/j.lfs.2020.118482
46. Markousis-Mavrogenis G, Tromp J, Ouwerkerk W, Devalaraja M, Anker SD, Cleland JG, et al. The clinical significance of interleukin-6 in heart failure: results from the BIOSTAT-CHF study. *Eur J Heart Fail.* (2019) 21:965–73. doi: 10.1002/ejhf.1482
47. van der Pol A, van Gilst WH, Voors AA, van der Meer P. Treating oxidative stress in heart failure: past, present and future. *Eur J Heart Fail.* (2019) 21:425–35. doi: 10.1002/ejhf.1320
48. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet.* (2020) 395:1417–8. doi: 10.1016/S0140-6736(20)30937-5
49. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med.* (2020) 383:120–8. doi: 10.1056/NEJMoa2015432
50. Gavriilaki E, Anyfanti P, Gavriilaki M, Lazaridis A, Douma S, Gkaliagkousi E. Endothelial dysfunction in COVID-19: lessons learned from coronaviruses. *Curr Hypertens Rep.* (2020) 22:63. doi: 10.1007/s11906-020-01078-6
51. RECOVERY Collaborative Group, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, et al. Dexamethasone in hospitalized patients with Covid-19. *N Engl J Med.* (2021) 384:693–704. doi: 10.1056/NEJMoa2021436
52. Feldmann M, Maini RN, Woody JN, Holgate ST, Winter G, Rowland M, et al. Trials of anti-tumour necrosis factor therapy for COVID-19 are urgently needed. *Lancet.* (2020) 395:1407–9. doi: 10.1016/S0140-6736(20)30858-8
53. WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group, Sterne JAC, Murthy S, Diaz JV, Slutsky AS, Villar J, et al. Association between administration of systemic corticosteroids and mortality among critically ill patients with COVID-19: a meta-analysis. *JAMA.* (2020) 324:1330–41. doi: 10.1001/jama.2020.17023
54. Ameri P, Inciardi RM, Di Pasquale M, Agostoni P, Bellasi A, Camporotondo R, et al. Pulmonary embolism in patients with COVID-19: characteristics and outcomes in the Cardio-COVID Italy multicenter study. *Clin Res Cardiol.* (2021) 110:1020–8. doi: 10.1007/s00392-020-01766-y
55. Kupferschmidt K, Cohen J. Race to find COVID-19 treatments accelerates. *Science.* (2020) 367:1412–3. doi: 10.1126/science.367.6485.1412
56. Chatre C, Roubille F, Vernhet H, Jorgensen C, Pers YM. Cardiac complications attributed to chloroquine and hydroxychloroquine: a systematic review of the literature. *Drug Saf.* (2018) 41:919–31. doi: 10.1007/s40264-018-0689-4
57. Tleyjeh IM, Kashour Z, AlDosary O, Riaz M, Tlayjeh H, Garbati MA, et al. Cardiac toxicity of chloroquine or hydroxychloroquine in patients with COVID-19: a systematic review and meta-regression analysis. *Mayo Clin Proc Innov Qual Outcomes.* (2021) 5:137–50. doi: 10.1016/j.mayocpiqo.2020.10.005
58. RECOVERY Collaborative Group, Horby P, Mafham M, Linsell L, Bell JL, Staplin N, et al. Effect of hydroxychloroquine in hospitalized patients with covid-19. *N Engl J Med.* (2020) 383:2030–40. doi: 10.1056/NEJMoa2022926
59. Nguyen LS, Dolladille C, Drici MD, Fenioux C, Alexandre J, Mira JP, et al. Cardiovascular toxicities associated with hydroxychloroquine and azithromycin: an analysis of the world health organization pharmacovigilance database. *Circulation.* (2020) 142:303–5. doi: 10.1161/CIRCULATIONAHA.120.048238
60. Varga ZV, Ferdinandy P, Liaudet L, Pacher P. Drug-induced mitochondrial dysfunction and cardiotoxicity. *Am J Physiol Heart Circ Physiol.* (2015) 309:H1453–67. doi: 10.1152/ajpheart.00554.2015
61. Frattini S, Maccagni G, Italia L, Metra M, Danzi GB. Coronavirus disease 2019 and cardiovascular implications. *J Cardiovasc Med (Hagerstown).* (2020) 21:725–32. doi: 10.2459/JCM.0000000000001068
62. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science.* (2020) 367:1260–3. doi: 10.1126/science.abb2507
63. Lindner D, Fitzek A, Bräuninger H, Aleshcheva G, Edler C, Meissner K, et al. Association of cardiac infection with SARS-CoV-2 in confirmed COVID-19 autopsy cases. *JAMA Cardiol.* (2020) 5:1281–5. doi: 10.1001/jamacardio.2020.3551
64. Bailey AL, Dmytrenko O, Greenberg L, Bredemeyer AL, Ma P, Liu J, et al. SARS-CoV-2 infects human engineered heart tissues and models COVID-19 myocarditis. *JACC*

Basic Transl Sci. (2021) 6:331–45. doi: 10.1016/j.jacbts.2021.01.002
65. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science.* (2020) 367:1444–8. doi: 10.1126/science.abb2762
66. Patel VB, Zhong JC, Grant MB, Oudit GY. Role of the ACE2/angiotensin 1-7 axis of the renin-angiotensin system in heart failure. *Circ Res.* (2016) 118:1313–26. doi: 10.1161/CIRCRESAHA.116.307708
67. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of coronaviruses on the cardiovascular system: a review. *JAMA Cardiol.* (2020) 5:831–40. doi: 10.1001/jamacardio.2020.1286
68. Kochi AN, Tagliari AP, Forleo GB, Fassini GM, Tondo C. Cardiac and arrhythmic complications in patients with COVID-19. *J Cardiovasc Electrophysiol.* (2020) 31:1003–8. doi: 10.1111/jce.14479
69. Giustino G, Croft LB, Stefanini GG, Bragato R, Silbiger JJ, Vicenzi M, et al. Characterization of myocardial injury in patients with COVID-19. *J Am Coll Cardiol.* (2020) 76:2043–55. doi: 10.1016/j.jacc.2020.08.069
70. Zaccone G, Tomasoni D, Italia L, Lombardi CM, Metra M. Myocardial involvement in COVID-19: an interaction between comorbidities and heart failure with preserved ejection fraction. A further indication of the role of inflammation. *Curr Heart Fail Rep.* (2021) 18:99–106. doi: 10.1007/s11897-021-00509-y
71. Deng Q, Hu B, Zhang Y, Wang H, Zhou X, Hu W, et al. Suspected myocardial injury in patients with COVID-19: evidence from front-line clinical observation in Wuhan, China. *Int J Cardiol.* (2020) 311:116–21. doi: 10.1016/j.ijcard.2020.03.087
72. Wei JF, Huang FY, Xiong TY, Liu Q, Chen H, Wang H, et al. Acute myocardial injury is common in patients with COVID-19 and impairs their prognosis. *Heart.* (2020) 106:1154–9. doi: 10.1136/heartjnl-2020-317007
73. Lombardi CM, Carubelli V, Iorio A, Inciardi RM, Bellasi A, Canale C, et al. Association of troponin levels with mortality in italian patients hospitalized with coronavirus disease 2019: results of a multicenter study. *JAMA Cardiol.* (2020) 5:1274–80. doi: 10.1001/jamacardio.2020.3538
74. Calvo-Fernández A, Izquierdo A, Subirana I, Farré N, Vila J, Durán X, Markers of myocardial injury in the prediction of short-term COVID-19 prognosis. *Rev Esp Cardiol.* (2021) 74:576–83. doi: 10.1016/j.rec.2020.09.011
75. Fan Q, Zhu H, Zhao J, Zhuang L, Zhang H, Xie H, et al. Risk factors for myocardial injury in patients with coronavirus disease 2019 in China. *ESC Heart Fail.* (2020) 7:4108–17. doi: 10.1002/ehf2.13022
76. He X, Wang L, Wang H, Xie Y, Yu Y, Sun J, et al. Factors associated with acute cardiac injury and their effects on mortality in patients with COVID-19. *Sci Rep.* (2020) 10:20452. doi: 10.1038/s41598-020-77172-1
77. Almeida Junior GLG, Braga F, Jorge JK, Nobre GF, Kalichsztein M, Faria PMP, et al. Prognostic value of troponin-T and B-type natriuretic peptide in patients hospitalized for COVID-19. *Arq Bras Cardiol.* (2020) 115:660–6. English, Portuguese. doi: 10.36660/abc.20200385
78. Manocha KK, Kirzner J, Ying X, Yeo I, Peltzer B, Ang B, et al. Troponin and other biomarker levels and outcomes among patients hospitalized with COVID-19: derivation and validation of the HA2T2 COVID-19 mortality risk score. *J Am Heart Assoc.* (2021) 10:e018477. doi: 10.1161/JAHA.120.018477
79. Bardají A, Carrasquer A, Sánchez-Giménez R, Lal-Trehan N, Del-Moral-Ronda V, Peiró ÓM, et al. Prognostic implications of myocardial injury in patients with and without COVID-19 infection treated in a university hospital. *Rev Esp Cardiol.* (2021) 74:24–32. doi: 10.1016/j.rec.2020.08.027
80. Efros O, Barda N, Meisel E, Leibowitz A, Fardman A, Rahav G, et al. Myocardial injury in hospitalized patients with COVID-19 infection-Risk factors and outcomes. *PLoS ONE.* (2021) 16:e0247800. doi: 10.1371/journal.pone.0247800
81. Ali J, Khan FR, Ullah R, Hassan Z, Khattak S, Lakhta G, et al. Cardiac troponin i levels in hospitalized COVID-19 patients as a predictor of severity and outcome: a retrospective cohort study. *Cureus.* 13:e14061. doi: 10.7759/cureus.14061
82. Tanboga IH, Canpolat U, Özcan Çetin EH, Kundi H, Turan S, Celik O, et al. The prognostic role of cardiac troponin in hospitalized COVID-19 patients. *Atherosclerosis.* (2021) 325:83–8. doi: 10.1016/j.atherosclerosis.2021.04.006
83. Wibowo A, Pranata R, Akbar MR, Purnomowati A, Martha JW. Prognostic performance of troponin in COVID-19: a diagnostic meta-analysis and meta-regression. *Int J Infect Dis.* (2021) 105:312–8. doi: 10.1016/j.ijid.2021.02.113
84. Dong N, Cai J, Zhou Y, Liu J, Li F. End-stage heart failure with COVID-19: strong evidence of myocardial injury by 2019-nCoV. *JACC Heart Fail.* (2020) 8:515–7. doi: 10.1016/j.jchf.2020.04.001
85. Inciardi RM, Lupi L, Zaccone G, Italia L, Raffo M, Tomasoni D, et al. Cardiac involvement in a patient with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:819–24. doi: 10.1001/jamacardio.2020.1096
86. Sala S, Peretto G, Gramegna M, Palmisano A, Villatore A, Vignale D, et al. Acute myocarditis presenting as a reverse Tako-Tsubo syndrome in a patient with SARS-CoV-2 respiratory infection. *Eur Heart J.* (2020) 41:1861–2. doi: 10.1093/eurheartj/ehaa286
87. Doyen D, Moceri P, Ducreux D, Dellamonica J. Myocarditis in a patient with COVID-19: a cause of raised troponin and ECG changes. *Lancet.* (2020) 395:1516. doi: 10.1016/S0140-6736(20)30912-0
88. Tavazzi G, Pellegrini C, Maurelli M, Belliato M, Sciutti F, Bottazzi A, et al. Myocardial localization of coronavirus in COVID-19 cardiogenic shock. *Eur J Heart Fail.* (2020) 22:911–5. doi: 10.1002/ejhf.1828
89. Zeng JH, Liu YX, Yuan J, Wang FX, Wu WB, Li JX, et al. First case of COVID-19 complicated with fulminant myocarditis: a case report and insights. *Infection.* (2020) 48:773–7. doi: 10.1007/s15010-020-01424-5
90. Kim IC, Kim JY, Kim HA, Han S. COVID-19-related myocarditis in a 21-year-old female patient. *Eur Heart J.* (2020) 41:1859. doi: 10.1093/eurheartj/ehaa288
91. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med.* (2020) 8:420–2. doi: 10.1016/S2213-2600(20)30076-X
92. Basso C, Leone O, Rizzo S, De Gaspari M, van der Wal AC, Aubry MC, et al. Pathological features of COVID-19-associated myocardial injury: a multicentre cardiovascular pathology study. *Eur Heart J.* (2020) 41:3827–35. doi: 10.1093/eurheartj/ehaa664
93. Menter T, Haslbauer JD, Nienhold R, Savic S, Hopfer H, Deigendesch N, et al. Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. *Histopathology.* (2020) 77:198–209. doi: 10.1111/his.14134
94. Lax SF, Skok K, Zechner P, Kessler HH, Kaufmann N, Koelblinger C, et al. Pulmonary arterial thrombosis in COVID-19 with fatal outcome: results from a prospective, single-center, clinicopathologic case series. *Ann Intern Med.* (2020) 173:350–61. doi: 10.7326/M20-2566
95. Buja LM, Wolf DA, Zhao B, Akkanti B, McDonald M, Lelenwa L, et al. The emerging spectrum of cardiopulmonary pathology of the coronavirus disease 2019 (COVID-19): report of 3 autopsies from Houston, Texas, and review of autopsy findings from other United States cities. *Cardiovasc Pathol.* (2020) 48:107233. doi: 10.1016/j.carpath.2020.107233
96. Duarte-Neto AN, Monteiro RAA, da Silva LFF, Malheiros DMAC, de Oliveira EP, Theodoro-Filho J, et al. Pulmonary and systemic involvement in COVID-19 patients assessed with ultrasound-guided minimally invasive autopsy. *Histopathology.* (2020) 77:186–97. doi: 10.1111/his.14160
97. Bradley BT, Maioli H, Johnston R, Chaudhry I, Fink SL, Xu H, et al. Histopathology and ultrastructural findings of fatal COVID-19 infections in Washington State: a case series. *Lancet.* (2020) 396:320–32. doi: 10.1016/S0140-6736(20)31305-2
98. Rapkiewicz AV, Mai X, Carsons SE, Pittaluga S, Kleiner DE, Berger JS, et al. Megakaryocytes and platelet-fibrin thrombi characterize multi-organ thrombosis at autopsy in COVID-19: a case series. *EClinicalMedicine.* (2020) 24:100434. doi: 10.1016/j.eclinm.2020.100434
99. Grosse C, Grosse A, Salzer HJF, Dünser MW, Motz R, Langer R. Analysis of cardiopulmonary findings in COVID-19 fatalities: high incidence of pulmonary artery thrombi and acute suppurative bronchopneumonia. *Cardiovasc Pathol.* (2020) 49:107263. doi: 10.1016/j.carpath.2020.107263
100. Hanley B, Naresh KN, Roufosse C, Nicholson AG, Weir J, Cooke GS, et al. Histopathological findings and viral tropism in UK patients with severe fatal COVID-19: a post-mortem study. *Lancet Microbe.* (2020) 1:e245–53. doi: 10.1016/S2666-5247(20)30115-4
101. Oprinca GC, Muja LA. Postmortem examination of three SARS-CoV-2-positive autopsies including histopathologic and

immunohistochemical analysis. *Int J Legal Med.* (2021) 135:329–39. doi: 10.1007/s00414-020-02406-w
102. Escher F, Pietsch H, Aleshcheva G, Bock T, Baumeier C, Elsaesser A, et al. Detection of viral SARS-CoV-2 genomes and histopathological changes in endomyocardial biopsies. *ESC Heart Fail.* (2020) 7:2440–7. doi: 10.1002/ehf2.12805
103. Kawakami R, Sakamoto A, Kawai K, Gianatti A, Pellegrini D, Nasr A, et al. Pathological evidence for SARS-CoV-2 as a cause of myocarditis: JACC review topic of the week. *J Am Coll Cardiol.* (2021) 77:314–25. doi: 10.1016/j.jacc.2020.11.031
104. Haslbauer JD, Tzankov A, Mertz KD, Schwab N, Nienhold R, Twerenbold R, et al. Characterisation of cardiac pathology in 23 autopsies of lethal COVID-19. *J Pathol Clin Res.* (2021) 7:326–37. doi: 10.1002/cjp2.212
105. Bearse M, Hung YP, Krauson AJ, Bonanno L, Boyraz B, Harris CK, et al. Factors associated with myocardial SARS-CoV-2 infection, myocarditis, and cardiac inflammation in patients with COVID-19. *Mod Pathol.* (2021) 34:1345–57. doi: 10.1038/s41379-021-00790-1
106. Fox SE, Falgout L, Vander Heide RS. COVID-19 myocarditis: quantitative analysis of the inflammatory infiltrate and a proposed mechanism. *Cardiovasc Pathol.* (2021) 107361. doi: 10.1016/j.carpath.2021.107361. [Epub ahead of print].
107. Dolhnikoff M, Ferreira Ferranti J, de Almeida Monteiro RA, Duarte-Neto AN, Soares Gomes-Gouvêa M, Viu Degaspare N, et al. SARS-CoV-2 in cardiac tissue of a child with COVID-19-related multisystem inflammatory syndrome. *Lancet Child Adolesc Health.* (2020) 4:790–4. doi: 10.1016/S2352-4642(20)30257-1
108. Halushka MK, Vander Heide RS. Myocarditis is rare in COVID-19 autopsies: cardiovascular findings across 277 postmortem examinations. *Cardiovasc Pathol.* (2021) 50:107300. doi: 10.1016/j.carpath.2020.107300
109. Azzawi M, Hasleton PS, Kan SW, Hillier VF, Quigley A, Hutchinson IV. Distribution of myocardial macrophages in the normal human heart. *J Anat.* (1997) 191:417–23. doi: 10.1046/j.1469-7580.1997.19130417.x
110. Rossi MA, Celes MR, Prado CM, Saggioro FP. Myocardial structural changes in long-term human severe sepsis/septic shock may be responsible for cardiac dysfunction. *Shock.* (2007) 27:10–8. doi: 10.1097/01.shk.0000235141.05528.47
111. Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A, et al. COVID-19 and cardiovascular disease. *Circulation.* (2020) 141:1648–655. doi: 10.1161/CIRCULATIONAHA.120.046941
112. Zhou R. Does SARS-CoV-2 cause viral myocarditis in COVID-19 patients? *Eur Heart J.* (2020) 41:2123. doi: 10.1093/eurheartj/ehaa392
113. Peretto G, Sala S, Caforio ALP. Acute myocardial injury, MINOCA, or myocarditis? Improving characterization of coronavirus-associated myocardial involvement. *Eur Heart J.* (2020) 41:2124–5. doi: 10.1093/eurheartj/ehaa396
114. Marshall M, Ferguson ID, Lewis P, Jaggi P, Gagliardo C, Collins JS, et al. Symptomatic acute myocarditis in seven adolescents following Pfizer-BioNTech COVID- 19 vaccination. *Pediatrics.* (2021) e2021052478. doi: 10.1542/peds.2021-052478. [Epub ahead of print].
115. Mansour J, Short RG, Bhalla S, Woodard PK, Verma A, Robinson X, et al. Acute myocarditis after a second dose of the mRNA COVID-19 vaccine: a report of two cases. *Clin Imaging.* (2021) 78:247–9. doi: 10.1016/j.clinimag.2021.06.019
116. Muthukumar A, Narasimhan M, Li QZ, Mahimainathan L, Hitto I, Fuda F, et al. In depth evaluation of a case of presumed myocarditis following the second dose of COVID-19 mRNA vaccine. *Circulation.* (2021). doi: 10.1161/CIRCULATIONAHA.121.056038. [Epub ahead of print].
117. Larson K, Ammirati E, Adler ED, Cooper LT, Hong KN, Saponara G, et al. Myocarditis after BNT162b2 and mRNA-1273 vaccination. *Circulation.* (2021). doi: 10.1161/CIRCULATIONAHA.121.055913. [Epub ahead of print].
118. Rosner CM, Genovese L, Tehrani BN, Atkins M, Bakhshi H, Chaudhri S, et al. Myocarditis temporally associated with COVID-19 vaccination. *Circulation.* (2021). doi: 10.1161/CIRCULATIONAHA.121.055891. [Epub ahead of print].
119. Albert E, Aurigemma G, Saucedo J, Gerson DS. Myocarditis following COVID-19 vaccination. *Radiol Case Rep.* (2021) 16:2142–5. doi: 10.1016/j.radcr.2021.05.033
120. Abu Mouch S, Roguin A, Hellou E, Ishai A, Shoshan U, Mahamid L, et al. Myocarditis following COVID-19 mRNA vaccination. *Vaccine.* (2021) 39:3790–3. doi: 10.1016/j.vaccine.2021.05.087
121. Cines DB, Bussel JB. SARS-CoV-2 vaccine-induced immune thrombotic thrombocytopenia. *N Engl J Med.* (2021) 384:2254–6. doi: 10.1056/NEJMe2106315
122. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. *N Engl J Med.* (2021) 384:2092–101. doi: 10.1056/NEJMoa2104840
123. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination. *N Engl J Med.* (2021) 384:2124–30. doi: 10.1056/NEJMoa2104882
124. Muir KL, Kallam A, Koepsell SA, Gundabolu K. Thrombotic thrombocytopenia after Ad26.COV2.S vaccination. *N Engl J Med.* (2021) 384:1964–5. doi: 10.1056/NEJMc2105869
125. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. *JAMA.* (2021) 325:2448–56. doi: 10.1001/jama.2021.7517
126. American Heart Association/American Stroke Association Stroke Council Leadership. Diagnosis and management of cerebral venous sinus thrombosis with vaccine-induced thrombotic thrombocytopenia. *Stroke.* (2021) 57:2478–82. doi: 10.1161/STROKEAHA.121.035564
127. Bikdeli B, Chatterjee S, Arora S, Monreal M, Jimenez D, Krumholz HM, et al. Cerebral venous sinus thrombosis in the US population, after adenovirus-based SARS-CoV-2 vaccination, and After COVID-19. *J Am Coll Cardiol.* (2021) 78:408–11. doi: 10.1016/j.jacc.2021.06.001. [Epub ahead of print].
128. Alvarez-Garcia J, Jaladanki S, Rivas-Lasarte M, Cagliostro M, Gupta A, Joshi A, et al. New heart failure diagnoses among patients hospitalized for COVID-19. *J Am Coll Cardiol.* (2021) 77:2260–2. doi: 10.1016/j.jacc.2021.03.006
129. Szekely Y, Lichter Y, Taieb P, Banai A, Hochstadt A, Merdler I, et al. Spectrum of cardiac manifestations in COVID-19: a systematic echocardiographic study. *Circulation.* (2020) 142:342–53. doi: 10.1161/CIRCULATIONAHA.120.047971
130. Mahmoud-Elsayed HM, Moody WE, Bradlow WM, Khan-Kheil AM, Senior J, Hudsmith LE, et al. Echocardiographic findings in patients with COVID-19 pneumonia. *Can J Cardiol.* (2020) 36:1203–7. doi: 10.1016/j.cjca.2020.05.030
131. Schott JP, Mertens AN, Bloomingdale R, O'Connell TF, Gallagher MJ, Dixon S, et al. Transthoracic echocardiographic findings in patients admitted with SARS-CoV-2 infection. *Echocardiography.* (2020) 37:1551–6. doi: 10.1111/echo.14835
132. Goerlich E, Metkus TS, Gilotra NA, Wu KC, Cingolani OH, Hays AG. Prevalence and clinical correlates of echo-estimated right and left heart filling pressures in hospitalized patients with coronavirus disease 2019. *Crit Care Explor.* (2020) 2:e0227. doi: 10.1097/CCE.0000000000000227
133. Hadzibegovic S, Lena A, Churchill TW, Ho JE, Potthoff S, Denecke C, et al. Heart Failure with preserved ejection fraction according to the HFA-PEFF score in COVID-19 patients: clinical correlates and echocardiographic findings. *Eur J Heart Fail.* (2021). doi: 10.1002/ejhf.2210. [Epub ahead of print].
134. Lassen MCH, Skaarup KG, Lind JN, Alhakak AS, Sengeløv M, Nielsen AB, et al. Echocardiographic abnormalities and predictors of mortality in hospitalized COVID-19 patients: the ECHOVID-19 study. *ESC Heart Fail.* (2020) 7:4189–97. doi: 10.1002/ehf2.13044
135. Goerlich E, Gilotra NA, Minhas AS, Bavaro N, Hays AG, Cingolani OH. Prominent longitudinal strain reduction of basal left ventricular segments in patients with coronavirus disease-19. *J Card Fail.* (2021) 27:100–4. doi: 10.1016/j.cardfail.2020.09.469
136. Stöbe S, Richter S, Seige M, Stehr S, Laufs U, Hagendorff A. Echocardiographic characteristics of patients with SARS-CoV-2 infection. *Clin Res Cardiol.* (2020) 109:1549–66. doi: 10.1007/s00392-020-01727-5
137. Baycan OF, Barman HA, Atici A, Tatlisu A, Bolen F, Ergen P, et al. Evaluation of biventricular function in patients with COVID-19 using

speckle tracking echocardiography. *Int J Cardiovasc Imaging*. (2021) 37:135–44. doi: 10.1007/s10554-020-01968-5
138. Li Y, Li H, Zhu S, Xie Y, Wang B, He L, et al. Prognostic value of right ventricular longitudinal strain in patients with COVID-19. *JACC Cardiovasc Imaging*. (2020) 13:2287–99. doi: 10.1016/j.jcmg.2020.04.014
139. Huang C, Huang L, Wang Y, Li X, Ren L, Gu X, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet Lond Engl*. (2021) 397:220–32. doi: 10.1016/S0140-6736(20)32656-8
140. Townsend L, Dyer AH, Jones K, Dunne J, Mooney A, Gaffney F, et al. Persistent fatigue following SARS-CoV-2 infection is common and independent of severity of initial infection. *PLoS ONE*. (2020) 15:e0240784. doi: 10.1371/journal.pone.0240784
141. van den Borst B, Peters JB, Brink M, Schoon Y, Bleeker-Rovers CP, Schers H, et al. Comprehensive health assessment three months after recovery from acute COVID-19. *Clin Infect Dis Off Publ Infect Dis Soc Am*. (2020) ciaa1750. doi: 10.1093/cid/ciaa1750. [Epub ahead of print].
142. Puntmann VO, Carerj ML, Wieters I, Fahim M, Arendt C, Hoffmann J, et al. Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19). *JAMA Cardiol*. (2020) 5:1265–73. doi: 10.1001/jamacardio.2020.3557
143. Rajpal S, Tong MS, Borchers J, Zareba KM, Obarski TP, Simonetti OP, et al. Cardiovascular magnetic resonance findings in competitive athletes recovering from COVID-19 infection. *JAMA Cardiol*. (2021) 6:116. doi: 10.1001/jamacardio.2020.4916
144. Kotecha T, Knight DS, Razvi Y, Kumar K, Vimalesvaran K, Thornton G. Patterns of myocardial injury in recovered troponin-positive COVID-19 patients assessed by cardiovascular magnetic resonance. *Eur Heart J*. (2021).
145. Daniels CJ, Rajpal S, Greenshields JT, Rosenthal GL, Chung EH, Terrin M, et al. Prevalence of clinical and subclinical myocarditis in competitive athletes with recent SARS-CoV-2 infection: results from the big ten COVID-19 cardiac registry. *JAMA Cardiol*. (2021) e212065. doi: 10.1001/jamacardio.2021.2065. [Epub ahead of print].
146. Brito D, Meester S, Yanamala N, Patel HB, Balcik BJ, Casaclang-Verzosa G, et al. High prevalence of pericardial involvement in college student athletes recovering from COVID-19. *JACC Cardiovasc Imaging*. (2021) 14:541–55. doi: 10.1016/j.jcmg.2020.10.023
147. Özer S, Candan L, Özyildiz AG, Turan OE. Evaluation of left ventricular global functions with speckle tracking echocardiography in patients recovered from COVID-19. *Int J Cardiovasc Imaging*. (2021) 37:2227–33. doi: 10.1007/s10554-021-02211-5
148. Weckbach LT, Curta A, Bieber S, Kraechan A, Brado J, Hellmuth JC, et al. Myocardial inflammation and dysfunction in COVID-19-associated myocardial injury. *Circ Cardiovasc Imaging*. (2021) 14:e012220. doi: 10.1161/CIRCIMAGING.120.011713

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 12 August 2021
doi: 10.3389/fcvm.2021.686328

Myoglobin Offers Higher Accuracy Than Other Cardiac-Specific Biomarkers for the Prognosis of COVID-19

*Jia-Sheng Yu†, Ru-Dong Chen†, Ling-Cheng Zeng, Hong-Kuan Yang and Hua Li**

Department of Neurosurgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Although sporadic studies have shown that myoglobin may have better prognostic performance than other cardiac markers in COVID-19, a comprehensive comparative study is lacking. Herein, we retrospectively analyzed the clinical and laboratory data of COVID-19 patients admitted to the Guanggu Campus of Wuhan Tongji Hospital from February 9, 2020 to March 30, 2020, intending to compare the prognostic accuracy of three commonly used cardiac markers on COVID-19 mortality. Our results revealed that abnormal increases in myocardial biomarkers were associated with a significantly increased risk of in-hospital mortality with COVID-19. Interestingly, myoglobin, a non-cardiac-specific biomarker, also expressed in skeletal myocytes, had even higher prognostic accuracy than cardiac-specific biomarkers such as high-sensitivity troponin I (hs-TnI) and creatine kinase-MB (CK-MB). More importantly, multivariate Cox analysis showed that myoglobin, rather than hs-TnI or CK-MB, was independently prognostic for in-hospital mortality in COVID-19. These results were further confirmed by subgroup analyses of patients with severe and critical illnesses and those without a history of cardiovascular disease. Our findings suggest that myoglobin may be a reliable marker of illness reflecting general physiological disturbance and help to assess prognosis and treatment response in patients with COVID-19.

Keywords: COVID-19, myoglobin, myocardial biomarkers, in-hospital mortality, rhabdomyolysis

OPEN ACCESS

Edited by:
Shuyang Zhang, Peking Union Medical College Hospital (CAMS), China

Reviewed by:
Luis Alberto Gonano, Universidad Nacional de La Plata, Argentina
Paulo M. Dourado, University of São Paulo, Brazil

***Correspondence:**
Hua Li
huali@hust.edu.cn

†These authors have contributed equally to this work

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *26 March 2021*
Accepted: *19 July 2021*
Published: *12 August 2021*

Citation:
Yu J-S, Chen R-D, Zeng L-C, Yang H-K and Li H (2021) Myoglobin Offers Higher Accuracy Than Other Cardiac-Specific Biomarkers for the Prognosis of COVID-19. Front. Cardiovasc. Med. 8:686328. doi: 10.3389/fcvm.2021.686328

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a global health concern (1). Despite the advent of vaccines that have slowed the spread of the virus, COVID-19 may likely become an endemic disease that will coexist with humans for a long time. Patients with COVID-19 may deteriorate to multi-organ dysfunction including myocardial failure (2–5). Increases in troponin and creatine kinase-MB (CK-MB), two of the most sensitive indicators of myocardial injury, have been demonstrated to be associated with a more severe clinical course and to correlate with poorer outcomes in COVID-19 (6–9). However, in our previous study and studies by other investigators, multifactorial analysis combining most clinical indicators showed that hypersensitive troponin I (hs-TnI) and CK-MB were not independent predictors of

mortality in COVID-19. In contrast, myoglobin (MYO), a non-cardiac-specific indicator also expressed in skeletal muscle cells, was independent prognostic (10–15). The reasons underlying this curious phenomenon were not fully understood, but a comprehensive comparison of the predictive capabilities of myocardial biomarkers can help clarify the mechanisms involved and may contribute to our understanding of the significance of cardiac injury in COVID-19. This study retrospectively analyzed the clinical and laboratory data of patients with COVID-19 admitted to the Guanggu Campus of Wuhan Tongji Hospital between February 9, 2020 and March 30, 2020, to compare the prognostic accuracy of three commonly used cardiac markers for in-hospital mortality of COVID-19. Exploring their prognostic roles is of great significance for identifying useful biomarkers for early diagnosis, prognosis, and therapeutic response assessment in clinical practice.

METHODS

Study Design and Participants

This study was a retrospective study conducted in the Guanggu Campus of Tongji hospital (Wuhan), Tongji Medical College, Huazhong University of Science and Technology, which was a designated hospital for patients with COVID-19 during the outbreak of novel coronavirus disease in Wuhan, China. The diagnosis of COVID-19 was confirmed by RNA testing of the SARS-CoV-2 in the on-site clinical laboratory according to the WHO interim guidance. 1,284 patients with laboratory-confirmed COVID-19 between February 9, 2020 and March 30, 2020 were initially enrolled. Patients aged < 18 years or lacking cardiac biomarker results (e.g., MYO, CK-MB, and hs-TnI) were excluded. Ultimately, 1,229 patients were enrolled in this study. The study was approved by the ethics committee of Tongji hospital, Huazhong University of Science and Technology (TJ-IRB20210317). Written informed consent was waived due to the use of de-identified retrospective data.

Data Collection and Follow-Up

All clinical, laboratory and outcome data were available and were collected according to the electronic medical records using a standardized data collection form. Data collection for early laboratory results was defined using the first examination at admission (within 24 h of admission). Data collection for the late-stage laboratory results was defined using the last assessment during hospitalization. All data were verified by two physicians blinded to the patient's identity.

Demographic characteristics, medical history, and physical examination findings at admission, and early and late laboratory results during hospitalization were collected. Demographic characteristics obtained for the study included age and gender. Medical history included hypertension (HP), diabetes (DM), chronic liver disease (CLD), coronary heart disease (CHD), cerebrovascular disease, chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), and cancer. Physical examination findings included first body temperature, respiratory rate, pulse rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), and saturation of pulse oxygen (SpO_2). Laboratory findings included hs-TnI, CK-MB, MYO, neutrophil (NEU), lymphocyte (LYM), high sensitivity C-reactive protein (hs-CRP), interleukin 6 (IL-6), D-dimer, fibrinogen (FIB), alanine aminotransferase (ALT), albumin (ALB), creatinine (Cr), estimated glomerular filtration rate (EGFR), and glucose (GLU) levels. All clinical samples were tested in the same laboratory using the same standard.

All patients were followed up from admission to discharge to observe the risks of in-hospital mortality. Survival time was defined as the time from hospital admission to discharge or death. The follow-up data were obtained from reviewing medical records by trained researchers using a double-blind method.

Myocardial Injury Biomarkers

In this study, we focused on three commonly used biomarkers of myocardial injury (hs-TnI, CK-MB, and MYO). At our institution, these three biomarkers have been integrated as a test package into the laboratory test panel for COVID-19 patients. Serum levels of hs-TnI, CK-MB, and MYO were measured by chemiluminescence microparticle immunoassays on the Architect i2000SR platform (Abbott Laboratories, Chicago, IL) according to manufactures' instructions. The values were considered elevated if they were above the upper limit of normal (ULN) levels, which were defined as the 99th upper percentile of the biomarker distribution in the normal population. According to our laboratory normal ranges, the ULN levels of hs-TnI, CK-MB, and MYO were 34.2 pg/ml, 5.2 ng/ml, and 154.9 ng/ml, respectively, in male patients, and were 15.6 pg/ml, 3.1 ng/ml, and 106 ng/ml in female patients.

Clinical Classifications

According to the Guidance for Corona Virus Disease 2019: Prevention, Control, Diagnosis, and Management edited by the National Health Commission of the People's Republic of China (16), all cases were identified into four categories of mild cases, ordinary cases, severe cases, and critical cases based on the clinical conditions at the time of admission. (1) Mild cases: the clinical symptoms are mild, and no pneumonia manifestation can be found in imaging. (2) Ordinary cases: patients have symptoms like fever and respiratory tract symptoms, and pneumonia manifestation can be seen in imaging. (3) Severe cases: meeting any of the following: respiratory distress, RR $\geq$ 30 breaths/min; the oxygen saturation is $<$93% at a rest state; arterial partial pressure of oxygen (PaO_2)/oxygen concentration (FiO_2) $\leq$ 300 mmHg (1 mmHg = 0.133 kPa). Patients with $>$50% lesions progression within 24 to 48 h in pulmonary imaging were treated as severe cases. (4) Critical cases: meeting any of the following: respiratory failure occurs, mechanical ventilation is needed; shock occurs; or complicated with other organ failures that require monitoring and treatment in the intensive care unit.

Statistical Analysis

Continuous variables were presented as mean $\pm$ standard deviation or median (inter-quartile range, IQR), as appropriate. Categorical variables were presented as *n* (%). Event frequencies were compared by chi-square test. Differences in cardiac biomarker levels between the early- and late-stage groups

TABLE 1 | Baseline characteristics of the overall study population and patients stratified based on mortality.

Characteristics	Total (n = 1229)	Alive (n = 1163)	Died (n = 66)	P-value
Age (yrs.), median (IQR)	62 (51-70)	61 (50-69)	71 (67-81)	**<0.001**
Male, *n* (%)	588 (47.8)	541 (46.5)	47 (71.2)	**<0.001**
Comorbidities, *n* (%)				
History of HP-*n* (%)	433 (35.2)	404 (34.7)	29 (43.9)	0.128
History of DM-*n* (%)	207 (16.8)	193 (16.6)	14 (21.2)	0.330
Chronic liver disease-*n* (%)	20 (1.6)	19 (1.6)	1 (1.5)	0.941
History of CHD-*n* (%)	95 (7.7)	88 (7.6)	7 (10.6)	0.368
Stroke history-*n* (%)	49 (4.0)	42 (3.6)	7 (10.6)	**0.005**
Chronic kidney disease-*n* (%)	31 (2.5)	25 (2.1)	6 (9.1)	**<0.001**
History of COPD-*n* (%)	10 (0.8)	9 (0.8)	1 (1.5)	0.514
Cancer-*n* (%)	41 (3.3)	37 (3.2)	4 (6.1)	0.205
Physical examination on admission, median (IQR)				
Temperature (°C)	36.5 (36.2-36.9)	36.5 (36.2-36.9)	36.6 (36.4-37.1)	**0.021**
Pulse (/min)	89 (80-100)	89 (80-100)	92 (83-102)	0.275
Respire (/min)	20 (19-22)	20 (19-22)	21 (20-30)	**0.001**
SBP (mmHg)	132 (120-145)	132 (120-145)	130 (117-144)	0.336
DBP (mmHg)	80 (72-90)	80 (73-90)	78 (70-87)	0.106
SpO_2 (%)	97 (95-98)	97 (95-98)	92 (88-97)	**<0.001**
Hospital stay-days, median (IQR)	21 (14-32)	21 (14-33)	14 (9-21)	**<0.001**

p-values were calculated between the alive and died groups by Mann-Whitney U-test or chi-square test, as appropriate. IQR, interquartile range; HP, hypertension; DM, diabetes; CHD, coronary heart disease; COPD, chronic obstructive pulmonary disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; SpO_2, percutaneous oxygen saturation. The bold p values mean that they are of significance in statistics.

were compared using the Wilcoxon signed-ranks test (two-tailed). Other comparisons between the two groups were made using the independent samples t-test (normally distributed continuous variables) or the Mann-Whitney U-test (non-normally distributed continuous variables).

To better understand the overall performance of myocardial biomarkers, we performed both standard receiver-operating characteristic (ROC) analysis and time-dependent ROC curve analysis (17, 18). The area under the ROC curve (AUC) was calculated to evaluate the performance of each biomarker. The optimal cut-off point was assessed by standard ROC analysis and determined using the maximization of the Youden's index. Time-dependent AUC curves with 95% confidence intervals (CIs) were performed to compare the prognostic accuracy of cardiac biomarkers.

Cumulative survival curves for in-hospital death were assessed using Kaplan-Meier product-limit estimation with log-rank tests. Univariate Cox proportional hazards regression and mixed-effects Cox models were used to calculate hazard ratios (HRs) for risk factors.

Multivariate Cox proportional hazards model analysis was performed to identify independent prognostic factors associated with in-hospital mortality in patients with COVID-19. Considering the possible collinearity issues, the least absolute shrinkage and selection operator (LASSO) regression was previously performed to screen potential prognostic factors (19). Variables with non-zero coefficients in the LASSO regression were selected for further proportional hazard assumption (Schoenfeld test) to assess the applicability of the variables to the multivariate Cox model (20). If the proportional hazard assumption was not violated ($p > 0.05$), the multivariate Cox proportional hazard model was then performed. Only variables with a p-value < 0.05 in the multivariate analysis were considered as independent prognostic factors. The predictive performance of the multivariate Cox model was evaluated by Harrell's concordance index (C-index).

The cases with missing biomarker data were excluded listwise with statistics software. Statistical analyses were performed using SPSS 22.0 (SPSS, Chicago, IL, USA) and R software version 3.6.3 (http://www.r-project.org). p-value < 0.05 was considered statistically significant.

RESULTS

Baseline Characteristics and Laboratory Results of Patients Stratified by Mortality

Of the 1,229 eligible patients, 66 (5.4%) died during follow-up. **Table 1** presents the basic clinical characteristics and physical examination findings for the overall study population and for patients stratified by mortality. Patients who died were older, more likely to be male, and more likely to have a history of stroke and chronic kidney disease than those who were alive. Besides, they had lower SpO_2 and DBP levels and higher body temperature and respire rate levels at admission (**Table 1**).

Table 2 shows the levels of early and late laboratory parameters in the overall study population, survivors, and non-survivors. Non-survivors had lower LYM, ALB, and EGFR and

TABLE 2 | Laboratory findings at the early and late stages of hospitalization in overall study patients and patients stratified based on mortality.

Characteristics	N	Total	Alive	Died	*P*-value
Laboratory results at early stage, median (IQR)					
Hs-TnI (pg/ml)	1,229	3.1 (1.9-8.0)	2.8 (1.9-7.4)	25.8 (8.0-223.6)	**<0.001**
CK-MB (ng/ml)	1,229	0.7 (0.5-1.2)	0.7 (0.4-1.1)	2.4 (1.0-4.4)	**<0.001**
MYO (ng/ml)	1,229	35.6 (26.1-59.6)	34.5 (25.6-53.9)	169.2 (95.7-368.5)	**<0.001**
NEU (10∧9/L)	1,229	3.68 (2.67-5.17)	3.59 (2.63-4.97)	7.50 (4.91-11.87)	**<0.001**
LYM (10∧9/L)	1,229	1.34 (0.94-1.79)	1.38 (1.00-1.82)	0.60 (0.43-0.88)	**<0.001**
Hs-CRP (mg/L)	1,228	5.2 (1.2-37.1)	4.1 (1.1-31.1)	89.1 (44.9-144.5)	**<0.001**
IL6 (pg/ml)	1,095	3.76 (1.76-11.92)	3.50 (1.66-9.66)	58.95 (24.55-167.55)	**<0.001**
D-dimer (μg/ml FEU)	1,214	0.56 (0.24-1.36)	0.51 (0.23-1.14)	5.61 (1.71-21.00)	**<0.001**
FIB (g/L)	1,221	4.06 (3.20-5.41)	4.02 (3.20-5.35)	4.79 (2.94-6.09)	0.081
ALT (U/L)	1,229	21.0 (13.0-36.0)	20.0 (13.0-35.0)	26.0 (17.8-44.5)	**0.004**
ALB (g/L)	1,229	37.4 (33.3-41.5)	37.6 (33.8-41.7)	31.3 (27.8-34.6)	**<0.001**
Cr (μmol/L)	1,229	67 (56-82)	67 (56-80)	88 (66-117)	**<0.001**
EGFR (ml/min/1.73m∧2)	1,229	93.2 (79.3-103.5)	93.7 (80.6-104.0)	67.4 (49.1-89.4)	**<0.001**
GLU (mmol/L)	1,223	5.57 (4.97-7.02)	5.51 (4.94-6.83)	7.24 (5.96-10.30)	**<0.001**
Laboratory results at late stage, median (IQR)					
Hs-TnI (pg/ml)	1,229	2.3 (1.9-5.8)	2.2 (1.9-5.0)	144.9 (31.3-544.9)	**<0.001**
CK-MB (ng/ml)	1,229	0.6 (0.4-1.0)	0.6 (0.4-0.9)	4.8 (1.9-10.2)	**<0.001**
MYO (ng/ml)	1,229	30.2 (23.5-43.5)	29.5 (23.1-39.9)	672.7 (279.9-1200.0)	**<0.001**
NEU (10∧9/L)	1,229	3.25 (2.51-4.19)	3.16 (2.49-4.03)	11.09 (6.8-17.61)	**<0.001**
LYM (10∧9/L)	1,229	1.58 (1.25-1.96)	1.61 (1.29-1.99)	0.44 (0.29-0.77)	**<0.001**
Hs-CRP (mg/L)	1,228	1.6 (0.7-5.3)	1.5 (0.6-4.2)	114.7 (72.4-198.0)	**<0.001**
IL6 (pg/ml)	1,094	3.14 (1.57-7.75)	2.96 (1.51-6.35)	377.60 (75.19-1698.50)	**<0.001**
D-dimer (μg/ml FEU)	1,215	0.40 (0.22-0.94)	0.37 (0.22-0.79)	6.68 (3.34-16.05)	**<0.001**
FIB (g/L)	1,221	3.59 (2.99-4.43)	3.57 (2.99-4.30)	4.52 (3.02-5.84)	**0.003**
ALT (U/L)	1,226	19.0 (12.0-30.0)	19.0 (12.0-30.0)	29.0 (18.0-48.0)	**<0.001**
ALB (g/L)	1,216	39.6 (36.5-42.0)	39.8 (36.8-42.1)	31.1 (28.1-34.3)	**<0.001**
Cr (μmol/L)	1,226	68 (57-81)	67 (57-80)	121 (76-166)	**<0.001**
EGFR (ml/min/1.73m∧2)	1,223	93.2 (80.6-103.0)	93.8 (82.4-103.4)	47.2 (32.2-82.6)	**<0.001**
GLU (mmol/L)	1,223	5.25 (4.79-6.29)	5.21 (4.78-6.06)	8.51 (6.32-11.32)	**<0.001**

p-values were calculated between alive and died groups by Mann-Whitney U-test. n, number; IQR, interquartile range; Hs-TnI, high sensitivity troponin-I; CK-MB, creatine kinase-MB; MYO, myoglobin; NEU, neutrophil; LYM, lymphocytes; Hs-CRP, high sensitivity C-reactive protein; IL6, interleukin 6; FIB, fibrinogen; ALT, alanine aminotransferase; ALB, albumin; Cr, creatinine; EGFR, estimated glomerular filtration rate; GLU, glucose.
The bold p values mean that they are of significance in statistics.

higher levels of hs-TnI, CK-MB, MYO, NEU, hs-CRP, IL-6, D-dimer, ALT, Cr, and GLU in the early stages of hospitalization compared to survivors. These trends could also be found in the later levels of laboratory results (**Table 2**).

In terms of dynamic characteristics, the levels of hs-TnI, CK-MB, MYO, NEU, hs-CRP, IL-6, D-dimer, FIB, ALT, and GLU in survivors decreased significantly in the late stage of the disease. In contrast, in non-survivors, their levels increased significantly in the late stage (see **Supplementary Table 1**).

Myocardial Injury Biomarker Levels in COVID-19 Patients

Figures 1A-C demonstrate the levels of myocardial injury biomarkers. As shown, myocardial biomarker levels were significantly higher in non-survivors compared to survivors in both early and late stages. In terms of dynamic changes, myocardial biomarker levels were significantly decreased in survivors while elevated in non-survivors.

There were 18 (1.5%), 912 (74.2%), 231 (18.8%), and 68 (5.5%) patients with mild, ordinary, severe, and critical conditions, respectively. Myocardial biomarker levels in patients stratified according to clinical classification are shown in **Figures 1D-F**. Patients in the severe group had higher levels of hs-TnI and MYO in the early stages and higher levels of hs-TnI in the late stages compared to the mild/ordinary group. Patients in the critical group had significantly higher myocardial biomarker levels in both early and late stages than in the severe group.

The percentages of patients with myocardial marker levels below the cut-off (for definition, see later section), between the cut-off and ULN, and above the ULN are illustrated in **Supplementary Figures 1A,B**. The percentages of deaths in patients with myocardial marker levels below the cut-off, between

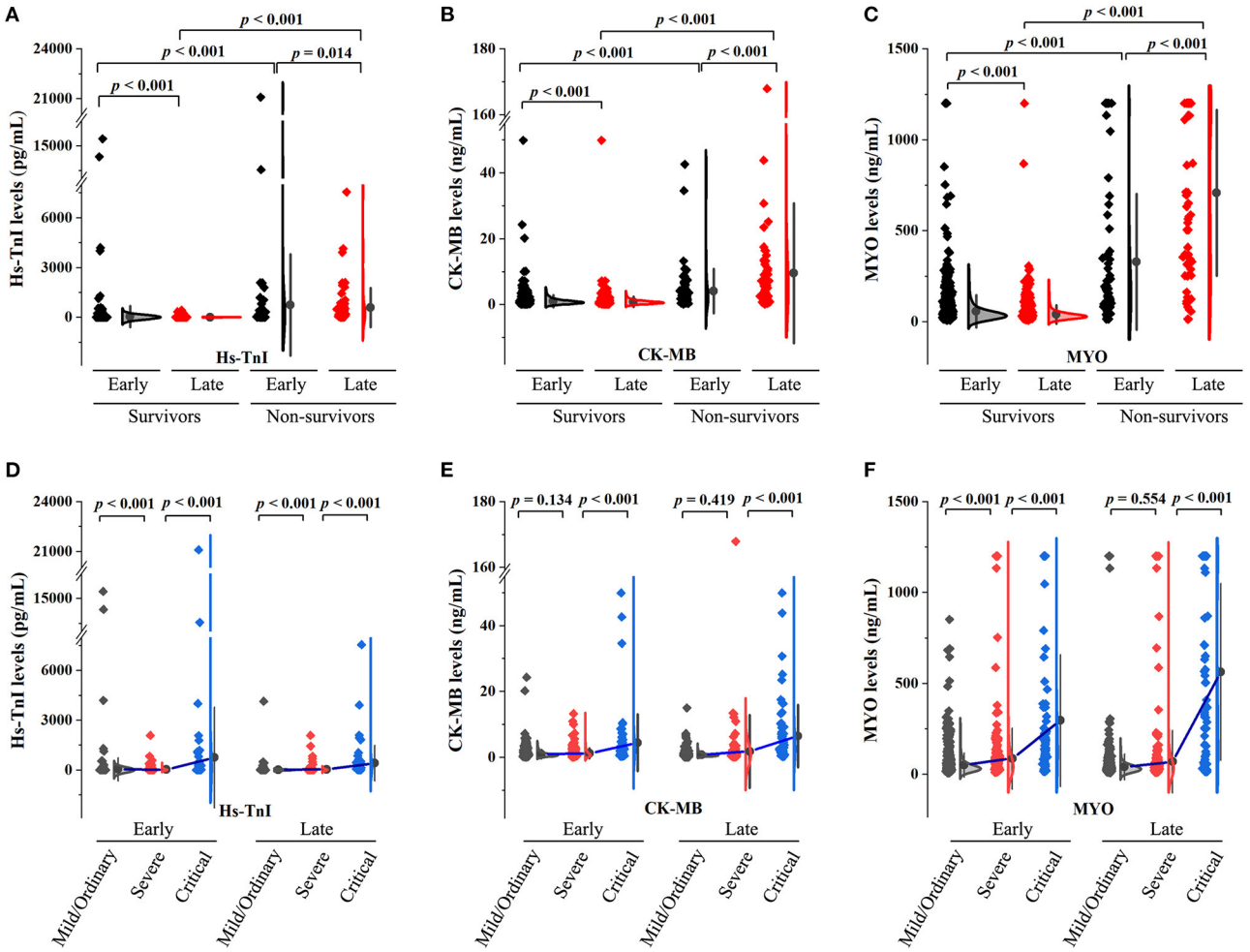


FIGURE 1 | Summary of myocardial biomarker levels in patients with COVID-19. **(A–C)** The early- and late-stage levels of myocardial biomarkers in patients stratified by mortality. **(D–F)** The early- and late-stage levels of myocardial biomarkers in patients stratified by clinical classifications.

the cut-off and ULN, and above the ULN were shown in **Supplementary Figures 1C,D**.

Supplementary Figure 2 shows the correlation matrix heat map for 16 clinical parameters in the early and late stages of the disease. MYO levels were significantly correlated with creatinine, EGFR, hs-CRP, IL-6, hs-TnI, CK-MB, and age. Hs-TnI levels were significantly correlated with ALB, LYM, EGFR, hs-CRP, IL-6, and D-dimer.

Associations of Elevated Myocardial Biomarker Levels With In-hospital Mortality of COVID-19

To evaluate the associations between in-hospital mortality of COVID-19 and covariates including myocardial injury markers, we performed Cox regression analysis where the Wald χ^2 and *p*-values were calculated. After adjusting for age, gender, and comorbidities (HP, DM, CLD, CHD, CKD, COPD, stroke, and cancer), hs-TnI, CK-MB, and MYO were significantly associated with in-hospital mortality of COVID-19. Notably, the Wald χ^2 values for MYO were relatively higher than those for hs-TnI and CK-MB (**Supplementary Table 2**).

To assess the association of each myocardial marker elevation above ULN with in-hospital mortality, Cox regression-derived hazard ratios were calculated. After adjusting for age, gender, and comorbidities, the HRs for the risks of in-hospital mortality for elevated early-stage levels of Hs-cTnI, CK-MB, and MYO were 7.47 (95% CI, 4.39-12.72, $p < 0.001$), 10.37 (95% CI, 5.87-18.30, $p < 0.001$), and 7.96 (95% CI, 4.75-13.35, $p < 0.001$). Adjusted HRs for elevated late-stage levels of Hs-cTnI, CK-MB, and MYO were 36.35 (95% CI, 20.13-65.66, $p < 0.001$), 27.37 (95% CI, 16.19-46.28, $p < 0.001$), and 77.54 (95% CI, 39.09-153.82, $p < 0.001$) (see **Supplementary Table 3**).

Kaplan-Meier curves between groups categorized by ULN are shown in **Supplementary Figure 3**. As illustrated, patients with hs-TnI, CK-MB, and MYO levels above the ULN had significantly decreased survival rates.

TABLE 3 | Overall performance of the myocardial biomarkers according to the standard ROC analysis.

	Early stage			Late stage		
	Hs-TnI	**CK-MB**	**MYO**	**Hs-TnI**	**CK-MB**	**MYO**
N	1,229	1,229	1,229	1,229	1,229	1,229
Mortality	66	66	66	66	66	66
AUC (95% CI)	0.84 (0.78-0.89)	0.78 (0.72-0.85)	0.89 (0.84-0.94)	0.94 (0.90-0.98)	0.92 (0.87-0.96)	0.96 (0.92-1.00)
cut-off	7.9 pg/ml	1.2 ng/ml	80.8 ng/ml	15.7 pg/ml	1.5 ng/ml	98.0 ng/ml
Sensitivity (95% CI)	0.77 (0.65-0.87)	0.71 (0.59-0.82)	0.85 (0.74-0.93)	0.89 (0.79-0.96)	0.85 (0.74-0.93)	0.91 (0.81-0.97)
Specificity (95% CI)	0.77 (0.75-0.80)	0.76 (0.74-0.79)	0.86 (0.84-0.88)	0.94 (0.93-0.96)	0.91 (0.89-0.93)	0.96 (0.95-0.97)
PPV (95% CI)	0.16 (0.14-0.27)	0.15 (0.13-0.23)	0.26 (0.23-0.43)	0.48 (0.41-0.70)	0.35 (0.31-0.54)	0.56 (0.49-0.78)
NPV (95% CI)	0.98 (0.97-0.99)	0.98 (0.96-0.98)	0.99 (0.98-0.99)	0.99 (0.99-1.00)	0.99 (0.98-0.99)	1.00 (0.99-1.00)
DLR.Positive (95% CI)	3.42 (2.89-4.05)	2.99 (2.49-3.60)	6.13 (5.14-7.31)	16.00 (12.45-20.55)	9.58 (7.76-11.83)	22.50 (16.83-30.07)
DLR.Negative (95% CI)	0.29 (0.19 - 0.46)	0.38 (0.26 - 0.55)	0.18 (0.10-0.31)	0.11 (0.06-0.23)	0.17 (0.09-0.29)	0.10 (0.04-0.20)
FP	263	277	161	65	103	47
FN	15	19	10	7	10	6

N, number; AUC, area under the ROC curves; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; DLR, diagnostic likelihood ratio; FP, false positive; FN, false negative; Hs-TnI, high sensitivity troponin-I; CK-MB, creatine kinase-MB; MYO, myoglobin.

Prognostic Performance of Myocardial Injury Biomarkers in Predicting In-hospital Mortality of COVID-19

The standard ROC curves were conducted to compare the relative accuracy, sensitivity, specificity, and positive and negative predictive value of each biomarker. The best cut-off values were calculated based on the ROC analysis. Our results show that using the cut-off values determined by the ROC curves improved prognostic accuracy over the use of ULN values.

Table 3 summarizes the overall performance of the myocardial biomarkers. When incorporating the early levels of myocardial biomarkers, the AUC was 0.89 (95% CI 0.84-0.94) for MYO, 0.84 (95% CI 0.78-0.89) for hs-TnI, and 0.78 (95% CI 0.72-0.85) for CK-MB. The optimal cut-offs were 80.8 ng/ml for MYO with a sensitivity of 84.8% and a specificity of 86.2%, 7.9 pg/ml for hs-TnI with a sensitivity of 77.3% and a specificity of 77.4%, and 1.2 ng/ml for CK-MB with a sensitivity of 71.2% and a specificity of 76.2% (**Table 3**).

When incorporating the late levels of myocardial biomarkers for ROC analysis, the AUCs were 0.96 (95% CI 0.92-1.00) for MYO, 0.94 (95% CI 0.90-0.98) for hs-TnI, and 0.92 (95% CI 0.87-0.96) for CK-MB, respectively. The optimal cut-off point was 98.0 ng/ml for MYO with a sensitivity of 90.9% and a specificity of 96.0%, 15.7 pg/ml for hs-TnI with a sensitivity of 89.4% and a specificity of 94.4%, and 1.5 ng/ml for CK-MB with a sensitivity of 84.8% and a specificity of 91.1% (**Table 3**).

Time-dependent ROC curve analysis was performed based on early levels of myocardial biomarkers to better understand the performance of each myocardial biomarker (see **Figure 2**). The AUC values for predicting 14-day survival for MYO, hs-TnI, and CK-MB were 0.88, 0.82, and 0.84, respectively (**Figure 2D**). For predicting 28-day survival, the AUC values for MYO, hs-TnI, and CK-MB were 0.84, 0.77, and 0.77, respectively (**Figure 2E**). The time-dependent AUC curves for myocardial markers are shown in **Figure 2F**. As shown, the AUC values of MYO were superior to those of hs-TnI and CK-MB throughout the follow-up period (**Figure 2F**).

Patients with biomarker levels above the newly established cut-off value had a significantly higher risk of in-hospital mortality than patients with biomarker levels below the cut-off value. After adjusting for confounders including age, sex, comorbidities (HP, DM, CLD, CHD, CKD, COPD, stroke, and cancer), and disease severity indicators (hs-CRP, D-dimer, NEU) in a multivariate Cox model, the HRs for risk of mortality with increased early levels of hs-TnI, CK-MB, and MYO were 2.31 (95% CI, 1.20-4.48, $p = 0.013$), 1.82 (95% CI, 1.01-3.31, $p = 0.048$), and 8.31 (95% CI, 3.94-17.52, $p < 0.001$), respectively. Adjusted HRs for late levels of hs-TnI, CK-MB, and MYO were 18.19 (95% CI, 7.87-42.05, $p < 0.001$), 8.22 (95% CI, 3.81-17.71, $p < 0.001$), and 34.41 (95% CI, 14.15-83.68, $p < 0.001$), respectively (**Table 4**).

Kaplan-Meier curves showed an early separation of mortality curves for patients with biomarker levels above the ULN and patients with biomarker levels between the cut-off and the ULN, indicating a significantly increased risk of death in these patients (**Figure 3**).

Overall, these results suggest that all three commonly used cardiac biomarkers are significantly associated with in-hospital mortality. MYO provided better prognostic performance than hs-TnI and CK-MB in predicting mortality of COVID-19.

MYO, Rather Than hs-TnI or CK-MB, Was an Independent Prognostic Factor Associated With In-hospital Mortality of COVID-19

To identify independent prognostic factors associated with COVID-19 mortality, we further developed multivariate Cox models. Potential confounders including age, sex, comorbidities

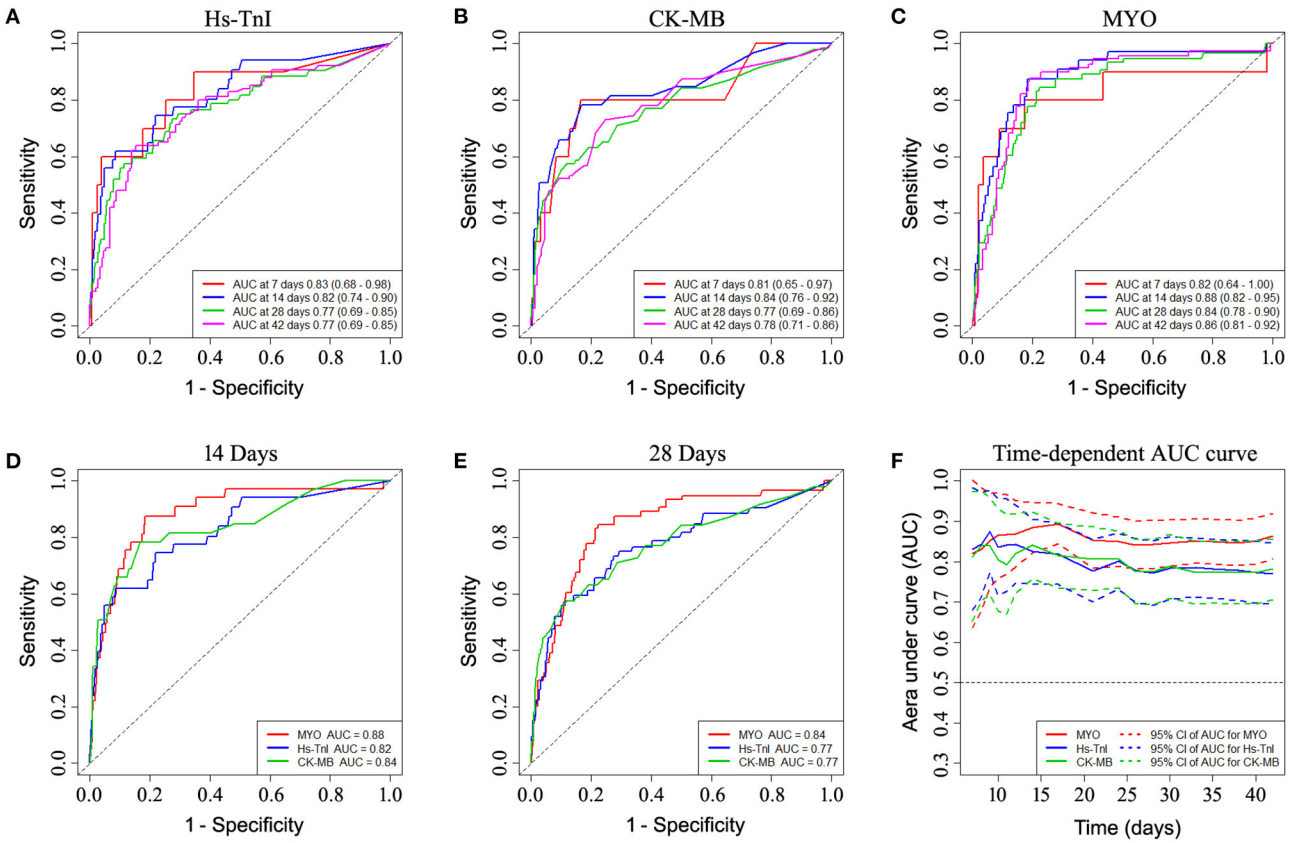


FIGURE 2 | Time-dependent ROC curve analysis based on the early-stage levels of myocardial biomarkers showing the prognostic performance of myocardial biomarkers. **(A–C)** ROC performance of myocardial biomarkers at different times during follow-up. **(D,E)** Comparison of the prognostic performance of each biomarker in predicting 14-day and 28-day mortality. **(F)** Time-dependent AUC curves of myocardial markers showed superior AUC values for myoglobin over hs-TnI and CK-MB throughout the follow-up period. ROC, receiver operating characteristic curve; AUC, area under curve.

(HP, DM, CLD, CHD, CKD, COPD, stroke, and cancer), physical examination on admission (temperature, respiratory rate, pulse, SBP, DBP, and SpO_2), and laboratory parameters (hs-TnI, CK-MB, MYO, NEU, LYM, hs-CRP, IL-6, D-dimer, FIB, ALT, ALB, Cr, EGFR, and GLU) were controlled for. LASSO regression was previously performed to address possible collinearity issues.

When incorporating variables including age, sex, comorbidities, physical examinations, and early-stage laboratory results in the LASSO regression, five variables (MYO, NEU, hs-CRP, IL-6, and D-dimer) with non-zero coefficients were identified (see **Figure 4A**). They all had p-values < 0.05 in the multivariate model, suggesting an independent prognostic effect of early levels of MYO, NEU, hs-CRP, IL-6, and D-dimer on COVID-19 mortality (see **Figure 4B**).

Three variables (MYO, hs-CRP, and D-dimer) were identified when incorporating variables including age, sex, comorbidities, physical examinations, and late-stage laboratory results in the LASSO regression (**Figure 4C**). They were all found to have p-values < 0.05, indicating that late levels of MYO, hs-CRP, and D-dimer had an independent prognostic effect on mortality in COVID-19 (see **Figure 4D**).

Subgroup Analyses Confirming the Strong Prognostic Ability of MYO for In-hospital Mortality in COVID-19

Given that COVID-19 patients with underlying cardiovascular disease are more likely to exhibit elevated myocardial marker levels than patients without cardiovascular disease (6), we performed a subgroup analysis in patients without a history of cardiovascular disease (see **Supplementary Figure 4**). The results confirmed that MYO provided better prognostic performance than hs-TnI and CK-MB and that MYO was an independent factor associated with in-hospital mortality in COVID-19. We further performed a subgroup analysis of patients with severe or critical conditions. Similarly, the results showed that MYO provided a better prognostic performance with an independent prognostic effect on in-hospital mortality (**Figure 5**).

DISCUSSION

The novel finding of this study is that among myocardial biomarkers, MYO had a higher prognostic performance for

TABLE 4 | Associations of increased myocardial marker levels above cut-offs with in-hospital mortality of COVID-19.

		Crude		Model 1		Model 2	
		HR (95% CI)	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
Early-stage levels	**Hs-TnI**						
	≤cut-off	Ref		Ref		Ref	
	>cut-off	7.85 (4.46-13.81)	<0.001	5.34 (2.89-9.88)	<0.001	2.31 (1.20-4.48)	0.013
	CK-MB						
	≤cut-off	Ref		Ref		Ref	
	>cut-off	5.98 (3.60-9.92)	<0.001	3.73 (2.18-6.37)	<0.001	1.82 (1.01-3.31)	0.048
	MYO						
	≤cut-off	Ref		Ref		Ref	
	>cut-off	23.88 (12.18-46.82)	<0.001	19.36 (9.65-38.84)	<0.001	8.31 (3.94-17.52)	<0.001
Late-stage levels	**Hs-TnI**						
	≤cut-off	Ref		Ref		Ref	
	>cut-off	67.15 (32.05-140.68)	<0.001	63.54 (28.93-139.57)	<0.001	18.19 (7.87-42.05)	<0.001
	CK-MB						
	≤cut-off	Ref		Ref		Ref	
	>cut-off	40.99 (21.91-76.69)	<0.001	31.46 (16.53-59.87)	<0.001	8.22 (3.81-17.71)	<0.001
	MYO						
	≤cut-off	Ref		Ref		Ref	
	>cut-off	116.72 (53.14-256.36)	<0.001	108.64 (47.23-249.88)	<0.001	34.41 (14.15-83.68)	<0.001

The variables were categorized into two groups according to the cut-off of each biomarker. The biomarkers were included as dichotomous variables in the univariable COX regression analysis.

Model 1: Adjusted for age, sex, and co-existing diseases (hypertension, diabetes, coronary heart disease, chronic obstructive pulmonary disease, chronic liver disease, stroke history, chronic kidney disease, and cancer history).

Model 2: Model 1 plus variables related to disease severity (neutrophil count, D-Dimer, and high sensitivity C-reactive protein).

HR, hazard ratio; CI, confidence interval; Hs-TnI, high sensitivity troponin-I; CK-MB, creatine kinase-MB; MYO, myoglobin.

in-hospital mortality than hs-TnI and CK-MB, which had been largely ignored in previous studies. MYO had the most enormous AUC value among cardiac biomarkers in the ROC analysis. More importantly, MYO, but not hs-TnI or CK-MB, was an independent factor associated with the risk of in-hospital mortality. These results are consistent with multivariate regression analyses in our and others' previous studies showing that elevated serum myoglobin concentrations were an independent predictor of in-hospital mortality in patients with COVID-19 (10, 12–15).

Comparative studies investigating the ability of myocardial biomarkers to predict COVID-19 mortality have been reported sparsely. In a multicenter retrospective study, Qin et al. evaluated the associations and prognostic power of circulating cardiac injury markers with COVID-19 outcomes. They found that increases in MYO had the highest overall performance in predicting the risk of COVID-19 mortality, followed by NT-proBNP, hs-TnI, and CK-MB (21). Herein, we focused on three commonly used cardiac biomarkers, which were tested as an integrated test package for COVID-19 patients at our institution. Analyses based on these tests could help reduce the statistical bias associated with inconsistent sampling times. In addition, we performed subgroup and dynamic analyses of early and late laboratory data and obtained consistent results confirming that MYO provides a better prognostic performance and has an independent prognostic effect on in-hospital mortality.

These findings are interesting because MYO, a non-cardiac-specific biomarker expressed also in skeletal myocytes, provides even higher prognostic accuracy than cardiac-specific biomarkers such as hs-TnI and CK-MB. The origins are unclear by far. Undeniably, elevated MYO together with hs-TnI and CK-MB is indicative of myocardial cell injury after COVID-19. Rhabdomyolysis, a potential late complication of SARS-CoV-2 infection, is another possible mechanism for the elevation of MYO. Rhabdomyolysis has been reported to be an important factor contributing to poor outcomes in COVID-19 patients (22–25). Patients with rhabdomyolysis usually present with acute renal impairment, as evidenced by elevated creatinine levels (26). Our data showed that the MYO levels in COVID-19 patients were significantly correlated with creatinine levels with a correlation coefficient of 0.46. In contrast, the correlation coefficients for hs-TnI and CK-MB with creatinine were 0.18 and 0.16, respectively. These results suggest that MYO may reflect the severity of disease in COVID-19 patients who develop rhabdomyolysis. However, given that the overall prevalence of rhabdomyolysis in COVID-19 patients is only 2.2% (25), the robust prognostic potency of MYO cannot be attributed entirely to the development of rhabdomyolysis. It is more likely that MYO may be a marker of illness reflecting general physiological disturbance including

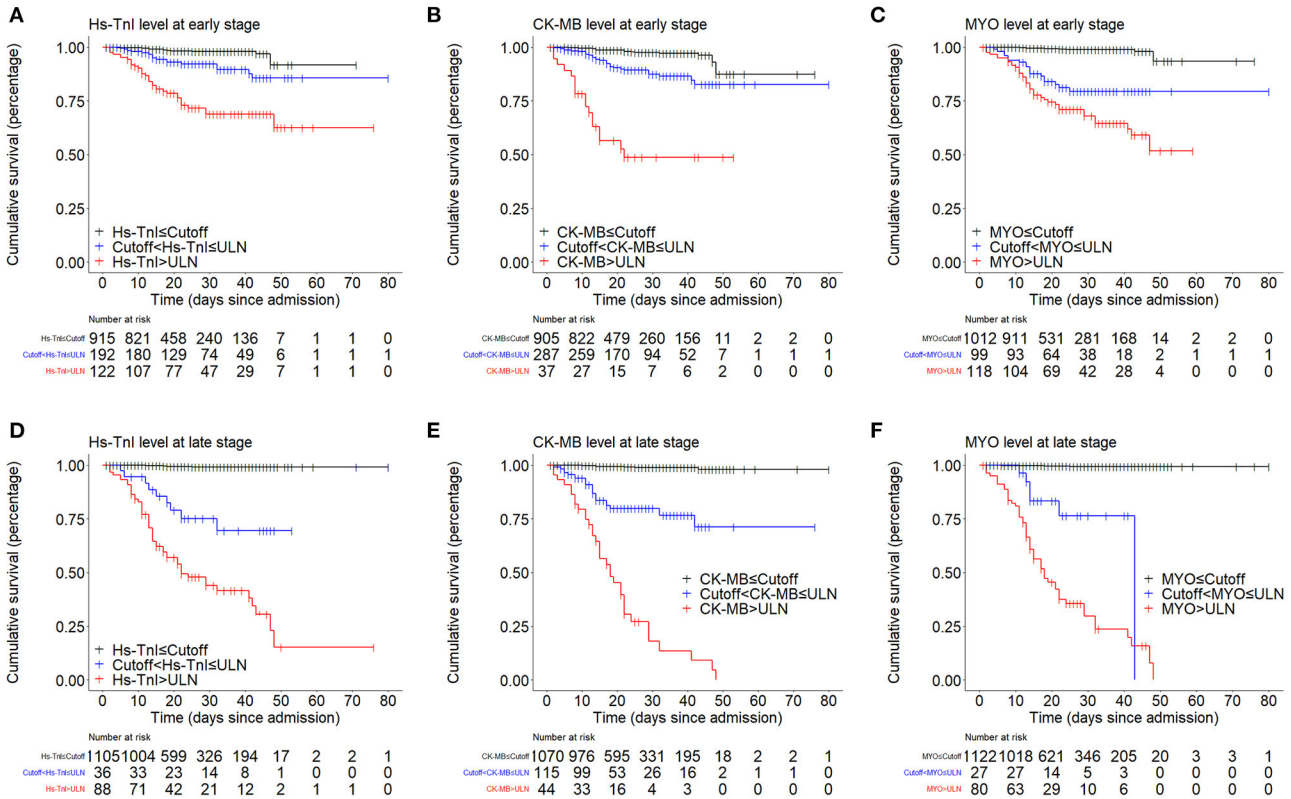


FIGURE 3 | Kaplan-Meier curves showing the cumulative survival rates of patient groups divided by cut-off and ULN. **(A–C)** Kaplan-Meier curve analysis based on the early-stage levels of myocardial biomarkers. **(D–F)** Kaplan-Meier curve analysis based on the late-stage levels of myocardial biomarkers. ULN, upper limit of normal.

myocardial injury, acute systemic hypoxia, and rhabdomyolysis during COVID-19 infection, and thus has high accuracy in predicting in-hospital mortality.

Another finding of this study is that although the cardiac-specific biomarkers hs-TnI and CK-MB were correlated with in-hospital mortality, they were not independent prognostic in the multivariate COX analysis. By far, the mechanisms underlying elevated cardiac biomarkers after COVID-19 infection are not fully understood. Myocarditis, stress cardiomyopathy, acute heart failure, and direct viral damage were considered potential etiologies (27). Recent literature data show that troponin release is relatively modest and slightly elevated in overall SARS-CoV-2 infected patients. Only 8-12% of positive cases had hs-TnI concentrations higher than ULN (4). In the present study, we obtained comparable results, with 9.9% of patients with hs-TnI levels higher than ULN in the early stage and 7.2% in the late stage. Considering the generally mild myocardial injury in overall COVID-19 patients, the myocardial injury may not be the major cause of poor prognosis in COVID-19 patients.

Given that many patients with severe COVID-19 infection exhibit concomitant elevations in cardiac biomarkers and inflammatory factors such as hs-CRP and IL-6, it is suggested that the myocardial inflammatory response is the underlying pathophysiology (28). The elevation of cardiac biomarkers and other inflammatory biomarkers raises the possibility that this reflects a cytokine storm, which may clinically present as fulminant myocarditis (4, 27, 29). In addition, increased prothrombotic and procoagulant responses following SARS-CoV-2 infection can lead to increased frequency of pulmonary embolism and worsening hypoxemia, leading to cardiac injury and heart failure (4, 30). The present study showed that hs-TnI levels were highly correlated with inflammation-related factors such as hs-CRP and IL-6 and coagulation indicators such as D-dimer, which, together with MYO, were independent predictors of mortality. Therefore, it is reasonable to assume that for most patients with COVID-19, the myocardial injury may be secondary to inflammatory injury and coagulation abnormalities following viral infection.

Despite the values of these findings, our study has some limitations. First, this study is retrospective in nature. The presence of selection bias associated with patient selection cannot be excluded. Second, the level of laboratory variables may be affected by the fact that the time from diagnosis to hospital admission varies among patients. Finally, this is a single-center study. The current sample size is small for the total patients with COVID-19 worldwide. Further studies involving larger populations and multiple centers are needed to confirm the results.

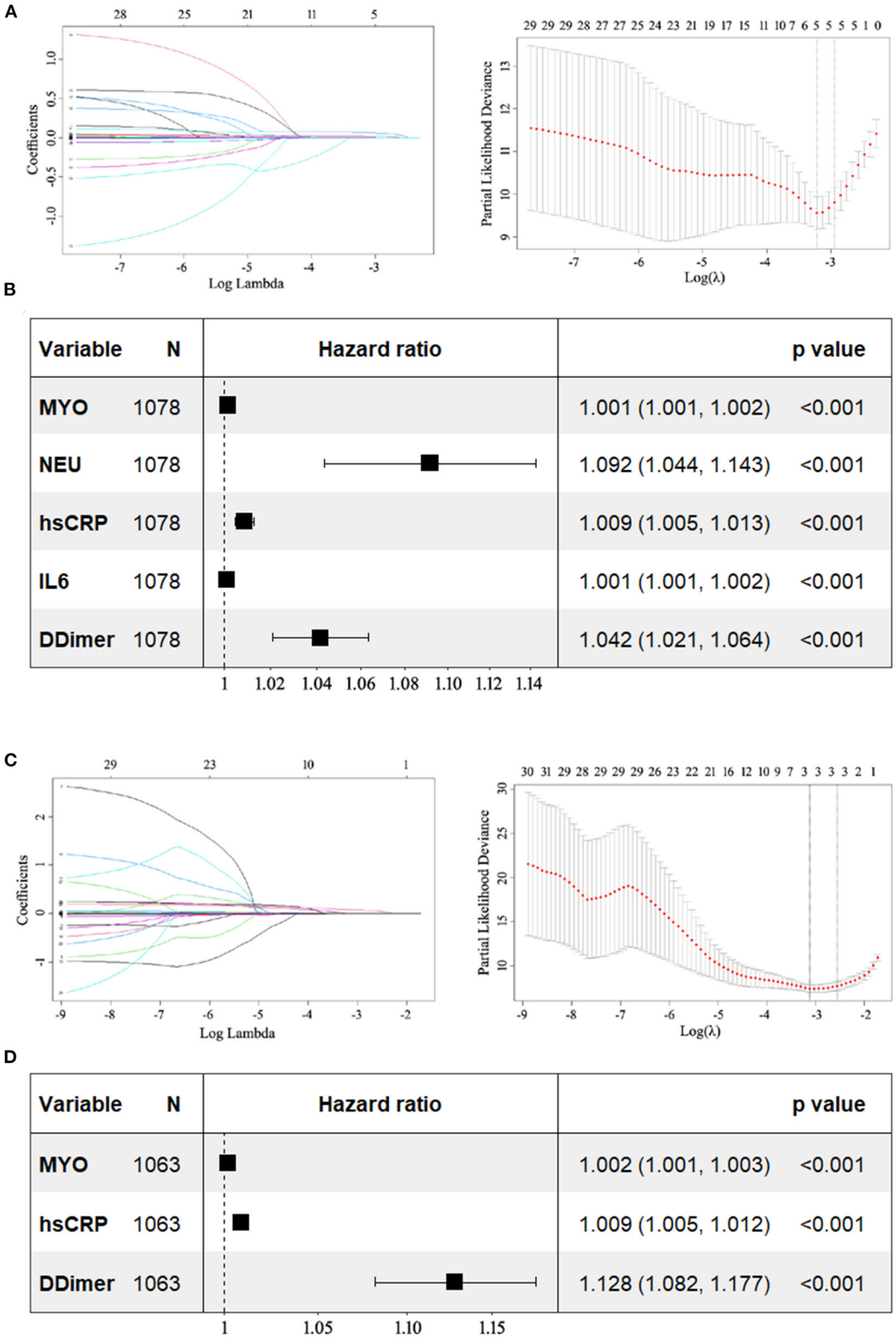


FIGURE 4 | Forest plots showing the results of multivariate Cox analysis. The potential prognostic factors included in the multivariate analysis were previously identified by LASSO regression. **(A)** LASSO coefficient profiles of the 30 variables including age, sex, comorbidities, physical examinations, and early-stage results of

(Continued)

FIGURE 4 | laboratory parameters. Dotted vertical lines were drawn at the optimal values by using the minimum criteria and the 1 SE of the minimum criteria (the 1-SE criteria). Five variables with non-zero coefficients (MYO, NEU, hs-CRP, IL-6, and D-dimer) were identified. **(B)** In multivariate analysis, all five variables had *p*-values < 0.05. **(C)** LASSO coefficient profiles of the 30 variables including age, sex, comorbidities, physical examinations, and late-stage results of laboratory parameters. Three variables (MYO, hs-CRP, and D-dimer) with non-zero coefficients were identified. **(D)** In multivariate analysis, they all had *p*-values less than 0.05. LASSO, least absolute shrinkage and selection operator; MYO, myoglobin; NEU, neutrophil; hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin 6.

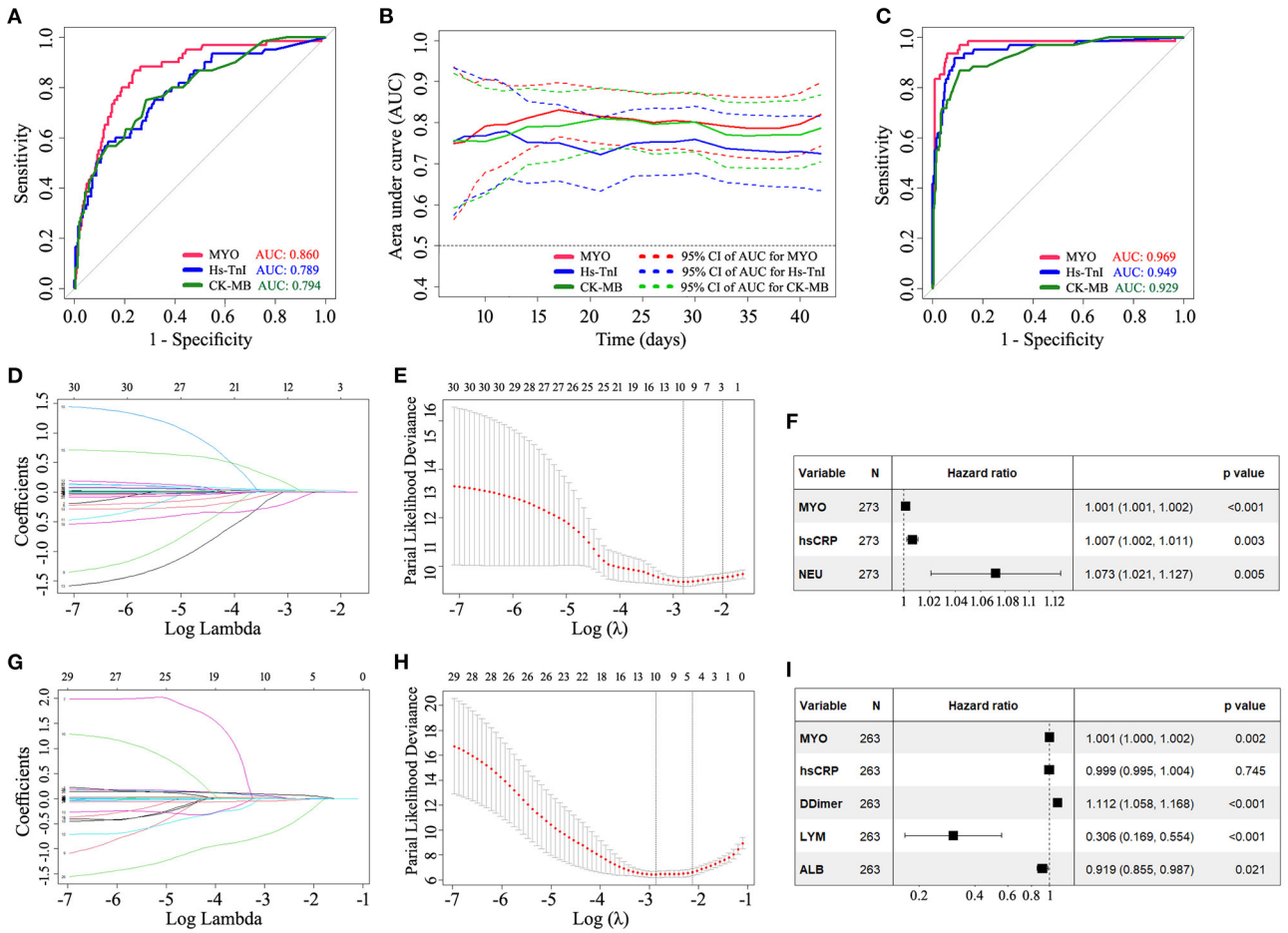


FIGURE 5 | Subgroup analysis demonstrating the prognostic ability of MYO in COVID-19 patients with severe or critical conditions. The results confirmed that MYO provided a better prognostic performance than hs-TnI and CK-MB and had an independent prognostic effect on in-hospital mortality. **(A)** Standard ROC curve analysis based on the early-stage levels of biomarkers. **(B)** Time-dependent AUC curves based on the early levels of cardiac markers. **(C)** Standard ROC curve analysis based on the late-stage levels of biomarkers. **(D–F)** LASSO regression and multivariate COX analysis based on early levels of biomarkers. **(G–I)** LASSO regression and multivariate COX analysis based on late levels of biomarkers. LASSO, least absolute shrinkage and selection operator; MYO, myoglobin; NEU, neutrophil; hs-CRP, high sensitivity C-reactive protein; LYM, lymphocyte; ALB, albumin; N, number; AUC, area under curve.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of Tongji hospital, Huazhong University of Science and Technology. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HL conceived the study and wrote the manuscript. J-SY, R-DC, and HL collected the data. R-DC, H-KY, and L-CZ analyzed the data. J-SY and HL modified the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by Grants from the National Natural Science Foundation of China (81601008).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.686328/full#supplementary-material

Supplementary Figure 1 | (A,B) Percentages of patients with myocardial marker levels below the cut-off, between the cut-off and ULN, and above the ULN in early **(A)** and late **(B)** stages. **(C,D)** Percentages of deaths in patients with myocardial marker levels below the cut-off, between the cut-off and ULN, and above the ULN in early **(C)** and late **(D)** stages. ULN, upper limit of normal.

Supplementary Figure 2 | Correlation matrix heat map of 16 clinical parameters in early **(A)** and late **(B)** stages of the disease. Spearman's correlation coefficient was used to calculate the correlation of the variables.

Supplementary Figure 3 | Kaplan-Meier curves showing the cumulative survival of patients divided by ULN. **(A-C)** Kaplan-Meier curve analysis based on the early-stage levels of myocardial biomarkers. **(D-F)** Kaplan-Meier curve analysis based on the late-stage levels of myocardial biomarkers. ULN, upper limit of normal.

Supplementary Figure 4 | Subgroup analysis demonstrating the prognostic ability of MYO in COVID-19 patients without a history of cardiovascular disease. The results showed that MYO provided a better prognostic performance with an independent prognostic effect on in-hospital mortality. **(A)** Standard ROC curve analysis based on the early-stage levels of biomarkers. **(B)** Time-dependent AUC curves based on the early levels of cardiac markers. **(C)** Standard ROC curve analysis based on the late-stage levels of biomarkers. **(D-F)** LASSO regression and multivariate COX analysis based on early levels of biomarkers. **(G-I)** LASSO regression and multivariate COX analysis based on late levels of biomarkers. LASSO, least absolute shrinkage and selection operator; MYO, myoglobin; NEU, neutrophil; hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin 6; *N*, number; AUC, area under curve.

Supplementary Table 1 | Laboratory results at the early and late stages in the overall study population, survivors and non-survivors.

Supplementary Table 2 | Effects of variables on in-hospital mortality analyzed by univariate Cox proportional-hazards regression.

Supplementary Table 3 | Association of elevated myocardial marker levels above ULN with in-hospital mortality of COVID-19.

Supplementary Table 4 | Multivariate Cox analysis of prognostic factors identified by LASSO regression.

REFERENCES

1. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med.* (2020) 382:1199–207. doi: 10.1056/NEJMoa2001316
2. Chen C, Li H, Hang W, Wang DW. Cardiac injuries in coronavirus disease 2019 (COVID-19). *J Mol Cell Cardiol.* (2020) 145:25–9. doi: 10.1016/j.yjmcc.2020.06.002
3. Lala A, Johnson KW, Januzzi JL, Russak AJ, Paranjpe I, Richter F, et al. Prevalence and impact of myocardial injury in patients hospitalized with COVID-19 infection. *J Am Coll Cardiol.* (2020) 76:533–46. doi: 10.1101/2020.04.20.20072702
4. Liu PP, Blet A, Smyth D, Li H. The science underlying COVID-19: implications for the cardiovascular system. *Circulation.* (2020) 142:68–78. doi: 10.1161/CIRCULATIONAHA.120.047549
5. Shi S, Qin M, Cai Y, Liu T, Shen B, Yang F, et al. Characteristics and clinical significance of myocardial injury in patients with severe coronavirus disease 2019. *Eur Heart J.* (2020) 41:2070–9. doi: 10.1093/eurheartj/ehaa408
6. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017
7. Lippi G, Lavie CJ, Sanchis-Gomar F. Cardiac troponin I in patients with coronavirus disease 2019 (COVID-19): evidence from a meta-analysis. *Prog Cardiovasc Dis.* (2020) 63:390–1. doi: 10.1016/j.pcad.2020.03.001
8. Parohan M, Yaghoubi S, Seraji A. Cardiac injury is associated with severe outcome and death in patients with coronavirus disease 2019 (COVID-19) infection: a systematic review and meta-analysis of observational studies. *Eur Heart J Acute Cardiovasc Care.* (2020) 9:665–77. doi: 10.1177/2048872620937165
9. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* (2020) 5:802–10. doi: 10.1001/jamacardio.2020.0950
10. Guo L, Xiong W, Liu D, Feng Y, Wang P, Dong X, et al. The mncp-spi score predicting risk of severe covid-19 among mild-pneumonia patients on admission. *Infect Drug Resist.* (2020) 13:3593. doi: 10.2147/IDR.S263157
11. He F, Luo Q, Lei M, Fan L, Shao X, Huang G, et al. Risk factors for severe cases of COVID-19: a retrospective cohort study. *Aging.* (2020) 12:15730–40. doi: 10.18632/aging.103803
12. Su M, Wang Y, Peng J, Wu M-J, Deng W, Yang Y-S. Elevated cardiac biomarkers are associated with increased mortality for inpatients with COVID-19: a retrospective case-control study. *J Clin Anesth.* (2020) 65:109894. doi: 10.1016/j.jclinane.2020.109894
13. Zhang C, Qin L, Li K, Wang Q, Zhao Y, Xu B, et al. A novel scoring system for prediction of disease severity in COVID-19. *Front Cell Infect Microbiol.* (2020) 10:318. doi: 10.3389/fcimb.2020.00318
14. Chen H, Chen R, Yang H, Wang J, Hou Y, Hu W, et al. Development and validation of a nomogram using on admission routine laboratory parameters to predict in-hospital survival of patients with COVID-19. *J Med Virol.* (2021) 93:2332–9. doi: 10.1002/jmv.26713
15. Yu JS, Pan NN, Chen RD, Zeng LC, Yang HK, Li H. Cardiac biomarker levels and their prognostic values in COVID-19 patients with or without concomitant cardiac disease. *Front Cardiovasc Med.* (2021) 7:599096. doi: 10.3389/fcvm.2020.599096
16. Liang X, Feng Z, Li L. *Guidance for Corona Virus Disease 2019: Prevention, Control, Diagnosis and Management*. Beijing: People's Medical Publishing House (2020).
17. Heagerty PJ, Lumley T, Pepe MS. Time-dependent ROC curves for censored survival data and a diagnostic marker. *Biometrics.* (2000) 56:337–44. doi: 10.1111/j.0006-341X.2000.00337.x
18. Kamarudin AN, Cox T, Kolamunnage-Dona R. Time-dependent ROC curve analysis in medical research: current methods and applications. *BMC Med Res Methodol.* (2017) 17:53. doi: 10.1186/s12874-017-0332-6
19. Sauerbrei W, Royston P, Binder H. Selection of important variables and determination of functional form for continuous predictors in multivariable model building. *Stat Med.* (2007) 26:5512–28. doi: 10.1002/sim.3148
20. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika.* (1994) 81:515–26. doi: 10.1093/biomet/81.3.515
21. Qin JJ, Cheng X, Zhou F, Lei F, Akolkar G, Cai J, et al. Redefining cardiac biomarkers in predicting mortality of inpatients with COVID-19. *Hypertension.* (2020) 76:1104–12. doi: 10.1161/HYPERTENSIONAHA.120.15528
22. Jin M, Tong Q. Rhabdomyolysis as potential late complication associated with COVID-19. *Emerg Infect Dis.* (2020) 26:1618. doi: 10.3201/eid2607.200445
23. Rivas-García S, Bernal J, Bachiller-Corral J. Rhabdomyolysis as the main manifestation of coronavirus disease 2019. *Rheumatology.* (2020) 59:2174–6. doi: 10.1093/rheumatology/keaa351
24. Singh B, Kaur P, Mechineni A, Maroules M. Rhabdomyolysis in COVID-19: report of four cases. *Cureus.* (2020) 12:e10686. doi: 10.7759/cureus.10686

25. Geng Y, Ma Q, Du YS, Peng N, Yang T, Zhang SY, et al. Rhabdomyolysis is associated with in-hospital mortality in patients with COVID-19. *Shock.* (2021). doi: 10.21203/rs.3.rs-90892/v1
26. Samies NL, Pinninti S, James SH. Rhabdomyolysis and acute renal failure in an adolescent with coronavirus disease 2019. *J Pediatric Infect Dis Soc.* (2020) 9:507–9. doi: 10.1093/jpids/piaa083
27. Sandoval Y, Januzzi JL Jr, Jaffe AS. Cardiac troponin for assessment of myocardial injury in COVID-19: JACC review topic of the week. *J Am Coll Cardiol.* (2020) 76:1244–58. doi: 10.1016/j.jacc.2020.06.068
28. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny OJJC. Potential effects of coronaviruses on the cardiovascular system: a *review*. *JAMA Cardiol.* (2020) 5:831–40. doi: 10.1001/jamacardio.2020.1286
29. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol.* (2020) 17:259–60. doi: 10.1038/s41569-020-0360-5
30. Shi W, Lv J, Lin L. Coagulopathy in COVID-19: focus on vascular thrombotic events. *J Mol Cell Cardiol.* (2020) 146:32–40. doi: 10.1016/j.yjmcc.2020.07.003

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 16 August 2021
doi: 10.3389/fcvm.2021.676592

Decrease in the Number of Patients Presenting With ST-Segment Elevation Myocardial Infarction Across Catheterization Centers in Indonesia During the Coronavirus Disease 2019 Pandemic

Doni Firman[1], Arwin Saleh Mangkuanom[1], Nanda Iryuza[1], Ismir Fahri[2], I Made Junior Rina Artha[3], Erwin Mulia[4], Muhammad Syukri[5], Emir Yonas[6], Raymond Pranata[7] and Amir Aziz Alkatiri[1]*

[1] Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Indonesia, National Cardiovascular Center Harapan Kita, Jakarta, Indonesia, [2] Department of Cardiology and Vascular Medicine, Mohammad Yunus General Hospital, Bengkulu, Indonesia, [3] Department of Cardiology and Vascular Medicine, Faculty of Medicine, Udayana University, Sanglah General Hospital, Denpasar, Indonesia, [4] Department of Cardiology and Vascular Medicine, Bumi Waras Hospital, Lampung, Indonesia, [5] Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Andalas, DR. M. Djamil General Hospital, Padang, Indonesia, [6] Faculty of Medicine, Universitas YARSI, Jakarta, Indonesia, [7] Faculty of Medicine, Universitas Pelita Harapan, Banten, Indonesia

OPEN ACCESS

Edited by:
Shuyang Zhang, Peking Union Medical College Hospital (CAMS), China

Reviewed by:
Johannes Tobias Neumann, University Heart and Vascular Center Hamburg (UHZ), Germany
Timothy Henry, Christ Hospital, United States

***Correspondence:**
Doni Firman
dr.donifirman@gmail.com

Specialty section:
This article was submitted to Cardiovascular Epidemiology and Prevention, a section of the journal Frontiers in Cardiovascular Medicine

Received: *05 March 2021*
Accepted: *21 July 2021*
Published: *16 August 2021*

Citation:
Firman D, Mangkuanom AS, Iryuza N, Fahri I, Artha IMJR, Mulia E, Syukri M, Yonas E, Pranata R and Alkatiri AA (2021) Decrease in the Number of Patients Presenting With ST-Segment Elevation Myocardial Infarction Across Catheterization Centers in Indonesia During the Coronavirus Disease 2019 Pandemic. Front. Cardiovasc. Med. 8:676592. doi: 10.3389/fcvm.2021.676592

Background: The coronavirus disease 2019 (COVID-19) pandemic has become a global problem, put a heavy burden on the health care system, and resulted in many fatalities across the globe. A reduction in the number of cardiac emergencies, especially ST-segment elevation myocardial infarction (STEMI), is observed worldwide. In this study, we aimed to analyze the trends of cases and presentation of STEMI across several cardiac catheterization centers in Indonesia.

Method: This retrospective study was performed by combining medical record data from five different hospitals in Indonesia. We compared data from the time period between February to June 2019 with those between February and June 2020. Patients who were diagnosed with STEMI and underwent primary percutaneous coronary intervention (PPCI) procedures were included in the study.

Results: There were 41,396 emergency department visits in 2019 compared with 29,542 in 2020. The number of patients with STEMI declined significantly from 338 in 2019 to 190 in 2020. Moreover, the total number of PPCI procedures reduced from 217 in 2019 to 110 in 2020. The proportion of PPCI was not significantly reduced (64.2 vs. 57.9%). The majority of the patients were men, with a mean age of 54 years in 2019 and 55 years in 2020. We observed a significantly longer door-to-balloon time in 2020 than in 2019 ($p < 0.001$). We also observed a difference in the door-to-balloon time and ischemic time between the two periods.

Conclusion: We observed a decline in the number of patients presenting with STEMI to our centers. However, we observed no significant decline in the percentage of PPCI performed across our centers during this pandemic.

Keywords: STEMI, COVID-19, case, decrease, pandemic (COVID-19), cardiovascular

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has become a global problem that has put a heavy burden on the health care system and resulted in many fatalities (1, 2). Each country has different policies to limit the transmission of COVID-19. When the disease began to spread in Indonesia in February 2020, many health services were disrupted because hospitals or other health care facilities had to change their service system policies to reduce the spread of the virus.

The pandemic also resulted in a significant reduction in the number of patients seeking medical care either in the emergency department or outpatient clinic. A reduction in the number of cardiac emergencies, especially ST-segment elevation myocardial infarction (STEMI), was observed worldwide (3–9). Data on 115,716 adult emergency department visits to 108 emergency departments in the United States showed a significant reduction in the incidence of most of the serious cardiovascular events, with the exception of STEMI, in the year 2020 compared with that in 2019 (10). Similarly, no decrease in the number of patients presenting with STEMI was observed in France, and the authors of the study hypothesized that the pandemic probably dissuaded "non-critical" patients, but not those requiring reperfusion (11). In Greece, the number of patients presenting to the emergency department with acute coronary syndrome (ACS) in 2020 was significantly reduced compared to that in the previous year (12). Similarly, the number of patients presenting with cardiovascular emergencies in Germany decreased in 2020 (13). The number of immediate/early percutaneous coronary interventions (PCIs) for non-ST-segment elevation myocardial infarction (NSTEMI) in China significantly decreased in 2020 compared to that in the previous year (14).

Several possible causes for a decrease in the number of STEMI cases in Indonesia have been proposed; one of them is the fear of visiting the hospital during the COVID-19 pandemic. In this study, we aimed to analyze the trends of cases and presentation of STEMI across several cardiac catheterization centers in Indonesia.

MATERIALS AND METHODS

This was a retrospective study involving 190 patients with STEMI in 2020 and 338 patients in 2019. This study was approved by the Ethical Committee of the National Cardiovascular Center Harapan Kita (LB 02.01/VII/KEP 070/2018) and adhered to the declaration of Helsinki. Data were obtained from five hospitals in Indonesia, namely the National Cardiovascular Center Harapan Kita (Jakarta, Indonesia), Mohammad Yunus General Hospital (Bengkulu, Indonesia), DR. M. Djamil General Hospital (Padang, Indonesia), Bumi Waras Hospital (Bandar Lampung, Indonesia), and Sanglah General Hospital (Denpasar, Indonesia). These hospitals are referral centers for PCI and cardiovascular care in their respective regions. The National Cardiovascular Center Harapan Kita is a national tertiary hospital for cardiovascular disease, while the rest are general hospitals with cardiac intervention facilities. We retrieved medical records data from two different periods: (i) between February 1, 2019, and June 30, 2019; and (ii) between February 1, 2020, and June 30, 2020. Patients who were diagnosed with STEMI and underwent primary PCI (PPCI) procedures were included in the study. We also included data of patients with STEMI without PPCI and overall presentation to the emergency departments of the participating hospitals for comparative purposes.

During the second observation period of this study, most of the patients underwent rapid test for COVID-19 antibody. The number of polymerase chain reaction tests performed on these patients was very small due to the unavailability at the time. Thus, the diagnosis of COVID-19 was mainly based on rapid test for COVID-19 antibody. The data retrieved included demography, medication, procedural data, in-hospital outcome, length of hospital stay, angiographic characteristics, total ischemic time, patient's delay, hospital, and system delay. Thrombolysis in myocardial infarction (TIMI) flow grade was assessed angiographically by the physician performing the procedure. During the PCI procedure, door-to-wire crossing time was recorded as a surrogate for reperfusion per the 2017 European Society of Cardiology guidelines for the management of acute myocardial infarction (AMI) in patients presenting with ST-segment elevation. Continuous variables are presented as median [25th percentile, 75th percentile]. Categorical variables are presented as frequency and percentage.

In this paper, we present continuous data as median, minimum and maximum, and mean + standard deviation as appropriate. Percentage is used to present discrete data. We used Statistical Package for the Social Sciences (SPSS) software version 25 for Windows (SPSS, Chicago, Illinois, USA). Baseline analysis of patients' demography and comorbidity. The independent samples *t*-test, the chi-square test, and Fisher's exact test were used to compare variables as appropriate.

We compared sex, age, diabetes, hypertension, smoking status, and Killip class between 2019 and 2020 data. Clinical parameters and procedural data were analyzed using the *t*-test to compare percentages, onset, time interval since STEMI onset, door-to-balloon time, ischemic time, and LVEF. We also analyzed adverse effects and complications including bleeding, initiation of CPR, stroke, mortality, and length of hospital stay.

RESULTS

The baseline characteristics are illustrated in **Tables 1**, **2**, the complications and outcomes are presented in **Table 3**. There were 41,396 and 29,542 emergency department visits in 2019 and 2020, respectively. The number of patients with STEMI declined significantly from 338 cases in 2019 to 190 cases in 2020. Moreover, the number of PPCI procedures decreased from a total of 217 in 2019, to 110 in 2020. The majority of the patients were men, with a mean age of 54 years in 2019 and 55 years in 2020 as shown in **Table 1**. There were no significant differences regarding the presence of hypertension and diabetes mellitus between the two periods. In the pandemic period, the number of patients who presented directly to the PCI center's emergency department declined sharply compared to referred patients from the other health care facilities ($p < 0.001$). Furthermore, the onset

TABLE 1 | Baseline characteristics of all study subjects.

Characteristic	2019 ($n = 338$)	2020 ($n = 190$)	*P*-Value
Male (%)	294 (87)	167 (87.9)	0.787
Age, mean (SD), years	55.31 (11.34)	55.17 (10.50)	0.888
Hypertension (%)	196 (58.0)	103 (54.20)	0.412
Diabetes (%)	131 (38.8)	79 (41.6)	0.578
Smoker (%)	193 (57.1)	85 (44.7)	0.007
Killip Class I (%)	197 (58.3)	103 (54.2)	0.092
Presentation of Cases (Overall)			
Patient self-presenting to PCI Capable Center (%)	178 (52.7)	64 (33.7)	<0.001
Referred from other hospital (%)	160 (47.3)	126 (66.3)	<0.001
Procedures for patients with STEMI			
Conservative treatment (%)	55 (16.3)	33 (17.4)	0.299
Fibrinolysis (%)	66 (19.5)	47 (24.7)	0.299
Primary PCI (%)	217 (64.2)	110 (57.9)	0.299

of symptoms to arrival was found to be similar in between the two periods. Nevertheless, we observed a significantly longer door-to-balloon period and ischemic time in the pandemic period than in 2019 ($p < 0.001$ and $p = 0.041$, respectively) (**Tables 1**, **2**).

We observed a trend of decrease in the number and percentage of STEMI cases and rates of PPCI in 2020 compared with 2019. A total of 74 cases with STEMI were reported from the five catheterization centers in March 2020 compared to 107 cases in March 2019 and the trend continues until the end of observation in June. Overall, compared with the number of patients with STEMI in 2019, we noted a trend for decrease in 2020, reaching the lowest point in June 2020 when there were only 34 patients with STEMI compared to 60 cases in June 2019. We observed no significant change in the percentage of PPCI performed across our centers. However, the percentage of patients undergoing conservative treatment was higher in June 2020 compared with that in the previous year (33.3 and 11.7%, respectively) (**Figures 1**–**3**).

DISCUSSION

During the COVID-19 pandemic, a significant decrease in the number of hospitalizations of patients with ACS was seen in the United Kingdom (UK), Ireland, Italy, and the United States. Data from NHS facilities across the UK show a hospitalization rate reduction of 40% for ACS, 35% for AMI, 23% for STEMI, and 42% for NSTEMI. Similar observations were reported from Italy, with ∼50% reduction in patients with AMI presenting to the hospital in every region of the country. Moreover, a 26.5 and 65.1% reduction in patients with STEMI, and NSTEMI, respectively, and a significantly increased case fatality rate for STEMI compared with those in the previous year were observed (RR 3.3, 95% confidence interval [CI] 1.7–6.6; $P < 0.001$) (8, 15–18).

A lower number of patients with STEMI presented at our PCI centers during the pandemic. Therefore, we might assume that patients were reluctant to come to a hospital with a PCI center, which usually is larger and more crowded with patients, to avoid COVID-19 transmission and tended to visit a less crowded hospital or other health care providers. Compared with 2019, we observed an increase in the door-to-balloon time (97.79 vs. 125.56 min, $p < 0.001$) and ischemic time (447.33 vs. 488.57 min, $p = 0.041$) in 2020. Lockdown and stay at home order may cause patient delay in seeking medical care, Indonesia did not impose lockdown, which may explain only small difference (although statistically significant) between the ischemic time in 2019 and 2020. Solomon et al., in a study using data from the Kaiser Permanente Oakland Medical Center, observed a decrease in the number of hospitalizations of patients with STEMI in conjunction with the first reported death due to COVID-19 in California, 2 weeks before the implementation of the shelter in place order (18). This finding shows that social restriction policies might play a smaller role than previously believed in decreasing the rate of presentation to hospitals. Contrary to the observations in the US, in the data from NHS facilities across the UK, a decrease in admissions for ACS occurred at least 2 weeks before the first death due to COVID-19. In response to this, the British Heart Foundation and the British Cardiovascular Society launched public campaigns to encourage patients with heart attack symptoms to visit a hospital (17).

We observed a reduction in the proportion of smokers among admitted patients with STEMI in 2020 compared with that in 2019 (57.1 vs. 44.7% $p = 0.007$). In our view, this does not necessarily signify a reduction in the number of smokers, but rather it is an index of the reduction in the number of patients admitted to PCI centers. As supported by the finding of a reduced percentage of patients who presented themselves to the hospital for STEMI in 2020 compared with that in 2020 (34.5 vs. 58.1%, $p < 0.001$), the majority of admitted patients were referred from other hospitals during the COVID-19 pandemic (41.9 vs. 65.5%, $p < 0.001$), which showed an increase from that in 2019. In contrast, during 2019, the majority of admissions for PPCI were voluntary presentations to PCI centers. Our findings were similar to those of a study by Mafham et al. regarding the decrease in admissions for STEMI in the UK during the COVID-19 pandemic. Our findings show the reluctance of

TABLE 2 | Baseline and procedural characteristics of study subjects who underwent primary PCI.

Characteristic	2019 ($n = 217$)	2020 ($n = 110$)	*P*-Value	Difference between means (95% CI)
Male (%)	194 (89.4)	97 (88.2)	0.713	
Age, mean (SD), years	54.01 (10.19)	55.00 (10.06)	0.405	
Hypertension (%)	137 (63.1)	73 (66.3)	0.326	
Diabetes (%)	125 (57.6)	71 (64.5)	0.235	
Smoker (%)	140 (64.5)	51 (26.8)	<0.001	
Killip Class I (%)	197 (90.8)	103 (93.6)	0.257	
Presentation of Cases				
Patient self-presenting to PCI Capable center (%)	126 (58.1)	38 (34.5)	<0.001	
Referred from other hospital (%)	91 (41.9)	72 (65.5)	<0.001	
Clinical Parameters				
STEMI Onset, mean (SD), minutes	349.60 (158.48)	363 (171.24)	0.611	13.4 (−50.9–24.1)
Door-to-balloon time, mean (SD), minutes	97.79 (60.29)	125.56 (66.35)	<0.001	27.7(−42.1–13.4)
Ischemic Time, mean (SD), minutes	447.33 (164.75)	488.57 (185.26)	0.041	41.24 (−80.81–1.65)
LVEF, Mean (SD)	48.13 (12.86)	50.69 (13.59)	0.199	0.2 (−5.7–0.6)
Anterior MI (%)	116 (53.5)	56 (50.9)	0.317	
Procedural Data				
Radial (%)	171 (78.8)	92 (83.6)	0.243	
TIMI 0 Flow, Pre (%)	181 (83.4)	93 (84.5)	0.638	
TIMI 3 Flow, Post (%)	180 (82.9)	103 (93.6)	0.027	
Stent number, mean (SD)	1.14 (0.38)	1.15 (0.41)	0.844	

TABLE 3 | Outcomes of interests.

Complication and Outcome	2019 ($n = 338$)	2020 ($n = 190$)	*P*-Value	Difference between means (95% CI)
Bleeding, Yes (%)	8 (2.4)	2 (1.1)	0.505	
CPR, Yes (%)	14 (4.1)	9 (4.7)	0.648	
Stroke Event, Yes (%)		1 (0)	0.336	
Mortality, Alive (%)	206 (60.9)	104 (54.7)	1.000	
Length of Stay, mean (SD), days	5.27 (3.12)	5.29 (3.15)	0.935	0.02 (−0.74–0.69)

patients with STEMI to voluntarily seek medical care and present themselves to cardiovascular centers compared with the previous year, possibly due to fear of contracting COVID-19. Mafham et al. also associated this reduction in patients with STEMI in their study with the increased news coverage of COVID-19 in the media, and supported the hypothesis that this is caused by the widespread fear of COVID-19 (17).

We found markedly longer door-to-balloon time in 2020 compared with that in 2019 (125.56 vs. 97.79 min, $p < 0.001$). This delay was due to several reasons. First, patients were examined thoroughly for signs and symptoms of COVID-19 before entering the ED. Second, after patients entered the ED, several examinations were performed to minimize the risk of COVID-19 transmission such as completing the COVID-19 epidemiological form, chest x-ray scan, complete blood count, rapid immunological test for COVID-19, and chest CT-scan if necessary. Third, since most hospitals in Indonesia were not well-prepared for a dedicated isolated catheterization laboratory (cath lab), the donning process of the personal protective equipment (PPE) for the operator and scrub nurses might have prolonged the door-to-balloon time. Furthermore, we acknowledge that the exercise of precautions, such as travel and contact history interviews and chest x-ray images taken before patient transfer to the cath lab, may be possible causes of delay. However, this measure is crucial, especially because most cath labs are positively ventilated and performing procedures in this environment might facilitate the spread of disease if patients are not screened thoroughly (5).

Our observation regarding the increase in door-to-balloon time during the COVID-19 pandemic was consistent with results of a study by Tam et al., who also observed an increase in the symptom to the first medical contact time, door to device time, and cath lab arrival to device time compared with those in the years 2018 and 2019. The authors reported a minimum increase of 226.5 min from symptom onset to first medical contact time, and a minimum of increase of 11 min in the cath lab arrival to device time. Interestingly, in non-office-hours presentations, a decrease in the door to device time compared to that in previous years was noted.

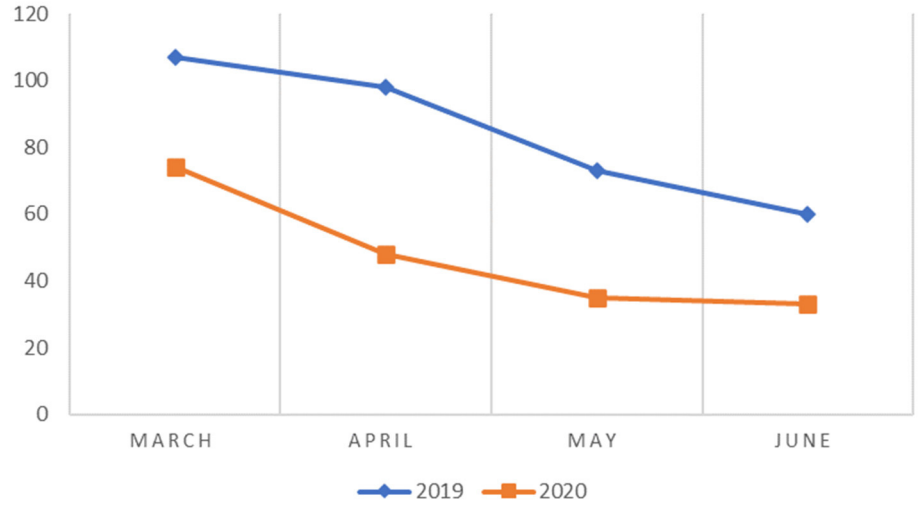


FIGURE 1 | Total STEMI Cases before and during COVID 19 pandemic.

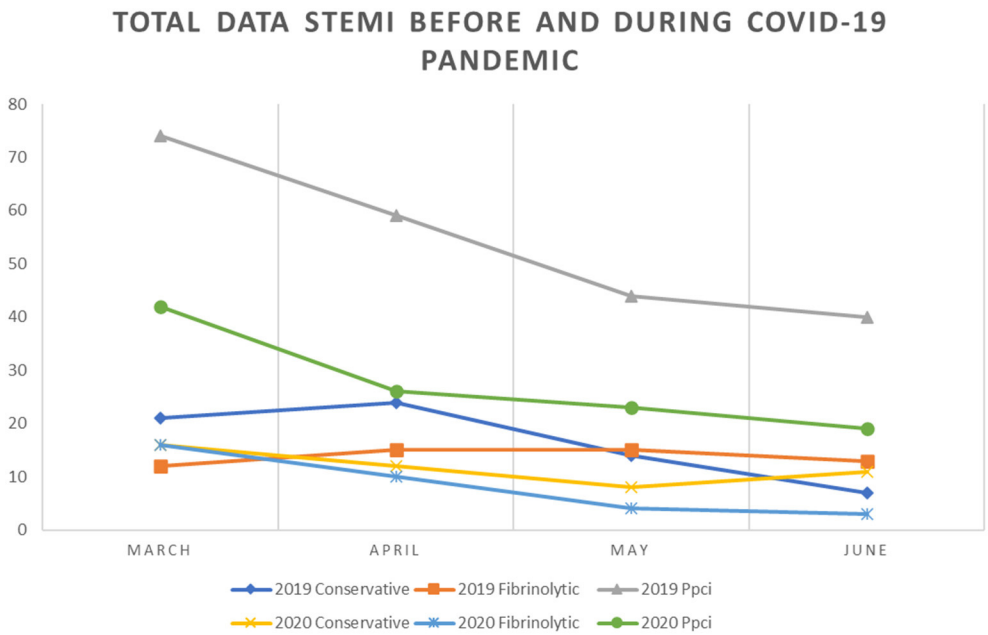


FIGURE 2 | Comparison between conservative, fibrinolytic and primary PCI management of STEMI in 2019 and 2020.

In these trying times, we observed a shift in reperfusion strategies for treating STEMI while adapting to the highly contagious nature of COVID-19. A protocol from Sichuan provincial hospital advocates thrombolysis in an isolation ward for all COVID-19 positive patients with STEMI, with no contraindication for thrombolysis in patients with onset times within 12 h; an elective PCI is considered subsequently for this group of patients. Stable patients with onset time >12 h are evaluated for PCI, while unstable patients with severe pneumonia are given conservative treatment in an isolation ward (19). Similar guidelines, primarily focusing on the utilization of thrombolysis for COVID-19 positive patients with STEMI, were also proposed by the Peking Union Medical College Hospital, while for NSTEMI patients, they recommended that the treatment of COVID-19 pneumonia by an infectious diseases specialist should take precedence (20). However,

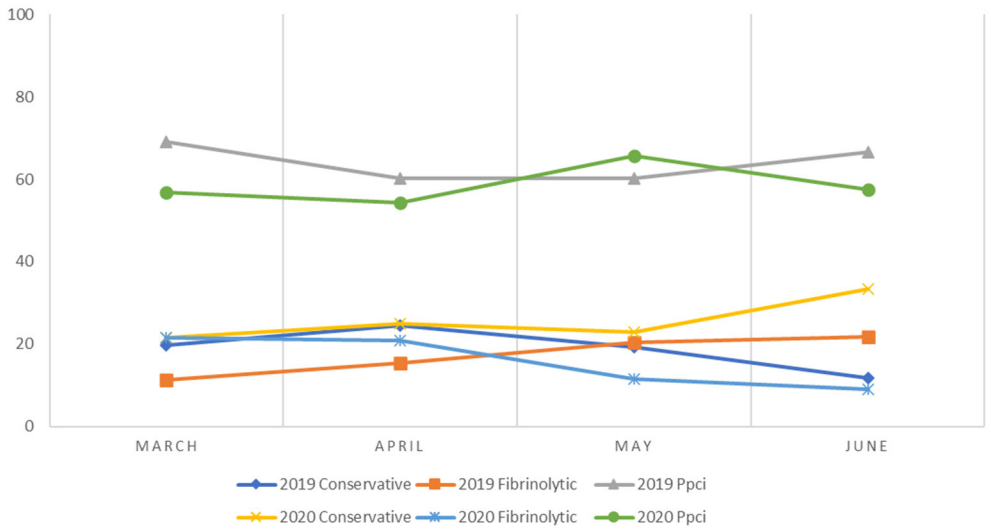


FIGURE 3 | Percentage of STEMI Cases before and during COVID 19 Pandemic.

guidelines from the Society for Cardiovascular Angiography and Interventions (SCAI) Emerging Leader Mentorship advocate PCI for COVID-19 positive patients with STEMI, albeit with several considerations such as screening before arrival at the cath lab and optimal use of PPE for staff. In its guidelines, SCAI acknowledges the possibility of overload of the healthcare system during this pandemic; however, systemic fibrinolytic therapy is only advocated for low-risk STEMI (inferior STEMI without right ventricular involvement or lateral myocardial infarction without hemodynamic compromise). For patients with high-risk STEMI, SCAI advocates the use of PCI as the primary modality; however, it should only be performed on the culprit vessel. PCI should be performed on a non-culprit vessel only if the lesion is deemed unstable or in the case of multiple culprit vessels (21). The recommendation of SCAI is in accordance with that of The European Society of Cardiology in their European Association of Percutaneous Coronary Intervention (EAPCI) position statement on invasive management of ACS during the COVID-19 pandemic. In this statement, PPCI remains the preferred reperfusion strategy in PCI centers, provided that it fits within the time frame (120 min from onset of symptoms). EAPCI also states that all patients with STEMI should be managed as COVID-19 positive. If considerable delay in reperfusion strategy is anticipated due to implementation of protective measures, fibrinolysis should be performed, given that there are no contraindications. EAPCI also recommends complete revascularization when appropriate and left ventricular angiogram in place of echo to evaluate left ventricular function (22). Additionally, a position statement from SCAI, ACC, and ACEP also recommend PPCI as the standard of care in patients with STEMI (23). We observed a statistically significant slight increase in the proportion of patients with post procedural TIMI3 flow (53.3 vs. 54.2%, $p = 0.027$). Total ischemic time and time-to-treatment delays for urgent cardiovascular interventions are expected to be longer during the pandemic as a consequence of screening and other policies related to the pandemic (24). This finding was also noted in the present study, wherein there was an increase in ischemic time during the period of study in 2020 compared with that during the period of study in 2019 (488.57 vs. 447.33 min, $p = 0.041$).

Currently, at all catheterization centers in our study, we perform PPCI on all COVID-19 positive patients with STEMI with onset <12 h. We also perform PPCI on patients with STEMI with onset >12 h who are clinically unstable. Currently, catheterization of all patients with ACS is performed in dedicated specialized isolated cath labs. After PCI, patients with reactive severe acute respiratory syndrome coronavirus 2 antibody tests, absolute lymphopenia, neutrophil to lymphocyte ratio >3.13, C-reactive protein >10, or infiltration on chest x-ray scanning are admitted to the isolation ward, whereas patients with none of the above are admitted to the regular ward.

Although we observed trends for lower rates of bleeding and C-reactive protein in this study, we did not observe an increase in mortality during the COVID-19 pandemic compared with that in the previous year (5.5 and 5.1% for 2020 and 2019, respectively, $p < 0.001$). In contrast, a study from Italy observed a significant increase in fatality rates of STEMI during the COVID-19 pandemic (13.7 and 4.1% for 2020 and 2019, respectively, RR = 3.3, 95% CI: 1.7–6.6; $P < 0.001$) (15).

The reduction in the presentation and admission rates for STEMI in Indonesia and other countries might not represent a true decrease in incidence of STEMI in the general population. This phenomenon might represent reluctance of patients to seek medical care due to fear of contracting COVID-19. We observed that self-presenting patients decreased while transfer

patients increased, both for total number and the percent with PCI, this be due to the characteristics of the hospitals. Hospitals included in this study are large government referral hospital and were assigned as COVID-19 treatment hospitals. It is possible that patients were more reluctant to present to these hospitals and chose less crowded hospitals that turns out to be non-PCI capable hospital, thus reducing self-presentation to our designated hospitals while concomitantly increasing the number of referrals.

Public health counseling of the community by medical practitioners plays an important role in combating the paranoia of contracting COVID-19 in case of an emergency. Patients with complaints suggestive of underlying myocardial infarction will gain the most benefit from timely medical attention to prevent sequelae in the future (25). Patients with STEMI will gain the most benefit of timely medical attention, since this subgroup of patients has the highest risk of out of hospital cardiac arrest without proper medical treatment (6, 26, 27).

Currently, Indonesia imposes no lockdown on its population. However, large scale social restrictions were imposed nationally and regionally by provincial governments in areas with a high prevalence of COVID-19. They included measures such as closing public places, restricting public transport, and limiting travel to and from the restricted regions. This policy was enacted in March 2020 after confirmation of 117 cases of COVID-19 on the 15th of March 2020; the first case of COVID-19 in Indonesia was reported on the 2nd of March 2020.

The enactment of this policy, which restricts travel, might have hindered patients from reaching cardiovascular centers for proper management of their complaints. Reports from the UK also indicate that the enforcement of lockdown/social distancing measures might further influence public perception into the reluctance of going to medical centers even when the need arises. This phenomenon might be what is currently happening in Indonesia. Ideally, efforts should be made to encourage the public to seek proper medical care when the need arises (17). Another potential cause of decreased STEMI and other acute cardiovascular diseases is the reduced pollution and change in lifestyle due to "work from home" and other policies (28, 29).

Study Limitation

Due to the abrupt and fast increase in COVID-19 cases in Indonesia during the study period (February–June 2020), our centers were not prepared for COVID-19 testing and diagnosis at the outset of this pandemic. Testing kits using Reverse Transcriptase Polymerase Chain Reaction were unavailable until August 2020, while rapid testing for COVID-19 using serum immunoglobulin assay was available only at certain centers. In most cases, an epidemiological contact form was used to supplement these testing kits. Unfortunately, we were unable to obtain precise data regarding the results of rapid tests.

In conclusion, we observed a decline in the number of patients presenting with STEMI to our centers. However, we observed no significant decline in the percentage of PPCI being performed across our centers during this pandemic. We also observed prolongation of the door-to-balloon time and ischemic time compared with those in the same month in the previous year. Although delays might be harmful to patients, this delay was caused by preliminary screening of patients with STEMI for COVID-19 at the emergency department.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee of the National Cardiovascular Center Harapan Kita (LB 02.01/VII/KEP 070/2018). The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

DF: conceptualization, methodology, and writing—review and editing. AM, IF, IA, EM, and MS: writing—review and editing. NI: formal analysis. EY and RP: writing—original draft, writing—review and editing, and visualization. AA: supervision, project administration, and writing—review and editing. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Coughlan JJ, Chongprasertpon N, Arockiam S, Arnous S, Kiernan TJ. COVID-19 and STEMI: a snapshot analysis of presentation patterns during a pandemic. *IJC Hear Vasc.* (2020) 30:100546. doi: 10.1016/j.ijcha.2020.100546
2. Hamadeh A, Aldujeli A, Briedis K, Tecson KM, Sanz-Sánchez J, Al dujeili M, et al. Characteristics and outcomes in patients presenting with COVID-19 and ST-segment elevation myocardial infarction. *Am J Cardiol.* (2020) 131:1–6. doi: 10.1016/j.amjcard.2020.06.063
3. Gluckman TJ, Wilson MA, Chiu ST, Penny BW, Chepuri VB, Waggoner JW, et al. Case rates, treatment approaches, and outcomes in acute myocardial infarction during the coronavirus disease 2019 pandemic. *JAMA Cardiol.* (2020) 5:1419–24. doi: 10.1001/jamacardio.2020.3629
4. Rodriguez-Leor O, Cid-Alvarez B. ST-segment elevation myocardial infarction care during COVID-19. *JACC Case Rep.* (2020) 2:1625–7. doi: 10.1016/j.jaccas.2020.04.011
5. Tam CCF, Cheung KS, Lam S, Wong A, Yung A, Sze M, et al. Impact of coronavirus disease 2019 (COVID-19) outbreak on ST-segment-elevation myocardial infarction care in Hong Kong, China. *Circ Cardiovasc Qual Outcomes.* (2020) 13:e006631. doi: 10.1161/CIRCOUTCOMES.120.006631
6. Pranata R, Lim MA, Yonas E, Siswanto BB, Meyer M. Out-of-hospital cardiac arrest prognosis during the COVID-19 pandemic. *Intern Emerg Med.* (2020) 15:875–7. doi: 10.1007/s11739-020-02428-7
7. Bergonti M. Emergency Room visits in the COVID-19 pandemic. *Eur Heart J.* (2021) 42:369–70. doi: 10.1093/eurheartj/ehaa938

8. Garcia S, Albaghdadi MS, Meraj PM, Schmidt C, Garberich R, Jaffer FA, et al. Reduction in ST-segment elevation cardiac catheterization laboratory activations in the United States during COVID-19 pandemic. *J Am Coll Cardiol.* (2020) 75:2871–2. doi: 10.1016/j.jacc.2020.04.011
9. Garcia S, Stanberry L, Schmidt C, Sharkey S, Megaly M, Albaghdadi MS, et al. Impact of COVID-19 pandemic on STEMI care: an expanded analysis from the United States. *Catheter Cardiovasc Interv*. (2020). doi: 10.1002/ccd.29154. [Epub ahead of print].
10. Pines JM, Zocchi MS, Black BS, Celedon P, Carlson JN, Moghtaderi A, et al. The effect of the COVID-19 pandemic on emergency department visits for serious cardiovascular conditions. *Am J Emerg Med.* (2021) 47:42–51. doi: 10.1016/j.ajem.2021.03.004
11. Montagnon R, Rouffilange L, Agard G, Benner P, Cazes N, Renard A. Impact of the COVID-19 pandemic on emergency department use: focus on patients requiring urgent revascularization. *J Emerg Med.* (2021) 60:229–36. doi: 10.1016/j.jemermed.2020.09.042
12. Oikonomou E, Aznaouridis K, Barbetseas J, Charalambous G, Gastouniotis I, Fotopoulos V, et al. Hospital attendance and admission trends for cardiac diseases during the COVID-19 outbreak and lockdown in Greece. *Public Health.* (2020) 187:115–9. doi: 10.1016/j.puhe.2020.08.007
13. Schwarz V, Mahfoud F, Lauder L, Reith W, Behnke S, Smola S, et al. Decline of emergency admissions for cardiovascular and cerebrovascular events after the outbreak of COVID-19. *Clin Res Cardiol.* (2020) 109:1500–6. doi: 10.1007/s00392-020-01688-9
14. Liu S, Song C, Yin D, Wang Y, Zhao Y, Yang W, et al. Impact of public health emergency response to COVID-19 on management and outcome for NSTEMI patients in Beijing: a single-center historic control. *Catheter Cardiovasc Interv.* (2021) 97:E475–83. doi: 10.1002/ccd.29145
15. De Rosa S, Spaccarotella C, Basso C, Calabrò MP, Curcio A, Filardi PP, et al. Reduction of hospitalizations for myocardial infarction in Italy in the COVID-19 era. *Eur Heart J.* (2020) 41:2083–8. doi: 10.1093/eurheartj/ehaa409
16. De Filippo O, D'Ascenzo F, Angelini F, Bocchino PP, Conrotto F, Saglietto A, et al. Reduced rate of hospital admissions for ACS during Covid-19 outbreak in Northern Italy. *N Engl J Med.* (2020) 383:88–9. doi: 10.1056/NEJMc2009166
17. Mafham MM, Spata E, Goldacre R, Gair D, Curnow P, Bray M, et al. COVID-19 pandemic and admission rates for and management of acute coronary syndromes in England. *Lancet.* (2020) 396:381–9. doi: 10.1016/S0140-6736(20)31356-8
18. Solomon MD, McNulty EJ, Rana JS, Leong TK, Lee C, Sung S-H, et al. The Covid-19 pandemic and the incidence of acute myocardial infarction. *N Engl J Med.* (2020) 383:691–3. doi: 10.1056/NEJMc2015630
19. Zeng J, Huang J, Pan L. How to balance acute myocardial infarction and COVID-19: the protocols from Sichuan Provincial People's Hospital. *Intensive Care Med.* (2020) 46:1111–3. doi: 10.1007/s00134-020-05993-9
20. Jing ZC, Zhu HD, Yan XW, Chai WZ, Zhang S. Recommendations from the peking union medical college hospital for the management of acute myocardial infarction during the COVID-19 outbreak. *Eur Heart J.* (2020) 41:1791–4. doi: 10.1093/eurheartj/ehaa258
21. Szerlip M, Anwaruddin S, Aronow HD, Cohen MG, Daniels MJ, Dehghani P, et al. Considerations for cardiac catheterization laboratory procedures during the COVID-19 pandemic perspectives from the Society for Cardiovascular Angiography and Interventions Emerging Leader Mentorship (SCAI ELM) Members and Graduates. *Catheter Cardiovasc Interv.* (2020) 96:586–97. doi: 10.1002/ccd.28887
22. Chieffo A, Stefanini GG, Price S, Barbato E, Tarantini G, Karam N, et al. EAPCI position statement on invasive management of acute coronary syndromes during the COVID-19 pandemic. *EuroIntervention.* (2020) 16:233–46. doi: 10.4244/EIJY20M05_01
23. Mahmud E, Dauerman HL, Welt FGP, Messenger JC, Rao SV, Grines C, et al. Management of acute myocardial infarction during the COVID-19 pandemic: a position statement from the society for cardiovascular angiography and interventions (SCAI), the American College of Cardiology (ACC), and the American College of Emergency Physicians (ACEP). *J Am Coll Cardiol.* (2020) 76:1375–84. doi: 10.1002/ccd.28946
24. Chieffo A, Stefanini GG, Price S, Barbato E, Tarantini G, Karam N, et al. EAPCI position statement on invasive management of acute coronary syndromes during the COVID-19 pandemic. *Eur Heart J.* (2020) 41:1839–51. doi: 10.1093/eurheartj/ehaa381
25. Lim MA, Huang I, Yonas E, Vania R, Pranata R. A wave of non-communicable diseases following the COVID-19 pandemic. *Diabetes Metab Syndr Clin Res Rev.* (2020) 14:979–80. doi: 10.1016/j.dsx.2020.06.050
26. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent st-segment elevation: task force for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation of. *Eur Heart J.* (2016) 37:267–315. doi: 10.1093/eurheartj/ehv320
27. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. *Eur Heart J.* (2018) 39:119–77. doi: 10.5603/KP.2018.0041
28. Pranata R, Vania R, Tondas AE, Setianto B, Santoso A. A time-to-event analysis on air pollutants with the risk of cardiovascular disease and mortality: a systematic review and meta-analysis of 84 cohort studies. *J Evid Based Med.* (2020) 13:102–15. doi: 10.1111/jebm.12380
29. July J, Pranata R. Impact of the coronavirus disease pandemic on the number of strokes and mechanical thrombectomies: a systematic review and meta-analysis. *J Stroke Cerebrovasc Dis.* (2020) 29:105185. doi: 10.1016/j.jstrokecerebrovasdis.2020.105185

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 19 August 2021
doi: 10.3389/fcvm.2021.710946

Impact of Angiotensin-Converting Enzyme Inhibitors and Angiotensin Receptor Blockers on the Inflammatory Response and Viral Clearance in COVID-19 Patients

Linna Huang[1,2,3†], Ziying Chen[1,2,3,4†], Lan Ni[5], Lei Chen[6], Changzhi Zhou[7], Chang Gao[8], Xiaojing Wu[1,2,3], Lin Hua[9], Xu Huang[1,2,3], Xiaoyang Cui[1,2,3], Ye Tian[1,2,3], Zeyu Zhang[1,2,3] and Qingyuan Zhan[1,2,3]*

[1] Center for Respiratory Diseases, China-Japan Friendship Hospital, Beijing, China, [2] Department of Pulmonary and Critical Care Medicine, China-Japan Friendship Hospital, Beijing, China, [3] National Clinical Research Center for Respiratory Diseases, Beijing, China, [4] Peking University Health Science Center, Beijing, China, [5] Department of Pulmonary and Critical Care Medicine, Zhongnan Hospital of Wuhan University, Wuhan, China, [6] Department of Pulmonary and Critical Care Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [7] Department of Pulmonary and Critical Care Medicine, The Central Hospital of Wuhan, Wuhan, China, [8] Department of Critical Care Medicine, The First Affiliated Hospital of Soochow University, Suzhou, China, [9] School of Biomedical Engineering, Capital Medical University, Beijing, China

OPEN ACCESS

Edited by:
Hendrik Tevaearai Stahel, Bern University Hospital, Switzerland

Reviewed by:
Vicente Estrada, San Carlos University Clinical Hospital, Spain
Frank Davis, University of Michigan, United States

***Correspondence:**
Qingyuan Zhan
drzhanqy@163.com

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

Received: *17 May 2021*
Accepted: *23 July 2021*
Published: *19 August 2021*

Citation:
Huang L, Chen Z, Ni L, Chen L, Zhou C, Gao C, Wu X, Hua L, Huang X, Cui X, Tian Y, Zhang Z and Zhan Q (2021) Impact of Angiotensin-Converting Enzyme Inhibitors and Angiotensin Receptor Blockers on the Inflammatory Response and Viral Clearance in COVID-19 Patients. Front. Cardiovasc. Med. 8:710946. doi: 10.3389/fcvm.2021.710946

Objectives: To evaluate the impact of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) on the inflammatory response and viral clearance in coronavirus disease 2019 (COVID-19) patients.

Methods: We included 229 patients with confirmed COVID-19 in a multicenter, retrospective cohort study. Propensity score matching at a ratio of 1:3 was introduced to eliminate potential confounders. Patients were assigned to the ACEI/ARB group ($n = 38$) or control group ($n = 114$) according to whether they were current users of medication.

Results: Compared to the control group, patients in the ACEI/ARB group had lower levels of plasma IL-1β [(6.20 ± 0.38) vs. (9.30 ± 0.31) pg/ml, $P = 0.020$], IL-6 [(31.86 ± 4.07) vs. (48.47 ± 3.11) pg/ml, $P = 0.041$], IL-8 [(34.66 ± 1.90) vs. (47.93 ± 1.21) pg/ml, $P = 0.027$], and TNF-α [(6.11 ± 0.88) vs. (12.73 ± 0.26) pg/ml, $P < 0.01$]. Current users of ACEIs/ARBs seemed to have a higher rate of vasoconstrictive agents (20 vs. 6%, $P < 0.01$) than the control group. Decreased lymphocyte counts [(0.76 ± 0.31) vs. (1.01 ± 0.45)*10^9/L, $P = 0.027$] and elevated plasma levels of IL-10 [(9.91 ± 0.42) vs. (5.26 ± 0.21) pg/ml, $P = 0.012$] were also important discoveries in the ACEI/ARB group. Patients in the ACEI/ARB group had a prolonged duration of viral shedding [(24 ± 5) vs. (18 ± 5) days, $P = 0.034$] and increased length of hospitalization [(24 ± 11) vs. (15 ± 7) days, $P < 0.01$]. These trends were similar in patients with hypertension.

Conclusions: Our findings did not provide evidence for a significant association between ACEI/ARB treatment and COVID-19 mortality. ACEIs/ARBs might decrease proinflammatory cytokines, but antiviral treatment should be enforced, and

hemodynamics should be monitored closely. Since the limited influence on the ACEI/ARB treatment, they should not be withdrawn if there was no formal contraindication.

Keywords: ACE inhibitor, ARB, inflammatory response, viral clearance, COVID-19

INTRODUCTION

Up to March 31, 2020, the total number of patients with coronavirus disease 2019 has risen sharply to nearly 700,000 globally, with a mortality rate of nearly 5%. Meanwhile, this epidemic seems to be spreading at an exponential rate and has become an urgent public health emergency of international concern.

Several large retrospective studies have revealed that pre-existing cardiovascular disease and diabetes were the most frequent comorbidities of coronavirus disease 2019 (COVID-19) patients (1–3); these patients even had a higher risk of mortality (4, 5) than those with underlying respiratory disease. Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are widely prescribed for these patients. ACEIs/ARBs have an impact on the renin-angiotensin system (RAS) and are postulated to attenuate pulmonary and systemic inflammatory responses, reducing the severity and mortality of viral pneumonia-related acute respiratory distress syndrome (6–8), ultimately by angiotensin-converting enzyme 2 (ACE2) upregulation through the ACE2-Ang-(1-7)-Mas axis (9).

The molecular biology of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is well-established, as it appears to bind to its target cells through ACE2, which is expressed by epithelial cells of the lung, to enable it to infect host cells (10, 11). The expression of ACE2 is substantially increased in patients who are treated with ACE inhibitors and ARBs (12), which promotes SARS-CoV-2 entry into the body, increasing the risk of developing COVID-19 (13, 14).

The controversial pathogenesis as well as the mixed results of several clinical studies (15, 16) of pneumonia with other pathogens made it difficult for physicians to determine whether the use of ACE inhibitors or ARBs should be terminated in patients with COVID-19.

To date, the actual impact of ACE inhibitor and ARB prescriptions on COVID-19 patients has not been assessed in current studies. Therefore, we aimed to evaluate the clinical manifestations and outcomes, especially inflammatory responses and viral clearance, by a multicenter, retrospective cohort study.

MATERIALS AND METHODS

Study Design and Population

We retrospectively included patients with microbiologically confirmed cases of COVID-19 according to the World Health Organization (WHO) (17) and official Chinese guidelines (18) in a multicenter retrospective cohort study performed at three tertiary hospitals in Wuhan, Hubei Province, China (Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology; Zhongnan Hospital of Wuhan University; and the Central Hospital of Wuhan) from February 15, 2020 to March 25, 2020. Patients included in our study were all assessed for eligibility on the basis of positive SARS-CoV-2 nucleic acid testing results by reverse transcription-polymerase chain reaction (RT-PCR) with nasopharyngeal swab samples. However, it was not possible to determine whether the patients had pneumonia, as not all were available for CT scans.

Exclusion Criteria

(1) Patients younger than 18 years old.

(2) Patients still hospitalized at the end of the study.

All patients were treated according to the standard protocols for antiviral, antibiotic, glucocorticoid, and Chinese medicine treatments.

The ethics committee of China-Japan Friendship Hospital approved this study (2020-21-K16). Written informed consent was waived due to the rapid emergence of this infectious disease.

Group Division

We divided the patients into two groups. The ACEI/ARB group included patients who were current users of ACE inhibitors or ARB medication, while non-current users were included as the control group. Patients in the ACEI/ARB group were further divided into subgroups of a continued medication group and a terminated medication group according to the application of ACE inhibitors or ARBs during hospitalization.

Data Collection and Analysis

We collected data on the following parameters from the hospital electronic medical record systems, nursing records, laboratory examination systems, and radiological examinations and obtained standardized data collection forms: demographic characteristics, comorbidities, medication history within 1 month, symptoms at admission, laboratory finding changes from day 1 to day 14, radiological manifestations, treatment during hospitalization and outcome data that contained the rate of in-hospital death and progression, the duration of viral shedding, the length of hospital stay and the time from onset to death or discharge. The primary outcome was mortality at discharge, while the secondary outcomes we observed included the duration of hospital stay, the duration of viral shedding and the differences in inflammatory cytokines.

Patients with cardiovascular disease and diabetes are often taking a combination of medications with statins (19) and oral hypoglycemic agents, especially thiazolidinediones, which have been reported to have an impact on the level of ACE2 by several studies (14, 20). To further control for potential confounders, data on the use of statins, thiazolidinediones and other antihypertensive agents (α receptor blocking agents, β receptor blocking agents, calcium channel blockers and diuretics)

prior to admission in each group were calculated within 90 days (6).

Two researchers also independently reviewed the data collection forms to double check the data collected. Any missing or uncertain records of the epidemiological, medication and symptom data were collected and clarified through direct communication with patients and their families.

We compared the two groups in terms of the above aspects to identify the differences between current users and non-users prior to admission. Then, among the current users of ACEIs/ARBs, an analysis was conducted by comparing the dynamic changes in indicators involved in immune status and inflammatory reactions, as well as the outcomes between patients who continued and terminated medication during hospitalization. As hypertension itself could activate the RAS, patients with hypertension were excluded to avoid potential confounders. A comparison of the immune status, inflammatory reactions and outcomes between the ACEI/ARB and control groups in patients without hypertension was conducted.

Cytokine and Chemokine Measurement

To evaluate the impact of coronavirus and additional ACE inhibitors or ARBs on the production of cytokines or chemokines in the acute phase of the illness, plasma cytokines and chemokines [interleukin 1β (IL-1β), IL-2R, IL-6, IL-8, IL-10, and tumor necrosis factor α (TNF-α)] were measured using chemiluminescent immunoassays (CLIAs) (CFDA approved) by Siemens IMMULITE 1000 for patients according to the manufacturer's instructions.

Definitions

Medications classified as ACE inhibitors were benazepril, perindopril and fosinopril, while the ARBs of the included patients were candesartan, irbesartan, valsartan, olmesartan, telmisartan, and losartan.

Patients were considered a current user of medication if they had a supply of medication to last until the date of hospitalization assuming an 80% compliance rate (6, 21). The patients who did not meet the definition were regarded as non-current users. ACE inhibitors or ARBs were considered to be continued if they were given more than 50% of the days during hospitalization (8); otherwise, they were considered to be terminated.

In-hospital progression was defined as a decline in PaO_2/FiO_2 of more than 100 mmHg or the need for invasive positive pressure ventilation (IPPV) and/or extracorporeal membrane oxygenation (ECMO) during hospitalization.

The duration of viral shedding was defined as the duration of the SARS-CoV-2 RNA test result becoming negative from positive. All patients were routinely reexamined for SARS-CoV-2 nucleic acid testing every 5 days to assess whether it had turned negative.

Shock was defined according to the interim guidance of the WHO for novel coronavirus (22). Acute kidney injury (AKI) was identified and classified on the basis of the highest serum creatinine level or urine output criteria according to the Kidney Disease Improving Global Outcomes Classification (KDIGO) (22, 23). Respiratory failure, coagulation and liver failure were defined as a Sequential Organ Failure Assessment (SOFA) score greater than or equal to two points.

Statistical Analysis

Descriptive statistics included proportions for categorical variables and the mean (standard deviation) or median (interquartile range) for continuous variables. Data were unadjusted unless specifically stated otherwise.

Processing of Missing Data

When the missing rate of vital variables involved in our study was <15%, we used SAS predictive mean matching imputation to replace missing values within each variable, while the variables were abandoned when the missing rate reached 20%.

Processing of the Unbalanced Sample Size: Propensity Score Matching

The propensity score matching (PSM) method was applied at a ratio of 1:3 between the ACEI/ARB group and the control group. The Sequential Organ Failure Assessment (SOFA) score, Charlson's comorbidity index (CCI), and body mass index (BMI) were matched variables in PSM to derive the cohort. The overall balance test was conducted to confirm that the baseline data of the two groups matched successfully.

Proportions were compared using χ^2 or Fisher's exact tests, and continuous variables were compared using the *t*-test or Wilcoxon rank sum test, as appropriate. Statistical significance was defined as a two-tailed *P*-value of $\leq$0.05. SAS software, version 9.4 (SAS Institute Inc.) was used for all analyses.

RESULTS

From February 15, 2020 to March 25, 2020, a total of 229 patients with confirmed cases of COVID-19 were admitted; 51 patients were current users of ACEIs/ARBs, while the other 178 patients were non-current users of the medication. The PSM method was applied at a ratio of 1:3 between the ACEI/ARB group ($n = 38$) and the control group ($n = 114$). The SOFA score and CCI were matched variables in PSM to derive the cohort. Thirteen cases in the ACEI/ARB group and 64 cases in the control group were not matched successfully. The overall balance test was with no significant difference between the two groups ($P = 0.872$). Among the patients with ACEI/ARB medication, 18 continued medication during hospitalization, while the other 20 terminated medication (**Figure 1**). The mean age was 57 ± 12 years, male patients accounted for 52% ($n = 79$), the SOFA score was 1.5 (1–2.3) points, and the CCI was 1 (1–2) prior to admission.

Comparisons of Baseline Prior Hospitalization Between the ACEI/ARB and Control Groups

The ACEI/ARB group included more patients with hypertension (67 vs. 22%, $P < 0.01$) than the control group. The demographic characteristics, other comorbidities, severity of the condition and possible medication histories might have influenced the ACE2 level but did not differ significantly between the two groups. No significant difference was found between the two groups in

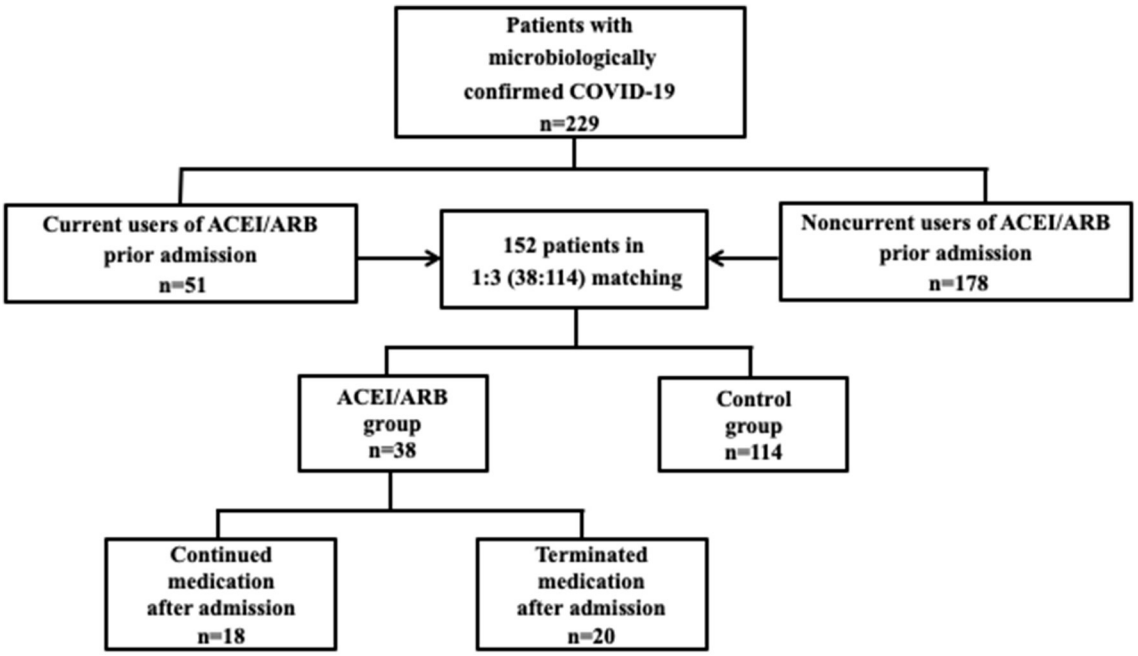


FIGURE 1 | Flowchart. A flowchart illustrated the enrollment of patients in our study. From February 15, 2020 to March 25, 2020, a total of 229 patients with confirmed cases of COVID-19 were admitted; 51 patients were current users of ACEIs/ARBs, while the other 178 patients were non-current users of the medication. The PSM method was applied at a ratio of 1:3 between the ACEI/ARB group ($n = 38$) and the control group ($n = 114$). The SOFA score and CCI were matched variables in PSM to derive the cohort. Among the patients with ACEI/ARB medication, 18 continued medication during hospitalization, while the other 20 terminated medication.

time from onset to hospitalization and to COVID-19 diagnosis (**Table 1**).

Comparisons of Clinical Symptoms, Laboratory Examinations, and Radiological Manifestations on Admission Between the ACEI/ARB and Control Groups

The symptoms, including fever, cough, hemoptysis, dyspnea, fatigue/myalgia and diarrhea, as well as vital signs, with the exception of systolic blood pressure, were not significantly different between the ACEI/ARB group and the control group. Although systolic blood pressure was lower in the study group (116 ± 14 vs. 124 ± 13 mmHg, $P = 0.031$), it was within the normal range. For laboratory examinations, patients with ACE inhibitor or ARB medication had lower lymphocyte counts [(0.76 ± 0.31) vs. (1.01 ± 0.45) $*10^9$/L, $P = 0.027$] than the control group (**Table 2**).

The first measurements of the inflammatory factors, including IL-1β, IL-2R, IL-6, IL-8, IL-10, and TNFα, were taken within 3 days of admission; while the most (97%, 147/152) were within 24 h. The time from COVID-19 diagnose to measurements was (3 ± 2) days. Besides, as the missing rate reached 12–15%, SAS predictive mean matching imputation was applied to replace missing values in each group. The missing rates of IL-2R, serum ferritin, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were as high as 25–35%; therefore, they were abandoned in the statistical analysis. Patients in the ACEI/ARB group had slightly lower levels of proinflammatory cytokines, including IL-1β [(6.20 ± 0.38) vs. (9.30 ± 0.31) pg/ml, $P = 0.020$], IL-6 [(31.86 ± 4.07) vs. (48.47 ± 3.11) pg/ml, $P = 0.041$], IL-8 [(34.66 ± 1.90) vs. (47.93 ± 1.21) pg/ml, $P = 0.027$], and TNF-α [(6.11 ± 0.88) vs. (12.73 ± 0.26) pg/ml, $P < 0.01$], and higher levels of the anti-inflammatory cytokine IL-10 [(9.91 ± 0.42) vs. (5.26 ± 0.21) pg/ml, $P = 0.012$] than the control group (**Table 2**).

Comparison of Organ Function, Treatment and Outcomes During Hospitalization Between the ACEI/ARB and Control Groups

Current users of ACEIs/ARBs seemed to have a higher rate of vasoconstrictive agent application (18 vs. 7%, $P < 0.01$) than the control group; however, the percentages of respiratory failure, shock, AKI, coagulation failure, and liver failure were not different between the two groups. In addition, the necessities for invasive IPPV and ECMO were not decreased in the ACEI/ARB group (**Table 3**).

The duration of viral shedding [(24 ± 5) vs. (18 ± 5) days, $P = 0.034$], length of hospital stay [(24 ± 11) vs. (15 ± 7) days, $P < 0.01$], and time from onset to death or discharge [(32 ± 10) vs. (25 ± 7) days, $P < 0.01$] were longer in the ACEI/ARB group than in the control group, while no difference was found in the rate of in-hospital progression or death (**Table 3**).

TABLE 1 | Baseline variables in the two groups prior to admission.

	All ($n = 152$)	ACEI/ARB group ($n = 38$)	Control group ($n = 114$)	*P*
Age, years, mean ± SD	57 ± 12	57 ± 11	58 ± 18	0.671
Gender (men), number (%)	79 (52%)	19 (51%)	60 (53%)	0.533
Body mass index, kg/m^2, mean ± SD	21.0 ± 6.9	21.1 ± 6.4	21.0 ± 7.0	0.838
Comorbidities, number (%)				
Hypertension	55 (36%)	30 (67%)	25 (22%)	<0.001[b]
Diabetes	37 (24%)	10 (27%)	27 (24%)	0.217
Coronary heart disease	17 (11%)	6 (16%)	11 (10%)	0.071
Chronic heart failure	6 (4%)	2 (5%)	4 (4%)	0.622
Underlying lung disease	18 (12%)	7 (18%)	11 (10%)	0.094
Chronic kidney disease	2 (1%)	1 (3%)	1 (1%)	0.512
Chronic liver dysfunction	3 (2%)	0 (0%)	3 (3%)	0.425
Malignancy	3 (2%)	0 (0%)	3 (3%)	0.186
History of smoking, number (%)	23 (15%)	8 (21%)	15 (13%)	0.081
Other medication history within 90 days, number (%)				
Corticosteroids	0 (0%)	0 (0%)	0 (0%)	1
Immunosuppressants	0 (0%)	0 (0%)	0 (0%)	1
Statins	21 (14%)	6 (16%)	15 (13%)	0.214
Thiazolidinediones	1 (1%)	0 (0%)	1 (1%)	0.996
α receptor blocking agent	4 (3%)	1 (3%)	3 (3%)	0.820
β receptor blocking agent	19 (13%)	5 (13%)	14 (12%)	0.731
CCB	19 (13%)	5 (13%)	14 (12%)	0.731
Diuretics	16 (11%)	4 (11%)	12 (11%)	1
SOFA Score, points (IQR)	1.5 (1–2.3)	1.5 (1–2.5)	1.5 (1–2)	0.879
CCI, points (IQR)	1 (1–2)	1 (1–2)	1 (1–2)	1
Treatment before hospital, number (%)				
Methylprednisolone	10 (7%)	3 (8%)	7 (6%)	0.091
Antibiotic therapy	92 (61%)	22 (58%)	70 (61%)	0.429
Antiviral therapy	102 (67%)	22 (57%)	80 (70%)	0.239
Time from onset to hospital admission, days, mean ± SD	10 ± 6	11 ± 3	10 ± 6	0.296
Time from onset to diagnosis, days, mean ± SD	7 ± 5	7 ± 5	7 ± 2	0.8

[b]*P < 0.01; CCB, calcium channel blocker; SOFA, Sequential Organ Failure Assessment; CCI, Charlson's Comorbidity Index (18).*

Subgroup Analyses: Comparison Between Patients Who Continued and Terminated Medication During Hospitalization

Among the patients in the ACEI/ARB group, 18 continued medication during hospitalization, while the other 20 terminated medication for several reasons. The baseline variables were with no significant difference between the two groups (**Supplementary Table 1**). The dynamic changes in lymphocytes and inflammatory factors at the first, seventh, and fourteenth days after hospitalization as well as the outcomes were compared between the two groups. The missing rates of IL-2R and IL-8 at seven days and 14 days after admission were extremely high and were not included in the analysis. Patients with continued use of ACEIs/ARBs had consistently lower levels of lymphocytes, IL-1β, IL-6, and TNF-α but maintained higher levels of IL-10 on the seventh and fourteenth days than patients who terminated medication during hospitalization. However, the patients who terminated the medication had a trend of elevated lymphocyte counts [day 1, day 7, day 14: (0.82 ± 0.47) vs. (1.41 ± 0.74) vs. (1.69 ± 0.45)*10^9/L, $P = 0.029$] and IL-1β [day 1, day 7, day 14: (6.03 ± 3.19) vs. (10.78 ± 6.88) vs. (13.75 ± 5.26) pg/ml, $P < 0.01$] from the first day to the fourteenth day (**Figure 2**, **Supplementary Table 2**).

The duration of viral shedding [(27 ± 4) vs. (21 ± 5) days, $P = 0.032$], length of hospital stay [(26 ± 10) vs. (20 ± 3) days, $P = 0.044$], and time from onset to death or discharge [(34 ± 9) vs. (29 ± 10) days, $P = 0.019$] were longer in the continued medication group than in the terminated medication group. The rates of in-hospital progression and death were not significantly different between the two groups (**Table 4**).

Subgroup Analyses: A Comparison of the Immune Status, Inflammatory Reactions and Outcomes Between the ACEI/ARB and Control Groups in Patients With Hypertension

Among 55 patients with hypertension, 30 patients were divided into the study group (ACEI/ARB group), and the other 25 patients were in the control group.

Compared with the control group, the patients in the study group had lower levels of IL-1β [(6.33 ± 0.56) vs. (8.27 ± 0.14)

TABLE 2 | Clinical, laboratory findings, and radiological manifestations in the two groups on admission.

	All ($n = 152$)	ACEI/ARB group ($n = 38$)	Control group ($n = 114$)	*P*
Initial symptoms, number (%)				
Fever (≥37.3°C)	140 (92%)	35 (92%)	105 (92%)	0.981
Cough	109 (72%)	27 (70%)	82 (72%)	0.866
Productive cough	60 (39%)	16 (42%)	44 (39%)	0.605
Hemoptysis	3 (2%)	1 (3%)	2 (2%)	0.263
Dyspnea	78 (51%)	20 (53%)	58 (51%)	0.432
Fatigue or myalgia	67 (44%)	16 (43%)	51 (45%)	0.619
Diarrhea	46 (30%)	12 (31%)	34 (30%)	0.764
Initial signs, mean ± SD				
Highest temperature, °C	38.4 ± 0.7	38.5 ± 1.1	38.3 ± 0.4	0.461
Respiratory rate, breaths/min	23 ± 3	22 ± 3	23 ± 3	0.709
Heart rate, beats/min	96 ± 11	97 ± 8	96 ± 14	0.338
Systolic blood pressure, mmHg	123 ± 10	116 ± 14	124 ± 13	0.031[a]
SpO_2, %	94 ± 4	93 ± 3	94 ± 4	0.741
FiO_2, %	40 ± 18	42 ± 15	40 ± 17	0.302
Laboratory examination, mean ± SD				
Blood routine				
WBC, $*10^9$/L	5.94 ± 3.00	6.27 ± 3.21	5.80 ± 2.97	0.085
Neutrophil count, $*10^9$/L	4.40 ± 2.99	5.21 ± 3.29	4.39 ± 3.01	0.097
Lymphocytes, $*10^9$/L	0.89 ± 0.40	0.76 ± 0.31	1.01 ± 0.45	0.027[a]
Biochemical examination				
ALT, U/L	43 ± 4	42 ± 4	43 ± 4	0.747
AST, U/L	40 ± 5	44 ± 4	40 ± 5	0.841
TBIL, mmol/L	11.3 ± 5.2	11.0 ± 5.9	11.4 ± 5.0	0.660
Scr, μmol/L	79.2 ± 2.7	77.5 ± 2.2	80.1 ± 3.6	0.915
LDH, U/L	295 ± 89	301 ± 77	294 ± 91	0.617
TnT, pg/ml	11 ± 1	12 ± 1	11 ± 1	0.770
NT-proBNP, pg/ml	401 ± 55	411 ± 55	397 ± 51	0.528
Inflammatory factors				
IL-1β, pg/ml	8.02 ± 0.33	6.20 ± 0.38	9.30 ± 0.31	0.020[a]
IL-2R, U/ml	796.02 ± 27.40	724.25 ± 52.30	807.23 ± 26.21	0.246
IL-6, pg/ml	47.11 ± 3.26	31.86 ± 4.07	48.47 ± 3.11	0.041[a]
IL-8, pg/ml	46.03 ± 1.85	34.66 ± 1.90	47.93 ± 1.21	0.027[a]
IL-10, pg/ml	6.37 ± 0.37	9.91 ± 0.42	5.26 ± 0.21	0.012[b]
TNF-α, pg/ml	11.21 ± 0.44	6.11 ± 0.88	12.73 ± 0.26	<0.001[b]
PCT, ng/ml	0.27 ± 0.07	0.26 ± 0.03	0.29 ± 0.08	0.619
Coagulation function				
PT, s	14 ± 3	14 ± 1	14 ± 1	0.995
APTT, s	42 ± 5	44 ± 3	42 ± 5	0.881
D-Dimer, μg/ml	2.19 ± 0.44	2.33 ± 0.47	2.12 ± 0.46	0.448
Chest CT manifestations, number (%)				
Bilateral lesion	82 (54%)	19 (49%)	63 (55%)	0.374
GGO	89 (59%)	19 (49%)	70 (61%)	0.310
Consolidation	36 (24%)	11 (29%)	25 (22%)	0.229

[a]$P < 0.05$; [b]$P < 0.01$; SpO_2, saturation of peripheral oxygen; FiO_2, fraction of inspiration; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; Scr, creatinine; LDH; lactate dehydrogenase; TnT, troponin T; NT-proBNP, N-terminal pro-brain natriuretic peptide; IL-1β, interleukin-1β; IL-2R, interleukin-2R; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; TNF-α, tumor necrosis factor-α; PCT, procalcitonin; PT, prothrombin time; APTT, activated partial thromboplastin time; GGO, ground-glass opacity.

pg/ml, $P = 0.026$], IL-6 [(40.16 ± 12.59) vs. (52.33 ± 14.09) pg/ml, $P = 0.030$], and IL-8 [(31.60 ± 2.97) vs. (42.83 ± 3.27) pg/ml, $P = 0.030$] on admission. Regarding clinical outcomes, the duration of viral shedding [(26 ± 6) vs. (19 ± 4) days, $P = 0.029$] and time from onset to death or discharge [(30 ± 10) vs. (24 ± 8) days, $P = 0.031$] were longer in the study group than in the

TABLE 3 | Organ function, treatments and outcomes in the two groups during hospitalization.

	All (n = 152)	ACEI/ARB group (n = 38)	Control group (n = 114)	P
Organ failure*, number (%)				
Respiratory failure	25 (16%)	8 (20%)	17 (15%)	0.092
Shock	13 (9%)	4 (11%)	8 (7%)	0.060
AKI	15 (10%)	4 (11%)	11 (10%)	0.829
Coagulation failure	3 (2%)	1 (3%)	2 (2%)	0.664
Liver failure	15 (10%)	4 (11%)	11 (10%)	0.796
Treatment, number (%)				
Antibiotics	105 (69%)	24 (64%)	81 (71%)	0.461
Antiviral treatment	145 (95%)	36 (92%)	109 (96%)	0.334
Glucocorticoids	49 (32%)	11 (30%)	38 (33%)	0.612
Intravenous immunoglobin	36 (24%)	9 (23%)	27 (24%)	0.552
Standard oxygen therapy	132 (87%)	35 (92%)	97 (85%)	0.080
HFNO	28 (18%)	7 (18%)	21 (18%)	0.927
NPPV	18 (12%)	5 (12%)	13 (11%)	0.327
IPPV	17 (11%)	4 (11%)	13 (11%)	0.629
ECMO	4 (3%)	1 (3%)	3 (3%)	0.994
Vasoconstrictive agents	15 (10%)	7 (18%)	8 (7%)	<0.01[b]
Outcome				
In-hospital progression[#], number (%)	28 (18%)	6 (16%)	22 (19%)	0.326
In-hospital death, number (%)	15 (10%)	4 (10%)	11 (10%)	0.983
Hospital length of stay, days, mean ± SD	17 ± 8	24 ± 11	15 ± 7	<0.01[b]
Duration of viral shedding, days, mean ± SD	19 ± 3	24 ± 5	18 ± 5	0.034[a]
Time from onset to death or discharge, days, mean ± SD	27 ± 9	32 ± 10	25 ± 7	<0.01[b]

*[a]$P < 0.05$; [b]$P < 0.01$; *Shock was defined according to the interim guidance of the WHO for novel coronavirus (22, 23). AKI was identified and classified on the basis of the highest serum creatinine level or urine output criteria according to kidney disease, improving global outcome classification (23, 24). Respiratory failure, coagulation and liver failure were defined as a SOFA score greater than or equal to two points. [#]Defined as a decline in PaO_2/FiO_2 > 100 mmHg or the need for IPPV and/or ECMO during hospitalization. AKI, acute kidney injury; HFNO, high flow nasal oxygenation; NPPV, noninvasive positive pressure ventilation; IPPV, invasive positive pressure ventilation; ECMO, extracorporeal membrane oxygenation.*

control group; however, no difference was detected in the rate of in-hospital progression and death between the two groups.

DISCUSSION

To our knowledge, this is the first study to thoroughly evaluate the inflammatory responses and viral clearance of COVID-19 patients treated with ACEIs/ARBs by a multicenter, retrospective cohort control study and to allow dynamic observation of inflammatory responses by continuous monitoring from the first to the fourteenth day after admission.

The major findings of our study were that ACEIs/ARBs inhibited the proinflammatory response but promoted the anti-inflammatory response and persistently decreased lymphocytes, thus extending the duration of viral shedding and the length of hospital stay. Antiviral treatments should be enforced in those patients. In addition, since current users of ACEIs/ARBs seem to have a higher necessity of vasoconstrictive agents, hemodynamics should be monitored closely during medication use. The message to the physician was that the influence on the ACEI/ARB treatment was limited, and they should not be withdrawn if there was no formal contraindication.

Inflammation is mediated by proinflammatory cytokines and anti-inflammatory cytokines. Inappropriate elevated expression of proinflammatory cytokines can result in sepsis, tissue destruction, or death (21, 24). Our study revealed that the plasma levels of IL-1β, IL-6, IL-8, and TNF-α in patients taking ACEI/ARBs were lower than those in patients not without medication; in addition, persistently lower levels of proinflammatory factors were maintained in patients who continued medication during hospitalization, which was consistent with the previous experimental results by Gullestad et al. (25) with the conclusion that high-dose enalapril was associated with a significant decrease in IL-6 activity in patients with severe chronic heart failure. The specific organ and systemic inflammatory responses were postulated to attenuate through a reduction in the level of cytokines, which might be explained by the attenuating effects of ACE inhibitors through the deactivation of the ACE-AngII-AT1 axis but the stimulation of the ACE2-Ang-(1-7)-Mas axis in a feedback mechanism (9, 26, 27) as a negative regulator with attenuated cytokines and thus protecting the patients from organ injury. Consequently, some authors (28, 29) have speculated that the use of ACEIs/ARBs might actually be a potentially beneficial intervention in those with COVID-19.

Apart from organ protection by attenuating the inflammatory response, basic investigation has shown that bradykinin and substance P produced by ACE inhibitors sensitize the sensory

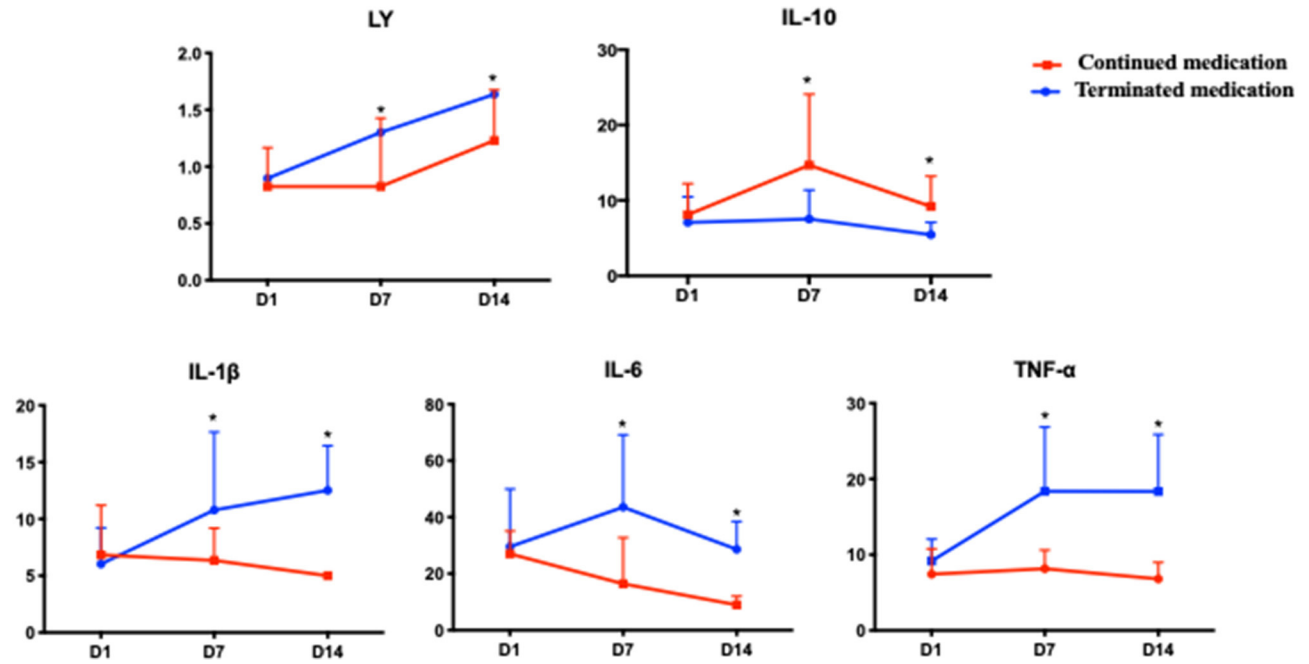


FIGURE 2 | The dynamic changes in the lymphocyte counts and inflammatory factors between patients who continued and those who terminated ACEIs/ARBs during hospitalization. Patients with continued use of ACEIs/ARBs had consistently lower levels of lymphocytes, IL-1β, IL-6, and TNF-α but maintained higher levels of IL-10 on the seventh and fourteenth days than patients who terminated medication during hospitalization. However, the patients who terminated the medication had a trend of elevated lymphocyte counts and IL-1β from the first day to the fourteenth day. *$p < 0.01$.

TABLE 4 | Outcomes in patients who continued and those who terminated ACEIs/ARBs during hospitalization.

Outcomes	Continued ACEIs/ARBs ($n = 18$)	Terminated ACEIs/ARBs ($n = 20$)	*P*
In-hospital progression[#]	3 (17%)	3 (15%)	0.611
In-hospital death	2 (11%)	2 (10%)	0.709
Duration of viral shedding, days	27 ± 4	20 ± 5	0.032[a]
Hospital length of stay, days	26 ± 10	20 ± 3	0.044[a]
Time from onset to death or discharge, days	34 ± 9	29 ± 10	0.019[a]

[a]*P < 0.05;* [#]*Defined as a decline in PaO_2/FiO_2 > 100 mmHg or the need for IPPV and/or ECMO during hospitalization.*

nerves of the airways and enhance the cough reflex (30, 31), which plays a protective role against pathogens. These two mechanics made it possible to improve the outcome in patients with pneumonia. Mortensen et al. (6) found a significant decrease in mortality, the length of hospital stay, and mechanical ventilation in patients taking ACE/ARBs who were hospitalized with pneumonia compared to a matched cohort. A meta-analysis (32) that included 19 studies noted that patients taking ACE inhibitors were associated with a significant approximately one-third reduction in the risk of pneumonia compared with controls. In addition, a recent study (8) by Christopher Henry also observed lower rates of death and intubation with continued use of ACE inhibitors than with terminated use (OR = 0.25; 95% CI, 0.09–0.64) throughout the hospital stay in cases of viral pneumonia not due to coronavirus. Unfortunately, our study did not find decreased mortality in patients with current use of ACEI/ARBs, even though we analyzed patients with continued medication during hospitalization and combined with hypertension to avoid potential confounding factors. The most likely explanation was that our study included a small number of patients, while most of their patients had mild cases as determined by SOFA scores and without excessive inflammatory reactions, which was the target for ACE inhibitors or ARBs.

What noteworthy was that ACEI/ARBs increased the necessity of vasoconstrictive agents. It could be explained by the nature of the antihypertensive agents and came as a revelation to us that the hemodynamics should be monitored closely during medication.

Our research also revealed that ACE inhibitors or ARBs led to prolonged viral shedding and extended the length of hospitalization. SARS-CoV-2 appears to bind to its target cells through angiotensin-converting enzyme 2 (ACE2). ACE inhibitors or ARBs upregulate ACE2 receptor expression in humans (33) by blocking the classic ACE pathway; thus, it is theoretically possible that the pre-existing use of these drugs

might predispose a person to infection with a greater viral load of SARS-CoV-2 (13). This hypothesis was supported by the evidence of Ferrario that there was a 4.7-fold increase in cardiac ACE2 mRNA by an ACE inhibitor (34). Decreased lymphocyte counts and elevated plasma levels of IL-10 were also important discoveries in patients with ACEI/ARBs. Moreover, the lymphocyte counts in patients with continued use of medication during hospitalization recovered slowly, as observed by successive monitoring on the first to fourteenth days. The immune status was weakened by lymphocytopenia and elevated anti-inflammatory cytokines in patients taking ACEI/ARBs, which might be another reason for the slow viral clearance. As the important criterion for discharge was the negative conversion of the SARS-CoV-2, prolonged viral shedding led to an extended length of hospitalization. This might be the defect of the ACEI/ARBs and might explain the mixed results and controversy about their prescription in COVID-19 patients. For this reason, antiviral therapy in patients taking ACEI/ARBs should be reinforced, and their viral load should be monitored closely.

An autopsy report revealed that mononuclear inflammatory infiltration dominated by lymphocytes was observed in the lungs, but no virus inclusion bodies were found (35). We could then propose a hypothesis that cytokines released by inflammatory storms secondary to viral infection might be more important in the death of critically ill patients with COVID-19 than the viral infection itself in a certain period. From this perspective, it is possible that ACEI/ARBs might improve the outcome in critically ill patients with excessive inflammatory responses or severe multiple organ failure; when the inflammatory storm gradually diminishes, the focus of therapy should be on clearance of the virus and the enhancement of the immune system. Prospective cohort and randomized controlled trials are needed to confirm this hypothesis and examine potential mechanisms of action.

Our study was limited by the small number of patients included and by not strictly excluding confounding factors. We especially noticed that the number of patients with hypertension was much higher in the ACEI/ARB group, which might be an important confounding factor. However, by subgroup analyze in patients with hypertension, we found similar results. The prospective randomized controlled studies designed by increasing the sample size and strictly excluding potential confounders to explore the impact of ACE/ARBs on inflammatory responses, viral clearance and the mortality in COVID-19 patients should be encouraged in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of China-Japan Friendship Hospital approved this study. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception and design of the study or to the data acquisition, analysis, or interpretation, reviewed and approved the final manuscript, and significantly contributed to this study. QZ took full responsibility for the integrity of the submission and publication and was involved in the study design. LHuang and ZC involved in data collection, had full access to all of the data in the study, took responsibility for the integrity of the data and were responsible for data verification, as well as the drafting of the manuscript. LHua took the responsibility for statistical analysis and the accuracy of the data analysis. Others involved in data collection, had full access to all of the data in the study, and took responsibility for the integrity of the data.

FUNDING

The study was supported by the grants from the Zhejiang University special scientific research fund for COVID-19 prevention and control (2020XGZX008). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.710946/full#supplementary-material

REFERENCES

1. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med.* (2020) 8:e26. doi: 10.1016/S2213-2600(20)30079-5
2. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of Covid-19 in China. *N Engl J Med.* (2020) 382:1859–62. doi: 10.1056/NEJMc2005203
3. Zhang JJ, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy.* (2020) 75:1730–41. doi: 10.1111/all.14238
4. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med.* (2020) 180:934–43. doi: 10.1001/jamainternmed.2020.0994
5. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3
6. Mortensen EM, Pugh MJ, Copeland LA, Restrepo MI, Cornell JE, Anzueto A, et al. Impact of statins and angiotensin-converting enzyme inhibitors on mortality of subjects hospitalised with pneumonia. *The Eur Respir J.* (2008) 31:611–7. doi: 10.1183/09031936.00162006

7. Wu A, Good C, Downs JR, Fine MJ, Pugh MJ, Anzueto A, et al. The association of cardioprotective medications with pneumonia-related outcomes. *PLoS ONE.* (2014) 9:e85797. doi: 10.1371/journal.pone.0085797
8. Henry C, Zaizafoun M, Stock E, Ghamande S, Arroliga AC, White HD. Impact of angiotensin-converting enzyme inhibitors and statins on viral pneumonia. *Proceedings.* (2018) 31:419–23. doi: 10.1080/08998280.2018.1499293
9. Tan WSD, Liao W, Zhou S, Mei D, Wong WF. Targeting the renin-angiotensin system as novel therapeutic strategy for pulmonary diseases. *Curr Opin Pharmacol.* (2018) 40:9–17. doi: 10.1016/j.coph.2017.12.002
10. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell.* (2020) 181:281–92.e6. doi: 10.1016/j.cell.2020.02.058
11. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell.* (2020) 181:271–80.e8. doi: 10.1016/j.cell.2020.02.052
12. Li XC, Zhang J, Zhuo JL. The vasoprotective axes of the renin-angiotensin system: physiological relevance and therapeutic implications in cardiovascular, hypertensive and kidney diseases. *Pharmacological Res.* (2017) 125(Pt A):21–38. doi: 10.1016/j.phrs.2017.06.005
13. Thomson G. COVID-19: social distancing, ACE2 receptors, protease inhibitors and beyond? *Int J Clin Pract.* (2020) 74:e13503. doi: 10.1111/ijcp.13503
14. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir Med.* (2020) 8:e21. doi: 10.1016/S2213-2600(20)30116-8
15. Okaishi K, Morimoto S, Fukuo K, Niinobu T, Hata S, Onishi T, et al. Reduction of risk of pneumonia associated with use of angiotensin I converting enzyme inhibitors in elderly inpatients. *Am J Hypertens.* (1999) 12(8 Pt 1):778–83. doi: 10.1016/S0895-7061(99)00035-7
16. Van de Garde EM, Souverein PC, van den Bosch JM, Deneer VH, Leufkens HG. Angiotensin-converting enzyme inhibitor use and pneumonia risk in a general population. *Eur Respir J.* (2006) 27:1217–22. doi: 10.1183/09031936.06.00110005
17. World Health Organization. *Clinical Management of Severe Acute Respiratory Infection When Novel Coronavirus (nCoV) Infection is Suspected: Interim Guidance.* (2020). Available online at: https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected
18. National Health Commission of the People's Republic of China. *Chinese Management Guideline for COVID-19 (Version 7.0).* (2020). Available online at: http://211.136.65.146/cache/www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989/files/ce3e6945832a438eaae415350a8ce964.pdf?ich_args2=65-11155015043898_f32a0b9f969d8670abb9ee3d42d8e898_10001002_9c896c2ad2caf0d59239518939a83798_0ad7c3e0d3161a7f69a04f61b3ce861b.
19. Chopra V, Rogers MA, Buist M, Govindan S, Lindenauer PK, Saint S, et al. Is statin use associated with reduced mortality after pneumonia? A systematic review and meta-analysis. *Am J Med.* (2012) 125:1111–23. doi: 10.1016/j.amjmed.2012.04.011
20. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *J Virol.* (2020) 94:e00127-20. doi: 10.1128/JVI.00127-20
21. Mortensen EM, Nakashima B, Cornell J, Copeland LA, Pugh MJ, Anzueto A, et al. Population-based study of statins, angiotensin II receptor blockers, and angiotensin-converting enzyme inhibitors on pneumonia-related outcomes. *Clin Infect Dis.* (2012) 55:1466–73. doi: 10.1093/cid/cis733
22. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
23. Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. *KDIGO Clinical Practice Guideline for Acute Kidney Injury.* (2012). Available online at: https://kdigo.org/wp-content/uploads/2016/10/KDIGO-2012-AKI-Guideline-English.pdf (accessed January 23, 2020).
24. Nathan C. Points of control in inflammation. *Nature.* (2002) 420:846–52. doi: 10.1038/nature01320
25. Gullestad L, Aukrust P, Ueland T, Espevik T, Yee G, Vagelos R, et al. Effect of high- versus low-dose angiotensin converting enzyme inhibition on cytokine levels in chronic heart failure. *J Am Coll Cardiol.* (1999) 34:2061–7. doi: 10.1016/S0735-1097(99)00495-7
26. Duprez DA. Role of the renin-angiotensin-aldosterone system in vascular remodeling and inflammation: a clinical review. *J Hypertension.* (2006) 24:983–91. doi: 10.1097/01.hjh.0000226182.60321.69
27. Marchesi C, Paradis P, Schiffrin EL. Role of the renin-angiotensin system in vascular inflammation. *Trends Pharmacol Sci.* (2008) 29:367–74. doi: 10.1016/j.tips.2008.05.003
28. Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci.* (2020) 63:364–74. doi: 10.1007/s11427-020-1643-8
29. Sun M, Yang JM, Sun YP, Su GH. Inhibitors of RAS might be a good choice for the therapy of COVID-19 pneumonia. *Zhonghua Jie He He Hu Xi Za Zhi.* (2020) 43:219–22. doi: 10.3760/cma.j.issn.1001-0939.2020.03.016
30. Fox AJ, Lalloo UG, Belvisi MG, Bernareggi M, Chung KF, Barnes PJ. Bradykinin-evoked sensitization of airway sensory nerves: a mechanism for ACE-inhibitor cough. *Nat Med.* (1996) 2:814–7. doi: 10.1038/nm0796-814
31. Tomaki M, Ichinose M, Miura M, Hirayama Y, Kageyama N, Yamauchi H, et al. Angiotensin converting enzyme (ACE) inhibitor-induced cough and substance P. *Thorax.* (1996) 51:199–201. doi: 10.1136/thx.51.2.199
32. Caldeira D, Alarcao J, Vaz-Carneiro A, Costa J. Risk of pneumonia associated with use of angiotensin converting enzyme inhibitors and angiotensin receptor blockers: systematic review and meta-analysis. *BMJ.* (2012) 345:e4260. doi: 10.1136/bmj.e4260
33. Vuille-dit-Bille RN, Camargo SM, Emmenegger L, Sasse T, Kummer E, Jando J, et al. Human intestine luminal ACE2 and amino acid transporter expression increased by ACE-inhibitors. *Amino Acids.* (2015) 47:693–705. doi: 10.1007/s00726-014-1889-6
34. Turgeon RD, Kolber MR, Loewen P, Ellis U, McCormack JP. Higher versus lower doses of ACE inhibitors, angiotensin-2 receptor blockers and beta-blockers in heart failure with reduced ejection fraction: systematic review and meta-analysis. *PLoS ONE.* (2019) 14:e0212907. doi: 10.1371/journal.pone.0212907
35. Wang H-j, Du S-h, Yue X, Chen C-x. Review and prospect of pathological features of corona virus disease. *Fa Yi Xue Za Zhi.* (2020) 36:16–20. doi: 10.12116/j.issn.1004-5619.2020.01.004

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 09 September 2021
doi: 10.3389/fcvm.2021.732518

Association Between Cardiovascular Risk Factors and the Severity of Coronavirus Disease 2019: Nationwide Epidemiological Study in Korea

Kyoung Ae Kong[1†], *Sodam Jung*[2†], *Mina Yu*[3], *Junbeom Park*[2] *and In Sook Kang*[2*]

[1] Department of Preventive Medicine, College of Medicine, Ewha Womans University, Seoul, South Korea, [2] Division of Cardiology, Department of Internal Medicine, Ewha Womans University Mokdong Hospital, College of Medicine, Ewha Womans University, Seoul, South Korea, [3] Division of Nephrology, Department of Internal Medicine, Ewha Womans University Seoul Hospital, College of Medicine, Ewha Womans University, Seoul, South Korea

OPEN ACCESS

Edited by:
Shuyang Zhang,
Peking Union Medical College Hospital (CAMS), China

Reviewed by:
Christoph Sinning,
University Heart and Vascular Center Hamburg (UHZ), Germany
Giulio Francesco Romiti,
Sapienza University of Rome, Italy

***Correspondence:**
In Sook Kang
pinkvision@ewha.ac.kr

[†]These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Cardiovascular Epidemiology and Prevention, a section of the journal Frontiers in Cardiovascular Medicine

Received: 29 June 2021
Accepted: 13 August 2021
Published: 09 September 2021

Citation:
Kong KA, Jung S, Yu M, Park J and Kang IS (2021) Association Between Cardiovascular Risk Factors and the Severity of Coronavirus Disease 2019: Nationwide Epidemiological Study in Korea. Front. Cardiovasc. Med. 8:732518. doi: 10.3389/fcvm.2021.732518

Background: Acute respiratory viral infections can result in cardiovascular involvement, with such patients having a significantly higher mortality rate than those without cardiovascular involvement. Due to the ongoing coronavirus disease 2019 (COVID-19) pandemic, it is important to determine whether cardiovascular risk factors are associated with the severity of COVID-19.

Methods: These nationwide data were provided by the Korea Disease Control and Prevention Agency. We defined a patient as having a "critical illness" if they required more than invasive mechanical ventilation and "fatal illness" if they died.

Results: Among the total 5,307 patients, 2,136 (40.8%) were male. The critical illness rate was 5.1% (males: 6.7, females: 4.0%) and the fatality rate was 4.54%. The multivariable analysis showed that age ≥60 years, male sex, diabetes mellitus, hypertension, heart failure, chronic kidney disease, cancer, and dementia were independent risk factors for critical illness. The risk scoring model showed the significance of multiple risk factors. Patients with four risk factors; old age (≥60 years), male sex, hypertension, and diabetes mellitus had a more than a 100 times higher risk for severe COVID-19 than those without these risk factors (OR; 95% confidence interval, 104; 45.6–240.6 for critical, 136.2; 52.3–3547.9 for fatal illness).

Conclusions: This study demonstrated that cardiovascular risk factors are also significant risk factors for severe COVID-19. In particular, patients who have multiple cardiovascular risk factors are more likely to progress to severe COVID-19. Therefore, early and appropriate treatment of these patients is crucial.

Keywords: COVID-19, SARS-CoV-2, cardiovascular disease, risk factor, mortality

INTRODUCTION

The risk of myocardial infarction is known to be proportional to the severity of an acute respiratory infection (1). Acute viral pneumonia can result in cardiovascular diseases, such as heart failure, acute myocardial infarction, arrhythmia, and myocarditis. Patients with cardiovascular involvement have a significantly higher mortality rate than those without cardiovascular involvement (1–4).

Since the end of 2019, coronavirus disease 2019 (COVID-19) caused by a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread to more than 200 countries around the world. It has displayed high transmission power, severity, and mortality. The clinical manifestation of COVID-19 is broad, ranging from no symptoms to fever, acute respiratory distress syndrome, multiple organ failure, and death (5). Many countries around the world have been struggling to contain COVID-19, and so far, no definite treatment has been developed. Therefore, it is important to assess the risk factors that affect the severity and fatality of COVID-19.

Excess mortality was reported in the United States during the influenza pandemic. Although the association with secondary infections was not identified, cardiovascular event may have been a contributing factor (1, 6). A recent observational study showed that underlying cardiovascular diseases, such as coronary artery disease, congestive heart failure, and arrhythmia were associated with an increased risk of in-hospital death by COVID-19 (7). Early on in the pandemic, studies in China showed that people with underlying conditions such as hypertension, diabetes mellitus, cerebrovascular disease, or cardiovascular disease were more likely to be admitted to the intensive care unit (5).

Hence, in this study, we focused and analyzed the clinical implications of cardiovascular risk factors and the presence or absence of cardiovascular disease on the outcome and severity of COVID-19. Research on cardiovascular risk factors, including hypertension and diabetes mellitus, which are prevalent in the entire population, and the effect of sex and age on the severity of COVID-19 are not only important to cardiologists, but are significant public health topics. Further, this study can provide important prognostic information for patients.

The previous studies are mostly conducted early on in the pandemic and have limited study populations. The Republic of Korea reduced the spread of COVID-19 by proactively and systematically identifying patients with COVID-19 based on the national health insurance system, which is a single-payer, compulsory subscription system. Accordingly, we have a good basis for analyzing the characteristics of the clinical features of COVID-19 using data from across Korea. Here, we analyzed whether cardiovascular disease and/or the associated risk factors affect the severity of COVID-19 using data provided by the Korea Disease Control and Prevention Agency (KDCA).

MATERIALS AND METHODS

Data Source and Study Population

This is a retrospective cohort study, using nationwide data from the Republic of Korea. Study candidates are the patients with COVID-19 who had been hospitalized, among the patients released from isolation or died as of April 30, 2020. Since the first confirmed case of COVID-19 in Korea on January 19, 2020, the KDCA has actively tracked almost all patients and their contacts in an attempt to control the spread of COVID-19. Further, cumulative statistics are released daily on a public web site (http://ncov.mohw.go.kr/en/) and through the media. The KDCA developed a registry of confirmed COVID-19 cases and provided the anonymized data to select researchers. The data includes only COVID-19 patients that had been released from isolation or died until 30 April 2020. We analyzed the data received from the KDCA via encrypted, remote access.

A brief summary of the COVID-19 related quarantine issues in Korea from January 19 to April 30, 2020 is as follows. The approximate total population of the Republic of Korea was 51,780,579 in April 2020 (8). The total number of COVID-19 tests conducted was 623,069 and the number of confirmed cases was 10,774 as of April 30, 2020 (**Figure 1A**). Among the 10,774 confirmed cases, 1,073 (9.96%) were foreign patients. In total, 9,072 patients had been released from isolation, and 248 had died (fatality rate: 2.73%). Of the 9,072 patients who had been released from isolation, 8,976 had accessible medical records. Of these, 5,350 were hospitalized, 3,450 were admitted to community treatment centers, and 176 were isolated at home. Most of the patients who were isolated at home or community treatment center had asymptomatic or mild disease (9). If disease progressed to moderate or severe condition, they were transferred to hospitals. Of the people who initially entered a community treatment center, approximately 270–280 patients were eventually hospitalized (**Figure 1B**) (10). The KDCA allowed select researchers temporary access to the anonymized data of 5,628 patients (under granted permission).

A total of 5,628 raw data points corresponding to inpatients from the KDCA were initially reviewed (**Figure 1B** in the pink box). Finally, 5,307 patients were analyzed after excluding 272 patients under the age of 20 years, 19 pregnant women, 26 without clinical severity information, and four without comorbidity information.

The data included the presence of diabetes mellitus, hypertension, heart failure, chronic heart disease, asthma, chronic obstructive pulmonary disease, chronic kidney disease, malignancy, chronic liver disease, rheumatic/autoimmune disease, and dementia, but did not show the duration of disease and medication history. The co-morbid condition was collected through history taking by medical personnel with questionnaire. There was no available detailed information, disease status and treatment regimens for COVID-19 in the given data.

Study Definitions

A confirmed case was defined as a patient who had tested positive for SARS-CoV-2 after a real-time reverse transcription-polymerase chain reaction (RT-PCR) test with respiratory specimens: upper respiratory specimens (nasopharyngeal and oropharyngeal swabs), with or without a lower respiratory specimen (sputum), regardless of their clinical manifestations (2). To be released from isolation or discharged, patients had to be:

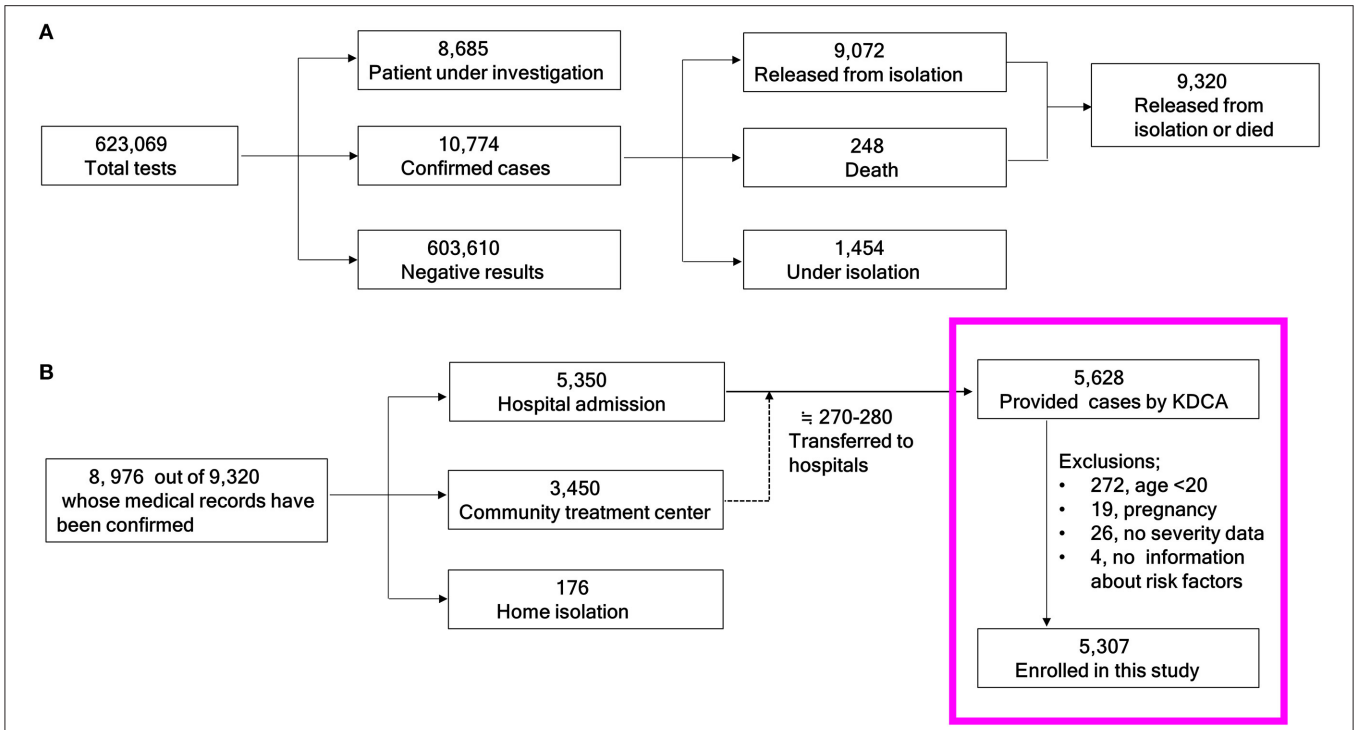


FIGURE 1 | Schema of the study population. **(A)** From the total number of people tested for COVID-19 to the cumulative numbers of those who were released from isolation or died. **(B)** Flow chart of the current study candidates. KDCA, Korea Disease Control and Prevention Agency.

(1) afebrile without symptoms for 10 days and/or (2) have two negative RT-PCR results at least a 24-h interval (11).

Disease severity was defined according to the KDCA and World Health Organization guidelines (11, 12) as follows: level 1, no limitation of daily activities; level 2, limitation of daily activities but no need for oxygen therapy; level 3, oxygen therapy via a nasal cannula; level 4, oxygen therapy via a facial mask; level 5, high-flow supplemental oxygen therapy or non-invasive mechanical ventilation; level 6, needs invasive mechanical ventilation; level 7, multi-organ failure or needs extracorporeal membrane oxygenation (ECMO) therapy; level 8, death. Levels 6–8 were defined as critical illness, whereas 8 was defined as fatal illness. In this study, critical illness is a broader concept that includes fatal illness, and fatal illness refers to a mortality case. The severity evaluation was based on patients with the most severe condition during their hospital stay. For example, fatal illness refers to death of patients regardless of whether they received level 1 or 7 treatment. All fatality cases were made to correspond to level 8. Both critical and fatal illness were considered to be severe COVID-19.

Information on comorbidities was reviewed to determine whether patients had previously been diagnosed with specific comorbidities. Body temperature and body mass index were the initial findings on hospital admission.

Statistical Analysis

The baseline characteristics of the subjects were described as a frequency and proportion for categorical data. The chi-square test was used to compare the categorical variables. The values of continuous variables were expressed as the median and interquartile range (IQR; Q1, Q3). The Mann-Whitney *U*, or Wilcoxon rank-sum tests were performed for body temperature. Univariate and multivariable logistic regression models were applied to evaluate the risk factors of critical and fatal illness. Age was given as a categorical variable in units of 10 years. There were no critical or death cases reported in the 20–29-year age (20s) group. So, age group was categorized as <40 (20s + 30s) years, 40s, 50s, 60s, 70s, and ≥ 80 years for logistic regression, and the 60s used as the reference.

Multivariable logistic regression was used to analyze the independent risk of critical and fatal illness after adjusting for several comorbid diseases: diabetes mellitus, hypertension, heart failure, chronic heart disease (other than hypertension and heart failure), bronchial asthma, chronic obstructive lung disease (COPD), chronic kidney disease (CKD), chronic liver disease, rheumatic disease, cancer (excluding cured cases), and dementia.

Utilizing the cardiovascular risk factors, a model for criticality and fatality prediction was made with age ≥ 60, male sex, medical history of diabetes mellitus, and hypertension as one point each. Theses ranged from a minimum of zero to a maximum of four points.

Next, the criticality and fatality prediction models were analyzed by logistic regression model, odds ratios (ORs) and c-statistics were obtained. The c-statistics were equivalent to the area under the receiver operating characteristic (ROC) curve, based on the predicted probability of the outcomes (the critical or

TABLE 1 | Baseline characteristics of the study population.

Characteristics	Overall (n = 5,307)	Critical illness (n = 271)	Fatal illness (n = 241)	Incidence (%)	
	N (column %)	*N* (column %)	*N* (column %)	Critical	Fatal
Age (years)					
20–29	1,104 (20.8)	0 (0)	0 (0)	0	0
30–39	549 (10.3)	3 (1.1)	2 (0.8)	0.5	0.4
40–49	738 (13.9)	2 (0.7)	2 (0.8)	0.3	0.3
50–59	1,141 (21.5)	21 (7.7)	15 (6.2)	1.8	1.3
60-69	906 (17.1)	45 (16.6)	34 (14.1)	5.0	3.8
70–79	545 (10.3)	82 (30.3)	73 (30.3)	15	13.4
≥80	324 (6.1)	118 (43.5)	115 (47.7)	36.4	35.5
Female	3,144 (59.2)	127 (46.9)	114 (47.3)	4	3.6
Male	2,163 (40.8)	144 (53.1)	127 (52.7)	6.7	5.9
Body temperature (°C) Median (Q1,Q3)	36.9 (36.5, 37.3)	37.0 (36.6, 37.9)	37.0 (36.5, 37.9)		
Cough*					
Yes	2,231 (42.0)	92 (33.9)	81 (33.6)	4.1	3.6
No	3,075 (57.9)	179 (66.1)	169 (66.4)	5.8	5.2
Sputum*					
Yes	1,549 (29.2)	79 (29.2)	72 (29.9)	5.1	4.6
No	3,757 (70.8)	192 (70.8)	169 (70.1)	5.1	4.5
Sore throat*					
Yes	839 (15.8)	14 (5.2)	13 (5.4)	1.7	1.5
No	4,467 (84.2)	257 (94.8)	228 (94.6)	5.8	5.1
Shortness of breath*					
Yes	658 (12.4)	134 (49.4)	113 (46.9)	20.4	17.2
No	4,648 (87.6)	137 (50.6)	128 (53.1)	2.9	2.8
Diarrhea*					
Yes	504 (9.5)	20 (7.4)	18 (7.5)	4	3.6
No	4,802 (90.5)	251 (92.6)	223 (92.5)	5.2	4.6
Systolic BP†					
<120	1,201 (22.6)	66 (24.4)	58 (24.1)	5.5	4.8
120–129	1,076 (20.3)	33 (12.2)	28 (11.6)	3.1	2.6
130–139	1,039 (19.6)	36 (13.3)	32 (13.3)	3.5	3.1
140–159	1,381 (26.0)	77 (28.4)	68 (28.2)	5.6	4.9
≥160	507 (9.6)	41 (15.1)	37 (15.4)	8.1	7.3
BMI (kg/m^2)‡					
<18.5	191 (3.6)	16 (5.9)	16 (6.6)	8.4	8.4
18.5–22.9	1,741 (32.8)	55 (20.3)	46 (19.1)	3.2	2.6
23.0–24.9	1,005 (18.9)	25 (9.2)	20 (8.3)	2.5	2
24.9–29.9	1,011 (19.1)	49 (18.2)	39 (16.2)	4.8	3.9
≥30	193 (3.6)	7 (2.6)	5 (2.1)	3.6	2.6
Diabetes mellitus					
Yes	684 (12.9)	106 (39.1)	98 (40.7)	15.5	14.3
No	4,623 (87.1)	165 (60.9)	143 (59.3)	3.6	3.1
Hypertension					
Yes	1,197 (22.6)	164 (60.5)	144 (59.8)	13.7	12.0
No	4,110 (77.4)	107 (39.5)	97 (40.2)	2.6	2.4
Heart failure					
Yes	59 (1.1)	20 (7.4)	18 (7.5)	33.9	30.5
No	5,248 (98.9)	251 (92.6)	223 (92.5)	4.8	4.2

(Continued)

TABLE 1 | Continued

Characteristics	Overall (n = 5,307)	Critical illness (n = 271)	Fatal illness (n = 241)	Incidence (%)	
	N (column %)	*N* (column %)	*N* (column %)	Critical	Fatal
Chronic heart disease					
Yes	179 (3.4)	29 (10.7)	26 (10.8)	16.2	14.5
No	5,112 (96.3)	242 (89.3)	215 (89.2)	4.7	4.2
Missing	16 (0.3)	0	0	0	0
Asthma					
Yes	126 (2.4)	13 (4.8)	13 (5.4)	10.3	10.3
No	5,181 (97.6)	258 (95.2)	228 (94.6)	5	4.4
COPD					
Yes	38 (0.7)	9 (3.3)	8 (3.3)	23.7	21.1
No	5,269 (99.3)	262 (96.7)	233 (96.7)	5	4.4
Chronic kidney disease					
Yes	55 (1.0)	18 (6.6)	16 (6.6)	32.7	29.1
No	5,252 (99.0)	253 (93.4)	225 (93.4)	4.8	4.3
Cancer					
Yes	145 (2.7)	22 (8.1)	22 (9.1)	15.2	15.2
No	5,162 (97.3)	249 (91.9)	219 (90.9)	4.8	4.2
Chronic liver disease[§]					
Yes	83 (1.6)	7 (2.6)	7 (2.9)	8.4	8.4
No	4,912 (92.6)	264 (91.9)	234 (97.1)	5.4	4.8
Rheumatic disease[‖]					
Yes	38 (0.7)	3 (1.1)	3 (1.2)	7.9	7.9
No	4,951 (89.8)	268 (98.9)	238 (98.8)	5.4	4.8
Dementia[¶]					
Yes	224 (4.2)	76 (28.0)	75 (31.1)	33.9	33.5
No	4,768 (89.8)	195 (72.0)	166 (68.9)	4.1	3.5
Severity**					
Level 1	4,179 (78.7)	0 (0)	0 (0)	0	0
Level 2	314 (5.9)	0 (0)	0 (0)	0	0
Level 3	468 (8.8)	0 (0)	0 (0)	0	0
Level 4	43 (0.8)	0 (0)	0 (0)	0	0
Level 5	32 (0.6)	0 (0)	0 (0)	0	0
Level 6	19 (0.4)	19 (7.0)	0 (0)	100	0
Level 7	11 (0.2)	11 (4.1)	0 (0)	100	0
Level 8	241 (4.5)	241 (88.9)	241 (100)	100	100

*COPD, chronic obstructive lung disease. *missing n = 1, [†]missing n = 103, [‡]missing n = 1,166, [§]missing n = 312, [‖]missing n = 318, [¶]missing n = 315, **Severity level 1, no limitation of daily activities; level 2, limitation of daily activities but no need oxygen therapy; level 3, oxygen therapy via nasal cannula; level 4, oxygen therapy via facial mask; level 5, high-flow supplemental oxygen therapy or non-invasive mechanical ventilation; level 6, the need for invasive mechanical ventilation; level 7, multi-organ failure or the need for extracorporeal membrane oxygenation therapy; level 8, death.*

fatal disease) in the logistic regression models with the risk score as independent variable. In this model, each score was treated as binary category of 0 or 1.

For risk score validation, we performed internal validation using bootstrap resampling. To evaluate the performance of compensating overfitting of logistic regression and the risk score model, a total of 1,000 random bootstrap samples were generated for replacement of the original data, and each bootstrap sample size was the same scale as the original data. Then the means and 95% confidence intervals of bootstrap samples were calculated. The c-statistic difference between original data and bootstrap samples was defined as optimism. Optimism-corrected c-statistic can be obtained by subtracting the estimated mean of the optimism estimate value from the c-index in the original sample.

A P-value of <0.05 was considered statistically significance. The statistical analysis was performed using the SAS software (version 9.4, SAS Institute, Cary, NC, USA).

Ethics Statement

This study was deemed exempt from ethical review and the requirement for informed consent was waived by the Ewha Womans University Mokdong hospital Institutional

Review Board (EUMC2020-07-002) because all of the data were fully anonymized and did not include personally identifiable information.

RESULTS

The baseline demographic and clinical characteristics are presented in **Table 1**. Among the 5,307 patents, 2,136 (40.8%) were male, the rate of critical illness was 5.1% (male: 6.7, female: 4.0%; $P < 0.001$), and the fatality of the study group was 4.54%. Number of cases is highest in the 20s and 50s, but no critical illness or fatal illness was in the 20s. Meanwhile, critical illness started to rise steeply from the age of 50s, reaching 43.5% in the 80s.

Clinical symptoms including cough, sputum, sore throat and diarrhea did not differ according to severity, but shortness of breath was more frequently reported in the critical illness than in the non-critical illness (20.4 vs. 2.9%) patients. Patients with systolic blood pressures of <120 or ≥140 mmHg were more likely to be critically ill than those with a systolic blood pressure between 120 and 140 mmHg. Underweight patients with a body mass index of <18.5 kg/m^2 also showed a higher in the critical illness than in the non-critical illness patients.

Patients with chronic diseases (diabetes mellitus, hypertension, CKD), cardiovascular diseases (heart failure, chronic stable heart disease), respiratory disorder (asthma, COPD), cancer, and/or dementia presented higher rate of critical illness than those without. No significant difference in severity was seen between patients with chronic liver disease or rheumatic disorder and those without. Among the 271 critically ill patients, 19 survived with invasive ventilation and 11 ECMO, whereas the remaining 241 did not survived.

Figure 2 shows the disease frequency and critical illness ratio according to sex and age. There were some differences between males and females in the distribution of disease and severity according to age. For example, despite the high frequency of COVID-19 in patients in their 20s, no cases of critical illness were found. In addition, the rate of critical illness increased significantly with age, but this characteristic was more prominent in males.

Logistic analyses were performed to evaluate the risk factors for critical and fatal illness (**Table 2**). In the univariate analyses of **Table 2**, all variables were significantly related to both critical and fatal illness except for chronic liver disease and rheumatic disease. Importantly, age was an important risk factor, with those aged <50 years having less risk. Those age ≥70 years had sharply increased ORs. In particular, in patients aged ≥80 years, heart failure, CKD, and dementia had a high OR above 7.0. In the multivariable model 1 in **Table 2**, asthma and COPD lost their significance for critical and fatal illnesses. Heart failure showed a decreased odds ratio as 2.13 (1.12–4.05) for critical illness and lost the significance of 1.94 (0.99–3.77) for fatal illness.

In addition, we performed multivariable analyses with four major cardiovascular risk factors (model 2). The results showed similar ORs with model 1 in **Table 2**. However, ORs of those with age ≥80 was markedly elevated and hypertension lost statistical power of 1.29 (0.94–1.77) for fatal illness.

Model 1 and model 2 showed good performance for prediction of critical illness (original c-statistics, 0.905 and 0.902; optimism-corrected c-statistics, 0.899 and 0.900) and fatal illness (0.917 and 0.912; 0.912 and 0.910). Interestingly, model 2 showed excellent performance similar to model 1. Furthermore, all the values of model 2; bootstrap, original, and corrected, c-statistics showed ≥0.9 for critical and fatal illness.

According to the result of model 2, we calculated risk score with simplified four variables: age ≥60 years, male sex,

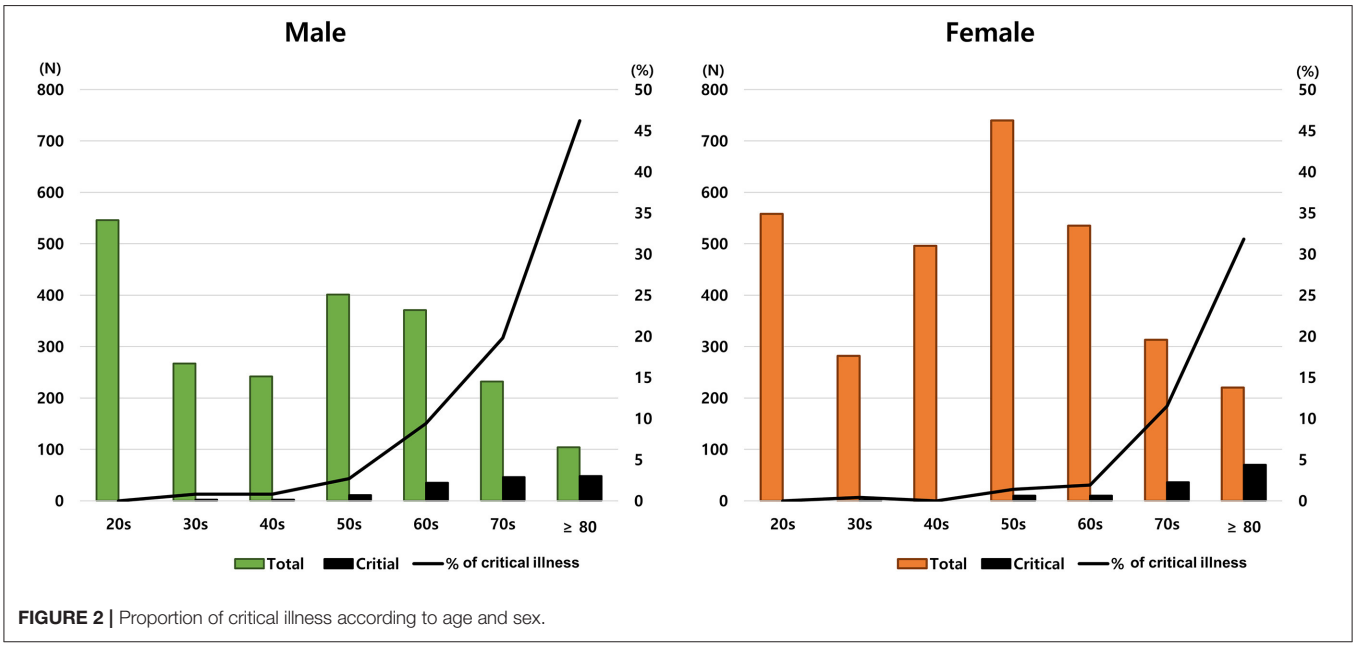


FIGURE 2 | Proportion of critical illness according to age and sex.

TABLE 2 | Logistic analyses and c-statistics for critical and fatal illness.

	Critical illness Odds ratio (95% CI)			Fatal illness Odds ratio (95% CI)		
	Univariate	Multivariable		Univariate	Multivariable	
		Model 1	Model 2		Model 1	Model 2
Age (years)						
<40*	0.04 (0.01–0.11)	0.05 (0.02–0.18)	0.05 (0.01–0.15)	0.03 (0.01–0.13)	0.05 (0.01–0.21)	0.04 (0.01–0.17)
40s	0.05 (0.01–0.22)	0.08 (0.02–0.32)	0.07 (0.02–0.29)	0.07 (0.02–0.29)	0.11 (0.03–0.44)	0.09 (0.02–0.39)
50s	0.36 (0.21–0.61)	0.45 (0.26–4.16)	0.43 (0.25–0.73)	0.34 (0.19–0.63)	0.43 (0.23–0.80)	0.41 (0.22–0.75)
60s	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
70s	3.39 (2.32–4.96)	2.80 (1.89–4.16)	3.10 (2.11–4.58)	3.97 (2.60–6.05)	3.29 (2.13–5.10)	3.68 (2.39–5.65)
≥80	10.96 (7.53–15.96)	7.18 (4.65–11.07)	11.66 (7.87–17.27)	14.11 (9.35–21.29)	9.40 (5.87–15.05)	15.51 (10.09–23.83)
Male (vs. Female)	1.89 (1.48–2.42)	2.47 (1.85–3.31)	2.35 (1.77–3.11)	1.85 (1.42–2.40)	2.51 (1.84–3.43)	2.34 (1.73–3.16)
Diabetes mellitus	3.80 (2.93–4.92)	1.84 (1.36–2.50)	1.89 (1.40–2.55)	4.02 (3.06–5.28)	2.07 (1.50–2.87)	2.09 (1.52–2.86)
Hypertension	4.35 (3.37–5.6)	1.49 (1.10–2.01)	1.49 (1.11–2.00)	4.14 (3.17–5.41)	1.30 (0.94–1.79)	1.29 (0.94–1.77)
Heart failure	7.96 (4.57–13.85)	2.13 (1.12–4.05)		7.72 (4.36–13.66)	1.94 (0.99–3.77)	
CHD	3.10 (2.04–4.71)	2.13 (1.12–4.05)		3.08 (1.99–4.78)	1.02 (0.60–1.72)	
Asthma	2.01 (1.11–3.64)	1.43 (0.70–2.92)		2.30 (1.27–4.16)	1.71 (0.83–3.53)	
COPD	4.62 (2.17–9.87)	1.23 (0.48–3.16)		4.50 (2.04–9.93)	1.04 (0.38–2.83)	
CKD	7.93 (4.43–14.19)	2.75 (1.33–5.72)		7.54 (4.13–13.77)	2.54 (1.19–5.43)	
Cancer	2.83 (1.77–4.54)	2.41 (1.38–4.20)		3.24 (2.02–5.22)	2.89 (1.64–5.10)	
CLD	1.37 (0.62–3.00)	1.10 (0.46–2.68)		1.55 (0.71–3.42)	1.32 (0.54–3.23)	
Rheumatic disease	1.18 (0.36–3.86)	1.91 (0.52–6.99)		1.34 (0.41–4.39)	2.38 (0.64–8.82)	
Dementia	9.42 (6.90–12.87)	2.32 (1.57–3.42)		10.94 (7.96–15.03)	2.58 (1.74–3.84)	
Bootstrap†		0.908 (0.891–0.923)	0.903 (0.886–0.919)		0.920 (0.904–0.934)	0.913 (0.896–0.928)
Original, Corrected‡		0.905, 0.899	0.902, 0.900		0.917, 0.912	0.912, 0.910

<40, 20–39 years; CHD, chronic heart disease; COPD, chronic obstructive lung disease; CKD, chronic kidney disease; CLD, chronic liver disease. †Mean of c-statistics (and 95% confidence interval) of bootstrap samples; ‡C-statistics of original data, the optimism corrected c-statistics.*

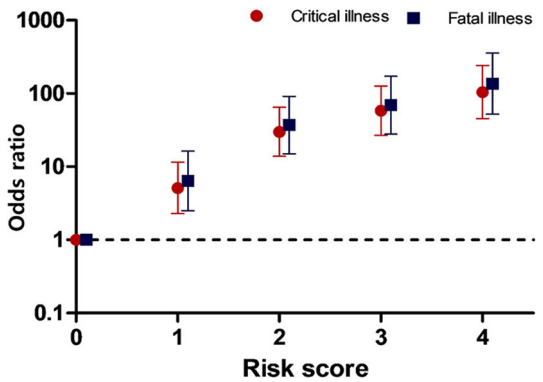


FIGURE 3 | Odds ratios for critical and fatal illness according to the risk score. The scores represent the number of risk factors.

diabetes mellitus, and hypertension, which are also known to be cardiovascular risk factors. Each one risk factor was calculated as one point. **Figure 3** shows that the OR for the disease severity increased as the number of risk scores increased relative to the zero point. The ORs (95% confidence interval) for critical and fatal illness were as followings: score 1; 5.1 (2.3–11.5) and 6.4 (2.5–16.3), score 2; 29.9 (13.9–64.7) and 37.2 (15.0–91.9), score 3; 58.4 (36.9–12.8) and 69.2 (27.9–171.8), and 4; 104 (45.6–240.6), and 136.2 (52.3–354.9), respectively. This risk scoring model showed good model fitness (original c = 0.8300 and 0.8321, corrected c = 0.8303 and 0.8324) for critical and fatal illness, respectively.

DISCUSSION

This study demonstrated that cardiovascular risk factors are also significant risk factors for severe COVID-19. In particular, age ≥60 years was shown to be a strong risk factor; the risk of severe COVID-19 significantly increased by 10 times in those aged ≥80 years compared to those in their 60s. Similar to heart disease, male had a higher risk, and more than twice the odds ratio for critical COVID-19 than female. Hypertension was a risk factor for critical illness rather than fatal illness which was same to heart failure. Additionally, dementia and cancer were found to be poor prognostic factors. Respiratory diseases, such as asthma and COPD were not found to be significant risk factors. Regarding the risk score model, the risk of critical or fatal illness increased sharply according to every increase in score compared to those without risk factors (risk score zero). Therefore, the more risk factors a patient has, the greater their likelihood of progressing to severe COVID-19.

Cardiovascular risk factors are known as smoking, hypertension, diabetes, obesity, physical inactivity, age, male sex (13, 14). However, the data we have only contain diabetes, hypertension, age, sex among the cardiovascular risk factors. Therefore, the risk score was calculated only for the risk factors included in the data. Nonetheless, one interesting thing about this study is that prediction performance of model 2, which included four cardiovascular risk factors (age; six grouped, sex, DM, hypertension), showed as good as that of model 1, which was included 13 variables. When the age groups were simplified as binary group based on the age of 60, the prediction performance was decreased from 0.900 to 0.830 (optimism corrected c) for critical illness. However, which was also good performance. The purpose of this study was to show an association between CV risk factors and the severe COVID-19, rather than developing a new scoring system for predicting severe COVID-19. Through this, we aimed to bring health care providers and patients themselves to have attention of the deleterious effects of multiple CV risk factors in the COVID-19 pandemic era. We intentionally simplified the scoring system as much as possible and included well-known highly prevalence disease.

Previous studies have shown an association between the Middle East respiratory syndrome (MERS) and SARS with acute myocarditis, myocardial infarction, and heart failure as well as a relationship of COVID-19 and myocardial injury (2, 3, 15). These viral infections are all caused by CoV. Furthermore, SARS-CoV-2 has similar pathogenicity to MERS-CoV, which can induce damage to the cardiovascular system, and as a result, can increase the difficulty and complexity of patient treatment (16). There are two implications for this. First is the importance of comorbidities on the prognosis of viral infection. In particular, hypertension and diabetes have been reported as common comorbidities in COVID-19, SARS, and MERS, especially among those with more severe disease (5, 17, 18). In a cohort of 138 hospitalized patients with COVID-19, the reported rate of hypertension was 31% (58% in patients requiring intensive care), and diabetes was 10% (22% in patients requiring intensive care) (5). In the current study, 22.6% had hypertension (60.5% in those with critical illness), 12.9% had diabetes mellitus (39.1% in those with critical illness). Second, it is important to determine whether myocardial damage occurs during viral infection. Data from China showed that elevated level of cardiac biomarker-troponin T was related to increased mortality of patients with COVID-19 regardless of cardiovascular disease (4), and almost 12% of patients without known cardiovascular disease had elevated troponin levels or experienced cardiac arrest during hospitalization (19). We suggest, these results that elevated troponin in other studies is associated with mortality may indirectly explain the mechanism of disease severity and mortality in our study.

Potential mechanisms for the association between acute viral infection and increased myocardial damage are as follows: (1) type 1 myocardial infarction, which is caused by atherosclerotic plaque rupture or coronary thrombosis related acute inflammation; (2) type 2 myocardial infarction, which is related to the mismatch of oxygen demand and supply, and (3) direct effect of the virus and inflammation on the cardiac cells (1, 16, 20). A cardiac metabolic mismatch may be induced by the aggravation of coronary artery stenosis by toxin-mediated vasoconstriction in individuals who already have coronary artery stenosis due to chronic atherosclerotic plaques, particularly in the elderly (1). The current study showed that cardiovascular risk factors are also risk factors for severe COVID-19. However, data regarding cardiac biomarkers, which can evaluate myocardial damage, was not available. Therefore, it can only be presumed that the poor prognosis of patients with multiple cardiovascular risk factors is related to myocardial damage.

Previous studies have shown that elevated cardiac troponin and pro-brain natriuretic peptide are each independently associated with poor outcomes in patients with COVID-19 patients (4, 21). However, there is scarce evidence as to which patients are associated with elevated cardiac biomarkers. The COVID-19 pandemic is still driven by virus mutations, and it is a high possibility that a subsequent global pandemic will be repeated by various respiratory viruses. Therefore, research on COVID-19 and the cardiovascular system should be continued to improve patients' prognoses. Through this study, we identified that patients with multiple CV risk factors are associated with severe COVID-19. Through future follow-up studies, it is important to investigate whether the risk score model/multiple CV risk factors in this study is associated with the proportional increase of cardiac troponin or pro-brain natriuretic peptide or new-onset atrial fibrillation which reflecting cardiac complications and poor outcome of COVID-19 patients.

This study showed that CKD, cancer, and dementia are also risk factors. Dementia is a disease that is more prevalent in older individuals. However, it was still found to be a significant risk factor even after adjusting for age and other comorbid conditions. Several studies have found that CKD is related to an increased risk of mortality from COVID-19 (5, 12). In studies form Europe and America, the mortality of CKD patients was higher than that of the normal group, and inversely proportional to the glomerular filtration rate (11, 22). There are several reasons as to why renal dysfunction worsens COVID-19. First, there is a decrease in immune function in uremic patients (23, 24). A previous study showed that in hemodialysis patients infected with COVID-19, the absolute number of natural killer cells is smaller, and the ratio differs from that in COVID-19 patients without dialysis (25). Second, patients with CKD are known to have a higher risk of cardiovascular disease than patients with normal renal function (26). The mortality rate and risk rate from cardiovascular disease are high, and it is considered to be one of the reasons for the high COVID-19 mortality rate in patients with CKD. In this study, we did not have data on the stage of CKD, and whether patients were undergoing dialysis or had previously had a kidney transplant. We did not include CKD in the risk score model due to heterogeneity and these limitations. However, CKD should be considered an important risk factor for severe COVID-19.

In general, patients with an underlying respiratory disease appear to have a poor prognosis for respiratory infections (27). Previous studies have shown that COPD has a significant effect on the prognosis of COVID-19 pneumonia. As yet, this association has not been confirmed in patients with asthma. In

previous meta-analyses, COPD was found to increase the risk of severe COVID-19 with an odds ratio of 4.38 and a relative risk of 1.88, compared to those without COPD (28, 29). However, in the present study, COPD and asthma were not found to affect the criticality and fatality of COVID-19. Since the above studies were meta-analyses, there are methodological differences from the current study. In addition, in this study, only 38 (0.7%) patients had COPD, which might have underpowered the relationship. However, 123 (2.4%) patients had asthma in this study, which also showed no relationship. Hence, further research is needed to determine whether respiratory disease is a risk factor for severe COVID-19.

The current study has some limitations. First, since no cardiac biomarkers were available, such as cardiac troponin or pro-brain natriuretic peptide, that can reflect myocardial damage or heart failure, it is unclear as to whether the disease severity associated with cardiovascular risk factors is directly related to actual heart damage. Second, specific disease condition was not available in this study. For example, we could not know whether the result related to chronic heart disease is due to which of ischemic heart disease, valvular heart disease, and cardiomyopathy. In addition, our data did not include information on atrial fibrillation, which was known to be one of the poor prognostic factors associated with COVID-19 (30). There is no information about the stage of CKD, with the data only indicating whether a patient did or did not have CKD. Third, there was no information about the time or duration for the event (for the critical illness) and censored data. Some variables had missing values and we did not replace the missing values. However, the result of multivariable logistic analysis might not be affected by the missing values. There was no significance difference between the result of multivariable logistic analysis with the limited risk factors which were cardiovascular risk factors and heart diseases (**Table 2**). Further, the missing data did not affect the result of risk score model of **Figure 3**.

This study has a strong point as nation-wide cohort study which minimizing selection bias. The data is based on the unique single-payer, compulsory subscription system of the Republic of Korea and infectious disease control system integrated by the government through KDCA. Hence, the study could be good reference to explore the situation of a single country and elucidate interracial differences for future investigation. Another novelty of this study is the study population is younger and predominantly females compared to other large series on COVID-19. Since young patients and female patients were included in the analysis, it can be considered to apply to a wider range of populations.

In conclusion, we have described the clinical characteristics and disease severity of hospitalized patients with confirmed COVID-19 in the Republic of Korea, using nationwide data from 5,307 patients. Our results showed that those over 60 years, of the male sex, or those with heart failure, cardiovascular risk factors; hypertension, diabetes mellitus, and CKD have an increased risk of severe COVID-19. Further, the risk scoring model showed the significance of multiple risk factors. Those with four risk factors, old age ($\geq$60 years), male sex, hypertension, and diabetes mellitus, had odds ratio more than 100 of severe COVID-19 than those without these risk factors, although it should be taken into account that it can be statistically exaggerated due to relatively small numbers of patients. In addition, dementia and cancer were also found to be related to severe COVID-19.

DATA AVAILABILITY STATEMENT

Data cannot be shared publicly for protecting personal information by the Korea Disease Control and Prevention Agency (KDCA). Broad information regarding Korean COVID-19 statistics are released daily on a public web site (http://ncov.mohw.go.kr/en/) and through the media by KCDA. You may contact KDCA and/or the corresponding author for the detailed data in current study.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ewha Womans University Mokdong Hospital Institutional Review. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

KK and IK: conceptualization and data curation. KK: formal analysis. KK, SJ, MY, JP, and IK: investigation and validation. SJ and IK: methodology and writing—original draft. SJ: visualization. KK, SJ, and IK: writing—review and editing. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We acknowledge all the health-care workers involved in the diagnosis and treatment of COVID-19 patients in South Korea. We thank Korea Disease Control and Prevention Agency, National Medical Center and the Health Information Manager in hospitals for their effort in collecting the medical records.

REFERENCES

1. Musher DM, Abers MS, Corrales-Medina VF. Acute infection and myocardial infarction. *N Engl J Med.* (2019) 380:171–6. doi: 10.1056/NEJMra1808137
2. Duan J, Wu Y, Liu C, Yang C, Yang L. Deleterious effects of viral pneumonia on cardiovascular system. *Eur Heart J.* (2020) 41:1833–8. doi: 10.1093/eurheartj/ehaa325
3. Xiong T-Y, Redwood S, Prendergast B, Chen M. Coronaviruses and the cardiovascular system: acute and long-term implications. *Eur Heart J.* (2020) 41:1798–800. doi: 10.1093/eurheartj/ehaa231
4. Tao G, Guo T, Fan Y, Chen M, Wu X, Zhang L, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiology.* (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017

5. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *JAMA*. (2020) 323:1061–9. doi: 10.1001/jama.2020.1585
6. Dahal S, Jenner M, Dinh L, Mizumoto K, Viboud C, Chowell G. Excess mortality patterns during 1918-1921 influenza pandemic in the state of Arizona, USA. *Ann Epidemiol.* (2018) 28:273–80. doi: 10.1016/j.annepidem.2017.12.005
7. Mehra MR, Desai SS, Kuy S, Henry TD, Patel AN. Cardiovascular disease, drug therapy, and mortality in Covid-19. *N Engl J Med.* (2020) 382:e102. doi: 10.1056/NEJMc2021225
8. Vaduganathan M, Vardeny O, Michel T, McMurray JJV, Pfeffer MA, Solomon SD. Renin–angiotensin–aldosterone system inhibitors in patients with Covid-19. *N Engl J Med.* (2020) 382:1653–9. doi: 10.1056/NEJMsr2005760
9. Kang E, Lee SY, Jung H, Kim MS, Cho B, Kim YS. Operating protocols of a community treatment center for isolation of patients with coronavirus disease, South Korea. *Emerg Infect Dis.* (2020) 26:2329–37. doi: 10.3201/eid2610.201460
10. Bangalore S, Sharma A, Slotwiner A, Yatskar L, Harari R, Shah B, et al. ST-segment elevation in patients with covid-19 — a case series. *N Engl J Med.* (2020) 382:2478–80. doi: 10.1056/NEJMc2009020
11. Henry BM, Lippi G. Chronic kidney disease is associated with severe coronavirus disease 2019 (COVID-19) infection. *Int Urol Nephrol.* (2020) 52:1193–4. doi: 10.1007/s11255-020-02451-9
12. Marshall JC, Murthy S, Diaz J, Adhikari N, Angus DC, Arabi YM, et al. A minimal common outcome measure set for COVID-19 clinical research. *Lancet Infect Dis.* (2020) 20:e192–e7. doi: 10.1016/S1473-3099(20)30483-7
13. Bots SH, Peters SAE, Woodward M. Sex differences in coronary heart disease and stroke mortality: a global assessment of the effect of ageing between 1980 and 2010. *BMJ Glob Health.* (2017) 2:e000298. doi: 10.1136/bmjgh-2017-000298
14. Thomas A. Gaziano DP, Gaziano JM. Global burden of cardiovascular disease. In: Mann DL, Zipes DP, Libby P, Bonow RO, editors. *Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine*. 10th ed. Philadelphia: Elsevier (2014). p 1–20.
15. Tavazzi G, Pellegrini C, Maurelli M, Belliato M, Sciutti F, Bottazzi A, et al. Myocardial localization of coronavirus in COVID-19 cardiogenic shock. *Eur J Heart Fail.* (2020) 22:911–5. doi: 10.1002/ejhf.1828
16. Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A, et al. COVID-19 and cardiovascular disease. *Circulation.* (2020) 141:1648–55. doi: 10.1161/CIRCULATIONAHA.120.046941
17. Booth CM, Matukas LM, Tomlinson GA, Rachlis AR, Rose DB, Dwosh HA, et al. Clinical features and short-term outcomes of 144 patients with SARS in the Greater Toronto Area. *JAMA*. (2003) 289:2801–9. doi: 10.1001/jama.289.21.JOC30885
18. Badawi A, Ryoo SG. Prevalence of comorbidities in the Middle East respiratory syndrome coronavirus (MERS-CoV): a systematic review and meta-analysis. *Int J Infect Dis.* (2016) 49:129–33. doi: 10.1016/j.ijid.2016.06.015
19. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol.* (2020) 17:259–60. doi: 10.1038/s41569-020-0360-5
20. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth Universal definition of myocardial infarction. *J Am Coll Card.* (2018) 72:2231–64. doi: 10.1093/eurheartj/ehy655
21. Caro-Codón J, Rey JR, Buño A, Iniesta AM, Rosillo SO, Castrejon-Castrejon S, et al. Characterization of NT-proBNP in a large cohort of COVID-19 patients. *Eur J Heart Fail.* (2021) 23:456–64. doi: 10.1002/ejhf.2095
22. Uribarri A, Núñez-Gil IJ, Aparisi A, Becerra-Muñoz VM, Feltes G, Trabattoni D, et al. Impact of renal function on admission in COVID-19 patients: an analysis of the international HOPE COVID-19 (Health Outcome Predictive Evaluation for COVID 19) Registry. *J Nephrol.* (2020) 33:737–45. doi: 10.1007/s40620-020-00790-5
23. Betjes MGH. Immune cell dysfunction and inflammation in end-stage renal disease. *Nat Rev Nephrol*. (2013) 9:255–65. doi: 10.1038/nrneph.2013.44
24. D'Marco L, Puchades MJ, Romero-Parra M, Gimenez-Civera E, Soler MJ, Ortiz A, et al. Coronavirus disease 2019 in chronic kidney disease. *Clin Kidney J.* (2020) 13:297–306. doi: 10.1093/ckj/sfaa104
25. Arslan H, Musabak U, Ayvazoglu Soy EH, Kurt Azap O, Sayin B, Akcay S, et al. Incidence and immunologic analysis of coronavirus disease (COVID-19) in hemodialysis patients: a single-center experience. *Exp Clin Transplant*. (2020) 18:275–83. doi: 10.6002/ect.2020.0194
26. Major RW, Cheng MRI, Grant RA, Shantikumar S, Xu G, Oozeerally I, et al. Cardiovascular disease risk factors in chronic kidney disease: a systematic review and meta-analysis. *PLoS ONE.* (2018) 13:e0192895. doi: 10.1371/journal.pone.0192895
27. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, et al. Diagnosis and treatment of adults with community-acquired pneumonia. An official clinical practice guideline of the American Thoracic Society and Infectious Diseases Society of America. *Am J Respir Crit Care Med.* (2019) 200:e45–e67. doi: 10.1164/rccm.201908-1581ST
28. Zhao Q, Meng M, Kumar R, Wu Y, Huang J, Lian N, et al. The impact of COPD and smoking history on the severity of COVID-19: a systemic review and meta-analysis. *J Med Virol.* (2020) 92:1915–21. doi: 10.1002/jmv.25889
29. Alqahtani JS, Oyelade T, Aldhahir AM, Alghamdi SM, Almehmadi M, Alqahtani AS, et al. Prevalence, severity and mortality associated with copd and smoking in patients with COVID-19: a rapid systematic review and meta-analysis. *PLoS ONE.* (2020) 15:e0233147. doi: 10.1371/journal.pone.0233147
30. Romiti GF, Corica B, Lip GYH, Proietti M. Prevalence and impact of atrial fibrillation in hospitalized patients with COVID-19: a systematic review and meta-analysis. *J Clin Med.* (2021) 10:2490. doi: 10.3390/jcm10112490

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

SYSTEMATIC REVIEW
published: 13 October 2021
doi: 10.3389/fcvm.2021.720129

Prevalence of Atrial Fibrillation and Associated Mortality Among Hospitalized Patients With COVID-19: A Systematic Review and Meta-Analysis

Zuwei Li[1†], Wen Shao[2†], Jing Zhang[3], Jianyong Ma[4], Shanshan Huang[2], Peng Yu[2], Wengen Zhu[5*] and Xiao Liu[6,7*]*

[1] Cardiology Department, The Affiliated Hospital of Jiangxi University of Chinese Medicine, Nanchang, China, [2] Endocrine Department, The Second Affiliated Hospital of Nanchang University, Nanchang, China, [3] Anesthesiology Department, The Second Affiliated Hospital of Nanchang University, Nanchang, China, [4] Department of Pharmacology and Systems Physiology, University of Cincinnati College of Medicine, Cincinnati, OH, United States, [5] Department of Cardiology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, [6] Cardiology Department, The Sun Yat-sen Memorial Hospital of Sun Yat-sen University, Guangzhou, China, [7] Guangdong Province Key Laboratory of Arrhythmia and Electrophysiology, Guangzhou, China

Background: Epidemiological studies have shown that atrial fibrillation (AF) is a potential cardiovascular complication of coronavirus disease 2019 (COVID-19). We aimed to perform a systematic review and meta-analysis to clarify the prevalence and clinical impact of AF and new-onset AF in patients with COVID-19.

Methods: PubMed, Embase, the Cochrane Library, and MedRxiv up to February 27, 2021, were searched to identify studies that reported the prevalence and clinical impact of AF and new-onset AF in patients with COVID-19. The study was registered with PROSPERO (CRD42021238423).

Results: Nineteen eligible studies were included with a total of 21,653 hospitalized patients. The pooled prevalence of AF was 11% in patients with COVID-19. Older (≥60 years of age) patients with COVID-19 had a nearly 2.5-fold higher prevalence of AF than younger (<60 years of age) patients with COVID-19 (13 vs. 5%). Europeans had the highest prevalence of AF (15%), followed by Americans (11%), Asians (6%), and Africans (2%). The prevalence of AF in patients with severe COVID-19 was 6-fold higher than in patients with non-severe COVID-19 (19 vs. 3%). Furthermore, AF (OR: 2.98, 95% CI: 1.91 to 4.66) and new-onset AF (OR: 2.32, 95% CI: 1.60 to 3.37) were significantly associated with an increased risk of all-cause mortality among patients with COVID-19.

Conclusion: AF is quite common among hospitalized patients with COVID-19, particularly among older (≥60 years of age) patients with COVID-19 and patients with severe COVID-19. Moreover, AF and new-onset AF were independently associated with an increased risk of all-cause mortality among hospitalized patients with COVID-19.

Keywords: atrial fibrillation, COVID-19, death, prevalence, meta-analysis

OPEN ACCESS

Edited by:
Shimon Rosenheck, Hebrew University of Jerusalem, Israel

Reviewed by:
Andree Kurniawan, University of Pelita Harapan, Indonesia
Xiangyi Kong, Chinese Academy of Medical Sciences and Peking Union Medical College, China

***Correspondence:**
Peng Yu
yupeng_jxndefy@163.com
Wengen Zhu
zhuwg6@mail.sysu.edu.cn
Xiao Liu
liux587@mail.sysu.edu.cn

[†]These authors share first authorship

Specialty section:
This article was submitted to Cardiac Rhythmology, a section of the journal Frontiers in Cardiovascular Medicine

Received: *11 June 2021*
Accepted: *15 September 2021*
Published: *13 October 2021*

Citation:
Li Z, Shao W, Zhang J, Ma J, Huang S, Yu P, Zhu W and Liu X (2021) Prevalence of Atrial Fibrillation and Associated Mortality Among Hospitalized Patients With COVID-19: A Systematic Review and Meta-Analysis. Front. Cardiovasc. Med. 8:720129. doi: 10.3389/fcvm.2021.720129

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the pathogen of coronavirus disease 2019 (COVID-19), which emerged in December 2019 and has since caused a global epidemic. As of November 14, 2020, over 50 million cases of COVID-19 infection have been reported worldwide, resulting in more than 1 million deaths. Previous studies have confirmed that pneumonia is not only an infectious disease affecting the respiratory system, but it also has a significant impact on the cardiovascular system, leading to heart failure, arrhythmias, and myocardial ischemia (1–3). In addition to fever as the primary symptom, there are also initial clinical manifestations of the cardiovascular system among patients with COVID-19, (4, 5) indicating that cardiovascular diseases are potential complications of COVID-19 (6, 7).

Atrial fibrillation (AF) is the most common arrhythmia and can lead to stroke, peripheral embolization, heart failure, and other unfavorable outcomes (8). The prevalence of AF is between approximately 2.3% and 3.4% in the general population (9, 10). However, for patients with pulmonary disease, critical illness, or systemic inflammatory response syndrome, the prevalence and clinical impact of AF are even more substantial (11–13).

More recently, numerous epidemiological studies have shown an increased risk of AF and new-onset AF among patients with COVID-19 but have yielded inconsistent results (14–32). Moreover, accumulating literature has demonstrated that AF or new-onset AF might be significantly associated with the worst outcomes (e.g., mortality) in patients with COVID-19 (21, 25, 29). Subsequently, several meta-analyses have examined the relationship between COVID-19 and AF (33–36). However, these studies focused on arrhythmias or AF and only examined the association between AF and pooled unfavorable outcomes among patients with COVID-19. It is not clear whether AF increases the risk of death among patients with COVID-19. Furthermore, no studies to date have assessed the prevalence and clinical impact of new-onset AF in patients with COVID-19.

To help clinicians understand the potential damage to the cardiovascular system caused by COVID-19 and strengthen the monitoring and preservation of cardiac function, we conducted a systematic review and meta-analysis of observational studies to clarify the prevalence and clinical impact of AF and new-onset AF in patients with COVID-19.

METHODS

Protocol Registration and Search Strategy

This study was registered with PROSPERO (International prospective register of systematic reviews. https://www.crd.york.ac.uk/PROSPERO/ -registration number-CRD 42021238423). We performed this meta-analysis following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (**Supplemental Table 1**) (37).

Two authors (W. L. and X. L.) independently conducted the database search, selection, data extraction, and statistical analysis. Four databases were searched for all related studies, including PubMed, Embase, the Cochrane Library, and MedRxiv (https://www.medrxiv.org/), up to February 27, 2021. No language restrictions were applied. The following search terms were used for all databases: ("2019-novel coronavirus" OR "SARS-CoV-2" OR "COVID-19" OR "2019-nCoV" OR "COVID 19" OR "severe acute respiratory syndrome coronavirus 2") AND ("atrial fibrillation" OR "atrial fibrillations" OR "auricular fibrillation" OR "auricular fibrillations"). In addition, the conference abstracts and bibliographies of related literature were scanned to obtain other articles that might meet the requirements.

Selection Criteria and Study Selection

Studies were included if they met the following inclusion criteria: (1) patients in the literature were adults (>18 years of age) who were diagnosed with COVID-19 according to polymerase chain reaction (PCR) tests and had sinus rhythm at admission according to a 12-lead electrocardiogram (ECG); (2) studies reported the prevalence of AF during hospital admission and/or the association between AF and outcomes (e.g., all-cause mortality) in patients with COVID-19; and (3) articles were cohort or nested case–control studies. Accordingly, studies with the following conditions were excluded: (1) reviews, meta-analyses, congress abstracts, practice guidelines, patents, cases, editorials, replies, or comments; and (2) data of the articles remained unavailable after contacting the corresponding authors for further information.

The initial search results were imported into EndNote X8.2 software (Thomson Reuters, New York, NY) for management. Subsequently, duplications were eliminated automatically and manually. First, we examined the citation titles and abstracts. After the preliminary screening, we retrieved full reports that were likely to meet the predefined inclusion criteria. Any inconsistency was resolved through discussion (W. S. and X. L.) until a consensus was reached.

Data Collection and Quality Assessment

Data were extracted based on prespecified inclusion criteria. The following information was abstracted: study characteristics (first author's name, publication year, country, and study design), patient characteristics (sample size, age, sex, and medications), exposure (AF diagnosis and number of episodes during hospitalization), and outcomes (number of events, adjusted OR/RRs and the corresponding 95% CI, and adjustments).

For studies that reported the prevalence of AF, the Joanna Briggs Institute critical appraisal checklist was used to assess the study quality. For studies that reported the association between AF and outcomes in patients with COVID-19, the Newcastle–Ottawa quality scale (NOS) was applied. Case-control studies were appraised on selection, comparability, and exposure, while cohort studies were appraised on selection, comparability, and outcomes. Studies with an NOS of ≥6 stars were considered moderate- to high-quality articles.

Statistical Analysis

RevMan software, version 5.3 (The Cochrane Collaboration 2014, Nordic Cochrane Center Copenhagen, Denmark) and Stata software (Version 14.0, Stata Corp LP, College Station, Texas, USA) were both applied in data analysis. To determine the

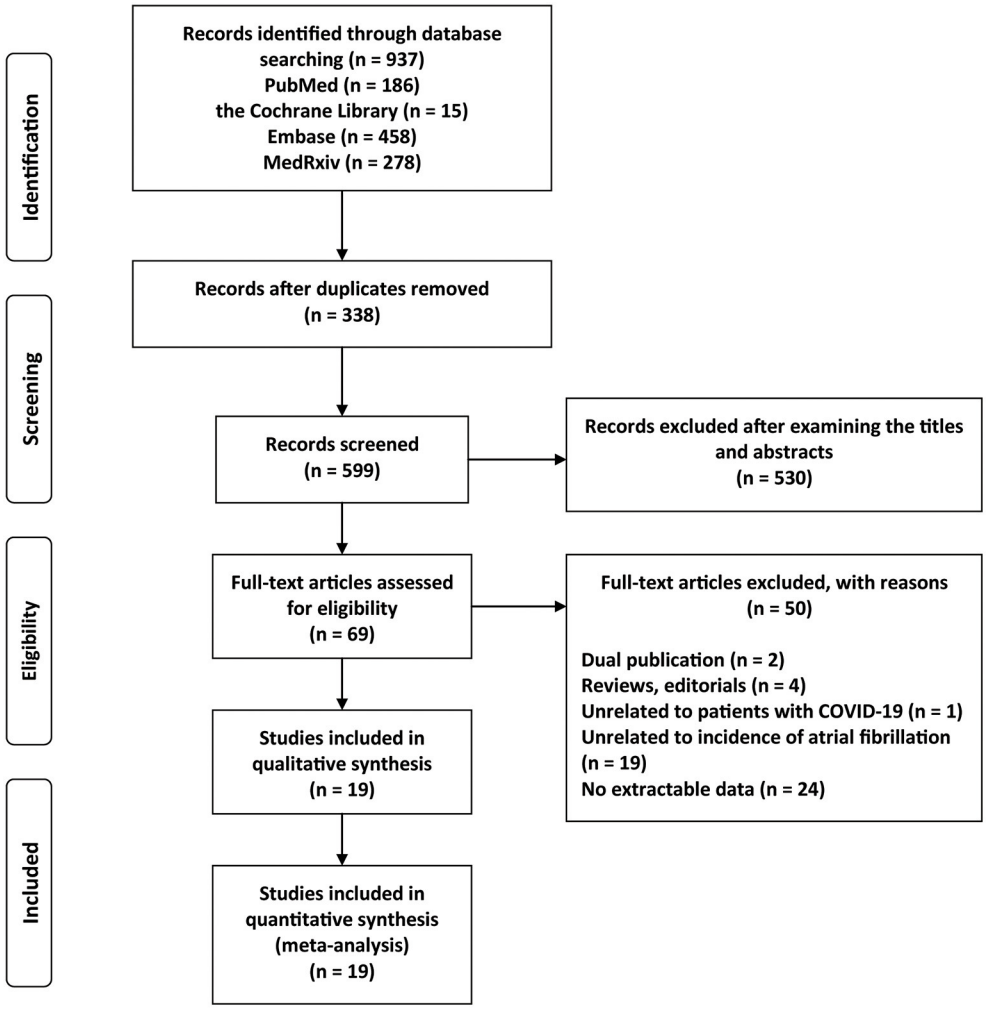


FIGURE 1 | Flow chart of the study selection process.

prevalence of AF in patients with COVID-19, the exact binomial (Clopper–Pearson) method was used to calculate 95% confidence intervals (CIs). Estimates were standardized using the Freeman–Tukey double arcsine transformation. To elucidate the clinical impact of AF in patients with COVID-19, we pooled the crude odds ratios (ORs) for categorical outcomes using the inverse-variance method. The crude ORs were calculated by events and total numbers of patients in the AF groups and control groups. Moreover, we estimated the adjusted effect size by calculating the natural logarithm of the OR (log [OR]) and its standard error (SElog [OR]). The ORs were shown with 95% CIs. We evaluated the degree of heterogeneity among the included studies using the χ^2 statistic (with a *P*-value of 0.10 considered significant) and the I^2 test (25%, 50%, and 75% represent low, moderate, and high heterogeneity, respectively) (38). We used the random effect model in this study to improve the reliability of our results considering the potential heterogeneity.

Subgroup analyses were performed to research possible modulated factors influencing our primary meta-analysis results, including age, region, study design, sample size, cases of AF, and severity. We defined patients with severe COVID-19 as those who were admitted to the ICU, while patients who were not admitted to the ICU were considered to have non-severe COVID-19. Additionally, patients with a history of AF were excluded from the analysis of the prevalence of new-onset AF. Publication bias was assessed using funnel plots, Egger's test, and Begg's test. To appraise the robustness and reliability of the primary study outcomes, we also carried out sensitivity analyses by omitting each study in turn. All statistical tests were double-sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Literature Search

The study selection process is shown in **Figure 1**. A total of 937 citations were identified through the initial database search. After a quick screening of the title and abstract, 69 articles remained. We further excluded 50 articles after the full-text review for the

TABLE 1 | Characteristics of included studies in this meta-analysis.

References, country	Sample of size, N	AF diagnosis	Study design	Mean age (years), Male %	History of AF, N	AF cases, N	New-onset AF cases, N	Outcomes reported	Medication (%)	Adjustments
Aajal et al. (20), Morocco	100	ECG	Prospective cohort	55.3, 37	22	2	NR	Prevalence	NR	–
Angeli et al. (23), Italy	50	ECG	Retrospective cohort	64, 72	NR	3	NR	Prevalence	Hydroxychloroquine: 82.0; Macrolides: 56.0; Lopinavir-Ritonavir: 54.0	–
Bhatla et al. (21), USA	700	ECG	Retrospective cohort	50, 45	39	25	NR	Prevalence, Mortality	Hydroxychloroquine: 24.6; Remdesivir: 8.1	None
Chen et al. (39), USA	143	ECG	Retrospective cohort	67, 62.2	19	13	13	Prevalence	NR	–
Colon et al. (14), USA	115	ECG	Retrospective cohort	56, 53.9	6	12	NR	Prevalence	Remdesivir/Placebo Trial: 7.0; Hydroxychloroquine: 6.1; Azithromycin: 43.5	–
Coromilas et al. (32), USA	4,526	ECG	Retrospective cohort	62.8, 57	408	509	NR	Prevalence	Hydroxychloroquine: 57.6; Azithromycin: 49.8; Antiviral: 15.3; IL-6 Inhibitor: 9.6; Anticoagulation: 29.4	–
Iacopino et al. (27), Italy	30	ECG	Prospective cohort	75.2, 66.7	8	10	10	Prevalence	None: 10.0; Antibiotic therapy: 6.7; Hydroxychloroquine+antiviral: 46.7; Hydroxychloroquine+ antiviral+azithromycin: 6.7; Hydroxychloroquine: 30.0; Monoclonal antibodies: 6.7; Low molecular weight heparins: 100.0	–
Kelesoglu et al. (25), Turkey	658	ECG	Retrospective cohort	54, 56.6	NR	33	33	Prevalence Mortality	NR	–
Linschoten et al. (18), Netherlands	3011	ECG	Retrospective cohort	67, 62.8	NR	142	NR	Prevalence	NR	–

(Continued)

TABLE 1 | Continued

References, country	Sample of size, N	AF diagnosis	Study design	Mean age (years), Male %	History of AF, N	AF cases, N	New-onset AF cases, N	Outcomes reported	Medication (%)	Adjustments
Mountantonakis et al. (15), USA	9,564	ECG	Retrospective cohort	64.8, 58.9	687	1,687	1,109	Prevalence Mortality	NR	Matching for age, gender, smoking, race, medical history, lactate, WBC magnesium, procalcitonin,d-dimer, ferritin, CRP, creatinine, bun, AST, lymphocyte count, ALT, ALT phos, serum glucose, potassium, sodium
Oates et al. (31), USA	77	ECG	Retrospective cohort	69, 55	4	5	4	Prevalence	Hydroxychloroquine: 87.0; Azithromycin: 60.0; Remdesivir: 4.0; Tocilizumab: 4.0	–
Peltzer et al. (28), USA	1,053	ECG	Retrospective cohort	62, 62	94	166	101	Prevalence Mortality	Hydroxychloroqine: 70.8; Remdesivir: 4.9; Steroids: 22.9; IL-6 inhibitor: 6.2; Intravenous gamma globulin: 0.9	Age, sex, race, renal disease, hypoxia, heart failure, CAD, hypertension, diabetes, pulmonary disease, renal disease,immunosuppression, smoking status, and cancer
Rav-Acha et al. (16), Israel	390	ECG	Retrospective cohort	57.5, 55.4	21	20	16	Prevalence Mortality	Azithromycin: 24.2; Hydroxychloroquine: 37.9; QT prolonging drug: 17.2	None
Russo et al. (19), Italy	414	ECG	Retrospective cohort	66.9, 61.1	72	71	50	Prevalence Mortality	NR	None
Saleh et al. (22), USA	201	ECG	Prospective cohort	58.5, 57.2	14	17	17	Prevalence	Hydroxychloroquine/ Chloroquine: 40.8; (Hydroxychloroquine/ Chloroquine) + Azithromycin: 59.2	–
Sanz et al. (26), Spain	160	ECG	Prospective cohort	65.7, 60	30	12	12	Prevalence Mortality	NR	None
Wetterslev et al. (17), Denmark	155	ECG	Retrospective cohort	66, 72.9	NR	52	NR		NR	–
Yenercag et al. (24), Turkey	140	ECG	Retrospective cohort	51.7, 49.3	NR	13	NR	Prevalence	NR	–
Zylla et al. (29), Germany	166	ECG	Retrospective cohort	64.1, 65.1	NR	11	NR	Prevalence Mortality	Hydroxychloroquine: 44.6; Hydroxychloroquine + azithromycin: 16.3; Anticoagulation therapy: 30.7	None

AF, atrial fibrillation; ECG, electrocardiogram; NR, not reported. WBC, white blood cell; CAD, coronary artery disease; CRP, C-reactive protein; BUN, blood urea nitrogen; AST, Aspartate aminotransferase, ALT, Alanine aminotransferase.

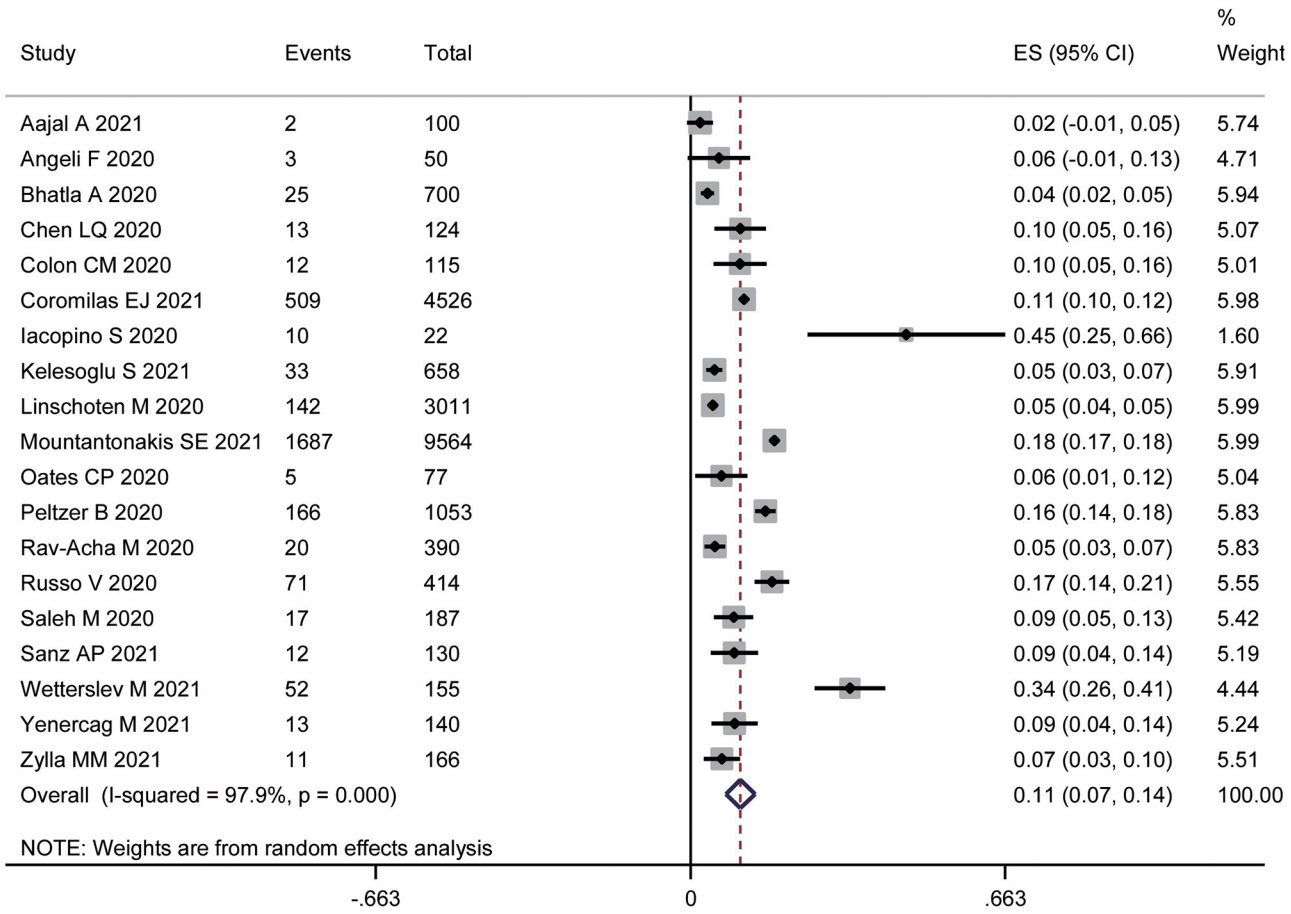


FIGURE 2 | Forest plot of the prevalence of atrial fibrillation in patients with COVID-19.

following reasons: (1) dual publication ($n = 2$); (2) editorials or review articles ($n = 4$); (3) unrelated to patients with COVID-19 ($n = 1$); (4) unrelated to the prevalence of AF ($n = 19$); and (5) no extractable data ($n = 24$). As a result, we included 19 eligible studies (14–32).

Study Characteristics and Study Quality

The basic characteristics are shown in **Table 1**. Among the 19 included studies, (14–32) the publishing years ranged from 2020 to 2021. Overall, a total of 21,653 hospitalized patients were included, with 12,700 (58.7%) being men (ranging from 37.0 to 72.9%). The number of individuals ranged from 30 to 9,564, with the mean age of the participants ranging from 50.0 years to 75.2 years. Among the included studies, the diagnosis of AF was based on electrocardiograms. Three reports were from Asia, (16, 24, 25) 1 from Africa, (20) 7 from Europe, (17–19, 23, 26, 27, 29) and 8 from America (14, 15, 21, 22, 28, 30–32). Apart from 4 prospective cohort studies, (20, 22, 26, 27) the remaining 15 articles were designed as retrospective cohort studies (14–19, 21, 23–25, 28–32).

Based on the Joanna Briggs Institute Critical Appraisal Checklist, all 19 studies (14–32) that reported the prevalence of AF met a minimum of six of the nine criteria, which meant that these articles applied rigorous methodology (**Supplemental Table 2**). In accordance with the NOS, all 8 studies (15, 16, 19, 21, 25, 26, 28, 29) that involved the association between AF and outcomes of patients with COVID-19 were viewed as moderate to high quality, with a score range of 6–8 (**Supplemental Table 3**).

The Prevalence of AF in Patients With COVID-19

Nineteen studies (14–32) with a total of 21,582 participants reported the prevalence of AF in hospitalized patients with COVID-19. The pooled prevalence of AF was 11% (95% CI: 7% to 14%), with high heterogeneity ($I^2 = 97.9\%$) (**Figure 2**).

In the subgroup analysis, older (mean age ≥60 years) patients with COVID-19 showed a nearly 2.5-fold higher prevalence of AF than younger (mean age <60 years) patients with COVID-19 (ES: 13 vs. 5%, P for subgroup difference <0.001) (**Figure 3A**). Europeans had the highest prevalence of AF (ES: 15%), followed by Americans (ES: 11%), Asians (ES: 6%), and Africans (ES: 2%) (P for subgroup difference <0.001) (**Figure 3B**). Furthermore, the prevalence of AF in patients with severe COVID-19 was

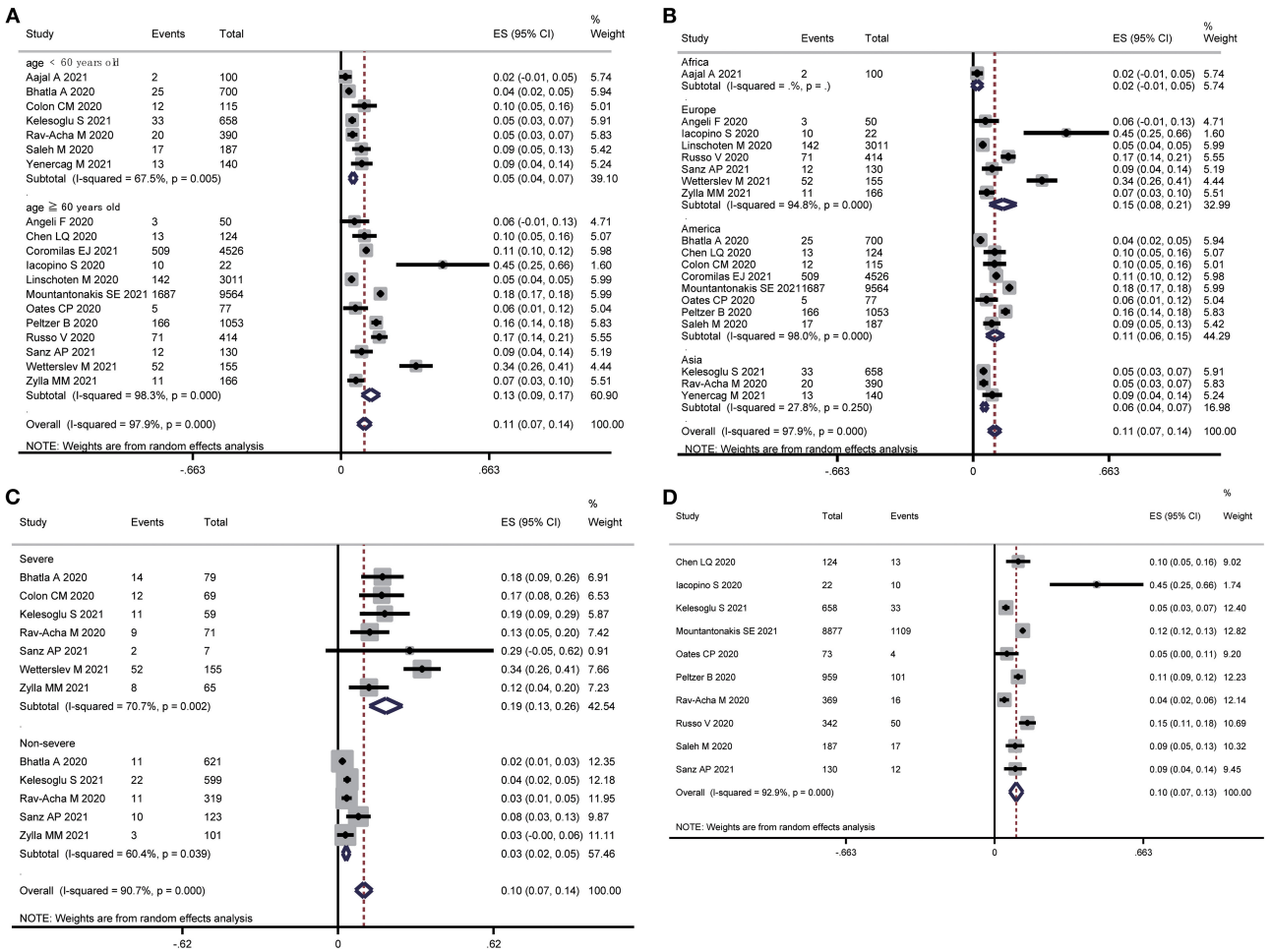


FIGURE 3 | Subgroup analysis of the prevalence of atrial fibrillation in patients with COVID-19. **(A)** Age subgroup. **(B)** Region subgroup. **(C)** Severity subgroup. **(D)** New-onset atrial fibrillation subgroup.

6-fold higher than in patients with nonsevere COVID-19 (ES: 19 vs. 3%, *P* for subgroup difference <0.001) (**Figure 3C**). Ten articles (15, 16, 19, 22, 25–28, 30, 31) provided data on the prevalence of new-onset AF (ES: 10%, 95% CI: 7% to 13%, I^2 = 92.9%) (**Figure 3D**). There was no significant difference in the study design ($P = 0.92$), sample size ($P = 0.74$), or cases of AF ($P = 0.20$) (**Table 2**).

The Impact of AF on All-Cause Mortality in Patients With COVID-19

Eight articles (15, 16, 19, 21, 25, 26, 28, 29) with a total of 13,075 participants reported the association between AF and all-cause mortality in patients with COVID-19. Ultimately, of the 2,025 patients in the AF group, 1,024 patients died (50.6%). There were 11,050 patients in the control group, with 3,242 deaths (29.3%). As presented in **Figure 4A**, AF was significantly associated with an increased risk of all-cause mortality among patients with COVID-19 (crude OR: 2.98, 95% CI: 1.91 to 4.66, I^2 = 77%). Moreover, the pooled result of the multivariate analysis (15, 28) did not change (adjusted OR: 1.65, 95% CI: 1.16 to 2.35, I^2 = 59%) (**Figure 4B**).

Additionally, 6 publications (15, 16, 19, 25, 26, 28) with a total of 11,335 participants reported the association between new-onset AF and all-cause mortality in patients with COVID-19. There was a strong association between new-onset AF and all-cause mortality among hospitalized patients with COVID-19 (crude OR: 2.32, 95% CI: 1.60 to 3.37, I^2 = 54%) (**Figure 5A**). Consistently, the pooled multivariate analysis (15, 28) showed similar results (adjusted OR: 2.01, 95% CI: 1.12 to 3.62, $P = 0.02$, $I^2 = 82\%$) (**Figure 5B**).

As shown in **Supplemental Figure 1**, the funnel plot, Egger's test ($p = 0.19$), and Begg's test ($p = 0.99$) showed no statistically significant potential publication bias, although publication bias was not suggested when the included studies was limited ($N < 10$). Sensitivity analyses performed by omitting each study

TABLE 2 | Subgroup analysis of prevalence of AF in patient with COVID-19.

Items		Number of studies	ES (95%CI)	P	$P^{*}_{h(\%)}$	$P^{\#}$
Result of primary analysis		19	0.105 (0.074–0.136)	<0.001	97.9	–
Mean age	<60 years	7	0.054 (0.037–0.072)	<0.001	67.5	<0.001
	≥60 years	12	0.133 (0.093–0.174)	<0.001	98.3	–
Study design	Retrospective	15	0.106 (0.072–0.140)	<0.001	98.3	0.92
	Prospective	4	0.102 (0.028–0.176)	0.007	88.2	–
Sample size	<300	11	0.110 (0.067–0.154)	<0.001	87.4	0.74
	≥ 300	8	0.100 (0.055–0.145)	<0.001	99.1	–
Cases of AF	<15	9	0.082 (0.049–0.116)	<0.001	73.6	0.20
	≥ 15	10	0.117 (0.076–0.159)	<0.001	98.9	–
Region	Europe	7	0.146 (0.080–0.212)	<0.001	94.8	<0.001
	America	8	0.107 (0.064–0.150)	<0.001	98.0	–
	Asia	3	0.055 (0.039–0.071)	<0.001	27.8	–
	Africa	1	0.020 (−0.007–0.047)	0.153	–	–
Severity of illness	Severe	7	0.191 (0.125–0.257)	<0.001	70.7	<0.001
	Non-severe	5	0.033 (0.018–0.047)	<0.001	60.4	–
Incidence of new-onset AF		10	0.097 (0.067–0.126)	<0.001	92.9	–

*AF, atrial fibrillation. *P for within-group heterogeneity, #P for subgroup difference.*

indicated that our results were stable and reliable, with a range from 2.61 (95% CI: 1.69 to 4.02) to 3.55 (95% CI: 2.14 to 5.91) (**Supplemental Figure 2**).

DISCUSSION

Overall, 19 studies were included in this study with a total of 21,653 hospitalized patients. The pooled prevalence of AF approached 11% in patients with COVID-19. Our results demonstrated that AF is quite common among hospitalized patients with COVID-19, particularly among older patients (≥60 years of age), North American and European patients, and patients with severe COVID-19. Furthermore, AF and new-onset AF were significantly associated with an increased risk of all-cause mortality among hospitalized patients with COVID-19.

Our results seemed to agree with previous studies, (33–36) while there were essential differences between the present meta-analysis and others. Two previous meta-analyses (34, 35) did not specify the type of arrhythmia in COVID-19, which meant that those studies mainly focused on arrhythmias instead of each subtype, such as AF. Our meta-analysis extended these studies and had two important strengths. This is the most comprehensive study to assess the prevalence of AF, as well as new-onset AF, among hospitalized patients with COVID-19, and our results showed that new-onset AF was also independently associated with an increased risk of mortality by excluding data from patients with a prior history of AF. More importantly, our subgroup analyses first revealed regional differences in the prevalence of AF among hospitalized patients with COVID-19 and the correlation between AF and severe COVID-19.

Compared with the prevalence of arrhythmias in hospitalized patients with community-acquired pneumonia (7%, 95% CI: 6 to 9%), (40) this study showed a higher prevalence of AF in hospitalized patients with COVID-19 (11%, 95% CI: 7 to 14%). The exact pathophysiology underlying AF in COVID-19 may be multifactorial and remains elusive. At present, some studies preliminarily speculate that SARS-CoV-2 is similar to SARS-CoV, which may cause a series of cascade reactions leading to pneumonia by combining with angiotensin-converting enzyme-2 (ACE2) in the human respiratory tract and lung tissue (41). The ACE2 receptor is also widely expressed in the cardiovascular system (42). Theoretically, the cardiovascular system is also a potential target organ of SARS-CoV-2 (43). Therefore, ACE2-related signaling pathways may play a key role in myocardial injury, which may affect atrial remodeling and increase susceptibility to AF (44). Moreover, inflammatory factor storms may be the mechanism of disease progression (45). Various inflammatory factors have been proven to be closely related to the development of AF. It has been reported that even mild tension in rat atrial tissue pretreated with IL-6 can lead to the occurrence of AF (46). In addition to the direct damage to myocardial cells caused by virus infection and the systemic inflammatory response syndrome induced by the virus, metabolic abnormalities, (47) hypoxemia, (48–50) respiratory failure, and usage of certain antiviral drugs (51, 52) also play roles in the pathogenesis of AF.

The potential mechanism by which AF contributes to increased mortality in patients with COVID-19 is yet to be determined. Coagulation abnormalities, cardiac injury, and stroke are possible mechanisms. For example, patients with AF had marked elevations in troponin, brain natriuretic peptide, C-reactive protein, and D-dimer, which may be

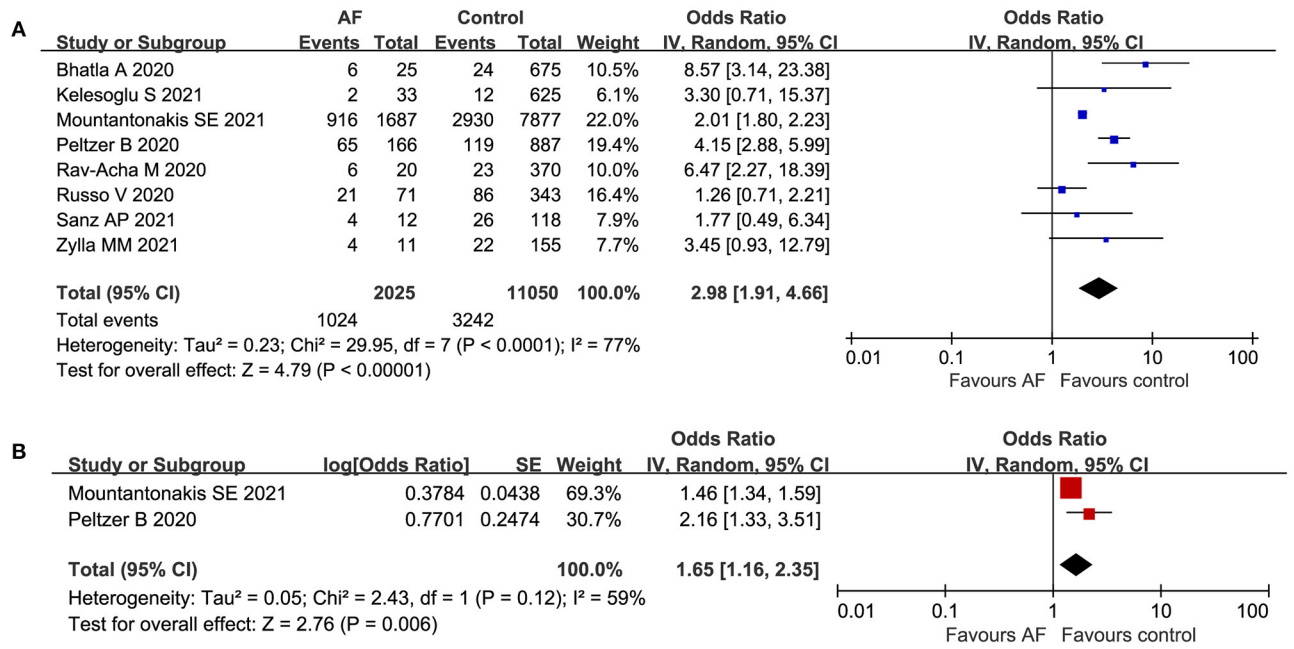


FIGURE 4 | Forest plot of the association between atrial fibrillation and all-cause mortality in patients with COVID-19. **(A)** Crude effect size of the association between atrial fibrillation and all-cause mortality in patients with COVID-19. **(B).** Adjusted effect size of the association between atrial fibrillation and all-cause mortality in patients with COVID-19.

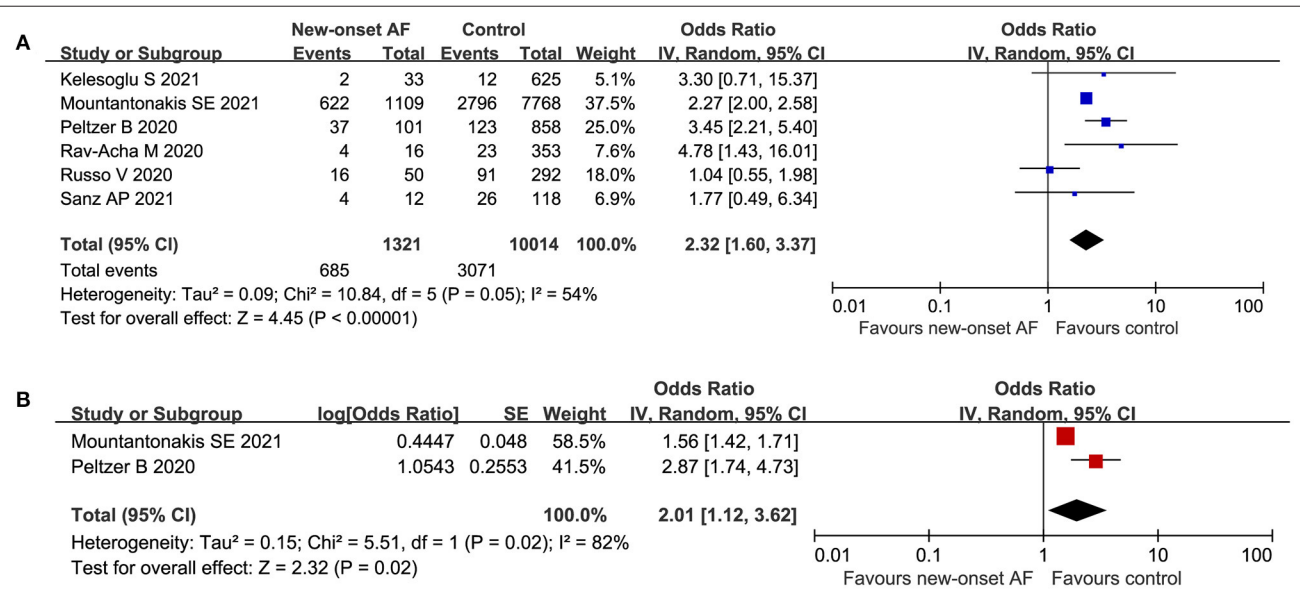


FIGURE 5 | Forest plot of the association between new-onset atrial fibrillation and all-cause mortality in patients with COVID-19. **(A)** Crude effect size of the association between new-onset atrial fibrillation and all-cause mortality in patients with COVID-19. **(B)** Adjusted effect size of the association between new-onset atrial fibrillation and all-cause mortality in patients with COVID-19.

the manifestations of cardiac injury, worsening cardiac function, and inflammatory response (28). Furthermore, hypercoagulability is an important feature in COVID-19, and AF could contribute to poor cardiac output, exacerbate the hypercoagulable state, and eventually lead to increased mortality (53).

Our prognosis analysis showed that in-hospital mortality was significantly higher among patients with AF than among

patients without AF. After adjustment for age, race, body mass index, and comorbidities, AF and new-onset AF were independently associated with a higher risk of all-cause mortality among patients with COVID-19. Moreover, it was notable that a few studies reported that new-onset AF was associated with longer hospital stays, more bleeding events, and more embolic events. These consistent findings indicated that AF and new-onset AF were associated with poor prognosis in patients with COVID-19. Therefore, clinicians should be more attentive to patients with COVID-19 and AF, optimize the clinical management of the disease, and implement more effective treatment regimens. Although no specific therapies have been recommended for patients with COVID-19 with AF to date, anticoagulant therapy may be useful. Systemic anticoagulants were reported to reduce mortality in hospitalized patients with COVID-19 (54). Similarly, low-molecular-weight heparin treatment was associated with lower 28-day mortality in patients with COVID-19 who had symptoms of coagulation disorders (55). In addition, several potential agents have been proposed for the treatment of patients with severe COVID-19, such as the interleukin-6 receptor antagonist tocilizumab (56, 57) and corticosteroids (58). Considering the strong link between inflammation and AF, the effect of these agents on the prevention of AF in patients with severe COVID-19 should be studied further.

Considering the high prevalence of AF among patients with COVID-19 and its poor prognostic implications, clinicians should recognize AF in patients with COVID-19. Careful electrocardiographic monitoring is advisable in patients with COVID-19 to detect AF early. Additionally, screening for AF should be performed in patients with COVID-19 and respective risk factors, particularly in older patients (≥60 years of age), North American and European patients, and patients with severe COVID-19. Moreover, our results highlight the importance of utilizing AF and new-onset AF as clinical markers of in-hospital mortality and poor prognosis in hospitalized patients with COVID-19. Future investigations will need to further explore the association between COVID-19 and AF and to evaluate the safest and most effective strategies for clinical treatment and management of the disease.

Study Limitations

There are several limitations to the present systematic review and meta-analysis that need to be discussed. First, all the included studies were observational studies which cannot prove causality. Most of the studies were retrospective (79%) cohort. Hence, further well-designed, large-scale, prevalence studies are warranted to assessed the prevalence of AF in patient with COVID-19, as well as the potential difference in region, severity and age. Second, a high degree of heterogeneity was observed in our results. Although meta-regression was not performed, the subgroup analysis showed the heterogeneity might derived from region, age or severity (**Table 2**). Third, many studies did not adjust for clinical confounding factors regarding the outcome of death. However, the positive association between AF and all-cause mortality persisted in the adjusted subgroup, suggesting that our results were relatively stable. Fourth, all the included participants were inpatients, rather than community patients, which may overestimate the prevalence and clinical impact of AF on patients with COVID-19. Fifth, many articles did not report specific drugs for treatment, so we cannot address the effects of these factors on the association between AF and poor prognosis in patients with COVID-19. Sixth, it is well known that AF significantly contributes to the incidence of stroke; however, stroke was not assessed in the present meta-analysis. Nevertheless, studies have shown that stroke is an uncommon complication of COVID-19, and there is no significant association between cerebrovascular disease and fatal outcomes in patients with COVID-19, suggesting that the prognostic damage caused by AF might be independent of stroke (39, 59, 60). Finally, there was only a small number of studies from Asia and Africa. In light of varying population characteristics among different regions, more studies from Asia and Africa are needed to confirm the regional differences in the prevalence of COVID-19.

CONCLUSIONS

AF is quite common among hospitalized patients with COVID-19, particularly among older patients (≥60 years of age), North American and European patients, and patients with severe COVID-19. Moreover, AF and new-onset AF were independently associated with an increased risk of all-cause mortality among hospitalized patients with COVID-19. Our results should be confirmed by further well-designed, prospective studies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

XL, WZ, and PY were responsible for the entire project and revised the draft. ZL and WS performed the study selection, data extraction, statistical analysis, and interpretation of the data. WS and XL drafted the first version of the manuscript. All authors participated in the interpretation of the results and prepared the final version of the manuscript.

FUNDING

This work was supported by the National Natural Science Foundation of China (PY, 81760050 and 81760048; XL, 82100347) and the Jiangxi Provincial Natural Science Foundation for Youth Scientific Research (PY, 20192ACBL21037), as well

as the Young Teachers' Basic Scientific Research Business Expenses Project (WZ, 20ykpy72), the China Postdoctoral Science Foundation (WZ, 2020M673016), and the China National Postdoctoral Program for Innovative Talents (WZ, BX20200400).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.720129/full#supplementary-material

REFERENCES

1. Corrales-Medina VF, Musher DM, Shachkina S, Chirinos JA. Acute pneumonia and the cardiovascular system. *Lancet.* (2013) 381:496–505. doi: 10.1016/S0140-6736(12)61266-5
2. Corrales-Medina VF, Musher DM, Wells GA, Chirinos JA, Chen L, Fine MJ. Cardiac complications in patients with community-acquired pneumonia: incidence, timing, risk factors, and association with short-term mortality. *Circulation.* (2012) 125:773–81. doi: 10.1161/CIRCULATIONAHA.111.040766
3. Perry TW, Pugh MJ, Waterer GW, Nakashima B, Orihuela CJ, Copeland LA, et al. Incidence of cardiovascular events after hospital admission for pneumonia. *Am J Med.* (2011) 124:244–51. doi: 10.1016/j.amjmed.2010.11.014
4. Driggin E, Madhavan MV, Bikdeli B, Chuich T, Laracy J, Biondi-Zoccai G, et al. Cardiovascular considerations for patients, health care workers, and health systems during the COVID-19 pandemic. *J Am Coll Cardiol.* (2020) 75:2352–71. doi: 10.1016/j.jacc.2020.03.031
5. Liu K, Fang YY, Deng Y, Liu W, Wang MF, Ma JP, et al. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. *Chin Med J (Engl).* (2020) 133:1025–31. doi: 10.1097/CM9.0000000000000744
6. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017
7. Kang Y, Chen T, Mui D, Ferrari V, Jagasia D, Scherrer-Crosbie M, et al. Cardiovascular manifestations and treatment considerations in COVID-19. *Heart.* (2020) 106:1132–41. doi: 10.1136/heartjnl-2020-317056
8. January CT, Wann LS, Calkins H, Chen LY, Cigarroa JE, Cleveland JC Jr, et al. 2019 AHA/ACC/HRS focused update of the 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a report of the American college of cardiology/American heart association task force on clinical practice guidelines and the heart rhythm society in collaboration with the society of thoracic surgeons. *Circulation.* (2019) 140:e125–51. doi: 10.1161/CIR.0000000000000665
9. Ball J, Carrington MJ, McMurray JJ, Stewart S. Atrial fibrillation: profile and burden of an evolving epidemic in the 21st century. *Int J Cardiol.* (2013) 167:1807–24. doi: 10.1016/j.ijcard.2012.12.093
10. Lip GYH, Brechin CM, Lane DA. The global burden of atrial fibrillation and stroke: a systematic review of the epidemiology of atrial fibrillation in regions outside North America and Europe. *Chest.* (2012) 142:1489–98. doi: 10.1378/chest.11-2888
11. Chiang CE, Naditch-Brûlé L, Murin J, Goethals M, Inoue H, O'Neill J, et al. Distribution and risk profile of paroxysmal, persistent, and permanent atrial fibrillation in routine clinical practice: insight from the real-life global survey evaluating patients with atrial fibrillation international registry. *Circ Arrhythm Electrophysiol.* (2012) 5:632–9. doi: 10.1161/CIRCEP.112.970749
12. Zoni-Berisso M, Lercari F, Carazza T, Domenicucci S. Epidemiology of atrial fibrillation: European perspective. *Clin Epidemiol.* (2014) 6:213–20. doi: 10.2147/CLEP.S47385
13. McManus DD, Rienstra M, Benjamin EJ. An update on the prognosis of patients with atrial fibrillation. *Circulation.* (2012) 126:e143–6. doi: 10.1161/CIRCULATIONAHA.112.129759
14. Colon CM, Barrios JG, Chiles JW, McElwee SK, Russell DW, Maddox WR, et al. Atrial Arrhythmias in COVID-19 Patients. *JACC Clin Electrophysiol.* (2020) 6:1189–90. doi: 10.1016/j.jacep.2020.05.015
15. Mountantonakis SE, Saleh M, Fishbein J, Gandomi A, Lesser M, Chelico J, et al. Atrial fibrillation is an independent predictor for in-hospital mortality in patients admitted with SARS-CoV-2 infection. *Heart Rhythm.* (2021). doi: 10.1016/j.hrthm.2021.01.018
16. Rav-Acha M, Orlev A, Itzhaki I, Zimmerman SF, Fteiha B, Bohm D, et al. Cardiac arrhythmias among hospitalized Coronavirus 2019 (COVID-19) patients: prevalence, characterization, and clinical algorithm to classify arrhythmic risk. *Int J Clin Pract.* (2020) 75:e13788. doi: 10.22541/au.160071287.74177510
17. Wetterslev M, Jacobsen PK, Hassager C, Jøns C, Risum N, Pehrson S, et al. Cardiac arrhythmias in critically ill patients with coronavirus disease 2019: a retrospective population-based cohort study. *Acta Anaesthesiol Scand.* (2021) 65:770–7. doi: 10.1111/aas.13806
18. Linschoten M, Peters S, van Smeden M, Jewbali LS, Schaap J, Siebelink HM, et al. Cardiac complications in patients hospitalised with COVID-19 *Eur Heart J Acute Cardiovasc Care.* (2020) 9:817–23. doi: 10.1177/2048872620974605
19. Russo V, Di Maio M, Mottola FF, Pagnano G, Attena E, Verde N, et al. Clinical characteristics and prognosis of hospitalized COVID-19 patients with incident sustained tachyarrhythmias: A multicenter observational study. *Eur J Clin Invest.* (2020) 50:e13387. doi: 10.1111/eci.13387
20. Aajal A, El Boussaadani B, Hara L, Benajiba C, Boukouk O, Benali M, et al. The consequences of the lockdown on cardiovascular diseases. *Ann Cardiol Angeiol (Paris).* (2021). doi: 10.1016/S0735-1097(21)04491-0
21. Bhatla A, Mayer MM, Adusumalli S, Hyman MC, Oh E, Tierney A, et al. COVID-19 and cardiac arrhythmias. *Heart Rhythm.* (2020) 17:1439–44. doi: 10.1016/j.hrthm.2020.06.016
22. Saleh M, Gabriels J, Chang D, Soo Kim B, Mansoor A, Mahmood E, et al. Effect of Chloroquine, Hydroxychloroquine, and Azithromycin on the Corrected QT Interval in Patients With SARS-CoV-2 Infection. *Circ Arrhythm Electrophysiol.* (2020) 13:e008662. doi: 10.1161/CIRCEP.120.008662
23. Angeli F, Spanevello A, De Ponti R, Visca D, Marazzato J, Palmiotto G, et al. Electrocardiographic features of patients with COVID-19 pneumonia. *Eur J Intern Med.* (2020) 78:101–6. doi: 10.1016/j.ejim.2020.06.015
24. Yenerçag M, Arslan U, Seker OO, Dereli S, Kaya A, Dogduş M, et al. Evaluation of P-wave dispersion in patients with newly diagnosed coronavirus disease 2019. *J Cardiovasc Med.* (2021) 22:197–203. doi: 10.2459/JCM.0000000000001135
25. Kelesoglu S, Yilmaz Y, Ozkan E, Calapkorur B, Gok M, Dursun ZB, et al. New onset atrial fibrilation and risk faktors in COVID-19. *J Electrocardiol.* (2021) 65:76–81. doi: 10.1016/j.jelectrocard.2020.12.005
26. Pardo Sanz A, Salido Tahoces L, Ortega Pérez R, González Ferrer E, Sánchez Recalde Á, Zamorano Gómez JL. New-onset atrial fibrillation during COVID-19 infection predicts poor prognosis. *Cardiol J.* (2021) 28:34–40. doi: 10.5603/CJ.a2020.0145
27. Iacopino S, Placentino F, Colella J, Pesce F, Pardeo A, Filannino P, et al. New-onset cardiac arrhythmias during COVID-19 hospitalization. *Circ Arrhythm Electrophysiol.* (2020) 13:e009040. doi: 10.1161/CIRCEP.120.009040
28. Peltzer B, Manocha KK, Ying X, Kirzner J, Ip JE, Thomas G, et al. Outcomes and mortality associated with atrial arrhythmias among patients hospitalized with COVID-19. *J Cardiovasc Electrophysiol.* (2020) 31:3077–85. doi: 10.1111/jce.14770
29. Zylla MM, Merle U, Vey JA, Korosoglou G, Hofmann E, Muller M, et al. Predictors and Prognostic Implications of Cardiac Arrhythmias in Patients Hospitalized for COVID-19. *J Clin Med.* (2021) 10:133. doi: 10.3390/jcm10010133
30. Chen LQ, Burdowski J, Marfatia R, Weber J, Gliganic K, Diaz N, et al. Reduced cardiac function is associated with cardiac injury and mortality risk in hospitalized COVID-19 patients. *Clin Cardiol.* (2020) 43:1547–54. doi: 10.1002/clc.23479
31. Oates CP, Turagam MK, Musikantow D, Chu E, Shivamurthy P, Lampert J, et al. Syncope and presyncope in patients with COVID-19. *Pacing Clin Electrophysiol.* (2020) 43:1139–48. doi: 10.1111/pace.14047

32. Coromilas EJ, Kochav S, Goldenthal I, Biviano A, Garan H, Goldbarg S, et al. Worldwide survey of COVID-19 associated arrhythmias. *Circ Arrhythm Electrophysiol.* (2021).
33. Yang H, Liang X, Xu J, Hou H, Wang Y. Meta-analysis of atrial fibrillation in patients With COVID-19. *Am J Cardiol.* (2021) 144:152–6. doi: 10.1016/j.amjcard.2021.01.010
34. Liao SC, Shao SC, Cheng CW, Chen YC, Hung MJ. Incidence rate and clinical impacts of arrhythmia following COVID-19: a systematic review and meta-analysis of 17,435 patients. *Crit Care.* (2020) 24:690. doi: 10.1186/s13054-020-03368-6
35. Pellicori P, Doolub G, Wong CM, Lee KS, Mangion K, Ahmad M, et al. COVID-19 and its cardiovascular effects: a systematic review of prevalence studies. *Cochrane Database Syst Rev.* (2021) 3:Cd013879. doi: 10.1002/14651858.CD013879
36. Mulia EPB, Maghfirah I, Rachmi DA, Julario R. Atrial arrhythmia and its association with COVID-19 outcome: a pooled analysis. *Diagnosis (Berl).* (2021). doi: 10.1515/dx-2020-0155
37. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg.* (2010) 8:336–41. doi: 10.1016/j.ijsu.2010.02.007
38. Higgins J, Thomas J, Chandler J, Cumpston M, Li T, Page M, et al. Handbook for Systematic Reviews of Interventions version 6.0 (updated August 2019). Available online at: www.training.cochrane.org/handbook. doi: 10.1002/9781119536604
39. Chen J, Bai H, Liu J, Chen G, Liao Q, Yang J, et al. Distinct clinical characteristics and risk factors for mortality in female inpatients with coronavirus disease 2019 (COVID-19): a sex-stratified, large-scale cohort study in Wuhan, China. *Clin Infect Dis.* (2020) 71:3188–95. doi: 10.1093/cid/ciaa920
40. Tralhão A, Póvoa P. Cardiovascular events after community-acquired pneumonia: a global perspective with systematic review and meta-analysis of observational studies. *J Clin Med.* (2020) 9:414. doi: 10.3390/jcm9020414
41. Gralinski LE, Baric RS. Molecular pathology of emerging coronavirus infections. *J Pathol.* (2015) 235:185–95. doi: 10.1002/path.4454
42. Liu X, Long C, Xiong Q, Chen C, Ma J, Su Y, et al. Association of angiotensin converting enzyme inhibitors and angiotensin II receptor blockers with risk of COVID-19, inflammation level, severity, and death in patients with COVID-19: a rapid systematic review and meta-analysis. *Clin Cardiol.* (2020). doi: 10.1002/clc.23421. [Epub ahead of print].
43. Chen L, Li X, Chen M, Feng Y, Xiong C. The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. *Cardiovasc Res.* (2020) 116:1097–100. doi: 10.1093/cvr/cvaa078
44. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci China Life Sci.* (2020) 63:457–60. doi: 10.1007/s11427-020-1637-5
45. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
46. Mitrokhin VM, Mladenov MI, Kamkin AG. Effects of interleukin-6 on the bio-electric activity of rat atrial tissue under normal conditions and during gradual stretching. *Immunobiology.* (2015) 220:1107–12. doi: 10.1016/j.imbio.2015.05.003
47. Kwenandar F, Japar KV, Damay V, Hariyanto TI, Tanaka M, Lugito NPH, et al. Coronavirus disease 2019 and cardiovascular system: a narrative review. *Int J Cardiol Heart Vasc.* (2020) 29:100557. doi: 10.1016/j.ijcha.2020.100557
48. Stevenson IH, Roberts-Thomson KC, Kistler PM, Edwards GA, Spence S, Sanders P, et al. Atrial electrophysiology is altered by acute hypercapnia but not hypoxemia: implications for promotion of atrial fibrillation in pulmonary disease and sleep apnea. *Heart Rhythm.* (2010) 7:1263–70. doi: 10.1016/j.hrthm.2010.03.020
49. Linz D, Schotten U, Neuberger HR, Böhm M, Wirth K. Negative tracheal pressure during obstructive respiratory events promotes atrial fibrillation by vagal activation. *Heart Rhythm.* (2011) 8:1436–43. doi: 10.1016/j.hrthm.2011.03.053
50. Linz D, Hohl M, Ukena C, Mahfoud F, Wirth K, Neuberger HR, et al. Obstructive respiratory events and premature atrial contractions after cardioversion. *Eur Respir J.* (2015) 45:1332–40. doi: 10.1183/09031936.00175714
51. Lazzerini PE, Boutjdir M. Capecchi PCOVID-19 L Arrhythmic Risk, and Inflammation: Mind the Gap! *Circulation.* (2020) 142:7–9. doi: 10.1161/CIRCULATIONAHA.120.047293
52. Cvetkovic RS, Goa KL. Lopinavir/ritonavir: a review of its use in the management of HIV infection. *Drugs.* (2003) 63:769–802. doi: 10.2165/00003495-200363080-00004
53. Abou-Ismail MY, Diamond A, Kapoor S, Arafah Y, Nayak L. The hypercoagulable state in COVID-19: Incidence, pathophysiology, and management. *Thromb Res.* (2020) 194:101–15. doi: 10.1016/j.thromres.2020.06.029
54. Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. *J Am Coll Cardiol.* (2020) 75:2950–73. doi: 10.1016/j.jacc.2020.04.031
55. Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost.* (2020) 18:1094–9. doi: 10.1111/jth.14817
56. Hariyanto TI, Hardyson W, Kurniawan A. Efficacy and safety of tocilizumab for coronavirus disease 2019 (Covid-19) patients: a systematic review and meta-analysis. *Drug Res (Stuttg).* (2021) 71:265–74. doi: 10.1055/a-1336-2371
57. T. Ivan Hariyanto, Kurniawan A. Tocilizumab administration is associated with the reduction in biomarkers of coronavirus disease 2019 infection. *J Med Virol.* (2021) 93:1832–6. doi: 10.1002/jmv.26698
58. Zhao M. Cytokine storm and immunomodulatory therapy in COVID-19: Role of chloroquine and anti-IL-6 monoclonal antibodies. *Int J Antimicrob Agents.* (2020) 55:105982. doi: 10.1016/j.ijantimicag.2020.105982
59. Aggarwal G, G. Lippi, and B. Michael Henry, Cerebrovascular disease is associated with an increased disease severity in patients with Coronavirus Disease 2019 (COVID-19): a pooled analysis of published literature. *Int J Stroke.* (2020) 15:385–9. doi: 10.1177/1747493020921664
60. Siow I, Lee KS, Zhang JJY, Saffari SE, Ng A, Young B. Stroke as a Neurological complication of COVID-19: a systematic review and meta-analysis of incidence, outcomes and predictors. *J Stroke Cerebrovasc Dis.* (2021) 30:105549. doi: 10.1016/j.jstrokecerebrovasdis.2020.105549

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 13 October 2021
doi: 10.3389/fcvm.2021.750887

Evaluation of Endothelial Dysfunction and Inflammatory Vasculopathy After SARS-CoV-2 Infection—A Cross-Sectional Study

Philipp Jud[1], Paul Gressenberger[1], Viktoria Muster[1], Alexander Avian[2], Andreas Meinitzer[3], Heimo Strohmaier[4], Harald Sourij[5], Reinhard B. Raggam[1], Martin Helmut Stradner[6], Ulrike Demel[6], Harald H. Kessler[7], Kathrin Eller[8] and Marianne Brodmann[1]*

[1] Division of Angiology, Department of Internal Medicine, Medical University of Graz, Graz, Austria, [2] Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz, Graz, Austria, [3] Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria, [4] Department Center of Medical Research, Medical University of Graz, Graz, Austria, [5] Division of Endocrinology, Department of Internal Medicine, Medical University of Graz, Graz, Austria, [6] Division of Rheumatology and Immunology, Department of Internal Medicine, Medical University of Graz, Graz, Austria, [7] Diagnostic and Research Institute of Hygiene, Microbiology and Environmental, Medical University of Graz, Graz, Austria, [8] Division of Nephrology, Department of Internal Medicine, Medical University of Graz, Graz, Austria

OPEN ACCESS

Edited by:
Mingxing Xie,
Huazhong University of Science and Technology, China

Reviewed by:
Adriana Georgescu,
Institute of Cellular Biology and Pathology (ICBP), Romania
Jian Xu,
University of Oklahoma Health Sciences Center, United States

***Correspondence:**
Philipp Jud
philipp.jud@medunigraz.at

Specialty section:
This article was submitted to Atherosclerosis and Vascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *31 July 2021*
Accepted: *15 September 2021*
Published: *13 October 2021*

Citation:
Jud P, Gressenberger P, Muster V, Avian A, Meinitzer A, Strohmaier H, Sourij H, Raggam RB, Stradner MH, Demel U, Kessler HH, Eller K and Brodmann M (2021) Evaluation of Endothelial Dysfunction and Inflammatory Vasculopathy After SARS-CoV-2 Infection—A Cross-Sectional Study. Front. Cardiovasc. Med. 8:750887. doi: 10.3389/fcvm.2021.750887

Background: Rising data suggest that COVID-19 affects vascular endothelium while the underlying mechanisms promoting COVID-19-associated endothelial dysfunction and inflammatory vasculopathy are largely unknown. The aim was to evaluate the contribution of COVID-19 to persisting vascular injury and to identify parameters linked to COVID-19-associated endothelial dysfunction and inflammatory vasculopathy.

Methods: In a cross-sectional design, flow-mediated dilation (FMD), nitroglycerine-related dilation (NMD), pulse-wave velocity (PWV), augmentation index, intima-media thickness (IMT), compounds of the arginine and kynurenine metabolism, homocysteine, von Willebrand factor (vWF), endothelial microparticles (EMP), antiendothelial cell antibodies, inflammatory, and immunological parameters, as well as nailfold capillary morphology were measured in post-COVID-19 patients, patients with atherosclerotic cardiovascular diseases (ASCVD) and healthy controls without prior or recent SARS-CoV-2 infection.

Results: Post-COVID-19 patients had higher values of PWV, augmentation index, IMT, asymmetric and symmetric dimethylarginine, vWF, homocysteine, CD31+/CD42b– EMP, C-reactive protein, erythrocyte sedimentation rate, interleukin-6, and β-2-glycoprotein antibodies as well as lower levels of homoarginine and tryptophan compared to healthy controls (all with $p < 0.05$). A higher total number of pathologically altered inflammatory conditions and higher rates of capillary ramifications, loss, caliber variability, elongations and bushy capillaries with an overall higher microangiopathy evolution score were also observed in post-COVID-19 patients (all with $p < 0.05$). Most parameters of endothelial dysfunction and inflammation were comparably altered in post-COVID-19 patients and patients with ASCVD, including FMD and NMD.

Conclusion: COVID-19 may affect arterial stiffness, capillary morphology, EMP and selected parameters of arginine, kynurenine and homocysteine metabolism as well as of inflammation contributing to COVID-19-associated endothelial dysfunction and inflammatory vasculopathy.

Keywords: COVID-19, endothelial dysfunction, inflammation, vasculopathy, capillary changes

INTRODUCTION

COVID-19 caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has evolved into a pandemic since it was detected in the end of 2019. A higher mortality rate was reported among patients with preexisting cardiovascular diseases compared to patients without an underlying disease and cardiovascular diseases seem to be risk factors for severe SARS-CoV-2 infection (1–3). Additionally, there are rising data suggesting that SARS-CoV-2 affects directly and indirectly endothelial cells, thus leading to endothelial injury and dysfunction thereby contributing to thromboembolism, vasculitic changes and abnormal nailfold capillaroscopy (4–9). The underlying mechanisms promoting COVID-19-associated endothelial dysfunction and inflammatory vasculopathy are yet still largely unknown while potential dysregulation of the renin-angiotensin-aldosterone system, immunothrombosis, and direct endothelial infection have been proposed (10–12).

Endothelial dysfunction may be a key contributor of vasculopathy due to underlying functional and structural changes of endothelial cells, and numerous parameters have been attributed to endothelial dysfunction. Flow-mediated dilation (FMD), pulse-wave velocity (PWV), and intima-media thickness (IMT) represent widely used, non-invasive indicators of vascular reactivity, arterial stiffness, and morphological changes of large arteries (13–15). All have been thoroughly evaluated in atherosclerotic cardiovascular diseases (ASCVD) as predictors for cardiovascular events and mortality (16–18). Additionally, homocysteine, kynurenine and compounds of the arginine metabolism, like homoarginine, asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA), are important mediators of endothelial dysfunction representing further predictors of cardiovascular mortality (19–22). Moreover, endothelial microparticles (EMP), which are released during apoptosis or activation of endothelial cells, as well as antiendothelial cell antibodies (AECA) may be associated with endothelial injury and activation, thus contributing to vasculopathy and endothelial dysfunction (23, 24).

Data about the respective parameters of endothelial dysfunction and inflammatory vasculopathy are largely lacking in COVID-19. Furthermore, data investigating if SARS-CoV-2 infection may cause persistent endothelial and vascular immunopathologic changes are also very limited. The aim of this study was to investigate if previous SARS-CoV-2 infection contributes to persisting endothelial dysfunction, inflammatory vasculopathy, macro-, and microvascular changes and to compare these findings to patients with ASCVD and healthy controls without SARS-CoV-2 infection.

MATERIALS AND METHODS

Study Population and Design

Post-COVID-19 patients diagnosed between March and April 2020 and inpatient treatment at the division of Angiology of the Medical University of Graz were screened *via* charts review for study inclusion and invited to participate. For every COVID-19 subject, one sex-matched healthy volunteer was recruited as well as one age—(± 1 year) and sex-matched subject with known ASCVD was also screened for study inclusion and invited to participate in the study (**Figure 1**). Overall, 42 subjects participated that study which were subdivided into three respective groups with 14 subjects per group. Inclusion criterion for the group of patients with COVID-19 was a known prior SARS-CoV-2 infection. Inclusion criterion for the ASCVD group was the presence of at least one detected, asymptomatic or symptomatic ASCVD, either coronary artery disease, or cerebrovascular disease, or lower extremity arterial disease (LEAD) or upper extremity arterial disease (UEAD). Exclusion criteria for all three cohorts were age < 18 years, any type of preexisting connective tissues disease or vasculitis, existing autoimmune diseases, recent pregnancy, recent malignancies and any acute infections, including foot ulcers or necrosis, at time of enrollment. For the group of COVID-19 subjects, preexisting history of diabetes mellitus, asymptomatic and symptomatic ASCVD, including angina pectoris, myocardial infarction, stroke, intermittent claudication, rest pain, and/or necrosis or ulcers of the lower or upper extremity, were additional exclusion criteria. All subjects were instructed to withhold potentially vasodilatory medications, including calcium channel blockers, phosphodiesterase-5 inhibitors, or prostanoids, and anticoagulation at least 24 h prior to study measurements. All participating patients with ASCVD and healthy controls underwent measurement of COVID-19 immunoglobulin (Ig) G

Abbreviations: ACP, anti-citrullinated protein; ADMA, asymmetric dimethylarginine; AECA, antiendothelial cell antibodies; ANA, antinuclear antibodies; ANCA, anti-neutrophil cytoplasmic antibodies; ASCVD, atherosclerotic cardiovascular diseases; CRP, C-reactive protein; CSURI, capillaroscopic skin ulcer risk index; ELISA, enzyme-linked immunosorbent assay; EMP, endothelial microparticles; ENA, extractable nuclear antigen; ESR, erythrocyte sedimentation rate; FMD, flow-mediated dilation; GAB, global arginine bioavailability; Ig, immunoglobulin; IL-6, interleukin 6; IMT, intima-media thickness; LEAD, lower extremity arterial disease; MES, microangiopathy evolution score; NMD, nitroglycerine-related dilation; NVC, nailfold video capillaroscopy; PCR, polymerase chain reaction; PWV, pulse-wave velocity; SAA, serum amyloid A; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; SDMA, symmetric dimethylarginine; UEAD, upper extremity arterial disease; vWF, von Willebrand factor.

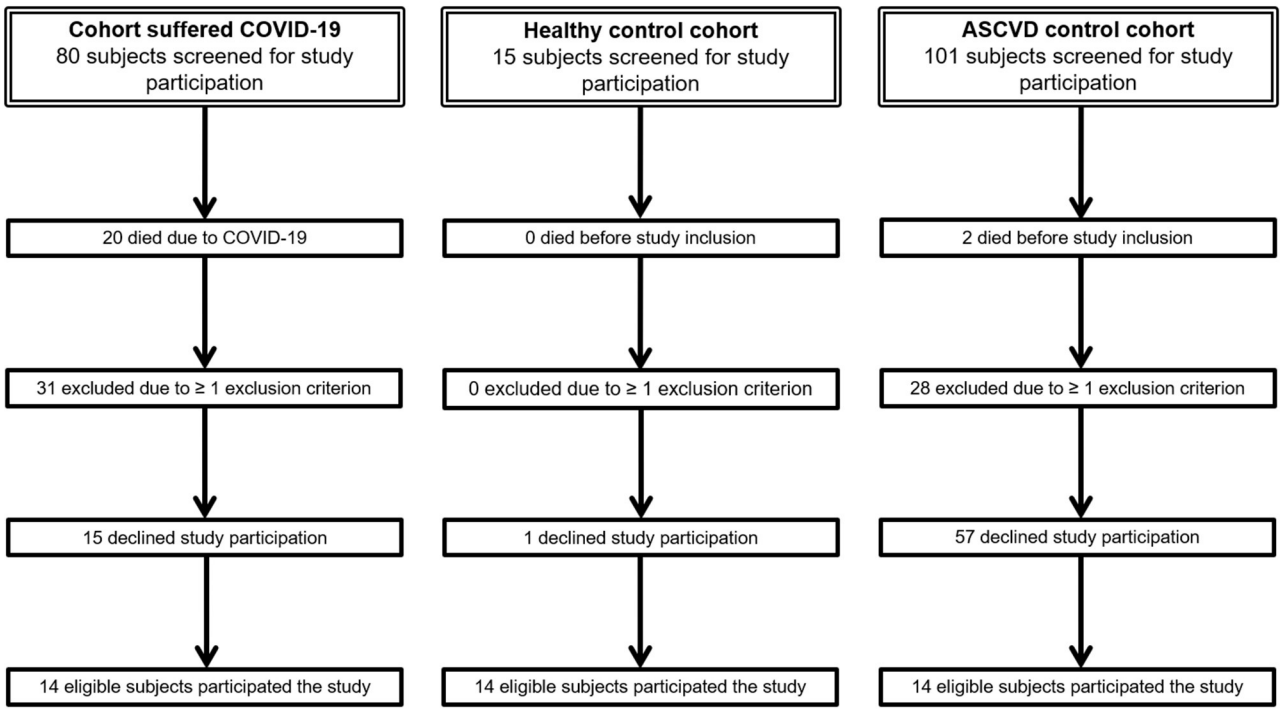


FIGURE 1 | Flow chart of study recruitment.

antibodies and detection of SARS-CoV-2 RNA by polymerase chain reaction (PCR) testing within 3 days prior to start of the study in order to exclude a preexisting or recent SARS-CoV-2 infection. SARS-CoV-2 IgG antibodies were measured by the LIAISON® SARS-CoV-2 S1/S2 IgG (DiaSorin, Saluggia, Italy). This fully automated test allows detection and quantitation of IgG antibodies against S1/S2 antigens of SARS-CoV-2. For detection of SARS-CoV-2 RNA, oropharyngeal swabs were collected by using the Copan ESwab collection system containing 1 ml of transport medium. Samples were tested for SARS-CoV-2 RNA at the Molecular Diagnostics Laboratory, Medical University of Graz, within 12 h of arrival. Presence of SARS-CoV-2 RNA was determined by real-time PCR using the SARS-CoV-2 Test for use on the cobas® 6800/8800 Systems (Roche Molecular Diagnostics, Pleasanton, USA). With this assay, selective amplification of target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primers for ORF1a/b nonstructural region that is unique to SARS-CoV-2. In addition, a conserved region in the structural protein envelope E-gene is chosen for pan-Sarbecovirus detection. The pan-Sarbecovirus detection set also detects SARS-CoV-2 virus. No study subject had received COVID-19 vaccines prior to study measurements.

Between September 2020 and March 2021, parameters of endothelial dysfunction, immune-inflammatory parameters, and capillary morphology of the nailfold were investigated. Primary endpoint was the difference of FMD between post-COVID-19 patients, patients with ASCVD and healthy controls. Secondary endpoints were differences of nitroglycerine-related dilation (NMD), PWV, IMT, homocysteine, compounds of the arginine metabolism, kynurenine, tryptophan, von Willebrand factor (vWF), EMP, AECA, immune-inflammatory parameters and capillary morphology of the nailfold between patients with previous SARS-CoV-2 infection, ASCVD and healthy controls. After signing the informed consent form, blood sampling or biochemical analysis were obtained followed by medical history evaluating cardiovascular risk factors. Subsequently, pulse-wave analysis and measurements of IMT, FMD, NMD and capillary changes by nailfold video capillaroscopy (NVC) were performed. Measurements of pulse-wave analysis, IMT, FMD, NMD, laboratory parameters, and NVC were performed in the morning between 7:00 a.m. and 9:00 a.m. after an overnight fast in a temperature-controlled (22–24°C) and quiet room.

Pulse-Wave Analysis, Intima-Media Thickness, and Flow-Mediated Dilation

Pulse-wave analysis including aortic PWV, augmentation index and pulse pressure was measured and calculated *via* the oscillometric device Mobil-O-Graph® (I.E.M., Aachen, Germany) by automated analysis. After obtainment of blood samples and a rest of 5 min, size-adjusted cuff was placed on the right upper arm about 2–4 cm above the ante-cubital fossa in supine position and subsequent pulse-wave analysis was performed. The patients were requested not to speak and not to move over the whole pulse-wave analysis. PWV of >10 m/s was defined as pathologic PWV (17).

Measurement of the IMT of the common carotid, axillary and superficial femoral artery was assessed in supinely positioned patients. After further rest of 5 min, both common carotid, axillary, and femoral arteries were examined in a longitudinal plane using a high-resolution linear array probe with 8–13 MHz (Siemens ACUSON S2000™, Siemens Healthcare Corp., Erlangen, Germany). The thickness of the intimal and medial layers of the vascular wall was measured on frozen longitudinal images in at least 1-cm-long segment of the artery. Three IMT measurements were performed per subject and per anatomic location while the mean value of the three measurements of the respective location was recorded.

All FMD measurements were performed by the same trained technician according to recent guidelines (13). All recommendations of those guidelines were fulfilled regarding subject preparation, protocol, and operator-dependent factors while sublingual administration of 0.4 mg glyceryl trinitrate was used instead of recommended 25 μg glyceryl trinitrate. Guideline recommendations for technique and analysis, including continuous measurement of velocity and diameter using simultaneous live duplex ultrasound and the use of continuous edge-detection and wall tracking software calculating peak diameter and shear rate stimulus, could not be fulfilled since such a software was not available during the study. Instead, offline analysis by a blinded observer was performed. A blood pressure cuff was placed below the antecubital fossa on the forearm and the baseline diameter of brachial artery was examined in a longitudinal plane between 2 and 7 cm proximal to the antecubital fossa. Three end-diastolic diameters between two intimal layers were measured ECG-gated during image acquisition in a one-centimeter-long segment of the brachial artery. Afterwards, the cuff was inflated >50 mmHg above the resting systolic pressure for 5 min and then deflated. The postischemic diameter of the brachial artery was measured 60 s after cuff release. FMD was defined as the change in postischemic diameter as a percentage of the baseline diameter. After a rest of 15 min, NMD was performed. Diameter of the brachial artery was recorded similar to the technique described for FMD before and 5 min after sublingual administration of 0.4 mg glyceryl trinitrate spray. All FMD and NMD measurements were performed using a conventional ultrasound scanner (Siemens ACUSON S2000™, Siemens Healthcare Corp., Erlangen, Germany) with an 8–13 MHz linear array transducer. Additionally, values of FMD < 7% and of NMD < 15.6% were defined as pathologic FMD and NMD values according to proposed reference values (25, 26).

Biochemical Analyses

Fasting blood samples for evaluation of L-arginine, homoarginine, citrulline, ornithine, ADMA, and SDMA, kynurenine, tryptophan, vWF, homocysteine, AECA, and EMP and immune-inflammatory parameters were obtained. Present leukocytosis, lymphopenia, hypocomplementemia, elevated levels of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum amyloid A (SAA), interleukin 6 (IL-6), antinuclear antibodies (ANA), extractable nuclear antigen (ENA) antibodies, antiphospholipid antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), anti-citrullinated protein (ACP) antibodies, rheumatoid factor, and cytoplasmic antibodies, as well as decreased and increased levels of Ig were additionally recorded. Detailed list of the respective immune-inflammatory parameters is shown in **Table 1**.

TABLE 1 | Immune-inflammatory parameters and cut-off values indicating potentially inflammatory conditions.

White blood cells (WBC)	Antinuclear antibodies (ANA)
C-reactive protein (CRP)	Extractable nuclear antigen (ENA) antibodies
Erythrocyte sedimentation rate (ESR)	Lupus anticoagulant
Serum amyloid A (SAA)	Cardiolipin and β-2-glycoprotein antibodies
Complement factors C3 and C4	Anti-neutrophil cytoplasmic antibodies (ANCA)
Interleukin 6 (IL-6)	Anti-citrullinated protein (ACP) antibodies
Immunoglobulin (Ig) A, G, M	Rheumatoid factor
IgG subclasses 1–4	Cytoplasmic antibodies
Definitions of pathologically altered inflammatory conditions	
Leucocytosis > 11.3×10^9/L	Elevated antiphospholipid antibodies: Lupus anticoagulant > 45 s
Lymphopenia < 20%	Cardiolipin antibodies > 10 U/mL
Elevated CRP > 5 mg/L	β-2-glycoprotein antibodies > 10 U/mL
Elevated ESR > 20 mm/h	Elevated ANCA: MPO-ANCA > 5 U/mL
Elevated SAA > 6.4 mg/L	PR3-ANCA > 10 U/mL
Hypocomplementemia	c-ANCA ≥ 1:80
C3 < 0.9 g/L	p-ANCA titer ≥ 1:80
C4 < 0.1 g/L	x-ANCA ≥ 1:80
Elevated IL-6 > 7.0 pg/mL	Elevated ACP antibodies > 10 U/mL
Elevated ANA titer ≥ 1:80	Elevated rheumatoid factor > 20 U/mL
Elevated ENA antibodies > 1 U/mL	Positive cytoplasmic antibodies
Decreased Ig	Elevated Ig
IgA < 0.7 g/L	IgA > 4 g/L
IgG < 7 g/L	IgG > 16 g/L
IgM < 0.4 g/L	IgM > 2.3 g/L
IgG_1 < 4.05 g/L	IgG_1 > 10.11 g/L
IgG_2 < 1.69 g/L	IgG_2 > 7.86 g/L
IgG_3 < 0.11 g/L	IgG_3 > 0.85 g/L
IgG_4 < 0.03 g/L	IgG_4 > 2.01 g/L

Blood sample for measurement of parameters of the arginine and kynurenine metabolism as well as AECA were centrifuged at 4,000 × g for 10 min at 15°C temperature within 1 h after blood sampling obtainment. The supernatant was collected and divided into aliquots of 1 ml, which were stored at −80°C until final analysis. Amino acids and metabolites were measured by high-performance liquid chromatography as described elsewhere (27–29). L-arginine/ADMA, L-arginine/SDMA, homoarginine/ADMA, homoarginine/SDMA, L-arginine/ornithine, citrulline/L-arginine, citrulline/ornithine, global arginine bioavailability (GAB) ratio, defined as ratio of L-arginine over ornithine plus citrulline, and kynurenine/tryptophan were calculated by division of the respective parameter. AECA were measured by enzyme-linked immunosorbent assay (ELISA) method using

a qualitative ELISA kit (Cusabio Technology, Wuhan, China) according to the user manual.

EMP were measured according to the recommendations for the analysis of extracellular vesicles published by Cossarizza et al. (30). Blood samples were collected in 5 ml citrate tubes after discarding the first 2 ml of blood without venous stasis and kept in upright position. Within 1 h after obtaining blood sampling, the plasma was centrifuged at 2,500 × g for 15 min at room temperature to obtain platelet-poor plasma. One milliliter of the supernatant was centrifuged again at 2,500 × g for 15 min at room temperature to obtain platelet-free plasma. The supernatant was collected and divided into aliquots of 0.1 ml, which were snap-frozen in liquid nitrogen and stored at −80°C until further analysis. A platelet-free plasma aliquot was thawed in a water bath at 37°C and immediately processed for fluorescence staining. Twenty-five microliters of platelet-free plasma was mixed with fluorochrome-labeled anti-human CD31, CD42b, CD51, CD54, CD62E, CD105, and CD144 antibodies (Biolegend, San Diego, USA) and incubated for 1.5 h at 4°C in the dark, followed by incubation with fluorescein-isothiocyanate-labeled lactadherin (CellSystems, Troisdorf, Germany) for another 30 min. Lactadherin binds specifically to phosphatidylserine on the outer surface of extracellular vesicles. Corresponding fluorochrome-labeled isotype antibodies were used as negative controls. After incubation, the samples were diluted 1:50 with 0.22 μm filtered phosphate buffered saline prior to flow cytometric analysis. EMP were identified as events that are positive for the above-mentioned markers and negative for CD42b. CD42b was used to distinguish EMP from platelet-derived microparticles (31). A microparticle gate was established using fluorescent 1 μm silica beads (Kisker Biotech, Steinfurt, Germany) for size calibration.

The remaining laboratory parameters were measured in sera and plasma samples of the patients at a single central lab of the Medical University of Graz.

Nailfold Video Capillaroscopy and Capillary Changes

NVC of the second to the fifth finger on both hands was performed in sitting position after pulse-wave analysis (Skinview, Optometron Ltd., Ismaning, Germany). Morphological changes of the capillaries, including microhemorrhages, capillary edema, capillary ramifications, bushy capillaries, capillary loss, giant capillaries, capillary ectasia, tortuous capillaries, capillary caliber variability, elongated capillaries, capillary thrombosis and disorganization of the microvascular array were recorded and a semi-quantitative rating scale to score each capillary abnormality was adopted (0 = no changes; 1 = <33% of capillary changes; 2 = 33–66% of capillary changes; 3 = more than 66% of capillary changes, per linear millimeter). The score values from the eight digits were added together and divided by eight resulting in the final score values. Microvascular disease activity was assessed by capillaroscopic skin ulcer risk index (CSURI) and microangiopathy evolution score (MES) (32, 33). Microvascular changes were also quantified into early, active and late pattern, as defined by Cutolo et al. (34).

Statistical Analysis

Categorical variables were represented by frequency and percentages. Continuous variables were given as median and interquartile range or as mean ± standard deviation (SD). Normal distribution was examined *via* Shapiro–Wilk test. In case of normally distributed data, two-sided *t*-test was used and for non-normally distributed data Mann–Whitney *U*-test was utilized. $P < 0.05$ were assumed as statistically significant and statistical analyses were executed *via* SPSS version 26.0.

Ethical Approval

The study was approved by the Institutional Review Board of the Medical University Graz, Austria (EK 32-502 ex 19/20). All patients gave their written informed consent.

RESULTS

Fourteen post-COVID-19 patients (7 male, 50%) with a mean age (± SD) of 68.7 (±12.0) years, 14 sex-matched healthy controls with a mean age (± SD) of 30.7 (±4.2) years, and 14 sex- and age-matched patients with ASCVD and a mean age (± SD) of 66.9 (±10.9) years participated in that study. Age-matching was impossible for two patients with ASCVD due to a high refusal rate of study participation (**Figure 1**). No subject of the healthy controls and ASCVD controls had a positive COVID-19 PCR or COVID-19 antibody testing. Patients characteristics are shown in **Table 2**.

Endothelial Dysfunction and Macrovascular Changes

No difference between all three groups were found for FMD and NMD. Post-COVID-19 patients had a higher rate of pathologic aortic PWV with >10 m/s ($p = 0.001$) and higher values of aortic PWV, augmentation index, IMT of the common carotid, axillary and superficial femoral artery, ADMA, SDMA, kynurenine/tryptophan ratio, vWF antigen and activity, homocysteine and CD31+/CD42b– EMP compared to healthy controls ($p < 0.001$; $p = 0.009$; $p < 0.001$; $p < 0.001$; $p < 0.001$; $p = 0.001$; $p = 0.043$; $p = 0.001$; $p = 0.002$; $p = 0.004$; $p = 0.004$; $p = 0.020$, respectively). In the group of post-COVID-19 patients, values of those respective parameters were comparable to patients with ASCVD without significant differences, except for IMT of the axillary artery, which was lower ($p = 0.017$), and for CD31+/CD42b– EMP, which were higher ($p = 0.012$) in the COVID-19 group. Significantly lower values of homoarginine, tryptophan, L-arginine/ADMA, homoarginine/ADMA, and homoarginine/SDMA ratio were found in post-COVID-19 patients compared to healthy controls ($p = 0.004$; $p = 0.027$; $p < 0.001$; $p < 0.001$; $p = 0.002$, respectively), which were again comparable to the values of patients with established ASCVD. Ornithine was lower and L-arginine/ornithine and GAB ratio were higher in post-COVID-19 patients compared to patients with ASCVD ($p = 0.001$; $p = 0.020$; $p = 0.022$, respectively). AECA, CD54+/CD42b–,

TABLE 2 | Patients' characteristics.

	COVID-19 ($n = 14$)	ASCVD ($n = 14$)	Controls ($n = 14$)
Patients, *n* (%)			
Female	7 (50.0%)	7 (50.0%)	7 (50.0%)
Male	7 (50.0%)	7 (50.0%)	7 (50.0%)
Age (years), mean (± SD)	68.7 ± 12.0*	66.9 ± 10.9†	30.7 ± 4.2
Duration after SARS-CoV-2 infection (weeks), mean (± SD)	28.6 ± 3.0	–	–
COVID-19 phenotype, *n* (%)			
COVID-19 pneumonia	14 (100.0)	–	–
COVID-19 ARDS	3 (21.4)	–	–
Disease duration of ASCVD (weeks), median (25th–75th percentile)	–	293.3 (62.1–529.9)	–
Prior familial ASCVD, *n* (%)	5 (35.7)	9 (64.3)	4 (28.6)
BMI (kg/m^2), mean (± SD)	29.4 ± 8.3*	27.6 ± 4.5†	23.8 ± 3.2
HbA_{1c} (mmol/mol), median (25th–75th percentile)	39 (33–42)*	41 (37–47)†	33 (32–34)
eGFR (ml/min/1.73 m^2), median (25th–75th percentile)	84.4 (72.2–90.7)*	76.7 (65.1–89.1)†	103.4 (97.6–115.7)
Current sport activity, *n* (%)	8 (57.1)*	5 (35.7)†	13 (92.9)
Times per week (*n*), median (25th–75th percentile)	2 (0–3)*	0 (0–3)†	4 (2–5)
Duration per week (min), median (25th–75th percentile)	30 (0–60)‡	0 (0–45)†	50 (30–60)
Previous history, *n* (%)			
COPD	1 (7.1%)	3 (21.4)	0 (0.0)
Smoking			
Current	0 (0.0)*‡	5 (35.7)	4 (28.6)
Ex	6 (42.9)	6 (42.9)	4 (28.6)
Non-smokers	8 (57.1)	3 (21.4)	6 (42.9)
Bronchial asthma	0 (0.0)	0 (0.0)	0 (0.0)
Arterial hypertension	6 (42.9)*‡	13 (92.9)†	0 (0.0)
Diabetes mellitus	0 (0.0)‡	4 (28.6)†	0 (0.0)
Atrial fibrillation	1 (7.1)	2 (14.3)	0 (0.0)
Hypercholesterolemia	6 (42.9)*‡	12 (85.7)†	0 (0.0)
Hypertriglyceridemia	2 (14.3)‡	7 (50.0)†	0 (0.0)
CKD	1 (7.1)	3 (21.4)	0 (0.0)
Inactive malignancy	4 (28.6)*	1 (7.1)	0 (0.0)
Coronary artery disease	0 (0.0)‡	8 (57.1)†	0 (0.0)
Myocardial infarction	0 (0.0)‡	4 (28.6)†	0 (0.0)
Cerebrovascular disease	0 (0.0)‡	11 (78.6)†	0 (0.0)
Stroke	0 (0.0)	2 (14.3)	0 (0.0)
Upper extremity arterial disease	0 (0.0)	3 (21.4)	0 (0.0)
Lower extremity arterial disease	0 (0.0)‡	13 (92.9)†	0 (0.0)
Renal artery disease	0 (0.0)	1 (7.1)	0 (0.0)
Mesenteric artery disease	0 (0.0)	1 (7.1)	0 (0.0)
PCI/PTA	0 (0.0)‡	11 (78.6)†	0 (0.0)
Drug therapy, *n* (%)			
ACE inhibitors	3 (21.4)	4 (28.6)†	0 (0.0)
ARB	2 (14.3)	5 (35.7)†	0 (0.0)
Beta blockers	3 (21.4)‡	9 (64.3)†	0 (0.0)
Calcium antagonists	2 (14.3)	5 (35.7)†	0 (0.0)
Diuretics	1 (7.1)	4 (28.6)†	0 (0.0)
Other antihypertensives	0 (0.0)	0 (0.0)	0 (0.0)
Antiplatelet therapy	0 (0.0)‡	9 (64.3)†	0 (0.0)
Oral anticoagulation	2 (14.3)	3 (21.4)	0 (0.0)
Statins	0 (0.0)‡	10 (71.4)†	0 (0.0)
PCSK-9 inhibitors	0 (0.0)	2 (14.3)	0 (0.0)
Metformin	0 (0.0)‡	4 (28.6)†	0 (0.0)

(Continued)

TABLE 2 | Continued

	COVID-19 ($n = 14$)	ASCVD ($n = 14$)	Controls ($n = 14$)
Other oral antihyperglycemic agents	0 (0.0)	2 (14.3)	0 (0.0)
Insulin	0 (0.0)	0 (0.0)	0 (0.0)

ACE, angiotensin-converting enzyme; ARB, Angiotensin receptor blockers; ARDS, acute respiratory distress syndrome; ASCVD, atherosclerotic cardiovascular diseases; BMI, body mass index; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; PCI, percutaneous coronary intervention; PCSK-9, proprotein convertase subtilisin/kexin type 9; PTA, percutaneous transluminal angioplasty.

$^{*}p < 0.05$ between group with previous COVID-19 and healthy controls.

$^{\dagger}p < 0.05$ between group with ASCVD and healthy controls.

$^{\ddagger}p < 0.05$ between group with previous COVID-19 and group with ASCVD.

CD62E+/CD42b–, CD105+/CD42b–, and CD144+/CD42b– EMP were undetectable in all three groups (**Table 3**).

Inflammation

Higher values of CRP, ESR, IL-6, and β-2-glycoprotein antibodies as well as higher frequencies of CRP elevation and any Ig decrease were observed for post-COVID-19 patients compared to healthy controls ($p = 0.009$; $p = 0.007$; $p = 0.004$; $p = 0.031$; $p = 0.007$; $p = 0.015$, respectively). Again, these parameters were comparable between the ASCVD und the COVID-19 cohort, without statistically significant differences. Post-COVID-19 patients revealed higher levels of PR3-ANCA, IgM, and IgG_2 compared to patients with ASCVD ($p = 0.016$; $p = 0.011$; $p = 0.036$, respectively), but not to healthy controls. Healthy controls had lower levels of C3 and C4 and higher rates of hypocomplementemia than patients with previous COVID-19 and with ASCVD (all with $p < 0.05$). Post-COVID-19 patients had a higher total number of pathologically altered inflammatory conditions compared to healthy controls ($p = 0.016$), but not to patients with ASCVD ($p = 0.385$ (**Table 4**).

Microvascular Changes

Capillary ramifications, loss, caliber variability, and elongations were more frequently observed in post-COVID-19 patients compared to patients with ASCVD ($p = 0.015$; $p = 0.034$; $p = 0.047$; $p = 0.020$, respectively) and capillary ramifications, loss, caliber variability and bushy capillaries were more frequently compared to healthy controls ($p = 0.015$; $p = 0.034$; $p = 0.003$; $p = 0.014$, respectively). Using a semi-quantitative rating scale, significantly higher score values were achieved for capillary ramifications, capillary loss and elongated capillaries in the group with previous COVID-19 compared to the group with ASCVD ($p = 0.016$; $p = 0.035$; $p = 0.028$, respectively). Higher score values were also observed for capillary ramifications, loss, caliber variability, elongation and bushy capillaries compared to healthy controls ($p = 0.016$; $p = 0.035$; $p = 0.003$; $p = 0.028$; $p = 0.018$, respectively). Total MES was higher in post-COVID-19 patients compared to patients with ASCVD ($p = 0.048$) and to healthy controls ($p = 0.040$) (**Table 5**).

DISCUSSION

We could demonstrate substantial differences of selected pathways contributing to endothelial dysfunction in patients 6 months after SARS-CoV-2 infection. Although no differences were observed for markers of vascular reactivity, post-COVID-19 patients had an increased arterial stiffness, distinct alterations of the arginine and kynurenine metabolism, and higher values of IMT, vWF, homocysteine, and CD31+/CD42b– EMP compared to healthy controls. Additionally, many of the respective parameters, including also FMD and NMD, were altered to an extent comparable with the values of patients with clinically relevant ASCVD; 78.6% of those had a prior endovascular intervention. Furthermore, capillary changes have been observed more frequently in post-COVID-19 patients compared to healthy controls and the group of ASCVD including also a higher MES. Changes for most of the respective parameters have previously been described in patients mainly with acute COVID-19 while data about persistent changes after suffered COVID-19 are very limited (9, 35–40).

Pathophysiological mechanisms contributing to endothelial dysfunction in COVID-19 are largely unknown. Direct and indirect endothelial damage due to SARS-CoV-2 by binding to the angiotensin-converting-enzyme-2 receptor and by acute systemic inflammation have been proposed (4, 41). While direct infection of endothelial cells by SARS-CoV-2 may be unlikely, as there is lacking evidence of expression of angiotensin-converting-enzyme-2 receptor on human endothelial cells, indirect endothelial damage by release of inflammatory mediators may affect several pathways contributing to endothelial dysfunction, including nitric oxide or kynurenine metabolism, resulting subsequently in impaired FMD and increased arterial stiffness (22, 42–45). Our findings support the hypothesis of indirect endothelial damage caused by systemic inflammation. On the one hand, post-COVID-19 patients revealed numerous altered parameters of endothelial dysfunction, and subclinical inflammation expressed by elevated levels of CRP, ESR, and IL-6 as well as by a higher total number of pathologically altered inflammatory conditions (17, 25, 26). Respective inflammatory changes of post-COVID-19 patients were again similar to those observed in patients with ASCVD. Furthermore, although FMD and NMD did not differ between the three cohorts, post-COVID-19 patients had similar values of FMD and NMD compared to patients with ASCVD and

TABLE 3 | Parameters of endothelial dysfunction.

	COVID-19 ($n = 14$)	ASCVD ($n = 14$)	Controls ($n = 14$)
FMD (%), mean (± SD)	4.44 ± 2.90	3.17 ± 2.95	4.58 ± 3.48
<7%, *n* (%)	10 (71.4%)	11 (78.6%)	10 (71.4%)
NMD (%), mean (± SD)	16.78 ± 6.32	17.11 ± 9.23	20.60 ± 8.46
<15.6%, *n* (%)	5 (38.5%)	7 (50.0%)	3 (21.4%)
Aortic PWV (m/s), median (25th–75th percentile)	10.75 (8.10–11.45)*	9.95 (8.40–11.60)†	5.70 (5.38–6.05)
>10m/s, *n* (%)	8 (57.1%)*	7 (50.0%)†	0 (0.0%)
Augmentation index (%), median (25th–75th percentile)	22 (10–40)*	33 (24–39)†	4 (1–11)
Pulse pressure (mmHg), median (25th–75th percentile)	47 (35–50)	52 (48–67)	49 (41–53)
IMT (mm), median (25th–75th percentile)			
IMT common carotid artery average	0.59 (0.52–0.68)*	0.72 (0.60–1.01)†	0.44 (0.40–0.45)
IMT axillary artery average	0.58 (0.45–0.64)*‡	0.71 (0.59–0.88)†	0.40 (0.39–0.46)
IMT superficial femoral artery average	0.54 (0.47–0.62)*	0.55 (0.43–0.61)†	0.40 (0.36–0.40)
ADMA (μmol/L), median (25th–75th percentile)	0.76 (0.65–0.79)*	0.80 (0.72–0.83)†	0.60 (0.62–0.65)
SDMA (μmol/L), median (25th–75th percentile)	0.73 (0.65–0.86)*	0.84 (0.65–1.07)†	0.65 (0.62–0.70)
L-arginine (μmol/L), median (25th–75th percentile)	119.74 (113.40–142.29)	136.64 (120.34–149.80)	132.50 (107.62–143.44)
Homoarginine (μmol/L), median (25th–75th percentile)	1.59 (1.16–2.31)*	1.59 (1.41–2.21)†	2.36 (1.90–3.45)
Citrulline (μmol/L), median (25th–75th percentile)	34.59 (31.13–38.69)	39.21 (32.46–50.94)	32.11 (25.91–40.24)
Ornithine (μmol/L), median (25th–75th percentile)	66.17 (63.33–73.63)‡	94.30 (80.98–114.48)†	64.25 (38.44–78.30)
L-arginine/ADMA ratio, median (25th–75th percentile)	173.33 (143.28–188.47)*	165.88 (151.23–192.94)†	207.22 (200.43–224.48)
L-arginine/SDMA ratio, median (25th–75th percentile)	167.33 (132.87–188.57)	153.82 (125.35–201.30)†	190.40 (167.97–220.01)
Homoarginine/ADMA ratio, median (25th–75th percentile)	2.16 (1.47–2.90)*	2.02 (1.75–2.82)†	3.75 (2.99–5.67)
Homoarginine/SDMA ratio, median (25th–75th percentile)	2.01 (1.39–3.20)*	2.04 (1.38–2.93)†	3.79 (2.89–5.19)
L-arginine/ornithine ratio, median (25th–75th percentile)	1.88 (1.53–2.11)‡	1.49 (1.10–1.78)†	2.22 (1.46–2.82)
Citrulline/L-arginine ratio, median (25th–75th percentile)	0.28 (0.21–0.31)	0.31 (0.22–0.38)	0.27 (0.22–0.28)
Citrulline/ornithine ratio, median (25th–75th percentile)	0.50 (0.39–0.56)	0.43 (0.40–0.46)	0.59 (0.39–0.66)
GAB ratio, median (25th–75th percentile)	1.20 (1.06–1.42)‡	0.95 (0.77–1.17)†	1.38 (1.07–1.71)
Kynurenine (μmol/L), median (25th–75th percentile)	2.45 (2.00–3.14)	2.96 (2.37–3.23)†	2.21 (2.00–2.39)
Tryptophan (μmol/L), median (25th–75th percentile)	54.40 (49.97–59.15)*	59.52 (54.33–66.96)	61.76 (56.25–70.70)
Kynurenine/tryptophan ratio, median (25th–75th percentile)	0.050 (0.040–0.053)*	0.045 (0.040–0.050)†	0.030 (0.030–0.040)
vWF antige *n* (%), mean (± SD)	138.6 ± 14.1*	137.8 ± 11.1†	109.3 ± 25.3
vWF activity (%), mean (± SD)	168.5 ± 60.8*	177.9 ± 55.3†	110.5 ± 31.5
Homocysteine (μmol/L), median (25th–75th percentile)	12.3 (10.5–14.8)*	9.7 (6.6–14.8)	9.0 (8.6–10.4)
AECA, *n* (%)			
Positive	0 (0.0)	0 (0.0)	0 (0.0)
Negative	14 (100.0)	14 (100.0)	14 (100.0)
EMP (U/μl)			
CD31+/CD42b–	201.25 (158.88–279.50)*‡	115.50 (90.88–169.75)	137.50 (73.00–171.38)
CD51+/CD42b–	13.50 (5.25–49.25)	27.75 (19.38–39.63)	22.25 (17.88–28.50)
CD54+/CD42b–	–§	–§	–§
CD62E+/CD42b–	–§	–§	–§
CD105+/CD42b–	–§	–§	–§
CD144+/CD42b–	–§	–§	–§

ADMA, asymmetric dimethylarginine; AECA, antiendothelial cell antibodies; ASCVD, atherosclerotic cardiovascular diseases; EMP, endothelial microparticles; FMD, flow-mediated dilation; GAB, global arginine bioavailability; IMT, intima-media thickness; NMD, nitroglycerine-related dilation; PWV, pulse-wave velocity; SDMA, symmetric dimethylarginine; vWF, von Willebrand factor.

**$p < 0.05$ between group with previous COVID-19 and healthy controls.*

†$p < 0.05$ between group with ASCVD and healthy controls.

‡$p < 0.05$ between group with previous COVID-19 and group with ASCVD.

§not detectable.

also the number of post-COVID-19 with pathologic FMD and NMD values according to proposed reference values were similar compared to patients with ASCVD (25, 26). Interestingly, FMD and NMD values of our healthy control cohort were also comparable to FMD and NMD values of post-COVID-19 and ASCVD patients, which may be attributed

TABLE 4 | Immune-inflammatory parameters.

	COVID-19 ($n = 14$)	ASCVD ($n = 14$)	Controls ($n = 14$)
WBC (10^9/L), median (25th–75th percentile)	6.0 (5.7–6.6)	6.8 (5.1–8.2)	5.3 (3.9–7.0)
Leukocytosis, *n* (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Lymphocytes (10^9/L), median (25th–75th percentile)	1.5 (1.1–1.7)	1.5 (1.3–2.2)	1.6 (1.2–1.9)
Lymphopenia, *n* (%)	3 (21.4%)	5 (35.7%)	1 (7.1%)
CRP (mg/dL), median (25th–75th percentile)	2.5 (0.8–7.8)*	1.9 (0.8–3.8)†	0.7 (0.5–1.3)
CRP elevation, *n* (%)	6 (42.9%)*	3 (21.4%)	0 (0.0%)
ESR (mm/h), median (25th–75th percentile)	7 (5–10)*	9 (4–15)†	2 (2–5)
ESR elevation > 20, *n* (%)	1 (7.1%)	0 (0.0%)	0 (0.0%)
SAA (mg/L), median (25th–75th percentile)	4.5 (2.2–9.3)	5.7 (3.3–8.2)	4.5 (1.1–6.0)
SAA elevation > 6.4, *n* (%)	5 (35.7%)	5 (35.7%)	3 (21.4%)
Complement factors (g/L), median (25th–75th percentile)			
C3	1.136 (1.086–1.302)*	1.245 (1.102–1.336)†	1.001 (0.858–1.190)
C4	0.193 (0.170–0.243)*	0.216 (0.146–0.245)†	0.158 (0.140–0.197)
Hypocomplementemia, *n* (%)	0 (0.0)*	0 (0.0)†	4 (28.6)
IL-6 (pg/mL), median (25th–75th percentile)	2.5 (1.8–4.0)*	3.1 (1.6–4.7)†	1.4 (1.4–1.7)
Elevated IL-6, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)
Positive ANA titer ≥ 1:80, *n* (%)	5 (35.7)	4 (28.6)	4 (28.6)
ENA (U/mL), median (25th–75th percentile)	0.0 (0.0–0.1)	0.1 (0.0–0.1)	0.1 (0.0–0.2)
Elevated ENA, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)
Antiphospholipid antibodies			
Lupus anticoagulant (sec), median (25th–75th percentile)	32.8 (31.2–38.5)	35.3 (33.8–37.4)	33.7 (31.6–35.3)
Elevated lupus anticoagulant, *n* (%)	0 (0.0)	1 (7.1)	0 (0.0)
Total level of cardiolipin antibodies including IgA, IgG, IgM cardiolipin antibodies (U/mL), median (25th–75th percentile)	1.7 (0.1–3.9)	3.1 (2.5–4.4)†	0.5 (0.3–1.1)
Elevated cardiolipin antibodies, *n* (%)	2 (14.3)	0 (0.0)	0 (0.0)
Total level of β-2-glycoprotein antibodies including IgA, IgG, IgM β-2-glycoprotein antibodies (U/mL), median (25th–75th percentile)	2.2 (1.6–2.7)*	2.3 (1.9–2.4)†	1.6 (1.5–1.8)
Elevated β-2-glycoprotein antibodies, *n* (%)	1 (7.1)	0 (0.0)	0 (0.0)
Any elevated antiphospholipid antibody, *n* (%)	2 (14.3)	1 (7.1)	0 (0.0)
ANCA			
MPO-ANCA (U/mL), median (25th–75th percentile)	0.8 (0.6–1.1)	0.8 (0.8–1.0)†	1.2 (0.8–1.3)
Elevated MPO-ANCA, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)
PR3-ANCA (U/mL), median (25th–75th percentile)	0.4 (0.4–1.9)‡	0.4 (0.4–0.4)†	0.5 (0.4–2.4)
Elevated PR3-ANCA, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)
Positive c-ANCA titer ≥ 1:80, *n* (%)	0 (0.0)	1 (7.1)	0 (0.0)
Positive p-ANCA titer ≥ 1:80, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)
Positive x-ANCA titer ≥ 1:80, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)
ACP antibodies (U/mL), median (25th–75th percentile)	0.9 (0.5–1.1)	0.8 (0.5–1.1)	0.9 (0.6–1.1)
Elevated ACP antibodies, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)
Rheumatoid factor (U/mL), median (25th–75th percentile)	0 (0–7)	0 (0–1)	0 (0–8)
Elevated rheumatoid factor, *n* (%)	0 (0.0)	0 (0.0)	1 (7.1)
Positive cytoplasmic antibodies, *n* (%)	1 (7.1)	1 (7.1)	0 (0.0)
Ig (g/L), median (25th–75th percentile)			
IgA	1.88 (1.07–2.82)	1.77 (1.60–2.42)	1.52 (1.18–2.00)
<0.7, *n* (%)	1 (7.1)	1 (7.1)	0 (0.0)
> 4, *n* (%)	1 (7.1)	1 (7.1)	1 (7.1)
IgG	10.55 (8.47–12.10)	9.13 (8.25–10.30)	9.61 (9.13–10.80)
<7, *n* (%)	1 (7.1)	1 (7.1)	0 (0.0)
> 16, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)

(Continued)

TABLE 4 | Continued

	COVID-19 ($n = 14$)	ASCVD ($n = 14$)	Controls ($n = 14$)
IgM	1.03 (0.72–1.24)‡	0.55 (0.49–0.76)†	0.83 (0.57–1.04)
<0.4, *n* (%)	0 (0.0)	1 (7.1)	0 (0.0)
> 2.3, *n* (%)	0 (0.0)	1 (7.1)	0 (0.0)
IgG_1	6.44 (5.02–8.14)	6.11 (5.33–7.72)	5.99 (5.41–7.45)
<4.05, *n* (%)	0 (0.0)	1 (7.1)	0 (0.0)
> 10.11, *n* (%)	0 (0.0)	0 (0.0)	1 (7.1)
IgG_2	3.00 (2.12–4.31)‡	2.27 (1.77–2.72)†	3.46 (2.5–3.88)
<1.69, *n* (%)	0 (0.0)	2 (14.3)	0 (0.0)
> 7.86, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)
IgG_3	0.35 (0.17–0.41)	0.28 (0.25–0.43)	0.32 (0.27–0.41)
<0.11, *n* (%)	2 (14.3)	0 (0.0)	0 (0.0)
> 0.85, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)
IgG_4	0.41 (0.13–1.04)	0.23 (0.11–1.02)	0.45 (0.27–0.54)
<0.03, *n* (%)	2 (14.3)	0 (0.0)	0 (0.0)
>2.01, *n* (%)	1 (7.1)	0 (0.0)	0 (0.0)
Any Ig elevation, *n* (%)	2 (14.3)	2 (14.3)	2 (14.3)
Any Ig decrease, *n* (%)	5 (35.7)*	4 (28.6)†	0 (0.0)
Total number of pathologically altered inflammatory conditions, median (25th−75th percentile)	2 (1–3)*	2 (1–2)	1 (0–2)

ACP, anti-citrullinated protein; ANA, antinuclear antibodies; ANCA, anti-neutrophil cytoplasmic antibodies; ASCVD, atherosclerotic cardiovascular diseases; CRP, C-reactive protein; ENA, extractable nuclear antigen; ESR, erythrocyte sedimentation rate; Ig, immunoglobulin; IL-6, interleukin 6; SAA, serum amyloid A; WBC, white blood cells.

*$p < 0.05$ between group with previous COVID-19 and healthy controls.

†$p < 0.05$ between group with ASCVD and healthy controls.

‡$p < 0.05$ between group with previous COVID-19 and group with ASCVD.

to other subject-related factors influencing vascular reactivity, like smoking, physical activity, mental stress, alcohol intake or hormonal changes during physiological menstrual cycle (13). The association between inflammation and atherosclerosis is well-established and it may be possible that persisting changes of inflammatory parameters caused by SARS-CoV-2 may affect endothelial cells similarly (46). On the other hand, the occurrence of persisting capillary changes in post-COVID-19 patients also suggests an interaction *via* inflammation and immunological pathways. Capillary changes have mainly been described in autoimmune disorders, especially in systemic sclerosis (32–34). Interestingly, post-COVID-19 patients had a higher prevalence of capillary ramifications and capillary loss, which are typically seen in long-lasting systemic sclerosis, while no capillary pattern suggestive for systemic sclerosis were observed. Compared to the study of Natalello et al. (9), we could observe less capillary edema, thrombosis and ectasia but higher rates of capillary ramifications, bushy capillaries and capillary loss. Additionally, higher rates of capillary caliber variability and elongations were observed and higher scores using semi-quantitative rating scale of respective capillary changes and total MES were found in post-COVID-19 patients. As connective tissues diseases or vasculitides were an exclusion criterion, it can be assumed that SARS-CoV-2 affects substantially and persistently microvasculature. Finally, endothelial damage caused by SARS-CoV-2 *per se* without interaction *via* inflammatory pathways may also be a potential pathophysiologic explanation for COVID-19-associated endothelial dysfunction and vasculopathy. Associations between EMP and parameters of the arginine metabolism to other viruses, like parvovirus B19 or human immunodeficiency virus, have previously been described (47, 48). Furthermore, ADMA and CD31+/CD42b– EMP have been associated with capillary changes in systemic sclerosis (49, 50). Therefore, direct but yet unknown interactions of SARS-CoV-2 to nitric oxide metabolism or endothelial homeostasis may also contribute to the persistent endothelial dysfunction and vasculopathy observed in our study.

Limitations of our study are that this study included a limited number of patients and the fact that we did not measure the above-named parameters before, during and after SARS-CoV-2 infection to evaluate potential changes. Therefore, it can be only hypothesized that persisting endothelial damage is caused directly or indirectly due to COVID-19. However, measuring endothelial function in people before COVID-19 is challenging, given that one would need to assess a large number of people to ascertain that a subgroup will have a SARS-CoV-2 infection. Additionally, while no post-COVID-19 patient had any preexisting ASCVD, most of them had at least one atherosclerotic cardiovascular risk factor. Although those cardiovascular risk factors were not significantly different or overrepresented in post-COVID-19 patients, a potential bias affecting the results on endothelial dysfunction due to present cardiovascular risk factors cannot be definitely excluded. Furthermore, the large age difference between the control group and the two patient groups need to be mentioned which may affect several measured parameters. However, the aim of this study was to compare parameters of endothelial

TABLE 5 | Capillary changes.

	COVID-19 ($n = 14$)	ASCVD ($n = 14$)	Controls ($n = 14$)
Microhemorrhages			
n, (%)	8 (57.1)	6 (42.9)	3 (21.4)
Points, median (25th–75th percentile)	0.125 (0.000–0.250)	0.000 (0.000–0.125)	0.000 (0.000–0.031)
Capillary edema			
n, (%)	0 (0.0)	0 (0.0)	0 (0.0)
Points, median (25th–75th percentile)	0.000 (0.000–0.000)	0.000 (0.000–0.000)	0.000 (0.000–0.000)
Capillary ramifications			
n, (%)	5 (35.7)*‡	0 (0.0)	0 (0.0)
Points, median (25th–75th percentile)	0.000 (0.000–0.125)*‡	0.000 (0.000–0.000)	0.000 (0.000–0.000)
Bushy capillaries			
n, (%)	7 (50.0)*	3 (21.4)	1 (7.1)
Points, median (25th–75th percentile)	0.063 (0.000–0.156)*	0.000 (0.000–0.031)	0.000 (0.000–0.000)
Capillary loss			
n, (%)	4 (28.6)*‡	0 (0.0)	0 (0.0)
Points, median (25th–75th percentile)	0.000 (0.000–0.469)*‡	0.000 (0.000–0.000)	0.000 (0.000–0.000)
Giant capillaries (≥50 μm)			
n, (%)	0 (0.0)	0 (0.0)	0 (0.0)
Points, median (25th–75th percentile)	0.000 (0.000–0.000)	0.000 (0.000–0.000)	0.000 (0.000–0.000)
Capillary ectasia (≥25 μm)			
n, (%)	1 (7.1)	2 (14.3)	0 (0.0)
Points, median (25th–75th percentile)	0.000 (0.000–0.000)	0.000 (0.000–0.000)	0.000 (0.000–0.000)
Tortuous capillaries			
n, (%)	12 (85.7)	8 (57.1)	9 (64.3)
Points, median (25th–75th percentile)	0.500 (0.125–1.375)	0.375 (0.000–1.063)	0.125 (0.000–0.531)
Capillary caliber variability			
n, (%)	7 (50.0)*‡	2 (14.3)	0 (0.0)
Points, median (25th–75th percentile)	0.063 (0.000–0.281)*	0.000 (0.000–0.000)	0.000 (0.000–0.000)
Elongated capillaries			
n, (%)	8 (57.1)‡	2 (14.3)	3 (21.4)
Points, median (25th–75th percentile)	0.125 (0.000–0.469)*‡	0.000 (0.000–0.000)	0.000 (0.000–0.031)
Capillary thrombosis			
n, (%)	0 (0.0)	1 (7.1)	0 (0.0)
Points, median (25th–75th percentile)	0.000 (0.000–0.000)	0.000 (0.000–0.000)	0.000 (0.000–0.000)
Disorganization of microvascular array			
n, (%)	12 (85.7)	8 (57.1)	8 (57.1)
Points, median (25th–75th percentile)	1.063 (0.250–2.156)	0.375 (0.000–1.313)	0.313 (0.000–1.125)
Early pattern, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)
Active pattern, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)
Late pattern, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)
CSURI (points)	–§	–§	–§
MES (points), median (25th–75th percentile)	1.630 (0.250–2.438)*‡	0.375 (0.000–1.313)	0.315 (0.00–1.125)

ASCVD, atherosclerotic cardiovascular diseases; CSURI, capillaroscopic skin ulcer risk index; MES, microangiopathy evolution score.
$p < 0.05$ between group with previous COVID-19 and healthy controls.
†$p < 0.05$ between group with ASCVD and healthy controls.
‡$p < 0.05$ between group with previous COVID-19 and group with ASCVD.
§*not detectable.*

dysfunction and inflammation in post-COVID-19 patients between a healthy group without suspected alterations and a group of patients with suspected altered parameters to rank the potential influence of COVID-19 on endothelial dysfunction and inflammatory vasculopathy. Therefore, young, healthy and sex-matched controls were used instead of age-matched controls.

Strengths of our study are that all parameters were measured together in one study cohort with balanced sex and age distribution and a quite homogenous COVID-19 phenotype.

Another strength of our study is that we included a healthy control and a sex- and age-matched ASCVD control group to discriminate the impact of COVID-19 on endothelial dysfunction and inflammatory vasculopathy, which has not been done before in studies investigating endothelial function in people who had COVID-19. In previous studies, different COVID-19 phenotypes and COVID-19 subjects with several cardiovascular comorbidities were commonly included (9, 34–39). A further strength is that all controls had no proven recent or prior SARS-CoV-2 infection at study measurement.

In conclusion, COVID-19 may contribute to enhanced endothelial dysfunction and disturbed vascular homeostasis *via* influence of EMP, inflammatory pathways as well as of arginine, kynurenine and homocysteine metabolism. Thus, changes of arterial stiffness, vascular reactivity and microvasculature may be promoted after SARS-CoV-2 infection. Further studies are needed to elucidate the underlying pathways of COVID-19 associated endothelial dysfunction and to clarify if those vascular changes are long-lasting and if COVID-19 may be even a potential risk factor for the development of atherosclerotic or inflammatory vascular diseases.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of the Medical University Graz. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PJ contributed to conception of the manuscript, subject recruitment, data acquisition, data interpretation, and writing of the manuscript. PG contributed to conception of the manuscript and data acquisition. VM and HSo contributed to subject recruitment. AA contributed to statistical analysis. AM, HSt, RR, MS, UD, and HK contributed to data analysis and interpretation. KE contributed to data acquisition. MB contributed to conception and supervision of the manuscript. All authors revised the manuscript and gave their final approval of the manuscript version to be published.

ACKNOWLEDGMENTS

We thank Gudrun Dimsity, Eva-Maria Pock, Cornelia Missbrenner, Sabrina Teschl, and Verena Zrim for their assistance in the measurements of parameters of endothelial dysfunction.

REFERENCES

1. Dou Q, Wei X, Zhou K, Yang S, Jia P. Cardiovascular manifestations and mechanisms in patients with COVID-19. *Trends Endocrinol Metab.* (2020) 31:893–904. doi: 10.1016/j.tem.2020.10.001
2. Figliozzi S, Masci PG, Ahmadi N, Tondi L, Koutli E, Aimo A, et al. Predictors of adverse prognosis in COVID-19: a systematic review and meta-analysis. *Eur J Clin Invest.* (2020) 50:e13362. doi: 10.1111/eci.13362
3. Fathi M, Vakili K, Sayehmiri F, Mohamadkhani A, Hajiesmaeili M, Rezaei-Tavirani M, et al. The prognostic value of comorbidity for the severity of COVID-19: a systematic review and meta-analysis study. *PLoS One.* (2021) 16:e0246190. doi: 10.1371/journal.pone.0246190
4. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet.* (2020) 395:1417–8. doi: 10.1016/S0140-6736(20)30937-5
5. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med.* (2020) 383:120–8. doi: 10.1056/NEJMoa2015432
6. Ren B, Yan F, Deng Z, Zhang S, Xiao L, Wu M, et al. Extremely high incidence of lower extremity deep venous thrombosis in 48 patients with severe COVID-19 in Wuhan. *Circulation.* (2020) 142:181–3. doi: 10.1161/CIRCULATIONAHA.120.047407
7. Schaller T, Hirschbühl K, Burkhardt K, Braun G, Trepel M, Märkl B, et al. Postmortem examination of patients with COVID-19. *JAMA.* (2020) 323:2518–20. doi: 10.1001/jama.2020.8907
8. McGonagle D, Bridgewood C, Ramanan AV, Meaney JFM, Watad A. COVID-19 vasculitis and novel vasculitis mimics. *Lancet Rheumatol.* (2021) 3:e224–e33. doi: 10.1016/S2665-9913(20)30420-3
9. Natalello G, De Luca G, Gigante L, Campochiaro C, De Lorenzis E, Verardi L, et al. Nailfold capillaroscopy findings in patients with coronavirus disease 2019: Broadening the spectrum of COVID-19 microvascular involvement. *Microvasc Res.* (2021) 133:104071. doi: 10.1016/j.mvr.2020.104071
10. Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong JC, Turner AJ, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. *Circ Res.* (2020) 126:1456–474. doi: 10.1161/CIRCRESAHA.120.317015
11. Iba T, Connors JM, Levy JH. The coagulopathy, endotheliopathy, and vasculitis of COVID-19. *Inflamm Res.* (2020) 69:1181–9. doi: 10.1007/s00011-020-01401-6
12. Teuwen LA, Geldhof V, Pasut A, Carmeliet P. COVID-19: the vasculature unleashed. *Nat Rev Immunol.* (2020) 20:389–91. doi: 10.1038/s41577-020-0343-0
13. Thijssen DHJ, Bruno RM, van Mil ACCM, Holder SM, Faita F, Greyling A, et al. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *Eur Heart J.* (2019) 40:2534–47. doi: 10.1093/eurheartj/ehz350
14. Stoner L, Young JM, Fryer S. Assessments of arterial stiffness and endothelial function using pulse wave analysis. *Int J Vasc Med.* (2012) 2012:903107. doi: 10.1155/2012/903107
15. Naqvi TZ, Lee MS. Carotid intima-media thickness and plaque in cardiovascular risk assessment. *JACC Cardiovasc Imaging.* (2014) 7:1025–1038. doi: 10.1016/j.jcmg.2013.11.014
16. Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF Jr, et al. Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation.* (2004) 109:613–9. doi: 10.1161/01.CIR.0000112565.60887.1E

17. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. *Eur Heart J.* (2018) 39:3021–104. doi: 10.1093/eurheartj/ehy339
18. Baldassarre D, Hamsten A, Veglia F, de Faire U, Humphries SE, Smit AJ, et al. Measurements of carotid intima-media thickness and of interadventitia common carotid diameter improve prediction of cardiovascular events: results of the IMPROVE (Carotid Intima Media Thickness [IMT] and IMT-Progression as Predictors of Vascular Events in a High Risk European Population) Study. *J Am Coll Cardiol.* (2012) 60:1489–99. doi: 10.1016/j.jacc.2012.06.034
19. Zhu M, Mao M, Lou X. Elevated homocysteine level and prognosis in patients with acute coronary syndrome: a meta-analysis. *Biomarkers.* (2019) 24:309–16. doi: 10.1080/1354750X.2019.1589577
20. Vogl L, Pohlhammer J, Meinitzer A, Rantner B, Stadler M, Peric S, et al. Serum concentrations of L-arginine and L-homoarginine in male patients with intermittent claudication: a cross-sectional and prospective investigation in the CAVASIC Study. *Atherosclerosis.* (2015) 239:607–14. doi: 10.1016/j.atherosclerosis.2015.02.019
21. Schlesinger S, Sonntag SR, Lieb W, Maas R. Asymmetric and symmetric dimethylarginine as risk markers for total mortality and cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. *PLoS One.* (2016) 11:e0165811. doi: 10.1371/journal.pone.0165811
22. Zuo H, Ueland PM, Ulvik A, Eussen SJ, Vollset SE, Nygård O, et al. Plasma biomarkers of inflammation, the kynurenine pathway, and risks of all-cause, cancer, and cardiovascular disease mortality: the Hordaland Health Study. *Am J Epidemiol.* (2016) 183:249–58. doi: 10.1093/aje/kwv242
23. Werner N, Wassmann S, Ahlers P, Kosiol S, Nickenig G. Circulating CD31+/annexin V+ apoptotic microparticles correlate with coronary endothelial function in patients with coronary artery disease. *Arterioscler Thromb Vasc Biol.* (2006) 26:112–6. doi: 10.1161/01.ATV.0000191634.13057.15
24. Aslim E, Hakki Akay T, Bastürk B, Ozkan S, Gültekin B, Ozcobanoglu S, et al. The role of antiendothelial cell antibodies in the development and follow-up of coronary and peripheral arterial diseases. *Angiology.* (2008) 59:209–13. doi: 10.1177/0003319707304537
25. Maruhashi T, Kajikawa M, Kishimoto S, Hashimoto H, Takaeko Y, Yamaji T, et al. Diagnostic criteria of flow-mediated vasodilation for normal endothelial function and nitroglycerin-induced vasodilation for normal vascular smooth muscle function of the brachial artery. *J Am Heart Assoc.* (2020) 9:e013915. doi: 10.1161/JAHA.119.013915
26. Moens AL, Goovaerts I, Claeys MJ, Vrints CJ. Flow-mediated vasodilation: a diagnostic instrument, or an experimental tool? *Chest.* (2005) 127:2254–63. doi: 10.1378/chest.127.6.2254
27. Hervé C, Beyne P, Jamault H, Delacoux E. Determination of tryptophan and its kynurenine pathway metabolites in human serum by high-performance liquid chromatography with simultaneous ultraviolet and fluorimetric detection. *J Chromatogr B Biomed Appl.* (1996) 675:157–61. doi: 10.1016/0378-4347(95)00341-X
28. Schwarz EL, Roberts WL, Pasquali M. Analysis of plasma amino acids by HPLC with photodiode array and fluorescence detection. *Clin Chim Acta.* (2005) 354:83–90. doi: 10.1016/j.cccn.2004.11.016
29. Meinitzer A, Puchinger M, Winklhofer-Roob BM, Rock E, Ribalta J, Roob JM, et al. Reference values for plasma concentrations of asymmetrical dimethylarginine (ADMA) and other arginine metabolites in men after validation of a chromatographic method. *Clin Chim Acta.* (2007) 384:141–8. doi: 10.1016/j.cca.2007.07.006
30. Cossarizza A, Chang HD, Radbruch A, Acs A, Adam D, Adam-Klages S, et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition). *Eur J Immunol.* (2019) 49:1457–973. doi: 10.1002/eji.201970107
31. Deng F, Whang S, Zang L. Endothelial microparticles act as novel diagnostic and therapeutic biomarkers of circulatory hypoxia-related diseases: a literature review. *J Cell Mol Med.* (2017) 21:1698–710. doi: 10.1111/jcmm.13125
32. Sebastiani M, Manfredi A, Colaci M, D'amico R, Malagoli V, Giuggioli D, et al. Capillaroscopic skin ulcer risk index: a new prognostic tool for digital skin ulcer development in systemic sclerosis patients. *Arthritis Rheum.* (2009) 61:688–94. doi: 10.1002/art.24394
33. Sulli A, Secchi ME, Pizzorni C, Cutolo M. Scoring the nailfold microvascular changes during capillaroscopic analysis in systemic sclerosis patients. *Ann Rheum Dis.* (2008) 67:885–7. doi: 10.1136/ard.2007.079756
34. Cutolo M, Sulli A, Pizzorni C, Accardo S. Nailfold videocapillaroscopy assessment of microvascular damage in systemic sclerosis. *J Rheumatol.* (2000) 27:155–60.
35. Riou M, Oulehri W, Momas C, Rouyer O, Lebourg F, Meyer A, et al. Reduced flow-mediated dilatation is not related to COVID-19 severity three months after hospitalization for SARS-CoV-2 infection. *J Clin Med.* (2021) 10:1318. doi: 10.3390/jcm10061318
36. Schnaubelt S, Oppenauer J, Tihanyi D, Mueller M, Maldonado-Gonzalez E, Zejnilovic S, et al. Arterial stiffness in acute COVID-19 and potential associations with clinical outcome. *J Intern Med.* (2021) 290:437–43. doi: 10.1111/joim.13275
37. Szeghy RE, Province VM, Stute NL, Augenreich MA, Koontz LK, Stickford JL, et al. Carotid stiffness, intima-media thickness and aortic augmentation index among adults with SARS-CoV-2. *Exp Physiol.* (2021). doi: 10.1113/EP089481. [Epub ahead of print].
38. Hannemann J, Balfanz P, Schwedhelm E, Hartmann B, Ule J, Müller-Wieland D, et al. Elevated serum SDMA and ADMA at hospital admission predict in-hospital mortality of COVID-19 patients. *Sci Rep.* (2021) 11:9895. doi: 10.1038/s41598-021-89180-w
39. Hoechter DJ, Becker-Pennrich A, Langrehr J, Bruegel M, Zwissler B, Schaefer S, et al. Higher procoagulatory potential but lower DIC score in COVID-19 ARDS patients compared to non-COVID-19 ARDS patients. *Thromb Res.* (2020) 196:186–92. doi: 10.1016/j.thromres.2020.08.030
40. Ponti G, Roli L, Oliva G, Manfredini M, Trenti T, Kaleci S, et al. Homocysteine (Hcy) assessment to predict outcomes of hospitalized Covid-19 patients: a multicenter study on 313 Covid-19 patients. *Clin Chem Lab Med.* (2021) 59:e354–e7. doi: 10.1515/cclm-2021-0168
41. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet.* (2020) 395:1033–4. doi: 10.1016/S0140-6736(20)30628-0
42. McCracken IR, Saginc G, He L, Huseynov A, Daniels A, Fletcher S, et al. Lack of evidence of angiotensin-converting enzyme 2 expression and replicative infection by SARS-CoV-2 in human endothelial cells. *Circulation.* (2021) 143:865–8. doi: 10.1161/CIRCULATIONAHA.120.052824
43. Hingorani AD, Cross J, Kharbanda RK, Mullen MJ, Bhagat K, Taylor M, et al. Acute systemic inflammation impairs endothelium-dependent dilatation in humans. *Circulation.* (2000) 102:994–9. doi: 10.1161/01.CIR.102.9.994
44. Vlachopoulos C, Dima I, Aznaouridis K, Vasiliadou C, Ioakeimidis N, Aggeli C, et al. Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals. *Circulation.* (2005) 112:2193–200. doi: 10.1161/CIRCULATIONAHA.105.535435
45. Wang Q, Liu D, Song P, Zou MH. Tryptophan-kynurenine pathway is dysregulated in inflammation, and immune activation. *Front Biosci (Landmark Ed).* (2015) 20:1116–43. doi: 10.2741/4363
46. Baldassarre D, De Jong A, Amato M, Werba JP, Castelnuovo S, Frigerio B, et al. Carotid intima-media thickness and markers of inflammation, endothelial damage and hemostasis. *Ann Med.* (2008) 40:21–44. doi: 10.1080/07853890701645399
47. Bachelier K, Biehl S, Schwarz V, Kindermann I, Kandolf R, Sauter M, et al. Parvovirus B19-induced vascular damage in the heart is associated with elevated circulating endothelial microparticles. *PLoS One.* (2017) 12:e0176311. doi: 10.1371/journal.pone.0176311
48. Kurz K, Teerlink T, Sarcletti M, Weiss G, Zangerle R, Fuchs D. Asymmetric dimethylarginine concentrations decrease in patients with HIV infection under antiretroviral therapy. *Antivir Ther.* (2012) 17:1021–7. doi: 10.3851/IMP2304
49. Silva I, Teixeira A, Oliveira J, Almeida I, Almeida R, Águas A, et al. Endothelial dysfunction and nailfold videocapillaroscopy pattern as predictors of digital ulcers in systemic sclerosis: a Cohort Study and Review of the Literature. *Clin Rev Allergy Immunol.* (2015) 49:240–52. doi: 10.1007/s12016-015-8500-0

50. Michalska-Jakubus M, Kowal-Bielecka O, Smith V, Cutolo M, Krasowska D. Plasma endothelial microparticles reflect the extent of capillaroscopic alterations and correlate with the severity of skin involvement in systemic sclerosis. *Microvasc Res.* (2017) 110:24–31. doi: 10.1016/j.mvr.2016.11.006

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

BRIEF RESEARCH REPORT
published: 15 October 2021
doi: 10.3389/fcvm.2021.748396

Late Cardiac Pathology in Severe Covid-19. A Postmortem Series of 30 Patients

Ana Ferrer-Gómez[1,2†], *Héctor Pian-Arias*[1†], *Irene Carretero-Barrio*[1,2], *Antonia Navarro-Cantero*[1,2], *David Pestaña*[2,3], *Raúl de Pablo*[2,4,5], *José Luis Zamorano*[2,4,6,7], *Juan Carlos Galán*[4,8,9], *Belén Pérez-Mies*[1,2,4,10], *Ignacio Ruz-Caracuel*[1,4] *and José Palacios*[1,2,4,10*]

[1] Pathology Department, University Hospital Ramón y Cajal, Madrid, Spain, [2] Faculty of Medicine, Alcalá University, Alcalá de Henares, Spain, [3] Anaesthesiology and Surgical Critical Care Department, Hospital Universitario Ramón y Cajal, Madrid, Spain, [4] Instituto Ramón y Cajal for Health Research (IRYCIS), Madrid, Spain, [5] Medical Intensive Care Unit, Hospital Universitario Ramón y Cajal, Madrid, Spain, [6] Cardiology Department, Hospital Universitario Ramón y Cajal, Madrid, Spain, [7] Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain, [8] Microbiology Department, Hospital Universitario Ramón y Cajal, Madrid, Spain, [9] Centro de Investigación Biomédica en Red en Epidemiología y Salud Pública (CIBERESP), Madrid, Spain, [10] Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain

OPEN ACCESS

Edited by:
Andrew F. James,
University of Bristol, United Kingdom

Reviewed by:
Ashraf Roshdy,
Alexandria University, Egypt
Federico Quaini,
University of Parma, Italy

***Correspondence:**
José Palacios
jose.palacios@salud.madrid.org

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Cardiovascular Biologics and Regenerative Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *27 July 2021*
Accepted: *22 September 2021*
Published: *15 October 2021*

Citation:
Ferrer-Gómez A, Pian-Arias H, Carretero-Barrio I, Navarro-Cantero A, Pestaña D, de Pablo R, Zamorano JL, Galán JC, Pérez-Mies B, Ruz-Caracuel I and Palacios J (2021) Late Cardiac Pathology in Severe Covid-19. A Postmortem Series of 30 Patients. Front. Cardiovasc. Med. 8:748396. doi: 10.3389/fcvm.2021.748396

The role of SARS-CoV-2 as a direct cause in the cardiac lesions in patients with severe COVID-19 remains to be established. Our objective is to report the pathological findings in cardiac samples of 30 patients who died after a prolonged hospital stay due to Sars-Cov-2 infection. We performed macroscopic, histological and immunohistochemical analysis of the hearts of 30 patients; and detected Sars-Cov-2 RNA by RT-PCR in the cardiac tissue samples. The median age of our cohort was 69.5 years and 76.6% were male. The median time between symptoms onset and death was 36.5 days. The main comorbidities were arterial hypertension (13 patients, 43.3%), dyslipidemia (11 patients, 36.7%), cardiovascular conditions (8 patients, 26.7%), and obesity (8 patients, 26.7%). Cardiovascular conditions included ischemic cardiopathy in 4 patients (13.3%), hypertrophic cardiomyopathy in 2 patients (6.7%) and valve replacement and chronic heart failure in one patient each (3.3%). At autopsy, the most frequent histopathological findings were coronary artery atherosclerosis (8 patients, 26.7%), left ventricular hypertrophy (4 patients, 13.3%), chronic epicardial inflammation (3 patients, 10%) and adipose metaplasia (2 patients, 6.7%). Two patients showed focal myocarditis, one due to invasive aspergillosis. One additional patient showed senile amyloidosis. Sars-Cov-2 RNA was detected in the heart of only one out of 30 patients, who had the shortest disease evolution of the series (9 days). However, no relevant cardiac histological alterations were identified. In present series, cardiac pathology was only modest in most patients with severe COVID-19. At present, the contribution of a direct effect of SARS-CoV-2 on cardiac lesions remains to be established.

Keywords: COVID-19, SARS-CoV-2, heart, cardiac pathology, autopsy

INTRODUCTION

Coronavirus-19 disease (COVID-19), caused by the new coronavirus SARS-CoV-2 has become a global health challenge in our time (1). It is known that SARS-CoV-2 affects mainly the respiratory system, with a spectrum of clinical manifestations ranging from asymptomatic to mild illness with fever and fatigue (80% of symptomatic patients) (2). However, in the most severe cases, which represent around 5%, it can lead to respiratory distress syndrome that requires ventilatory support (3, 4). As cardiovascular complications including myocarditis, acute myocardial infarction, and exacerbation of heart failure are present in patients suffering from other respiratory viral infections, such as influenza virus each annual epidemic period, special attention has been paid to a possible heart involvement by SARS-CoV-2 since the beginning of the pandemic (5). In fact, reported clinical cardiac manifestation in COVID-19 patients included right heart dilatation and dysfunction, myocarditis, cardiac fibrosis, arrhythmias, endothelial dysfunction, dysautonomia, and thrombotic events (6). Moreover, it has been suggested that patients with a history of cardiovascular disease or cardiovascular risk factors such as hypertension, dyslipidemia or obesity are strongly associated to severe symptoms and higher mortality rate in patients infected by SARS-CoV-2 (2, 7). The possible pathophysiological mechanisms by which SARS-CoV-2 would cause damage to the myocardium and vascular endothelium include a direct myocardial injury due to viral invasion, a damage secondary to hypoxemia as consequence of respiratory failure, infarct secondary to thrombosis, as well as a dysregulated immunological response (known as cytokine storm) (8).

Since autopsies are the gold standard procedure to settle the underlying pathophysiology of the diseases (9), several autopsy series of patients who died from COVID-19 have been reported in the last months. In addition, some studies have reviewed the cardiac lesions reported in those series (10–12). However, our information regarding cardiac pathology is limited to about 700 hearts and, despite these studies, there is still limited and controversial data about the histopathological cardiac findings in patients with SARS-CoV-2 infection. Previous series are mostly formed by patients who died during the acute illness. Thus, the longest duration of illness in the cohorts reviewed by Roshdy et al. (10) was 52 days with a median duration from symptoms onset to death of 12 days (range, 0–52 days, $n = 98$), which means that the long-term evolution or complications of the disease were not covered by this review.

The objective of our study is to present the histopathological cardiac lesions in a series of 30 autopsies performed in patients who died by severe COVID-19 with relative long-term evolution from symptoms onset (median 36.5 days, range 9–108 days).

MATERIALS AND METHODS

Autopsy Procedure and Clinical Data

This is a retrospective analysis of the macroscopic and histological findings in the hearts of all autopsies performed on patients with COVID-19 in University Hospital Ramón y Cajal (Madrid, Spain), from April 2020 to April 2021 ($n = 30$), representing ∼3% of patients who died from COVID-19 during this period. The Research Ethics Committee approved the study (reference: Necropsias_Covid19; 355_20). All the deceased patients were diagnosed of SARS-CoV-2 infection confirmed by RT-PCR nasopharyngeal swab test. Demographic and clinical data were collected from the electronic medical records.

These consecutive autopsies were requested by the medical staff according to clinical interest. Most autopsies ($n = 25$, 83%) corresponded to patients with severe respiratory diseases and were requested by ICU staffs. Consequently, the series does not represent the complete spectrum of causes of death attributable to COVID-19 (**Table 1**). All autopsies were consented by patients' relatives and carried out according to safety protocols, in a negative pressure autopsy room, using personal protection equipment, as previously reported (13).

In the first 14 consecutive decedents, we took *in-corpore* representative sections from the heart, lungs, liver, kidney, pancreas, and bone marrow. In the rest of the patients, due to improved technical training, we extracted the complete heart and lung block, left kidney, spleen, and sections from the liver, pancreas and bone marrow. We extracted the brain in 10 patients.

After each procedure, the hearts were fixed in formalin for 24–48 h.

Histopathology and Immunohistochemistry

For histological study, in the 14 first autopsies, we only took 5 representative sections corresponding to the anterior, lateral and posterior left ventricle walls, the septum, and right ventricle wall. In the rest of the patients, the complete heart and great proximal vessels were studied following the protocol depicted in **Figure 1**.

Hematoxylin and eosin stain was performed in all sections. In addition, when inflammatory infiltrates were present, special histochemical stains such as PAS, Grocott or Gram were done to rule out microorganisms such as bacteria or fungi. Congo red stain was performed in suspected cases of amyloid deposit.

To analyze the immune infiltrates, we performed immunohistochemistry for CD20, CD3, CD8, CD4, and CD68 (Agilent, Santa Clara, CA, USA) in sections with inflammation. Transthyretin immunohistochemistry (Agilent, Santa Clara, CA, USA) was also done to confirm senile amyloidosis.

Sars-Cov-2 RNA Analysis

To investigate the presence of Sars-Cov-2 RNA, we took swabs samples from the left ventricle in 28 patients. In addition, we tested Sars-Cov-2 RNA in paraffin blocks from the left ventricle in all 30 patients, selecting those blocks with inflammatory infiltrates.

All swab samples were sent on the same day to the Microbiology Department for the detection of genomic SARS-CoV-2 RNA (gRNA). RNA extraction and reverse-transcription-polymerase-chain-reaction (RT-PCR) amplification were performed within 3 h after reception in the laboratory. RNA extraction was performed using MagmaxTM Core Nucleic Acid Purification Kit (Thermo Fisher, Waltham, United States) and gRNA SARS-CoV-2 was detected using TaqmanTM 2019 nCoV assay (Thermo Fisher, Waltham, United States).

TABLE 1 | Demographic, clinical, and laboratory findings.

Demographics	**Total**	**30 (100%)**
Male, *n* (%)		23 (76.67%)
Age	Median (IQR)	69.5 (12.25)
	Min, Max	52, 91
Weight	Median (IQR)	76.5 (14)
	Min, Max	53, 109
Days from initial symptoms	Median (IQR)	36.5 (17.5)
	Min, Max	9, 108
Hospitalization days	Median (IQR)	29.5 (18)
	Min, Max	3, 102
Patients admitted to ICU, *n* (%)		26 (86.67%)
ICU days	Median (IQR)	21 (15)
	Min, Max	12, 95
Mechanical ventilation, *n* (%)		26 (86.67%)
Comorbidities		
Hypertension, *n* (%)		13 (43.33%)
Dyslipidemia, *n* (%)		11 (36.67%)
Cardiovascular condition, *n* (%)		8 (26.67%)
• Ischemic cardiopathy		4 (50.00%, $n = 8$)
• Hypertrophic cardiomyopathy		2 (18.18%, $n = 8$)
• Valve replacement		1 (12.50%, $n = 8$)
• Heart failure		1 (12.50%, $n = 8$)
Obesity, *n* (%)		8 (26.67%)
Malignancy, *n* (%)		6 (20.00%)
Chronic obstructive pulmonary disease/SAHS, *n* (%)		5 (16.67%)
Neurologic condition, *n* (%)		3 (10.00%)
Diabetes mellitus, *n* (%)		2 (6.67%)
Hepatic condition, *n* (%)		2 (6.67%)
Immunosuppression, *n* (%)		1 (3.33%)
Clinical symptoms		
Fever at admission, *n* (%)		24 (80.00%)
Dyspnea at admission, *n* (%)		22 (73.33%)
Cough at admission, *n* (%)		17 (56.67%)
Asthenia at admission, *n* (%)		13 (43.33%)
Diarrhea at admission, *n* (%)		4 (13.33%)
Anosmia/Ageusia at admission, *n* (%)		4 (13.33%)
Nausea/Vomiting at admission, *n* (%)		3 (10.00%)
Temperature in Celsius at admission	Median (IQR)	36.5 (0.6)
	Min, Max	35.5, 38
Oxygen saturation at admission	Median (IQR)	88 (14.3)
	Min, Max	70, 99
Heart rate at admission	Median (IQR)	100 (25)
	Min, Max	70–141
Arrythmia, *n* (%)	Previously diagnosed	2 (6.67%)
	During hospitalization	6 (20%)
Department that requested the autopsy		
Anesthesiology department, *n* (%)		18 (60%)
Medical intensive care unit, *n* (%)		7 (23.33%)
Internal medicine department, *n* (%)		2 (6.67%)
Geriatrics department, *n* (%)		2 (6.67%)
Pneumology department, *n* (%)		1 (3.33%)

(Continued)

TABLE 1 | Continued

Demographics	Total		30 (100%)
Cause of death (according to autopsy report)			
Hypoxemia, *n* (%)			24 (80%)
Pancreatitis, *n* (%)			2 (6.67%)
Intestinal necrosis, *n* (%)			2 (6.67%)
Subarachnoid hemorrhage, *n* (%)			1 (3.33%)
Invasive aspergillosis, *n* (%)			1 (3.33%)
Laboratory test at admission (normal values)			
White cell count /μL (4–11 × 10^3)	Median (IQR)		11.1 (9.90)
	Min, Max		0.01, 21.9
% Neutrophils (45–75)	Median (IQR)		85.75 (20.28)
	Min, Max		18.2, 96.9
Lymphocytes/μL (1–4.5 × 10^3)	Median (IQR)		0.89 (0.47)
	Min, Max		0, 1.45
Creatinine mg/dL (0.3–1.3)	Median (IQR)		0.92 (0.37)
	Min, Max		0.48, 2.82
CRP mg/L (0–5)	Median (IQR)		188.20 (160.75)
	Min, Max		0, 0.8
Ferritin ng/mL (20–300)	Median (IQR)		1454.01 (1496.12)
	Min, Max		59.15, 5269.9
Lactate dehydrogenase U/L (140–240)	Median (IQR)		442 (274.25)
	Min, Max		333, 987
Platelets/μL (140–400 × 10^3)	Median (IQR)		195 (146.25)
	Min, Max		22.3, 599
Fibrinogen mg/dl (150–400)	Median (IQR)		740 (31.65)
	Min, Max		295.1,740
APTT% (76–128)	Median (IQR)		84.85 (21.25)
	Min, Max		16.1, 131.3
PT s (9.7–12, 6)	Median (IQR)		12.20 (1.18)
	Min, Max		10.2, 49.2
D-dimer ng/mL (0–500)	Median (IQR)		1,164 (2,098)
	Min, Max		152, 14,493.41
Troponin at admission ng/ml (0–0.1)	Median (IQR)		0.00 (0.00)
	Min, Max		0, 0.8
Highest troponin during hospitalization ng/ml (0–0.1)	Median (IQR)		0.00 (0.00)
	Min, Max		0, 10.4
Natriuretic peptide pg/mL (<100)	Median (IQR)		81.80 (125.30)
	Min, Max		0.4, 2,288
IL6 pg/mL (0)	Median (IQR)		62.15 (121.04)
	Min, Max		1.27, 589
IL10 pg/mL (0)	Median (IQR)		7.94 (6.79)
	Min, Max		0.5, 54.44
IL12 pg/mL (0)	Median (IQR)		1.19 (2.40)
	Min, Max		0, 6.66
Pathologic findings	**Partial heart examination (*n* = 14)**	**Complete heart examination (*n* = 16)**	**Total (*n* = 30)**
Heart			
Coronary artery atherosclerosis, *n* (%)	2 (14.29%)	6 (37.5%)	8 (26.67%)
Left ventricle hypertrophy, *n* (%)	3 (21.43%)	1 (6.25%)	4 (13.33%)
Chronic epicardial inflammation, *n* (%)	0	3 (18.75%)	3 (10%)
Myocarditis, *n* (%)	0	1 (6.25%)	1 (3.3%)

(Continued)

TABLE 1 | Continued

Pathologic findings	Partial heart examination ($n = 14$)	Complete heart examination ($n = 16$)	Total ($n = 30$)
Aspergillus myocarditis, *n* (%)	1 (7.14%)	0	1 (3.3%)
Senile amyloidosis, *n* (%)	1 (7.14%)	0	1 (3.3%)
Without significant alterations, *n* (%)	5 (35.71%)	8 (50%)	13 (43.33%)
Lung			
Patients with predominant pattern, *n* (%)	Normal lung		1 (3.3%)
	Exudative DAD		6 (20%)
	Proliferative/Organizing DAD		19 (63.3%)
	Fibrotic DAD		4 (13.3%)
Acute bronchopneumonia, *n* (%)			12 (40%)
Vascular thrombi, *n* (%)			20 (67%)
Endotheliitis, *n* (%)			13 (43%)

Diffuse alveolar damage (DAD).

For formalin-fixed-paraffin embedded (FFPE) samples, RNA was extracted from 10 sections of 5 μm obtained from paraffin blocks using RecoverAll Total Nucleic Acid Isolation Kit (Invitrogen), following the manufacturer's instructions. RNA quantity was measured fluorometrically with Qubit RNA high-sensitivity assay kit (Invitrogen, Waltham, MAS, USA).

Literature Review

We have performed a non-systematic PUBMED review of autopsy series published in English including 4 or more patients not previously reported in the reviews by Halushka and Vander Heide (11), Roshdy et al. (10) and Kawakami et al. (12).

RESULTS

Demographics

The main demographic, clinical and laboratory findings of the 30 patients are listed in **Table 1**. The median age of our cohort was 69.5 years (range 52–91). Twenty-three patients (76.6%) were male. The median time between admission and death was 29.5 days (range 3–102). The main comorbidities were arterial hypertension in 13 patients (43.3%), dyslipidemia in 11 patients (36.7%), cardiovascular conditions in 8 patients (26.7%), obesity in 8 patients (26.7%), and diabetes in 2 patients (6.67%). None of them were vaccinated.

Cardiovascular conditions included ischemic cardiopathy in 4 patients (13.3%), hypertrophic cardiomyopathy in 2 patients (6.7%) and mitral and aortic valve replacement and chronic heart failure in one patient each (3.3%). Two patients had a previous diagnosis of auricular flutter and auricular fibrillation, respectively. Six patients developed arrythmias during hospitalization, including supraventricular extrasystoles (6.7%), auricular flutter (3.3%), bundle branch block (3.3%), streaks of supraventricular tachycardia (3.3%), and self-limited periods of arrhythmias (3.3%).

Pathology

The main pathological findings of this series are presented in **Table 1**.

Cardiac Pathology

Macroscopic Findings

Mean post-fixation weight of the heart, in 16 (53.3%) patients in whom the complete organ was studied, was 474.2 g (range 310–720) (normal weight 365 ± 71 g). Regarding macroscopic findings, serous pericardial effusion was evidenced in 8 patients (26.6%), left ventricular hypertrophy (>1.5 cm in diameter) was present in 4 patients (13.3%), adipose myocardial replacement in 2 patients (6.6 %), and the presence of a macrothrombus in left atrium in one patient (3.3%). Two hearts showed post-surgical changes; one showed mitral and aortic valve replacement and another a coronary artery bypass grafting. In 14 patients (47%) no macroscopically relevant alterations were identified in the heart.

Histopathology

The most frequent histopathological finding was coronary artery atherosclerosis (8 patients, 26.7%). Three patients (10%) showed focal chronic epicardial inflammation, that consisted of a slight lymphocytic mononuclear inflammatory infiltrate associated with reactive mesothelium. This infiltrate was mainly composed of T lymphocytes (CD3+) with a predominant cytotoxic phenotype (CD8+) that exceed the CD3+/CD4+ population. One patient (3.3%) revealed myofibrils necrosis associated with abundant macrophage infiltration and occasional CD3+ lymphocytes in an area of ∼1 cm^2 in the left ventricle, consistent with the diagnosis of myocarditis (**Figures 2A,B**).

In a 55-year-old male patient, without any comorbidity and 37 days of hospitalization, we observed the presence of ring-shaped fungal structures within the myocardium, associated to myofibrils necrosis and inflammation. This patient received corticosteroids and antibiotics, but no antifungal treatment. The fungal structures were positive for PAS and Grocott techniques (**Figures 2C–E**). They were also identified in lung and kidney parenchyma, where they were identified as *Aspergillus*

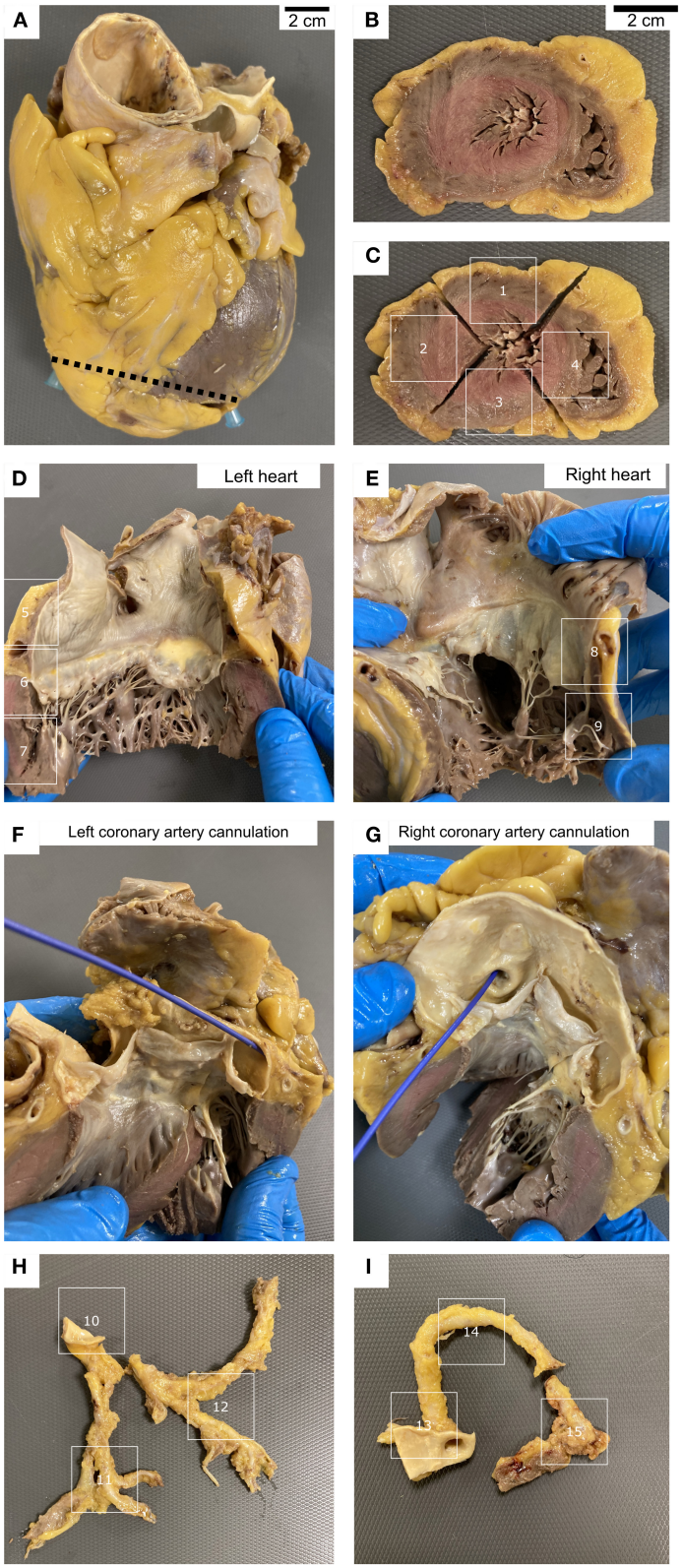


FIGURE 1 | Heart grossing protocol. **(A–C)** Apex sections. **(D)** Left atria and ventricle sections, including mitral valve. **(E)** Right atria and ventricle sections, including tricuspid valve. **(F–I)** Dissection of the coronary arteries. Cannulation of left **(F)** and right **(G)** coronary arteries. Left **(H)** and right **(I)** coronary arteries sections.

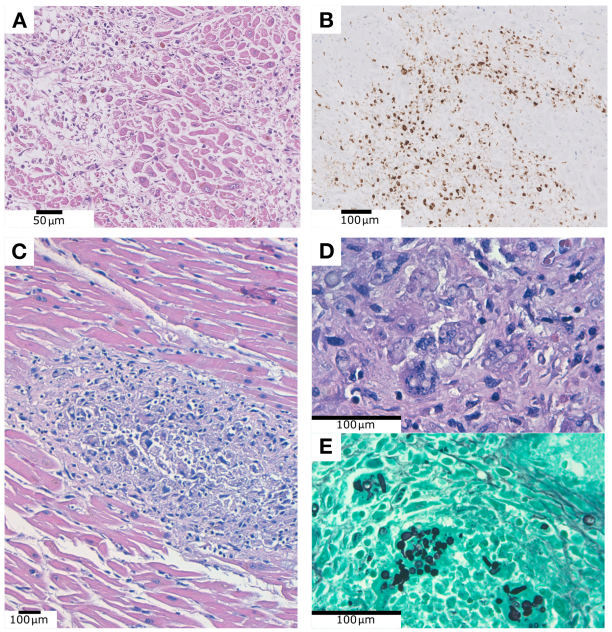


FIGURE 2 | (A) Myofibrils necrosis associated with abundant inflammatory infiltration. **(B)** Same case as **(A)**, showing CD68 positive macrophages infiltrating the myocardium. **(C)** Fungal structures within the myocardium, associated to myofibrils necrosis and inflammation. **(D)** Same case as **(C)**, PAS technique. **(E)** Same case as **(C)**, Grocott technique.

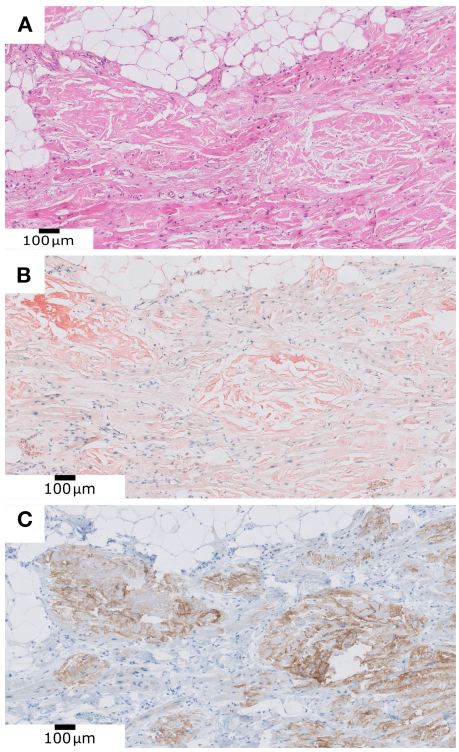


FIGURE 3 | (A) Deposit of eosinophilic and amorphous material compatible with amyloid within the myocardium. **(B)** Positive Congo Red histochemical stain. **(C)** Positive transthyretin immunohistochemistry.

fumigatus by postmortem microbiological culture and PCR. We did not observe intranuclear or intracytoplasmic inclusions in myocardial cells in none of our patients.

In a 90-year-old patient, suffering chronic cardiac failure, we evidenced a deposit of eosinophilic and amorphous material compatible with amyloid within the myocardium (**Figure 3**). This was confirmed with Congo red histochemical stain. This deposit was also identified in the cardiac and pulmonary vessels, pericardial adipose tissue, kidney and bone marrow. After performing immunohistochemical staining, the amyloid material was positive for transthyretin and negative for Kappa, Lambda and Amyloid AA, rendering the diagnosis of senile amyloidosis.

No histologically relevant alterations were found in 13 patients (43.3%), according to their age and clinical status.

Sars-Cov-2 RNA Analysis

All myocardial swabs except one [Cycle threshold (Ct) = 28] were negative for Sars-Cov2. The only positive case was also positive in the study of the FFPE tissue (Ct = 33), and Ct = 20 in nasopharyngeal sample obtained in the same autopsy. However, this patient did not show any relevant macroscopic or histopathological cardiac lesion. Interestingly, in spite of the absence of Sars-Cov-2 RNA in cardiac samples, all patients showed at least a positive result from the nasopharynx or lung in autopsy samples (manuscript in preparation).

Literature Review

Supplementary Table 1 compares the clinical and pathological findings reported by Halushka and Vander Heide (11) in their review of 293 cases and our review of 280 additional cases, including the 30 patients here reported.

DISCUSSION

In this study, we report the cardiovascular findings in the autopsies of 30 patients with severe COVID-19. Our results indicate a modest involvement of the heart in these patients, being the most frequent histopathological findings coronary artery atherosclerosis (8 patients, 26.7%), left ventricle hypertrophy (4 patients, 13.3%), chronic epicardial inflammation (3 patients, 10%), focal myocarditis (1 patient, 3.3%), and myocarditis due to Aspergillus (1 patient, 3.3%).

To the best of our knowledge, this is the autopsy series with the longest disease duration in which the heart has been histopathologically analyzed, and our results are in accordance with other studies with a shorter time of disease evolution.

Regarding duration of disease, in the review by Roshdy et al. (10), the median duration of prehospital symptoms ($n = 82$) and hospital stay ($n = 158$) were 5 (IQR, 2–7) and 6 days (IQR, 3–10), respectively. In total, the median duration from the onset of symptoms to death was 12 days (range, 0–52 days, $n = 98$).

In the review by Halushka and Vander Heide (11), the median number of days from diagnosis to fatality was 10 (range 1–51 days). In contrast, our series included patients with a median disease duration from symptoms onset to death of 36.5 and 29.5 days of hospitalization.

Pre-existing cardiovascular diseases are associated to a worse prognosis in patients with SARS-CoV-2 infection (6). In our series, eight patients had previous cardiac conditions, but the autopsy did not reveal other lesions than those related with the underlying disease. No differences were observed in the evolution of these cases in comparison to patients without previous cardiac conditions but presenting other comorbidity.

In addition to coronary atherosclerosis, the most frequent cardiac pathological finding was left ventricular hypertrophy in 4 patients. While two of them had a previous diagnosis of hypertrophic cardiomyopathy, the other two patients were not previously diagnosed of any cardiac disease. We did not find other pathological findings in the heart of these 4 patients.

We found focal and slight lymphocytic inflammatory infiltrates in the epicardium of 3 patients. However, the mere presence of these aggregates is not indicative of active pericarditis. They were mainly placed in the subepicardial adipose tissue and they were not associated with vessels. For this reason, we cannot argue in favor of a systemic endothelialitis involving epicardial lymphatic micro-vessels. Because in our series 26 of patients (86.7%) were treated with mechanical ventilation and we only found chronic pericarditis in 3 of them (10%), our data do not support an association between both facts. Although 8 patients showed different degrees of pericardial effusion, we related this finding to the common hemodynamic alterations in terminal ICU patients. Pericardial effusion has been reported in up to 94% of patients dying from COVID-19 without evidence of pericarditis (12). Although clinical studies have reported some cases of pericarditis secondary to SARS-CoV-2 (14–17), most autopsy studies have not found severe acute pericarditis.

One of the most controversial issue in COVID-19 cardiac pathology is to know if myocarditis is a common manifestation of the disease (7, 11, 18–20). In our series, only one patient (3.3%) showed focal myocarditis characterized by both myocyte necrosis and inflammation in absence of ischemic changes. The frequency of the diagnosis of myocarditis varies among series, probably due to different diagnostic criteria among authors. According to cardiac autopsy guidelines of the European Association of Cardiovascular Pathology (AECVP) (21), focal presence of myocardial inflammatory infiltrates in the myocardic tissue in the absence of myocyte necrosis is not enough evidence for diagnosis of myocarditis. It also maintains that small fibrosis foci have no pathological significance.

Following the mentioned restrictive criteria, it seems that myocarditis is not a frequent manifestation of severe COVID-19. Halushka and Vander Heide (11) reviewed 22 articles about cardiovascular findings in autopsies samples from COVID-19 patients and they concluded that although inflammatory infiltrates were present in the myocardium of 7% of the 277 patients, only 1.7% had complete histopathological evidence of myocarditis. In the partially overlapping review by Roshdy et al. (10) including 316 patients, clear myocarditis meeting the Dallas criteria was described in only five cases, whereas 35 additional patients had focal inflammatory infiltrates. In their review, Kawakami et al. (12) specifically discussed the role of myocarditis in COVID-19 patients. The authors reviewed literature findings (some series also reviewed by Halushka and Vander Heide (11) and by Roshdy et al. (10) and found that myocarditis was an uncommon pathologic diagnosis occurring in 4.5% of highly selected cases undergoing autopsy or endomyocardial biopsy. In their own series of 16 autopsied patients, the authors observed myocardial inflammatory infiltrates in 31% of the patients, which were not associated with myocardial necrosis. The authors concluded that given the extremely low frequency of myocarditis and the unclear therapeutic implications, the use of endomyocardial biopsy to diagnose myocarditis in the setting of COVID-19 is not recommended. A recent series (22), where 5% of the patients developed new onset myocarditis, confirms these results.

In our study, inflammatory infiltrates within the myocardium associated to myocyte necrosis were identified in only one patient (3.3%). In our review of 277 reported autopsied hearts (not included in previously reported reviews and including our 30 patients), 20 (7.2%) showed evidence of myocarditis; however, the frequency was highly variable, ranging from 0 out of 97 patients in the series reported by Bryce et al. (9), to 9 out of 9 patients in the series reported by del Nonno et al. (23).

The role of SARS-CoV-2 as the direct cause of viral myocarditis remains to be established, similarly as has been observed in other organs, such as the brain, in which no direct viral brain damage has been proven in large autopsy case series (24). In our study, myocardial PCR was performed in order to detect SARS-CoV-2 in the myocardium of all autopsies, but only one case became positive. However, all cases showed at least one positive result in samples taken during the autopsy from the nasopharynx or lungs. In the patient with the positive RT-PCR in cardiac samples, no relevant cardiac histological alterations were identified, whereas in the patient with focal myocarditis, no virus was detected. Several studies have investigated the presence of SARS-CoV-2 in the myocardium using different techniques. In the review by Roshdy et al. (10), 105 hearts were studied for the presence of SARS-CoV-2 and 50 (47%) were positive. In our review, which included 60 patients in whom the presence of SARS-CoV-2 in the myocardium was investigated, 17 (28%) had a positive result. Differences among series can be explain by the time of disease evolution. Thus, the median of hospital stay was 5 and 6 days in Roshdy's and our review, respectively, but 29.5 days in our series. In fact, the only positive patient in our series had an illness duration of 9 days from symptoms onset to death. According to these data, myocarditis as an immunologically mediated phenomena rather than direct viral damage cannot be excluded. In this sense, immune-mediated myocarditis has

been reported as a rare complication of COVID-19 mRNA vaccines (25).

One patient in this series had lesions of focal myocarditis due to Aspergillus fumigatus. Pulmonary aspergillosis can develop in severe COVID-19 patients. A review of 15 COVID-19-associated pulmonary aspergillosis (CAPA) clinical case series in the ICU reported 158 CAPA cases among 1,702 COVID-19 patients (9.3%, range between 0 and 33%). Only in four cases, CAPA was proven, while the majority had a probable or putative diagnosis (26). In a systematic review of autopsy series, the authors found 8 CAPA cases among 677 decedents (1.2%) (27). Cardiac lesions occurring in the setting of disseminated aspergillosis, as occurred in our patient, seem to be very unusual. Hanley et al. (14) reported a patient with an acute fungal pericarditis without characterization of the fungus.

Regarding thrombotic phenomena, SARS-CoV-2 infection has been associated with an increased thrombotic risk (28–30) and the presence of both micro and macrothrombi. We identified a patient with an atrial thrombus, but no cases of microthrombosis were observed. However, macro and microthrombosis were frequent in the lungs, even though all but three patients were being treated with prophylactic anticoagulation treatment. Thrombosis in COVID-19 patients has been related to the expression of ACE2 in endothelial tissue, which binds with SARS-CoV-2 causing direct endothelial damage and favoring thrombotic phenomena. The presence of thrombi has also been associated with an exaggerated immune response that triggers endothelial dysfunction and dysregulation. The frequency of both micro and macrothrombi varies largely among series (**Supplementary Table 1**). Thus, Pellegrini et al. (30) reported that 35% of patients in their series had myocardial necrosis and the most common cause associated with necrosis was the presence of microthrombi in 64% of cases. Bois et al. (31) also reported the presence of small vessel thrombosis in 80% of their patients. In contrast, other studies, including the series reported by Bryce et al. (9), who studied 97 patients, did not find heart vascular thrombosis. Since cardiac vascular pathology seems to be more frequent during the initial stage of the diseases (32), differences among series could be related, at least in part, with the time of evolution of the infection.

Regarding other histological findings, one of our elderly patients showed the presence of amyloid deposit within the myocardium that was positive for transthyretin. Other studies have identified the presence of cardiac amyloidosis in patients with COVID-19 (14, 33). Although not all series performed immunohistochemical studies, most cases, as the patient here reported and those reported by Menter et al. (34), are probably examples of senile amyloidosis. The frequency of cardiac amyloidosis is highly variable among series, ranging from 0 to 26.7%. In the reviews by Halushka and Vander Heide (11) and by Roshdy et al. (10) the frequency was 4 and 3.5%, respectively. In our own review, the frequency was 7.2%. Probably, differences among series were partially explained by the age of the patients included, since in our review the frequency of amyloidosis was high (14 to 26.7%) in the series in which the median age was 74 years or older but was low (0 to 7.3%) in those series with a median age lower than 70 years.

The limitations of our study include a relative low number of patients, who probably do not represent the complete spectrum of COVID-19 causes of dead. The use of two methods to study the hearts (partial and complete examination) is another limitation. However, we have not found differences between the pathological findings between both, except the presence of chronic epicardial inflammation, which has been more prevalent following the complete examination protocol. Finally, the lack of a control group of non-COVID-19 patients of similar age precludes any conclusion regarding if some lesions, such as collagen deposition, are increased in COVID-19 hearts.

Our series indicates that cardiac pathology is only modest in most patients and mainly consists of focal epicardial and myocardial inflammation, with little contribution of a direct effect of SARS-CoV-2. However, the frequency of these and other manifestations is highly variable among series suggesting that, in addition to biological variables, such as the time of evolution and methodological variables, like the extent of sampling, are responsible of these differences.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research Ethics Committee, Hospital Universitario Ramón y Cajal, Madrid, Spain (reference: Necropsias_Covid19; 355_20). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AF-G, HP-A, and JP contributed to conception and design of the study. AF-G, BP-M, and IR-C organized the database. IC-B, AN-C, DP, and JP wrote the first draft of the manuscript. RP, JZ, and JG wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

This work was supported by Instituto de Salud Carlos III (ISCIII) grant PI 19/01331, CIBERONC (grant CB16/12/00316), CIBERESP (grant CB06/02/0053), by the European Development Regional Fund. A way to achieve Europe (FEDER) and by MISP through the Investigator Initiated Studies Program (grant 2021/0224) and the Ramón y

Cajal Institute for Health Research (IRYCIS) (COVID-19 Grant, 2020/0290).

ACKNOWLEDGMENTS

We want to thank all members of the MACROCOVID and Pathology Departments from Hospital Universitario Ramón y Cajal (Madrid, Spain) for their constant support to the COVID-19 Autopsy Project. To Hospital Universitario Ramón y Cajal-IRyCIS Biobank (Madrid, Spain), for the management of tissue samples.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.748396/full#supplementary-material

REFERENCES

1. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3
2. Falasca L, Nardacci R, Colombo D, Lalle E, Di Caro A, Nicastri E, et al. Postmortem findings in Italian patients with COVID-19: a descriptive full autopsy study of cases with and without comorbidities. *J Infect Dis*. (2020) 222:1807–15. doi: 10.1093/infdis/jiaa578
3. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. (2020) 8:475–81. doi: 10.1016/S2213-2600(20)30079-5
4. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
5. Nguyen JL, Yang W, Ito K, Matte TD, Shaman J, Kinney PL. Seasonal influenza infections and cardiovascular disease mortality. *JAMA Cardiol*. (2016) 1:274–81. doi: 10.1001/jamacardio.2016.0433
6. Farshidfar F, Koleini N, Ardehali H. Cardiovascular complications of COVID-19. *JCI Insight*. (2021) 6:148980. doi: 10.1172/jci.insight.148980
7. Bansal M. Cardiovascular disease and COVID-19. *Diabetes Metab Syndr*. (2020) 14:247–50. doi: 10.1016/j.dsx.2020.03.013
8. Rozado J, Ayesta A, Morís C, Avanzas P. Fisiopatología de la enfermedad cardiovascular en pacientes con COVID-19. Isquemia, trombosis y disfunción cardiaca. *Rev Esp Cardiol*. (2020) 20:2–8. doi: 10.1016/S1131-3587(20)30028-5
9. Bryce C, Grimes Z, Pujadas E, Ahuja S, Beasley MB, Albrecht R, et al. Pathophysiology of SARS-CoV-2: the Mount Sinai COVID-19 autopsy experience. *Mod Pathol*. (2021) 34:1456–67. doi: 10.1038/s41379-021-00793-y
10. Roshdy A, Zaher S, Fayed H, Coghlan JG. COVID-19 and the heart: a systematic review of cardiac autopsies. *Front Cardiovasc Med*. (2021) 7:626975. doi: 10.3389/fcvm.2020.626975
11. Halushka MK, Vander Heide RS. Myocarditis is rare in COVID-19 autopsies: cardiovascular findings across 277 postmortem examinations. *Cardiovasc Pathol*. (2021) 50:107300. doi: 10.1016/j.carpath.2020.107300
12. Kawakami R, Sakamoto A, Kawai K, Gianatti A, Pellegrini D, Nasr A, et al. Pathological evidence for SARS-CoV-2 as a cause of myocarditis: JACC review topic of the week. *J Am Coll Cardiol*. (2021) 77:314–25. doi: 10.1016/j.jacc.2020.11.031
13. The COVID-19 Autopsy. The first COVID-19 autopsy in Spain performed during the early stages of the pandemic. *Rev Esp Patol*. (2020) 53:182–7. doi: 10.1016/j.patol.2020.05.004
14. Hanley B, Naresh KN, Roufosse C, Nicholson AG, Weir J, Cooke GS, et al. Histopathological findings and viral tropism in UK patients with severe fatal COVID-19: a post-mortem study. *Lancet Microbe*. (2020) 1:e245–53. doi: 10.1016/S2666-5247(20)30115-4
15. Sandino Pérez J, Aubert Girbal L, Caravaca-Fontán F, Polanco N, Sevillano Prieto Á, Andrés A. Pericarditis secundaria a infección por COVID-19 en un paciente trasplantado renal. *Nefrología*. (2021) 41:349–52. doi: 10.1016/j.nefro.2020.07.003
16. Eiros R, Barreiro-Perez M, Martin-Garcia A, Almeida J, Villacorta E, Perez-Pons A, et al. Pericarditis and myocarditis long after SARS-CoV-2 infection: a cross-sectional descriptive study in health-care workers. *medRxiv*. (2020) 2020.07.12.20151316. doi: 10.1101/2020.07.12.20151316
17. Blagojevic NR, Bosnjakovic D, Vukomanovic V, Arsenovic S, Lazic JS, Tadic M. Acute pericarditis and severe acute respiratory syndrome coronavirus 2: case report. *Int J Infect Dis*. (2020) 101:180–2. doi: 10.1016/j.ijid.2020.09.1440
18. Ho JS, Sia C-H, Chan MY, Lin W, Wong RC. Coronavirus-induced myocarditis: a meta-summary of cases. *Heart Lung J Crit Care*. (2020) 49:681–5. doi: 10.1016/j.hrtlng.2020.08.013
19. Hu H, Ma F, Wei X, Fang Y. Coronavirus fulminant myocarditis treated with glucocorticoid and human immunoglobulin. *Eur Heart J*. (2021) 42:206. doi: 10.1093/eurheartj/ehaa190
20. Sekhawat V, Green A, Mahadeva U. COVID-19 autopsies: conclusions from international studies. *Diagn Histopathol Oxf Engl*. (2021) 27:103–7. doi: 10.1016/j.mpdhp.2020.11.008
21. Basso C, Aguilera B, Banner J, Cohle S, d'Amati G, de Gouveia RH, et al. Guidelines for autopsy investigation of sudden cardiac death: 2017 update from the Association for European Cardiovascular Pathology. *Virchows Arch Int J Pathol*. (2017) 471:691–705. doi: 10.1007/s00428-017-2221-0
22. Buckley BJR, Harrison SL, Fazio-Eynullayeva E, Underhill P, Lane DA, Lip GYH. Prevalence and clinical outcomes of myocarditis and pericarditis in 718,365 COVID-19 patients. *Eur J Clin Invest*. (2021) 13:e13679. doi: 10.1111/eci.13679
23. del Nonno F, Frustaci A, Verardo R, Chimenti C, Nicastri E, Antinori A, et al. Virus-negative myopericarditis in human coronavirus infection. *Circ Heart Fail*. (2020) 13:e007636. doi: 10.1161/CIRCHEARTFAILURE.120.007636
24. Matschke J, Lütgehetmann M, Hagel C, Sperhake JP, Schröder AS, Edler C, et al. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. *Lancet Neurol*. (2020) 19:919–29. doi: 10.1016/S1474-4422(20)30308-2
25. Bozkurt B, Kamat I, Hotez PJ. Myocarditis with COVID-19 mRNA vaccines. *Circulation*. (2021) 144:471–84. doi: 10.1161/CIRCULATIONAHA.121.056135
26. Verweij PE, Brüggemann RJM, Azoulay E, Bassetti M, Blot S, Buil JB, et al. Taskforce report on the diagnosis and clinical management of COVID-19 associated pulmonary aspergillosis. *Intensive Care Med*. (2021) 47:819–34. doi: 10.1007/s00134-021-06449-4
27. Kula BE, Clancy CJ, Hong Nguyen M, Schwartz IS. Invasive mould disease in fatal COVID-19: a systematic review of autopsies. *Lancet Microbe*. (2021) 2:E405–14. doi: 10.1101/2021.01.13.21249761
28. Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res*. (2020) 191:145–7. doi: 10.1016/j.thromres.2020.04.013
29. Snell J. SARS-CoV-2 infection and its association with thrombosis and ischemic stroke: a review. *Am J Emerg Med*. (2021) 40:188–92. doi: 10.1016/j.ajem.2020.09.072
30. Pellegrini D, Kawakami R, Guagliumi G, Sakamoto A, Kawai K, Gianatti A, et al. Microthrombi as a major cause of cardiac injury in COVID-19: a pathologic study. *Circulation*. (2021) 143:1031–42. doi: 10.1161/CIRCULATIONAHA.120.051828
31. Bois MC, Boire NA, Layman AJ, Aubry M-C, Alexander MP, Roden AC, et al. COVID-19-associated nonocclusive fibrin microthrombi in the heart. *Circulation*. (2021) 143:230–43. doi: 10.1161/CIRCULATIONAHA.120.050754
32. Haslbauer JD, Tzankov A, Mertz KD, Schwab N, Nienhold R, Twerenbold R, et al. Characterization of cardiac pathology in 23 autopsies of

lethal COVID-19. *J Pathol Clin Res*. (2021) 7:326–37. doi: 10.1002/cjp2.212
33. Bradley BT, Maioli H, Johnston R, Chaudhry I, Fink SL, Xu H, et al. Histopathology and ultrastructural findings of fatal COVID-19 infections in Washington State: a case series. *Lancet Lond Engl*. (2020) 396:320–32. doi: 10.1016/S0140-6736(20)31305-2
34. Menter T, Haslbauer JD, Nienhold R, Savic S, Hopfer H, Deigendesch N, et al. Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. *Histopathology*. (2020) 77:198–209. doi: 10.1111/his.14134

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 22 October 2021
doi: 10.3389/fcvm.2021.755822

Post-ST-Segment Elevation Myocardial Infarction Follow-Up Care During the COVID-19 Pandemic and the Possible Benefit of Telemedicine: An Observational Study

Audrey A. Y. Zhang[1†], Nicholas W. S. Chew[1†], Cheng Han Ng[2], Kailun Phua[3], Yin Nwe Aye[1], Aaron Mai[2], Gwyneth Kong[2], Kalyar Saw[1], Raymond C. C. Wong[1,2], William K. F. Kong[1,2], Kian-Keong Poh[1,2], Koo-Hui Chan[1,2], Adrian Fatt-Hoe Low[1,2], Chi-Hang Lee[1,2], Mark Yan-Yee Chan[1,2], Ping Chai[1,2], James Yip[1,2], Tiong-Cheng Yeo[1,2], Huay-Cheem Tan[1,2] and Poay-Huan Loh[1,2]*

[1] Department of Cardiology, National University Heart Centre, National University Health System, Singapore, Singapore, [2] Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, [3] Department of Medicine, National University Hospital, Singapore

OPEN ACCESS

Edited by:
Mingxing Xie,
Huazhong University of Science and Technology, China

Reviewed by:
Eiichi Akiyama,
Kawaguchi Cardiovascular and Respiratory Hospital, Japan
Hendrik Tevaearai Stahel,
Bern University Hospital, Switzerland

***Correspondence:**
Nicholas W. S. Chew
nicholas_ws_chew@nuhs.edu.sg

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Coronary Artery Disease, a section of the journal Frontiers in Cardiovascular Medicine

Received: 09 August 2021
Accepted: 27 September 2021
Published: 22 October 2021

Citation:
Zhang AAY, Chew NWS, Ng CH, Phua K, Aye YN, Mai A, Kong G, Saw K, Wong RCC, Kong WKF, Poh K-K, Chan K-H, Low A-H, Lee C-H, Chan MY-Y, Chai P, Yip J, Yeo T-C, Tan H-C and Loh P-H (2021) Post-ST-Segment Elevation Myocardial Infarction Follow-Up Care During the COVID-19 Pandemic and the Possible Benefit of Telemedicine: An Observational Study. Front. Cardiovasc. Med. 8:755822. doi: 10.3389/fcvm.2021.755822

Background: Infectious control measures during the COVID-19 pandemic have led to the propensity toward telemedicine. This study examined the impact of telemedicine during the pandemic on the long-term outcomes of ST-segment elevation myocardial infarction (STEMI) patients.

Methods: This study included 288 patients admitted 1 year before the pandemic (October 2018–December 2018) and during the pandemic (January 2020–March 2020) eras, and survived their index STEMI admission. The follow-up period was 1 year. One-year primary safety endpoint was all-cause mortality. Secondary safety endpoints were cardiac readmissions for unplanned revascularisation, non-fatal myocardial infarction, heart failure, arrythmia, unstable angina. Major adverse cardiovascular events (MACE) was defined as the composite outcome of each individual safety endpoint.

Results: Despite unfavorable in-hospital outcomes among patients admitted during the pandemic compared to pre-pandemic era, both groups had similar 1-year all-cause mortality (11.2 vs. 8.5%, respectively, $p = 0.454$) but higher cardiac-related (14.1 vs. 5.1%, $p < 0.001$) and heart failure readmissions in the pandemic vs. pre-pandemic groups (7.1 vs. 1.7%, $p = 0.037$). Follow-up was more frequently conducted via teleconsultations (1.2 vs. 0.2 per patient/year, $p = 0.001$), with reduction in physical consultations (2.1 vs. 2.6 per patient/year, $p = 0.043$), during the pandemic vs. pre-pandemic era. Majority achieved guideline-directed medical therapy (GDMT) during pandemic vs. pre-pandemic era (75.9 vs. 61.6%, $p = 0.010$). Multivariable Cox regression demonstrated achieving medication target doses (HR 0.387, 95% CI 0.164–0.915, $p = 0.031$) and GDMT (HR 0.271, 95% CI 0.134–0.548, $p < 0.001$) were independent predictors of lower 1-year MACE after adjustment.

Conclusion: The pandemic has led to the wider application of teleconsultation, with increased adherence to GDMT, enhanced medication target dosing. Achieving GDMT was associated with favorable long-term prognosis.

Keywords: COVID-19, telemedicine, telehealth, ST-segment elevation myocardial infarction, pandemic

INTRODUCTION

The coronavirus-2019 (COVID-19) pandemic has demanded the rapid adaptation of healthcare operations in implementing measures to reduce the infectious rate but to also maintain the standard of patient care. Patients with cardiovascular disease are at increased risk of contracting the COVID-19 infection with a poorer outcome (1). The universally adopted strategy of social distancing as a measure to "flatten the curve" have resulted in a decrease in traditional physical consultations and the wider adaptation of teleconsultations. Teleconsultations, or telemedicine in general, offers virtual clinic consultations and monitoring which has gained traction as appropriate viable alternative for safe and efficient medical care. Its role has gained attention given the benefits of removing the risk of hospital exposure for these vulnerable patients during the pandemic. As the application of telemedicine expands, it becomes increasingly important to understand its impact on patient care and clinical outcomes.

During the pandemic, there has been a substantial reduction in patients presenting with ST-segment elevation myocardial infarction (STEMI) requiring primary percutaneous coronary intervention (PPCI) compared to the pre-pandemic era (2). Despite the decrease in PPCI case volume, the opposite effect of worse overall in-hospital STEMI performance metrics and short-term clinical outcomes were observed during the pandemic (3, 4). At present, little is known about the follow-up care of these STEMI patients during the pandemic and the potential role of telemedicine in the management of such patients following hospital discharge. This study is the first to examine the trend in teleconsultations for post-STEMI patients during the pandemic, and its association with optimal medical therapy, target medication doses, cardiovascular risk factor control and long-term clinical outcomes.

METHODS

Setting and Design

This is a retrospective single-center study of patients with STEMI who presented to a major PCI-capable hospital in Singapore, and survived the index STEMI admission. Consecutive patients were enrolled into two study groups according to the date of their index admission: (1) Pre-pandemic, from 1 October 2018 to 31 December 2018, and (2) pandemic, from 1 January 2020 to 31 March 2020. Those who did not survive the index admission were excluded from the study. There were no patients who were admitted during both study periods. The follow-up was 1 year following the index STEMI admission. For at least 1 year post-STEMI, the cardiologists of the center visit would traditionally follow up with these patients closely whilst on dual-antiplatelet therapy. It was highly unlikely for these patients to be followed up by other cardiologists outside of the center visit, although these patients might be followed up by doctors from other sub-specialties based on their comorbidities. The time period for the pre-pandemic group was carefully chosen to allow a control with the closest temporal proximity to the COVID-19 pandemic period, without its 1-year post-STEMI follow-up being affected by the pandemic.

During the pandemic, particularly when the Disease Outbreak Response was heightened to its second highest level on 7 February 2020, the standard post-STEMI care after hospital discharge had to be rapidly revamped with increased adaptation of telemedicine. This involved virtual consultations that were conducted via a secure audio-visual telecommunication system between the patients and healthcare providers. Patients were encouraged to subscribe to the hospital telemedicine service and were either provided with or used their own equipment to measure blood pressure, pulse rate and body weight. Patients were also offered remote vital signs monitoring conducted daily for 1 month post-STEMI. Prescriptions were optimized based on the virtual assessment and delivered to the patient's homes. The main goal of teleconsultation during the COVID-19 pandemic was not to provide superior care to the standard face-to-face consultations, but to provide these patients with "health maintenance strategy" individualized to their needs and risk factor control targets (5, 6). The teleconsultation integrated virtual consultations, symptomology assessment, evaluation of home monitoring vitals such as blood pressure, patient education, drug tolerance and adherence, quality of life, and anticoagulation tolerance (7). Physical face-to-face consultations were still conducted, albeit less frequently, during the pandemic and these consultations involved serum testing for cardiovascular risk factor control. Serum measurements of glycated A1c (HbA1c), low-density lipoprotein (LDL) cholesterol, creatinine, estimated glomerular filtration rates (eGFR) and international normalized ratio (INR) (as appropriate) were taken during the physical consultations. Hence, these study periods were carefully chosen to compare the effectiveness of telemedicine on post-STEMI care during the pandemic, vs. the standard post-STEMI care during the pre-pandemic era. Patients with recurrent STEMI presentations during subsequent study periods were excluded to avoid duplication. During the pandemic, the hospital was actively involved in the care for COVID-19 patients.

None of the patients in the study were diagnosed with COVID-19. In our institution, the COVID-19 patients would be co-managed by the pandemic and the Cardiology inpatient teams. Once the COVID-19 patients have been de-isolated with negative COVID-19 polymerase chain reaction tests, they

will be transferred under the Cardiology team's care. All patients, regardless of the COVID-19 status, will be reviewed outpatient in the Cardiology clinics. The COVID-19 status of the patients do not have any implications on their post-STEMI management.

Data Collection

Data on demographic and clinical characteristics were retrospectively collected from the hospital STEMI registry. This included past medical history, cardiovascular risk factors, presentation type, presentation route, complications during index admission, and medications on discharge. Angiographic data were also collected from the electronic medical records. Follow-up outpatient data on the number of outpatient consultations (including physical consultations, teleconsultations and cardiac rehabilitation), remote vital signs monitoring uptake, reported symptoms in clinic, and post-discharge medications were obtained. Serial measurements of Hba1c, LDL, and systolic blood pressure during the follow-up period were collected.

Guideline-directed medical therapy for STEMI was defined as being on dual antiplatelet therapy (aspirin and P2Y12 inhibitor), statin, β-blocker, with the option of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker (ACEI/ARB) if the post-STEMI left ventricular ejection fraction (LVEF) was $\leq$40% or the patient had diabetes mellitus (8, 9), unless these medications were clinically contraindicated in the individual. Patients who were on oral anticoagulation had to complete a month of triple antithrombotic therapy followed by concomitant oral anticoagulation and single antiplatelet, to be considered as being on guideline-directed medical therapy. β-blocker and angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blocker (ARB) doses were recorded on discharge and at follow-up clinic. Achieving target dose intensity of β-blocker and ACEI/ARB was based on the type and dose of the medication in accordance to a standardized algorithm as defined by our previous study (10). Guideline-directed medical therapy at follow-up was recorded in any of the outpatient clinic visits during the first year post-STEMI. The presence of guideline-directed medical therapy during the outpatient follow-up was used for the multivariable analyses. Our institution adopted the protocol for dual antiplatelet therapy in accordance to the European Society of Cardiology (11) and American College of Cardiology/American Heart Association (12) guidelines in administering a potent $P2Y_{12}$ inhibitor (prasugrel or ticagrelor), or clopidogrel if these are unavailable or contraindicated, and is usually prescribed before percutaneous coronary intervention is performed. Dual antiplatelet therapy was maintained over 12 months unless contraindicated.

Study Outcomes

All study outcomes were measured during the 1-year follow-up from the discharge date of the index admission. The primary safety endpoint was all-cause mortality. Secondary safety endpoints were cardiac readmissions for unplanned revascularisation, non-fatal MI, heart failure, arrhythmia, unstable angina, and major adverse cardiovascular events (MACE). MACE was defined as the composite outcome of each individual safety endpoints.

Secondary efficacy outcomes measured were (1) prescription of guideline-directed medical therapy, (2) achieving target dose intensities of β-blocker and ACEI/ARB (10), and (3) cardiovascular risk factor control (systolic blood pressure, LDL, and HbA1c).

Statistical Analyses

Categorical variables were described as percentages and continuous variables as mean with standard deviation (SD). Continuous variables were assessed with one-way analysis of variance (ANOVA). Categorical variables were evaluated with Pearson's chi-square test (or Fisher's Exact Test where appropriate). The multivariable Cox regression model was constructed to evaluate the association of telemedicine and 1-year MACE, as well as telemedicine and all-cause mortality, which included variables such as achieving medication target doses, guideline-directed medical therapy, remote vital signs monitoring, age, diabetes mellitus, chronic kidney disease, LVEF, smoking status, admission in the pandemic era, and presented with out-of-hospital cardiac arrest and/or cardiogenic shock. These co-variates were carefully chosen as they are traditional prognostic factors in STEMI patients.

Furthermore, *post-hoc* logistic regression was performed to evaluate the association of telemedicine and achieving guideline-directed medical therapy or medication target doses, which included co-variates such as age, smoking status, admission in the pandemic era, out-of-hospital cardiac arrest and cardiogenic shock, LVEF, gender, ethnicity, and presence of symptoms post-discharge. A p-value of < 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY. This study was conducted in accordance to the revised Declaration of Helsinki and approved by the institutional and local ethics committee (NHG DSRB No. 2013/00442). As the study involved retrospective analysis of clinically acquired data, the institutional review board waived the need for written patient consent.

RESULTS

Baseline Characteristics

Table 1 displays the baseline characteristics of the study population. A total of 320 patients with STEMI who underwent primary PCI were reviewed retrospectively from the local STEMI registry. A total of 17 patients were lost to follow-up, with 6 patients from the pre-pandemic era and 11 from the pandemic era. All patients included in the analysis completed 1-year of follow-up. There were 15 inpatient deaths, 9 and 6 of whom were from the pandemic and pre-pandemic eras, respectively. After excluding inpatient deaths in the index hospitalization, 288 patients who survived their index admission were recruited in the study analysis. There were 170 (59.0%) STEMI patients in the pandemic group, and 118 (41.0%) in the pre-pandemic group. Baseline demographic characteristics and past medical history were similar between both groups. There were more evolved MI

TABLE 1 | Baseline characteristics of study participants with ST-segment elevation myocardial infarction during index admission according to pre-pandemic or pandemic era.

	Total (n = 288)	Pandemic (n = 170)	Pre-pandemic (n = 118)	P-value
Demographic				
Age, years	59 (13)	59 (13)	58 (12)	0.626
Sex, female	46 (16.0)	29 (17.1)	17 (14.4)	0.546
Ethnicity				0.448
Chinese	142 (49.3)	88 (51.8)	54 (45.8)	
Malay	58 (20.1)	29 (17.1)	29 (24.6)	
Indian	66 (22.9)	39 (22.9)	27 (22.9)	
Other	22 (7.6)	14 (8.2)	8 (6.8)	
Medical history				
Smoking status				0.952
Non-smoker	130 (45.1)	78 (45.9)	52 (44.1)	
Active smoker	124 (43.1)	72 (42.4)	52 (44.1)	
Ex-smoker	34 (11.8)	20 (11.8)	14 (11.9)	
Hypertension	169 (58.7)	98 (57.6)	71 (60.2)	0.669
Diabetes	113 (39.2)	70 (41.2)	43 (36.4)	0.418
Hyperlipidaemia	179 (62.2)	100 (58.8)	79 (66.9)	0.162
Previous myocardial infarction	38 (13.2)	20 (11.8)	18 (15.3)	0.389
Previous PCI	45 (15.6)	21 (12.4)	24 (20.3)	0.066
Previous CABG	5 (1.7)	1 (0.6)	4 (3.4)	0.073
Stroke	14 (4.9)	8 (4.7)	6 (5.1)	0.883
Chronic kidney disease	23 (8.0)	15 (8.8)	8 (6.8)	0.529
Atrial fibrillation	8 (2.8)	4 (2.4)	4 (3.4)	0.598
Previous heart failure	9 (3.1)	6 (3.5)	3 (2.5)	0.636
Family history of premature CAD	37 (12.8)	28 (16.5)	9 (7.6)	**0.027**
Index admission				
Presentation type				**<0.001**
STEMI	248 (86.1)	136 (80.0)	112 (94.9)	
Evolved MI	23 (8.0)	23 (13.5)	0	
Out-of-hospital cardiac arrest	17 (5.9)	11 (6.5)	6 (5.1)	
Presentation route				0.156
Direct visit	199 (69.1)	112 (65.9)	87 (73.7)	
Interhospital transfers	89 (30.9)	58 (34.1)	31 (26.3)	
Complications				
Heart failure (Killip class 3)	34 (11.8)	26 (15.3)	8 (6.8)	**0.028**
Sepsis	23 (8.0)	18 (10.7)	5 (4.2)	**0.049**
New onset atrial fibrillation	16 (5.6)	14 (8.2)	2 (1.7)	**0.017**
Major bleed	27 (9.4)	18 (10.6)	9 (7.6)	0.397
Cardiogenic shock	21 (7.3)	13 (7.6)	8 (6.8)	0.781
Stroke	3 (1.0)	3 (1.8)	0	0.147
Acute kidney injury	53 (18.4)	27 (15.9)	26 (22.0)	0.185
Inotrope requirement	34 (11.8)	22 (12.9)	12 (10.2)	0.473
Requiring intubation	36 (12.5)	25 (14.7)	11 (9.3)	0.174
Requiring CABG	7 (2.5)	5 (3.2)	2 (1.7)	0.442
Length of stay, days	6 (7)	6 (8)	5 (5)	0.226
LVEF on discharge, %	46 (12)	44 (13)	49 (10)	**0.002**
Angiographic characteristics				
Radial access	210 (73.0)	128 (75.3)	82 (70.1)	0.451
Multivessel disease	140 (48.6)	85 (50.0)	55 (46.6)	0.571
Number of stents				0.616
0	36 (19.3)	30 (19.7)	6 (17.1)	

(Continued)

TABLE 1 | Continued

	Total ($n = 288$)	Pandemic ($n = 170$)	Pre-pandemic ($n = 118$)	*P*-value
1	120 (64.2)	95 (62.5)	25 (71.4)	
2	26 (13.9)	22 (14.5)	4 (11.4)	
3	5 (2.7)	5 (3.3)	0	
Door-to-balloon time, minutes	88 (145)	96 (172)	80 (103)	0.390
Discharge medications Aspirin	269 (93.4)	160 (94.1)	109 (92.4)	0.557
P2Y12 inhibitor	281 (97.6)	170 (100)	111 (94.1)	**0.001**
Oral anticoagulation	13 (4.5)	8 (4.7)	5 (4.2)	0.851
Betablocker	231 (82.5)	136 (84.0)	95 (80.5)	0.454
ACEI/ARB	191 (68.2)	113 (69.8)	78 (66.1)	0.517
Statin	269 (93.7)	157 (92.9)	112 (94.9)	0.488
Guideline-directed medical therapy	220 (76.4)	131 (77.1)	83 (75.4)	0.748

Categorical data presented as n (%). Continuous data presented as mean values (standard deviation).
ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; CABG, coronary artery bypass graft; CAD, Coronary artery disease; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention; STEMI, ST segment elevation myocardial infarction.
Statistically significant P values are highlighted in bold.

TABLE 2 | Characteristics of study participants with ST-segment elevation myocardial infarction during 1-year follow-up based on pre-pandemic or pandemic era.

	Total ($n = 288$)	Pandemic ($n = 170$)	Pre-pandemic ($n = 118$)	*P*-value
Outpatient consultations				
Total consultations	3.6 (3.1)	4.1 (3.5)	2.7 (2.2)	**<0.001**
Physical consultations	2.4 (1.7)	2.1 (1.6)	2.6 (1.7)	**0.043**
Teleconsultations	0.8 (1.7)	1.2 (1.9)	0.2 (1.1)	**0.001**
Cardiac rehabilitation	0.17 (0.74)	0.1 (0.7)	0.3 (0.7)	**<0.001**
Remote vital signs monitoring	97 (33.7)	59 (34.7)	38 (32.2)	0.659
Reported symptoms				
Typical chest pain	3 (1.2)	1 (0.7)	2 (1.9)	0.386
Atypical chest pain	22 (8.7)	12 (8.2)	10 (9.3)	0.741
Dyspnoea	21 (8.3)	10 (6.8)	11 (10.3)	0.320
Palpitations	3 (1.2)	3 (2.0)	0	0.137
Orthopnoea/PND/lower limb oedema	30 (11.8)	20 (13.6)	10 (9.3)	0.299
Post-discharge medications				
Aspirin	269 (93.4)	160 (94.1)	109 (92.4)	0.557
P2Y12 inhibitor	251 (87.8)	154 (90.6)	97 (83.6)	0.077
Oral anticoagulation	22 (7.6)	12 (7.1)	10 (8.5)	0.656
Beta-blocker	220 (76.9)	136 (80.0)	84 (72.4)	0.135
ACEI/ARB	202 (70.6)	126 (74.1)	76 (65.6)	0.117
Statin	263 (92.0)	158 (92.9)	105 (90.5)	0.459

Categorical data presented as n (%). Continuous data presented as mean values (standard deviation).
ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; PND, paroxysmal nocturnal dyspnea.
Total consultations include physical and teleconsultations.
Statistically significant P values are highlighted in bold.

(13.5% vs. none, $p < 0.001$) and out-of-hospital cardiac arrest (6.5 vs. 5.1%, $p < 0.001$) in the pandemic group compared to the pre-pandemic group. Those who were admitted during the pandemic had higher incidence of unfavorable inpatient clinical progress compared to those admitted during the pre-pandemic era, such as Killip class 3 heart failure (15.3 vs. 6.8%, $p = 0.028$), sepsis (10.7 vs. 4.2%, $p = 0.049$), new onset atrial fibrillation (8.2 vs. 1.7%, $p = 0.017$) and lower LVEF (44 vs. 49%, $p = 0.002$). Importantly, there was no difference in discharge medications between the pandemic and pre-pandemic groups, apart from P2Y12 inhibitor use (100 vs. 94.1%, $p = 0.001$, respectively). Of the 7 patients discharged without P2Y12 inhibitor, only 1 was on concomitant oral anticoagulation with aspirin. The prescription of guideline-directed medical therapy on discharge between both groups was similar ($p = 0.748$).

Telemedicine, Guideline-Directed Medical Therapy, Target Drug Dose Intensity, and Cardiovascular Risk Factor Control

The characteristics of study participants during follow-up are described in **Table 2**. The average number of physical consultations per patient over a 1-year period during the pandemic was lower than that in the pre-pandemic era (2.1 vs. 2.6 visits per patient per year, respectively, $p = 0.043$). Conversely, there was higher average number of teleconsultations per patient during the pandemic compared to the pre-pandemic era over the 1-year follow-up (1.2 vs. 0.2 teleconsultations per patient per year, respectively, $p = 0.001$). Cardiac rehabilitation visits were fewer during the pandemic compared to pre-pandemic era (mean of 0.1 vs. 0.3 per patient per year, respectively, $p < 0.001$).

During follow-up, all first visit post-myocardial infarction clinic consultations were physical consultations. The mean duration from discharge to first physical consultation was longer during the pandemic compared to pre-pandemic era (50 ± 39 vs. 39 ± 31 days, respectively, $p = 0.005$), with also longer mean duration between the first physical consultation to second physical consultation during the pandemic compared to pre-pandemic era (128 ± 84 vs. 98 ± 67 days, respectively, $p = 0.008$). There was no statistical difference in the uptake of remote vital signs monitoring between both study groups.

The pandemic era observed a significantly greater proportion of patients being on guideline-directed medical therapy (75.9%) compared to the pre-pandemic era (61.6%, $p = 0.010$) on follow-up. There was a trend towards achieving medication target doses in both β-blocker (19.4 vs. 15.3%, respectively, $p = 0.363$) and ACEI/ARB (9.5 vs. 5.9%, respectively, $p = 0.278$) during the pandemic compared to the pre-pandemic era.

We observed some differences in cardiovascular risk factor control and laboratory measurements from admission to outpatient surveillance between the pandemic and pre-pandemic periods. Firstly, LDL during index admission was similar in both pandemic and pre-pandemic groups (3.09 vs. 3.14 mmol/L, respectively, $p = 0.588$). Throughout the 1-year follow-up, similar improvement in LDL was achieved in the pandemic and pre-pandemic groups on the first clinic visit (1.81 vs. 1.52 mmol/L, respectively, $p = 0.124$), second visit (1.73 vs. 1.69 mmol/L, respectively, $p = 0.788$) and third visit (1.95 vs. 1.24 mmol/L, respectively, $p = 0.179$). Secondly, the percentage of patients with Hba1c ≥7% was similar between the pandemic and pre-pandemic eras during admission (30.7 vs. 29.0%, respectively, $p = 0.536$) and first clinic visit (29.4 vs. 32.6%,

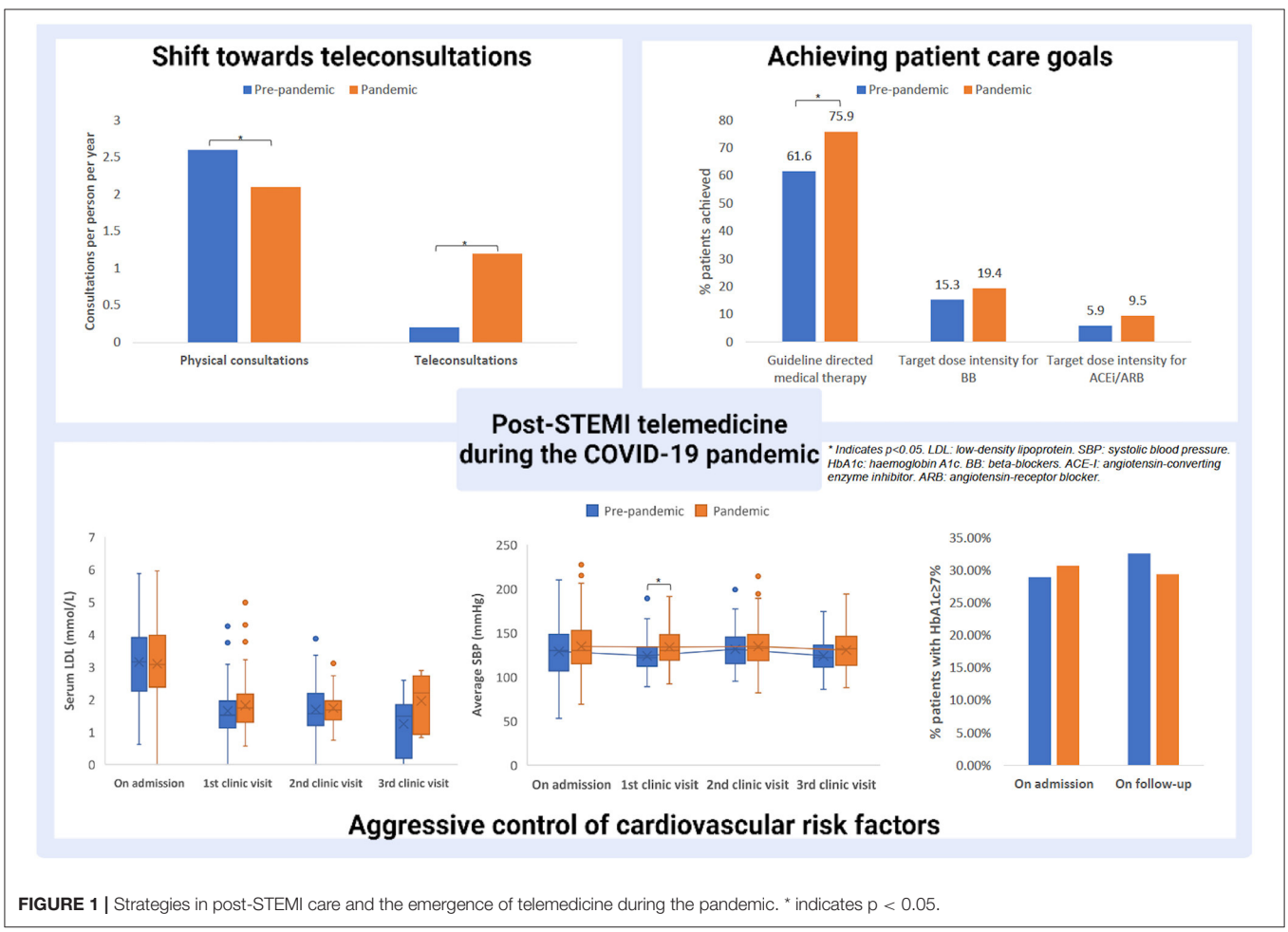


FIGURE 1 | Strategies in post-STEMI care and the emergence of telemedicine during the pandemic. * indicates p < 0.05.

respectively, $p = 0.734$). Thirdly, the average systolic blood pressure measured on discharge (134 vs. 128 mmHg, $p = 0.09$) and first clinic visit (133 vs. 123 mmHg, $p < 0.001$) was higher during the pandemic vs. the pre-pandemic eras; however such difference was no longer observed subsequently during the second (132 vs. 131 mmHg, $p = 0.235$) and third visit (130 vs. 123 mmHg, $p = 0.174$). These findings are summarized in **Figure 1**.

Study Safety End-Point

The 1-year all-cause mortality rates were similar between both groups ($p = 0.454$). However, there was an overall increased cardiac readmissions in the pandemic vs. the pre-pandemic era (14.1 vs. 5.1%, $p < 0.001$). There were increased heart failure readmissions in the pandemic (7.1%) compared to pre-pandemic era (1.7%, $p = 0.037$). No differences in unplanned revascularisation ($p = 0.787$), non-fatal MI ($p = 0.336$), arrhythmia ($p = 0.239$), unstable angina ($p = 0.701$) and MACE ($p = 0.112$) were observed between the two groups (**Table 3**).

On the multivariable Cox regression analysis, there was no significant association between teleconsultation and 1-year MACE [adjusted hazards ratio [aHR] 1.938, 95% confidence interval [CI] 0.896–4.190, $p = 0.093$]. Patients who achieved medication target doses (aHR 0.387, 95% CI 0.164–0.915, $p = 0.031$) and guideline-directed medical therapy (aHR 0.271, 95% CI 0.134–0.548, $p < 0.001$) were significantly associated with decreased rates of MACE after adjusting for important confounders (**Table 4**). There was also no significant association between teleconsultation and 1-year all-cause mortality (aHR 0.867, 95% CI 0.203–3.706, $p = 0.847$) after adjusting for important confounders (**Supplementary Material 1**)

In addition, the association between telemedicine and guideline-directed medical therapy or medication target doses was explored. *Post-hoc* multivariable logistic regression demonstrated that having teleconsultations was significantly associated with achieving guideline-directed medical therapy [odds ratio [OR] 3.472, 95% CI 1.537–7.843, $p = 0.003$] but not achieving medication target doses (OR 1.272, 95% CI 0.636–2.542, $p = 0.496$), after adjusting for important confounders.

DISCUSSION

The conventional post-STEMI care has been drastically affected by the COVID-19 pandemic, and healthcare institutions have been required to adapt quickly to the stringent infectious control measures without compromising STEMI care. To our knowledge, this study is the first to systematically examine real-world data of the impact of COVID-19 pandemic on the standard of follow-up care and outcomes of STEMI patients over the ensuing year following hospital discharge. Our study has revealed several important findings. Firstly, despite exclusion of those who died while inpatient, patients admitted with STEMI during the pandemic had worse in-hospital outcomes such as increased rates of sepsis, new onset atrial fibrillation, heart failure and reduced left ventricular ejection fraction, compared to the pre-pandemic counterparts. Yet, during the 1-year follow-up, both these groups of patients had similar rates of all-cause mortality, but there were more frequent overall cardiac readmissions and heart failure readmission among those admitted during the pandemic era. This was in conjunction with the wider adaptation of teleconsultations, albeit a reduction of physical consultations, during the pandemic. Secondly, there were significantly more patients achieving guideline-directed medical therapy during the pandemic compared to the pre-pandemic era. Thirdly, there was also a trend toward increased rate of achieving medication target doses of β-blocker and ACEI/ARB therapy during the pandemic vs. the pre-pandemic era. Despite this, patients in the pandemic era had substantially higher mean LDL levels

TABLE 3 | Safety and efficacy end-points of the study population during 1-year follow-up post-index ST-segment elevation myocardial infarction admission.

	Total ($n = 288$)	Pandemic ($n = 170$)	Pre-pandemic ($n = 118$)	*P*-value
Safety end-point				
All-cause mortality	29 (10.1)	19 (11.2)	10 (8.5)	0.454
Cardiac readmission				
Unplanned revascularisation	3 (1.0)	2 (1.2)	1 (0.8)	0.787
Non-fatal MI	5 (1.7)	4 (2.4)	1 (0.8)	0.336
Heart failure	14 (4.9)	12 (7.1)	2 (1.7)	**0.037**
Arrythmia	2 (0.7)	2 (1.2)	0	0.239
Unstable angina	6 (2.1)	4 (2.4)	2 (1.7)	0.701
Major adverse cardiac events	59 (20.4)	43 (25.2)	16 (13.6)	0.112
Efficacy end-point				
Guideline-directed medical therapy	202 (70.2)	129 (75.9)	72 (61.6)	**0.010**
Achieving target dose intensity				
ACEI/ARB	23 (8.0)	16 (9.5)	7 (5.9)	0.278
Beta-blocker	51 (17.7)	33 (19.4)	18 (15.3)	0.363

Categorical data presented as n (%).
ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; MI, myocardial infarction.
Statistically significant P values are highlighted in bold.

TABLE 4 | Cox regression for 1-year MACE in patients who survived index admission of STEMI.

Variables	Adjusted hazards ratio (95% confidence ratio)	*p*-value
Teleconsultation	1.938 (0.896–4.190)	0.093
Achieving medication target doses	0.387 (0.164–0.915)	**0.031**
Post-discharge guideline-directed medical therapy	0.271 (0.134–0.548)	**<0.001**
Remote vital signs monitoring	0.512 (0.216–1.213)	0.128
Age	1.024 (1.000–1.050)	0.055
Diabetes mellitus	1.369 (0.706–2.655)	0.353
Chronic kidney disease	3.057 (1.291–7.238)	**0.011**
Left ventricular ejection fraction	0.944 (0.921–0.969)	**<0.001**
Smoker/Ex-smoker	0.609 (0.297–1.246)	0.174
Out-of-hospital cardiac arrest/cardiogenic shock	0.842 (0.348–2.037)	0.702
Admission in pandemic era	1.905 (0.827–4.390)	0.130

Statistically significant P values are highlighted in bold.

on follow-up, albeit statistically non-significant, than those in the pre-pandemic era. Fourthly, for patients who survived the index STEMI admission, achieving medication target doses and guideline-directed medical therapy during the follow-up were independently associated with a lower 1-year MACE. Even though teleconsultation was not an independent predictor of MACE, our findings highlight that teleconsultation had a significant association with achieving guideline-directed medical therapy during follow-up.

As demonstrated by a recent meta-analysis on the global impact of the COVID-19 pandemic on STEMI care (3), short-term STEMI outcomes have been shown to be unfavorable with delayed symptom onset-to-door time, door-to-balloon time, lower LVEF on discharge, suboptimal reperfusion following PCI, increased duration of intensive care unit stay and increased in-hospital mortality during the pandemic era compared to the pre-pandemic era. Similarly, our study has shown worse STEMI metrics during the index admission even after excluding those who did not survive. There were higher overall cardiac related readmissions, particularly heart failure readmissions, in the pandemic compared to pre-pandemic eras. The study sample size, however, might be too small to detect small significant differences in readmission rates in the other subgroups. Despite this, our findings revealed similar 1-year follow-up mortality between both groups. Moreover, achieving medication target doses and guideline-directed medical therapy during follow-up are independent predictors of reducing the risk of MACE. Whether teleconsultation affects the overall outcome of patients with STEMI remains to be investigated. However, it allows for safer and regular follow-up during the pandemic, with drug optimisation for patients in the early post-STEMI period. Importantly, as demonstrated by the present study that patients admitting during the pandemic had worse clinical outcomes during the index admission, this could have increased the demand for closer outpatient surveillance with increased teleconsultations particularly for patients with worse severity of cardiac disease. This might be reflected by the large standard deviation of the average number of teleconsultations in this study. Telemedicine indeed offers a synergistic avenue, in conjunction with physical consultations, in enhancing more frequent surveillance which is particularly important during the pandemic whilst maintaining the stringent infection control measures. Beyond the pandemic, teleconsultation has been shown to be cost-effective particularly for patients with myocardial infarction, as this important window of follow-up helps ameliorate adverse post-STEMI remodeling, and reduces the potential for the detrimental consequences of chronic heart failure (13).

Our recent published data displayed an increase in STEMI cases during the pandemic compared to the pre-pandemic era, which was partly due to the our regional STEMI network strategy in centralizing primary PCI service at our hospital, taking advantage of the geographical proximity of healthcare hospitals within the West of Singapore allowing timely inter-hospital transfers (14). Our previous study (4) also demonstrated that no significant door-to-balloon delay in inter-hospital transfers between the pandemic and pre-pandemic periods. This allowed the other hospitals to divert resources in providing care for the COVID-19 cases. Moreover, patients admitted during the pandemic had higher incidence of heart failure, sepsis, atrial fibrillation and lower LVEF, compared to those in the pre-pandemic period, which might play a role on the follow-up requirements during the pandemic.

Although telemedicine is a viable alternative, it is not a complete replacement for physical face-to-face consultations. In our study, there was increased overall cardiac related and heart failure readmissions during the pandemic compared to the pre-pandemic era. This could partly be due to the increased in-hospital complications during the pandemic era, such as increased prevalence of Killip class 3 heart failure at presentation and lower LVEF, compared to the pre-pandemic era. However, one might speculate that this observation suggests the limitation of teleconsultation follow-up when it comes to patients at risk of heart failure especially during the early stage following STEMI since it is limited by the absence of face-to-face clinical examination of fluid status and the lack of traditional parameter measurements in clinics such as body weight (15, 16). Nevertheless, the increasing evidence for telemedicine in heart failure management appears promising with several reviews demonstrating significant reduction in heart failure-related hospital admission compared to the conventional care

(17–21). Various trials including Telemedical Interventional Monitoring in Heart Failure (TIM-HF I and TIM-HF II) have shown improved patient education, medication adherence rates, lower mortality, overall hospital admissions and heart failure admissions, with improved quality of life for patients and reduced healthcare costs with the use of telemedicine (22, 23). Hence, patients might require closer monitoring during early stage following STEMI especially those with unfavorable risk factors such as lower LVEF (10).

Teleconsultations allow rapid titration of guideline-directed medical therapy and the increased likelihood of achieving medication target doses. Despite the restrictions during the pandemic, patients were more likely to be on guideline-directed medical therapy with similar medication target dose intensities, compared to their pre-pandemic counterparts. Our study echoes previous landmark trials such that patients achieving guideline-directed medical therapy and target doses have significantly lower rate of MACE (24, 25), especially in the setting of reduced LVEF. Several reviews demonstrated significant benefits in telemedicine for HbA1c (26, 27) and LDL reductions (28, 29), although the evidence for telemedical interventions on lowering blood pressure and body mass index remains mixed (30–32). However, our study highlights the concerns regarding aggressive cardiovascular risk factor control during the pandemic. Even though we demonstrated non-significant differences between pandemic and pre-pandemic groups in terms of LDL control over the 1-year follow-up period, the absolute differences between the serial LDL levels are clinically significant. Clinicians need to be aware of the potentiality of inadequate cardiovascular risk factor control particularly in the pandemic when lifestyle and diet might be changed during the lockdown. Teleconsultation remains the cornerstone of post-STEMI care during the pandemic with timely consultations, prompt initiation and titration of optimal medical therapy, whilst ensuring social distancing and reducing the patient's exposure to the hospital. As it will take time for the telemedicine program to adapt and evolve with the dynamic demands of the pandemic, it is a possibility that there might be variations in follow-up efficacy and efficiency within each of the study groups. However, given the small study sample size, the correlation of monthly variations with clinical outcomes is likely to be underpowered to draw any conclusions. Nevertheless, these are invaluable lessons that we should take beyond the pandemic in reducing waiting and traveling time, and clinic delays, whilst maintaining the standard of post-STEMI care (33–35). The institution is constantly evolving its telemedicine programmes in conjunction with regular physical consultations, and also integrating allied health care practitioner-led remote intensive management in addition to the cardiologist-led standard care (10).

Further studies are needed to evaluate patient's perspective and potential hurdles of telemedicine. Potential hurdles to implementation of telemedicine include patient-related factors associated with older age, low health literacy, cognitive dysfunction, privacy and security concerns (6, 36, 37). In the face of constant evolution of modalities to deliver digital healthcare, the European Society of Cardiology recommends the development of specific training programs for patients, caregivers and medical staff to assist them in understanding the capabilities and limitations of telemedicine (36).

CLINICAL IMPLICATIONS

With enhanced pandemic control measures, there is a pressing need to reduce physical consultations. Telemedicine plays an important role during the pandemic to bridge this gap in providing adequate follow-up to ensure optimisation of medical therapy post-STEMI and maintaining intensive cardiovascular risk factor control (21). It has, at least in part, contributed to the comparable 1-year post-STEMI outcomes between the pandemic and pre-pandemic eras among our patients, despite the adverse in-hospital STEMI metrics observed during the pandemic. These lessons from the pandemic serve a vital and broader role for the future with the emergence of telemedicine in post-STEMI care.

LIMITATIONS

Although this study is the first to examine the feasibility, efficacy, and safety of telemedicine in post-STEMI care during the pandemic, our study has several limitations that merit consideration. Firstly, this is a single-center retrospective observational study with a small sample size, and hence it is not possible to infer causality between telemedicine and the observed clinical outcomes. Nevertheless, our study offers real-world data based on consecutive patients enrolled in our STEMI database, and it reflects the actual follow-up processes that transitioned from physical consultations to teleconsultations during the pandemic. Secondly, the care provided to our control group (pre-pandemic group) might not be representative of care standard that was in line with the current recommendations. Nevertheless, it was chosen as it was the most recent period possible during which the 1-year follow-up care was not affected by the pandemic. Thirdly, teleconsultation was not standardized across all attending physicians and follow-up intervals varied among the patients given the nature of the study and resource constraints during the pandemic. Fourthly, the general attitudes to health and the stresses faced by patients and healthcare providers may also differ during pre-pandemic and pandemic era. For example, the pandemic might motivate the adoption of healthier lifestyle and healthier choices; on the other hand, the social distancing and compulsory home isolation may compel a more sedentary lifestyle (38). New challenges for healthcare providers during the pandemic include the need to comply to social distancing while ensuring the rapport with patients and quality of care are not compromised, and also identifying patients at higher risk of complications in a remote setting (39). However, this study was not designed to evaluate these additional factors which might have an impact on clinical outcomes and cardiovascular risk factor control.

However, our study findings represent actual clinical practice based on the physician's clinical judgment and discretion. Moreover, telemedicine consists of both virtual telehealth clinics and the utility of digital healthcare technologies. However, our study was not designed to evaluate the deliverance of

digital healthcare. Overall, the results of the study need to be interpreted with caution, as the study observations might be related to the complex interplay between the COVID-19 pandemic, telemedicine and other non-measurable factors. This retrospective cohort provides, for the first time, real-world data of the dynamic change in hospital follow-up processes in STEMI follow-up with drastic decrease in physical consultations due to social distancing policies and the rapid emergence of telemedicine. With the inherent limitations of a real-world cohort study in this ever-changing landscape during a pandemic, the preliminary findings shed light on the invaluable lessons of teleconsultation adaptation, but controlling for external influences of the pandemic is evidently not possible. Furthermore, we were not able to evaluate if the number of total consultations correlated with improvement in outcomes as the number of consultations was determined by both the routine follow-up as well as the patient's individual need for closer surveillance.

CONCLUSION

Despite the unfavorable in-hospital STEMI metrics of patients admitted during the pandemic, their 1-year mortality rate was similar to those admitted during the pre-pandemic era. The pandemic led to wider adaptation of teleconsultation which might partly contribute to increased use of guideline-directed medical therapy and meeting medication target dosing. Guideline-directed medical therapy was associated with better outcomes regardless of telemedicine or the pandemic. Telemedicine, at its core, should not be considered a replacement of the traditional face-to-face doctor-patient interactions, but a synergistic extension of post-STEMI care. The invaluable lessons of telemedicine during the pandemic should be extended for future post-STEMI care.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

IRB INFORMATION

This study was approved by the local institution review board (NHG DSRB No. 2013/00442).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by NHG DSRB No. 2013/00442. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.755822/full#supplementary-material

REFERENCES

1. Ganatra S, Hammond SP, Nohria A. The novel coronavirus disease (COVID-19) threat for patients with cardiovascular disease and cancer. *JACC CardioOncol.* (2020) 2:350–55. doi: 10.1016/j.jaccao.2020.03.001
2. De Luca G, Verdoia M, Cercek M, Jenson LO, Vavlukis M, Calmac L, et al. Impact of COVID-19 pandemic on mechanical reperfusion for patients with STEMI. *J Am Coll Cardiol.* (2020) 76:2321–30. doi: 10.1016/j.jacc.2020.09.546
3. Chew NW, Ow ZGW, Teo VXY, Heng RRY, Ng CH, Lee CH, et al. The global impact of the COVID-19 pandemic on STEMI care: a systematic review and meta-analysis. *Can J Cardiol.* (2021) 37:1450–9. doi: 10.1016/j.cjca.2021.04.003
4. Chew NW, Sia CH, Wee HL, Loh JDB, Rastogi S, Kojodjojo P, et al. Impact of the COVID-19 pandemic on door-to-balloon time for primary percutaneous coronary intervention - results from the Singapore Western STEMI Network. *Circ J.* (2021) 85:139–49. doi: 10.1253/circj.CJ-20-0800
5. Cleland JGF, Clark RA, Pellicori P, Inglis SC. Caring for people with heart failure and many other medical problems through and beyond the COVID-19 pandemic: the advantages of universal access to home telemonitoring. *Eur J Heart Fail.* (2020) 22:995–8. doi: 10.1002/ejhf.1864
6. Tersalvi G, Winterton D, Cioffi GM, Ghidini S, Roberto M, Biasco L, et al. Telemedicine in heart failure during COVID-19: a step into the future. *Front Cardiovasc Med.* (2020) 7:612818. doi: 10.3389/fcvm.2020.612818
7. Nan J, Jia R, Meng S, Jin Y, Chen W, Hu H. The impact of the COVID-19 pandemic and the importance of telemedicine in managing acute st segment elevation myocardial infarction patients: preliminary experience and literature review. *J Med Syst.* (2021) 45:9. doi: 10.1007/s10916-020-01703-6
8. O'Gara PT, Kushner FG, Ascheim DD, Casey DE Jr, Chung MK, de Lemos JA, et al. 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation.* (2013) 127:e362–425. doi: 10.1161/CIR.0b013e3182742c84
9. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J.* (2018) 39:119–77. doi: 10.1093/eurheartj/ehx393
10. Chan MY, Koh KWL, Poh SC, Marchesseau S, Singh D, Han Y, et al. Remote postdischarge treatment of patients with acute myocardial infarction by allied health care practitioners vs standard care: the IMMACULATE randomized clinical trial. *JAMA Cardiol.* (2020) 6:830–5. doi: 10.1001/jamacardio.2020.6721
11. Valgimigli M, Bueno H, Byrne RA, Collet JP, Costa F, Jeppsson A, et al. 2017 ESC focused update on dual antiplatelet therapy in coronary artery disease developed in collaboration with EACTS: the Task Force for dual antiplatelet therapy in coronary artery disease of the European Society of Cardiology (ESC) and of the European Association for Cardio-Thoracic Surgery (EACTS). *Eur Heart J.* (2018) 39:213–60. doi: 10.1093/eurheartj/ehx419
12. Levine GN, Bates ER, Bittl JA, Brindis RG, Fihn SD, Fleisher LA, et al. 2016 ACC/AHA guideline focused update on duration of dual antiplatelet therapy

in patients with coronary artery disease. *Circulation.* (2016) 134:e123–55. doi: 10.1161/CIR.0000000000000404
13. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. *J Am Coll Cardiol.* (2017) 70:1–25. doi: 10.1016/j.jacc.2017.04.052
14. Phua K, Chew NWS, Sim V, Zhang AA, Rastogi S, Kojodjojo P, et al. One-year outcomes of patients with ST-segment elevation myocardial infarction during the COVID-19 pandemic. *J Thromb Thrombolysis.* (2021) 1–11 doi: 10.1007/s11239-021-02557-6
15. Tse G, Chan C, Gong M, Meng L, Zhang J, Su XL, et al. Telemonitoring and hemodynamic monitoring to reduce hospitalization rates in heart failure: a systematic review and meta-analysis of randomized controlled trials and real-world studies. *J Geriatr Cardiol.* (2018) 15:298–309. doi: 10.11909/j.issn.1671-5411.2018.04.008
16. Emani S. Remote monitoring to reduce heart failure readmissions. *Curr Heart Fail Rep.* (2017) 14:40–7. doi: 10.1007/s11897-017-0315-2
17. Conway A, Inglis SC, Clark RA. Effective technologies for noninvasive remote monitoring in heart failure. *Telemed J E Health.* (2014) 20:531–8. doi: 10.1089/tmj.2013.0267
18. Kotb A, Cameron C, Hsieh S, Wells G. Comparative effectiveness of different forms of telemedicine for individuals with heart failure (HF): a systematic review and network meta-analysis. *PLoS ONE.* (2015) 10:e0118681. doi: 10.1371/journal.pone.0118681
19. Piotrowicz E. The management of patients with chronic heart failure: the growing role of e-Health. *Expert Rev Med Devices.* (2017) 14:271–277. doi: 10.1080/17434440.2017.1314181
20. Yun JE, Park JE, Park HY, Lee HY, Park DA. Comparative effectiveness of telemonitoring versus usual care for heart failure: a systematic review and meta-analysis. *J Card Fail.* (2018) 24:19–28. doi: 10.1016/j.cardfail.2017.09.006
21. Carbo A, Gupta M, Tamariz L, Palacio A, Levis S, Nemeth Z, et al. Mobile technologies for managing heart failure: a systematic review and meta-analysis. *Telemed J E Health.* (2018). doi: 10.1089/tmj.2017.0269
22. Koehler F, Winkler S, Schieber M, Sechtem U, Stangl K, Böhm M, et al. Telemedical Interventional Monitoring in Heart Failure (TIM-HF), a randomized, controlled intervention trial investigating the impact of telemedicine on mortality in ambulatory patients with heart failure: study design. *Eur J Heart Fail.* (2010) 12:1354–62. doi: 10.1093/eurjhf/hfq199
23. Koehler F, Koehler K, Prescher S, Sechtem U, Stangl K, Böhm M, et al. Mortality and morbidity 1 year after stopping a remote patient management intervention: extended follow-up results from the telemedical interventional management in patients with heart failure II (TIM-HF2) randomised trial. *Lancet Digit Health.* (2020) 2:e16–24. doi: 10.1016/S2589-7500(19)30195-5
24. Dargie HJ. Effect of carvedilol on outcome after myocardial infarction in patients with left-ventricular dysfunction: the CAPRICORN randomised trial. *Lancet.* (2001) 357:1385–90. doi: 10.1016/S0140-6736(00)04560-8
25. Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. The Acute Infarction Ramipril Efficacy (AIRE) Study Investigators. *Lancet.* (1993) 342:821–8.
26. Eberle C, Stichling S. Clinical improvements by telemedicine interventions managing type 1 and type 2 diabetes: systematic meta-review. *J Med Internet Res.* (2021) 23:e23244. doi: 10.2196/23244
27. Eberle C, Stichling S. Effect of Telemetric interventions on glycated hemoglobin A1c and management of type 2 diabetes mellitus: systematic meta-review. *J Med Internet Res.* (2021) 23:e23252. doi: 10.2196/23252
28. Akbari M, Lankarani KB, Naghibzadeh-Tahami A, Tabrizi R, Honarvar B, Kolahdooz F, et al. The effects of mobile health interventions on lipid profiles among patients with metabolic syndrome and related disorders: a systematic review and meta-analysis of randomized controlled trials. *Diabetes Metab Syndr.* (2019) 13:1949–55. doi: 10.1016/j.dsx.2019.04.011
29. Lau D, McAlister FA. Implications of the COVID-19 pandemic for cardiovascular disease and risk-factor management. *Can J Cardiol.* (2021) 37:722–32. doi: 10.1016/j.cjca.2020.11.001
30. Parati G, Pellegrini D, Torlasco C. How digital health can be applied for preventing and managing hypertension. *Curr Hypertens Rep.* (2019) 21:40. doi: 10.1007/s11906-019-0940-0
31. Timpel P, Oswald S, Schwarz PEH, Harst L. Mapping the evidence on the effectiveness of telemedicine interventions in diabetes, dyslipidemia, and hypertension: an umbrella review of systematic reviews and meta-analyses. *J Med Internet Res.* (2020) 22:e16791. doi: 10.2196/16791
32. Huang JW, Lin YY, Wu NY. The effectiveness of telemedicine on body mass index: a systematic review and meta-analysis. *J Telemed Telecare.* (2019) 25:389–401. doi: 10.1177/1357633X18775564
33. Kronenfeld JP, Penedo FJ. Novel Coronavirus (COVID-19): telemedicine and remote care delivery in a time of medical crisis, implementation, and challenges. *Transl Behav Med.* (2021) 11:659–63. doi: 10.1093/tbm/ibaa105
34. Orlando JF, Beard M, Kumar S. Systematic review of patient and caregivers' satisfaction with telehealth videoconferencing as a mode of service delivery in managing patients' health. *PLoS ONE.* (2019) 14:e0221848. doi: 10.1371/journal.pone.0221848
35. Knox L, Rahman RJ, Beedie C. Quality of life in patients receiving telemedicine enhanced chronic heart failure disease management: a meta-analysis. *J Telemed Telecare.* (2017) 23:639–49. doi: 10.1177/1357633X16660418
36. Frederix I, Caiani EG, Dendale P, Anker S, Bax J, Böhm A, et al. ESC e-cardiology working group position paper: overcoming challenges in digital health implementation in cardiovascular medicine. *Eur J Prev Cardiol.* (2019) 26:1166–77. doi: 10.1177/2047487319832394
37. Walker RC, Tong A, Howard K, Palmer SC. Patient expectations and experiences of remote monitoring for chronic diseases: Systematic review and thematic synthesis of qualitative studies. *Int J Med Inform.* (2019) 124:78–85. doi: 10.1016/j.ijmedinf.2019.01.013
38. Esther T. Van der Werf, Martine Busch, Meik C. Jong, Hoenders HJR. Lifestyle changes during the first wave of the COVID-19 pandemic: a cross-sectional survey in the Netherlands. *BMC Public Health*. (2021) 21:1226. doi: 10.1186/s12889-021-11264-z
39. Verhoeven V, Tsakitzidis G, Philips H, Royen PV. Impact of the COVID-19 pandemic on the core functions of primary care: will the cure be worse than the disease? A qualitative interview study in Flemish GPs. *BMJ Open.* (2020) 10:e039674. doi: 10.1136/bmjopen-2020-039674

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

MINI REVIEW
published: 28 October 2021
doi: 10.3389/fcvm.2021.744482

COVID-19: The Cause of the Manifested Cardiovascular Complications During the Pandemic

Audditiya Bandopadhyay[1]*, *Alok Kumar Singh*[2] *and Gyaneshwer Chaubey*[1]*

[1] Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi, India, [2] M.D.D.M. (Cardiology), Senior Intervention Cardiologist, Lifeline Hospital, Varanasi, India

In the course of human history, we encountered several devastating waves of pandemics, affecting millions of lives globally and now the rapid and progressive spread of the novel SARS-CoV-2, causing Coronavirus disease (COVID-19) has created a worldwide wave of crisis. Profoundly straining national health care systems, it also significantly impacted the global economic stability. With the introduction of COVID-19 measures, mainly driven by immunization drives, casualties due to the virus were reported to decrease considerably. But then comes into play the post-Covid morbidities, along with their short and long-term effects on the elderly and the co-morbid population. Moreover, the pediatric population and the otherwise healthy cohort of the young athletes were also reported being largely affected by the varying amount of post-recovery virus-induced Cardiac manifestations, in the subsequent waves of the pandemic. Therefore, here we thrived to find answers to the seemingly unending series of questions that popped up with the advent of the disease, nevertheless, there still lies a blind spot in understanding the impacts of the disease on the Cardiovascular Health of an individual, even after the clinical recovery. Thus, along with the current data related to the diverse cardiovascular complications due to SARS-COV-2 infection, we suggest long-term 'Cardiac surveillance' for the COVID-19 recovered individuals.

Keywords: SARS-CoV-2, inflammation, myocardial damage, heart, CVD

OPEN ACCESS

Edited by:
Hendrik Tevaearai Stahel,
Bern University Hospital, Switzerland

Reviewed by:
Lisandra de Castro Brás,
East Carolina University, United States
Raj Sewduth,
VIB KU Leuven Center for Cancer Biology, Belgium

***Correspondence:**
Audditiya Bandopadhyay
audditiyab@gmail.com
Gyaneshwer Chaubey
gyaneshwer.chaubey@bhu.ac.in

Specialty section:
This article was submitted to Cardiovascular Genetics and Systems Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *20 July 2021*
Accepted: *06 October 2021*
Published: *28 October 2021*

Citation:
Bandopadhyay A, Singh AK and Chaubey G (2021) COVID-19: The Cause of the Manifested Cardiovascular Complications During the Pandemic. Front. Cardiovasc. Med. 8:744482. doi: 10.3389/fcvm.2021.744482

INTRODUCTION

In late 2019, a cluster of cases of "pneumonia of unknown origin," emerged, the epicenter of which was linked to the seafood wholesale market in Wuhan, China, that heralded the onset of Coronavirus disease (1). However, there are further reports suggesting that this virus was already circulating in China before the seafood market cluster event (https://www.sciencemag.org/news/2020/01/wuhan-seafood-market-may-not-be-source-novel-virus-spreading-globally). The disease spread rapidly to several countries around the globe and was already declared a pandemic by WHO. To date, a total of 187,086,096 confirmed cases of COVID−19 with a mortality of 4,042,921 have been reported (2). COVID−19 questioned the existence of mankind in the twenty-first century not just by crippling the global healthcare system but also contributing to the psychological and socio-economic burden on the entire humanity.

The family of seven known human Coronaviruses has long been associated with emerging respiratory distress syndromes and flu-like outbreaks. This is the reason behind the high occurrence of cases of pneumonia and bronchitis in patients with a severe COVID-19 infection. In the past

two decades, two recorded epidemics were caused by the same family of the virus—Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), in 2002–2003, and more recently, the Middle East Respiratory Coronavirus (MERS-CoV) in 2012, has widely been mentioned. Previously known human coronavirus variants, which were associated with the common cold—HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1, have not yet been found to be associated with heart abnormalities. But there are few reports of the patients suffering from the Middle East respiratory syndrome (MERS; caused by MERS-CoV) with myocarditis and a few cases of cardiac disease in the patients who suffered from SARS (caused by SARS-CoV) (3, 4). However, recent literature reported serious cardiovascular complications occurring in about 10–20% of hospitalized patients, apart from the respiratory effects of COVID-19; and the patients who suffered from pre-existing heart ailments may suffer either a heart attack or congestive heart failure (5). This deciphers distinct characteristics of SARS-CoV-2 in its comprehensive cardiac involvement, which could also be a consequence of the exposure of the virus to millions due to the pandemic. Reports also stated that COVID-19 triggered inflammation of the heart muscle—Myocarditis (6). The most recent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), displayed tropism for the heart and can lead to myocarditis (inflammation of the heart), necrosis of its cells, mimicking heart attacks, arrhythmias, and acute or protracted heart failure (muscle dysfunction) (3). These complications, which at times are the sole features of COVID-19 clinical presentation, have occurred even in the cases with milder symptoms and in people who did not experience any symptoms. Unsuspected cardiac involvement including sudden cardiac death, in such healthy and young athlete groups, has further elevated the concerns regarding our current knowledge about the impact of the disease on heart health.

STRUCTURAL ASPECTS OF COVID-19 VIRUS

The difference between SARS-CoV-2 and SARS is apparently a furin polybasic site that alters their structure, and when cleaved, broadens the types of cells (tropism) that the virus can infect (7). It is a large family of single positive-stranded, enveloped RNA virus that finds its host in several animals, and by methods not yet explained, they can pass from one species to another. The virus targets the angiotensin-converting enzyme 2 (ACE2) receptor throughout the body, which facilitates the entry of viral genetic material by the means of its spike protein, along with the assistance of the cellular serine protease transmembrane protease serine 2 (TMPRSS2), heparan sulfate, and other proteases, which cleaves the viral spikes protein and make the entry pathway for the viral genetic contents (8). So, the higher the ACE2 receptor's number of receptors in any cell, the higher the susceptibility for the viral entry and greater viral load possibility. The involvement of ACE2 in the regulation of blood volume, systemic vascular resistance, and thus cardiovascular homeostasis is monumental (**Figure 1**). Previous studies have shown its association with hypertension, stroke, dyslipidemia, and cardiovascular diseases, and kidney diseases (9–12). The heart also has a high level of ACE2 expression which makes it more susceptible to the SARS-COV-2 infection. The affinity of SARS-CoV-2to ACE2 is significantly higher than that of SARS (13), and thus it may perturb the angiotensin-renin pathway severely.

COVID-19 AND ITS SYSTEMIC IMPLICATIONS

The tropism to other organs beyond the lungs has been quoted in some studies from the autopsy specimens. It was found that the SARS-CoV-2 genomic RNA was the highest in the lungs. However, in the heart, kidney, and liver, considerable amounts of viral load were detected in 16 out of 22 deceased patients (14). In a report from (15), out of a series of an autopsy of 39 deceased COVID-19 patients, only 31% had a high viral load, i.e., above 1,000 copies, in the heart while ∼38% of the deceased was not found to possess a detectable viral load in the myocardium. Accordingly, SARS-CoV-2 infection can damage the heart in both direct and indirect ways. *In-vitro* studies have shown the ability of SARS-CoV-2 to infect the induced pluripotent stem cells (iPSCs) derived cardiomyocytes, causing the distinctive pattern of cell fragmentation along with the complete dissolution of the contractile machinery (16). In another iPSC study, SARS-CoV-2 infection leads to apoptosis, and ultimately the heartbeat ceases within 72 h of the viral exposure (17). Besides the direct involvement of the viral infection in the heart muscles, its entry into the endothelial lining of the blood vessels of the heart and multiple vesicular beds has also been reported. Another potential threat is the effects of secondary immune response in the infected heart and endothelial cells (endothelitis) which may include the dysregulation of the renin-angiotensin-aldosterone system modulating blood pressure; activation of pro-inflammatory responses including platelets, neutrophils, macrophages, and lymphocytes, the cytokine storm and a prothrombotic state (**Figure 1**).

There is a varying level of cardiovascular manifestations, oscillating from limited necrosis of cardiac cells leading to myocarditis to an often-fatal failure of the heart to pump sufficient blood leading to cardiogenic shock (18). One out of every five hospitalized COVID-19 patients suffering from cardiac injury reflects an accumulation of troponin (a cardiac muscle-specific marker) in blood and the same happens with those having pre-existing heart ailments. Also, for this kind of myocardial injury in-hospital mortality, troponin accumulation is an indicator of morbidity risk (19). Moreover, it has been observed that patients with higher troponin amounts also have increased levels of many inflammatory markers [including interleukin-6(IL-6), C- reactive protein, ferritin, lactate dehydrogenase (LDH), and an increased neutrophil count] and heart dysfunction (amino-terminal pro-B–type natriuretic peptide) (20). Conversely, an immunologic basis is likely as there is a possibility of myocarditis results from the hyperimmune response in order to tackle coronavirus by releasing excess cytokines. Cytokines could result in inflammation that damages the lungs and the heart alike. This

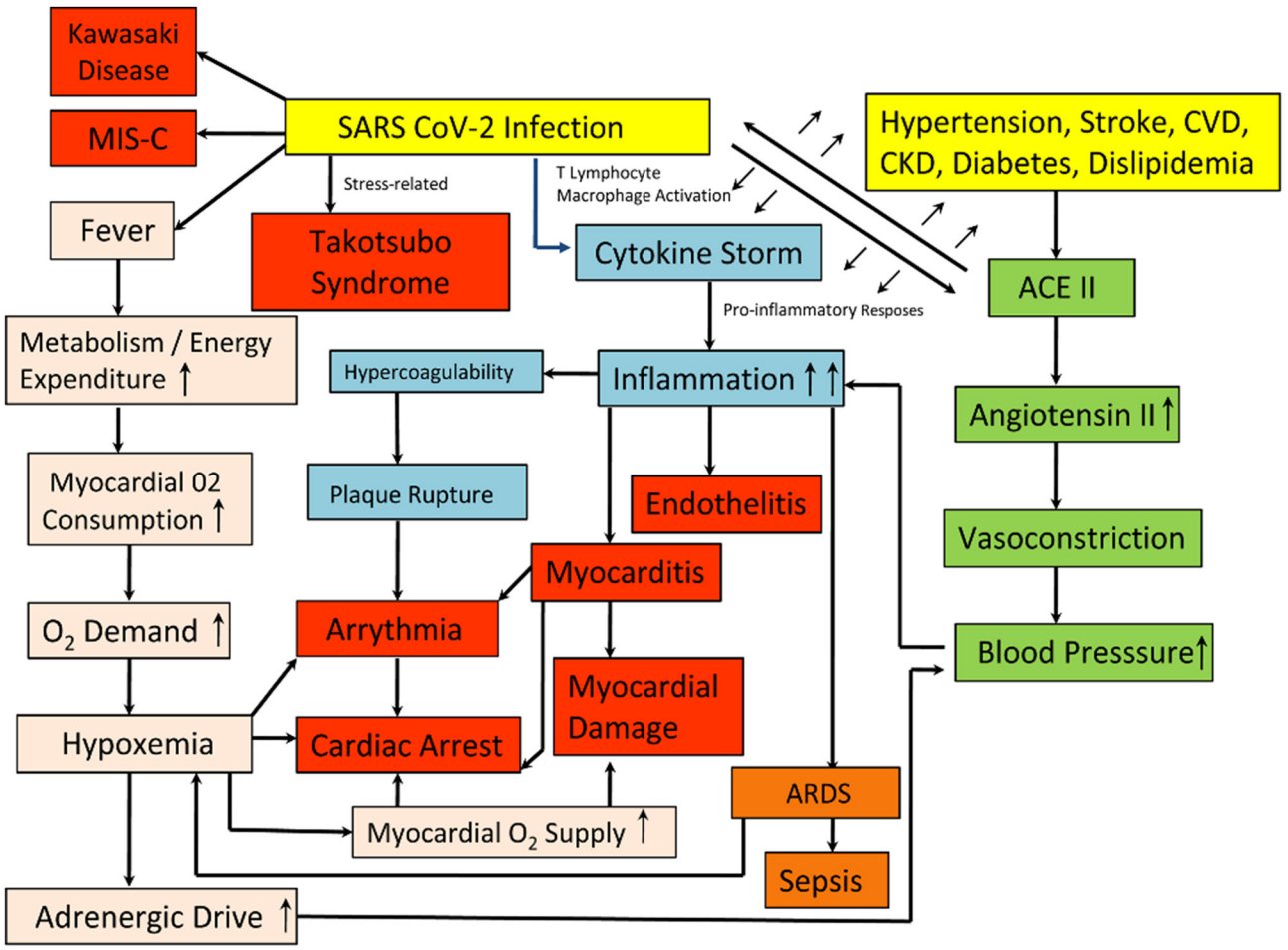


FIGURE 1 | Schematic representation of the pathways leading to post-COVID Heart conditions. The Yellow boxes represent the causes, whereas the Red and the Orange boxes depict the consequences of the infection on the heart and the lungs. The Pink, Blue, and the Green boxes depict the various systemic pathways involved, leading to the development of the conditions.

condition, known as a cytokine storm, is more serious in the elderly and the co-morbid population. However, it was also seen to affect the middle-aged population largely during the subsequent waves of the pandemic in India. It is the primary reason for the severe respiratory complications which lead to death in patients suffering from coronavirus (21). Cytokines promote blood coagulation and thus, interfere with the body's clot-busting system. Blood clots in coronary arteries in turn can block blood flow and cause heart attacks. A tendency for clotting, both in the microvasculature and large vessels, has been reported in multiple autopsy reports and in young COVID-19 patients with a history of stroke. Another relevant possibility could be the development of cardiac complications in some coronavirus patients, as a consequence of infections in their lungs. Insufficient oxygen increases the risk of arrhythmias. At the same time, fever caused by the virus increases the body's metabolism, thus the cardiac output. As a result, the patient's heart struggles with an elevated oxygen demand along with a reduced supply, causing an imbalance that leads to a myocardial injury. The causes of death might involve multiple organ dysfunctions in most cases, and therefore it is difficult to differentiate the myocardial injury as the sole reason for such cases. Schematic representation of the different pathways leading to the post-COVID conditions has been depicted in **Figure 1**.

CARDIOVASCULAR HEALTH WITH THE RISE OF COVID-19

With an ascent in the number of COVID-19 confirmed cases and the accumulating clinical data, in addition to the common presentation of respiratory failure, the cardiovascular manifestations induced by this viral infection have generated considerable concern (22). Huang et al. (23) reported that 12% of the patients with COVID-19 were diagnosed to have an acute myocardial injury, manifested due to the elevated levels of high-sensitive troponin I. This was further supported by reports stating 16.7% out of 138 hospitalized patients with COVID-19 had suffered from arrhythmias and 7.2% had an acute myocardial

injury (24). However, the plausible cause of the COVID-19 infection in the development of myocardial injury in the hospitalized patients suffering from underlying cardiovascular disease (CVD) is still unknown and it requires extensive study. Although still unclear, whether it is an after-effect of a hyperactive immune response against the virus or the virus itself leading to myocardial inflammation which is associated with cardiac function impairment and ventricular tachyarrhythmias.

Myocarditis is a diffuse pattern of inflammation of the heart, typically representing a variable admixture of injury and an inflammatory response to the injury, and may extend through all the three layers of the human heart to the pericardium, encompassing the heart. This is even more worrisome than the restricted pattern injury. The immunological and the inflammatory response is one of the most common observations at the autopsy studies after SARS-CoV-2 infections, unlike the SARS-associated myocarditis, which didn't show any lymphocyte infiltration. Conduction block and malignant ventricular arrhythmias, both of which can lead to cardiac arrest, can occur when myocytes, which synchronize electrical conduction, are involved. Besides in-hospital arrhythmias, numerous geographic regions with high COVID-19 dissemination have been reported to observe a steep increase in out-of-hospital cardiac arrest and sudden death. There has been a rise of 77% in the cases in Lombardy, Italy, as compared to the previous year (25). Due to a cluster of chest pain-like sensations, an irregular EKG, and high levels of cardiac-specific enzymes in the blood, myocarditis imitating a heart attack has been reported in individuals as young as 16 years old (3). Heart failure, acute cor pulmonale (right heart failure with potential pulmonary emboli), and cardiogenic shock can occur when there is significant and diffuse heart muscle injury. Other pathways that could also be responsible for COVID-19-related heart dysfunction, such as Takotsubo syndrome or the Broken heart syndrome (a transient stress-related illness that causes apical ballooning), ischemia caused by endocarditis, and related atherosclerotic plaque rupture with thrombosis were reported (3). Other causes included the multisystem inflammatory syndrome of children (MIS-C), although MIS-C reported here, was not only exclusive to children but also the same clinical features have been the subject of case reports in adults, such as in a 45-year-old (3).

Although the children were thought to be less susceptible to COVID-19, as compared to the adults, and while the majority of them with COVID-19 were asymptomatic or presented with only milder forms of the symptoms, the reports of COVID-19 associated severe inflammatory symptoms among the pediatric patients were not null (26–28). An unexpected cluster of eight children (aged 4–14 years) presenting with a hyperinflammatory syndrome with symptoms of Kawasaki Disease was reported in a case series from the United Kingdom (26). COVID-19 patients who underwent magnetic resonance imaging (MRI) or echocardiogram of the heart have recently revealed some fresh information concerning some cardiac involvement (29–31). In one such study, the left and the right ventricular abnormalities were reported in 479 out of 1,216 patients, and 397 patients, respectively, with evidence of new myocardial infarction in 36 of them. Myocarditis was reported in 35 and Takotsubo Cardiomyopathy in 19 patients in the same study. Severe cardiac disease (severe ventricular dysfunction or tamponade) was also observed in 15% of the patients. And in those without any pre-existing cardiac disease, the echocardiogram was abnormal in 46%, and 13% of the cases had severe disease. Patients were between 52 and 78 years old (30). In another study, 15 patients had abnormal CMR findings on conventional CMR sequences: myocardial edema was found in 54% of patients, and LGE was found in 31% of the patients reduced right ventricle performance which includes ejection fraction, cardiac index, and stroke volume per body surface area were found in patients with positive conventional CMR findings (31). A group of 100 individuals recovered from the illness, but 78 had cardiac abnormalities, including 12 of 18 patients who had no symptoms, and 60 showed continuing myocardial inflammation, which is consistent with myocarditis (29). These findings point to the necessity for more research on covid-19's long-term cardiovascular effects. The majority of over 1,200 individuals with COVID-19 in a large prospective cohort had echocardiographic abnormalities (30). This raises questions about whether heart involvement is considerably more common than previously thought, especially because at least 30–40% of SARS-CoV-2 infections are asymptomatic. Because all of these patients did not have a systematic cardiovascular assessment for any probable myocarditis or other heart abnormalities, which could explain some of the lingering symptoms, they may have hidden underlying cardiac pathology.

ROLE OF ANGIOTENSIN-CONVERTING ENZYME AND IT'S INHIBITORS IN COVID-19

Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), both of which are known to block the renin-angiotensin system (RAS), also might affect an individual's susceptibility to COVID-19 and further worsen its severity (32–35). Angiotensin II, the main effector molecule in the renin-angiotensin-aldosterone system (RAAS), is upregulated in many clinical conditions, for which inhibition of angiotensin II by RAAS inhibitors is a common therapeutic strategy. Angiotensin-converting enzyme (ACE) produces angiotensin II from angiotensin I, whereas ACE2 inactivates angiotensin II by converting it to angiotensin (1–7) (34). Therefore, ACE2 has been assumed to have a protective effect against cardiovascular disease and lung injury. It has been shown that the RAAS inhibitors may increase the ACE2 expression, thus raise concern among COVID-19 positive patients (33).

On the other hand, a study reported significant interactions between ethnicity and ACE inhibitors and ARBs for COVID-19 disease. The risk of COVID-19 disease associated with ACE inhibitors was shown to be higher in the Caribbean and Black African groups than the white group. Variations among the ethnic groups raise the possibility of ethnic-specific effects of ACE inhibitors/ARBs on COVID-19 disease susceptibility and severity (36). Another study found that the administration of ACEI/ARB drugs had a positive effect on reducing D-dimer and the number of people with fever (37). As a result of such

paradoxical issues of using ACEIs/ARBs during COVID-19, it is still an area requiring extended investigation to prove. However, in the setting of coronavirus disease, downregulation of ACE2 by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection might be involved in mediating cardiovascular damage, besides, the medications that have been proposed as treatments for COVID-19 such as hydroxychloroquine and azithromycin have pro-arrhythmic effects, AF, atrial fibrillation; VF, ventricular fibrillation; VT, ventricular tachycardia (38).

PRESENT SCENARIO

Previous studies have depicted the overall clinical attributes and epidemiological findings of patients with COVID-19, and a portion of it has shown that the condition of some patients with COVID-19 deteriorates rapidly. In contrast to the asymptomatic, a substantial proportion of people suffer a long-standing, often incapacitating illness, called long-COVID. Typical symptoms of this include fatigue, difficulty in breathing, chest pain, and abnormal heart rhythm (39, 40). While the patients with underlying Cardiovascular pathology, but without myocardial injury put up with a relatively favorable prognosis, myocardial injury is much more common in patients with COVID-19 and has been found to have a significant association with the fatalities due to COVID-19.

The most intriguing question that stirred up in this while is that why do certain individuals have a propensity for heart involvement after the SARS-CoV-2 infection? Studies deciphered that the infected patients who get myocarditis do not necessarily have any more virus in their bodies than those who do not foster the condition. The prediction once recognized a few months into the pandemic, was that the cardiac involvement would chiefly occur in patients with severe COVID-19. Clearly, it is found to be much more common than anticipated. However, the true incidence is unknown. Primarily, it is vital to determine any cause that drives the pathogenesis. Whether it represents an individual's inflammatory response, an autoimmune phenomenon or some other explanations are yet to be clarified.

Beyond the prevention of COVID-19 infections, the goal of averting cardiovascular involvement is paramount. The marked heterogeneity of the disease, ranging from lack of symptoms to fatality, is poorly understood. A newly emerged virus, widely circulating throughout the human population, with a panoply of manifestations, has made this especially daunting to untangle. It wouldn't surprise much in the future if the patients present with cardiomyopathy of unknown etiology and test positive for SARS-CoV-2 antibodies. However, attributing all such cardiomyopathy solely to the virus may be difficult, given the high prevalence of infections. A biopsy might be a necessity to identify any virus particles to support any causality.

These sudden after-effects could be attributed and studied to be validated at two different levels. First is the entry point for the virus, that is the ACE2 receptors and their variations among the individuals of certain ethnicities, which makes them more susceptible or resistant toward the virus. There have been studies concluding the polymorphism within the ACE2 gene within the populations that explains the outcome, on comparing the Western and the Indian populations, and their affinity with the East Asians (41).

The other points to be considered include the immunity of the individual and its effects after the entry of the virus. The reaction of the immunity toward the non-self determines its activity, and thus results in a hyperactive state of immune responses, that leads to systemic inflammation, which prevails for a much longer time, as compared to the symptoms themselves. The classification of asymptomatic people for COVID-19 is vague. And in many cases, the asymptomatic individuals are sometimes just the result of the symptoms getting masked due to ignorance or the socio-economic background of those individuals. They may have underlying inflammation-related pneumonia due to the disease and still not experience any level of hypoxia and thus, be considered asymptomatic. On the other hand, the body of the athletes, in practice, may demand more oxygen and experience the symptoms of hypoxia and thus, can lead to cardiac arrest due to pulmonary thromboembolism, as a consequence of dilated arteries due to the disease-related inflammation (42). The same demographic group of young and healthy, that is most common to lack the symptoms after SARS-CoV-2 infections, raises the question of how many athletes have an occult cardiac disease. Systematic assessment through some form of cardiac imaging and arrhythmia screening of athletes, who test positive for SARS-CoV-2, irrespective of symptoms, seems prudent until more is perceived. The authors in a study reported on a cohort, consisting of a large sample size of 2,461 athletes, of whom 1,597 (64.9%) had the complete comprehensive screening testing, including CMR imaging without prior selection, where they found that 37 (2.3%) of these athletes demonstrated diagnostic criteria for myocarditis by CMR imaging, including 20 without cardiovascular symptoms and with normal ECG, echocardiography, and troponin test results, who would not have been identified without CMR imaging (43). However, another subsequent study was published, where a cohort of 145 competitive athletes, who had tested positive for COVID-19 with either mild to moderate or, no symptoms, were evaluated approximately 15 days post-positive test result, using cardiac MRI, EEG, and serum markers of cardiac pathology, and only two were found to have MRI findings consistent with myocarditis. This led to conclude that its incidence following COVID-19 was much less prevalent than previously thought (44). Controversies remain until the results are validated further, on larger cohorts, considering ethnicity (ancestry) as a parameter, as that could play a vital role in the risk prediction of an individual.

FUTURE DIRECTION AND CONCLUSION

Long-term observation and prospective study design (Cardiac Surveillance) on the viability of treatments, explicit for myocardial injury are of utmost significance. Further, aggressive treatment may be considered for patients with myocardial injury. Therefore, monitoring of myocardial injury markers and cardiac function is of extreme importance, and attention should be paid to the early identification and comprehensive management of myocardial injury in such patients. But what has

so far driven populations to be more vulnerable to post-COVID morbidities?

We hypothesize it as the genetic variability among the individuals at these two tiers, making them more or less susceptible toward the mentioned long-standing ailments, which are probably more severe than the disease itself. There comes into play the role of genetic mapping. Genome-wide analysis (GWAS) and Whole-genome analysis (WGA) study designs would reveal and map a particular population at risk, would categorize the vulnerable groups to prioritize them at first, and thus manage the casualties due to the disease burden.

AUTHOR CONTRIBUTIONS

GC and AB conceived and designed the study. AB, GC, and AS constituted the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

GC was supported by Faculty IOE Grant BHU (6031). AB was supported by UGC-RET and CAS fellowship. No funders had a role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

REFERENCES

1. Pradhan RR, Yadav AK, Mandal S. Cardiovascular implications of coronavirus disease 2019 (COVID-19): a systematic review. (2020). doi: 10.21203/rs.3.rs-39929/v1
2. Cascella M, Rajnik M, Aleem A, Dulebohn S, Di Napoli R. Features, evaluation, and treatment of coronavirus (COVID-19). In: *StatPearls*. Treasure Island, FL: StatPearls Publishing (2021). Available online at: https://www.ncbi.nlm.nih.gov/books/NBK554776/
3. Topol EJ. COVID-19 can affect the heart. *Science*. (2020) 370:408–9. doi: 10.1126/science.abe2813
4. Xiong TY, Redwood S, Prendergast B, Chen M. Coronaviruses and the cardiovascular system: acute and long-term implications. *Eur Heart J*. (2020) 41:1798–800. doi: 10.1093/eurheartj/ehaa231
5. Lewis DKL. *How Does Cardiovascular Disease Increase the Risk of Severe Illness and Death From COVID-19*. Harvard Health Blog (2020).
6. Basu-Ray I, Adeboye A, Soos MP. Cardiac manifestations of coronavirus (COVID-19). In: *StatPearls*. Treasure Island, FL: StatPearls Publishing (2021). Available online at: https://www.ncbi.nlm.nih.gov/books/NBK556152/
7. Matheson NJ, Lehner PJ. How does SARS-CoV-2 cause COVID-19? *Science*. (2020) 369:510–1. doi: 10.1126/science.abc6156
8. Hikmet F, Méar L, Edvinsson Å, Micke P, Uhlén M, Lindskog C. The protein expression profile of ACE2 in human tissues. *Mol Syst Biol*. (2020) 16:e9610. doi: 10.15252/msb.20209610
9. Wu X, Zhu B, Zou S, Shi J. The association between ACE2 gene polymorphism and the stroke recurrence in Chinese population. *J Stroke Cerebrovasc Dis*. (2018) 27:2770–80. doi: 10.1016/j.jstrokecerebrovasdis.2018.06.001
10. Zhang Q, Cong M, Wang N, Li X, Zhang H, Zhang K, et al. Association of angiotensin-converting enzyme 2 gene polymorphism and enzymatic activity with essential hypertension in different gender: a case-control study. *Medicine*. (2018) 97:e12917. doi: 10.1097/MD.0000000000012917
11. Pan Y, Wang T, Li Y, Guan T, Lai Y, Shen Y, et al. Association of ACE2 polymorphisms with susceptibility to essential hypertension and dyslipidemia in Xinjiang, China. *Lipids Health Dis*. (2018) 17:1–9. doi: 10.1186/s12944-018-0890-6
12. Wang M, Zhang W, Zhou Y, Zhou X. Association between serum angiotensin-converting enzyme 2 levels and postoperative myocardial infarction following coronary artery bypass grafting. *Exp Ther Med*. (2014) 7:1721–7. doi: 10.3892/etm.2014.1640
13. Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, et al. Extrapulmonary manifestations of COVID-19. *Nat Med*. (2020) 26:1017–32. doi: 10.1038/s,41591-020-0968-3
14. Puelles VG, Lütgehetmann M, Lindenmeyer MT, Sperhake JP, Wong MN, Allweiss L, et al. Multiorgan and renal tropism of SARS-CoV-2. *N Engl J Med*. (2020) 383:590–2. doi: 10.1056/NEJMc2011400
15. Lindner D, Fitzek A, Bräuninger H, Aleshcheva G, Edler C, Meissner K, et al. Association of cardiac infection with SARS-CoV-2 in confirmed COVID-19 autopsy cases. *JAMA Cardiol*. (2020) 5:1281–5. doi: 10.1001/jamacardio.2020.3551
16. Perez-Bermejo JA, Kang S, Rockwood SJ, Simoneau CR, Joy DA, Silva AC, et al. SARS-CoV-2 infection of human iPSC-derived cardiac cells reflects cytopathic features in hearts of patients with COVID-19. *Sci Transl Med*. (2021) 13:eabf7872. doi: 10.1126/scitranslmed.abf7872
17. Sharma A, Garcia G Jr, Wang Y, Plummer JT, Morizono K, Arumugaswami V, et al. Human iPSC-derived cardiomyocytes are susceptible to SARS-CoV-2 infection. *Cell Rep Med*. (2020) 1:100052. doi: 10.1016/j.xcrm.2020.100052
18. Shafi AM, Shaikh SA, Shirke MM, Iddawela S, Harky A. Cardiac manifestations in COVID-19 patients-A systematic review. *J Card Surg*. (2020) 35:1988–2008. doi: 10.1111/jocs.14808
19. Gomila-Grange A, Espasa M, Moglia E. Cardiogenic shock caused by SARS-CoV-2 in a patient with serial negative nucleic acid amplification tests. *Case Report SN Comprehens Clin Med*. (2020) 2:1903–5. doi: 10.1007/s42399-020-00496-6
20. Bonow RO, Fonarow GC, O'Gara PT, Yancy CW. Association of coronavirus disease 2019 (COVID-19) with myocardial injury and mortality. *JAMA Cardiol*. (2020) 5:751–3. doi: 10.1001/jamacardio.2020.1105
21. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LF. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol*. (2020) 20:363–74. doi: 10.1038/s41577-020-0311-8
22. Kwenandar F, Japar KV, Damay V, Hariyanto TI, Tanaka M, Lugito NPH, et al. Coronavirus disease 2019 and cardiovascular system: a narrative review. *IJC Heart Vascul*. (2020) 2020:100557. doi: 10.1016/j.ijcha.2020.100557
23. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
24. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol*. (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017
25. Baldi E, Sechi GM, Mare C, Canevari F, Brancaglione A, Primi R, et al. Out-of-hospital cardiac arrest during the Covid-19 outbreak in Italy. *N Engl J Med*. (2020) 383:496–8. doi: 10.1056/NEJMc2010418
26. Qiu H, Wu J, Hong L, Luo Y, Song Q, Chen D. Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: an observational cohort study. *Lancet Infect Dis*. (2020) 20:689–96. doi: 10.1016/S1473-3099(20)30198-5
27. Riphagen S, Gomez X, Gonzalez-Martinez C, Wilkinson N, Theocharis P. Hyperinflammatory shock in children during COVID-19 pandemic. *Lancet*. (2020) 395:1607–8. doi: 10.1016/S0140-6736(20)31094-1
28. Verdoni L, Mazza A, Gervasoni A, Martelli L, Ruggeri M, Ciuffreda M, et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet*. (2020) 395:1771–8. doi: 10.1016/S0140-6736(20)31103-X
29. Puntmann VO, Carerj ML, Wieters I. Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19). *JAMA Cardiol*. (2020) 5:1265–73. doi: 10.1001/jamacardio.2020.3557
30. Dweck MR, Bularga A, Hahn RT, Bing R, Lee KK, Chapman AR, et al. Global evaluation of echocardiography in patients with COVID-19. *Eur Heart J Cardiovasc Imaging*. (2020) 21:949–58. doi: 10.1093/ehjci/jeaa178
31. Huang L, Zhao P, Tang D, Zhu T, Han R, Zhan C, et al. Cardiac involvement in patients recovered from COVID-2019 identified using

magnetic resonance imaging. *JACC Cardiovasc Imaging*. (2020) 13:2330–9. doi: 10.1016/j.jcmg.2020.05.004
32. Morales DR, Conover MM, You SC, Pratt N, Kostka K, Duarte-Salles T, et al. Renin-angiotensin system blockers and susceptibility to COVID-19: an international, open science, cohort analysis. *Lancet Digit Health*. (2021) 3:e98–114. doi: 10.1016/S2589-7500(20)30289-2
33. Vaduganathan M, Vardeny O, Michel T, McMurray JJ, Pfeffer MA, Solomon SD. Renin-angiotensin-aldosterone system inhibitors in patients with Covid-19. *New England Journal of Medicine*. (2020) 382:1653–9. doi: 10.1056/NEJMsr2005760
34. Nishiga M, Wang DW, Han Y, Lewis DB, Wu JC. COVID-19 and cardiovascular disease: from basic mechanisms to clinical perspectives. *Nat Rev Cardiol*. (2020) 17:543–58. doi: 10.1038/s41569-020-0413-9
35. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*. (2003) 426:450–4. doi: 10.1038/nature02145
36. Hippisley-Cox J, Young D, Coupland C, Channon KM, San Tan P, Harrison DA, et al. Risk of severe COVID-19 disease with ACE inhibitors and angiotensin receptor blockers: cohort study including 8.3 million people. *Heart*. (2020) 106:1503–11. doi: 10.1136/heartjnl-2020-317393
37. Xue Y, Sun S, Cai J, Zeng L, Wang S, Wang S, et al. Effects of ACEI and ARB on COVID-19 patients: a meta-analysis. *J Renin-Angioten Aldosterone Syst*. (2020) 21:1470320320981321. doi: 10.1177/1470320320981321
38. Sanders JM, Monogue ML, Jodlowski TZ, Cutrell JB. Pharmacologic treatments for coronavirus disease 2019 (COVID-19): a review. *JAMA*. (2020) 323:1824–36. doi: 10.1001/jama.2020.6019
39. Del Rio C, Collins LF, Malani P. Long-term health consequences of COVID-19. *JAMA*. (2020) 324:1723–4. doi: 10.1001/jama.2020.19719
40. Nalbandian A, Sehgal K, Gupta A, Madhavan MV, McGroder C, Stevens JS, et al. Post-acute COVID-19 syndrome. *Nat Med*. (2021) 27:601–15. doi: 10.1038/s41591-021-01283-z
41. Srivastava A, Pandey RK, Singh PP, Kumar P, Rasalkar AA, Tamang R, et al. Most frequent South Asian haplotypes of ACE2 share identity by descent with East Eurasian populations. *PLoS ONE*. (2020) 15:e0238255. doi: 10.1371/journal.pone.0238255
42. Davey MS, Davey MG, Hurley R, Hurley ET, Pauzenberger L. Return to play following COVID-19 infection-A systematic review of current evidence. *J Sport Rehabil*. (2021) 25:1–6. doi: 10.1123/jsr.2021-0028
43. Udelson JE, Rowin EJ, Maron BJ. Return to play for athletes after COVID-19 infection: the fog begins to clear. *JAMA Cardiol*. (2021) 6:997–9. doi: 10.1001/jamacardio.2021.2079
44. Starekova J, Bluemke DA, Bradham WS, Eckhardt LL, Grist TM, Kusmirek JE, et al. Evaluation for myocarditis in competitive student athletes recovering from coronavirus disease 2019 with cardiac magnetic resonance imaging. *JAMA Cardiol*. (2021) 6:945–50. doi: 10.1001/jamacardio.2020.7444

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 05 November 2021
doi: 10.3389/fcvm.2021.752237

The Prognostic Value of Cardiac Biomarkers and Echocardiography in Critical COVID-19

Bert Zwaenepoel[1†], Sebastiaan Dhont[1†], Eric Hoste[2,3], Sofie Gevaert[1] and Hannah Schaubroeck[2]*

[1] Department of Cardiology, Ghent University Hospital, Ghent University, Ghent, Belgium, [2] Department of Intensive Care Medicine, Ghent University Hospital, Ghent University, Ghent, Belgium, [3] Research Foundation – Flanders (FWO), Brussels, Belgium

Background: Early risk stratification is crucial in critically ill COVID-19 patients. Myocardial injury is associated with worse outcome. This study aimed to evaluate cardiac biomarkers and echocardiographic findings in critically ill COVID-19 patients and to assess their association with 30-day mortality in comparison to other biomarkers, risk factors and clinical severity scores.

Methods: Prospective, single-center, cohort study in patients with PCR-confirmed, critical COVID-19. Laboratory assessment included high sensitive troponin T (hs-cTnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP) on admission to ICU: a hs-cTnT $\geq$ 14 pg/mL and a NT-proBNP $\geq$ 450 pg/mL were considered as elevated. Transthoracic echocardiographic evaluation was performed within the first 48 h of ICU admission. The primary outcome was 30-day all-cause mortality. Predictive markers for mortality were assessed by ROC analysis and cut-off values by the Youden Index.

Results: A total of 100 patients were included. The median age was 63.5 years, the population was predominantly male (66%). At the time of ICU admission, 47% of patients had elevated hs-cTnT and 39% had elevated NT-proBNP. Left ventricular ejection fraction was below 50% in 19.1%. Elevated cardiac biomarkers (hs-cTnT P-value < 0.001, NT-proBNP P-value $= 0.001$) and impaired left ventricular function (P-value $= 0.011$) were significantly associated with mortality, while other biomarkers (D-dimer, ferritin, C-reactive protein) and clinical scores (SOFA) did not differ significantly between survivors and non-survivors. An optimal cut-off value to predict increased risk for 30-day all-cause mortality was 16.5 pg/mL for hs-cTnT (OR 8.5, 95% CI: 2.9, 25.0) and 415.5 pg/ml for NT-proBNP (OR 5.1, 95% CI: 1.8, 14.7).

Conclusion: Myocardial injury in COVID-19 is common. Early detection of elevated hs-cTnT and NT-proBNP are predictive for 30-day mortality in patients with critical COVID-19. These markers outperform other routinely used biomarkers, as well as clinical indices of disease severity in ICU. The additive value of routine transthoracic echocardiography is disputable and should only be considered if it is likely to impact therapeutic management.

Keywords: COVID-19, hs-cTnT, NT-proBNP, ICU, myocardial injury, myocardial biomarker

OPEN ACCESS

Edited by:
Michiaki Nagai, Hiroshima City Asa Hospital, Japan

Reviewed by:
Jeroen Peter Kooman, Maastricht University Medical Centre, Netherlands
Ayfer Colak, Izmir Tepecik Training and Research Hospital, Turkey

***Correspondence:**
Bert Zwaenepoel
bert.zwaenepoel@uzgent.be

†These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *02 August 2021*
Accepted: *18 October 2021*
Published: *05 November 2021*

Citation:
Zwaenepoel B, Dhont S, Hoste E, Gevaert S and Schaubroeck H (2021) The Prognostic Value of Cardiac Biomarkers and Echocardiography in Critical COVID-19. Front. Cardiovasc. Med. 8:752237. doi: 10.3389/fcvm.2021.752237

INTRODUCTION

Currently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected almost 230 million people, resulting in more than 4.6 million registered deaths (1). Based upon the severity of illness, the National Institutes of Health (NIH) proposes five categories of Coronavirus Disease 2019 (COVID-19): asymptomatic, mild, moderate, severe and critical. The latter contains individuals with respiratory failure, septic shock, and/or multiple organ dysfunction requiring intensive care (2).

Myocardial injury is defined by an elevation of cardiac troponin. In case of myocardial infarction, this elevation occurs in combination with clinical features of ischemia, e.g., electrocardiographic changes, ischemic symptoms or imaging of new loss of viable myocardium or new regional wall motion abnormalities (3). Myocardial injury is common in COVID-19. The presence of values above the upper reference limit (URL) for high sensitive troponin (hs-cTnT) in COVID-19 patients varies widely, ranging from 20% in cohorts of hospitalized patients to more than 50% in critically ill patients (4–7). Ischemic or non-ischemic causes can mediate myocardial injury. Ischemic cardiac damage can be subdivided in type 1 and type 2 ischemia. The underlying pathophysiology of type 1 ischemia in COVID-19 is not fully understood. On one hand, the inflammatory response due to a COVID-19 infection may lead to plaque instability by activating inflammatory cells and release of inflammatory mediators, causing oxidative stress. On the other hand, COVID-19 infection is associated with endothelialitis and a prothrombotic state (8–11). Type 2 ischemia can be attributed to several factors such as hypoxemia, vasopressor use and suboptimal fluid balance, leading to a demand-supply inequity of oxygen (10). Non-ischemic injury may find its origin in various mechanisms such as myocarditis, Takotsubo syndrome, arrythmias, pulmonary embolism, and septic shock (12–16).

Data about natriuretic peptides are more scarce, though up to 48% of critical COVID-19 patients present with elevated levels of N-terminal pro-brain natriuretic peptide (NT-proBNP), reflecting hemodynamic stress (17). The presence of circulating NT-pro-BNP in patients with critical COVID-19 can be attributed to several factors. Myocardial injury may lead to cardiac dysfunction and increased ventricular wall stress, which can be increased further by the use of mechanical ventilation and vasopressor agents (18). Hypoxia-induced pulmonary hypertension may further aggravate myocardial wall stress by increasing right ventricular afterload (19, 20).

Echocardiographic abnormalities, such as left ventricular systolic and diastolic dysfunction as well as right ventricular impairment, are observed in up to half of all COVID-19 patients undergoing echocardiography (21–24).

Elevated cardiac biomarkers and echocardiographic abnormalities, especially reduced ventricular contractility, are associated with worse clinical outcome including mortality in COVID-19 patients (10, 17, 18, 23, 25–27). As most of the published reports are retrospective studies, the current role of cardiac biomarkers and/or echocardiography in the prognostication of COVID-19 patients is still unclear. Different cardiac societies therefore have recommended against the routine use of these parameters for prognostic purposes (16, 28).

The purpose of this study was to prospectively evaluate the presence of elevated cardiac biomarkers and echocardiographic abnormalities in critical COVID-19 patients at the time of admission to the intensive care unit (ICU), to assess their association with 30-day all-cause mortality and to compare their prognostic performance to that of other biomarkers, risk scores and risk factors.

METHODS

Study Design, Data Collection, and Study Outcome

This prospective, single-center, cohort study was carried out at the ICU of the Ghent University Hospital in Belgium, a 1.061-beds tertiary care center, between April 2020 and April 2021. The study was approved by the local ethical committee (BC-07568, April 1st, 2020). Inclusion criteria were: age 18 years or older, inclusion within 48 h of ICU admission, severe COVID-19 as diagnosed by real-time reverse-transcriptase polymerase chain reaction assays, and informed consent of the patient or legal representative. At the time the study protocol was made and the study was started, there were no epidemiological data available about cardiac biomarkers and echocardiography in critical COVID-19. The necessary number of patients to demonstrate differences between survivors and non-survivors could therefore not be estimated and therefore no power analysis was made. It was decided to include all consecutive patients admitted to our ICU in the first and second COVID-19 wave which arose in Belgium.

Demographics, pre-existing comorbidities, chronic medication, administered medication on ICU, clinical risk-scores and ratios (total and respiratory sequential organ failure assessment score (SOFA) and PaO2/FiO2-ratio (P/F ratio) on admission were automatically abstracted from the electronic health record on the moment of admission. Laboratory assessment included hs-cTnT (electrochemiluminescence immunoassay (ECLIA), Roche Cobas 8000 e80, Roche Diagnostics, Switzerland), NT-proBNP (ECLIA, Roche Cobas 8000 e80, Roche Diagnostics, Switzerland), C-reactive protein (CRP) (photometric measurement, Architect c16000, Abbott, Abbott Laboratories, Illinois, United States), ferritin (chemiluminescent Microparticle Immunoassay (CMIA), Architect i2000SR, Abbott, Abbott Laboratories, Illinois, United States) and D-dimer (immunoturbidimetry, STA R Max2, Stago, France). The first value upon admission was withheld when several blood samples were taken within 1 day. The cut-off for hs-cTnT was 14 pg/ml (corresponding with levels above the 99th percentile of a normal reference population) and for NT-proBNP 450 pg/mL. Patients were continuously monitored with a 3-lead electrocardiogram (ECG) and an additional 12-lead ECG was obtained on a daily base.

During follow-up, the use of vasopressors, mechanical ventilation and/or venovenous extracorporeal membrane oxygenation was recorded. Bedside transthoracic

echocardiography was performed within the first 48 h of inclusion, using a portable ultrasound machine CX50 (Philips Medical Systems, Andover, MA). The left ventricular ejection fraction (LVEF) was estimated with eyeball-method (normal, midrange and reduced) because of two reasons. First, acquisition of good quality images is often hard to obtain in critically ill patients and therefore more sophisticated methods (e.g., Simpson biplane, speckle tracing) to estimate the LVEF are not always feasible. Second, visually estimated ejection fraction has already shown to be extremely effective, rapid and consistent with quantitative echocardiographic assessment and is therefore a feasible method in critically ill patients (29). Other parameters that were obtained are: LV end diastolic diameter (LVEDD), diastolic function (E/A ratio and E/e' septal), tricuspid annular plane systolic excursion (TAPSE), estimate systolic pulmonary arterial pressure (SPAP) using the maximal tricuspid regurgitation velocity with CW Doppler, valvular function and presence of pericardial fluid. Diastolic function was dichotomized according to indices of diastolic dysfunction and increased left ventricular pressure (E/A $>$ 1.5 and/or E/e' septal $>$ 14). Echocardiography was performed by six skilled sonographers, all images were stored in the Picture Archiving and Communication System (PACS) of the hospital. The primary outcome of the study was all-cause 30-day mortality.

Data Analysis

The statistical analysis was performed using SPSS statistics (Version 27.0, IBM Corp, Armonk, NY). Normality of the distribution of continuous variables was tested by the Shapiro Wilk test. Categorical variables are shown as frequencies, and continuous variables as mean (standard deviation) or median (interquartile range) based upon normality of distribution. Comparison of categorical variables was performed using Chi-squared tests and for comparison of continuous variables Mann-Whitney U tests was used. Predictive markers for mortality were assessed by receiver operating characteristic (ROC) analysis and cut-off values by the Youden Index. The latter is a frequently used summary measure of the ROC curve. It represents the effectiveness of a diagnostic marker and enables the selection of an optimal threshold value (30). Multivariate logistic regression analysis was conducted to investigate risk factors for mortality. Since the number of events of the independent variable 30-d mortality was 21, the number of covariates that were added into the regression analysis to explore adjusted OR's was limited to 1. All tests were 2-sided with $P < 0.05$ considered statistically significant.

RESULTS

Patient Characteristics, Comorbidities, Chronic Medication, and Outcomes

In the study period, a total of 265 critically ill COVID-19 patients were admitted to our ICU department. The main reasons for exclusion were: more than 48 h on ICU prior to inclusion, inability to provide informed consent in Dutch, French or English (as the informed consent forms were only in these languages available) and refusal to participate by the patient or his/her representative ($n = 165$). A total sample of 100 critically ill COVID-19 patients was finally included within 48 h of ICU admission. Included patients originated from emergency departments of surrounding hospitals ($n = 34$), our own emergency department ($n = 44$) or the pneumology ward ($n = 22$). A flow diagram to illustrate the patients' selection can be found in **Figure 1**.

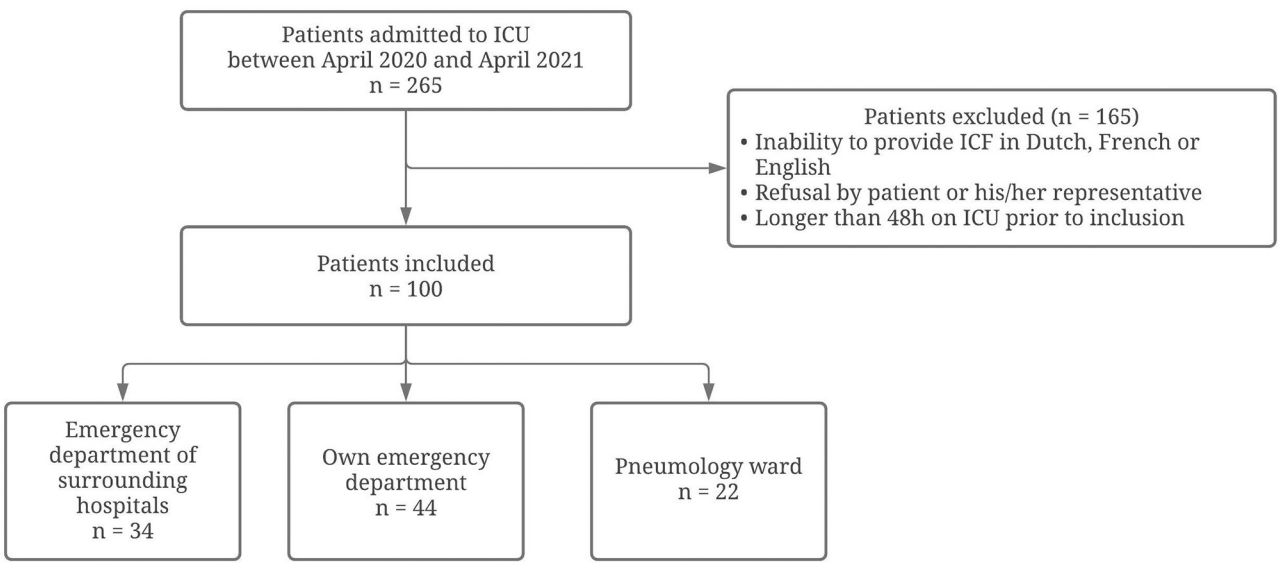


FIGURE 1 | Flow diagram on inclusion and exclusion of patients in the current study. Flow diagram reporting the numbers of included and excluded patients in the current study.

TABLE 1 | Baseline demographics, disease severity, laboratory assessments, and echocardiographic parameters of patients on admission to the intensive care unit.

Demographics ($n = 100$)	
Age (y)	63.5 (IQR 57.0–71.0)
Gender	
Male	66 (66.0 %)
Female	34 (34.0 %)
BMI (kg/m²)	28.7 (IQR 25.1–33.6)
Smoking history	
Never smoker	57 (57.0%)
Former smoker	36 (36.0%)
Active smoker	7 (7.0%)
Transferred from	
Emergency department	44 (44.0%)
Pneumology ward	22 (22.0%)
Surrounding hospital (not ICU)	34 (34.0%)
Comorbidities ($n = 100$)	
Arterial hypertension	42 (42.0%)
Kidney disease upon admission	
eGFR < 30 mL/min	3 (3.0%)
eGFR 30–60 mL/min	17 (17.0%)
eGFR > 60 mL/min	80 (80.0%)
Diabetes mellitus type 2	28 (28.0%)
Obstructive sleep apnea	6 (6.0%)
Chronic obstructive pulmonary disease / asthma	8 (8.0%)
Hypercholesterolemia	40 (40.0%)
Coronary artery disease	17 (17.0%)
Peripheral artery disease	6 (6.0%)
Chronic medication ($n = 100$)	
Use of statins	37 (37.0%)
Use of antidiabetic drugs	
Metformin	24 (24.0%)
SGLT2 inhibitor	2 (2.0%)
Other	15 (15.0%)
Use of antihypertensive drugs	
ACE-inhibitor	28 (28.0%)
Beta blocker	40 (40.0%)
Other	25 (25.0%)
Use of antithrombotic / anticoagulant drugs	
Aspirin	20 (20.0%)
P_2Y_{12} inhibitor	4 (4.0%)
NOAC or VKA	12 (12.0%)
Medication administered on ICU ($n = 100$)	
Dexamethasone	74 (74.0%)
Remdesivir	12 (12.0%)
Hydroxychlorquine	11 (11.0%)
Convalescent plasma	10 (10.0%)
Severity of illness ($n = 100$)	
Total SOFA-score on admission	3.0 (IQR 2.0–8.0)
Respiratory SOFA-score on admission	2.0 (IQR 2.0–3.0)
P/F ratio (IQR) on admission	96.3 (IQR 71.6–124.7)
Use of vasopressors during admission	54 (54.0 %)
Use of mechanical ventilation during admission	60 (60.0 %)
Use of vv-ECMO during admission	7 (7.0 %)
Inflammatory markers at time of inclusion ($n = 100$)	
CRP (mg/L)	136.5 (IQR 67.0–201.3)
D-dimer (ng/mL)	1020.0 (IQR 660.0–1795.0)
Ferritin (μg/L)	1139.0 (IQR 640.8–2346.8)
Cardiac biomarkers at time of inclusion ($n = 100$)	
hs-cTnT (μg/L)	
≥14 μg/L	47 (47.0 %)
<14 μg/L	53 (53.0 %)
NT-proBNP (pg/mL)	
≥450 pg/mL	39 (39.0 %)
<450 pg/mL	61 (61.0 %)
Echocardiography parameters at time of inclusion	
LVEF (%) ($n = 89$)	
Normal (>50%)	72 (80.9 %)
Midrange (40–50%)	16 (18.0 %)
Reduced (< 40%)	1 (1.1 %)
LVEDD (mm) ($n = 83$)	46.0 (IQR 43.0–51.0)
Diastolic function	
E/A ($n = 79$)	
<1.5	85 (85.0 %)
≥1.5	15 (15.0 %)
E/e' septal ($n = 72$)	
<14	60 (83.3 %)
≥14	12 (16.7 %)
Right ventricular function	
TAPSE ≥ 14 mm ($n = 77$)	73 (94.8 %)
Pulmonary artery pressure (mmHg) ($n = 51$)	24.0 (IQR 15.0–31.0)
Moderate to severe valvular dysfunction ($n = 87$)	7 (8.0 %)
Pericardial effusion ($n = 89$)	4.5 (4.5 %)

BMI, body mass index; vv-ECMO, venovenous extracorporeal membrane oxygenation; SOFA, Sequential Organ Failure Assessment; CRP, c-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic peptide; hs-cTnT, high sensitive troponin T; LVEDD, left ventricular end diastolic diameter; DT, deceleration time; TAPSE, tricuspid annular plane systolic excursion; LVEF, left ventricular ejection fraction; MR, mitral regurgitation; AR, aortic regurgitation; TR, tricuspid regurgitation; ICU, intensive care unit.

Baseline characteristics are presented in **Table 1**. Median age was 63.5 years, and the population was predominantly male (66%). None of the included patients were vaccinated against SARS-CoV-2. The mean body mass index (BMI) was 28.7 kg/m². Following comorbidities were recorded upon admission: arterial hypertension (42%), diabetes mellitus (28%), sleep apnea (6%), chronic obstructive pulmonary disease (COPD)/asthma (8%), coronary artery disease (17%), peripheral artery disease (6%), hypercholesterolemia (40%) and kidney disease upon admission (estimated glomerular filtration rate < 60 mL/min in 20% of patients). Chronic medication consisted of statins (37%), antidiabetic drugs (metformin 24%, SGLT2 inhibitors 2%, others 15%), antihypertensive drugs (ACE-inhibitors 28%, beta blockers 40%, others 25%) and antithrombotic/anticoagulant drugs (aspirin 20%, P2Y12 inhibitors 4%, NOAC/VKA 12%). During admission on ICU patients received additional treatment with dexamethasone (74%), remdesivir (12%), hydroxychloroquine

(11%) and convalescent plasma (10%). During admission 54% of patients required vasopressors, 60% of patients were mechanically ventilated and 7% of patients were supported with venovenous extracorporeal membrane oxygenation. On admission the median total SOFA-score was 3.0, with a respiratory SOFA-score of 2.0. The median P/F-ratio on admission was 96.3 mmHg. The median length of stay in ICU was 10 days. Within the first 30 days after inclusion 21 patients died (21%). Non-survivors were significantly older, were more often male and more often had kidney disease, sleep apnea, COPD/asthma and hypercholesterolemia. Respiratory SOFA, total SOFA and P/F ratio did not differ significantly between survivors and non-survivors.

Biomarkers

Biomarkers of inflammation (CRP and ferritin) and D-dimer did not differ significantly among survivors and non-survivors. Cardiac biomarkers were elevated in almost half of all included patients: hs-cTnT $\geq$ 14 pg/ml in 47%, and NT-proBNP $\geq$ 450 pg/ml in 39%. The level of these biomarkers was significantly higher in non-survivors (**Table 2**). **Figure 2** shows a ROC-curve for all 5 biomarkers with their respective area under the curve (AUC). CRP, ferritin and D-dimer were not associated with mortality, while the association of hs-cTnT (AUC: 0.79) and NT-proBNP (AUC: 0.71) was fair. Based on our data, we explored an optimal cut-off value for risk prediction for hs-cTnT and NT-proBNP. A value of 16.5 pg/ml for hs-cTnT corresponded with sensitivity and specificity for mortality of resp. 71.4 % and 48.6 %. The univariable odds ratio for 30-day all-cause mortality in patients with hs-cTnT $\geq$ 16.5 pg/ml was 8.5 (95% CI 2.9, 25.0). For NT-proBNP, an optimal cut-off value of 415.5 pg/ml corresponded with sensitivity and specificity for mortality of resp. 71.4 % and 38.5 %. The univariable odds ratio for 30-day all-cause mortality in patients with NT-proBNP $\geq$ 415.5 pg/ml was 5.1 (95% CI 1.8, 14.7). When adjusted for age, the adjusted odds ratio for 30-day all-cause mortality in patients with hs-cTnT $\geq$ 16.5 pg/ml was 7.1 (95% CI 2.3, 21.7; $P = 0.001$). For NT-proBNP a cut-off value of 415.5 pg/ml corresponded with an adjusted odds ratio of 3.5 (95% CI 1.1, 10.9; P 0.029). When adjusted for gender, the adjusted odds ratio for 30-day all-cause mortality in patients with hs-cTnT $\geq$ 16.5 pg/ml was 7.3 (95% CI 2.4, 21.9; $P < 0.001$). For NT-proBNP a cut-off value of 415.5 pg/ml corresponded with an adjusted odds ratio of 4.6 (95% CI 1.6, 13.4; P 0.006). When adjusted for kidney function (eGFR $<$ or $\geq$ 60 mL/min), the adjusted odds ratio for 30-day all-cause mortality in patients with hs-cTnT $\geq$ 16.5 pg/ml was 8.1 (95% CI 2.7, 24.6; $P < 0.001$). For NT-proBNP a cut-off value of 415.5 pg/ml corresponded with an adjusted odds ratio of 4.5 (95% CI 1.5, 13.2; P 0.006). When adjusted for SOFA-score, the adjusted odds ratio for 30-day all-cause mortality in patients with hs-cTnT $\geq$ 16.5 pg/ml was 8.1 (95% CI 2.7, 24.2; $P < 0.001$). For NT-proBNP a cut-off value of 415.5 pg/ml corresponded with an adjusted odds ratio of 4.8 (95% CI 1.6, 14.5; P 0.005). A survival analysis based upon the level of hs-cTnT and NT-proBNP on admission to ICU is presented in **Figure 3**. The unadjusted and adjusted odds ratios for 30-day all-cause mortality are in **Figures 4**, **5**.

TABLE 2 | Distribution of baseline demographics, disease severity, laboratory assessments, and echocardiographic parameters of patients between survivors and non-survivors.

	Survivors ($n = 79$)	Non-survivors ($n = 21$)	*P*-value
Demographics			
Age (y)	61.0 (IQR 52.0–71.0)	69.0 (IQR 66.5–72.0)	**0.008**
Sex			
Male	48 (60.8 %)	18 (85.7 %)	**0.032**
Female	31 (39.2 %)	3 (14.3 %)	
BMI (kg/m²)	28.9 (IQR 25.7–33.9)	25.8 (IQR 22.4–31.4)	**0.034**
Smoking history			0.338
No smoker	48 (60.8%)	9 (42.9%)	
Former smoker	26 (32.9%)	10 (47.6%)	
Active smoker	5 (6.3%)	2 (9.5%)	
Transferred from			0.826
Emergency department	36 (45.6%)	8 (38.1%)	
Pneumology ward	17 (21.5%)	5 (23.8%)	
Other hospital (not ICU)	26 (32.9%)	8 (38.1%)	
Length of stay ICU (d)	10.0 (IQR 5.0–16.0)	15.0 (IQR 6.5–24.0)	0.085
Comorbidities			
Arterial hypertension	33 (41.8 %)	9 (42.9 %)	0.929
Kidney disease upon admission			**0.031**
eGFR $<$ 30 mL/min	1 (1.3%)	2 (9.5%)	
eGFR 30–60 mL/min	11 (13.9%)	6 (28.6%)	
eGFR $>$ 60 mL/min	67 (84.8%)	13 (61.9%)	
Diabetes mellitus	22 (27.8 %)	6 (28.6 %)	0.948
Obstructive sleep apnea	2 (2.5%)	4 (19.0%)	**0.005**
Chronic obstructive pulmonary disease / asthma	2 (2.5%)	6 (28.6%)	**<0.001**
Hypercholesterolemia	27 (34.2%)	13 (61.9%)	**0.021**
Coronary artery disease	12 (15.2%)	5 (23.8%)	0.350
Peripheral artery disease	3 (3.8%)	3 (14.3%)	0.072
Chronic medication			
Use of statins	25 (31.6%)	12 (57.1%)	**0.031**
Use of antidiabetic drugs			
Metformin	20 (25.3%)	4 (19.0%)	0.550
SGLT2 inhibitor	0 (0.0%)	2 (9.5%)	**0.006**
Other	11 (13.9%)	4 (19.0%)	0.559
Use of antihypertensive drugs			
ACE-inhibitor	22 (27.8%)	6 (28.6%)	0.948
Beta blocker	31 (39.2%)	9 (42.9%)	0.764
Other	22 (27.8%)	3 (14.3%)	0.202
Use of antithrombotic / anticoagulant drugs			
Aspirin	13 (16.5%)	7 (33.3%)	0.086
P_2Y_{12} inhibitor	2 (2.5%)	2 (9.5%)	0.146
NOAC or VKA	7 (8.9%)	5 (23.8%)	0.061
Medication administered on ICU			
Dexamethasone	56 (70.9%)	18 (85.7%)	0.169

(Continued)

TABLE 2 | Continued

	Survivors ($n = 79$)	Non-survivors ($n = 21$)	*P*-value
Remdesivir	6 (7.6%)	6 (28.6%)	**0.009**
Hydroxychlorquine	10 (12.7%)	1 (4.8%)	0.304
Convalescent plasma	8 (10.1%)	2 (9.5%)	0.935
Severity of illness at time of inclusion			
Use of vasopressors	36 (45.6 %)	18 (85.7 %)	**0.001**
Use of mechanical ventilation	42 (53.2 %)	18 (85.7 %)	**0.007**
Use of vv-ECMO	4 (5.1 %)	3 (14.3 %)	0.141
Total SOFA-score	3.0 (IQR 2.0–8.0)	4.0 (IQR 2.0–11.5)	0.342
Respiratory SOFA-score	2.0 (IQR 2.0–3.0)	2.0 (IQR 2.0–3.0)	0.784
P/F ratio (IQR)	96.3 (IQR 70.2–120.6)	92.9 (IQR 74.5–153.5)	0.375
Inflammatory markers at time of inclusion			
CRP (mg/L)	137.1 (IQR 69.0–208.0)	125.5 (IQR 56.5–200.5)	0.496
D-dimer (ng/mL)	1025.0 (IQR 640.0–1740.0)	965.0 (IQR 625.0–2885.0)	0.912
Ferritin (μg/L)	1079.0 (IQR 661.0–2271.0)	1492.0 (IQR 603.0–3072.0)	0.469
Cardiac biomarkers at time of inclusion			
hs-cTnT (μg/L)			
≥16.5 μg/L	18 (22.8 %)	15 (71.4 %)	**< 0.001**
<16.5 μg/L	61 (77.2 %)	6 (28.6 %)	
NT-proBNP (pg/mL)			
≥415.5 pg/mL	26 (32.9 %)	15 (71.4 %)	**0.001**
<415.5 pg/mL	53 (67.1 %)	6 (28.6 %)	
Echocardiography parameters at time of inclusion			
LVEF (%)			
Normal (>50%)	62 (86.1 %)	10 (58.8 %)	**0.011**
Midrange (40–50%)	10 (13.9 %)	6 (35.3 %)	
Reduced (< 40%)	0 (0.0 %)	1 (5.9 %)	
LVEDD (mm)	47.0 (IQR 43.0–51.0)	46.0 (IQR 40.0–54.0)	1.000
Diastolic function			
E/A			
<1.5	55 (83.3 %)	12 (92.3 %)	0.410
≥1.5	11 (16.7 %)	1 (7.7 %)	
E/e' septal			
<14	52 (86.7 %)	8 (66.7 %)	0.090
≥14	8 (13.3 %)	4 (33.3 %)	
Right ventricular function			
TAPSE ≥ 14 mm	62 (96.9 %)	11 (84.6 %)	0.069
Pulmonary artery pressure (mmHg)	22.0 (IQR 11.8–30.0)	29.0 (IQR 26.0–37.0)	**0.043**
Moderate to severe valvular dysfunction	4 (5.6 %)	3 (18.8 %)	0.081
Pericardial effusion	3 (4.2 %)	1 (5.9 %)	0.759

BMI, body mass index; vv-ECMO, venovenous extracorporeal membrane oxygenation; SOFA, Sequential Organ Failure Assessment; CRP, c-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic peptide; hs-cTnT, high sensitive troponin T; LVEDD, left ventricular end diastolic diameter; TAPSE, tricuspid annular plane systolic excursion; LVEF, left ventricular ejection fraction; MR, mitral regurgitation; AR, aortic regurgitation; TR, tricuspid regurgitation; ICU, intensive care unit. Statistically significant result are marked in bold.

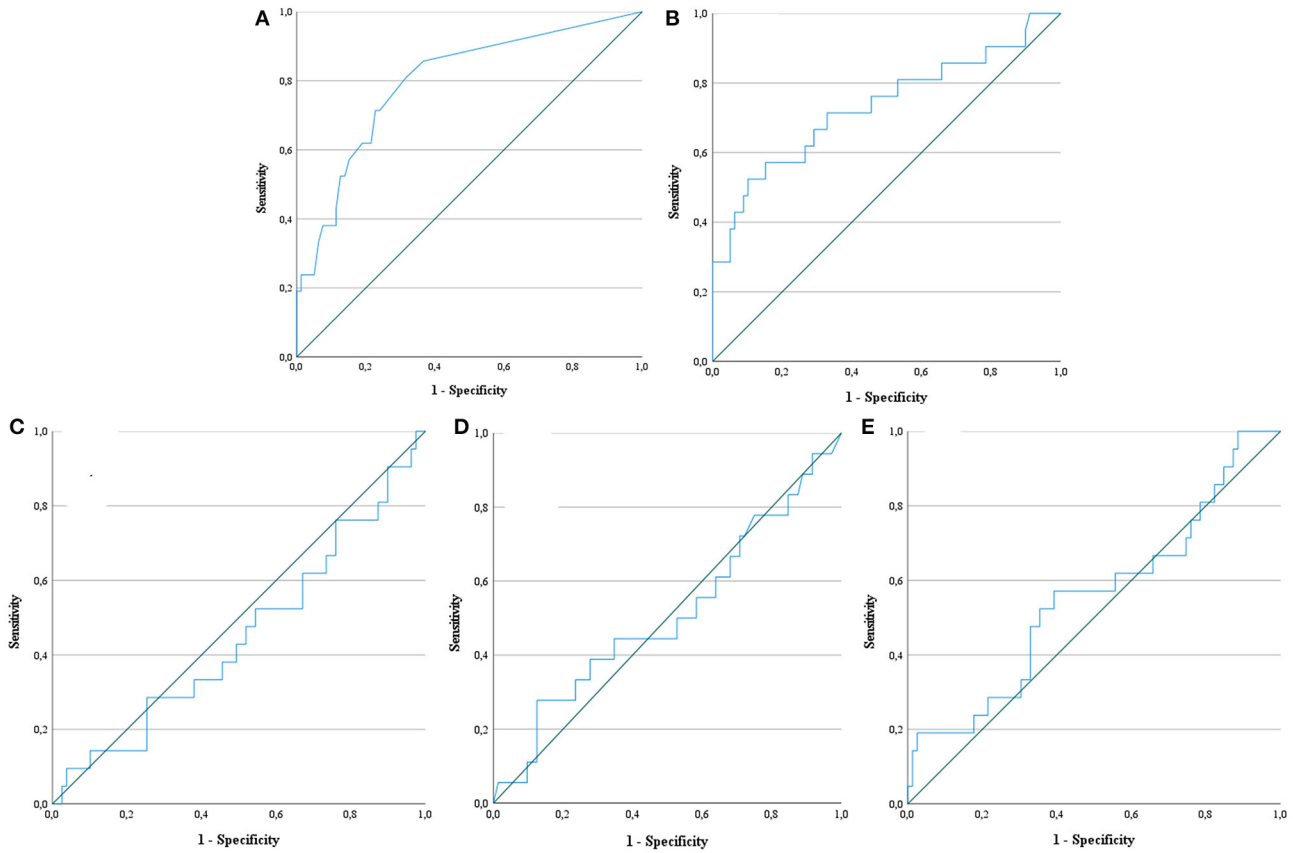
Echocardiography

Transthoracic echocardiography was not feasible in 11 patients (11%) due to poor visualization or prone ventilation. LVEF was reduced in 19.1% of patients (**Table 1**). One patient had a severely reduced LVEF of 25%, which was pre-existing due to a non-ischemic dilated cardiomyopathy. Sixteen patients (18.0%) had a mildly reduced LVEF. Of these, four patients (25%) were known with coronary artery disease and a pre-existing mildly reduced ejection fraction. For the other portion of patients there was no history of coronary artery disease and no previous echocardiography available. However, during admission none of the patients had evidence for acute ischemic signs on a continuous 3-lead ECG and daily 12-lead ECG. LVEF was significantly lower in those who ultimately died (**Table 2**). Levels of hs-cTnT and NT-proBNP were elevated in, respectively, 38.9 and 34.7% of patients with normal LVEF. Right ventricular function, evaluated by TAPSE, was normal (≥14 mm) in 94.8% of our cohort. After dichotomization between normal and abnormal TAPSE (≥ vs. <14 mm), patients with an abnormal RV function had higher mortality but this increase was not significant. There was no significant difference between survivors and non-survivors concerning diastolic function. The presence of moderate to severe valvular regurgitation (aortic, mitral, and tricuspid) or pericardial effusion did not differ significantly between the two groups. When adjusted for SOFA-score, the adjusted OR for 30-day all-cause mortality in patients with a reduced LVEF was 4.8 (95% CI 1.4, 16.2; *P* 0.011), which is represented in **Figure 5**. When adjusted for age, the adjusted OR for 30-day all-cause mortality in patients with a reduced LVEF was 3.7 (95% CI 1.1, 12.3; *P* 0.034). When adjusted for gender, the adjusted OR for 30-day all-cause mortality in patients with a reduced LVEF was 3.9 (95% CI 1.2, 12.9; *P* 0.026). Unadjusted OR's for 30-day all-cause mortality for all echocardiographic findings are shown in **Figure 4**.

DISCUSSION

This prospective study in critically ill COVID-19 patients has six important findings: (I) elevated levels of hs-cTnT and NT-proBNP upon admission are common and were found in, respectively, 47 and 39% of patients, (II) Elevated cardiac biomarkers are not necessarily linked to ventricular dysfunction as around 40% of patients with normal ejection fraction had either elevated levels of hs-cTnT and/or NT-proBNP, (III) Elevated levels of hs-cTnT, and to a lesser extent, NT-proBNP were associated with mortality, (IV) Serum levels of frequently used biomarkers (C-reactive protein, D-dimer and ferritin) and other clinical parameters of disease-severity (total SOFA, respiratory SOFA and P/F ratio) were not predictive for 30-day all-cause mortality, (V) Decreased LV function was associated with worse prognosis, whereas diastolic dysfunction and impaired RV function were not, (VI) cardiac ultrasound was not possible for various reasons in as much as 11% of this cohort of critical COVID-19 patients.

Whether cardiac biomarkers should be systematically measured as part of the workup for every hospitalized



	Area under the curve (AUC)	95% confidence interval	*P*-value
hs-cTnT	0.797	0.687–0.907	**<0.001**
NT-proBNP	0.732	0.594–0.870	**0.001**
CRP	0.451	0.309–0.594	0.496
D-dimer	0.508	0.349–0.668	0.912
Ferritin	0.552	0.406–0.697	0.469

FIGURE 2 | Receiver operating characteristic curve. Receiver operating characteristic (ROC) curves for five biochemical markers: high-sensitive troponin T **(A)**, N-terminal pro-brain natriuretic peptide **(B)**, C-reactive protein **(C)**, D-dimer **(D)** and ferritin **(E)**. hs-cTnT, high-sensitive troponin T; NT-proBNP, N-terminal pro-brain natriuretic peptide. Statistically significant result are marked in bold.

COVID-19 patient remains subject of debate. Currently, the European Society of Cardiology (ESC) and the American College of Cardiology (ACC) recommend against their routine use, while awaiting more evidence, as they warn for unnecessary diagnostic investigations, risk exposure and medical overuse (16, 28). Another reason to not currently recommend the routine use of cardiac biomarkers in prognostication is the belief that these markers would only be of limited incremental prognostic value to other markers of disease-severity (31). Recent research showed for example that higher D-dimer levels on admission to ICU seem to be independently associated with higher risk of death in critical COVID-19 (32). This, however, contrasts with the findings in our study and previous research. In an early report of 191 patients with COVID-19 in Wuhan, the univariable odds ratio for mortality when hs-cTnT was above the 99th percentile upper reference limit was 80.1 (95% CI, 10.3–620.4; $P < 0.0001$) regardless of underlying cardiovascular disease. This was higher than for all other biomarkers or scores tested, including D-dimer, ferritin and SOFA-score (33). Another study by Manocha et al. showed that hs-cTnT was the only independent predictor of mortality among the same five biomarkers (i.e., CRP, ferritin, D-dimer, NT-proBNP and hs-cTnT), whereas Shi et al. found statistical significance for both hs-cTnT and NT-proBNP (34, 35). Our results are in line with these findings and support the statement of Sandoval et al. that the use of cardiac biomarkers for prognostic purposes may help in risk-stratification (36). We furthermore agree that this should not necessarily lead to unnecessary diagnostic testing when it is accompanied by clear education about the goals and implications of potentially elevated biomarkers (36).

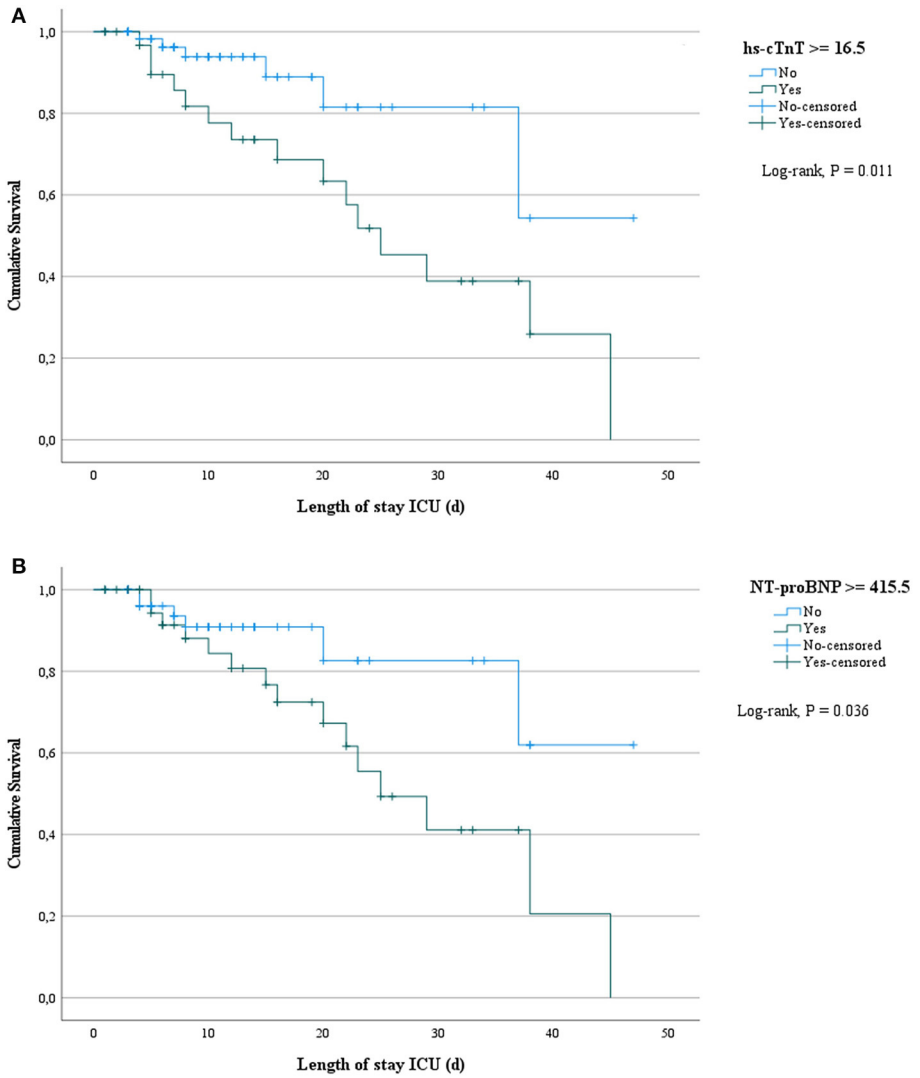


FIGURE 3 | Survival analysis based upon the level of hs-cTnT and NT-proBNP on admission to ICU. Survival analysis based upon both levels of high sensitive troponin T **(A)** and N-terminal pro-brain natriuretic peptide **(B)** on admission to ICU. hs-cTnT, high-sensitive troponin T; NT-proBNP, N-terminal pro-brain natriuretic peptide.

We observed a reduced left and right ventricular function in, respectively 17 and 5.2% of our patients. Previous large-scale research found similar results concerning reduced left ventricular function (20%), whereas right ventricular function was reduced in about 30% (23). Based on our data, reduced left ventricular systolic function was associated with mortality. However, right ventricular function, assessed with TAPSE, which only estimates longitudinal right ventricular function, was not. Due to the low number of patients with reduced right ventricular contractility one should interpret this finding with caution. In previous research, left- and right ventricular function, analyzed with strain measurements, were both correlated with poor outcome (23, 26, 37). Diastolic dysfunction, based upon E/A and E/e′ measurement, was not associated with higher odds for 30-day all-cause mortality. A prospective study of Szekely et al. showed similar results for E/A, though elevated E/e' in their cohort was associated with a higher hazard ratio for death. However, this result just narrowly met statistical significance (HR 1.08, 95% CI: 1.001, 1.2) (22). Overall, comparison of echocardiographic findings in COVID-19 subjects is difficult given the large heterogeneity in study populations and measurement approaches (37).

The fact that patients with elevated cardiac biomarkers did not necessarily have a reduced LVEF underlines the hypothesis that cardiac injury in COVID-19 may be due to a myriad of causes including direct myocardial injury of SARS-CoV-2 and indirect myocardial stress due to respiratory failure, thrombogenicity, sympathetic stimulation, cytokine release and endothelial dysfunction (31, 38–40). In recent research using cardiac magnetic resonance imaging (cMRI), COVID-19 patients with elevated hs-cTnT of unknown origin showed to have both ischemic and non-ischemic alterations on cMRI. However, in

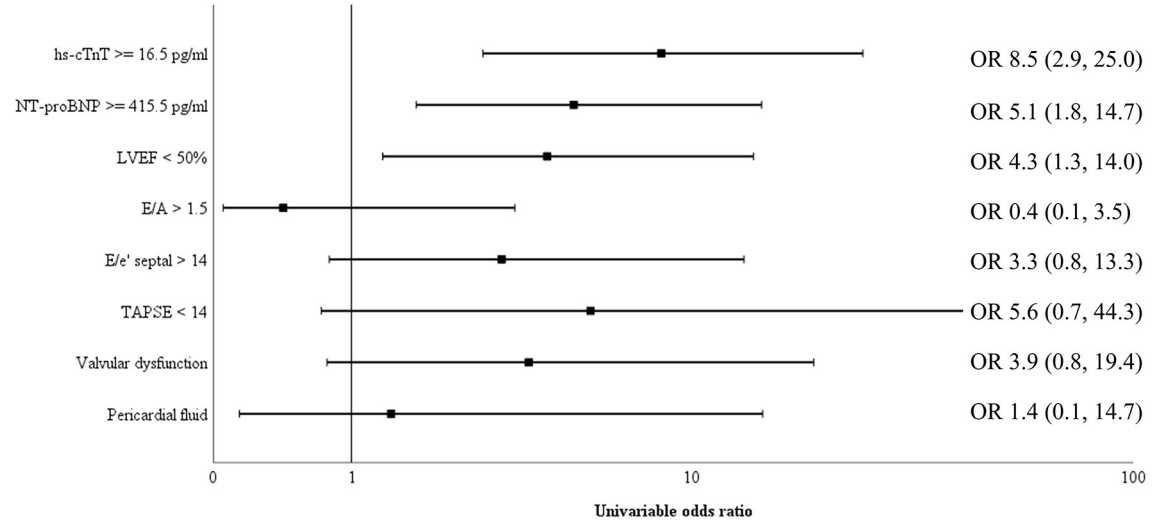


FIGURE 4 | Univariable odds ratio for 30-day all-cause mortality. Univariable odds ratio for 30-day all-cause mortality for cardiac biomarkers hs-cTnT and NT-proBNP as well as several echocardiographic measurements (reduced left ventricular ejection fraction (LVEF), increased E/e', increased E/A, decreased tricuspid annular plane systolic excursion (TAPSE), valvular dysfunction and pericardial fluid). Both elevated cardiac biomarkers above their respective cut-off value and a reduced LVEF had a significant higher odds ratios for 30-day all-cause mortality. NT-proBNP, N-terminal pro-brain natriuretic peptide; hs-cTnT, high sensitive troponin T; TAPSE, tricuspid annular plane systolic excursion; LVEF, left ventricular ejection fraction; OR, odds ratio.

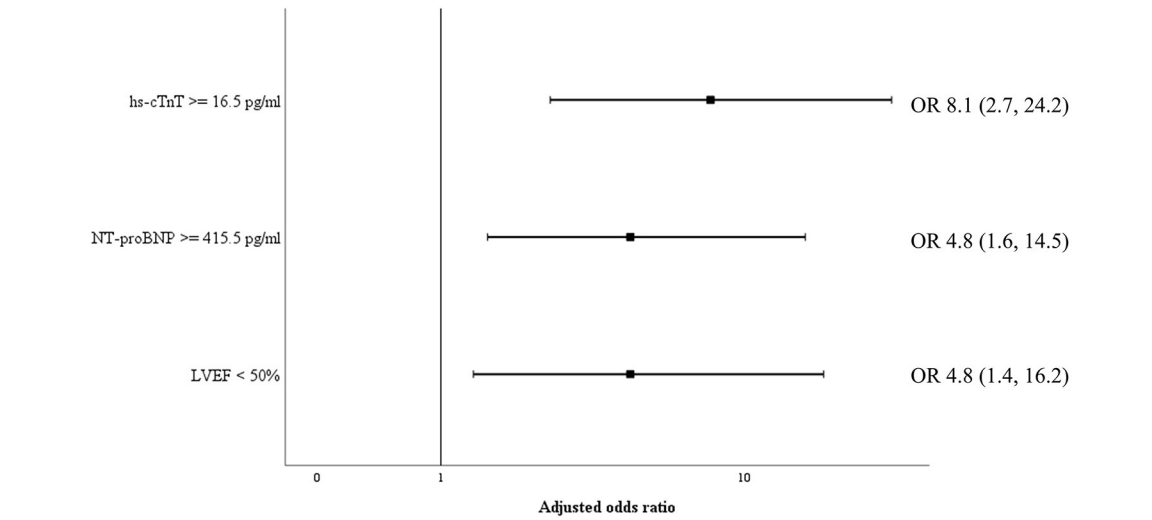


FIGURE 5 | Adjusted odds ratio for 30-day all-cause mortality. Odds ratio for 30-day all-cause mortality, adjusted for SOFA-score, for cardiac biomarkers hs-cTnT and NT-proBNP as well as reduced left ventricular ejection fraction. NT-proBNP, N-terminal pro-brain natriuretic peptide; hs-cTnT, high sensitive troponin T. LVEF, left ventricular ejection fraction; OR, odds ratio.

31% of cases even with cMRI no cause could be found and most subjects had a normal LVEF (93%) (41). As such, elevated cardiac biomarkers may represent disease severity in a more complete way than routine echocardiography.

Moreover, routine echocardiography is not always possible in real-world practice due to practical (poor visualization and prone ventilation) or logistic problems, which limits its use even more. In the present cohort echocardiography was not feasible in about one tenth of patients. Furthermore, it exposes health care personnel to contagious risks and may be more time-consuming due to disinfection protocols. Taken together, the additive value of routine echocardiography on top of the measurement of cardiac biomarkers is questionable, even though reduced left ventricular function may predict worse outcome. This is in line with the ESC guidance, which currently recommends against performing echocardiography in COVID-19 patients, unless it is likely to alter the management strategy (16).

The current study has some important strengths. First, the study population was critically ill and prospectively evaluated, which contrasts with most studies evaluating all hospitalized patients retrospectively. Second, the combination of a prospective assessment of biomarkers and echocardiography in the same study population is rather unique. To our knowledge, only two smaller similar series were previously published (42, 43). In these studies, LV dysfunction was common in patients with elevated serum levels of hs-cTnT, though also present in 12% of patients without elevated levels of hs-cTnT (42, 43). However, possible relationships between the levels of cardiac biomarkers or echocardiographic findings and outcome parameters were not studied.

Five study limitations should also be addressed. First, no serial data of cardiac biomarkers were obtained, although this could be of interest as dynamic changes and/or peak values during admission may add additive value in prognostication (36, 44). Second, extrapolation of these results should be done with caution as this was a single-center study in critical COVID-19 patients and criteria for admission to ICU may differ between hospitals. For instance, COVID-19 patients with mono-organ failure requiring high flow nasal cannula, as well as patients with established do-not-resuscitate orders were admitted to dedicated mid-care units and thus not included in the present study. Third, our study has a relatively small sample size and results must be validated in larger cohorts. Fourth, echocardiographic evaluation of LVEF was performed using eye-balling methodology and no other more advanced imaging techniques were obtained. Finally, the extent of preexisting cardiovascular disease was largely unknown and therefore no difference could be made between established cardiovascular disease and new COVID-19 induced cardiovascular abnormalities.

CONCLUSION

This study highlights the strong predictive value of the cardiac biomarkers hs-cTnT and NT-proBNP taken upon ICU admission in critically ill COVID-19 patients. They outperform other routinely used biomarkers, as well as clinical indices of disease severity in ICU in this specific cohort. Transthoracic echocardiography has several limitations and should therefore only be considered if it is likely to impact therapeutic management.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee, University Hospital Ghent C. Heymanslaan 10 9000 Ghent, Belgium Number: BC-07568 Date: April 1st, 2020. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HS, SG, and EH conceived the principal idea and critically revised the manuscript. BZ, SD, HS, and SG performed the echocardiography. BZ and SD were major contributors in data analysis and writing the manuscript. EH was prime investigator of the project and was a major contributor in the data analysis. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Els Vandecasteele and Dr. Fiona Tromp for performing echocardiography in part of the patient population, as well as Daisy Vermeiren, Stephanie Bracke, Jolien Van Hecke, and Anouska De Smeytere for the organization and logistic support during this project.

REFERENCES

1. Anon. COVID-19 Map. *Johns Hopkins Coronavirus Resource Center*. Available online at: https://coronavirus.jhu.edu/map.html (accessed July 28, 2021).
2. National Institutes of Health. *COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines.* (2021). Available online at: https://files.covid19treatmentguidelines.nih.gov/guidelines/covid19treatmentguidelines.pdf (accessed July 28, 2021).
3. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth universal definition of myocardial infarction (2018). *J Am Coll Cardiol*. (2018) 72:2231–64. doi: 10.1016/j.jacc.2018.08.1038
4. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol*. (2020) 5:802–10. doi: 10.1001/jamacardio.2020.0950
5. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ*. (2020) 368:m1091. doi: 10.1136/bmj.m1091
6. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol*. (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017
7. Stefanini GG, Chiarito M, Ferrante G, Cannata F, Azzolini E, Viggiani G, et al. Early detection of elevated cardiac biomarkers to optimise risk stratification in patients with COVID-19. *Heart*. (2020) 106:1512–8. doi: 10.1136/heartjnl-2020-317322
8. Koutsoukis A, Delmas C, Roubille F, Bonello L, Schurtz G, Manzo-Silberman S, et al. Acute coronary syndromes in the era of SARS-CoV-2 infection: a registry of the French Group of Acute Cardiac Care. *CJC Open*. (2020) 3:311–7. doi: 10.1016/j.cjco.2020.11.003
9. Musher DM, Abers MS, Corrales-Medina VF. Acute infection and myocardial infarction. *N Engl J Med*. (2019) 380:171–6. doi: 10.1056/NEJMra1808137
10. Bansal A, Kumar A, Patel D, Puri R, Kalra A, Kapadia SR, et al. Meta-analysis comparing outcomes in patients with and without cardiac injury and coronavirus disease 2019 (COVID 19). *Am J Cardiol*. (2020) 141:140–6. doi: 10.1016/j.amjcard.2020.11.009
11. Vrints CJM, Krychtiuk KA, Van Craenenbroeck EM, Segers VF, Price S, Heidbuchel H. Endothelialitis plays a central role in the pathophysiology of severe COVID-19 and its cardiovascular complications. *Acta Cardiol*. (2020) 76:109–24. doi: 10.1080/00015385.2020.1846921

12. Landesberg G, Jaffe AS, Gilon D, Levin PD, Goodman S, Abu-Baih A, et al. Troponin elevation in severe sepsis and septic shock: the role of left ventricular diastolic dysfunction and right ventricular dilatation*. *Crit Care Med*. (2014) 42:790–800. doi: 10.1097/CCM.0000000000000107
13. Thachil J, Tang N, Gando S, Falanga A, Cattaneo M, Levi M, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. *J Thromb Haemost*. (2020) 18:1023–6. doi: 10.1111/jth.14810
14. Siripanthong B, Nazarian S, Muser D, Deo R, Santangeli P, Khanji MY, et al. Recognizing COVID-19-related myocarditis: the possible pathophysiology and proposed guideline for diagnosis and management. *Hear Rhythm*. (2020) 17:1463–71. doi: 10.1016/j.hrthm.2020.05.001
15. Sala S, Peretto G, Gramegna M, Palmisano A, Villatore A, Vignale D, et al. Acute myocarditis presenting as a reverse Tako-Tsubo syndrome in a patient with SARS-CoV-2 respiratory infection. *Eur Heart J*. (2020) 41:1861–2. doi: 10.1093/eurheartj/ehaa286
16. The European Society for Cardiology E. *ESC Guidance for the Diagnosis and Management of CV Disease During the COVID-19 Pandemic*. Available online at: https://www.escardio.org/Education/COVID-19-and-Cardiology/ESCCOVID-19-Guidance (accessed July 28, 2021).
17. Caro-Codón J, Rey JR, Buño A, Iniesta AM, Rosillo SO, Castrejon-Castrejon S, et al. Characterization of NT-proBNP in a large cohort of COVID-19 patients. *Eur J Heart Fail*. (2021) 23:456–64. doi: 10.1002/ejhf.2095
18. Pranata R, Huang I, Lukito AA, Raharjo SB. Elevated N-terminal pro-brain natriuretic peptide is associated with increased mortality in patients with COVID-19: systematic review and meta-analysis. *Postgrad Med J*. (2020) 96:387–91. doi: 10.1136/postgradmedj-2020-137884
19. Sorrentino S, Cacia M, Leo I, Polimeni A, Sabatino J, Spaccarotella CAM, et al. B-type natriuretic peptide as biomarker of COVID-19 disease severity-a meta-analysis. *J Clin Med*. (2020) 9:2957. doi: 10.3390/jcm9092957
20. Mitaka C, Hirata Y, Nagura T, Tsunoda Y, Itoh M, Amaha K. Increased plasma concentrations of brain natriuretic peptide in patients with acute lung injury. *J Crit Care*. (1997) 12:66–71. doi: 10.1016/S0883-9441(97)90003-4
21. Dweck MR, Bularga A, Hahn RT, Bing R, Lee KK, Chapman AR, et al. Global evaluation of echocardiography in patients with COVID-19. *Eur Hear J Cardiovasc Imaging*. (2020) 21:949–58. doi: 10.1093/ehjci/jeaa178
22. Szekely Y, Lichter Y, Taieb P, Banai A, Hochstadt A, Merdler I, et al. Spectrum of cardiac manifestations in COVID-19: a systematic echocardiographic study. *Circulation*. (2020) 142:342–53. doi: 10.1161/CIRCULATIONAHA.120.047971
23. Karagodin I, Carvalho Singulane C, Woodward GM, Xie M, Tucay ES, Tude Rodrigues AC, et al. Echocardiographic correlates of in-hospital death in patients with acute COVID-19 infection: the World Alliance Societies of Echocardiography (WASE-COVID) study. *J Am Soc Echocardiogr Off Publ Am Soc Echocardiogr*. (2021) 34:819–30. doi: 10.1016/j.echo.2021.05.010
24. Cameli M, Pastore MC, Soliman Aboumarie H, Mandoli GE, D'Ascenzi F, Cameli P, et al. Usefulness of echocardiography to detect cardiac involvement in COVID-19 patients. *Echocardiography*. (2020) 37:1278–86. doi: 10.1111/echo.14779
25. Zhao B-C, Liu W-F, Lei S-H, Zhou B-W, Yang X, Huang T-Y, et al. Prevalence and prognostic value of elevated troponins in patients hospitalised for coronavirus disease 2019: a systematic review and meta-analysis. *J Intensive Care*. (2020) 8:88. doi: 10.1186/s40560-020-00508-6
26. Wibowo A, Pranata R, Astuti A, Tiksnadi BB, Martanto E, Martha JW, et al. Left and right ventricular longitudinal strains are associated with poor outcome in COVID-19: a systematic review and meta-analysis. *J Intensive Care*. (2021) 9:9. doi: 10.1186/s40560-020-00519-3
27. Li Y, Li H, Zhu S, Xie Y, Wang B, He L, et al. Prognostic value of right ventricular longitudinal strain in patients with COVID-19. *JACC Cardiovasc Imaging*. (2020) 13:2287–99. doi: 10.1016/j.jcmg.2020.04.014
28. Januzzi J. Troponin and BNP use in COVID-19. *J Am Coll Cardiol*. Available online at: https://www.acc.org/latest-in-cardiology/articles/2020/03/18/15/25/troponin-and-bnp-use-in-covid19 (accessed July 28, 2021).
29. Levitov A, Frankel HL, Blaivas M, Kirkpatrick AW, Su E, Evans D, et al. Guidelines for the appropriate use of bedside general and cardiac ultrasonography in the evaluation of critically ill patients-part II: cardiac ultrasonography. *Crit Care Med*. (2016) 44:1206–27. doi: 10.1097/CCM.0000000000001847
30. Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and its associated cutoff point. *Biom J*. (2005) 47:458–72. doi: 10.1002/bimj.200410135
31. Mueller C, Giannitsis E, Jaffe AS, Huber K, Mair J, Cullen L, et al. Cardiovascular biomarkers in patients with COVID-19. *Eur Hear J Acute Cardiovasc Care*. (2021) 10:310–9. doi: 10.1093/ehjacc/zuab009
32. Short SAP, Gupta S, Brenner SK, Hayek SS, Srivastava A, Shaefi S, et al. d-dimer and death in critically ill patients with coronavirus disease 2019. *Crit Care Med*. (2021) 49:e500–11. doi: 10.1097/CCM.0000000000004917
33. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3
34. Manocha KK, Kirzner J, Ying X, Yeo I, Peltzer B, Ang B, et al. Troponin and other biomarker levels and outcomes among patients hospitalized with COVID-19: derivation and validation of the HA(2)T(2) COVID-19 mortality risk score. *J Am Heart Assoc*. (2020) 10:e018477. doi: 10.1161/JAHA.120.018477
35. Shi S, Qin M, Cai Y, Liu T, Shen B, Yang F, et al. Characteristics and clinical significance of myocardial injury in patients with severe coronavirus disease 2019. *Eur Heart J*. (2020) 41:2070–9. doi: 10.1093/eurheartj/ehaa408
36. Sandoval Y, Januzzi JL, Jaffe AS. Cardiac troponin for assessment of myocardial injury in COVID-19: JACC review topic of the week. *J Am Coll Cardiol*. (2020) 76:1244–58. doi: 10.1016/j.jacc.2020.06.068
37. Messina A, Sanfilippo F, Milani A, Calabrò L, Negri K, Monge García MI, et al. COVID-19-related echocardiographic patterns of cardiovascular dysfunction in critically ill patients: A systematic review of the current literature. *J Crit Care*. (2021) 65:26–35. doi: 10.1016/j.jcrc.2021.05.010
38. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of coronaviruses on the cardiovascular system: a review. *JAMA Cardiol*. (2020) 5:831–40. doi: 10.1001/jamacardio.2020.1286
39. Bader F, Manla Y, Atallah B, Starling RC. Heart failure and COVID-19. *Heart Fail Rev*. (2021) 26:1–10. doi: 10.1007/s10741-020-10008-2
40. Kotecha T, Knight DS, Razvi Y, Kumar K, Vimalesvaran K, Thornton G, et al. Patterns of myocardial injury in recovered troponin-positive COVID-19 patients assessed by cardiovascular magnetic resonance. *Eur Heart J*. (2021) 42:1866–78. doi: 10.1093/eurheartj/ehab075
41. Knight DS, Kotecha T, Razvi Y, Chacko L, Brown JT, Jeetley PS, et al. COVID-19: myocardial injury in survivors. *Circulation*. (2020) 142:1120–2. doi: 10.1161/CIRCULATIONAHA.120.049252
42. Churchill TW, Bertrand PB, Bernard S, Namasivayam M, Churchill J, Crousillat D, et al. Echocardiographic features of COVID-19 illness and association with cardiac biomarkers. *J Am Soc Echocardiogr*. (2020) 33:1053–4. doi: 10.1016/j.echo.2020.05.028
43. Sud K, Vogel B, Bohra C, Garg V, Talebi S, Lerakis S, et al. Echocardiographic findings in patients with COVID-19 with significant myocardial injury. *J Am Soc Echocardiogr*. (2020) 33:1054–5. doi: 10.1016/j.echo.2020.05.030
44. Li C, Jiang J, Wang F, Zhou N, Veronese G, Moslehi JJ, et al. Longitudinal correlation of biomarkers of cardiac injury, inflammation, and coagulation to outcome in hospitalized COVID-19 patients. *J Mol Cell Cardiol*. (2020) 147:74–87. doi: 10.1016/j.yjmcc.2020.08.008

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

BRIEF RESEARCH REPORT
published: 09 November 2021
doi: 10.3389/fcvm.2021.745790

COVID-19 in Adults With Hypertrophic Cardiomyopathy

Milla E. Arabadjian[1], *Maria C. Reuter*[2], *Alexandra Stepanovic*[2], *Mark V. Sherrid*[2] *and Daniele Massera*[2]*

[1] New York University Rory Meyers College of Nursing, New York, NY, United States, [2] Hypertrophic Cardiomyopathy Program, Leon H. Charney Division of Cardiology, New York University Grossman School of Medicine, New York, NY, United States

Background: Individuals with cardiovascular disease are considered high risk for severe COVID-19. However, the clinical impact of COVID-19 in patients with hypertrophic cardiomyopathy (HCM) is unknown. The purpose of this study was to describe the clinical course and outcomes of COVID-19 in patients with HCM.

Methods: This retrospective observational study included adults with HCM and positive PCR/antibody test for SARS-CoV-2 at a large urban hospital system in the New York from January, 2020 to January, 2021.

Results: Seventy individuals were included, with a mean (SD) age of 60.1 (15.1) years, 39 (55.7%) of whom were male, and 42 (60%) white. Forty-five (65.3%) patients had obstructive HCM. Hypertension and obesity (BMI $\geq$ 30) were present in 45 (64.3%) and 37 (52.9%) patients, and the prevalence of atrial fibrillation, obstructive sleep apnea and diabetes was high. Common symptoms of COVID-19 were fever, cough, shortness of breath and fatigue, affecting 33 (47.1%), 33 (47.1%), 28 (40.0%), and 28 (40.0%) patients, respectively. Fourteen (20%) patients were hospitalized. The majority (45 [64.3%] patients) recovered without intervention. Two patients had non-fatal pulmonary embolisms, 1 had atrial fibrillation requiring electrical cardioversion and 1 had acute decompensated heart failure. Three (4.3%) patients required mechanical ventilation, two of whom died (case fatality rate 2.9%). A total of 15 (21.4%) patients were asymptomatic.

Conclusions: Our data suggest that in this diverse and high-risk group of patients with HCM, established risk factors for severe COVID-19, such as obesity, may be more important drivers of morbidity and mortality than the presence of HCM alone.

Keywords: COVID-19, hypertrophic cardiomyopathy, outcomes, risk, obesity

OPEN ACCESS

Edited by:
Hendrik Tevaearai Stahel, Bern University Hospital, Switzerland

Reviewed by:
Kenichi Hongo, Jikei University School of Medicine, Japan
Yosuke Miyazaki, Yamaguchi University, Japan

***Correspondence:**
Daniele Massera
Daniele.Massera@nyulangone.org

Specialty section:
This article was submitted to Heart Failure and Transplantation, a section of the journal Frontiers in Cardiovascular Medicine

Received: *22 July 2021*
Accepted: *20 September 2021*
Published: *09 November 2021*

Citation:
Arabadjian ME, Reuter MC, Stepanovic A, Sherrid MV and Massera D (2021) COVID-19 in Adults With Hypertrophic Cardiomyopathy. Front. Cardiovasc. Med. 8:745790. doi: 10.3389/fcvm.2021.745790

INTRODUCTION

Since the beginning of the COVID-19 pandemic, over 200 million cases with over 4 million deaths worldwide have been recorded (1). Cardiovascular disease has emerged as risk factor for increased morbidity and mortality (2–4). Heart failure in particular was shown to be associated with worse outcomes (5). Hypertrophic cardiomyopathy is the most common inherited cardiac disorder and is characterized by cardiac hypertrophy, left ventricular outflow obstruction in the majority of cases, and diastolic dysfunction (6). We were particularly concerned about outcomes of patients with hypertrophic cardiomyopathy (HCM) due to reports of ACE2 gene upregulation in septal myectomy specimens (7) — the ACE2 receptor being the entry point for the SARS-CoV-2

virus. However, the impact of preexisting HCM on the clinical course of COVID-19 is currently unknown. This study aimed to examine the outcomes of COVID-19 in patients with HCM.

MATERIALS AND METHODS

To address this question, we performed a retrospective cohort study of consecutive adult patients, age ≥18 years with HCM who underwent testing for COVID-19 PCR or antibodies at NYU Langone Health, a large urban healthcare system in the New York City area with hospitals in Manhattan, Brooklyn, and Nassau County between January 1, 2020 and January 6, 2021. Individuals were included in the study if they had either positive SARS-CoV-2 PCR or antibody testing. The study was approved by the NYU Grossman School of Medicine's Institutional Review Board and informed consent was waived.

Imaging studies were reviewed by one cardiologist (D.M.) to confirm HCM diagnosis by current guidelines (6) and to identify HCM structural characteristics including distribution of left ventricular hypertrophy (LVH), systolic anterior motion (SAM), left ventricular outflow tract (LVOT) obstruction, mid-ventricular obstruction, apical aneurysm, and mitral annular calcification (MAC). Clinical information was recorded, including medical therapy, history of septal myectomy or alcohol septal ablation, comorbid conditions, COVID-19 presenting symptoms, treatment, hospitalizations, and outcomes. Statistical analysis was performed using STATA 16 (StataCorp LLC, College Station, TX) (8). Data were expressed as means and standard deviation (SD) or medians (interquartile range) for continuous variables, and proportions for categorical variables, as appropriate. The unpaired *t*-test or the Mann Whitney U test were used to compare continuous variables, as appropriate, and the chi-squared or Fisher's exact test for categorical variables. A $p < 0.05$ was considered significant.

RESULTS

Demographics and HCM Characteristics

A total of 70 patients with HCM had positive COVID-19 testing, 58 (82.9%) of whom by PCR and 12 (17.1%) only by antibody testing. Median (IQR) age was 62 (51–71) years, 39 (55.7%) were male. There were 42 (60%) white, 19 (27%) Black, and 5 (7%) Asian individuals, of whom 10 (14%) were of Hispanic ethnicity. Individuals resided in the NYC metropolitan tri-state area (including Westchester County, Nassau County, Suffolk County, New Jersey and Connecticut) (**Figure 1**).

The majority of patients, 45 (64%), in this sample had obstructive HCM, defined as ≥30 mmHg LVOT or mid-ventricular gradient at rest or with provocation. Apical aneurysm was present in five (7.1%) patients, all of whom had a mid-systolic Doppler signal void (9). Asymmetric septal hypertrophy (ASH) was present in 60 (85.7%) individuals, Mean (SD) left ventricular ejection fraction was 69.6 (5.6) % and mean maximal LV wall thickness was 17.6 (4.2) mm. Fifteen patients (21.7%) had an implantable cardioverter defibrillator and one patient had a permanent pacemaker. Most patients were on standard HCM specific medical therapy including beta blockers and calcium channel blockers (**Table 1**). Disopyramide was used in 11 (15.9%)

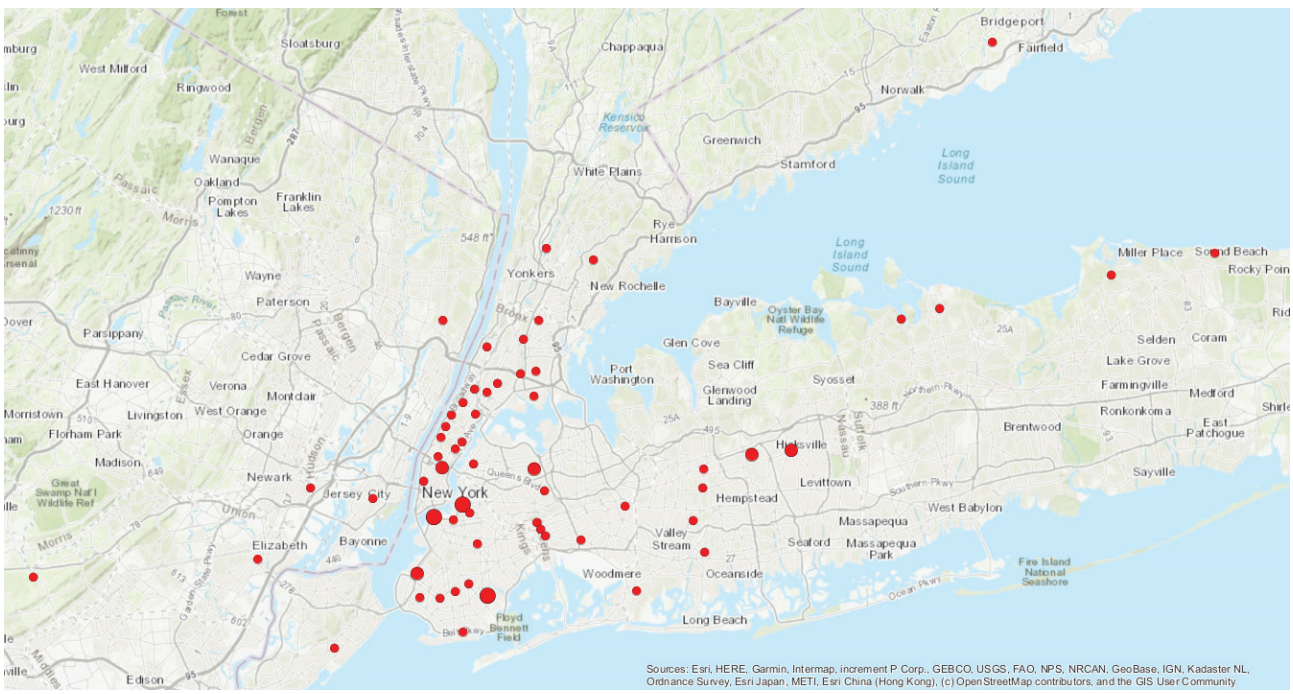


FIGURE 1 | Distribution of patients by zip code. One patient per zip code. Two patients per zip code. Three patients per zip code.

TABLE 1 | Demographics and baseline HCM characteristics.

	Total *N* = 70	Hospitalized *N*=14	Non-hospitalized *N* = 56	*p*-value
Age, median (IQR), years	62 (51–71)	64.5 (55–78)	61.5 (48.5–70)	0.155
Male sex, *N* (%)	39 (55.7)	6(42.9)	33 (58.9)	0.279
Race, *N* (%)				0.569
White	42 (60.0)	8 (57.1)	34 (60.7)	
Black/African American	19 (27.1)	6 (42.9)	13 (23.2)	
Asian	5 (7.1)	0 (0)	5 (8.9)	
Ethnicity, *N* (%)				0.967
Hispanic	10 (14.3)	2 (14.3)	8 (14.3)	
LV ejection fraction, median (IQR), %	70 (65–75)	72.5 (65–75)	70 (65–75)	0.208
Maximum LV thickness, median (IQR), mm	17 (15–20)	18 (16–20)	17 (15–20)	0.370
Type HCM, *N* (%)				0.373
Non-obstructive	23 (32.9)	6 (42.9)	17 (30.4)	
Obstructive	47 (67.1)	8 (57.1)	39 (69.6)	
Distribution of LV hypertrophy, *N* (%)				0.608
Asymmetric septal hypertrophy	60 (85.7)	11 (78.6)	48 (85.7)	
Concentric hypertrophy	4 (5.8)	0 (0)	4 (7.1)	
Apical hypertrophy	15 (21.4)	2 (14.3)	13 (23.2)	
Apical aneurysm	5 (7.1)	1 (7.1)	4 (7.1)	0.684
Pulmonary hypertension	4 (5.7)	3 (21.4)	1 (1.8)	0.023
≥ moderate mitral regurgitation, *N* (%)	4 (5.7)	0 (0)	4 (7.1)	0.401
≥ moderate aortic stenosis, *N* (%)	1 (1.4)	0 (0)	1 (1.8)	0.200
History of surgical septal myectomy, *N* (%)	15 (21.4)	1 (7.1)	14 (25)	0.135
Beta blocker, *N* (%)	58 (82.9)	11 (78.6)	47 (83.9)	0.634
Calcium channel blocker, *N* (%)	16 (22.9)	5 (35.7)	11 (20)	0.214
Disopyramide, *N* (%)	11 (15.7)	4 (28.6)	7 (12.5)	0.143
ACE/ARBs, *N* (%)	14 (20.0)	2 (14.3)	12 (21.4)	0.494
Amlodipine, *N* (%)	8 (11.4)	3 (21.4)	5 (8.9)	0.193
Diuretics, *N* (%)	22 (31.4)	5 (35.7)	17 (30.4)	0.699
Anti-coagulant therapy, *N* (%)	19 (27.1)	7 (50)	12 (21.4)	0.032
Anti-platelet agents, *N* (%)	22 (31.4)	4 (28.6)	18 (32.1)	0.506
Implantable cardioverter defibrillator, N (%)	15 (21.7)	2 (14.3)	13 (23.2)	0.374

patients and 15 (21.4%) had a prior history of surgical septal myectomy; none had alcohol septal ablation. A total of 26 (37.1%) individuals were taking QT-prolonging drugs.

Co-morbidities

The overall burden of co-morbidities was high (**Table 2**). The most common co-morbidities were hypertension in 45 (64.3%), obesity (BMI>30 kg/m^2) in 37 (52.9%), atrial fibrillation in 23 (32.3%), coronary artery disease in 17 (24.3%), obstructive sleep apnea in 15 (21.4%) and diabetes in 15 (21.4%) patients. Individuals in this sample had a median (IQR) of three (2–5) co-morbidities in addition to HCM. There were no significant differences in co-morbidities between racial/ethnic groups and between men and women (data not shown).

COVID-19 Course and Outcomes

Fifteen individuals (24.3%) in this cohort were asymptomatic. The most common symptoms included fever in 33 (47.1%), cough in 33 (47.1%), shortness of breath in 28 (40.0%) and fatigue in 28 (40.0%) patients (**Table 3**). The majority of patients, 45 (64.3%), did not seek or require medical interventions. Medical therapy included the use of antibiotics and steroids. Azithromycin was given to five (7.1%) patients and hydroxychloroquine to six (8.6%). Fourteen (20.0%) patients were hospitalized. There were no significant differences in demographics, HCM characteristics, or co-morbidity profile between the hospitalized and the non-hospitalized group. However, there were differences in presenting symptoms between the groups, as expected. The majority of hospitalized patients presented with shortness of breath, cough and fever than the non-hospitalized patients, 13 (92.9%) vs. 15 (26.8%), $p < 0.001$, 10 (71.4%) vs. 23 (41%), $p = 0.042$, and 10 (71.4%) vs. 23 (41%), $p = 0.042$, respectively. Furthermore, treatment differed between the hospitalized and non-hospitalized patients, with hospitalized patients utilizing more antibiotic therapy. Four of the hospitalized individuals required intensive care and three required intubation and mechanical ventilation. Two patients (2.9%) died during hospital admission, one woman and one man, both non-Hispanic whites, ages 69 and 71 years, and both obese, with BMI of 38 and 35.3 kg/m^2. The female patient had non-obstructive HCM (maximal wall thickness 23 mm), hypertension, hyperlipidemia, non-obstructive coronary artery disease, non-insulin dependent diabetes, COPD, liver steatosis, paroxysmal

TABLE 2 | Co-morbidities.

	Total *N* = 70	Hospitalized *N* = 14	Non-hospitalized *N* = 56	*p*-value
Hypertension, *N* (%)	45 (64.3)	11 (78.6)	34 (60.7)	0.212
Hyperlipidemia, *N* (%)	41 (58.6)	7 (50)	34 (60.7)	0.467
Coronary artery disease, *N* (%)	17 (24.3)	4 (28.6)	13 (23.2)	0.676
Atrial fibrillation, *N* (%)	23 (32.3)	5 (35.7)	18 (32.1)	0.799
Stroke or transient ischemic attack, *N* (%)	5 (7.1)	1 (7.1)	4 (7.1)	0.684
BMI, mean (SD), kg/m^2	31.6 (6.0)	32.1 (1.6)	31.2 (0.81)	0.805
BMI > 30, *N* (%)	37 (52.9)	9 (64.3)	28 (50.0)	0.338
DM, *N* (%)				0.591
Insulin dependent	3 (4.3)	1 (7.1)	2 (3.4)	
Non-insulin dependent	12 (14.3)	3 (21.4)	9 (16.1)	
Liver disease, *N* (%)				0.300
Hepatitis C	2 (2.9)	0 (0)	2 (3.6)	
Steatosis	3 (4.3)	2 (14.3)	1 (1.8)	
Chronic kidney disease, *N* (%)	7 (10.0)	3 (21.4)	4 (7.3)	0.142
Asthma or COPD, *N* (%)	9 (12.9)	3 (21.4)	6 (10.7)	0.253
Obstructive sleep apnea, *N* (%)	15 (21.4)	3 (21.4)	12 (21.4)	0.626
Cancer – active, *N* (%)	1 (1.4)	0 (0)	1 (1.8)	0.800
HIV, *N* (%)	1 (1.4)	0 (0)	1 (1.8)	0.800
Number of cardiac co-morbidities, median (IQR)	2 (1–3)	2 (1–3)	2 (1–3)	0.477
Number of total co-morbidities, median (IQR)	3 (2–5)	3.5 (2–5)	3 (2–5)	0.296

TABLE 3 | COVID-19 symptoms, treatment, outcomes.

	Total *N* = 70	Hospitalized *N* = 14	Non-hospitalized *N* = 56	*p*-value
COVID-19 diagnostic testing, *N* (%)				0.057
Positive SARS-CoV-2 PCR	58 (82.9)	14 (100)	44 (78.6)	
Positive SARS-CoV-2 IgG antibodies only	12 (17.1)	0 (0)	12 (21.4)	
Presenting symptoms, *N* (%)				
None	15 (21.4)	0 (0)	15 (26.8)	0.023
Fever	33 (47.1)	10 (71.4)	23 (41)	0.042
Cough	33 (47.1)	10 (71.4)	23 (41)	0.042
Shortness of breath	28 (40.0)	13 (92.9)	15 (26.8)	<0.001
Fatigue	28 (40.0)	3 (21.4)	25 (44.6)	0.113
GI distress	6 (8.6)	2 (14.3)	2 (7.1)	0.345
Loss of smell/taste	11 (15.7)	0 (0)	11 (19.6)	0.069
COVID-19 therapy, *N* (%)				
None/supportive	45 (64.3)	5 (35.7)	40 (71.4)	0.045
Azithromycin	5 (7.1)	4 (28.6)	1 (1.8)	0.005
Hydroxychloroquine	6 (8.6)	5 (35.7)	1 (1.8)	0.001
Non-macrolide antibiotics	6 (8.6)	3 (21.4)	3 (5.4)	0.090
Steroids	7 (10.0)	4 (28.6)	3 (5.4)	0.026
Remdesivir	1 (1.4)	1 (7.1)	0 (0)	0.200
COVID-19-associated complications in hospitalized patients, No. (%)				
Pulmonary embolism, *N* (%)	2 (2.9)			
Deep vein thrombosis, *N* (%)	1 (1.4)			
Thrombocytopenia, *N* (%)	1 (1.4)			
Atrial fibrillation requiring cardioversion, *N* (%)	1 (1.4)			
Acute decompensated heart failure	1 (1.4)			
ICU hospitalization, *N* (%)	4 (5.7)			
Intubated, *N* (%)	3 (4.3)			
Deceased, *N* (%)	2 (2.9)			

atrial fibrillation, chronic renal disease, and an implantable cardiac defibrillator for primary prevention of sudden cardiac death. The male patient had obstructive HCM with SAM, LVOT obstruction with a peak LVOT gradient at rest of 108 mmHg, moderate aortic valve stenosis and obstructive sleep apnea. In addition, two (2.9%) patients presented with non-fatal pulmonary embolisms, one of whom was also found to have a tibial deep vein thrombosis and presented with atrial fibrillation requiring electrical cardioversion. One patient (1.4%) presented with acute decompensated heart failure requiring intravenous diuretics.

DISCUSSION

This study is the first to examine the clinical course and outcomes of COVID-19 in patients with HCM, a significant proportion of whom had prior septal myectomy surgery and implantable cardioverter defibrillators. The hospital admission rate was high at 20%.The case fatality rate in this sample was similar to the general population (1). Both individuals who died had multiple co-morbid conditions associated with higher morbidity and mortality [2, 3.] Among hospitalized patients, the distribution of non-obstructive and obstructive HCM patients mirrors the distribution in unselected HCM cohorts (10). There were no significant differences in demographics, HCM characteristics, or COVID-19 risk factors between the hospitalized and not hospitalized group. One reason for this may be that only four patients required an ICU level of care. Moreover, a majority of the non-ICU hospitalized patients were treated with supportive care. It is likely that most were admitted for observation and monitoring of possible COVID-19 related deterioration, given that the presenting COVID-19 related symptom of the vast majority of hospitalized patients was shortness of breath. The overall low numbers of seriously ill HCM patients with COVID-19 in the sample preclude an adequate statistical analysis on risk profiles. Two patients presented with non-fatal pulmonary embolisms, a known complication of COVID-19 (11). Furthermore, even though one patient presented with acute decompensated heart failure, this is not uncommon in the setting of acute illness (12).

Prior reports have noted that there is ACE2 receptor upregulation in HCM tissue specimens (7), and ACE2 is the entry point of SARS-Cov-2. One small study examined cardiac samples from individuals with dilated cardiomyopathy, hypertrophic cardiomyopathy and healthy controls, which also supported upregulation of ACE2 in HCM tissue, but did not observe a difference in ACE2 expression between HCM patients taking ACE inhibitor medicines and those who did not (13). However, the clinical impact of this upregulation in HCM is unclear. Our study is the first to examine the clinical impact of COVID-19 on HCM patients in real world conditions. Our data suggest that HCM may not in itself contribute to worse clinical outcomes from COVID-19, above other established risk factors, such as age and obesity. Further studies in larger cohorts of patients with HCM and COVID-19 are needed.

Limitations of this study include the possibility of selection bias, underreporting and asymptomatic infections leading to a high admission rate. Furthermore, the relatively small sample size may limit the generalization of results. However, our cohort is representative of a diverse population. Last, as patients with HCM were deemed high risk for COVID-19 complications along with other types of cardiovascular disease, this awareness may have led to strict adherence with social distancing recommendations, which may have been the cause of a lower infection rate.

In conclusion, our data suggest that HCM in itself does not carry a higher risk of COVID-19 disease severity and complications. Established risk factors for severe COVID-19, such as age and obesity may be more influential.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by NYU Langone Health Institutional Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MA: conceptualization, methodology, formal analysis, investigation, data curation, and writing—original draft. MR: investigation, writing—review, and editing. AS: investigation. MS: writing—review and editing. DM: conceptualization, methodology, data curation, supervision, and writing—original draft. All authors on this manuscript have fulfilled the criteria for authorship as set forth by the ICMJE guidelines.

FUNDING

This study was supported by internal funds from the Leon H. Charney Division of Cardiology, Department of Medicine, NYU Langone Health.

REFERENCES

1. WHO. *WHO Coronavirus (Covid-19) Dashboard*. (2021). Available online at: https://covid19.who.int/. (Accessed April 22, 2020)

2. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3

3. Parohan M, Yaghoubi S, Seraji A, Javanbakht MH, Sarraf P, Djalali M. Risk factors for mortality in patients with coronavirus disease 2019 (COVID-19) infection: a systematic review and meta-analysis of observational studies. *Aging Male*. (2020) 23:1416–24. 1–9. doi: 10.1101/2020.04.09.20056291
4. Katz MH. Regardless of age, obesity and hypertension increase risks with COVID-19. *JAMA Intern Med*. (2021) 181:381. doi: 10.1001/jamainternmed.2020.5415
5. Alvarez-Garcia J, Lee S, Gupta A, Cagliostro M, Joshi AA, Rivas-Lasarte M, et al. Prognostic impact of prior heart failure in patients hospitalized with COVID-19. *J Am Coll Cardiol.* (2020) 76:2334–48. doi: 10.1016/j.jacc.2020.09.549
6. Ommen SR, Mital S, Burke MA, Day SM, Deswal A, Elliott P, et al. 2020 AHA/ACC guideline for the diagnosis and treatment of patients with hypertrophic cardiomyopathy: executive summary: a report of the American college of cardiology/American heart association joint committee on clinical practice guidelines. *J Am Coll Cardiol.* (2020) 76:3022–55. doi: 10.1016/j.jacc.2020.08.044
7. Bos JM, Hebl VB, Oberg AL, Sun Z, Herman DS, Teekakirikul P, et al. Marked up-regulation of ACE2 in hearts of patients with obstructive hypertrophic cardiomyopathy: implications for SARS-CoV-2–mediated COVID-19. *Mayo Clin Proc.* (2020) 95:1354–68. doi: 10.1016/j.mayocp.2020.04.028
8. StataCorp. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC (2019).
9. Po JRF, Kim B, Aslam F, Arabadjian M, Winson G, Cantales D, et al. Doppler systolic signal void in hypertrophic cardiomyopathy: apical aneurysm and severe obstruction without elevated intraventricular velocities. *J Am Society of Echocardiogr.* (2015) 28:1462–73. doi: 10.1016/j.echo.2015.08.015
10. Maron BJ. Clinical course and management of hypertrophic cardiomyopathy. *N Engl J Med*. (2018) 379:655–68. doi: 10.1056/NEJMra1710575
11. Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. *J Am Coll Cardiol.* (2020) 75:2950–73. doi: 10.1016/j.jacc.2020.04.031
12. Rey JR, Caro-Codón J, Rosillo SO, Iniesta ÁM, Castrejón-Castrejón S, Marco-Clement I, et al. Heart failure in COVID-19 patients: prevalence, incidence and prognostic implications. *Eur J Heart Fail.* (2020) 22:2205–15. doi: 10.1002/ejhf.1990
13. Tucker NR, Chaffin M, Bedi KC Jr, Papangeli I, Akkad AD, Arduini A, et al. Myocyte-specific upregulation of ACE2 in cardiovascular disease: implications for SARS-CoV-2–mediated myocarditis. *Circulation*. (2020) 142:708–10. doi: 10.1161/CIRCULATIONAHA.120.047911

Conflict of Interest: MA reports modest advisory board fees from MyoKardia, Inc. MR reports modest advisory board fees from MyoKardia, Inc. MS reports consulting fees from Celltrion. DM reports consulting fees from Bristol Myers Squibb.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 24 November 2021
doi: 10.3389/fcvm.2021.763827

Higher Incidence of Stroke in Severe COVID-19 Is Not Associated With a Higher Burden of Arrhythmias: Comparison With Other Types of Severe Pneumonia

Peter Jirak[1†], Zornitsa Shomanova[2†], Robert Larbig[3,4], Daniel Dankl[5], Nino Frank[5], Clemens Seelmaier[1], Dominyka Butkiene[4], Michael Lichtenauer[1], Moritz Mirna[1], Bernhard Strohmer[1], Jan Sackarnd[2], Uta C. Hoppe[1], Jürgen Sindermann[2], Holger Reinecke[2], Gerrit Frommeyer[3], Lukas J. Motloch[1‡] and Rudin Pistulli[2‡]*

[1] Clinic II for Internal Medicine, University Hospital Salzburg, Paracelsus Medical University, Salzburg, Austria, [2] Department of Cardiology I—Coronary and Peripheral Vascular Disease, Heart Failure, University Hospital Münster, Münster, Germany, [3] Department of Cardiology II—Electrophysiology, University Hospital Münster, Münster, Germany, [4] Division of Cardiology, Hospital Maria Hilf Mönchengladbach, Mönchengladbach, Germany, [5] Department of Anesthesiology, Perioperative Care, and Intensive Care Medicine, University Hospital Salzburg, Paracelsurs Medical University, Salzburg, Austria

OPEN ACCESS

Edited by:
Mingxing Xie,
Huazhong University of Science and Technology, China

Reviewed by:
Mate Vamos,
University of Szeged, Hungary
Jing Zhou,
Peking University First Hospital, China

***Correspondence:**
Peter Jirak
p.jirak@salk.at

[†] These authors have contributed equally to this work and share first authorship

[‡] These authors have contributed equally to this work and share senior authorship

Specialty section:
This article was submitted to Cardiac Rhythmology, a section of the journal Frontiers in Cardiovascular Medicine

Received: 24 August 2021
Accepted: 18 October 2021
Published: 24 November 2021

Citation:
Jirak P, Shomanova Z, Larbig R, Dankl D, Frank N, Seelmaier C, Butkiene D, Lichtenauer M, Mirna M, Strohmer B, Sackarnd J, Hoppe UC, Sindermann J, Reinecke H, Frommeyer G, Motloch LJ and Pistulli R (2021) Higher Incidence of Stroke in Severe COVID-19 Is Not Associated With a Higher Burden of Arrhythmias: Comparison With Other Types of Severe Pneumonia. Front. Cardiovasc. Med. 8:763827. doi: 10.3389/fcvm.2021.763827

Aims: Thromboembolic events, including stroke, are typical complications of COVID-19. Whether arrhythmias, frequently described in severe COVID-19, are disease-specific and thus promote strokes is unclear. We investigated the occurrence of arrhythmias and stroke during rhythm monitoring in critically ill patients with COVID-19, compared with severe pneumonia of other origins.

Methods and Results: This retrospective study included 120 critically ill patients requiring mechanical ventilation in three European tertiary hospitals, including n =60 COVID-19, matched according to risk factors for the occurrence of arrhythmias in $n = 60$ patients from a retrospective consecutive cohort of severe pneumonia of other origins. Arrhythmias, mainly atrial fibrillation (AF), were frequent in COVID-19. However, when compared with non-COVID-19, no difference was observed with respect to ventricular tachycardias (VT) and relevant bradyarrhythmias (VT 10.0 vs. 8.4 %, $p = ns$ and asystole 5.0 vs. 3.3%, $p = ns$) with consequent similar rates of cardiopulmonary resuscitation (6.7 vs. 10.0%, $p = ns$). AF was even more common in non-COVID-19 (AF 18.3 vs. 43.3%, $p = 0.003$; newly onset AF 10.0 vs. 30.0%, $p = 0.006$), which resulted in a higher need for electrical cardioversion (6.7 vs. 20.0%, $p = 0.029$). Despite these findings and comparable rates of therapeutic anticoagulation (TAC), the incidence of stroke was higher in COVID-19 (6.7.% vs. 0.0, $p = 0.042$). These events also happened in the absence of AF (50%) and with TAC (50%).

Conclusions: Arrhythmias were common in severe COVID-19, consisting mainly of AF, yet less frequent than in matched pneumonia of other origins. A contrasting higher incidence of stroke independent of arrhythmias also observed with TAC, seems to be an arrhythmia-unrelated disease-specific feature of COVID-19.

Keywords: COVID-19, arrhythmias, atrial fibrillation, stroke, pneumonia, ventricular tachycardia, anticoagulation

INTRODUCTION

The novel coronavirus disease COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a worldwide healthcare crisis with an overstrain of hospital resources (1, 2). Given its diverse cardiovascular involvement, further investigation of potential disease-specific processes is crucial to optimize its medical management (3–5). Although previous studies observed a high rate of cardiac injury in COVID-19 infections (3), two recent publications reported rates of cardiac injury to be similar to non-COVID-19 pneumonia, pointing against a COVID-19 specific cardiac involvement (6, 7). Similarly, the impact of COVID-19 on cardiac arrhythmias and thromboembolic events is also yet to be covered to the full extent. The arrhythmic burden is high in COVID-19 patients. The first investigation from Wang et al. reported cardiac arrhythmias in 17% of all their included patients and in 44.4% of those admitted to ICU (8). However, the missing definition of arrhythmias in that study should be taken into account when interpreting results (8). A recent work of Bertini et al. analyzed ECGs in critically ill COVID-19 patients and reported a high rate of ECG abnormalities (93%) with atrial fibrillation/flutter being the most common arrhythmia (22%) (9). In this context, the high incidence of stroke in COVID-19, as the most frequent thromboembolic complication of atrial fibrillation, attracts special attention (10–12). Similar investigations on thromboembolic events including stroke, deep vein thrombosis (DVT), and pulmonary embolism reported overall rates of up to 43% in critically ill COVID-19 patients (13–15). Of note, the majority of patients in those studies received at least a prophylactic anticoagulation (13–15). These findings suggest a potential correlation between cardiac arrhythmias and high rates of stroke and other thromboembolic events. Moreover, it remains unclear, whether the high arrhythmic burden in COVID 19 is the effect of unspecific proarrhythmogenic states promoted by cardiac injury as well as the systemic inflammatory burden, or whether a COVID-19 specific mechanism exists, which promotes cardiac arrhythmias. Given its considerable clinical impact, further investigation on COVID-19 associated arrhythmias and their potential link to thromboembolic events is urgently needed. Accordingly, in our multicentre study, we aimed for a comparative analysis of cardiac arrhythmias as well as stroke and other thromboembolic events in critically ill patients requiring ventilator therapy due to SARS-CoV-2 induced pneumonia matched to a historical cohort requiring respiratory support due to severe pneumonia of non-COVID-19 origin (non-COVID-19).

METHODS

The present retrospective study was conducted in three European tertiary centers in Germany and Austria (University Hospital Münster, Maria Hilf Hospital Mönchengladbach and the University Hospital Salzburg). The study was conducted in accordance with the Declaration of Helsinki and the standards of good clinical practice. All three local ethic committees approved the present study (University Hospital Münster Nr. 2020-306-f-S, Maria Hilf Hospital Mönchengladbach: Nr. 143/2020, and University Hospital Salzburg: Nr. 1071/2020).

Study Cohorts

A total of 120 patients were involved in this study (60 COVID-19 vs. 60 non-COVID-19). The COVID-19 cohort consisted of 60 consecutive patients with available ICU rhythm monitoring who suffered severe pneumonia. Severe pneumonia was defined as pneumonia-associated respiratory failure requiring mechanical ventilation [noninvasive ventilation (NIV) or invasive ventilation]; the term NIV in this study refers to mechanical ventilation involving end-expiratory and inspiratory positive air pressure support *via* a tightly fitted face mask or helmet, as opposed to invasive ventilation necessitating endotracheal intubation. All patients included in the study had some form of mechanical ventilation (patients who merely needed oxygen insufflations were not included) between March and May 2020. Patients were treated according to recent recommendations (1). All patients received anticoagulation during their ICU stay. A detailed description with regards to anticoagulation is given in the **Supplementary Methods** section. Patients with a history of hyperthyroid disease, of inherited arrhythmic disorders, and a history of persistent or permanent atrial fibrillation (AF) were excluded from the analyses. The diagnosis of COVID-19 was established in the presence of a positive result in real-time reverse transcription–polymerase chain reaction assay (performed according to the manufacturer) for COVID-19 and a chest radiography and/or computer tomography of the thorax indicative for COVID-19 related pneumonia according to current recommendations (2).

The control group was recruited from a consecutive collective of 1,222 patients suffering severe pneumonia of non-COVID-19 origin. All patients in the control group were treated between January 2014 and Mach 2020 at the ICU according to current intensive care guidelines (3). Patients from the control group requiring mechanical ventilation (non-invasive/invasive ventilation) were primarily matched to the COVID-19 population according to the medical history of paroxysmal AF. To account for potential confounders as proarrhythmic comorbidities, patients were further matched for known risk factors associated with cardiac arrhythmias. Matching was conducted stepwise and manually according to age, gender, heart failure, coronary artery disease, atrial flutter, diabetes mellitus, arterial hypertension, valvular heart disease, and previous stroke/TIA. If more than one candidate in the retrospective non-COVID-19 cohort fully fulfilled the matching criteria, the patient with the closest admission time point as compared with the time point of the beginning of the recruitment of the COVID-19 cohort (March 2020) was chosen for matching. To further validate the matching process, covariate imbalance was assessed. Standardized differences and omnibus test revealed no statistically significant covariate imbalance between the two investigated groups (**Supplementary Table 6**).

TABLE 1 | Baseline characteristics.

	COVID-19 ($n = 60$)		Non-COVID-19 (n=60)		p
	n	Mean ± SD, median (Q3–Q1) or %	n	Mean ± SD, median (Q3–Q1) or %	
Gender (female)	14/60	23.3%	14/60	23.3%	>0.999
Age (years)	60	66.5 ± 12.6	60	65.9 ± 11.61	0.813
BMI (kg/m^2)	51	27.7 (5.1)	50	25.6 (6.7)	0.493
Medical history					
Arterial hypertension	31/60	51.7%	33/60	55%	0.714
Coronary artery disease	9/60	15.0%	9/60	15.0%	>0.999
Peripheral vascular disease	4/60	6.7%	2/60	3.3%	0.679
Diabetes mellitus	13/60	21.7%	14/60	23.3%	0.827
Current smoking	10/60	16.7%	16/60	26.7%	0.184
Heart failure	7/60	11.7%	7/60	11.7%	>0.999
Valvular heart disease	3/60	5.0%	5/60	8.3%	0.717
Paroxysmal AF	9/60	15.0%	9/60	15.0%	>0.999
Atrial flutter	1/60	1.7%	0/60	0%	>0.999
Pulmonary arterial hypertension	2/60	3.3%	1/60	1.7%	>0.999
Obstructive lung disease	8/60	13.3%	12/60	20.0%	0.327
Structural lung disease	0/60	0%	1/60	1.7%	>0.999
Stroke/TIA	6/60	10.0%	3/60	5.0%	0.491
Medication					
Beta-blockers	18/60	30.0%	22/60	36.7%	0.439
NOAK/AOK	7/60	11.7%	8/60	13.3%	0.783
Amiodarone	0/60	0%	2/60	3.3%	0.496

AF, atrial fibrillation; BMI, body mass index; SD, standard deviation.
**p<0.05.*

Data Collection and Analyses

In all eligible patients, data were retrospectively collected from electronic medical records. Data obtained comprises demographics, medical history, laboratory examinations, comorbidities, complications, specific treatment measures, and outcomes, and also 12-lead ECGs at ICU admission and complete rhythm monitoring during ICU stay (continuous standard three-lead ECG during complete ICU stay). Laboratory samples were collected within the first hours after ICU admittance, and follow-up was conducted on a daily routine according to the need for clinical assessment. With regards to rhythm monitoring, baseline rhythm was evaluated and documented every hour during the entire ICU stay. Analyses of ECGs, classification of arrhythmias, and quantification of the duration of arrhythmias in the rhythm monitoring were analyzed and documented by a trained team of ICU nurses and physicians in one of the recruiting centers. Cardiac arrhythmias during ICU rhythm monitoring were classified according to current guidelines (4–6). AF was defined as the presence of an irregular rhythm with fibrillatory waves and no defined P-waves for at least 30 s during rhythm monitoring. Other SVTs were defined as regular rhythm when atrial and/or ventricular rates exceeded 100 bpm for at least 30 s during monitoring, consistent with atrial flutter, focal atrial tachycardia, atrioventricular nodal tachycardia, or atrioventricular tachycardia. Non-sustained ventricular tachycardia was defined as three or more consecutive ventricular beats occurring at a rate of ≥100 bpm and sustained ventricular tachycardia lasting ≥30 s. High grade atrioventricular block was defined as the presence of second- or third-degree heart block. Bradyarrhythmia absoluta was defined as the presence of an irregular rhythm with fibrillatory waves and no defined P-waves as well as heart rate <40/min for at least 30 s. Asystole was defined as the absence of electrical activity during rhythm monitoring lasting >6 s. New-onset AF was defined as AF during ICU monitoring in the absence of AF history, as indicated by the medical record of the patient.

Diagnosis of thromboembolic/thrombotic events including pulmonary embolism, thromboembolic stroke, and transient ischemic attack was established in agreement with current guidelines (7, 8). The diagnosis of thromboembolic stroke and transient ischemic attack of thromboembolic origin was verified by an experienced neurologist. Acquired data were independently reviewed and entered into the computer database by two blinded analysts. During ICU stay all recruited patients received standard prophylactic anticoagulation or therapeutic anticoagulation (TAC), if indicated, using low molecular weight heparin.

Statistical Analysis

Statistical analysis was conducted using R (version 4.0.2., R Core Team (2013), R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org/) using the packages "MatchIt," "optmatch" and "RItools," "stddiff," and also SPSS (Version 23.0, IBM, Armonk, New York, USA), and was carried out blindly by our statistical analytic team. Descriptive

TABLE 2 | Continuous rhythm monitoring during ICU stay.

	COVID-19 ($n = 60$)		Non-COVID-19 ($n = 60$)		p
	n	Median (Q3–Q1) or %	*n*	Median (Q3–Q1) or %	
Supraventricular tachyarrhythmias					
AF during ICU stay	11/60	18.3%	26/60	43.3%	0.003*
New-onset of AF	6/60	10.0%	18/60	30.0%	0.006*
Duration of total AF burden (minutes)	60	780.0 (1,680.0)	60	960.0 (4,035.0)	0.855
Other SVTs[$]	5/60	8.3%	8/60	13.3%	0.378
Ventricular tachyarrhythmias					
nsVT	4/60	6.7%	4/60	6.7%	>0.999
Sustained VT or VF	2/60	3.3%	1/60	1.7%	>0.999
Bradyarrhythmias					
High grade AVB[§]	0/60	0%	0/60	0%	>0.999
Asystole	3/60	5.0%	2/60	3.3%	>0.999
Bradyarrhytmia absoluta	0/60	0%	1/60	1.7%	>0.999
eCV	4/60	6.7%	12/60	20.0%	0.029*
Reason for eCV					
AF	3/60	5.0%	10/60	16.6%	0.040*
Other SVTs[$]	0/60	0%	1/60	1.7%	>0.999
Sustained VT or VF	1/60	1.7%	1/60	1.7%	>0.999

AF, atrial fibrillation; AVB, atrioventricular block; ICU, intensive care unit; eCV, electrical cardioversion; nsVT, non-sustained ventricular tachycardia; SVT, supraventricular tachycardia; VF, ventricular fibrillation; VT, ventricular tachycardia; [$]definition other SVT see method section; [§] For definition of high grade AVB see Method section;*$p < 0.05$.

statistics were obtained for all study variables. All categorical variables were compared by using the Fisher exact test. Ordinal data are presented as median (interquartile range [IQR]). Median values were compared using the Mann–Whitney-U test. Normal distribution of continuous variables was tested using the Kolmogorov–Smirnov test. According to results, continuous variables were compared using the independent student t-test or the Mann-Whitney U test, as appropriate. Continuous data are expressed as mean and standard deviation (SD) or median (interquartile range [IQR]) values. A $p < 0.05$ was regarded as statistically significant. Covariate imbalance was assessed by calculating standardized differences for the covariates age, gender, coronary artery disease, valvular heart disease, arterial hypertension, diabetes mellitus, atrial fibrillation, atrial flutter, stroke, and heart failure, and also by calculating an omnibus test and significant differences between the two investigated groups using Wilcoxon rank-sum test and χ^2 test."

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination of our research.

RESULTS

With regards to the assessment of covariate imbalance, standardized differences and omnibus test ($p = 0.556$) revealed no statistically significant differences between the two investigated groups (standardized differences >0.25 were considered significant covariate imbalance) (**Supplementary Table 6**).

The baseline characteristics of both patient cohorts are presented in **Table 1**. According to matching criteria, the same rates of heart failure, coronary artery disease, and paroxysmal AF were present in both groups at inclusion. Similarly, no significant differences were observed with regards to other comorbidities and predisposing risk factors for cardiac arrhythmias including arterial hypertension, diabetes mellitus and relevant valvular heart disease as well as sex and gender. No significant differences with regards to antiarrhythmics were observed (**Table 1**). Origin of pneumonia in the control group is depicted in **Supplementary Table 4**.

The analyses of the continuous rhythm monitoring during the ICU stay are presented in **Table 2** and **Figure 1**. Additionally, a separate analysis of patients displaying a QTc-time over 500 ms in the admission ECG is depicted in **Supplementary Table 5**. Expectedly, COVID-19 presented a high rate of cardiac arrhythmias. Nevertheless, when matched to non-COVID-19, rates of relevant ventricular tachyarrhythmias were similar (**Table 1**; **Figure 1D**). With regards to bradyarrhythmias, there was no significant difference in the incidence of high grade AVBs or asystole (**Table 1**; **Figure 1E**). Although the rates of AF diagnosed by 12-lead ECG at admission were similar in both groups (**Supplementary Table 1**), the incidence of AF during rhythm-monitoring was significantly higher in the non-COVID-19 population despite comparable risk factors for the development of arrhythmias. This was reflected by higher rates of AF during ICU stay, but similar AF duration during the monitoring period in affected patients was observed. The higher rates of AF also corresponded to a significantly

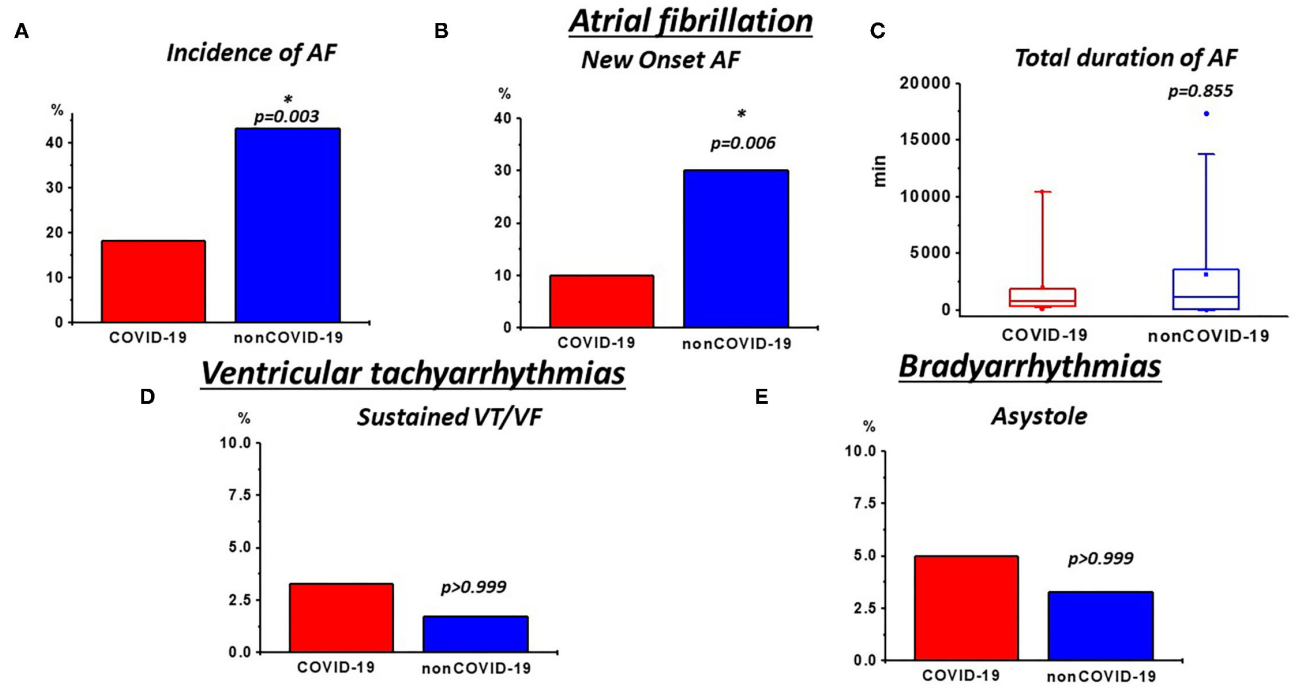


FIGURE 1 | Incidence of relevant cardiac arrhythmias during intensive care (ICU) rhythm monitoring in COVID-19 vs. non-COVID-19: **(A)** incidence of atrial fibrillation (AF) and **(B)** newly diagnosed AF was high in both groups. However, non-COVID-19 patients presented a higher burden of AF and newly diagnosed AF, **(C)** while the total duration of AF was not different in affected patients. **(D)** The incidence of sustained VTs/ventricular fibrillation (VF) was similar in both groups **(E)** and the frequency of asystole was also not significantly different. *$p < 0.050$.

higher necessity for electrical cardioversion in the non-COVID-19 group (**Table 2**). Furthermore, the incidence of newly diagnosed AF was significantly higher in non-COVID-19 indicating a more pronounced arrhythmic substrate in this population.

With regards to inflammatory activity and disease severity, non-COVID-19 revealed higher leucocytes and procalcitonin (PCT) levels. This was further accompanied by increased lactate levels and decreased pH (**Table 4**) in non-COVID-19. Consequently, while mortality was high in both groups, a significantly higher rate in the non-COVID-19 group was observed (**Table 3**), indicating a more pronounced critical patient status.

In contrast to these observations and in line with previous reports (9), we observed a higher rate of pulmonary embolisms in COVID-19 (**Table 3**). This observation was consistent with high stroke rates in COVID-19. Of note, despite a lower burden of AF as well as similarly high rates of anticoagulation and comparable CHA_2DS_2-Vasc scores, a significantly higher incidence of thrombotic strokes/TIA was revealed (**Table 3**; **Figure 2**). Of note, these events were also observed in patients receiving TAC and with continuous sinus rhythm (**Figure 2**; **Supplementary Table 2**), indicating disease-specific events that occur independently of cardiac arrhythmias.

DISCUSSION

The typical finding in severe COVID-19 disease is pneumonia accompanied by acute lung injury (10). In this context, many recent studies covered the topic of COVID-19-related cardiac injury (11, 12). Nevertheless, despite described cases of COVID-19 specific myocarditis, recent publications in critically ill patients, indicated that in this population cardiac injury is rather explained by the high inflammatory burden, similar to cardiac injury in other severe inflammatory processes such as in acute respiratory distress syndrome and severe pneumonia (11, 12). A further point of interest is the burden of arrhythmias in COVID-19 patients. Only a few studies on this topic have been published, pointing toward a high arrhythmic burden in this patient collective (13, 14). Nevertheless, comparable with findings on myocardial injury, arrhythmias and especially AF are a frequent finding in patients with severe pneumonia and sepsis (15). Accordingly, the present study aimed to further evaluate this issue.

To account for underlying medical conditions predisposing to cardiac arrhythmias, the study cohorts were matched for preexisting AF as well as age, gender, coronary artery disease, valvular heart disease, arterial hypertension, diabetes mellitus, atrial fibrillation, atrial flutter, stroke, and heart failure. Reliability of the matching process was further confirmed by analyzing

TABLE 3 | Patients' outcome and relevant therapies during ICU stay.

	COVID-19 ($n = 60$)		Non-COVID-19 ($n = 60$)		p
	n	Median (Q3–Q1) or %	*n*	Median (Q3–Q1) or %	
Outcome ICU					
Death	21/60	35.0%	34/60	56.7%	0.017*
Discharged from ICU	39/60	65.0%	26/60	43.3%	0.017*
Duration of ICU stay (days)	60	13.0 (18.0)	60	11.5 (17.0)	0.308
Required ICU therapy					
ECMO	9/60	15.0%	14/60	23.3%	0.246
Hemofiltration	17/60	28.3%	25/60	41.7%	0.126
Catecholamines	45/60	75.0%	53/60	88.3%	0.059
Required catecholamines	60	1.0 (1.0)	60	1.0 (1.0)	0.640
Ventilation therapy					
NIV	9/60	15.0%	7/60	11.7%	0.591
Intubation	51/60	85.0%	53/60	88.3%	0.591
Duration of intubation	60/60	9.0 (20.0)	60/60	5.0 (10.0)	0.711
Relevant bleedings	4/60	6.7%	4/60	6.7%	>0.999
CPR	4/60	6.7%	6/60	10.0%	0.509
Reason for CPR					
Asystole	1/60	1.7%	3/60	5.0%	0.619
VF/VT	1/60	1.7%	1/60	1.7%	>0.999
Pulseless electrical activity	2/60	3.3%	2/60	3.3%	>0.999
Therapeutic anticoagulation	24/60	40.0%	30/60	50.0%	0.271
Thrombosis/thromboembolic events					
Pulmonary embolism	10/60	16.7%	2/60	3.3%	0.015*
Peripheral thrombosis/thromboembolism	3/60	5.0%	5/60	8.3%	0.717
Stroke/TIA	4/60	6.7%	0/60	0%	0.042*

CPR, cardiopulmonary resuscitation; ECMO, extracorporal membrane oxygenation; ICU, intensive care unit; NIV, non-invasive ventilation; TIA, transient ischemic attack; VF, ventricular fibrillation; VT, ventricular tachycardia.
*$p < 0.05$.

covariate imbalance between the two investigated, showing no significant differences.

In contrast to former studies conducted on arrhythmias in COVID-19, the present project included monitoring data on arrhythmias for the entire ICU-stay in addition to standard 12-lead ECGs, thus allowing for a more precise analysis of the arrhythmic burden. To avoid potential interference of novel treatment options, such as dexamethasone, with our findings, patients were recruited during the first wave of the pandemic before publication of the "RECOVERY Trial in July 2020." The impact of COVID-19 disease on myocardial arrhythmias might be better reflected through this approach, since outcomes are not influenced by this treatment regime, which is now routinely applied in the involved study centers. Accordingly, COVID-19 specific therapy is low in the present patient collective as it was mostly experimental during this investigated period.

In the COVID-19 group, AF was the most frequent arrhythmia and was observed in 18.3% of all patients (**Table 2**; **Figure 1A**). It is in line with a recent publication by Bertini et al. (14), which reported an AF rate of about 22% in a similar patient collective, documented by ECG at hospital admission. Of note, the mean patient age in that study tended to be higher compared with our collectives, which might explain the slightly higher AF rate (14). Interestingly, the AF burden in the non-COVID-19 group in our study was even higher, ranging at around 43% and requiring a higher need for electrical cardioversion (**Table 2**; **Figure 1A**). This was also reflected by an increased incidence of new-onset of AF (**Table 2**; **Figure 1B**), indicating a more pronounced proarrhythmic substrate in this population. Of note, the rates of AF in our control group are in line with former studies conducted on AF in sepsis and septic shock, with rates of new onset of AF ranging between 7% and 46%, depending on disease severity (16, 17). Apart from AF, rates of other supraventricular and also ventricular tachyarrhythmias and relevant bradyarrhythmias with consequent need for cardiopulmonary resuscitation were similar in both COVID-19 and non-COVID-19 patients (**Tables 2**, **3**; **Figures 1D,E**). With respect to ventricular arrhythmias, one has to keep in mind the comparably low amount of heart failure (11.7%) and coronary artery disease (15%) in our patient collective resulting in a low percentage of patients with a predisposing myocardial substrate, which could facilitate ventricular tachycardias (VT).

Since inflammatory processes are known to increase the vulnerability for arrhythmias (15, 18), the higher inflammatory

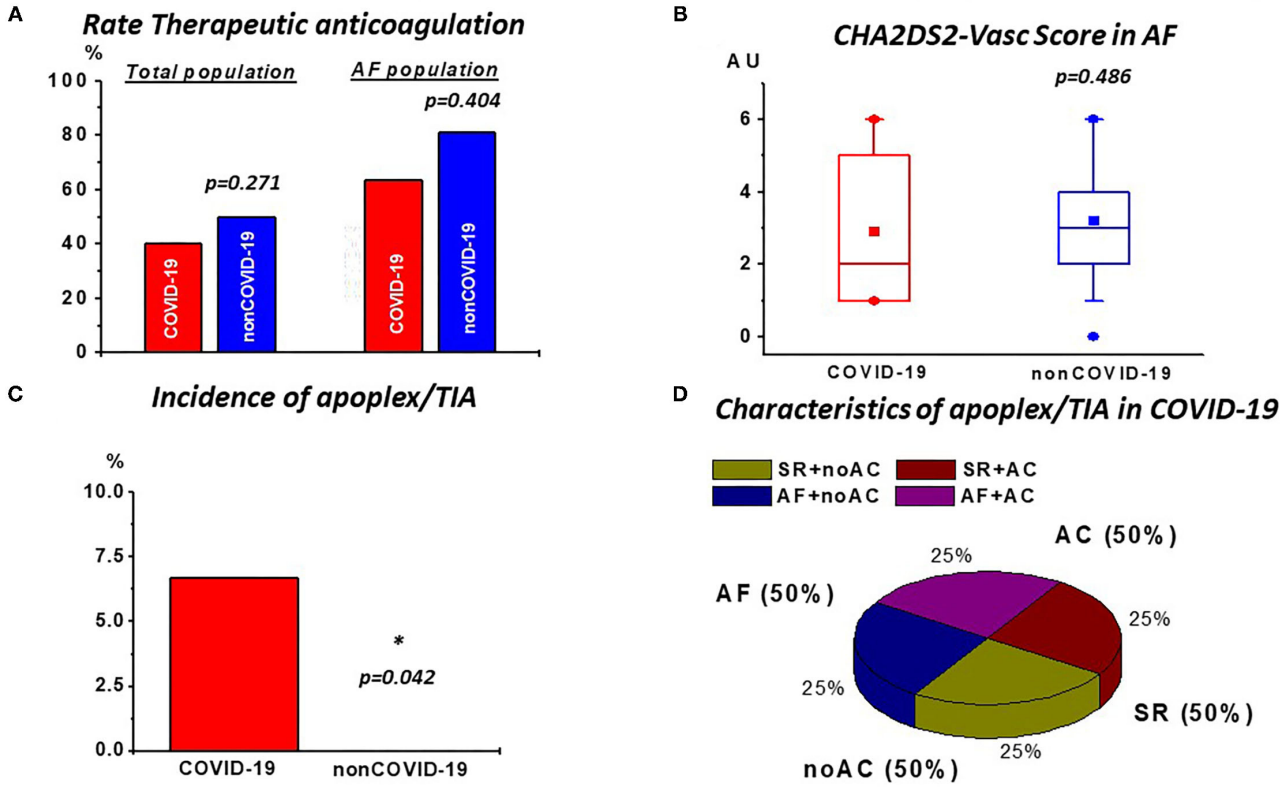


FIGURE 2 | Preventive strategies and characteristics of stroke/TIA events: **(A)** despite high but not different rates of therapeutic anticoagulation (TAC) in the total population and in patients with atrial fibrillation (AF) during rhythm monitoring as well as **(B)** similar CHA2DS2-Vasc Scores in this subgroup, **(C)** incidence of stroke/TIA events was significantly higher in COVID-19. **(D)** These events were also observed with AC (50%) and with continuous sinus rhythm (SR; 50%) during rhythm monitoring. noAC, no application of TAC. *$p < 0.05$.

burden and disease severity in the non-COVID-19 group, reflected by higher levels of leucocytes, PCT, lactate, and also lower pH levels with consequent higher mortality rates in non-COVID-19 (**Tables 3**, **4**), represents an important factor in this regard (15). Consequently, one might speculate that similar to other critically ill patients, in COVID-19, cardiac arrhythmias are primarily driven by inflammatory processes and disease burden, rather than by disease-specific effects of COVID-19.

While no significant increase of arrhythmias in the COVID-19 cohort was evident, thromboembolic events showed a significant increase compared with non-COVID-19 patients. This is reflected by a higher incidence of pulmonary embolism and stroke/TIA in our COVID-19 cohort (**Table 3**). Accordingly, this finding suggests a COVID-19 specific thromboembolic effect independent of arrhythmic burden. In AF, the most common observed arrhythmia in our study, TAC, is recommended according to preexisting risk factors with a high risk of thromboembolic strokes (6). This therapy is known to be efficient as indicated in our non-COVID-19 cohort with no stroke stoke/TIA events despite a higher incidence of AF (**Table 2**) but also with a high rate of TAC (**Figure 2A**). Whether in COVID-19, AF and the associated preexisting risk factors might further drive thromboembolic events, is still a matter of debate. Concerning our results, this seems questionable since the rate of neurologic events was higher in COVID-19 despite a lower incidence of AF, comparable CHA2DS2-Vasc scores (**Figures 2B,C**) and also the appearance of these events in patients with continuous sinus rhythm during monitoring (**Figure 2D**). This emphasizes the need for effective prevention strategies in critically ill COVID-19. However, in our study, the rate of TAC in critically COVID-19 was comparable with non-COVID-19, despite the lower rate of AF (**Figure 2A**). It could be argued, that, given the high incidence of thromboembolic events, more, if not all critical COVID-19 patients should receive effective anticoagulation. While a mortality benefit seems to be associated with anticoagulatory treatment in COVID-19, the clinical evidence for efficacy and safety of such an approach is a topic for ongoing investigations (19, 20). Importantly, we observed thrombotic/thromboembolic neurological events despite sufficient TAC (**Figure 2D**; **Supplementary Table 2**), indicating TAC to be probably less effective in this population. Thus, taken together our data emphasize that thromboembolic events seem to be a disease-specific in severe COVID-19 patients unrelated to the presence of arrhythmias.

TABLE 4 | Relevant laboratory markers during ICU stay.

	COVID-19 ($n = 60$)		Non-COVID-19 ($n = 60$)		p
	n	Median (Q3–Q1)	n	Median (Q3–Q1)	
Lactate (U/L)	60	2.6 (2.1)	60	3.5 (4.8)	0.017*
Min. pH	60	7.19 (0.1)	60	7.13 (0.1)	0.045*
Creatinine (mg/dl)	60	1.7 (2.1)	60	2.3 (2.6)	0.404
Min potassium (mmol/L)	60	3.4 (0.4)	60	3.3 (0.5)	0.720
Leukocytes (10^9/L)	60	14.8 (11.5)	60	20.2 (11.8)	0.002*
Min. lymphocytes (10^9/L)	60	4.4 (6.6)	45	4.9 (6.5)	0.712
CRP (ng/ml)	59	25.5 (17.7)	60	28.2 (15.4)	0.493
PCT (ng/ml)	60	1.9 (5.1)	57	3.0 (17.9)	0.013*
Interleukin 6 (pg/ml)	53	513.8 (2,395.2)	23	394.8 (1,080.6)	0.923
Fibrinogen (mg/dl)	34	672.5 (298)	54	602.5 (270.0)	0.175

CRP, C-reactive protein; Min., lowest level of laboratory biomarker obtained during the total period of ICU stay; PCT, procalcitonin. Relevant laboratory findings obtained during intensive care unit (ICU) stay. If not other indicated, the highest obtained value during the whole period of ICU stay is presented.
*$p < 0.05$.

LIMITATIONS

The present study has by design its limitations, while contributing novel clinical findings. Our sample size may be too small to detect differences in arrhythmias with low incidence, such as ventricular tachyarrhythmias and bradyarrhythmias. The heterogeneity of our comparison group, which consists of patients suffering from pneumonia of diverse origin might in part differ with regards to the pathogenetic mechanisms compared with COVID-19 pneumonia. Thus, the findings of the present study have to be considered as hypothesis generating. The passionate use of untested treatments in a number of COVID-19 patients (such as tocilizumab or hydroxychloroquine, **Supplementary Table 3**) might have affected the results, especially concerning arrhythmia burden due to effects on QT interval. However, while QT prolongation is suspected to promote this issue, QTc in our COVID-19 cohort was in the normal range with shorter QTc compared with non-COVID-19 (**Supplementary Table 1**). Instead of screening, diagnostic workups for thromboembolic events were only performed when clinically suspected and therefore, they are probably underestimated.

In summary, AF is common in severe COVID-19, but we found it to be less frequent than in severe pneumonia of non-COVID-19 origin. Arrhythmia might be mainly attributed to a high inflammatory activity and disease severity, instead of a COVID-19 specific mechanism. The contrasting higher incidence of stroke, despite the lower rate of AF, seems to be a disease-specific feature of critical COVID-19, consistent with high rates of pulmonary embolisms. Further research will hopefully clarify the potential role of TAC to prevent thromboembolic events, which are independent of AF.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University Hospital Münster: Nr. 2020-306-f-S, Maria Hilf Hospital Mönchengladbach: Nr. 143/2020, and University Hospital Salzburg: Nr. 1071/2020. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.763827/full#supplementary-material

REFERENCES

1. Alhazzani W, Møller MH, Arabi YM, Loeb M, Gong MN, Fan E, et al. Surviving sepsis campaign: guidelines on the management of critically ill adults with coronavirus disease 2019 (COVID-19). *Intensive Care Med.* (2020) 46:854–87. doi: 10.1007/s00134-020-06022-5
2. Rubin GD, Ryerson CJ, Haramati LB, Sverzellati N, Kanne JP, Raoof S, et al. The role of chest imaging in patient management during the COVID-19 pandemic: a multinational consensus statement from the Fleischner Society. *Radiology.* (2020) 7:2020201365. doi: 10.1148/radiol.2020201365
3. Papazian L, Aubron C, Brochard L, Chiche JD, Combes A, Dreyfuss D, et al. Formal guidelines: management of acute respiratory distress syndrome. *Ann Intensive Care.* (2019) 9:019–0540. doi: 10.1186/s13613-019-0540-9

4. Boriani G, Fauchier L, Aguinaga L, Beattie JM, Blomstrom Lundqvist C, Cohen A, et al. European Heart Rhythm Association (EHRA) consensus document on management of arrhythmias and cardiac electronic devices in the critically ill and post-surgery patient, endorsed by Heart Rhythm Society (HRS), Asia Pacific Heart Rhythm Society (APHRS), Cardiac Arrhythmia Society of Southern Africa (CASSA), and Latin American Heart Rhythm Society (LAHRS). *Europace.* (2019) 21:7–8. doi: 10.1093/europace/euy110
5. Cronin EM, Bogun FM, Maury P, Peichl P, Chen M, Namboodiri N, et al. 2019 HRS/EHRA/APHRS/LAHRS expert consensus statement on catheter ablation of ventricular arrhythmias. *Europace.* (2019) 21:1143–4. doi: 10.1093/europace/euz132
6. Katritsis DG, Boriani G, Cosio FG, Hindricks G, Jaïs P, Josephson ME, et al. European Heart Rhythm Association (EHRA) consensus document on the management of supraventricular arrhythmias, endorsed by Heart Rhythm Society (HRS), Asia-Pacific Heart Rhythm Society (APHRS), and Sociedad Latinoamericana de Estimulación Cardiaca y Electrofisiologia (SOLAECE). *Europace.* (2017) 19:465–511. doi: 10.1093/europace/euw301
7. Konstantinides SV, Meyer G, Becattini C, Bueno H, Geersing GJ, Harjola VP, et al. 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS). *Eur Heart J.* (2020) 41:543–603. doi: 10.1093/eurheartj/ehz405
8. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. 2018 guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke.* (2018) 49:e46–110. doi: 10.1161/STR.0000000000000158
9. Klok FA, Kruip M, van der Meer NJM, Arbous MS, Gommers D, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res.* (2020) 191:145–7. doi: 10.1016/j.thromres.2020.04.013
10. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med.* (2020) 382:1708–20. doi: 10.1056/NEJMoa2002032
11. Jirak P, Larbig R, Shomanova Z, Fröb EJ, Dankl D, Torgersen C, et al. Myocardial injury in severe COVID-19 is similar to pneumonias of other origin: results from a multicentre study. *ESC Heart Fail.* (2020) 17:13136. doi: 10.1002/ehf2.13136
12. Metkus TS, Sokoll LJ, Barth AS, Czarny MJ, Hays AG, Lowenstein CJ, et al. Myocardial injury in severe COVID-19 compared to non-COVID acute respiratory distress syndrome. *Circulation.* (2020) 13:050543. doi: 10.1161/CIRCULATIONAHA.120.050543
13. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA.* (2020) 7:1585. doi: 10.1001/jama.2020.1585
14. Bertini M, Ferrari R, Guardigli G, Malagù M, Vitali F, Zucchetti O, et al. Electrocardiographic features of 431 consecutive, critically ill COVID-19 patients: an insight into the mechanisms of cardiac involvement. *EP Europace.* (2020) 22:1848–54. doi: 10.1093/europace/euaa258
15. Shahreyar M, Fahhoum R, Akinseye O, Bhandari S, Dang G, Khouzam RN. Severe sepsis and cardiac arrhythmias. *Ann Transl Med.* (2018) 6:26. doi: 10.21037/atm.2017.12.26
16. Meierhenrich R, Steinhilber E, Eggermann C, Weiss M, Voglic S, Bögelein D, et al. Incidence and prognostic impact of new-onset atrial fibrillation in patients with septic shock: a prospective observational study. *Crit Care.* (2010) 14:10. doi: 10.1186/cc9057
17. Walkey AJ, Hammill BG, Curtis LH, Benjamin EJ. Long-term outcomes following development of new-onset atrial fibrillation during sepsis. *Chest.* (2014) 146:1187–95. doi: 10.1378/chest.14-0003
18. Boos CJ. Infection and atrial fibrillation: inflammation begets AF. *Eur Heart J.* (2020) 41:1120–22. doi: 10.1093/eurheartj/ehz953
19. Hadid T, Kafri Z, Al-Katib A. Coagulation and anticoagulation in COVID-19. *Blood Rev.* (2021) 47:8. doi: 10.1016/j.blre.2020.100761
20. McBane RD. 2nd, Torres Roldan VD, Niven AS, Pruthi RK, Franco PM, Linderbaum JA, et al. Anticoagulation in COVID-19: a systematic review, meta-analysis, and rapid guidance from Mayo clinic. *Mayo Clin Proc.* (2020) 95:2467–86. doi: 10.1016/j.mayocp.2020.08.030

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 24 November 2021
doi: 10.3389/fcvm.2021.775115

Cardiac Care of Non-COVID-19 Patients During the SARS-CoV-2 Pandemic: The Pivotal Role of CCTA

*Edoardo Conte[1,2], Saima Mushtaq[2], Maria Elisabetta Mancini[2], Andrea Annoni[2], Alberto Formenti[2], Giuseppe Muscogiuri[2], Margherita Gaudenzi Asinelli[2], Carlo Gigante[2], Carlos Collet[3], Jeroen Sonck[3,4], Marco Guglielmo[2], Andrea Baggiano[2], Nicola Cosentino[2], Marialessia Denora[2], Marta Belmonte[2], Cecilia Agalbato[2], Andrea Alessandro Esposito[5], Emilio Assanelli[2], Antonio L. Bartorelli[2,6], Mauro Pepi[2], Gianluca Pontone[2] and Daniele Andreini[2,7]**

[1] Department of Biomedical Sciences for Health, University of Milan, Milan, Italy, [2] Centro Cardiologico Monzino, Istituto di Ricerca e Cura a Carattere Scientifico, Milan, Italy, [3] Cardiovascular Center Aalst, Onze-Lieve-Vrouwziekenhuis Hospital, Aalst, Belgium, [4] Department of Advanced Biomedical Sciences, Federico II University of Naples, Naples, Italy, [5] Foundation Istituto di Ricerca e Cura a Carattere Scientifico Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy, [6] Department of Biomedical and Clinical Sciences "Luigi Sacco", University of Milan, Milan, Italy, [7] Department of Clinical Sciences and Community Health, Cardiovascular Section, University of Milan, Milan, Italy

Aim: The aim of this study is to evaluate the potential use of coronary CT angiography (CCTA) as the sole available non-invasive diagnostic technique for suspected coronary artery disease (CAD) during the coronavirus disease 2019 (COVID-19) pandemic causing limited access to the hospital facilities.

Methods and Results: A consecutive cohort of patients with suspected stable CAD and clinical indication to non-invasive test was enrolled in a hub hospital in Milan, Italy, from March 9 to April 30, 2020. Outcome measures were obtained as follows: cardiac death, ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI), and unstable angina. All the changes in medical therapy following the result of CCTA were annotated. A total of 58 patients with a mean age of 64 ± 11 years (36 men and 22 women) were enrolled. CCTA showed no CAD in 14 patients (24.1%), non-obstructive CAD in 30 (51.7%) patients, and obstructive CAD in 14 (24.1%) patients. Invasive coronary angiography (ICA) was considered deferrable in 48 (82.8%) patients. No clinical events were recorded after a mean follow-up of 376.4 ± 32.1 days. Changes in the medical therapy were significantly more prevalent in patients with vs. those without CAD at CCTA.

Conclusion: The results of the study confirm the capability of CCTA to safely defer ICA in the majority of symptomatic patients and to correctly identify those with critical coronary stenoses necessitating coronary revascularization. This characteristic could be really helpful especially when the hospital resources are limited

Keywords: atherosclerosis, COVID-19, cardiac CT, chest pain, coronary artery disease

OPEN ACCESS

Edited by:
Hendrik Tevaearai Stahel, Bern University Hospital, Switzerland

Reviewed by:
U. Joseph Schoepf, Medical University of South Carolina, United States
Filippo Cademartiri, Gabriele Monasterio Tuscany Foundation (CNR), Italy

***Correspondence:**
Daniele Andreini
daniele.andreini@ccfm.it

Specialty section:
This article was submitted to Cardiovascular Imaging, a section of the journal Frontiers in Cardiovascular Medicine

Received: *13 September 2021*
Accepted: *15 October 2021*
Published: *24 November 2021*

Citation:
Conte E, Mushtaq S, Mancini ME, Annoni A, Formenti A, Muscogiuri G, Gaudenzi Asinelli M, Gigante C, Collet C, Sonck J, Guglielmo M, Baggiano A, Cosentino N, Denora M, Belmonte M, Agalbato C, Esposito AA, Assanelli E, Bartorelli AL, Pepi M, Pontone G and Andreini D (2021) Cardiac Care of Non-COVID-19 Patients During the SARS-CoV-2 Pandemic: The Pivotal Role of CCTA. Front. Cardiovasc. Med. 8:775115. doi: 10.3389/fcvm.2021.775115

INTRODUCTION

The coronavirus disease 2019 (COVID-19) has rapidly and dramatically changed everyday life across the entire planet in an unprecedented way (1). In Italy, the first patient was presented at the end of February 2020 and was diagnosed nearby the metropolitan city of Milan in Lombardy, a region in the north of Italy (2). On March 7, 2020, almost all regions of northern Italy were locked down after the surge of the SARS-CoV-2 pandemic, and the public national healthcare system has been reorganized as a hub-and-spoke network (3). On Monday, March 9, 2020, the Centro Cardiologico Monzino, usually dedicated to cardiovascular care, was elected as a regional hub for cardiovascular emergencies, and all the non-urgent activities were suspended until April 30, 2020 (4).

Chest pain is a very common symptom that may subtend a wide range of clinical entities from non-cardiovascular and benign conditions to the acute coronary syndrome. Physical examination and rest ECG are the first steps in the clinical evaluation, but coronary artery disease (CAD) cannot be excluded in the patients with suspect symptoms by clinical assessment alone. Non-invasive diagnostic tests are recommended to establish the diagnosis and risk-stratify the patients (5). Before March 2020, the last version of ESC Guidelines on the management of chronic CAD recommended CCTA, stress cardiac magnetic resonance, and stress echocardiography at the same level of appropriateness (6–8). With the advent of the COVID-19 pandemic, cardiologists suddenly had to tackle a critical problem, namely, limited access to cardiovascular care and resources.

When compared to the pre-COVID era, during the first pandemic peak, in March 2020, non-invasive ischemic exercise/stress tests were not available in our center due to the extraordinary need to reorganize hospital activities and to the several concerns regarding the potential higher risk of contagion during exercise tests (due to hyperventilation and low-interpersonal distances without wide availability of the face mask and nasopharyngeal swab). Thus, CCTA was the sole test for patients with suspected CAD that remain available in a non-acute setting, even during the most severe first peak of the SARS-CoV-2 pandemic.

Thus, the aim of this manuscript is to describe the diagnostic and prognostic role that CCTA had in our hospital as the sole non-invasive diagnostic test for symptomatic patients with suspected stable CAD during an emergency pandemic when access to hospital facilities was limited.

MATERIALS AND METHODS

From March 9 to April 30th, during the peak of the COVID-19 pandemic, a consecutive cohort of the patients with high clinical suspicion of stable CAD and who underwent CCTA was enrolled in our cardiovascular dedicated hub hospital. It should be underlined that all the patients with highly suspected, but unknown, CAD evaluated at our center from March 9th and April 30 underwent CCTA as it was the only non-invasive test available for the suspected stable CAD in a non-acute setting, and invasive coronary angiography was almost entirely dedicated to the patients with the acute coronary syndrome. All the patients were evaluated for the presence of traditional cardiovascular risk factors, such as diabetes mellitus (glucose level of $>$ 7 mmol/l, or the need for insulin, or oral hypoglycemic agents), hypercholesterolemia (total cholesterol level $>$ 5 mmol/l or treatment with lipid-lowering drugs), hypertension (blood pressure $>$ 140/90 mmHg or use of antihypertensive medications), positive family history of CAD [presence of CAD in the first-degree relatives younger than 55 years (male) or 65 years (female)], and currently smoking (5). All the patients provided written informed consent, and the local ethics committee approved the study.

Patients underwent CCTA with a new generation 256-slice CT scanner (Revolution CT, GE Healthcare, Milwaukee, WI, USA) that was performed according to updated international guidelines (9, 10) with the following parameters: slice configuration 256 $\times$ 0.625 mm, gantry rotation time 280 ms, and prospective ECG triggering. Tube current and tube voltage were adapted to BMI. Patients received a 50 ml (for BMI $\leq$ 25 kg/m^2) or 60 ml (for BMI $>$ 25 kg/m^2) bolus of contrast medium (Iomeron 400 mg/ml, Bracco, Milan, Italy). All the patients received sublingual nitrates and betablockers (up to 25 mg of the intravenous metoprolol) before the CT scan.

Datasets of CCTA images were analyzed using vessel analysis software (CardioQ3 Package-GE Healthcare, Milwaukee, WI, USA). Reconstructed images were evaluated independently by two readers, both with over 10 years of clinical experience in the CCTA performance. Coronary arteries were divided into 16 segments according to the American Heart Association classification (11). In the case of motion artifacts with standard reconstruction, an additional reconstruction using an intracycle motion correction algorithm (a vendor-specific algorithm) was performed and analyzed. In case of image quality improvement after motion correction, the reconstructed image was used for analysis. Coronary segments were evaluated for the presence of critical stenoses, defined as coronary lumen narrowing exceeding 90%, and for the obstructive stenoses, defined as coronary lumen narrowing exceeding 50% (12). The presence of non-obstructive (from 0 to 50% stenosis) stenoses was recorded as well. For any disagreement in data analysis between the two readers, consensus agreement was achieved.

When a clinical significant coronary stenosis (defined as $>$70% stenosis on a proximal coronary segment or $>$90% stenosis on any coronary segment) was detected at CCTA, the referring physician (cardiologist) was informed and, if the clinically indicated, an invasive coronary angiography (ICA) was scheduled (**Figure 1**). As routinely performed, myocardial revascularization for the coronary lesion $<$90% stenosis was performed only after invasive fraction flow reserve (FFR) resulted in being $<$0.8. Clinical follow-up was recorded by telephone interview, and medical records were screened for the patients in whom ICA was considered deferrable or not indicated after CCTA. Outcome measures were obtained as follows: cardiac death, ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI), and unstable angina defined according to ESC guidelines (13).

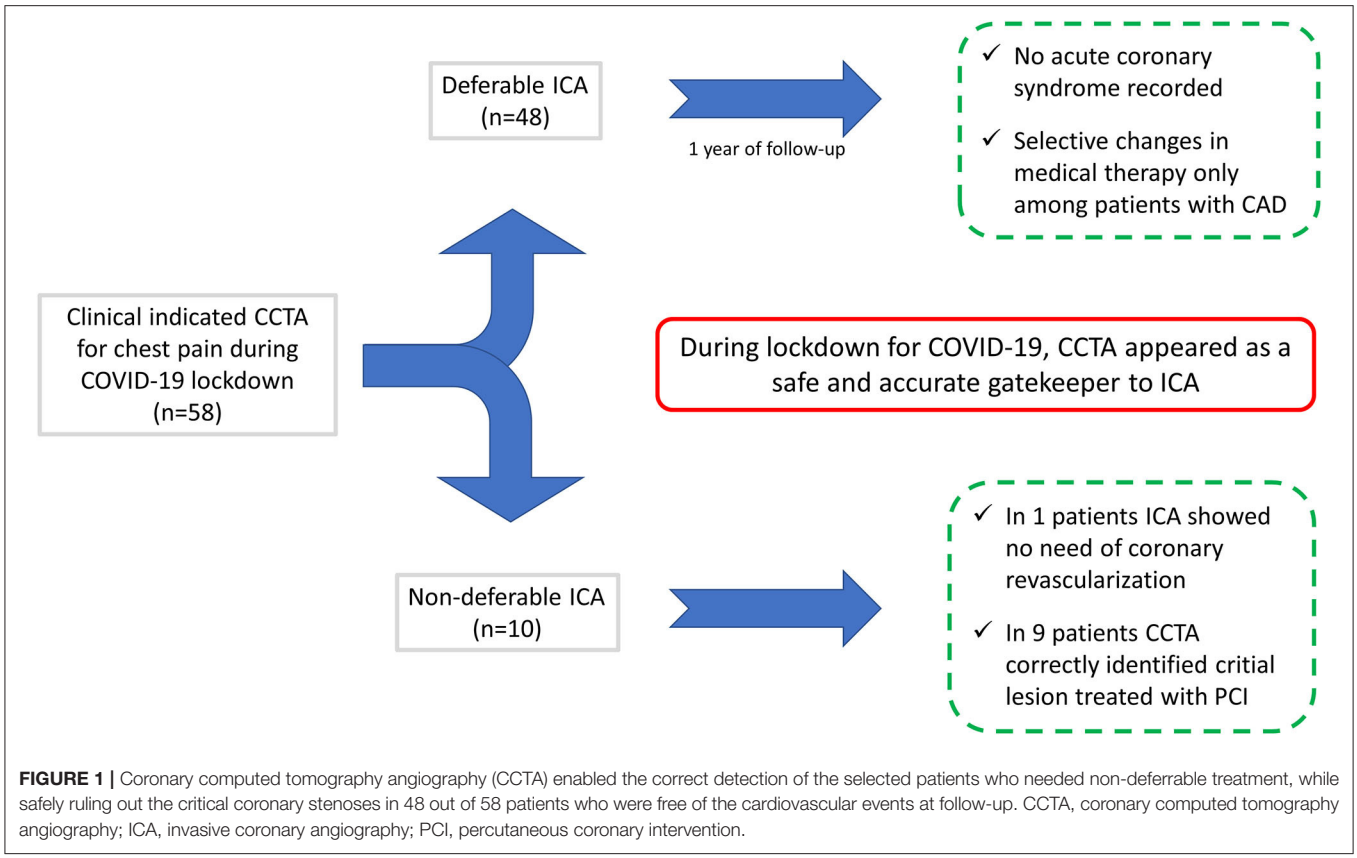


FIGURE 1 | Coronary computed tomography angiography (CCTA) enabled the correct detection of the selected patients who needed non-deferrable treatment, while safely ruling out the critical coronary stenoses in 48 out of 58 patients who were free of the cardiovascular events at follow-up. CCTA, coronary computed tomography angiography; ICA, invasive coronary angiography; PCI, percutaneous coronary intervention.

All the changes in medical therapy following the result of CCTA were annotated.

The effective dose (ED) of CCTA was calculated according to the European Working Group for Guidelines on Quality Criteria in CT. The dose-length product (DLP) was measured in mGy × cm in each patient. The ED was calculated as the DLP times a conversion coefficient for the chest ($K = 0.014$ mSv/mGy × cm) (14).

STATISTICAL ANALYSIS

Continuous variables were expressed as mean ± SD and discrete variables as absolute numbers and percentages. The Student's *t*-test was used to test differences in continuous variables between the two groups, and the chi-squared test or Fisher's exact test was used to assess differences regarding categorical data. Statistical significance was defined as a $p < 0.05$. Statistical analysis was performed using MedCalc Statistical Software version 19.2.1 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2020).

RESULTS

A total of 58 patients with a mean age of 64 ± 11 years (36 men and 22 women) were enrolled during the lockdown period for the COVID-19 pandemic. None of the patients suffered fever or respiratory symptoms suggestive of the SARS-CoV-2 infection. One patient showed bilateral ground-glass lung alterations on CCTA presumably due to the recent asymptomatic COVID-19 infection. Subsequent nasopharyngeal swab resulted negative for the SARS-CoV-2. Among the entire population enrolled, 10 (17.2%) patients underwent clinically indicated ICA according to CCTA findings, while ICA was considered deferrable in 48 (82.8%) patients. One patient was in atrial fibrillation during CCTA acquisition. A mean follow-up of 376.4 ± 32.1 days was obtained (**Figure 1**). No adverse events were recorded during or after CCTA. The mean radiation dose reached 4.7 mSv.

All the patients enrolled presented with symptoms highly suggestive for a new diagnosis of stable CAD, the mean pretest probability for CAD was 29.7% and resulted significantly higher among those who subsequently underwent ICA vs. those who did not (41.5 vs. 25.1%, respectively, $p < 0.001$) (**Table 1**). A total of 18 patients (31%) had typical chest pain that was significantly more prevalent among ICA vs. non-ICA group (80 vs. 20.8%, respectively, $p < 0.001$) (**Table 1**).

Coronary CT angiography showed no CAD in 14 patients (24.1%), non-obstructive CAD in 30 (51.7%) patients, and obstructive CAD in 14 (24.1%) patients. None of the patients with normal coronary arteries at CCTA was sent to the catheterization laboratory for non-deferrable ICA (**Table 1**). A total of 10 patients were sent to ICA based on the CCTA results and in all but one severe CAD was confirmed and treated accordingly. CCTA showed critical/subocclusive (>90% diameter stenosis) lesions in six patients. All underwent percutaneous revascularization after

TABLE 1 | Population characteristics.

	Total population ($n = 58$)	Deferrable ICA ($n = 48$)	Non-deferrable ICA ($n = 10$)	*p*
Clinical characteristics				
Age, mean ± SD	64.7 ± 11.6	64.3 ± 11	66.3 ± 14.7	0.597
Sex, *n* (%)	36 (62)	28 (58.3)	8 (80)	0.207
BMI, mean ± SD	26.4 ± 4.5	26.6 ± 4.5	25.7 ± 5.2	0.524
Hypertension, *n* (%)	33 (56.8)	26 (54.2)	7 (70)	0.637
Dyslipidemia, *n* (%)	28 (48.2	23 (47.9)	5 (50)	0.904
Family history, *n* (%)	20 (34.4)	17 (35.4)	3 (30)	0.745
Diabetes, *n* (%)	7 (12)	4 (8.3)	3 (30)	0.058
Active smoking, *n* (%)	7 (12)	6 (12.5)	1 (10)	0.826
Past smoking, *n* (%)	22 (37.9)	19 (39.6)	3 (30)	0.573
Typical chest pain, *n* (%)	18 (31)	10 (20.8)	8 (80)	<0.001
Atypical chest pain, *n* (%)	32 (55.2)	29 (60.4)	3 (30)	0.081
Non-cardiac chest pain, *n* (%)	3 (5.2)	3 (6.2)	0	0.421
Dyspnea, *n* (%)	5 (8.6)	6 (12.5)	0	0.228
Pretest probability of CAD (%), mean ± SD	27.9 ± 14.3	25.1 ± 12.3	41.5 ± 16.2	<0.001
CCTA				
No CAD, *n* (%)	14 (24.1)	14 (29.2)	0	<0.001
Non-obstructive CAD, *n* (%)	30 (51.8)	29 (60.4)	1 (10)	<0.001
Obstructive CAD, *n* (%)	14 (24.1)	5 (10.4)	9 (90)	<0.001
Stenosis >90%, *n* (%)	6 (10.3)	0 (0)	6 (60)	<0.001
Radiation dose (mSV), mean ± SD	4.7 ± 2.2	4.7 ± 2.1	4.6 ± 2.6	0.786

CCTA, coronary computed tomography angiography; CAD, coronary artery disease; ICA, invasive coronary angiography; BMI, body mass index.

ICA confirming the CCTA findings (**Figure 2**). The only patient who was not revascularized had a calcified non-high risk plaque of the proximal left anterior descending artery (LAD) and was referred to ICA due to typical angina with suspected left main CAD (**Table 2**).

In 48 patients, there was no clinical indication for ICA. Fourteen patients (29.2%) showed normal coronary arteries at CCTA while non-obstructive (0–50% stenosis) and obstructive CAD (more than 50% stenosis) was demonstrated in 29 (60.4%) and five (10.4%) patients, respectively. Of note, no clinical events were recorded among the patients in whom ICA was considered not indicated or deferrable. Moreover, medical therapy was changed in 16 patients, which led to the symptomatic improvement in 13 patients (81.2%). No therapy change was recorded among the patients in whom CCTA excluded coronary atherosclerosis. Of note, medical therapy changes were significantly more prevalent in the patients with obstructive or non-obstructive CAD at CCTA. In 41% of the patients with non-obstructive CAD, medical therapy was modified, and more specifically, in nine (31%) and in seven (24%) of them, aspirin and statin therapy were prescribed (**Figure 3**).

DISCUSSION

To the best of our knowledge, this study is the first to describe the potential use of CCTA as the solely available gatekeeper for ICA in stable patients with chest pain with high clinical suspicion of CAD during the lockdown phase of the COVID-19 pandemic. Even if limited by the low number of patients enrolled, the results of the study appeared to confirm the capability of CCTA to safely defer ICA in the majority of the symptomatic patients and to correctly identify those with critical coronary stenoses necessitating coronary revascularization. This resulted to be of the utmost importance taking into consideration the need to limit hospital access to non-COVID patients. Moreover, the identification of non-critical atherosclerosis enabled physicians to optimize medical therapy in a well-selected subgroup of patients (15).

The advent of SARS-CoV-2 infection dramatically changed cardiovascular care and management with healthcare resources mostly focused on the patients with COVID-19 (16). During the lockdown phase, people were advised to avoid, if possible, emergency departments that were overwhelmed by patients with COVID-19. Moreover, there was a general reluctance to go to the hospital for the SARS-CoV-2 infection fear. Consequently, a reduced rate of hospital admission was observed with a potential increase of cardiac mortality from ACS due to the lower medical referrals (17). On the contrary, in this cohort, no cardiovascular were recorded at mid-term follow-up among patients in which ICA was deferred, while all but one patient with non-deferable ICA according to CCTA underwent appropriate myocardial revascularization.

Thus, the results of this study suggest that, due to its high-negative predictive value for obstructive CAD (8), CCTA correctly identified the great majority of the patients in

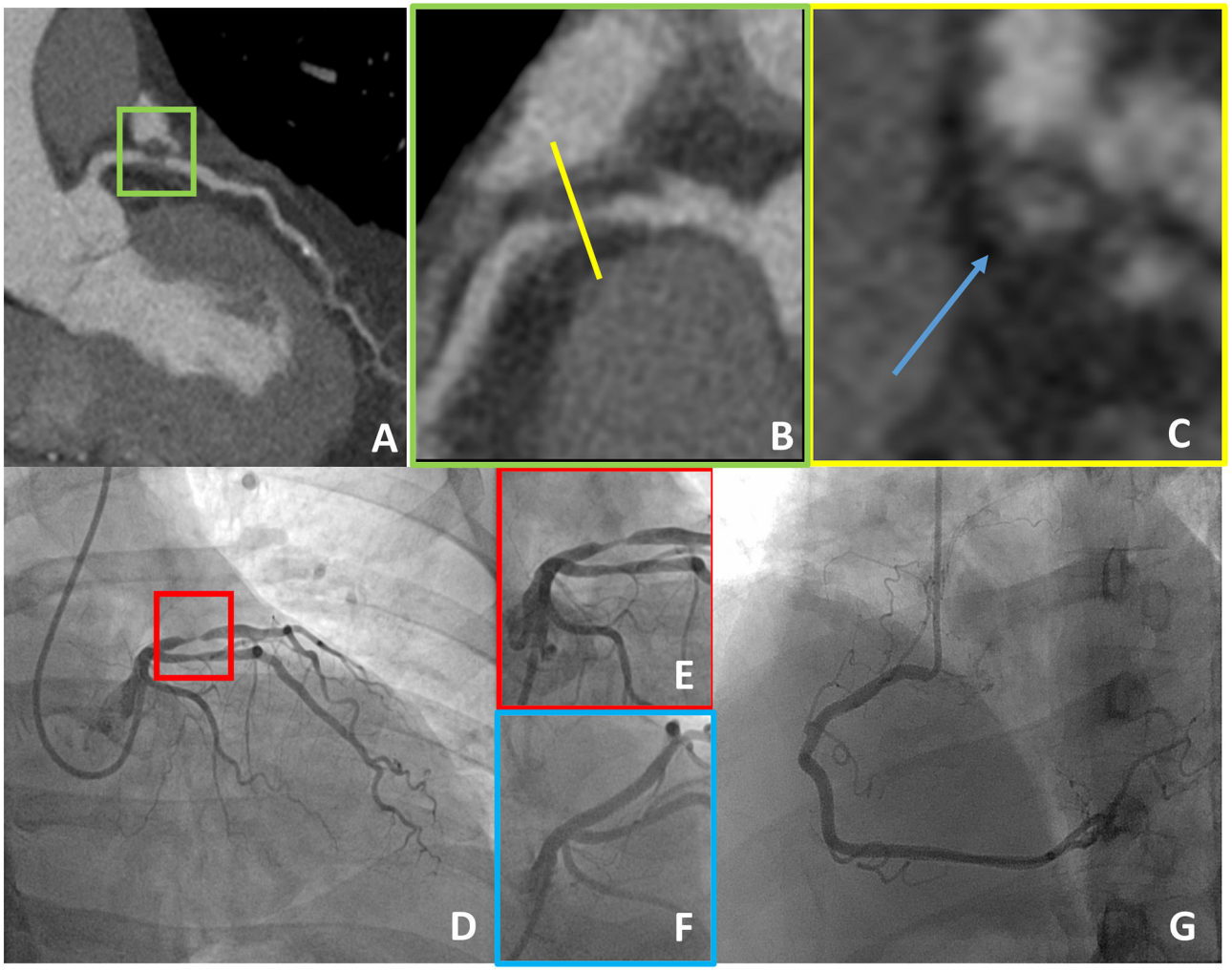


FIGURE 2 | A case example of a 55-year-old man with the typical chest pain in whom CCTA detected critical stenosis of the proximal LAD **(A,B)**, showing at the same time high-risk plaque features (positive remodeling and low-attenuation plaque as demonstrated in a short axis view, blue arrow in **C**). The patient underwent ICA that confirmed subocclusive disease of the proximal LAD that was treated with PCI **(D–G)**. CCTA, coronary computed tomography angiography; LAD, left anterior descending artery; ICA, invasive coronary angiography; PCI, percutaneous coronary intervention.

whom ICA could be apparently safely deferred (82% of the patients) avoiding overcrowded hospitals and emergency departments, especially during a pandemic surge. However, it should be underlined that both the low number of patients enrolled and the absence of long-term follow-up represents a limitation to this study. Of interest, identification of non-obstructive CAD at CCTA has prognostic value (18–20) and, as previously demonstrated, should lead to optimal medical therapy implementation, further improving the prognosis of the patient (15). In our study, no invasive imaging was recommended to patients with normal coronaries at CCTA, avoiding unnecessary hospitalization in time of the limited resources. On the contrary, 41% of the patients with non-obstructive CAD had their medical therapy optimized.

On the other hand, CCTA permitted correctly identifying patients with severe coronary stenoses necessitating non-deferrable treatment (18% of the patients). Upon the detection of severe disease by CCTA, the referring physician (cardiologist) was immediately informed, and patients were managed in a dedicated non-COVID-19 pathway and catheterization laboratory, lowering the probability of SARS-CoV-2 infection while providing at the same time the best treatment strategy and reducing the risk of subsequent ACS (21).

A COVID-19 pandemic is a generation-defining event, and cardiovascular imaging practice has been deeply impacted as well (22–26). The results of this observational study suggest that CCTA is an appropriate and safe tool for the non-invasive evaluation of the suspected CAD when facing limited access to cardiovascular care and resources. Indeed, compared with the other non-invasive diagnostic tools, CCTA requires only a minimal time of contact between patients and healthcare professionals.

TABLE 2 | Clinical, CCTA, and ICA characteristics of patients who underwent non-deferrable ICA.

Age and sex	Indication to CCTA	CCTA findings	High risk plaque features at CCTA	Clinical indication to ICA after CCTA results	ICA findings	Treatment
79 y/o, male	Typical chest pain	40% stenosis of LM and 70% stenosis of proximal LAD	No	Symptomatic patient with at least moderate coronary stenosis and typical angina	40% stenosis of LM and 50% stenosis of proximal LAD	Medical therapy for stable CAD
47 y/o male	Atypical chest pain	70% stenosis of mid-LAD	PRI, LAP	Symptomatic patient with severe stenosis at CCTA	70% stenosis of mid-LAD	Percutaneous revascularization and drug-eluting stent implantation on mid-LAD
48 y/o male	Atypical chest pain	75% stenosis of mid-LAD. Moderate stenosis of LCX and RCA	PRI, LAP	Symptomatic patient with severe stenosis at CCTA	75% stenosis of mid-LAD	Percutaneous revascularization and drug-eluting stent implantation on mid-LAD
73 y/o, male	Typical chest pain	75% stenosis of mid-LAD	PRI, LAP	Symptomatic patient with severe stenosis at CCTA	75% stenosis of mid-LAD	Percutaneous revascularization and drug-eluting stent implantation on mid-LAD
55 y/o, male	Typical chest pain	99% stenosis of proximal LAD	PRI, LAP	Symptomatic patient with severe stenosis at CCTA	99% stenosis of proximal LAD	Percutaneous revascularization and drug-eluting stent implantation on proximal LAD
72 y/o, male	Typical chest pain	95% stenosis of diagonal branch	PRI, LAP	Symptomatic patient with severe stenosis at CCTA	90% stenosis of diagonal branch	Percutaneous revascularization and drug-eluting stent implantation on diagonal branch
86 y/o, female	Typical chest pain	99% stenosis of proximal LAD. Moderate stenosis of LCX	PRI, SC	Symptomatic patient with severe stenosis at CCTA	99% stenosis of proximal LAD. Moderate stenosis of LCX	Percutaneous revascularization and drug-eluting stent implantation on proximal LAD
55 y/o, male	Typical chest pain	99% stenosis of proximal LAD.	PRI, LAP, NRS	Symptomatic patient with severe stenosis at CCTA	99% stenosis of proximal LAD.	Percutaneous revascularization and drug-eluting stent implantation on proximal LAD
85 y/o, male	Typical chest pain	90% stenosis of mid-RCA	PRI, LAP	Symptomatic patient with severe stenosis at CCTA	90% stenosis of mid-RCA	Percutaneous revascularization and drug-eluting stent implantation on mid-RCA
63 y/o, female	Typical chest pain	75% stenosis of mid LAD	LAP	Symptomatic patient with severe stenosis at CCTA	75% stenosis of mid-LAD	Percutaneous revascularization and drug-eluting stent implantation on proximal LAD

CCTA, Coronary computed tomography angiography; ICA, invasive coronary angiography; LAD, left anterior descendent artery; LCX, left circumflex; RCA, right coronary artery; PRI, positive remodeling index; LAP, low attenuation plaque; NRS, napkin ring sign.

STUDY LIMITATION

This study has several limitations. First, only the patients with a clinical indication underwent ICA, leading to the potentially underestimated false-negative results of CCTA. However, no clinical events were recorded during follow-up among the patients who did not undergo ICA. In this regard, it should be underlined that no further cardiac imaging was carried out in the follow-up period, thus, it was not possible to certainly exclude myocardial damage occurrence during follow-up; however, no major symptoms suspected for the cardiovascular events were recorded at follow-up.

Second, there the absence of another non-invasive control group, the low number of patients enrolled and the midterm follow-up may undermine the scientific strength of our findings, which should be considered as of speculative nature. Third, a larger cohort and a longer follow-up are needed for the validation of this report. Nevertheless, it should be considered

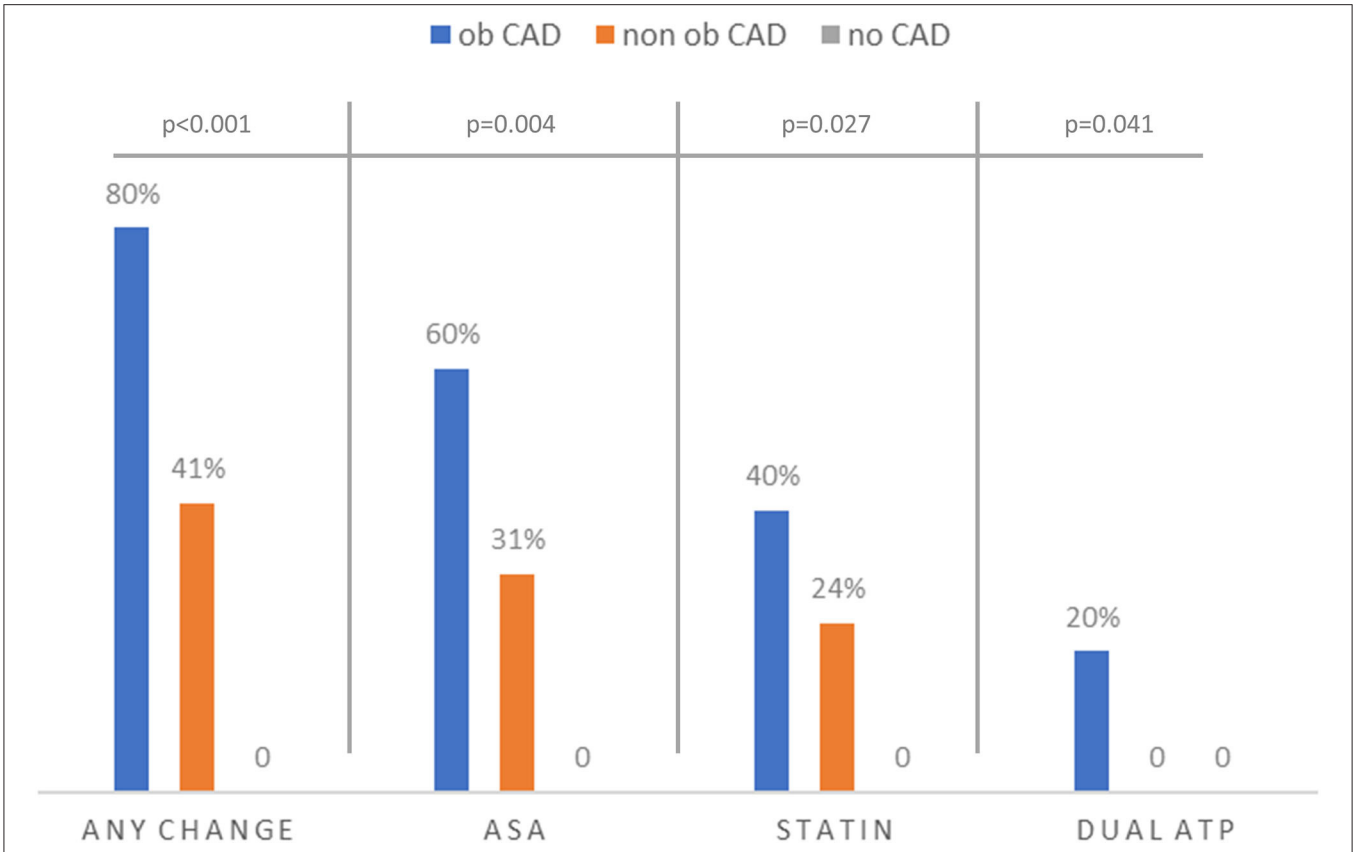


FIGURE 3 | Prevalence of medical therapy changes according to CCTA findings among patients who did not undergo ICA. Of note, no new drugs were introduced by the referring physician in patients who were free of coronary atherosclerosis, while a significantly higher rate of aspirin and statin new prescriptions was observed when non-obstructive or obstructive CAD was identified at CCTA. Ob CAD, obstructive CAD; non-ob CAD, non-obstructive CAD; CAD, coronary artery disease; DAPT, dual antiplatelet therapy.

that the present study has been performed during a global pandemic emergency with limited access to healthcare resources, and any control group randomly selected from the previous years could not be compared with the study population as the environmental conditions were totally different. Finally, we would like to highlight that the results of this study were obtained in a cardiovascular focused center using the last generation CT using postprocessing tools dedicated to the coronary analysis that may not be widely available, limiting the wide application study results in the different settings.

CONCLUSION

We describe the potentially pivotal role of CCTA in the diagnostic pathway of patients with non-COVID-19 with chest pain due to suspected CAD during the SARS-CoV-2 pandemic. This non-invasive imaging tool enhanced the selection of patients for the ICA and potential revascularization during a lockdown period characterized by increased mortality due to delayed or deferred hospitalization of patients with CAD. The high-negative predictive value of CCTA enables to safely defer in-hospital care. Indeed, patients with non-obstructive CAD could be identified and safely treated by the referring physicians (cardiologists). On the contrary, CCTA helps in identifying patients who necessitate ICA ensuring adequate resource utilization during the pandemic.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Centro Cardiologico Monzino, IRCCS. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EC and DA conceptualized the manuscript. EC wrote the first draft of the manuscript. SM, MM, AA, AF, and GM performed and analyzed CT images. MGA, CG, MG, MD,

MB, CA, and AB retrieved clinical data, follow-up information, and performed statistical analysis (data curation). CC, JS, and NC adjudicate events at follow-up. AE, EA, ALB, MP, GP, and DA provide senior expert advice and supervision. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Mapelli M. What COVID-19 is taking away from us. *Eur Heart J*. (2020) 41:2053–5. doi: 10.1093/eurheartj/ehaa374
2. Fagiuoli S, Lorini FL, Remuzzi G. Covid-19 Bergamo Hospital crisis unit. adaptations and lessons in the Province of Bergamo. *N Engl J Med*. (2020) 382:e71. doi: 10.1056/NEJMc2011599
3. Sebastiani G, Massa M, Riboli E. Covid-19 epidemic in Italy: evolution, projections and impact of government measures. *Eur J Epidemiol.* (2020) 35:341–5. doi: 10.1007/s10654-020-00631-6
4. Agostoni P, Mapelli M, Conte E, Baggiano A, Assanelli E, Apostolo A, et al. Cardiac patient care during a pandemic: how to reorganise a heart failure unit at the time of COVID-19. *Eur J Prev Cardiol*. (2020) 27:1127–32. doi: 10.1177/2047487320925632
5. Knuuti J, Wijns W, Saraste A, Capodanno D, Barbato E, Funck-Brentano C, et al. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. *Eur Heart J*. (2020) 41:407–77. doi: 10.1093/eurheartj/ehz425
6. *ESC Guidance for the Diagnosis and Management of CV Disease During the COVID-19 Pandemic*. Available online at: https://www.escardio.org/Education/COVID-19-and-Cardiology/ESC-COVID-19-Guidance (accessed Semptember 5, 2021).
7. Meah MN, Bing R, Newby DE. Primacy of coronary CT angiography as the gatekeeper for the cardiac catheterization laboratory. *Am Heart J.* (2020) 223:120–2. doi: 10.1016/j.ahj.2020.01.017
8. Haase R, Schlattmann P, Gueret P, Andreini D, Pontone G, Alkadhi H, et al. Diagnosis of obstructive coronary artery disease using computed tomography angiography in patients with stable chest pain depending on clinical probability and in clinically important subgroups: meta-analysis of individual patient data. *BMJ.* (2019) 365:l1945. doi: 10.1136/bmj.l1945
9. Conte E, Mushtaq S, Pontone G, Li Piani L, Ravagnani P, Galli S, et al. Plaque quantification by coronary computed tomography angiography using intravascular ultrasound as a reference standard: a comparison between standard and last generation computed tomography scanners. *Eur Heart J Cardiovasc Imaging.* (2020) 21:191–201. doi: 10.1093/ehjci/jez089
10. Andreini D, Mushtaq S, Pontone G, Conte E, Guglielmo M, Annoni A, et al. Diagnostic performance of coronary CT angiography carried out with a novel whole-heart coverage high-definition CT scanner in patients with high heart rate. *Int J Cardiol.* (2018) 257:325–31. doi: 10.1016/j.ijcard.2017.10.084
11. Austen WG, Edwards JE, Frye RL, Gensini GC, Goft VL, Griffith LS, et al. A reporting system on patients evaluated for coronary artery disease. Report of the Ad Hoc Committee for Grading of Coronary Artery Disease, Council on Cardiovascular Surgery, American Heart Association. *Circulation.* (1975) 51:5–40. doi: 10.1161/01.CIR.51.4.5
12. Leipsic J, Abbara S, Achenbach S, Cury R, Earls JP, Mancini GJ, et al. SCCT guidelines for the interpretation and reporting of coronary CT angiography: a report of the Society of Cardiovascular Computed Tomography Guidelines Committee. *J Cardiovasc Comput Tomogr.* (2014) 8:342–58. doi: 10.1016/j.jcct.2014.07.003
13. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). *Eur Heart J*. (2016) 37:267–315. doi: 10.1093/eurheartj/ehv320
14. Halliburton SS, Abbara S, Chen MY, Gentry R, Mahesh M, Raff GL, et al. SCCT guidelines on radiation dose and dose-optimization strategies in cardiovascular CT. *J Cardiovasc Comput Tomogr.* (2011) 5:198–224. doi: 10.1016/j.jcct.2011.06.001
15. Williams MC, Moss AJ, Dweck M, Adamson PD, Alam S, Hunter A, et al. Coronary artery plaque characteristics associated with adverse outcomes in the SCOT-HEART study. *J Am Coll Cardiol.* (2019) 73:291–301. doi: 10.1016/j.jacc.2018.10.066
16. Valdebenito M, Massalha E, Barbash IM, Maor E, Fefer P, Guetta V, et al. Transcatheter aortic valve implantation during the COVID-19 pandemic. *Am J Cardiol.* (2021) 145:97–101. doi: 10.1016/j.amjcard.2020.12.086
17. De Filippo O, D'Ascenzo F, Angelini F, Bocchino PP, Conrotto F, Saglietto A, et al. Reduced rate of hospital admissions for ACS during Covid-19 Outbreak in Northern Italy. *N Engl J Med.* (2020) 383:88–9. doi: 10.1056/NEJMc2009166
18. Cho I, Al'Aref SJ, Berger A, Ó Hartaigh B, Gransar H, Valenti V, et al. Prognostic value of coronary computed tomographic angiography findings in asymptomatic individuals: a 6-year follow-up from the prospective multicentre international CONFIRM study. *Eur Heart J.* (2018) 39:934–41. doi: 10.1093/eurheartj/ehx774
19. Andreini D, Magnoni M, Conte E, Masson S, Mushtaq S, Berti S, et al. Coronary plaque features on CTA can identify patients at increased risk of cardiovascular events. *JACC Cardiovasc Imaging.* (2019) 13:1704–17. doi: 10.1016/j.jcmg.2019.06.019
20. Conte E, Annoni A, Pontone G, Mushtaq S, Guglielmo M, Baggiano A, et al. Evaluation of coronary plaque characteristics with coronary computed tomography angiography in patients with non-obstructive coronary artery disease: a long-term follow-up study. *Eur Heart J Cardiovasc Imaging.* (2017) 18:1170–8. doi: 10.1093/ehjci/jew200
21. De Rosa S, Spaccarotella C, Basso C, Calabrò MP, Curcio A, Filardi PP, et al. Reduction of hospitalizations for myocardial infarction in Italy in the COVID-19 era. *Eur Heart J.* (2020) 41:2083–8. doi: 10.1093/eurheartj/ehaa409
22. Zoghbi WA, DiCarli MF, Blankstein R, Choi AD, Dilsizian V, Flachskampf FA, et al. Multimodality cardiovascular imaging in the midst of the COVID-19 pandemic: ramping up safely to a new normal. *J Am Coll Cardiol Img.* (2020) 13:1615–26. doi: 10.1016/j.jcmg.2020.06.001
23. Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A, et al. COVID-19 and cardiovascular disease. *Circulation.* (2020) 141:1648–55. doi: 10.1161/CIRCULATIONAHA.120.046941
24. Cosyns B, Lochy S, Luchian ML, Gimelli A, Pontone G, Allard SD, et al. The role of cardiovascular imaging for myocardial injury in hospitalized COVID-19 patients. *Eur Heart J Cardiovasc Imaging.* (2020) 21:709–14. doi: 10.1093/ehjci/jeaa136
25. Pontone G, Scafuri S, Mancini ME, Agalbato C, Guglielmo M, Baggiano A, et al. Role of computed tomography in COVID-19. *J Cardiovasc Comput Tomogr*. (2020) 5:27–36. doi: 10.1016/j.jcct.2020.08.013
26. Kadavath S, Mohan J, Ashraf S, Kassier A, Hawwass D, Madan N, et al. Cardiac catheterization laboratory volume changes during COVID-19-findings from a cardiovascular fellows consortium. *Am J Cardiol*. (2020) 130:168–9. doi: 10.1016/j.amjcard.2020.06.009

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 26 November 2021
doi: 10.3389/fcvm.2021.738814

Heart Failure Probability and Early Outcomes of Critically Ill Patients With COVID-19: A Prospective, Multicenter Study

Weibo Gao[1†], Jiasai Fan[2†], Di Sun[2†], Mengxi Yang[2], Wei Guo[3], Liyuan Tao[4], Jingang Zheng[2], Jihong Zhu[1], Tianbing Wang[3] and Jingyi Ren[2*]*

[1] Department of Emergency, Peking University People's Hospital, Beijing, China, [2] Department of Cardiology, Heart Failure Center, China-Japan Friendship Hospital, Beijing, China, [3] Trauma Center, Peking University People's Hospital, Beijing, China, [4] Research Center of Clinical Epidemiology, Peking University Third Hospital, Beijing, China

Background: The relationship between cardiac functions and the fatal outcome of coronavirus disease 2019 (COVID-19) is still largely underestimated. We aim to explore the role of heart failure (HF) and NT-proBNP in the prognosis of critically ill patients with COVID-19 and construct an easy-to-use predictive model using machine learning.

Methods: In this multicenter and prospective study, a total of 1,050 patients with clinical suspicion of COVID-19 were consecutively screened. Finally, 402 laboratory-confirmed critically ill patients with COVID-19 were enrolled. A "triple cut-point" strategy of NT-proBNP was applied to assess the probability of HF. The primary outcome was 30-day all-cause in-hospital death. Prognostic risk factors were analyzed using the least absolute shrinkage and selection operator (LASSO) and multivariate logistic regression, further formulating a nomogram to predict mortality.

Results: Within a 30-day follow-up, 27.4% of the 402 patients died. The mortality rate of patients with HF likely was significantly higher than that of the patient with gray zone and HF unlikely (40.8% vs. 25 and 16.5%, respectively, $P < 0.001$). HF likely [Odds ratio (OR) 1.97, 95% CI 1.13–3.42], age (OR 1.04, 95% CI 1.02–1.06), lymphocyte (OR 0.36, 95% CI 0.19–0.68), albumin (OR 0.92, 95% CI 0.87–0.96), and total bilirubin (OR 1.02, 95% CI 1–1.04) were independently associated with the prognosis of critically ill patients with COVID-19. Moreover, a nomogram was developed by bootstrap validation, and C-index was 0.8 (95% CI 0.74–0.86).

Conclusions: This study established a novel nomogram to predict the 30-day all-cause mortality of critically ill patients with COVID-19, highlighting the predominant role of the "triple cut-point" strategy of NT-proBNP, which could assist in risk stratification and improve clinical sequelae.

Keywords: COVID-19, heart failure, NT-ProBNP, nomogram, prognosis

OPEN ACCESS

Edited by:
Christoph Sinning, University Heart and Vascular Center Hamburg (UHZ), Germany

Reviewed by:
Siva K. Panguluri, University of South Florida, United States
Bastiaan Geelhoed, University Medical Center Groningen, Netherlands

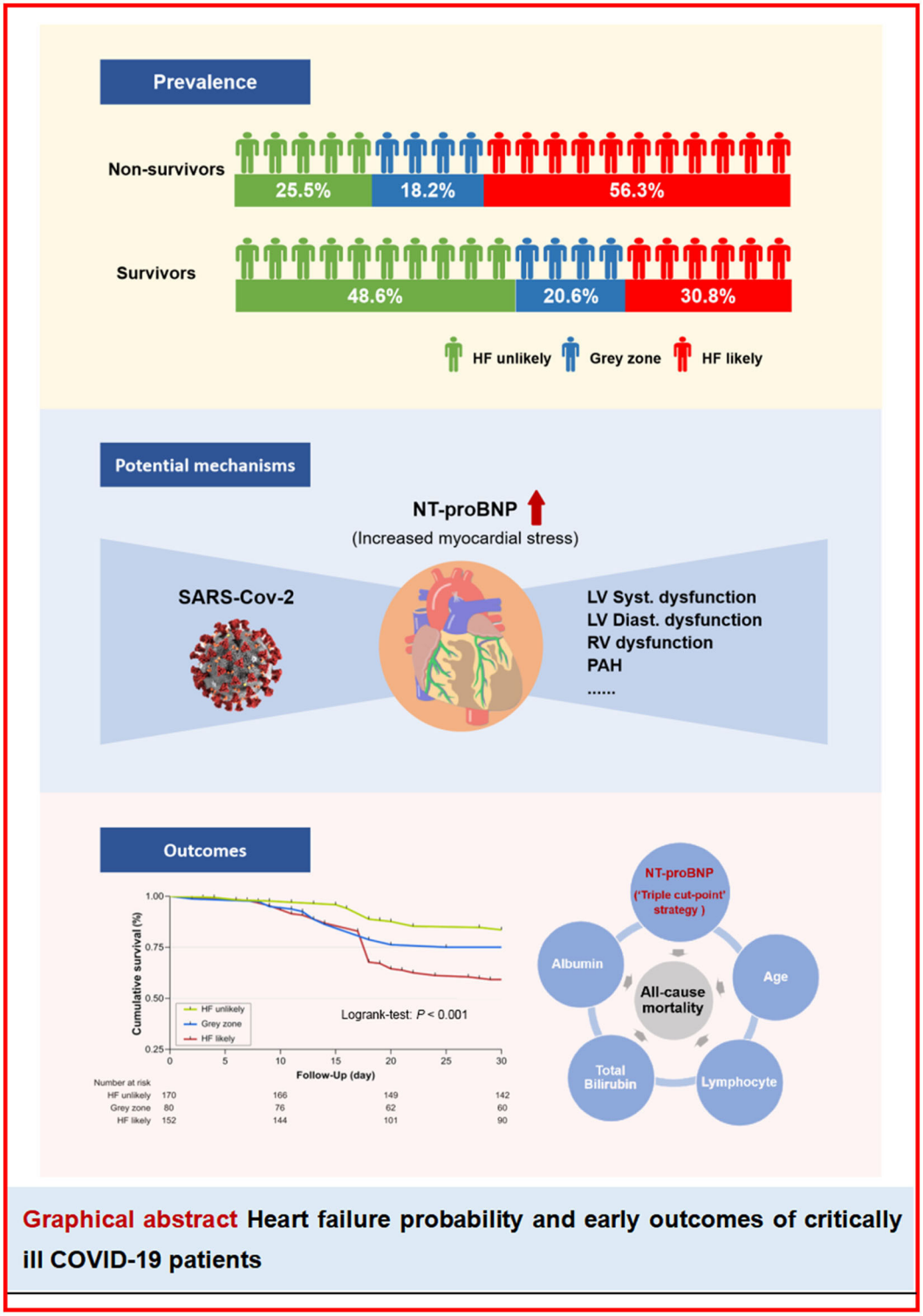
****Correspondence:***
Jingyi Ren
renjingyi1213@hotmail.com
Tianbing Wang
wangtianbing@pkuph.edu.cn

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Cardiovascular Genetics and Systems Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *09 July 2021*
Accepted: *28 October 2021*
Published: *26 November 2021*

Citation:
Gao W, Fan J, Sun D, Yang M, Guo W, Tao L, Zheng J, Zhu J, Wang T and Ren J (2021) Heart Failure Probability and Early Outcomes of Critically Ill Patients With COVID-19: A Prospective, Multicenter Study. Front. Cardiovasc. Med. 8:738814. doi: 10.3389/fcvm.2021.738814



Graphical abstract Heart failure probability and early outcomes of critically ill COVID-19 patients

GRAPHICAL ABSTRACT | Heart failure probability and early outcomes of critically ill COVID-19 patients. HF, heart failure; NT-proBNP, N-terminal probrain natriuretic peptide; LV, left ventricular; RV, right ventricular; PAH, pulmonary arterial hypertension.

INTRODUCTION

An outbreak of novel infectious pneumonia, now known as coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2, has been quickly spreading around the world since December 2019. To date, more than 168 million confirmed cases of COVID-19 have been identified worldwide, with over 3.49 million deaths. Despite the advancement of learning the etiology and clinical characteristics of COVID-19, there have been no effective strategies to wipe out the global COVID-19 epidemic, and it is still a public health threat.

The average mortality rate was estimated globally at 3.4% by the WHO, while it is 26–52% significantly higher for patients admitted to intensive care units (ICUs) (1, 2). Moreover, no medications have been proven definitely effective for curing COVID-19 (3). Thus, early evaluation and identification of individuals with high-risk mortality are of paramount importance to further guide optimal intervention strategies. Of note, critically ill patients with COVID-19 usually have multiple organ dysfunctions (4), among which cardiac involvement is prevalent, especially acute heart failure (AHF) (5). However, the role of AHF in the prognosis of COVID-19 has not been fully elucidated in prior studies, partially because comprehensive evaluations of cardiac dysfunction that utilize imaging examinations were usually unavailable in real-world practice. Hence, we proposed a "triple cut-point" strategy of N-terminal pro-brain natriuretic peptide (NT-proBNP) as a reliable and easy-to-use diagnostic tool for AHF in this study (6). Moreover, although previous studies have explored the risk factors of prognosis among critically ill patients with COVID-19 (7–9), a user-friendly and clinically relevant short-time outcome prediction model for patients with COVID-19 in ICU is still lacking.

Therefore, to address the gaps mentioned above, this multicenter study aims to (1) explore the potential prognostic value of the "triple cut-point" strategy of NT-proBNP and AHF in critically ill patients with COVID-19; and (2) construct and validate a simplified and effective nomogram to predict all-cause in-hospital death risk individually.

MATERIALS AND METHODS

Study Population

This multicenter, prospective, and observational study consecutively enrolled 1,050 patients with clinical suspicion of COVID-19 from four ICUs in Wuhan taken over by China-Japan Friendship Hospital, Peking University People's Hospital, Peking University First Hospital, and Peking University Third Hospital from January to May 2020. Patients who met one of the following criteria would be considered to be transferred to the ICU: (1) respiratory rate >30 breath/min; (2) blood oxygen (SpO_2) <93%; (3) PaO_2/FiO_2 <300 mmHg; (4) presented with respiratory failure; (5) presented with shock; or (6) other conditions that need to be monitored in the ICU. Patients were diagnosed as COVID-19 with a positive result of real-time reverse transcriptase-polymerase chain reaction assay from nasal swab specimens according to WHO guidance (10). Exclusion criteria included patients who were not diagnosed with COVID-19, younger than 18 years of age, and had incomplete data, or died within 24 h of admission to the ICU. As a result, 402 patients were included in the final analysis. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Peking University People's Hospital.

Data Collection

The clinical data from each patient were recorded by experienced physicians following ICU admission and included demographic features, preexisting comorbidities, symptoms, vital signs, and length of ICU stay. The comorbidities included hypertension, coronary heart disease (CHD), diabetes mellitus (DM), chronic kidney disease (CKD), asthma, chronic obstructive pulmonary disease, chronic bronchitis, transient ischemic attack, ischemic stroke, and hemorrhagic stroke.

All the patients, during hospitalization, were followed up for 30-days or until discharge or death. The primary outcome was 30-day all-cause death after admission.

Laboratory Measurements

Laboratory values were collected including complete blood count, high-sensitivity cardiac troponin I (hs-cTNI), NT-proBNP, biochemical tests, d-dimer, and procalcitonin (PCT). Complete blood count was measured with a Sysmex XN-9000 (Sysmex, Kobe, Japan) automatic hematology analyzer. Coagulation parameters, such as d-dimer, were measured with a Stago STA-R automatic blood coagulation analyzer (Stago, Paris, France). Biochemical tests, namely, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, albumin, blood urea nitrogen (BUN), and creatinine were performed using Roche Cobas 8000 automatic biochemical analyzer (Roche, Rotkreuz, Switzerland). Hs-cTNI was measured with an Abbott ARCHITECT i2000SR chemiluminescence immunoanalyzer (Abbott Laboratories, Illinois, United States). Elevated hs-cTNI was defined as plasma levels of hs-cTNI above the 99th-percentile upper reference limit. NT-proBNP was analyzed with a

TABLE 1 | Classification of patients using the "triple cut-point" strategy of NT-proBNP.

Setting	Cut-off levels of NT-proBNP (pg/mL)		
	Age < 50	Age 50–75	Age > 75
HF unlikely	<300		
Gray zone	300–450	300–900	300–1,800
HF likely	>450	>900	>1,800

NT-proBNP, N-terminal pro-B type natriuretic peptide; HF, heart failure.

Roche Cobas e602 electrochemical luminescence analyzer (Roche, Germany).

"Triple Cut-Point" Strategy of NT-proBNP

According to the recent guideline for HF, novel NT-proBNP cut-off values have been proposed to assist with AHF diagnosis. Hence, we classified the cases into three groups using this "triple cut-point" strategy of NT-proBNP to define the probability of AHF, as shown in **Table 1**. In detail, HF likely was defined as plasma NT-proBNP level > 450 pg/ml in patients below 50 years, >900 pg/ml in patients between 50 and 75 years, and >1,800 pg/ml in patients over 75 years (6). HF unlikely was defined as plasma NT-proBNP level <300 pg/ml regardless of age, while the stratified approach of 300 pg/ml to 450/900/1,800 pg/ml for ages <50/50–75/>75 years were considered as "gray zone."

Statistical Analysis

Continuous variables were presented as mean ± SD if normally distributed, and median and interquartile range otherwise. The differences between the two groups were compared by the Student *t*-test and Mann–Whitney U test appropriately. Categorical variables were shown as n (%) and compared by χ^2 test or Fisher exact test when necessary.

Kaplan–Meier survival estimates were calculated, and the log-rank test was performed to compare the groups in terms of survival. The least absolute shrinkage and selection operator (LASSO) method (glmnet package), which is appropriate for

TABLE 2 | Baseline characteristics of the cohort.

Demographics	Total *n* = 402 (100.0)	Survivors *n* = 292 (72.6)	Non-survivors *n* = 110 (27.4)	*P*-value
Age, years	67.5 ± 13.7	65.6 ± 13.6	72.4 ± 12.8	<0.001
Sex				0.070
Female, *n* (%)	183 (45.5)	141 (48.3)	42 (38.2)	
Male, *n* (%)	219 (54.5)	151 (51.7)	68 (61.8)	
Vital signs				
Temperature, °C	38.7 ± 3.7	38.6 ± 4.3	39.0 ± 1.0	0.332
Respiratory rate, breath/min	25.1 ± 6.1	25.0 ± 5.6	25.4 ± 7.2	0.603
SpO_2, %	91.2 ± 6.7	92.2 ± 5.7	88.6 ± 8.3	<0.001
Heart rate, beat/min	94.7 ± 17.0	93.7 ± 15.6	97.3 ± 20.0	0.002
SBP, mm/Hg	133.0 ± 23.0	132.6 ± 22.4	134.0 ± 24.5	0.570
DBP, mm/Hg	79.0 ± 14.4	78.9 ± 14.0	79.2 ± 15.3	0.883
Comorbidities				
Hypertension, *n* (%)	209 (52.0)	152 (52.1)	57 (51.8)	0.966
Coronary heart disease, *n* (%)	72 (17.9)	48 (16.4)	24 (21.8)	0.210
Diabetes mellitus, *n* (%)	96 (23.9)	69 (23.6)	27 (24.5)	0.848
Respiratory system diseases, *n* (%)	51 (12.7)	37 (12.7)	14 (12.7)	0.988
Chronic kidney disease, *n* (%)	37 (9.2)	25 (8.6)	12 (10.9)	0.468
Cerebrovascular diseases, *n* (%)	19 (4.7)	11 (3.8)	8 (7.3)	0.140
Cardiac comorbidities or risk factors, *n* (%)	258 (64.2)	187 (64.0)	71 (64.5)	0.925
No. of comorbidities ≥2, *n* (%)	139 (34.6)	95 (32.5)	44 (40.0)	0.161
Laboratory values				
Lymphocyte, $\times 10^9$ /L	0.8 (0.5, 1.1)	0.8 (0.7, 1.2)	0.6 (0.4, 0.8)	<0.001
Platelet, $\times 10^9$ /L	217.0 (141.8, 289.0)	235.0 (157.0, 307.2)	160 (106.0, 225.2)	<0.001
ALT, U/L	30.0 (16.4, 47.0)	28.5 (16.0, 46.0)	33.1 (18.0, 48.3)	0.291
AST, U/L	34.0 (21.0, 52.8)	29.2 (20.0, 50.8)	43.5 (26.4, 57.0)	<0.001
Albumin, g/L	32.2 ± 5.9	33.0 ± 5.2	29.9 ± 6.9	<0.001
Total bilirubin, μmol/L	12.2 (8.6, 16.9)	11.4 (8.3, 15.3)	14.5 (9.7, 20.4)	<0.001
eGFR, ml/ min/l.73 m^2	73.2 ± 31.7	76.0 ± 31.8	65.7 ± 30.2	0.003
Glucose, mmol/L	8.6 ± 4.1	8.3 ± 4.0	9.5 ± 5.8	0.021
D-dimer, μg/mL	2.5 (0.7, 20.1)	1.7 (0.5, 15.7)	7.3 (2.4, 20.1)	<0.001
PCT, ug/L	0.3 (0.1, 1.8)	0.2 (0.1, 1.2)	1.5 (0.3, 1.8)	<0.001
hs-cTNI, pg/mL	12.0 (2.4, 503.7)	10.2 (2.3, 80.1)	274.4 (6.0, 659.6)	<0.001
NT-proBNP, pg/mL	393.2 (121.5, 2774.8)	321.0 (105.0, 2774.8)	1563.5 (240.7, 2775.8)	<0.001

Data are expressed as mean ± SD, median (25th−75th percentile), or n (%). SpO_2, oxygen saturation; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; PCT, procalcitonin; hs-cTNI, higher sensitivity cardiac troponin I; NT-proBNP, N-terminal pro-brain natriuretic peptide.

regression of high-dimensional data, was used to select the most useful predictive variables from the data set. Then, the multivariate logistic regression analysis was performed to identify independent risk factors. Odds ratios (ORs) were shown with a 95% CI.

The nomogram was established based on the multivariate logistic regression analysis (rms package). A likelihood ratio test approach for model selection was performed. Nomogram performance was quantified with respect to discrimination and calibration. Discrimination (the ability of a nomogram to separate patients with all-cause in-hospital death) was quantified with the concordance index (C-index) and 95% CI. Calibration was assessed graphically by plotting the relationship between actual (observed) probabilities and predicted probabilities (calibration plot) by Hosmer goodness-of-fit test. The internal validation of performance was estimated with the bootstrapping method (500 replications). Integrated discrimination improvement (IDI) and net reclassification improvement (NRI) (survival package) were used to assess the improved ability of the "triple cut-point" strategy of NT-proBNP for the predictive value of the model.

All the tests were two-tailed, and a $P < 0.05$ was considered significant. The statistical analyses were performed with the SPSS version 25.0 software (SPSS Inc., Chicago, IL, United States), R programming language, and environment version 3.6.0 (http://cran.r-project.org).

RESULTS

Baseline Characteristics

This study finally included 402 critically ill patients with laboratory-confirmed COVID-19 and their baseline

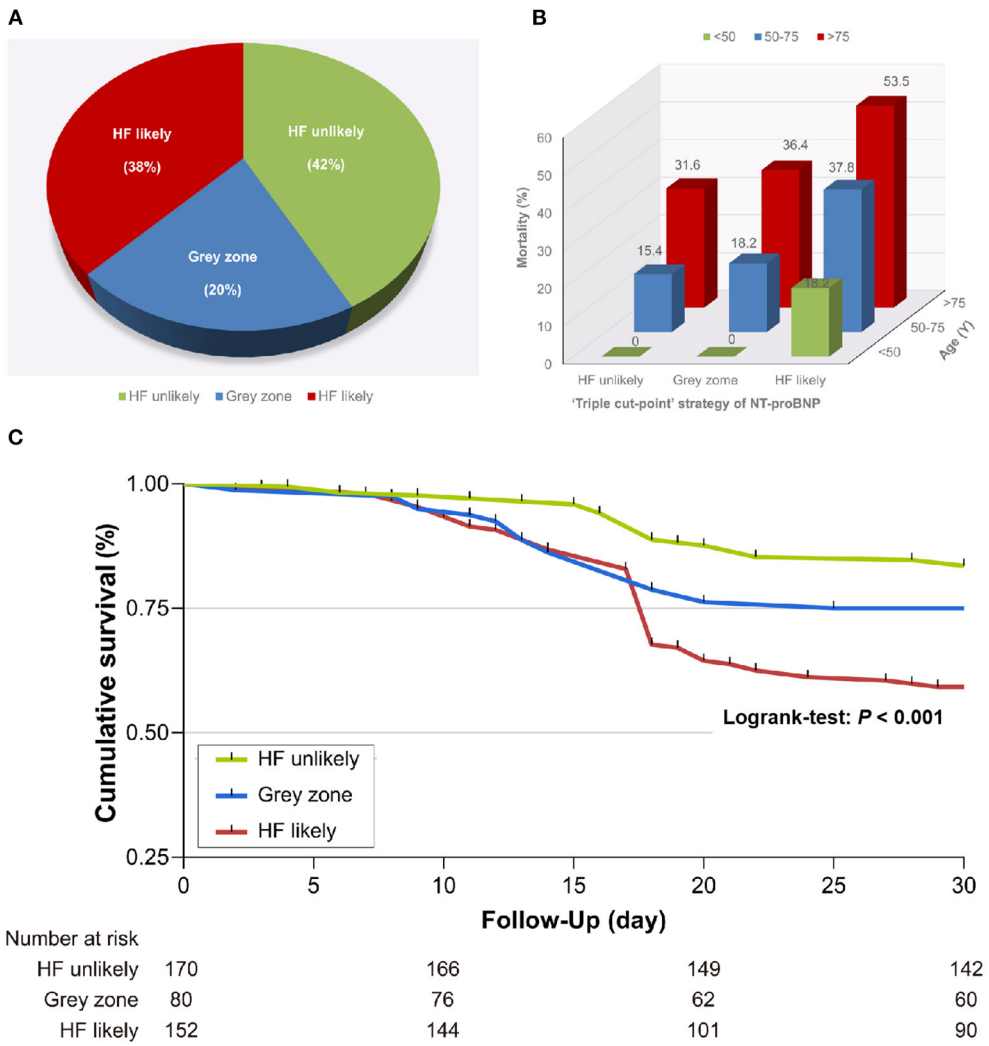


FIGURE 1 | Relationship between the "triple cut-point" strategy of N-terminal pro-brain natriuretic peptide (NT-proBNP) and death. **(A)** Distribution of the "triple cut-point" strategy of NT-proBNP ($n = 402$). **(B)** The mortality rate increased with aging and heart failure. **(C)** Kaplan–Meier survival curves stratified by the "triple cut-point" strategy of NT-proBNP. HF, heart failure.

characteristics are shown in **Table 2**. Overall, the mean age of the whole cohort was 67 years, and 54.5% ($n = 219$) were men. At the 30-day follow-up, 110 patients had died with a 27.4% mortality risk. Compared to the survivors, the non-survivors were more likely to be older, having decreased SpO_2 and elevated heart rate (HR) (all $P < 0.05$). Of note, there was no significance between the two groups regarding gender and comorbidities, irrespective of hypertension, CHD, DM, and respiratory system disease. Furthermore, we compared the laboratory data between the two groups and found that the non-survivors had significantly increased AST, total bilirubin, blood glucose, d-dimer, and PCT as well as decreased lymphocytes, platelets, and estimated glomerular filtration rate (eGFR) (all $P < 0.05$).

TABLE 3 | Distribution of "triple cut-point" strategy of NT-proBNP in critically ill patients with coronavirus disease 2019 (COVID-19).

Setting	Total $n = 402$ (100.0)	Survivors $n = 292$ (72.6)	Non-survivors $n = 110$ (27.4)	*P*-value
HF unlikely, *n* (%)	170 (42.3)	142 (48.6)	28 (25.5)	<0.001
Gray zone, *n* (%)	80 (19.9)	60 (20.6)	20 (18.2)	
HF likely, *n* (%)	152 (37.8)	90 (30.8)	62 (56.3)*	

Data are expressed as n (%). NT-proBNP, N-terminal pro-brain natriuretic peptide; HF, heart failure.

**The distribution of HF likely between survivors and non-survivors was confirmed to be significantly different by post-hoc test (p < 0.001).*

"Triple Cut-Point" Strategy of NT-proBNP and 30-Day Mortality

According to the "triple cut-point" strategy of NT-proBNP, the patients were divided into three groups, and the overall distribution of HF unlikely, gray zone, and HF likely was 170 (42.3%), 80 (19.9%), and 152 (37.8%), respectively (**Figure 1A**). As shown in **Table 3**, the non-survivor group has a significantly higher percentage of patients with HF likely (56.3 vs. 30.8%), and the distribution of the three groups (HF unlikely, gray zone, and HF likely) between the non-survivor and survivor groups was significantly different ($P < 0.001$). Otherwise, within the 30-day follow-up, we observed a mortality rate of 16.5 (28/170), 25 (20/80), and 40.8% (62/152) in group HF unlikely, gray zone, and HF likely, respectively ($P < 0.001$). Importantly, the mortality rate increased sharply, accompanied by the increased likelihood of AHF (**Figure 1B**). The Kaplan-Meier curves of short-time survival were shown in the central illustration, illustrating a significantly shorter mean survival time for patients with HF likely (**Figure 1C**). The overall cumulative risk of death at 30-days was significantly higher for the HF likely group than for HF unlikely and gray zone ($P < 0.001$).

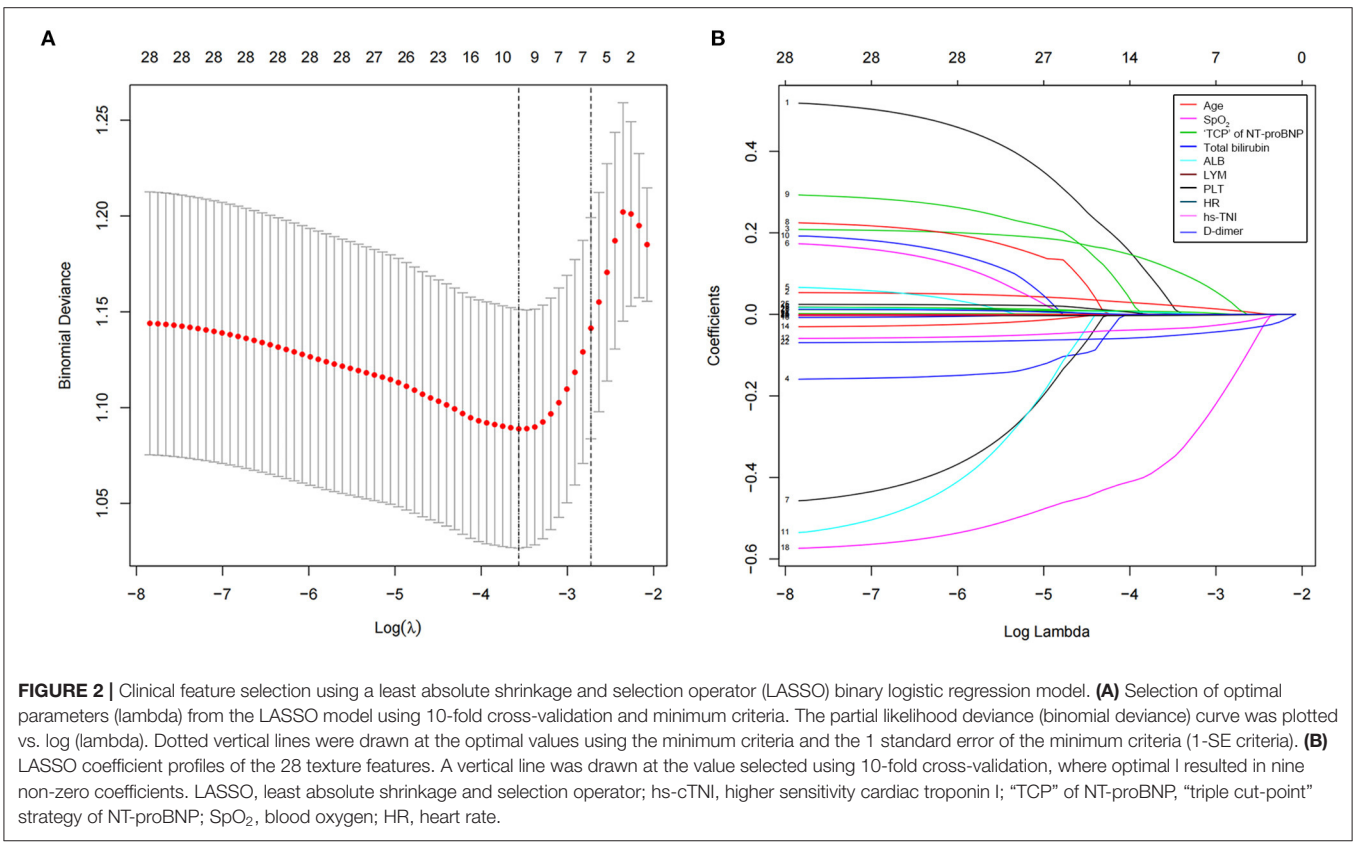


FIGURE 2 | Clinical feature selection using a least absolute shrinkage and selection operator (LASSO) binary logistic regression model. **(A)** Selection of optimal parameters (lambda) from the LASSO model using 10-fold cross-validation and minimum criteria. The partial likelihood deviance (binomial deviance) curve was plotted vs. log (lambda). Dotted vertical lines were drawn at the optimal values using the minimum criteria and the 1 standard error of the minimum criteria (1-SE criteria). **(B)** LASSO coefficient profiles of the 28 texture features. A vertical line was drawn at the value selected using 10-fold cross-validation, where optimal l resulted in nine non-zero coefficients. LASSO, least absolute shrinkage and selection operator; hs-cTNI, higher sensitivity cardiac troponin I; "TCP" of NT-proBNP, "triple cut-point" strategy of NT-proBNP; SpO_2, blood oxygen; HR, heart rate.

TABLE 4 | Multivariate logistic regression analyses of risk factors for 30-day mortality.

Variable	Univariate analysis*		Multivariate analysis	
	OR (CI 95%)	*P*-value	OR (CI 95%)	*P*-value
Age, years	1.042 (1.022, 1.061)	<0.001	1.040 (1.018, 1.061)	<0.001
Spo_2, %	0.926 (0.894, 0.959)	<0.001	—	
HR, beat/min	1.012 (1.000, 1.025)	0.060	—	
PLT, $\times 10^9$ /L	0.994 (0.992, 0.996)	<0.001	—	
LYM, $\times 10^9$ /L	0.224 (0.120, 0.418)	<0.001	0.361 (0.191, 0.681)	0.002
Albumin, g/L	0.878 (0.837, 0.921)	<0.001	0.915 (0.870, 0.963)	0.001
Total bilirubin, μmol/L	1.032 (1.010, 1.054)	0.005	1.022 (1.002, 1.042)	0.032
hs-cTNI, pg/mL	1.010 (1.001, 1.023)	0.048	—	
"TCP" of NT-proBNP		<0.001		0.013
HF unlikely	Reference	—	Reference	—
Gray zone	1.367 (0.718, 2.584)	0.343	1.011 (0.425, 1.725)	0.665
HF likely	2.773 (1.678, 4.583)	<0.001	1.970 (1.133, 3.424)	0.016
D-dimer, μg/mL	1.053 (1.027, 1.080)	<0.001	—	

**The variables of the univariate analysis were from the least absolute shrinkage and selection operator (LASSO) binary logistic regression model.*
Spo_2, blood oxygen; HR, heart rate; hs-cTNI, higher sensitivity cardiac troponin I; "TCP" of NT-proBNP, "triple cut-point" strategy of NT-proBNP.

Predictors of 30-Day in-hospital Death of Critically Ill Patients With COVID-19

The least absolute shrinkage and selection operator was used to select the potential prognostic factors from numerous parameters. Finally, 28 indexes were reduced to 10 potential predictors, namely, age, lymphocyte, platelet, total bilirubin, "triple cut-point" strategy of NT-proBNP, SpO_2, HR, albumin, hs-cTNI, and d-dimer, based on the 402 patients, and were indexes with non-zero coefficients in the LASSO regression model (**Figures 2A,B**). Furthermore, as shown in **Table 4**, the multivariate logistic regression analysis displays five independent predictors for the short-time fatal outcome, namely, HF likely (OR 1.97, 95% CI 1.133–3.424), older age (OR 1.04, 95% CI 1.018–1.061), lymphocyte (OR 0.361, 95% CI 0.191–0.681), total bilirubin (OR 1.022, 95% CI 1.002–1.042), and albumin (OR 0.915, 95% CI 0.87–0.963) (all $P < 0.05$).

Development and Validation of a Novel Nomogram for Predicting Prognosis

An optimal nomogram comprising all the above independent predictors was established to individualize the risk of 30-day in-hospital death (**Figure 3A**). The ratios of calculated β were used to decide the proportional prognostic effect of these variables. Projections from total points on the scales below indicated the estimated probability of death.

Performance accuracy was evaluated by the area under the curve (AUC) of the receiver operating characteristic (ROC) analysis. The AUC for in-hospital death was 0.781 (95% CI 0.733–0.827) (**Figure 3B**). The calibration curve of the nomogram for the probability of death demonstrated good agreement between prediction and observation in the primary cohort (**Figure 3C**). Hosmer-Lemeshow goodness-of-fit was satisfied ($P = 0.354$). The C-index for the prediction nomogram was 0.798 (95% CI 0.742–0.857). The decision curve analysis (DCA) for the clinical laboratory index nomogram is presented in **Figure 3D**. It showed that this nomogram had more benefits than the treat-all-patients scheme or the treat-none scheme in predicting the risk of 30-day in-hospital death of critically ill patients with COVID-19. Moreover, the bootstrap validation method was used to verify the predictive accuracy of the nomogram. The C-index for the nomogram of 30-day in-hospital death was 0.779 (95% CI 0.721–0.834), suggesting the accuracy of this predictive nomogram.

Incremental Predictive Value of "Triple Cut-Point" Strategy of NT-proBNP

To investigate the role of the "triple cut-point" strategy of NT-proBNP in the predictive value of the current model, NRI and IDI were calculated. Compared with the model without the "triple cut-point" strategy of NT-proBNP, the addition of the "triple cut-point" strategy of NT-proBNP resulted in a significantly improved discrimination [IDI 7.3% (95% CI 1.1–14.5%) and NRI 4.9% (95% CI 2.6–7.2%), both with $P < 0.05$].

DISCUSSION

In this prospective multicenter study, we recruited 402 critically ill patients with COVID-19 from four ICUs in China and established a novel nomogram to predict the 30-day all-cause mortality risk in these patients. To the best of our knowledge, there have been few risks score models for predicting the prognosis of critically ill patients with COVID-19. This study has developed a user-friendly and relatively personalized model incorporating five variables, age, "triple cut-point" strategy of NT-proBNP, albumin, lymphocyte count, and total bilirubin, to predict short-time mortality risk in critically ill Chinese patients with COVID-19, which could assist risk stratification and provide insights for timely interventions upon admission. Furthermore, it is highlighted that the "triple cut-point" strategy of NT-proBNP

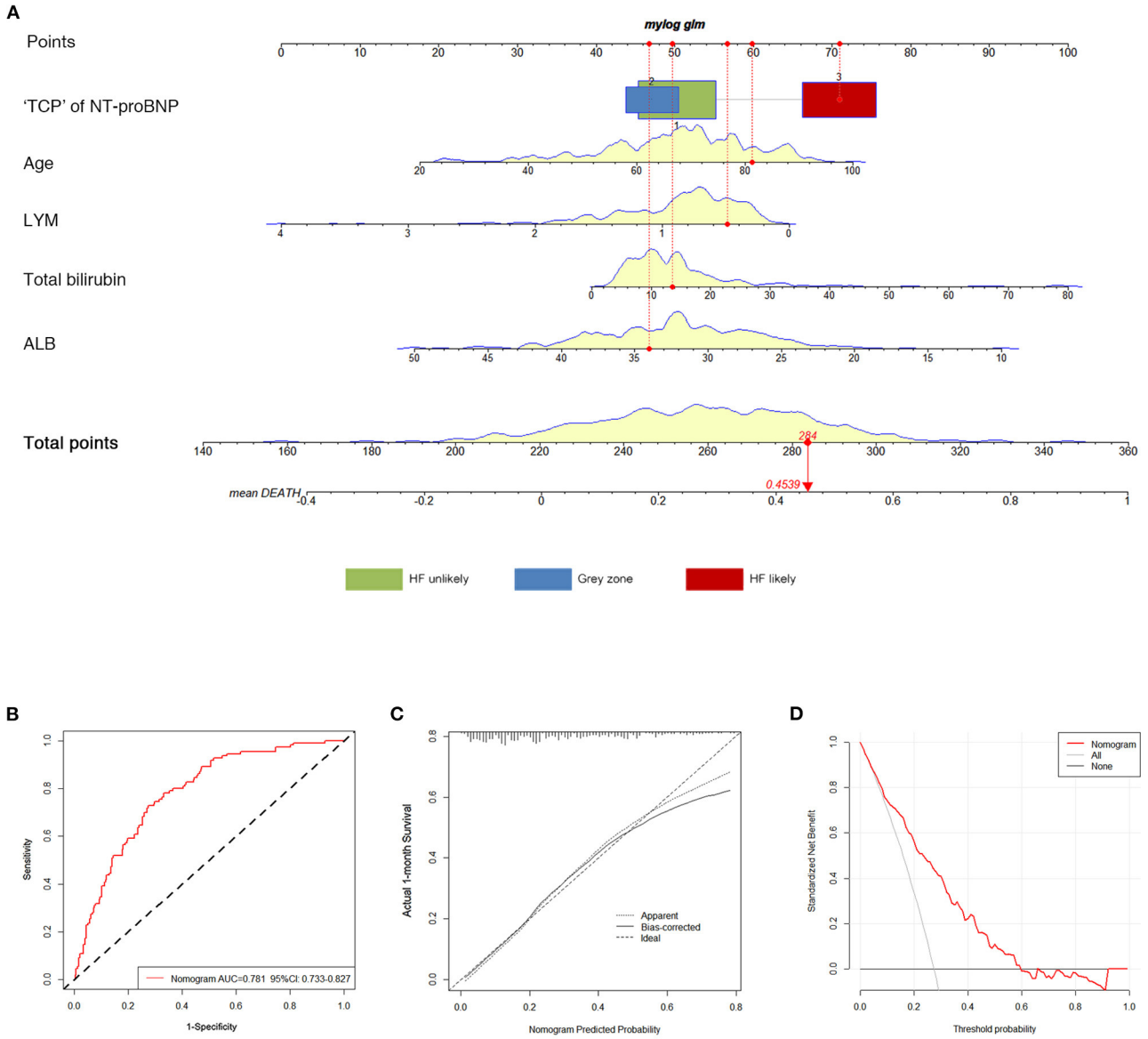


FIGURE 3 | Construction and validation of the nomogram for 30-day all-cause death. **(A)** The total nomogram point of each patient can be used to predict death risk on an individual basis. To predict patient death risk at 30-days, take the following as an example: an 81-year-old patient (60 points) who belonged to HF likely (71 points) had albumin of 34 g/L (46.5 points); his lymphocyte was.5 × 10^9 /L (56.5 points), and total bilirubin was 14 μmol/L (50 points) at admission. He has a total point score of 284, corresponding to a 45.39% risk of death at 30-days. **(B)** ROC curves of the nomogram. **(C)** Calibration plot of observed proportion vs. predicted probability of 30-day death of the nomogram. **(D)** DCA for the nomogram and the model with subtracting of HF. The y-axis measures the net benefit. The dotted pink line represents the nomogram. The thin gray line represents the assumption that all patients will die. The bold black line represents the assumption that no patient will die. "TCP" of NT-proBNP, "triple cut-point" strategy of NT-proBNP; HF, heart failure; AUC, the area under the curve; ROC, receiver operating characteristic; DCA, decision curve analysis.

demonstrated the predominant role of AHF in the clinical course and prognosis in COVID-19.

The mortality risk of COVID-19 has been proven high, with 28-day mortality ranging from 26–53.8% in critically ill adult patients worldwide (1, 11), indicating the imperative of proposing an easy-to-use prediction model to assist risk-stratify and therapeutic optimization in clinical practice for ICU patients. Emerging evidence has tried to explore the risk factors and construct diagnostic and prognostic models in COVID-19 populations (12, 13). However, previous reports regarding prognosis prediction have mainly focused on disease progression or mortality risk of the whole group without further distinguishing critically ill patients in ICU (14). The sample selection bias of the prior models could lead to poor adaptions. In line with existing data, this study reported a 27.6% mortality risk, and further constructed and validated a novel nomogram

for the prediction of 30-day all-cause death. Variables referring to older age, higher level of total bilirubin, lower level of lymphocyte count and albumin, and "triple cut-point" strategy of NT-proBNP were likely to recognize individuals who are at high risk with high sensitivity and specificity. More importantly, the quantitative appraisal made it possible to estimate the likelihood of death more accurately and individually with easy and rapid access in clinical practice.

It has been widely confirmed that cardiac involvement, such as cardiac injury, arrhythmias, myocarditis, and cardiac dysfunction, was prevalent and prognostic in hospitalized patients with COVID-19 (15–17), among which HF is responsible for substantial morbidity and mortality (10). New-onset HF was observed in nearly 23% of hospitalized patients with COVID-19 and as much as one-third of those admitted to the ICU (2, 9). Recent reports revealed that HF was the most frequent cause of death just after acute respiratory distress syndrome (ARDS) and sepsis, accounting for 27.4% of the proximate causes of death in patients with COVID-19 (18). However, the role of HF in the prognosis of critically ill patients with COVID-19 has not been fully elucidated, partially because of high diagnostic uncertainty. A complete diagnosis of HF usually includes symptoms, signs, biomarkers (BNP/NT-proBNP), and imaging examinations, while it is impractical and unavailable to evaluate cardiac function by echocardiography for each critically ill patient with COVID-19 in the clinical practice. Although BNP/NT-proBNP levels are easily interfered with and obscured by considerable factors, the utility of these biomarkers performed well in the emergency setting as an adjunct tool for the diagnosis and triage of dyspneic patients. As such, the guidance has recommended BNP/NT-proBNP as a diagnostic aid for HF with comparable diagnostic accuracy.

As the widely admitted biomarker in HF, NT-proBNP quantitatively reflects hemodynamic myocardial stress (19), indicating not only left ventricular (LV) systolic dysfunction but also cardiac abnormalities, such as LV diastolic dysfunction, right ventricular (RV) dysfunction, valvular dysfunction, increased pulmonary pressures, and atrial arrhythmias. Prior studies have observed ambiguous results that higher levels of BNP or NT-proBNP were found in patients with severe COVID-19 and that they were independently associated with high mortality, maybe because of single-center design, patient population selection bias, and small sample size (20–23). This multicenter study demonstrated that the non-survivors had a significantly higher level of NT-proBNP than the survivors (1,564 vs. 321 ng/ml), with reasonable sample size. Consistently, a recent study described the characterization of NT-proBNP in patients with COVID-19, and 48.5% of their cohort presented NT-proBNP levels above the recommended cut-off for the identification of HF (24).

Furthermore, considering the fact that the plasma level of NT-proBNP is largely affected by age and renal functions, it seems to be not rigorous enough to use NT-proBNP as a simple continuous variable alone to predict the prognosis of patients with COVID-19. Thus, we reclassified the subjects into three groups (HF likely, gray zone, and HF unlikely) according to the recent HF guidance as to the "triple cut-point" strategy of NT-proBNP, and observed that patients with HF likely occupied 37.8% of the total cohort, of which 56.3% were non-survivors (6). Moreover, we found that patients in the HF likely group had a significantly higher risk (OR 1.97, 95% CI 1.133–3.424) for 30-day all-cause death. Concerning the clinical presentations and biomarkers of HF on time would help make optimal individual treatment plans to prevent further deterioration efficiently.

It is worth noting that our prediction model did not incorporate troponin, as it was not independently associated with the outcome unexpectedly, while prior studies have suggested that troponin was a significant prognostic indicator in COVID-19 (15, 25). Similarly, Dong et al. conducted a retrospective study and built a nomogram assessing the 14-day and 21-day in-hospital survival of all the general patients with COVID-19. The final model was constructed based on hypertension, neutrophil-to-lymphocyte ratio, and NT-proBNP (26). Elevated troponin may be a possible confounder for NT-proBNP as they were postulated to share the same pathophysiological processes and found to be both elevated in pneumonia, sepsis, ARDS, and several other non-cardiac illnesses. Hence, we speculated that an accurate classification using the "triple cut-point" strategy of NT-proBNP may decrease the confounding effect of troponin. Notably, liver injuries, such as elevated total bilirubin and decreased albumin, have also been demonstrated to be common and associated with disease severity and poor outcomes for critically ill patients in this study, in accordance with previous studies (27). Furthermore, elevated total bilirubin may also be associated with cardiac dysfunctions as a significant and independent predictor of poor cardiovascular prognosis in patients with HF (28).

Critically ill conditions with COVID-19 were usually complicated by multiple organ dysfunctions with complex pathophysiological processes involving numerous parameters, including but not limited to hypoxemia, inflammation, thromboembolism, renal failure, and cardiac damage (29). Therefore, it is of vital importance to bring all reasonable possible variables into analysis and construct a scientific prediction model relying on appropriate statistical analysis awfully. In the current study, LASSO, a machine learning algorithm, was applied to shrink the regression coefficients from amounting clinical and laboratory indicators to 10 potential predictors. Thus, this algorithm could conquer common confusing collinearity issues and yield more robust results than traditional variable screening methods.

Some cautions should be considered when interpreting our results. First, although our study is observational and the sample size is relatively small, it has a multicenter and prospective design emphasizing critically ill patients. Further investigations with a larger sample size are warranted. Second, this study did not apply other diagnostic tools to make a complete HF diagnosis. However, it is impractical and unavailable to evaluate cardiac functions by echocardiography for each critically ill patient with COVID-19. Conversely, rapid measurements of NT-proBNP have substantial medical aids to fulfill the clinical need underlying this extraordinary stressful setting, although it should never be a stand-alone test for HF diagnosis. Third, our nomogram model lacks validation in an external

population. Nevertheless, internal verification indicated the predictive strength in our study.

CONCLUSIONS

In this study, we explored the independent predictors for short-time prognosis in critically ill patients with COVID-19 in China and established a novel nomogram to predict the 30-day all-cause mortality risk for the first time, highlighting the predominant role of the "triple cut-point" strategy of NT-proBNP. This easy-to-use prognostication nomogram can provide survival estimations and help identify patients with COVID-19 with a high-risk trajectory, further advancing clinical management and ultimately improving outcomes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Peking University People's Hospital. The ethics committee waived the requirement for written informed consent.

AUTHOR CONTRIBUTIONS

JR and TW conceived and designed the study and coordinated to complete the study. JR critically revised the manuscript. WG contributed to data collection and completed the project. JF and DS analyzed and interpreted the data and wrote the manuscript. LT assisted in performing statistical analysis. MY, WG, JZhe, and JZhu helped revise the manuscript for important intellectual content and language polishing. All the authors have read and approved the final version of the manuscript.

FUNDING

This study was supported by the National Natural Science Foundation of China (81770359 to JR), State Key Laboratory of Molecular Developmental Biology of China (2020-MDB-KF-17 to JR), Beijing Health Science and Technology Achievements and Appropriate Technology Promotion Project (BHTPP202004 to JR), Elite Medical Professionals project of China-Japan Friendship Hospital (ZRJY2021-BJ01 to JR), and China-Japan Friendship Hospital Scientific Research Funds (2019-2-QN-77 to DS).

ACKNOWLEDGMENTS

We sincerely acknowledge the healthcare workers from Zhongfaxincheng campus and Guanggu campus of Tongji Hospital Affiliated with Huazhong University of Science and Technology in Wuhan. They contributed tremendously to blocking the spread and prevalence of COVID-19 in Wuhan, Hubei Province, China, and the whole country.

REFERENCES

1. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. *JAMA.* (2020) 323:1574–81. doi: 10.1001/jama.2020.5394
2. Arentz M, Yim E, Klaff L, Lokhandwala S, Riedo FX, Chong M, et al. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington state. *JAMA.* (2020) 323:1612–4. doi: 10.1001/jama.2020.4326
3. Casadevall A, Joyner MJ, Pirofski L-A. A Randomized trial of convalescent plasma for COVID-19—potentially hopeful signals. *JAMA.* (2020) 324:455–7. doi: 10.1001/jama.2020.10218
4. Wang T, Du Z, Zhu F, Cao Z, An Y, Gao Y, et al. Comorbidities and multi-organ injuries in the treatment of COVID-19. *Lancet.* (2020) 395:e52. doi: 10.1016/S0140-6736(20)30558-4
5. Aboughdir M, Kirwin T, Abdul Khader A, Wang B. Prognostic value of cardiovascular biomarkers in COVID-19: a review. *Viruses.* (2020) 12:527. doi: 10.3390/v12050527
6. Mueller C, McDonald K, de Boer RA, Maisel A, Cleland JGF, Kozhuharov N, et al. Heart Failure Association of the European Society of Cardiology practical guidance on the use of natriuretic peptide concentrations. *Eur J Heart Fail.* (2019) 21:715–31. doi: 10.1002/ejhf.1494
7. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med.* (2020) 8:475–81. doi: 10.1016/S2213-2600(20)30079-5
8. Yu Y, Xu D, Fu SZ, Zhang J, Yang XB, Xu L, et al. Patients with COVID-19 in 19 ICUs in Wuhan, China: a cross-sectional study. *Crit Care.* (2020) 24:219–28. doi: 10.1186/s13054-020-02939-x
9. Haase N, Plovsing R, Christensen S, Poulsen LM, Brøchner AC, Rasmussen BS, et al. Characteristics, interventions, and longer term outcomes of COVID-19 ICU patients in Denmark—a nationwide, observational study. *Acta Anaesthesiol Scand.* (2021) 65:68–75. doi: 10.1111/aas.13701
10. WHO. *Clinical Management of Severe Acute Respiratory Infection When Novel Coronavirus (nCoV) Infection Is Suspected: Interim Guidance, 25 January 2020* (2020).
11. Xie J, Wu W, Li S, Hu Y, Hu M, Li J, et al. Clinical characteristics and outcomes of critically ill patients with novel coronavirus infectious disease (COVID-19) in China: a retrospective multicenter study. *Intensive CareMed.* (2020) 46:1863–72. doi: 10.1007/s00134-020-06211-2
12. Feng Z, Yu Q, Yao S, Luo L, Zhou W, Mao X, et al. Early prediction of disease progression in COVID-19 pneumonia patients with chest CT and clinical characteristics. *Nat Commun.* (2020) 11:4968–76. doi: 10.1038/s41467-020-18786-x
13. Wynants L, Van Calster B, Collins GS, Riley RD, Heinze G, Schuit E, et al. Prediction models for diagnosis and prognosis of covid-19: systematic review and critical appraisal. *BMJ.* (2020) 369:m1328. doi: 10.1136/bmj.m1328
14. Vultaggio A, Vivarelli E, Virgili G, Lucenteforte E, Bartoloni A, Nozzoli C, et al. Prompt predicting of early clinical deterioration of moderate-to-severe COVID-19 patients: usefulness of a combined score using IL-6 in a preliminary study. *J Allergy Clin Immunol Pract.* (2020) 8:2575–81.e2. doi: 10.1016/j.jaip.2020.06.013

15. Inciardi RM, Adamo M, Lupi L, Cani DS, Di Pasquale M, Tomasoni D, et al. Characteristics and outcomes of patients hospitalized for COVID-19 and cardiac disease in Northern Italy. *Eur Heart J.* (2020) 41:1821–9. doi: 10.1093/eurheartj/ehaa388
16. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *JAMA.* (2020) 323:1061–9. doi: 10.1001/jama.2020.1585
17. Liu PP, Blet A, Smyth D, Li H. The science underlying COVID-19: implications for the cardiovascular system. *Circulation.* (2020) 142:68–78. doi: 10.1161/CIRCULATIONAHA.120.047549
18. Zhang S, Guo M, Duan L, Wu F, Hu G, Wang Z, et al. Development and validation of a risk factor-based system to predict short-term survival in adult hospitalized patients with COVID-19: a multicenter, retrospective, cohort study. *Crit Care.* (2020) 24:438–50. doi: 10.1186/s13054-020-03123-x
19. Ibrahim NE, Burnett JC, Butler J, Camacho A, Felker GM, Fiuzat M, et al. Natriuretic peptides as inclusion criteria in clinical trials. *JACC Heart Fail.* (2020) 8:347–58. doi: 10.1016/j.jchf.2019.12.010
20. Gao L, Jiang D, Wen X, Cheng X, Sun M, He B, et al. Prognostic value of NT-proBNP in patients with severe COVID-19. *Respir Res.* (2020) 21:83–9. doi: 10.1186/s12931-020-01352-w
21. Pranata R, Huang I, Lukito AA, Raharjo SB. Elevated N-terminal pro-brain natriuretic peptide is associated with increased mortality in patients with COVID-19: systematic review and meta-analysis. *Postgrad Med J.* (2020) 96:387–91. doi: 10.1136/postgradmedj-2020-137884
22. Sorrentino S, Cacia M, Leo I, Polimeni A, Sabatino J, Spaccarotella CAM, et al. B-type natriuretic peptide as biomarker of COVID-19 disease severity—a meta-analysis. *J Clin Med.* (2020) 9:2957–63. doi: 10.3390/jcm9092957
23. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* (2020) 5:802–10. doi: 10.1001/jamacardio.2020.0950
24. Caro-Codón J, Rey JR, Buño A, Iniesta AM, Rosillo SO, Castrejon-Castrejon S, et al. Characterization of NT-proBNP in a large cohort of COVID-19 patients. *Eur J Heart Fail* (2021) 23:456–64. doi: 10.1002/ejhf.2095
25. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with Coronavirus Disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:811–18. doi: 10.1001/jamacardio.2020.1017
26. Dong YM, Sun J, Li YX, Chen Q, Liu QQ, Sun Z, et al. Development and validation of a nomogram for assessing survival in patients with COVID-19 pneumonia. *Clin Infect Dis.* (2020) 72:652–60. doi: 10.1093/cid/ciaa963
27. Bloom PP, Meyerowitz EA, Reinus Z, Daidone M, Gustafson J, Kim AY, et al. Liver biochemistries in hospitalized patients with COVID-19. *Hepatology.* (2021) 73:890–900. doi: 10.1002/hep.31326
28. Suzuki K, Claggett B, Minamisawa M, Packer M, Zile MR, Rouleau J, et al. Liver function and prognosis, and influence of sacubitril/valsartan in patients with heart failure with reduced ejection fraction. *Eur J Heart Fail.* (2020) 22:1662–71. doi: 10.1002/ejhf.1853
29. Gupta S, Hayek SS, Wang W, Chan L, Mathews KS, Melamed ML, et al. Factors associated with death in critically ill patients with coronavirus disease 2019 in the US. *JAMA Intern Med.* (2020) 180:1–12. doi: 10.1001/jamainternmed.2020.3596

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 30 November 2021
doi: 10.3389/fcvm.2021.745758

Long COVID 19 Syndrome: Is It Related to Microcirculation and Endothelial Dysfunction? Insights From TUN-EndCOV Study

Salma Charfeddine[1,2], Hassen Ibn Hadj Amor[3], Jihen Jdidi[2,4], Slim Torjmen[1,2], Salma Kraiem[3], Rania Hammami[1,2], Amine Bahloul[1,2], Nesrine Kallel[2,5], Nedia Moussa[2,5], Imen Touil[6], Aiman Ghrab[7], Jamel Elghoul[2,8], Zineb Meddeb[9], Yamina Thabet[9], Samir Kammoun[1,2], Kamel Bouslama[9], Sami Milouchi[2,7], Salem Abdessalem[10] and Leila Abid[1,2]*

[1] Cardiology Department, Hedi Chaker University Hospital Sfax, Sfax, Tunisia, [2] University of Medicine of Sfax, University of Sfax, Sfax, Tunisia, [3] Department of Cardiology, Tahar Sfar Hospital Mahdia, Sfax, Tunisia, [4] Department of Preventive Medicine, Hedi Chaker University Hospital Sfax, Sfax, Tunisia, [5] Department of Pneumology, Hedi Chaker University Hospital Sfax, Sfax, Tunisia, [6] Department of Pneumology, Tahar Sfar Hospital Mahdia, Mahdia, Tunisia, [7] Department of Cardiology, Habib Bourguiba Hospital Medenine, Sfax, Tunisia, [8] Department of Pneumology, Habib Bourguiba Hospital Medenine, Sfax, Tunisia, [9] Department of Internal Medicine, Mongi Slim LaMarsa Hospital, Tunis, Tunisia, [10] Clinic Pasteur Tunis, Tunis, Tunisia

The COVID-19 disease is a multisystem disease due in part to the vascular endothelium injury. Lasting effects and long-term sequelae could persist after the infection and may be due to persistent endothelial dysfunction. Our study focused on the evaluation of endothelial quality index (EQI) by finger thermal monitoring with E4 diagnosis Polymath in a large cohort of long COVID-19 patients to determine whether long-covid 19 symptoms are associated with endothelial dysfunction. This is a cross-sectional multicenter observational study with prospective recruitment of patients. A total of 798 patients were included in this study. A total of 618 patients (77.4%) had long COVID-19 symptoms. The mean EQI was 2.02 ± 0.99 $IC_{95\%}$ [1.95–2.08]. A total of 397 (49.7%) patients had impaired EQI. Fatigue, chest pain, and neuro-cognitive difficulties were significantly associated with endothelium dysfunction with an EQI < 2 after adjustment for age, sex, diabetes, hypertension, dyslipidemia, coronary heart disease, and the severity of acute COVID-19 infection. In multivariate analysis, endothelial dysfunction (EQI < 2), female gender, and severe clinical status at acute COVID-19 infection with a need for oxygen supplementation were independent risk factors of long COVID-19 syndrome. Long COVID-19 symptoms, specifically non-respiratory symptoms, are due to persistent endothelial dysfunction. These findings allow for better care of patients with long COVID-19 symptoms.

Keywords: COVID-19, long COVID-19 syndrome, endothelial function, microcirculation, endothelium

OPEN ACCESS

Edited by:
Hendrik Tevaearai Stahel, Bern University Hospital, Switzerland

Reviewed by:
Ayman Al Haj Zen, Hamad Bin Khalifa University, Qatar
Shunsuke Katsuki, Kyushu University Hospital, Japan
Sarvesh Chelvanambi, Brigham and Women's Hospital and Harvard Medical School, United States

****Correspondence:***
Salma Charfeddine
selma_charfeddine@yahoo.fr

Specialty section:
This article was submitted to Atherosclerosis and Vascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *22 July 2021*
Accepted: *28 October 2021*
Published: *30 November 2021*

Citation:
Charfeddine S, Ibn Hadj Amor H, Jdidi J, Torjmen S, Kraiem S, Hammami R, Bahloul A, Kallel N, Moussa N, Touil I, Ghrab A, Elghoul J, Meddeb Z, Thabet Y, Kammoun S, Bouslama K, Milouchi S, Abdessalem S and Abid L (2021) Long COVID 19 Syndrome: Is It Related to Microcirculation and Endothelial Dysfunction? Insights From TUN-EndCOV Study. Front. Cardiovasc. Med. 8:745758. doi: 10.3389/fcvm.2021.745758

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was detected in China in December 2019 (1, 2). Up to the time of writing, more than 149 million people worldwide have been infected, and over 3 million people have died from the coronavirus disease 2019 (COVID-19) (3). The COVID-19 disease is a multisystem disease due to in part the vascular endothelium injury it causes (4, 5). Lasting effects and long-term sequelae could persist after the infection, affecting patients' return to work and quality-of-life (6). The most prevalent ongoing symptoms are fatigue, dyspnea, chest pain, joint pain, palpitations, anosmia and dysgeusia, hair loss, cognitive symptoms, and psychosocial distress (6–11). Some studies suggest that Long-COVID 19 symptoms may be due to persistent endothelial dysfunction (12). In fact, the SARS-CoV-2 infection of endothelial cells is associated with changes in cell morphology and endothelial cells apoptosis that could persist several weeks after the acute infection. The Working Group on Atherosclerosis and Vascular Biology together with the Council of Basic Cardiovascular Science of the European Society of Cardiology provided a Position Statement on the importance of the endothelial function study in convalescent patients for early detection and prevention of long-term cardiovascular complications (4).

Our study focused on endothelial function evaluation by finger thermal monitoring (FTM) of endothelial quality with an E4-diagnose device (Polymath Company) (13) in a large cohort of long COVID-19 patients.

The study objectives were defined as the assessment of long COVID 19 symptoms' prevalence, endothelial function in recovered COVID-19 patients, and its link to long COVID-19 symptoms.

MATERIALS AND METHODS

Study Design

This was a cross-sectional multicenter observational study with prospective recruitment of patients. The recruiting period extended from January 20 to May 10, 2021. The study protocol was recorded in the Pan African Clinical Trials Registries (PACTR) with trial ID PACTR202102867544936. The study had the local Ethics and Investigation Committee approval, being designated with approval number CPP SUD 0299/2020.

Participants

Patients were recruited by local health authorities relying on the COVID-19 registry. After being informed about the study and potential risks, all eligible patients–recovered from confirmed severe acute SARS-CoV2 infection and having given written informed consent–underwent a comprehensive non-invasive assessment of endothelial and cardiac function during their long-COVID19 infection period. Inclusion criteria were as follows: age >18 years, both sexes, written informed consent, and a recent diagnosis of COVID-19 infection in the past 2 weeks−6 months proven by RT-PCR analysis of nasopharyngeal swabs positivity or viral rapid test. Important exclusion criteria included: Diseases carrying a life-expectancy of <1 year according to clinical judgment, pregnancy and breastfeeding, and foreseen inability to attend scheduled visits.

The long COVID-19 symptoms were defined by persistent symptoms 4 weeks after the start of acute COVID-9 infection (14). These symptoms were assessed simultaneously with the microvascular circulation and endothelial function at the inclusion. An objective evaluation of reported symptoms was performed. Shortness of breath was defined by a New York Heart Association class of dyspnea $\geq$ 2 (15). Fatigue was evaluated according to the modified fatigue severity scale (16). We used the Mini-Mental State Examination (MMSE) to assess cognitive performances. Cognitive difficulties were defined by an MMSE score $<$ 24 (17).

Test Methods

Assessment of Microvascular Circulation and Endothelial Function

All investigation sites used the E4-diagnose device (Polymath Company. Tunisia) with a fully automated and standardized post occlusion reactive hyperemia procedure (PORH). The E4-diagnose is a non-invasive, high resolution (0.002°C) skin temperature measuring device. It consists of a portable microcontroller (MCU) unit, two accurate finger temperature sensors, and an integrated wrist cuff. All the automated procedures and calculations are fully processed by the embedded MCU firmware. A dedicated PC software views, stores, and exports the data. The tests were carried out in a dimmed and quiet room. The ambient temperature (between 22–24°C) was maintained consistently during the test. The patient was fasting with no smoking nor heavy physical activity, for 4 h prior to the test. At least 20 min were allowed for acclimatization and subjects were kept in a relaxing sitting position. Systolic blood pressure was checked to be <160 mmHg and index fingers temperature above 27°C. The integrated wrist cuff is placed on the dominant forearm and both finger sensors are gently fixed to both index fingers.

The standard protocol is reported in **Supplementary Material**, **Supplementary Figures 1**, **2**.

During TUN-EndCOV study, EQI was selected as the best parameter to reflect the endothelial function relying on the classification below:

- EQI $\geq$ 2: Good endothelial function
- EQI $<$ 2: Endothelial dysfunction

The cut-off of two was in accordance with reported previous data (18) and after statistical validation (**Supplementary Material**).

The group endothelial dysfunction was divided into two subgroups with a cut-off of 1.5 for further analysis.

Echocardiographic Evaluation

A complete echocardiographic evaluation of the systolic and diastolic left ventricle (LV) function was performed. The LV global longitudinal strain (LVGLS) was determined by the speckle tracking analysis.

TABLE 1 | Baseline characteristics of the study population.

	Total population (*N* = 798)	Post COVID 19 symptoms (*N* = 618)	No post COVID 19 symptoms (*N* = 180)	*p*-value
Clinical characteristics				
Age (years)	49.94 ± 14.2	50.03 ± 14.2	49.65 ± 14.3	0.75
BMI (kg/m2)	28.34 ± 4.7	28.35 ± 4.6	28.32 ± 4.8	0.94
Females (*n*, %)	483 (60.5)	389 (62.9)	94 (52.2)	**0.01**
Diabetes (*n*, %)	189 (23.7)	153 (24.8)	36 (20.0)	0.18
Hypertension (*n*, %)	269 (33.7)	207 (33.5)	62 (34.4)	0.81
Dyslipidemia (*n*, %)	84 (10.5)	69 (11.2)	15 (8.3)	0.27
Smoking (*n*, %)	57 (7.1)	41 (6.6)	16 (8.9)	0.30
CV risk factors				
0	397 (49.7)	305 (49.4)	92 (51.1)	0.68
1	195 (24.4)	157 (25.4)	38 (21.1)	
2	130 (16.3)	89 (15.9)	32 (17.8)	
≥3	76 (9.5)	58 (9.4)	18 (10.0)	
Heart failure (*n*, %)	4 (0.5)	3 (0.5)	1 (0.6)	0.64
Coronary heart disease (*n*, %)	34 (4.3)	25 (4.0)	9 (5.0)	0.57
Pulmonary disease (*n*, %)	48 (6.0)	42 (6.8)	6 (3.3)	0.08
Chronic medications before trial				
Aspirin (*n*, %)	70 (8.8)	55 (8.9)	15 (8.3)	0.81
ACE inhibitors (*n*, %)	103 (12.9)	84 (13.6)	19 (10.6)	0.28
ARBs (*n*, %)	58 (7.3)	53 (8.6)	5 (2.8)	**0.008**
Bblockers (*n*, %)	84 (10.5)	56 (9.1)	29 (16.1)	**0.007**
Statins (*n*, %)	107 (13.4)	87 (14.1)	20 (11.1)	0.30
Calcium channel blockers (*n*, %)	37 (4.6)	29 (4.7)	8 (4.4)	0.88
Nitrates (*n*, %)	6 (0.8)	3 (0.5)	3 (1.7)	0.10
Severity of COVID 19 infection				
Moderate or severe symptoms (need of oxygen) (*n*, %)	185 (23.2)	159 (25.7)	26 (14.4)	**0.002**
Extend of lesions at thoracic CT scan				
≥50 % (*n*, %)	36 (4.5)	32 (5.2)	4 (2.2)	0.09
Endothelial function parameters				
EQI	2.02 ± 0.9	1.99 ± 0.9	2.09 ± 1.0	0.24
Flow_ratio	5.02 ± 3.2	5.02 ± 3.2	5.00 ± 3.3	0.93
Peak_time	51.86 ± 29.9	50.97 ± 28.5	54.95 ± 34.4	0.12
Half_time_decay	36.10 ± 17.5	35.74 ± 16.6	37.13 ± 19.9	0.42
Endothelial dysfunction (EQI <2)	397 (49.7)	319 (51.6)	78 (43.3)	**0.05**
Echocardiographic parameters				
LVEF (%)	61.00 ± 5.3	60.94 ± 5.3	61.30 ± 5.1	0.65
LVGLS (%)	−16.96 ± 2.7	−16.84 ± 2.6	−17.63 ± 2.8	0.07
E-wave velocity (cm/sec)	79.33 ± 19.0	79.21 ± 19.2	79.91 ± 18.2	0.81
A-wave velocity (cm/sec)	74.15 ± 18.2	73.74 ± 18.5	76.11 ± 16.7	0.40
E' velocity (cm/sec)	13.81 ± 3.9	13.64 ± 3.7	14.57 ± 5.0	0.13
E/E'	6.09 ± 2.1	6.08 ± 2.0	6.14 ± 2.5	0.87
sPAP (mmHg)	21.64 ± 7.2	21.77 ± 7.4	21.06 ± 6.4	0.53
Elevated LV filling pressure (*n*, %)	2 (0.3)	1 (0.2)	1 (0.6)	0.4

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blockers; BMI, body mass index; CV, cardiovascular; EQI, endothelium quality index; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; E, transmitral early diastolic peak velocity; A, late diastolic peak velocity; E', early relaxation velocity on tissue Doppler; sPAP, systolic pulmonary artery pressure. Bold values mean statistically significant difference.

Statistical Analysis

Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 23.0. The complete database is maintained by the study team. Categorical variables were expressed as frequencies and percentages and continuous variables as mean and SD. Differences in percentages were assessed using the chi-2 test and their means using the Student's t-test. Estimates of risk ratios were presented with 95% Confidence Intervals (CI). All variables that were statistically significant at univariate analysis and those considered of relevant clinical interest with a risk of error of 10% were included in a multivariable model (logistic regression) to identify the

TABLE 2 | Adjusted associations of different long COVID 19 symptoms to endothelial dysfunction.

	Total population ($n = 798$)	EQI ≥ 2 ($n = 401$)	EQI < 2 ($n = 397$)	*p*-value	$OR_{95\%}$	Adjusted* $OR_{95\%}$
≥ 1 symptom (*n*, %)	618 (77.4)	299 (74.5)	319 (80.3)	0.05	1.39 [0.99–1.39]	1.45 [1.02–2.07], $p = 0.03$
Chest pain, dyspnea or fatigue (*n*, %)	546 (68.4)	256 (63.8)	296 (73)	0.005	1.53 [1.13– 2.07]	1.50 [1.09-2.07], $p = 0.01$
Fatigue (*n*, %)	337 (42.2)	155 (38.7)	182 (45.8)	0.04	1.34 [1.01–1.78]	1.36 [1.01, 1.83], $p = 0.038$
Chest pain (*n*, %)	162 (20.3)	62 (15.5)	100 (25.2)	0.001	1.84 [1.29–2.62]	1.94 [1.34–2.80], $p < 0.001$
Palpitations (*n*, %)	139 (17.4)	78 (19.5)	61 (15.4)	0.12	0.75 [0.52–1.08]	0.84 [0.57–1.24], $p = 0.40$
Shortness of breath (*n*, %)	331 (41.5)	159 (39.7)	172 (43.3)	0.29	1.16 [0.87–1.54]	1.12 [0.83- 1.52], $p = 0.44$
Cough (*n*, %)	136 (17)	63 (15.7)	73 (18.4)	0.31	1.20 [0.83–1.75]	1.15 [0.78–1.69], $p = 0.47$
Headaches (*n*, %)	176 (22.1)	86 (21.4)	90 (22.7)	0.67	1.07 [0.76–1.50]	1.26 [0.88–1.79], $p = 0.19$
Anosmia (*n*, %)	28 (3.5)	15 (3.7)	13 (3.3)	0.72	0.87 [0.40–1.85]	1.06 [0.47 – 2.35], $p = 0.88$
Gastro-intestinal syndrome (*n*, %)	47 (5.9)	18 (4.5)	29 (7.3)	0.09	1.67 [0.91–3.07]	1.62 [0.86–3.05], $p = 0.13$
Neuro-cognitive difficulties (*n*, %)	97 (12.2)	44 (11.0)	53 (13.4)	0.30	1.25 [0.81–1.91]	1.62 [1.03–2.55], $p = 0.036$
Sleep disorders	76 (9.5)	34 (8.5)	42 (10.6)	0.31	1.27 [0.79–2.05]	1.45 [0.88–2.40], $p = 0.13$

**Adjusted to age, sex, diabetes, hypertension, dyslipidemia, coronary heart disease, and severe clinical status of COVID 19 infection with the need for oxygen.*
EQI, endothelium quality index; OR, odds ratio.

independent predictors of endothelial dysfunction and long COVID 19 symptoms and to determine adjusted Odds Ratio (OR). Receiver operating characteristic (ROC) analysis was carried out to determine the cut-off value of continuous variables associated with endothelial dysfunction. A $p < 0.05$ was considered statistically significant.

RESULTS

A total of 798 patients were included in this study. Patients were included at an average time of 68.934 ± 3.1 [28–186] days. The mean age was 49.94 ±14.2 years. Women accounted for 60.5 % of patients (483 of 798). Hypertension was the most common chronic health condition, reported in 33%, followed by diabetes in 23.7%. A total of 618 patients (77.4%) had long COVID 19 symptoms. The mean EQI was 2.02 ± 0.99 $IC_{95\%}$ [1.95–2.08]. A total of 397 (49.7%) patients had impaired EQI. The demographics, clinical characteristics, long-term medications, endothelial function, and echocardiographic parameters of the study population at inclusion according to the occurrence of long COVID 19 symptoms were reported in **Table 1**.

Among long COVID-19 symptoms, fatigue was the most common symptom reported in 42.2%, followed by shortness of breath in 41.5%, headaches in 22.1%, and chest pain in 20.3%.

Long COVID-19 symptoms were associated with endothelium dysfunction with an EQI<2 (**Table 2**). Fatigue, chest pain, and neuro-cognitive difficulties were significantly associated with an EQI <2 after adjustment for age, sex, diabetes, hypertension, dyslipidemia, coronary heart disease, and the severity of acute COVID-19 infection (need for oxygen) (**Table 2**). Long COVID-19 symptoms were not associated with the severity of the endothelial dysfunction in the subgroups analysis.

In multivariate analysis, endothelial dysfunction (EQI < 2), female gender, and severe clinical status at acute COVID-19 infection with a need for oxygen supplementation were independent risk factors of long COVID-19 syndrome (**Table 3**).

Endothelial dysfunction was significantly associated with the older age, body mass index (BMI), male gender, cardiovascular risk factors, the severity of symptoms during the acute phase of COVID-19 infection, the extension of pulmonary lesions during the COVID-19 infection, and reduced LV GLS (**Table 4**).

TABLE 3 | Associated factors to long COVID-19 syndrome in multivariate analysis.

Variable	Odds Ratio, 95 CI%	*P*
EQI < 2	1.522 (1.072–2.160)	0.019
Female gender	1.913 (1.340–2.731)	$<10^{-3}$
Severe clinical status of COVID 19 infection with need to oxygen	2.394 (1.495–3.833)	$<10^{-3}$
B-blockers	0.489 (0.296–0.806)	0.005

EQI, endothelium quality index.

According to ROC analysis, 45 years old, 25kg/m2, and−16% were the optimal cut-off values respectively for age, BMI, and LVGLS associated with endothelial dysfunction (**Supplementary Figure 3**). In multivariate analysis, age ≥ 45 years old, reduced LVGLS<-16%, and dyslipidemia were significantly associated with endothelial dysfunction (**Table 5**).

DISCUSSION

COVID-19 is a multisystem disease due to in part endothelium damage (4, 5). SARS-CoV-2 infects the host using the angiotensin-converting enzyme 2 (ACE2) receptors, which are expressed in several organs, including the lung, heart, kidney, intestine, and also expressed by endothelial cells, causing a distinguishable and distinct systemic endotheliitis (5, 19).

Lasting sequalae, symptoms, signs, or abnormal clinical parameters persisting 4 weeks or more after COVID-19 infection onset were commonly defined as "long COVID-19" (14, 20–22).

While it is well established that endothelial dysfunction is associated with poor prognosis in acute phase COVID19, its link with long COVID 19 symptoms is still questionable.

TABLE 4 | Baseline characteristics in the study population according to endothelium quality index.

	Total population ($n = 798$)	EQI ≥ 2 ($n = 401$)	Impaired EQI < 2 ($n = 397$)	*p*-value
Clinical characteristics				
Age (years)	49.94 ± 14.2	47.21 ± 14.5	52.71 ± 13.4	$<10^{-3}$
BMI (kg/m^2)	28.34 ± 4.7	27.55 ± 4.6	29.14 ± 4.6	$<10^{-3}$
Females (*n*, %)	483 (60.5)	279 (69.6)	204 (51.4)	$<10^{-3}$
Diabetes (*n*, %)	189 (23.7)	75 (18.7)	114 (28.7)	0.001
Hypertension (*n*, %)	269 (33.7)	112 (27.9)	157 (39.5)	0.001
Dyslipidemia (*n*, %)	84 (10.5)	26 (6.5)	58 (14.6)	$<10^{-3}$
Smoking (*n*, %)	57 (7.1)	28 (7.0)	29 (7.3)	0.86
CV risk factors				
0	365 (45.7)	213 (53.1)	152 (38.3)	$<10^{-3}$
1	223 (27.9)	108 (26.9)	115 (29.0)	
2	125 (15.7)	48 (12.0)	77 (19.4)	
≥3	85 (10.7)	32 (8.0)	53 (13.4)	
Heart failure (*n*, %)	4 (0.5)	1 (0.2)	3 (0.8)	0.31
Coronary heart disease (*n*, %)	34 (4.3)	10 (2.5)	24 (6.0)	0.01
Pulmonary disease (*n*, %)	48 (6.0)	26 (6.5)	22 (5.5)	0.57
Chronic medications before trial				
Aspirin (*n*, %)	70 (8.8)	19 (4.7)	51 (12.8)	$<10^{-3}$
ACE inhibitors (*n*, %)	103 (12.9)	41 (10.2)	62 (15.6)	0.02
ARBs (*n*, %)	58 (7.3)	19 (4.7)	39 (9.8)	0.006
Bblockers (*n*, %)	84 (10.5)	34 (8.5)	50 (12.6)	0.05
Statins (*n*, %)	107 (13.4)	31 (7.7)	76 (19.1)	$<10^{-3}$
Severity of COVID 19 infection				
Moderate or severe symptoms (need of oxygen) (*n*, %)	185 (23.2)	72 (18.0)	113 (28.5)	$<10^{-3}$
Extend of lesions at thoracic CT scan				
≥50 % (*n*, %)	36 (4.5)	11 (2.7)	25 (6.3)	0.01
Echocardiography				
LVEF (%)	61.00 ± 5.3	61.93 ± 5.3	60.16 ± 5.1	0.004
LVGLS (%)	−16.96 ± 2.7	−18.19 ± 2.3	−15.89 ± 2.5	$<10^{-3}$
E-wave velocity (cm/sec)	79.33 ± 19.0	83.49 ± 16.8	75.50 ± 20.1	$<10^{-3}$
A-wave velocity (cm/sec)	74.15 ± 18.2	73.69 ± 17.5	74.58 ± 18.9	0.68
E' velocity (cm/sec)	13.81 ± 3.9	14.79 ± 4.0	12.9 ± 3.6	$<10^{-3}$
E/E'	6.09 ± 2.1	5.95 ± 1.7	6.22 ± 2.4	0.28
sPAP (mmHg)	21.64 ± 7.2	20.97 ± 7.7	22.26 ± 6.7	0.13
Elevated LV filling pressure	2 (0.3)	0	2 (0.5)	0.24

ACEI, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor antagonists (ARBs); BMI, body mass index; CV, cardiovascular; EQI, endothelium quality index; LV, left ventricle, LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; E, transmitral early diastolic peak velocity; A, late diastolic peak velocity; E', early relaxation velocity on tissue Doppler; sPAP, systolic pulmonary artery pressure.

This study (1) reported long COVID-19 data in a large cohort of patients and (2) focused on endothelial dysfunction as a possible mechanism of underlying persistent long COVID-19 symptoms.

Our findings support that long COVID 19 syndrome is frequent. Recent reviews and metanalysis evaluated and summarized the best available evidence on the frequency of long COVID-19 (6, 11) (**Table 6**). Persistent long COVID-19 symptoms were reported until 6 months after the acute phase (23).

Different risk factors such as old age, a high number of comorbidities, severe clinical status, hospital admission, and oxygen supplementation at the acute COVID-19 phase were reported as potentially associated with long COVID-19 symptoms (8, 10, 24–26). TUN-EndCOV study showed that persistent symptoms especially chest pain, fatigue, and

TABLE 5 | Associated factors to endothelial dysfunction in multivariate analysis.

Variable	Odds Ratio, 95 CI%	*p*
Age > 45 years old	2.046 (1.151–3.635)	0.01
Reduced LVGLS	4.540 (2.451–8.410)	$<10^{-3}$
Dyslipidemia	2.834 (1.042–7.713)	0.04

BMI, body mass index; LVGLS, left ventricle global longitudinal strain.

TABLE 6 | Prevalence of long COVID-19 symptoms in the literature.

	TUN-End-COV study	Systematic review and metanalysis of Lopez Leon et al. (6)	Systematic review of Cabrera Martimbianco et al. (11)
At least one symptom (%)	77.4	80	4.7–80
Fatigue (%)	42.2	58	6.6–64.0
Chest pain (%)	20.3	16	0.4–89
Palpitations (%)	17.4	22	13
Shortness of breath (%)	41.5	24	5.5–61
Cough (%)	17	19	1.8–59.0
Headaches (%)	22.1	44	2.0–39.0
Anosmia (%)	3.5	-	0–26.2
Gastro-intestinal syndrome (%)	5.9	12	1.3–33.3
Neuro-cognitive difficulties (%)	12.2	43	18–57.1

neurocognitive symptoms (non-respiratory symptoms) during the long COVID-19 period were mainly associated with endothelial dysfunction, even after adjustment for age, sex, diabetes, hypertension, dyslipidemia, coronary heart disease, and severe clinical status of COVID-19 infection with the need for oxygen. In multivariate analysis, endothelial dysfunction was an independent risk factor of long COVID-19 syndrome. A recent case reported a non-amelioration of vascular reactivity 3 weeks after acute COVID-19 infection (27). A small-sized pilot study in patients with critical COVID-19 suggested that microvascular function assessed by Laser Speckle Contrast Imaging may not be fully recovered 3 months after disease onset (28).

The beta-blockers treatment was associated with reduced long COVID-19 symptoms. In fact, some papers suggested that beta-adrenergic blockers may be associated with beneficial effects during the acute COVID-19 infection by decreasing the SARS-CoV-2 virus entry, inhibiting NLRP3 inflammasome, reducing IL-6 and so that decreasing the consequent cardio-vascular and pulmonary acute COVID-19 complications (29). These mechanisms may also explain the long-term cardio-pulmonary COVID-19 symptoms. Furthermore, this benefit could be explained in part by beta-blockers pleiotropic effects on the endothelium (reduction of the myocardial oxygen consumption, and anti-oxidant properties) and their effect on rate control (30).

In this large cohort of long COVID-19 population, endothelial function was evaluated by an FTM of the endothelium quality with E4-diagnose device (Polymath Company. Tunisia) (13). It has been shown that the FTM can be used as a reproducible and operator-independent test for the non-invasive measurement of endothelial function in a controlled environment (31). In the largest report to date on any fingertip-based measurement of vascular reactivity and endothelial function, the vascular reactivity index (VRI) values were inversely correlated with age and male gender (18). Even so, the distribution of VRI values in the elderly population and between genders clearly showed a sizable number of good and intermediate scores (18). These findings support the clinical utility of FTM as a test that can differentiate good vascular function from poor vascular function, regardless of the characteristics of the patient (18). In the present study, older age and subclinical LV systolic dysfunction measured by LVGLS were an independently associated factor to impaired EQI. In previous studies, subclinical myocardial deformation with reduced LVGLS was reported at one-month follow-up in one out of every three patients recovered from COVID 19 infection, even in those without myocardial injury (32). The alteration of LVGLS associated with endothelial dysfunction following COVID-19 infection may be due to different factors: (1) the cardiomyocyte inflammation due to viral infiltration (33) and immune mechanisms (34, 35) (2) the hypoxia due to respiratory failure (34, 36), and (3) the myocardial injury due to microvascular dysfunction (19, 37, 38). This finding could explain the persistent chest pain during the long COVID-19.

This is the largest and the first study up to date that focused on the non-invasive evaluation of endothelial function by FTM during the long COVID-19. An important finding is the probable association of "non-respiratory" long COVID-19 symptoms especially chest pain, fatigue, and neuro-cognitive difficulties to endothelial dysfunction, highlighting the importance of an early evaluation of the endothelium quality for appropriate management. Understanding the pathophysiology and underlying mechanisms of persistent symptoms in long COVID-19 patients is required to guide investigations, management and improve patient prognosis.

Further studies are required to characterize long COVID-19 vascular sequelae in order to develop a planned monitoring program and adequate treatment.

Limitations and Perspectives

One limitation of the study could be that reported symptoms were collected by a physician and not through self-reports, so probably only significant symptoms were reported. Furthermore, a global evaluation of the quality of life of the patients was not reported. Another study limitation could be the absence of data on whether patients with endothelial dysfunction had any subclinical preexistent endothelial dysfunction before COVID-19 infection especially in the elderly. In fact, age was one of the most important factors associated with endothelial dysfunction in our study. Longitudinal follow-up of individuals with long COVID-19 syndrome and endothelial dysfunction is warranted to better understand the pathophysiology underlying the long-term persistence and to guide therapeutic intervention. Randomized studies are requested to study the effect of treatment with action on the endothelium function such as Beta-blockers, ACE inhibitors, ARBs, and statins on the long COVID-19 symptoms. Finally, lack of control data regarding endothelial function evaluation in patients with similar demographic characteristics without COVID-19 infection could be one of the study limitations.

CONCLUSION

There is increasing evidence regarding the link between endothelial dysfunction and persistent long COVID-19

symptoms. Risk stratification of long COVID-19 patients may be important to their management. The evaluation of endothelium quality by FTM to non-invasively detect endothelial dysfunction needs to be studied further to improve the management of long COVID-19 patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by CPP SUD 0299/2020. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

LA, SA, SC, and HI: conception and design of the study and literature review. JJ, SC, SA, and LA: analysis and interpretation of the data. SC: drafting of the manuscript. LA and SA: revising and editing the manuscript. All authors: data collection.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.745758/full#supplementary-material

REFERENCES

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet Lond Engl*. (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
2. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. (2020) 382:727–33. doi: 10.1056/NEJMoa2001017
3. Johns Hopkins Coronavirus Resource Center. (2021). Available online at: https://coronavirus.jhu.edu/ (accessed September 26, 2021).
4. Evans PC, Rainger GE, Mason JC, Guzik TJ, Osto E, Stamataki Z, et al. Endothelial dysfunction in COVID-19: a position paper of the ESC Working Group for atherosclerosis and vascular biology, and the esc council of basic cardiovascular science. *Cardiovasc Res*. (2020) 116:2177–84. doi: 10.1093/cvr/cvaa230
5. Osuchowski MF, Winkler MS, Skirecki T, Cajander S, Shankar-Hari M, Lachmann G, et al. The COVID-19 puzzle: deciphering pathophysiology and phenotypes of a new disease entity. *Lancet Respir Med*. (2021) 9:P622–642. doi: 10.1016/S2213-2600(21)00218-6
6. Lopez-Leon S, Wegman-Ostrosky T, Perelman C, Sepulveda R, Rebolledo P, Cuapio A, et al. More than 50 long-term effects of COVID-19: a systematic review and meta-analysis. *Sci Rep*. (2021) 11:16144. doi: 10.21203/rs.3.rs-266574/v1
7. Yelin D, Margalit I, Yahav D, Runold M, Bruchfeld J. Long COVID-19-it's not over until? *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. (2021) 27:506–8. doi: 10.1016/j.cmi.2020.12.001
8. Xiong Q, Xu M, Li J, Liu Y, Zhang J, Xu Y, et al. Clinical sequelae of COVID-19 survivors in Wuhan, China: a single-centre longitudinal study. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. (2021) 27:89–95. doi: 10.1016/j.cmi.2020.09.023
9. Carfì A, Bernabei R, Landi F, Gemelli. Against COVID-19 post-acute care study group. persistent symptoms in patients after acute COVID-19. *JAMA*. (2020) 324:603–5. doi: 10.1001/jama.2020.12603
10. Carvalho-Schneider C, Laurent E, Lemaignen A, Beaufils E, Bourbao-Tournois C, Laribi S, et al. Follow-up of adults with noncritical COVID-19 two months after symptom onset. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. (2021) 27:258–63. doi: 10.1016/j.cmi.2020.09.052
11. Cabrera Martimbianco AL, Pacheco RL, Bagattini ÂM, Riera R. Frequency, signs and symptoms, and criteria adopted for long COVID: a systematic review. *Int J Clin Pract*. (2021) 75:e14357. doi: 10.1111/ijcp.14357
12. Østergaard L, SARS. CoV-2 related microvascular damage and symptoms during and after COVID-19: Consequences of capillary transit-time changes, tissue hypoxia and inflammation. *Physiol Rep*. (2021) 9:e14726. doi: 10.14814/phy2.14726
13. polymath-Diagnose User Manual.pdf.
14. Shah W, Hillman T, Playford ED, Hishmeh L. Managing the long term effects of covid-19: summary of NICE, SIGN, and RCGP rapid guideline. *BMJ*. (2021) 372:n136. doi: 10.1136/bmj.n136
15. Yap J, Lim FY, Gao F, Teo LL, Lam CSP, Yeo KK. Correlation of the New York heart association classification and the 6-minute walk distance: a systematic review. *Clin Cardiol*. (2015) 38:621–8. doi: 10.1002/clc.22468
16. Kos D, Kerckhofs E, Carrea I, Verza R, Ramos M, Jansa J. Evaluation of the Modified Fatigue Impact Scale in four different European countries. *Mult Scler Houndmills Basingstoke Engl*. (2005) 11:76–80. doi: 10.1191/1352458505ms1117oa
17. Pangman VC, Sloan J, Guse L. An examination of psychometric properties of the mini-mental state examination and the standardized mini-mental state examination: implications for clinical practice. *Appl Nurs Res ANR*. (2000) 13:209–13. doi: 10.1053/apnr.2000.9231
18. Naghavi M, Yen AA, Lin AWH, Tanaka H, Kleis S. New indices of endothelial function measured by digital thermal monitoring of vascular reactivity: data from 6084 patients registry. *Int J Vasc Med*. (2016) 2016:1348028. doi: 10.1155/2016/1348028
19. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. *The Lancet*. (2020) 395:1417–8. doi: 10.1016/S0140-6736(20)30937-5
20. Tenforde MW, Kim SS, Lindsell CJ, Billig Rose E, Shapiro NI, Files DC, et al. Symptom duration and risk factors for delayed return to usual health among outpatients with COVID-19 in a multistate health care systems network - United States, March-June 2020. *MMWR Morb Mortal Wkly Rep*. (2020) 69:993–8. doi: 10.15585/mmwr.mm6930e1
21. Silva Andrade B, Siqueira S, de Assis Soares WR, de Souza Rangel F, Santos NO, Dos Santos Freitas A, et al. Long-COVID and post-COVID health complications: an up-to-date review on clinical conditions and their possible molecular mechanisms. *Viruses*. (2021) 13:700. doi: 10.3390/v13040700
22. Townsend L, Dowds J, O'Brien K, Sheill G, Dyer AH, O'Kelly B, et al. Persistent poor health post-COVID-19 is not associated with respiratory complications or initial disease severity. *Ann Am Thorac Soc*. (2021) 18:997–1003. doi: 10.1513/AnnalsATS.202009-1175OC
23. Ghosn J, Piroth L, Epaulard O, Le Turnier P, Mentré F, Bachelet D, et al. Persistent COVID-19 symptoms are highly prevalent 6 months after hospitalization: results from a large prospective cohort. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. (2021) 27:1041.e1–e4. doi: 10.1016/j.cmi.2021.03.012
24. Bellan M, Soddu D, Balbo PE, Baricich A, Zeppegno P, Avanzi GC, et al. Respiratory and psychophysical sequelae among patients with COVID-19 four months after hospital discharge. *JAMA Netw Open*. (2021) 4:e2036142. doi: 10.1001/jamanetworkopen.2020.36142

25. Huang C, Huang L, Wang Y, Li X, Ren L, Gu X, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet Lond Engl.* (2021) 397:220–32. doi: 10.1016/S0140-6736(20)32656-8
26. Jacobs LG, Gourna Paleoudis E, Lesky-Di Bari D, Nyirenda T, Friedman T, Gupta A, et al. Persistence of symptoms and quality of life at 35 days after hospitalization for COVID-19 infection. *PLoS ONE.* (2020) 15:e0243882. doi: 10.1371/journal.pone.0243882
27. Jud P, Kessler HH and Brodmann M. Case report: changes of vascular reactivity and arterial stiffness in a patient with covid-19 infection. *Front Cardiovasc Med*. (2021) 8:671669. doi: 10.3389/fcvm.2021.671669
28. Tehrani S, Gille-Johnson P. Microvascular dysfunction in patients with critical COVID-19, a pilot study. *Shock*. (2021) 56:964–8. doi: 10.1097/SHK.0000000000001803
29. Vasanthakumar N. Beta-Adrenergic Blockers as a Potential Treatment for COVID-19 Patients. *BioEssays News Rev Mol Cell Dev Biol.* (2020) 42:e2000094. doi: 10.1002/bies.202000094
30. Kunadian V, Chieffo A, Camici PG, Berry C, Escaned J, Maas AHEM, et al. An EAPCI expert consensus document on ischaemia with non-obstructive coronary arteries in collaboration with European Society of Cardiology Working Group on coronary pathophysiology & microcirculation endorsed by coronary vasomotor disorders international study group. *Eur Heart J.* (2020) 41:3504–20. doi: 10.1093/eurheartj/ehaa503
31. Ahmadi N, McQuilkin GL, Akhtar MW, Hajsadeghi F, Kleis SJ, Hecht H, et al. Reproducibility and variability of digital thermal monitoring of vascular reactivity. *Clin Physiol Funct Imaging*. (2011) 31:422–8. doi: 10.1111/j.1475-097X.2011.01037.x
32. Özer S, Candan L, Özyildiz AG, Turan OE. Evaluation of left ventricular global functions with speckle tracking echocardiography in patients recovered from COVID-19. *Int J Cardiovasc Imaging*. (2021) 37:2227-33. doi: 10.1007/s10554-021-02211-5
33. Akhmerov A, Marbán E. COVID-19 and the Heart. *Circ Res*. (2020) 126:1443–55. doi: 10.1161/CIRCRESAHA.120.317055
34. Chen C, Zhou Y, Wang DW. SARS-CoV-2: a potential novel etiology of fulminant myocarditis. *Herz*. (2020) 45:230–2. doi: 10.1007/s00059-020-04909-z
35. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of coronaviruses on the cardiovascular system: a review. *JAMA Cardiol*. (2020) 5:831–40. doi: 10.1001/jamacardio.2020.1286
36. Inciardi RM, Lupi L, Zaccone G, Italia L, Raffo M, Tomasoni D, et al. Cardiac involvement in a patient with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:819–24. doi: 10.1001/jamacardio.2020.1096
37. Tajbakhsh A, Gheibi Hayat SM, Taghizadeh H, Akbari A, Inabadi M, Savardashtaki A, et al. COVID-19 and cardiac injury: clinical manifestations, biomarkers, mechanisms, diagnosis, treatment, and follow up. *Expert Rev Anti Infect Ther*. (2021) 19:345–57. doi: 10.1080/14787210.2020.1822737
38. Fox SE, Li G, Akmatbekov A, Harbert JL, Lameira FS, Brown JQ, et al. Unexpected features of cardiac pathology in COVID-19 infection. *Circulation*. (2020) 142:1123–5. doi: 10.1161/CIRCULATIONAHA.120.049465

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

PERSPECTIVE
published: 15 December 2021
doi: 10.3389/fcvm.2021.787761

Promoting a Syndemic Approach for Cardiometabolic Disease Management During COVID-19: The CAPISCO International Expert Panel

Wael Al Mahmeed[1], Khalid Al-Rasadi[2], Yajnavalka Banerjee[3], Antonio Ceriello[4], Francesco Cosentino[5], Massimo Galia[6], Su-Yen Goh[7], Peter Kempler[8], Nader Lessan[9], Nikolaos Papanas[10], Ali A. Rizvi[11,12], Raul D. Santos[13,14], Anca P. Stoian[15], Peter P. Toth[16], Manfredi Rizzo[12,15,17] and The CArdiometabolic Panel of International experts on Syndemic COvid-19 (CAPISCO)*

[1] Cleveland Clinic, Heart and Vascular Institute, Abu Dhabi, United Arab Emirates, [2] Medical Research Center, Sultan Qaboos University, Muscat, Oman, [3] Department of Biochemistry, Mohamed Bin Rashid University, Dubai, United Arab Emirates, [4] IRCCS MultiMedica, Milan, Italy, [5] Unit of Cardiology, Karolinska Institute and Karolinska University Hospital, University of Stockholm, Stockholm, Sweden, [6] Department of Biomedicine, Neurosciences and Advanced Diagnostics (Bind), University of Palermo, Palermo, Italy, [7] Department of Endocrinology, Singapore General Hospital, Singapore, Singapore, [8] Department of Medicine and Oncology, Semmelweis University, Budapest, Hungary, [9] Imperial College London Diabetes Centre, The Research Institute, Abu Dhabi, United Arab Emirates, [10] Second Department of Internal Medicine, Diabetes Center, University Hospital of Alexandroupolis, Democritus University of Thrace, Alexandroupolis, Greece, [11] Department of Medicine, University of Central Florida College of Medicine, Orlando, FL, United States, [12] Division of Endocrinology, Diabetes and Metabolism, University of South Carolina School of Medicine, Columbia, IN, United States, [13] Heart Institute (InCor) University of Sáo Paulo Medical School Hospital, Sáo Paulo, Brazil, [14] Hospital Israelita Albert Einstein, Sáo Paulo, Brazil, [15] Faculty of Medicine, Diabetes, Nutrition and Metabolic Diseases, Carol Davila University, Bucharest, Romania, [16] Cicarrone Center for the Prevention of Cardiovascular Disease, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [17] Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (Promise), University of Palermo, Palermo, Italy

OPEN ACCESS

Edited by:
Xiaofeng Yang,
Temple University, United States

Reviewed by:
Owais Bhat,
Virginia Commonwealth University, United States
Jahaira Lopez-Pastrana,
Temple University, United States

***Correspondence:**
Manfredi Rizzo
manfredi.rizzo@unipa.it

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *01 October 2021*
Accepted: *22 November 2021*
Published: *15 December 2021*

Citation:
Al Mahmeed W, Al-Rasadi K, Banerjee Y, Ceriello A, Cosentino F, Galia M, Goh S-Y, Kempler P, Lessan N, Papanas N, Rizvi AA, Santos RD, Stoian AP, Toth PP, Rizzo M and The CArdiometabolic Panel of International experts on Syndemic COvid-19 (CAPISCO) (2021) Promoting a Syndemic Approach for Cardiometabolic Disease Management During COVID-19: The CAPISCO International Expert Panel. Front. Cardiovasc. Med. 8:787761. doi: 10.3389/fcvm.2021.787761

Efforts in the fight against COVID-19 are achieving success in many parts of the world, although progress remains slow in other regions. We believe that a syndemic approach needs to be adopted to address this pandemic given the strong apparent interplay between COVID-19, its related complications, and the socio-structural environment. We have assembled an international, multidisciplinary group of researchers and clinical practitioners to promote a novel syndemic approach to COVID-19: the CArdiometabolic Panel of International experts on Syndemic COvid-19 (CAPISCO). This geographically diverse group aims to facilitate collaborative-networking and scientific exchanges between researchers and clinicians facing a multitude of challenges on different continents during the pandemic. In the present article we present our "manifesto", with the intent to provide evidence-based guidance to the global medical and scientific community for better management of patients both during and after the current pandemic.

Keywords: diabetes, cardiovascular diseases, complications, COVID-19, pandemic, syndemic

INTRODUCTION

There is a bidirectional pathophysiologic relationship between coronavirus disease 2019 (COVID-19) and cardiometabolic diseases, and individuals at risk of the latter require careful consideration as the global pandemic continues to take its toll. Diabetes, obesity, and cardiovascular disease are associated with an increased risk for severe forms of COVID-19 and resulting death (1–6). At the same time, patients with COVID-19 infection are more prone to the development of new-onset diabetes mellitus (7). Investigators from different areas have emphasized the clinical relevance of the increased incidence of diabetes after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (8, 9). Furthermore, COVID-19 is associated with cardiovascular injury attributed to heightened inflammation, endothelial dysfunction and microthrombi formation (10–13). Endothelial dysfunction plays an important role in the pathogenesis of COVID-19, particularly in patients with pre-existing hypertension, diabetes, obesity and cardiovascular diseases (14). The endothelium, and particularly pulmonary endothelium, seems to be a key target organ in COVID-19 patients and its dysfunction has been shown to cause an impaired organ perfusion that can generate acute myocardial injury, renal failure, and a procoagulant state resulting in thromboembolic events (14–16).

It has been shown that SARS-CoV-2 can induce several pro-inflammatory cytokines (17) and that patients with severe COVID-19 develop a "cytokine storm syndrome" (18). Since these first observations it is become clear that the same cytokines that induce aberrant endothelial function may also trigger the acute phase response, which, in combination with local endothelial dysfunction, can lead to clinical consequences (14); indeed, inflammatory cytokines have a major role in both diabetes and cardiovascular diseases (19). Other authors have shown that COVID-19 is associated with myocardial damage such as myocarditis, arrhythmia and reduced left ventricular ejection fraction (20), all of which are associated to increased mortality risk (21). Plasma cardiac biomarkers, such as high sensitivity troponin, creatine kinase and N-terminal pro-B-type natriuretic peptide, are also associated with COVID-19 severity in adults and children (22, 23).

Beyond the direct effect of COVID-19 on the cardiovascular system and metabolic homeostasis, subjects with pre-existing cardiometabolic issues appear to be at a significantly higher risk of complications owing to reduced physical activity, altered eating behaviors, and lack of access to healthcare (24). This encapsulates the so-called "indirect" impact of COVID-19 and indeed a higher incidence of cardiovascular complications and fatalities has been documented secondary to the pandemic, for example in Italy (25). Even in New York City, emergency calls for cardiac arrests rose exponentially in the weeks when COVID-19 infections approached their zenith (26). Beyond deaths attributed directly to COVID-19, a large contribution to the excess mortality reported (27) is attributable to the indirect factors, including the disruption of the proper management of many clinical conditions–including cardiometabolic diseases–by the rapid conversion of entire hospitals or clinical units to deliver COVID-19-specific care (28).

This situation has been exacerbated in some geographical areas by increased unemployment, economic collapse, and widespread poverty (29). Indeed, increasing socio-economic disparities have come to the forefront in many populations during the pandemic, rendering people more vulnerable to economic, nutritional, social, and medical insecurity, particularly during prolonged periods of necessary government-imposed restrictions or lockdowns (30). In addition, the spread of the virus and the related complications and fatalities have been facilitated among subjects with the poorest socio-economic conditions and those living in overcrowded areas (29–31). Socio-economic inequalities are of increasing relevance during the ongoing vaccination campaigns as they contribute to disparities in care across different ethnic populations and geographical areas (32).

IMPORTANCE OF A SYNDEMIC APPROACH

The enormous efforts by many different organizations and individuals involved in the fight against COVID-19 have had some success in many parts of the world. However, this has not been universal and progress has been slow in many other regions. We believe that a syndemic approach needs to be adopted for addressing this pandemic (33) given the strong apparent interplay between COVID-19, its related complications, and the socio-structural environment. The term *syndemic* (from ancient Greek: *syn*, together; *demos*, people) emphasizes the relevance of biological, social, economic, and environmental factors in the health of individuals and populations (29). Physicians have an obligation to understand their patients' social, economic, and environmental situations and to utilize the tools available in existing health systems to improve their access to care. It is also expected that many health systems will continue to be under significant economic pressure, which may contribute to a reduced quality of care for patients with chronic conditions.

Another challenge is represented by the so-called *long-COVID syndrome*, which is a clinical condition present in subjects who have either recovered from COVID-19 but still report lasting effects of the infection or have had the usual symptoms for far longer than would be expected (34). Of increasing current interest are the neurological and neuropsychiatric complications (35), since several studies have reported a broad spectrum of symptoms, from the milder manifestations of memory loss, sleep disorders and impaired concentration to more serious cognitive decline, major depression or persistent delirium (36). Thus, there is an urgent need to better understand the long-term effects of COVID-19 on brain function, behavior and cognition. As a component of a holistic approach to the management of patients with COVID-19, mental health assessment should be included.

CAPISCO: AN INTERNATIONAL, MULTIDISCIPLINARY COLLABORATION

We believe it is crucial to improve interactions between specialists working in different disciplines, since insufficient

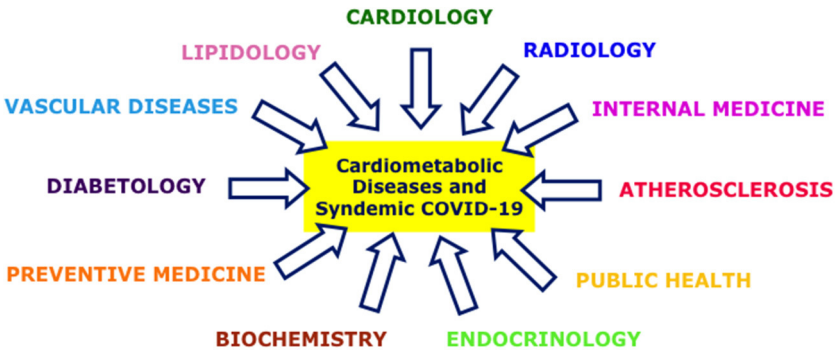


FIGURE 1 | A novel multi-disciplinary syndemic approach to cardiometabolic diseases during COVID-19.

cooperation has contributed to the indirect impact of COVID-19 (37). Furthermore, the pandemic has adversely medical education in many ways, for example owing to shifts to distance-learning modalities and decline in clinical clerkship due to the cancelation of routine patient appointments and surgical procedures and a transition to greater use of telemedicine (38). In response to these challenges we have recently assembled the Cardiometabolic Panel of International Experts on Syndemic COVID-19 (CAPISCO), a group of international researchers and clinical practitioners from many different disciplines including (but not limited to) diabetology, endocrinology, cardiology, lipidology, internal medicine, radiology, preventive medicine, public health and biochemistry–providing a multi-disciplinary representation in a novel approach to COVID-19 (**Figure 1**). We also emphasized geographical diversity when convening the group in order to facilitate collaborative-networking and scientific exchanges between researchers and clinicians facing a multitude of challenges in different continents during the pandemic.

Members of CAPISCO intend to collaboratively investigate:

1. how patients with cardiometabolic diseases and its complications are currently being managed and treated and the extent to which the pandemic impacts proper management, with a view to identifying, categorizing and defining innovative strategies to overcome potential barriers and disparities;
2. why differences in COVID-19 mortality rates have been reported among various countries, beyond the prevalence of the disease *per se*, with the aim to elucidate the social, economic and environmental factors potentially impacting clinical outcomes;
3. whether telemedicine is a reliable and useful tool to deliver high-quality patient care in light of experience gained during the pandemic; across different geographical areas we aim to investigate: what was successfully implemented and how, what was not successfully implemented and why, and what still needs to be improved;
4. how to assess the burden and late consequences of delayed management of cardiometabolic disease and other conditions due to COVID-19.

Ultimately, the overarching aim of CAPISCO is to give evidence-based guidance for the management of patients with cardiometabolic diseases during and after COVID-19 based on a syndemic approach. In terms of methodology, we plan to use a systematic approach, including systematic literature searches, formal quality-grading and analysis of collected studies, resulting in graded levels of recommendations. We also intend to make a roadmap plan of further research avenues once the data from the above indicated tools becomes available; this may also involve cooperation with health authorities and other international organizations.

CONCLUSIONS

In conclusion, we believe it is crucial to view COVID-19 through a syndemic lens to properly tackle the interlinked public health, medical, social and economic challenges that amplify each other in this crisis. The acronym of our expert panel, CAPISCO, is meaningful, since the word "*capisco*" means in Italian, "*I understand*". CAPISCO contributions will promote a holistic approach for all patients with cardiometabolic diseases based on solid, validated scientific research and clinical expertise. We hope that physicians around the world will be able to use them to help benefit clinical care, follow-up, and monitoring of their patients during and after the COVID-19 pandemic.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

This article is the result of three expert panel meetings conducted virtually between May and July 2021. MR and WA, co-chairs of CAPISCO, prepared the first draft of this article, which was

first critically discussed and reviewed with AR, AC, and AS, and then extensively reviewed by all the other members. All authors have equally contributed to the final manuscript and are listed alphabetically.

REFERENCES

1. Ceriello A, Standl E, Catrinoiu D, Itzhak B, Lalic NM, Rahelic D, et al. Issues of cardiovascular risk management in people with diabetes in the COVID-19 era. *Diabetes Care.* (2020) 43:1427–32. doi: 10.2337/dc20-0941
2. Khera A, Baum SJ, Gluckman TJ, Gulati M, Martin SS, Michos ED, et al. Continuity of care and outpatient management for patients with and at high risk for cardiovascular disease during the COVID-19 pandemic: A scientific statement from the American Society for Preventive Cardiology. *Am J Prev Cardiol.* (2020) 1:100009. doi: 10.1016/j.ajpc.2020.100009
3. Schnell O, Cos X, Cosentino F, Forst T, Giorgino F, Heersprink HJL, et al. Report from the CVOT Summit 2020: new cardiovascular and renal outcomes. *Cardiovasc Diabetol.* (2021) 20:75. doi: 10.1186/s12933-021-01254-1
4. Atallah B, El Lababidi R, Jesse W, Noor L, AlMahmeed W. Adapting to the COVID-19 pandemic at a quaternary care hospital in the Middle East Gulf region. *Heart Views.* (2020) 21:133–40.
5. Ceriello A, Stoian AP, Rizzo M. COVID-19 and diabetes management: What should be considered? *Diabetes Res Clin Pract.* (2020) 163:108151. doi: 10.1016/j.diabres.2020.108151
6. Stoian AP, Banerjee Y, Rizvi AA, Rizzo M. Diabetes and the COVID-19 pandemic: How Insights from recent experience might guide future management. *Metab Syndr Relat Disord.* (2020) 18:173–75. doi: 10.1089/met.2020.0037
7. Papachristou S, Stamatiou I, Stoian AP, Papanas N. New-onset diabetes in COVID-19: Time to frame its fearful symmetry. *Diabetes Ther.* (2021) 12:461–4. doi: 10.1007/s13300-020-00988-7
8. Shrestha DB, Budhathoki P, Raut S, Adhikari S, Ghimire P, Thapaliya S, et al. New-onset diabetes in COVID-19 and clinical outcomes: A systematic review and meta-analysis. *World J Virol.* (2021) 10:275–87. doi: 10.5501/wjv.v10.i5.275
9. Mameli C, Scaramuzza A, Macedoni M, Marano G, Frontino G, Luconi E, et al. Type 1 diabetes onset in Lombardy region, Italy, during the COVID-19 pandemic: The double-wave occurrence. *EClinicalMedicine.* (2021) 39:101067. doi: 10.1016/j.eclinm.2021.101067
10. Puntmann VO, Carerj ML, Wieters I, Fahim M, Arendt C, Hoffmann J, et al. Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:1265–73. doi: 10.1001/jamacardio.2020.3557
11. Ferrari F, Martins VM, Teixeira M, Santos RD, Stein R. COVID-19 and Thromboinflammation: is there a role for statins? *Clinics (Sáo Paulo).* (2021) 76:e2518. doi: 10.6061/clinics/2021/e2518
12. Libby P, Lüscher T. COVID-19 is, in the end, an endothelial disease. *Eur Heart J.* (2020) 41:3038–44. doi: 10.1093/eurheartj/ehaa623
13. Pellegrini D, Kawakami R, Guagliumi G, Sakamoto A, Kawai K, Gianatti A, et al. Microthrombi as a major cause of cardiac injury in COVID-19: a pathologic study. *Circulation.* (2021) 143:1031–42. doi: 10.1161/CIRCULATIONAHA.120.051828
14. Ionescu M, Stoian AP, Rizzo M, Serban D, Nuzzo D, Mazilu L, et al. The Role of Endothelium in COVID-19. *Int J Mol Sci.* (2021) 22:11920. doi: 10.3390/ijms222111920
15. Kaur S, Tripathi DM, Yadav A. The enigma of endothelium in COVID-19. *Front Physiol.* (2020) 11:989. doi: 10.3389/fphys.2020.00989
16. Sardu C, Gambardella J, Morelli MB, Wang X, Marfella R, Santulli G. Hypertension, thrombosis, kidney failure, and diabetes: Is COVID-19 an endothelial disease? A comprehensive evaluation of clinical and basic evidence. *J Clin Med.* (2020) 9:1417. doi: 10.3390/jcm9051417
17. Conti P, Ronconi G, Caraffa A, Gallenga CE, Ross R, Frydas I. e al. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. *J Biol Regul Homeost Agents.* (2020) 34:327–31.
18. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al. Across Speciality Collaboration, UK COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet.* (2020) 395:1033–4. doi: 10.1016/S0140-6736(20)30628-0
19. Abate N, Sallam HS, Rizzo M, Nikolic D, Obradovic M, Bjelogrlic P, et al. Resistin: an inflammatory cytokine. Role in cardiovascular diseases, diabetes and the metabolic syndrome. *Curr Pharm Des.* (2014) 20:4961–9. doi: 10.2174/1381612819666131206103102
20. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of coronaviruses on the cardiovascular system: a review. *JAMA Cardiol.* (2020) 5:831–40. doi: 10.1001/jamacardio.2020.1286
21. Silverio A, Di Maio M, Scudiero F, Russo V, Esposito L, Attena E, et al. Clinical conditions and echocardiographic parameters associated with mortality in COVID-19. *Eur J Clin Invest.* (2021) 51:e13638. doi: 10.1111/eci.13638
22. Zhu Z, Wang M, Lin W, Cai Q, Zhang L, Chen D, et al. Cardiac biomarkers, cardiac injury, and comorbidities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. *Immun Inflamm Dis.* (2021) 9:1071–100. doi: 10.1002/iid3.471
23. Zhao Y, Patel J, Huang Y, Yin L, Tang L. Cardiac markers of multisystem inflammatory syndrome in children (MIS-C) in COVID-19 patients: A meta-analysis. *Am J Emerg Med.* (2021) 49:62–70. doi: 10.1016/j.ajem.2021.05.044
24. Bornstein SR, Rubino F, Ludwig B, Rietzsch H, Schwarz PEH, Rodionov RN, et al. Consequences of the COVID-19 pandemic for patients with metabolic diseases. *Nat Metab.* (2021) 3:289–92. doi: 10.1038/s42255-021-00358-y
25. De Rosa S, Spaccarotella C, Basso C, Calabrò MP, Curcio A, Filardi PP, et al. Società Italiana di Cardiologia and the CCU Academy investigators group. Reduction of hospitalizations for myocardial infarction in Italy in the COVID-19 era. *Eur Heart J.* (2020) 41:2083–8. doi: 10.1093/eurheartj/ehaa409
26. Wessler BS, Kent DM, Konstam MA. Fear of coronavirus disease 2019 – an emerging cardiac risk. *JAMA Cardiol.* (2020) 5:981–2. doi: 10.1001/jamacardio.2020.2890
27. Rizzo M, Foresti L, Montano N. Comparison of reported deaths from COVID-19 and increase in total mortality in Italy. *JAMA Intern Med.* (2020) 180:1250–2. doi: 10.1001/jamainternmed.2020.2543
28. Fadaak R, Davies JM, Blaak MJ, Conly J, Haslock J, Kenny A, et al. Rapid conversion of an in-patient hospital unit to accommodate COVID-19: An interdisciplinary human factors, ethnography, and infection prevention and control approach. *PLoS ONE.* (2021) 16:e0245212. doi: 10.1371/journal.pone.0245212
29. Fronteira I, Sidat M, Magalhães JP, de Barros FPC, Delgado AP, Correia T, et al. The SARS-CoV-2 pandemic: A syndemic perspective. *One Health.* (2021) 12:100228. doi: 10.1016/j.onehlt.2021.100228
30. Padmanabhan S. The COVID-19 pan-syndemic – will we ever learn? *Clin Infect Dis.* (2020) 73:e2976–7. doi: 10.1093/cid/ciaa1797
31. Available online at: https://www.nytimes.com/2021/05/04/world/oxygen-shortage-covid.html (accessed September 30th, 2021).
32. Available online at: https://www.kff.org/coronavirus-covid-19/issue-brief/latest-data-on-covid-19-vaccinationsrace-ethnicity/ (accessed September 30th, 2021).
33. Horton R. Offline: COVID-19 is not a pandemic. *Lancet.* (2020) 396:874. doi: 10.1016/S0140-6736(20)32000-6
34. Mahase E. Covid-19: What do we know about "long covid"? *BMJ.* (2020) 370:m2815. doi: 10.1136/bmj.m2815
35. Nuzzo D, Vasto S, Scalisi L, Cottone S, Cambula G, Rizzo M, et al. Post-Acute COVID-19 Neurological Syndrome: A New Medical Challenge. *J Clin Med.* (2021) 10:1947. doi: 10.3390/jcm10091947
36. Pantelis C, Jayaram M, Hannan AJ, Wesselingh R, Nithianantharajah J, Wannan CM, et al. Neurological, neuropsychiatric and neurodevelopmental complications of COVID-19. *Aust N Z J Psychiatry.* (2021) 55:750–62. doi: 10.1177/0004867420961472
37. Ceriello A. Lessons from COVID-19: How human behaviour may influence the science. *Diabetes Res Clin Pract.* (2020) 169:108491. doi: 10.1016/j.diabres.2020.108491

38. Naidoo N, Akhras A, Banerjee Y. Confronting the challenges of anatomy education in a competency-based medical curriculum during normal and unprecedented times (COVID-19 pandemic): pedagogical framework development and implementation. *JMIR Med Educ.* (2020) 6:e21701. doi: 10.2196/21701

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

REVIEW
published: 23 December 2021
doi: 10.3389/fcvm.2021.648290

Acute Myocardial Infarction During the COVID-19 Pandemic: An Update on Clinical Characteristics and Outcomes

*Olga Toscano [1], Nicola Cosentino [1,2], Jeness Campodonico [1], Antonio L. Bartorelli [1,3] and Giancarlo Marenzi [1]**

[1] Centro Cardiologico Monzino, I.R.C.C.S., Milan, Italy, [2] Department of Clinical Sciences and Community Health, Cardiovascular Section, University of Milan, Milan, Italy, [3] Department of Biomedical and Clinical Sciences "Luigi Sacco," University of Milan, Milan, Italy

The outbreak of coronavirus disease 2019 (COVID-19) has rapidly become a worldwide pandemic. On top of respiratory complications, COVID-19 is associated with major direct and indirect cardiovascular consequences, with the latter probably being even more relevant, especially in the setting of time-dependent cardiovascular emergencies. A growing amount of data suggests a dramatic decline in hospital admissions for acute myocardial infarction (AMI) worldwide during the COVID-19 pandemic, mostly since patients did not activate emergency medical systems because hospitals were perceived as dangerous places regarding the infection risk. Moreover, during the COVID-19 pandemic, patients with AMI had a significantly higher in-hospital mortality compared to those admitted before COVID-19, potentially due to late arrival to the hospital. Finally, no consensus has been reached regarding the most adequate healthcare management pathway for AMI and shared guidance on how to handle patients with AMI during the pandemic is still needed. In this review, we will provide an update on epidemiology, clinical characteristics, and outcomes of patients with AMI during the COVID-19 pandemic, with a special focus on its collateral cardiac impact.

Keywords: acute myocardial infarction, COVID-19, epidemiology, clinical characteristic, outcome

OPEN ACCESS

Edited by:
Mingxing Xie,
Huazhong University of Science and Technology, China

Reviewed by:
Andrew F. James,
University of Bristol, United Kingdom
Carmine Morisco,
University of Naples Federico II, Italy
Alessandro Iadanza,
University of Siena, Italy

****Correspondence:***
Giancarlo Marenzi
giancarlo.marenzi@ccfm.it

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *31 December 2020*
Accepted: *26 November 2021*
Published: *23 December 2021*

Citation:
Toscano O, Cosentino N, Campodonico J, Bartorelli AL and Marenzi G (2021) Acute Myocardial Infarction During the COVID-19 Pandemic: An Update on Clinical Characteristics and Outcomes. Front. Cardiovasc. Med. 8:648290. doi: 10.3389/fcvm.2021.648290

INTRODUCTION

Coronavirus disease 2019 (COVID-19), a novel viral respiratory illness due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), became a global pandemic in 2020 (1). As we continued to fight against the infectious disease and the rapid contagion of the virus, we understood that, besides being primarily a respiratory illness, COVID-19 has potential direct and indirect cardiac sequelae that were initially underestimated (2, 3). Indeed, several reports have described relevant cardiac complications in patients with COVID-19 with or without the prior cardiovascular disease (2, 3). Of note, even the latter patients are more likely to have an acute myocardial infarction (AMI), heart failure, and life-threatening arrhythmias due to a direct impact of SARS-CoV-2 infection on the cardiovascular system (4). However, the pandemic might have even had more severe indirect sequelae. In particular, despite all the great efforts made by the international health authorities and national governments to fight the infection, the patients with COVID-19 surge in demand for Intensive Care Unit (ICU) admission has been overwhelming (5, 6). As a consequence,

also Intensive Cardiac Care Units (ICCU) have been dedicated to the treatment of patients with pneumonia and severe acute respiratory syndrome. Thus, the tremendous pressure exerted on the healthcare system by the viral pandemic compromised proven therapies for acute cardiovascular emergencies, such as AMI (7, 8). Another serious issue during the COVID-19 outbreak has been the reluctance of patients with chest pain to go to the hospital due to the fear of viral infection, even to the point of not seeking care at all or late in the course of AMI (9–11). These indirect effects of the pandemic have negatively affected the outcomes of patients with AMI, regardless of whether they were affected by SARS-CoV-2 infection or not.

In this review, we will provide an update on epidemiology, clinical characteristics, and outcomes of patients with AMI during the COVID-19 pandemic, with a special focus on its collateral impact on AMI. With the SARS-CoV-2 infection still not being under control, understanding and addressing the relationship between COVID-19 and AMI is critical if we want to prevent a further increase in mortality and a new heart failure pandemic wave.

POSSIBLE MECHANISMS LINKING COVID-19 TO AMI

Several mechanisms associated with COVID-19 may be involved in AMI. Type 1 AMI can be triggered in patients with COVID-19 by a pro-inflammatory state, which may promote destabilization of a coronary atherosclerotic plaque, a phenomenon already observed during influenza outbreaks (12). Notably, viral infections have been shown to activate inflammatory cells of the coronary plaque and to upregulate metalloproteinases and peptidases, which, in turn, may disrupt plaque cap exposing the highly thrombogenic core to the blood (13). Another potential mechanism is the mismatch between reduced oxygen supply and increased myocardial oxygen demand due to sympathetic system activation, tachycardia, hypotension, and hypoxemia in the setting of acute respiratory insufficiency, which may be responsible for Type 2 AMI (14). Moreover, other mechanisms related to specific features of SARS-CoV-2 infection have been advocated to explain AMI in patients with COVID-19. In particular, the endothelial and microvascular injuries induced by SARS-CoV-2 may further enhance inflammation, resulting in coronary vasospasm, thrombosis, and myocardial perfusion defects (15). Moreover, the low platelet count often described in patients with COVID-19 suggests an increased consumption due to great platelet activation and thrombus formation. Indeed, the cytokine storm associated with viral infection induces, together with the imbalance of endothelial function, significant activation of platelets, granulocytes, and microvesicles, which, in turn, produce tissue factors (16). Of note, it has also been demonstrated that plasma microvesicles-associated thrombin generation can still be present in patients with COVID-19 despite prophylactic anticoagulation (16).

Another possible mechanism implicated in the association between SARS-CoV-2 and AMI is the pro-inflammatory state. Since the association between infection and acute coronary atherothrombosis has been established for a variety of pathogens and sites of infection, it is likely that the causal agent and the host response could have a crucial role in eliciting an inflammatory pattern that may trigger AMI. Atherosclerotic plaques contain inflammatory cells that proliferate, secrete cytokines, and stimulate smooth muscle cells to form a fibrous cap. Thus, an inflammatory status generates circulating cytokines that may activate inflammatory cells in atherosclerotic plaques, enhancing plaque vulnerability and the possibility of its rupture, leading to coronary thrombosis (14).

Of note, there are multiple reports of microvascular involvement in different organs of patients with COVID-19, leading to ischemic stroke (17), deep vein thrombosis (18), pulmonary embolism (19), and arterial thrombotic events (20).

The COVID-19 has more far-reaching cardiovascular implications than the pathophysiological effects of the disease *per se*. In fact, all countries have developed containment strategies based on social distancing, and it is well-known that the lack of human relationships and reduced interaction with other people are major risk factors for cardiovascular mortality. A previous meta-analysis includes 181,000 subjects demonstrated that the risk for AMI increases by almost 30% in lonely and socially isolated people (21). The adult cohort studies reported initial evidence of a clinically meaningful increase in anxiety, depression, mental health disturbance, and disruption of well-being during the lockdown for SARS-CoV-2 spread containment, all of which have been associated with an increased AMI risk (22).

EPIDEMIOLOGY OF AMI DURING THE COVID-19 PANDEMIC

In the early period of the pandemic, many healthcare workers noticed a reduction in hospital admissions for AMI. This finding was largely consistent across continents and, although initially based on self-reported perceptions (23), it was then supported by objective evidence from worldwide registries, suggesting a 25–40% decrease in AMI admissions during the outbreak (**Table 1**). Xiang et al. (24) looked into the China Chest Pain Center Database to evaluate the impact of the COVID-19 pandemic on ST-elevation myocardial infarction (STEMI) admission in the 4 weeks before and after January 24, 2020 (the start date of the COVID-19 outbreak in China). They found an approximately 25% drop in the weekly number of patients hospitalized for STEMI during the COVID-19 outbreak nationwide, and about a 60% drop in Hubei province. In a multicenter, observational survey, De Rosa et al. (25) collected data from 54 ICCU across Italy during 1-week period at the beginning of the COVID-19 outbreak. A halving in AMI admissions was registered during the 2020 week compared with the equivalent 2019 week. Because of deep regional variations in COVID-19 involvement in Italy, with the north being the most affected area, the country was divided into three macro-areas (north, central, and south Italy), and the authors still found a similar decline in AMI admissions among these macro-areas. Similarly, a Spanish report compared the activity of 81 ICCU a week before the pandemic with

TABLE 1 | Characteristics of the studies investigating the admission rate for acute myocardial infarction during the COVID-19 pandemic.

First author [Ref#]	AMI type	Country	COVID-19 period considered	Patients (*n*)	Control period considered	Patients (*n*)	Percent change in AMI admissions
Xiang et al. (24)	STEMI	China	27 Dec 2019–23 Jan 2020	15,729	24 Jan−20 Feb 2020	11,598	−26%
De Rosa et al. (25)	STEMI/NSTEMI	Italy	12 Mar−19 Mar 2020	319	12 Mar−19 Mar 2019	618	−48%
Rodriguez-Leor et al. (26)	STEMI	Spain	16 Mar−22 Mar 2020	260	24 Jan−1 Mar 2020	433	−40%
Garcia et al. (27)	STEMI	United States	1 Mar−31 Mar 2020	138	1 Jan 2019–29 Feb 2020	>180/month	−38%
Mafham et al. (28)	STEMI/NSTEMI	England	1 Jan−24 May 2020	1,813/week	1 Jan−31 Dec 2019	3,017/week	−40%
Mesnier et al. (29)	STEMI/NSTEMI	France	16 Mar−12 Apr 2020	481	17 Feb−15 Mar 2020	686	−30%
Papafaklis et al. (30)	ACS	Greece	2 Mar−12 Apr 2020	771	2 Mar−12 Apr 2019	1,077	−38%
Solomon et al. (31)	STEMI/NSTEMI	United States	4 Mar−14 Apr 2020	516	4 Mar−14 Apr 2019	735	−30%
Mohammad et al. (32)	STEMI/NSTEMI	Sweden	1 Mar−7 May 2020	36/day	1 Mar−7 May 2015-2019	45/day	−20%
Gluckman et al. (33)	STEMI/NSTEMI	United States	23 Feb−28 Mar 2020	860	30 Dec 2018–22 Feb 2020	-	−19%
Wilson et al. (34)	STEMI	United Kingdom	19 Feb−8 Apr 2020	388	19 Feb−8 Apr 2017-2019	-	−51%

ACS, acute coronary syndrome; NSTEMI, non-ST elevation myocardial infarction; STEMI, ST-elevation myocardial infarction.

that of a week during the pandemic. The authors observed a significant reduction in ICCU activity mainly due to a marked decrease in STEMI hospitalization, with a concerning 40% decline in primary percutaneous coronary intervention (PCI) (26). Likewise, during the early phase (March 2020) of the COVID-19 pandemic, a 38% reduction of primary PCI activity was reported in nine high-volume catheterization laboratories of the United States (27). The same authors confirmed the marked reduction in interventional activity during April 2020 in a survey of 18 United States STEMI centers. Interestingly, the decline in hospital admissions for STEMI was seen in all geographic areas of the United States, irrespective of COVID-19 incidence, implementation of lockdown, and level of SARS-CoV-2 testing (35). Finally, another survey of more than 3,000 health professionals from 141 countries, endorsed by the European Society of Cardiology (ESC), showed an important decline in patients admitted to hospital for AMI during the pandemic (23). Notably, the responses received showed that 80% of health professionals felt that there had been a decrease in presentations, with the large majority of participants reporting at least a 40% reduction. Later on, nationwide analysis of acute coronary syndrome admissions conducted in other geographical areas that had lockdown restrictions, such as England (28), France (29), Greece (30), and California (31), showed the same concerning trend. Finally, Mohammad et al. (32) recorded a nationwide significant decline in AMI presentation during the COVID-19 pandemic as compared to the corresponding period of previous years (2015–2019) also in Sweden, that, unlike other countries, did not impose mandatory lockdown.

Several causes may explain the reduction in AMI admissions, such as patient reluctance to go to the hospital for fear of being exposed to SARS-CoV-2 or to overload an already strained health service, and delay in response of a congested ambulance and emergency service. The hypothesis that patients avoided access to the emergency departments because of contagion fear is supported by the lack of significant differences in AMI admission among the Italian macro-areas assessed by De Rosa et al. (25), despite great discrepancies in COVID-19 spread across the country. Swedish results are consistent with this data, showing that AMI admissions declined, when compared to previous years, even when areas as the COVID-19 hotspot in Stockholm were excluded from the analysis (32). However, we cannot exclude that some patients with AMI, who experienced dyspnea, only misjudged the symptom as COVID-19 related and chose to remain at home, without seeking care. Furthermore, social distancing and improved hygiene might have attenuated the spreading of influenza, a widely recognized AMI trigger (12). Another suggested hypothesis is the arrangement of healthcare resources during the pandemic with deferral of less urgent cases. In line with this theory, De Rosa et al. (25) showed less reduction in hospitalization for STEMI compared with non-STEMI (NSTEMI), a finding also reported by Mesnier et al. (29) in a French registry. Finally, it has been suggested that the widespread working from home, especially after the implementation of lockdown measures, may have contributed to decrease stress-induced AMI. However, as indicated both by the United States and English data, the drop in AMI admissions preceded the start of the lockdown by 2 weeks and 1 month,

respectively, thus suggesting that the above-mentioned condition might have played a minor role (36).

The reluctance of patients with AMI to go to the hospital due to the fear of being exposed to SARS-CoV-2 is also suggested by the significant increase in out-of-hospital cardiac arrests (OHCA) reported during the COVID-19 outbreak. This association was first observed in New York City, particularly, from March 30 to April 5, 2020. Indeed, during this period, there were 1,990 OHCA calls, a rate four times higher than that reported during the same time interval a year before (37). The dramatic fact was that this was associated with an eight times higher mortality. Later, Baldi et al. (38) compared the number of OHCA occurring in four Italian provinces with the highest rate of COVID-19 cases in the first 40 days of the outbreak to the same period of the previous year. The analysis showed a strong association between the cumulative incidence of OHCA and COVID-19 disease. Furthermore, they observed that the 60% increase in OHCA in 2020 compared to the same period in 2019 paralleled the time course of the COVID-19 outbreak. A similar significant increase of OHCA during the pandemic was also observed in an American cross-sectional study (37). Of note, this study reported that patients with OHCA presented more frequently with asystole and pulseless electrical activity than ventricular fibrillation or ventricular tachycardia. In addition, the rate of spontaneous circulation recovery was significantly lower during the COVID-19 period than in 2019. However, none of the above studies reported data regarding AMI diagnosis or history of coronary artery disease in the patients included in the analyses. One more piece of information comes from the study of Rashid et al. (39) who showed an almost double incidence of OHCA during a defined COVID-19 period compared to a pre-COVID-19 period in a cohort of patients hospitalized with AMI, substantiating the concerns that reduced AMI admissions may have resulted in an increased risk of OHCA.

CLINICAL CHARACTERISTICS AND OUTCOMES OF PATIENTS WITH AMI DURING COVID-19

To date, evidence concerning the clinical characteristics and in-hospital outcomes of AMI patients during the COVID-19 pandemic is limited, and it mainly derives from single-center experiences, with most studies reporting partial details on patient baseline risk, comorbidities, and clinical outcomes (**Table 2**) (28, 41).

Clinical observations made in England about the characteristics of patients with AMI during the pandemic lockdown showed that they were younger, less frequently diabetics, and less likely to have a history of prior cerebrovascular disease, as compared to those admitted during the previous year (28). Similar data were found in a retrospective cross-sectional study analyzing patients with STEMI and NSTEMI admitted between December 30, 2018 and May 16, 2020 in 49 hospitals in the Providence St. Joseph Health (PSJH) system that spreads across Alaska, California, Montana, Oregon, Texas, and Washington. This study showed that patients hospitalized during a defined COVID-19 period were younger and more likely to be Asian or Native American than the ones hospitalized before (33). On the other hand, a Swedish registry reported no difference (both at a nationwide level and in Stockholm) in age, gender, and comorbidities except for lower rates of prior AMI and coronary artery bypass grafting in patients with AMI during the pandemic (32). In line with the Swedish observation, both a French registry by Mesnier et al. (29) and a single-center German study by Primessnig et al. (42) showed that age, gender, and prevalence of risk factors did not differ between the pre-pandemic and pandemic period in patients with AMI. In northern California, patients presenting with AMI during the COVID-19 outbreak were less likely to have a history of coronary artery disease compared to patients presenting during the pre-COVID-19 period (31). However, there was not any difference in terms of demographic characteristics and comorbidities in the two periods.

An observation common to studies was that during the pandemic a higher percentage of patients were admitted with STEMI as compared to NSTEMI (25, 28, 40). Indeed, a large database of 99 English hospitals showed that, on average, hospitalization for NSTEMI was reduced by 50% and by 25% for STEMI (28). Likewise, a multicenter observational survey examining 319 consecutive patients with AMI in the week with the highest peak of COVID-19 spread in Italy reported a decrease in hospital admission by 27% for STEMI and by 65% for NSTEMI (25). The greater reduction in NSTEMI admissions might have several explanations. There is the chance that patients with NSTEMI did not seek medical help because their symptoms were less frequently characterized by precordial pain or chest discomfort, thus increasing their reluctance to expose themselves to the in-hospital risk of COVID-19 infection. In line with this hypothesis, data from the Lombardy region in Italy showed that, during the COVID-19 pandemic, patients with AMI presented more frequently with dyspnea and atypical symptoms (40). In addition, an association between increasing age and pre-existing comorbidities and a poorer outcome following COVID-19 infection was largely emphasized by the media at the start of the pandemic, affecting the choice of some patients with NSTEMI to remain at home, since they considered themselves at high risk in case of infection due of their older age and concomitant illnesses.

An important observation made during the COVID-19 pandemic was that patients with STEMI had greater enzymatic infarct size, as assessed by the peak of troponin or creatine kinase levels (42, 43), lower left ventricular ejection fraction (34, 42), higher intracoronary thrombotic burden (44), and, therefore, more frequent in-hospital complications. Indeed, a higher rate of cardiogenic shock, need for inotropic and mechanical hemodynamic support, and an increased incidence of life-threatening ventricular arrhythmias after successful revascularization of the culprit artery were found in patients with AMI admitted during the COVID-19 pandemic, with higher early mortality (10, 11, 25, 33, 42, 45). In particular, De Rosa et al. (25) found that in-hospital mortality for STEMI increased to 14% during the pandemic as compared to a 4% rate in the same period of 2019. In their work, De Rosa et al. found that major complications (cardiogenic shock, life-threatening arrhythmias,

TABLE 2 | Characteristics of the studies investigating the clinical impact of the COVID-19 pandemic on patients with acute myocardial infarction.

First author [Ref#]	Study population	Age (years)	Gender (males)	Mortality pandemic period	Mortality pre-pandemic period	AMI complications pandemic period	AMI complications pre-pandemic period
Cosentino et al. (10)	STEMI	64 ± 12	83%	19%	5%	CS 21%	CS 9%
Xiang et al. (24)	STEMI	63 ± 13	75%	5%	4%	AHF 14%	AHF 13%
De Rosa et al. (25)	STEMI/NSTEMI	68 ± 9	76%	10%	3%	16%*	7%*
Mesnier et al. (29)	STEMI/NSTEMI	65 ± 13	74%	5%	3%	Killip III–IV 9%	Killip III–IV 8%
Papafaklis et al. (30)	ACS	64 (56–74)	79%	3.3%	2.7%	CS 6.1%	CS 5.2%
Mohammad et al. (32)	STEMI/NSTEMI	70 (61–77)	67%	12%	6%	Killip III–IV 2.4%	Killip III–IV 2.4%
Gluckman et al. (33)	STEMI/NSTEMI	67 ± 13	68%	5%	5%	-	-
Carugo et al. (40)	STEMI/NSTEMI	69 (58–77)	77%	9%	-	CS 8%	-
Wilson et al. (34)	STEMI	63	68%	15%	11%	CS 18%	CS 19%

ACS, acute coronary syndrome; AHF, acute heart failure; AMI, acute myocardial infarction; CS, cardiogenic shock; NSTEMI, non-ST elevation myocardial infarction; STEMI, ST-elevation myocardial infarction.

**Cardiogenic shock, life-threatening arrhythmias, cardiac rupture/ventricular septal defect, or severe functional mitral regurgitation.*

cardiac rupture, and severe mitral regurgitation) were also increased from 10% of the previous year to 19%. Moreover, a study carried out in London found that not only higher in-hospital mortality in patients with STEMI but also a raised length of stay during the peak of the pandemic (1 march to 30 April 2020) compared to those observed during the corresponding 2019 period (46).

The pandemic caused significant disruption in AMI workflow, with a 39% increase in time from symptom onset to coronary angiography and a 31% increase in the time from first medical contact to coronary revascularization. Gluckman et al. (33) evaluated in-hospital outcomes in 15,000 patients admitted for AMI at PSJH by dividing them into three periods: before COVID-19 (from December 30, 2018 to February 22, 2020), the early period of the pandemic (from February 23, 2020 to March 28, 2020), and late period of the pandemic (from March 28, 2020 to May 16, 2020). Besides reporting a decrease in AMI hospitalization of 19%, the study found that patients with AMI had a 50% increased risk of in-hospital death during the late period of the pandemic, even after adjusting for patient characteristics. In particular, based on the PSJH all-cause in-hospital mortality risk model, the observed/expected ratio for mortality related to all AMIs (STEMI and NSTEMI) was significantly increased in both the early period (at 1.27) and the late period of the pandemic (at 1.23). Our initial clinical experience is consistent with these worrying data. Of note, since the first patient was diagnosed with COVID-19 in Italy on February 20, 2020, we observed a significantly worse outcome in patients with STEMI when compared with that of the same time in the previous year (10). Notably, although the two cohorts were similar in terms of age, rate of diabetes mellitus, and history of previous AMI, we observed a two-fold longer time from symptom onset to hospital presentation (7.5 vs. 3.1 h) and a three-fold higher rate of cardiogenic shock (21 vs. 9%) and in-hospital cardiac mortality (19 vs. 5%) during the COVID-19 outbreak when compared with the same period of 2019. Similar figures have also been reported by other registries across countries (**Figure 1**). Thus, despite the limited time of these observations, initial reports indicate increased mortality in patients with AMI during the pandemic. However, the mechanisms underlying the worse short-term outcome cannot be deduced from these experiences. Yet, the significant delay in hospital presentation of patients with STEMI reported during COVID-19 may have resulted in a higher rate of mechanical complications and, consequently, in-hospital mortality. Of note, the long delay in the management of patients with STEMI was observed since the very first COVID-19 outbreak in Far East countries. A single-center study from Hong Kong that includes seven consecutive patients requiring primary PCI for STEMI during COVID-19 in January 2020 found longer median times from symptom onset to myocardial reperfusion when compared with the previous year (318 vs. 82 min) (47).

Besides the fear of infection, the late presentation of patients with AMI during the pandemic may also be related to delays in the field, with a longer response time of the emergency medical services due to safety precautions and changes in standard procedures. In-hospital delays may also be playing a role as evaluation in the emergency department and treatment in the catheterization laboratory entail lengthy procedures due to patient triage and donning of personal protective equipment (25, 40). Further work is needed to determine what factors contributed most to the decreased and delayed AMI presentation and increased mortality. In particular, a recent study reviewed all available information on the incidence of STEMI hospitalizations during the COVID-19 pandemic, worldwide, focusing on the possible factors underlying discrepant results (48). This study confirmed that during the first peak of the COVID-19 pandemic, there has been a significant decrease in STEMI hospitalizations worldwide. However, the magnitude of decrease was of a lesser extent than initially described. Most importantly, through a meta-analytical approach of a significant number of reports that includes >100,000 cases from 57 countries, and systematic assessment of various health-related metrics, substantial differences emerged among studies and countries, probably due to different functioning of hospital services and different hub-and-spoke approaches to STEMI,

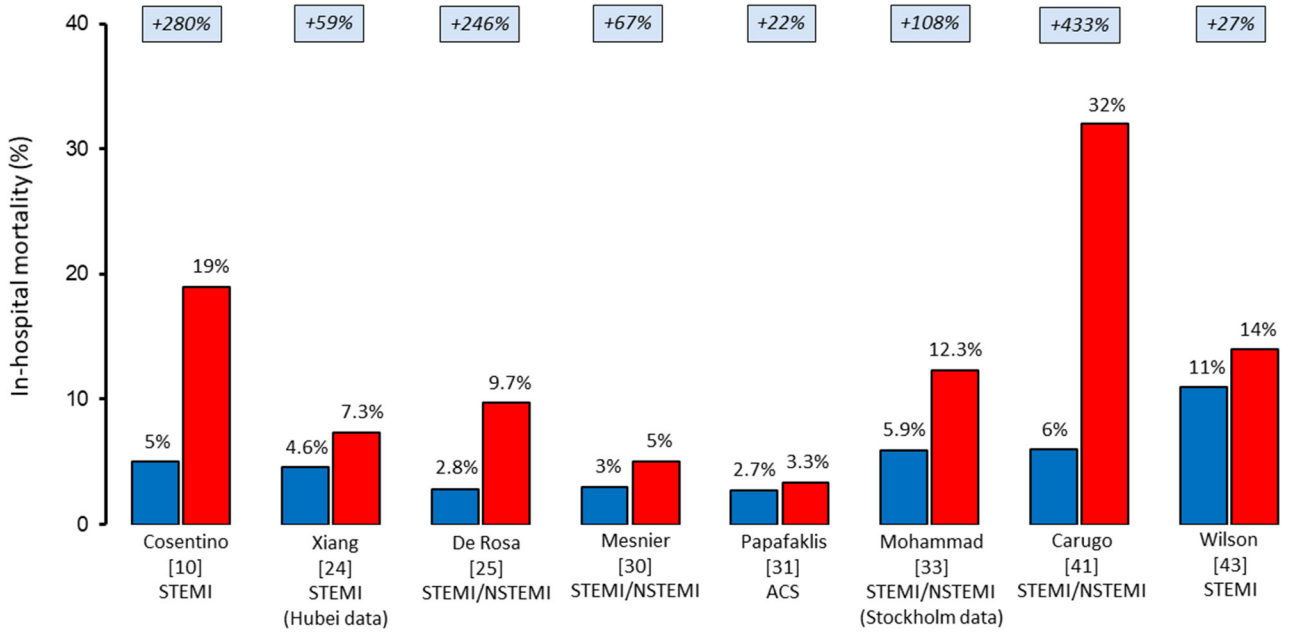


FIGURE 1 | In-hospital mortality rates of patients hospitalized with acute myocardial infarction before (blue) and during (red) the COVID-19 pandemic. ACS, acute coronary syndrome; NSTEMI, non-ST-elevation myocardial infarction; STEMI, ST-elevation myocardial infarction.

along with adequate public information during the pandemic (48). As different phases of the COVID-19 pandemic took shape, other investigations have been published to update the epidemiological picture of AMI, both at a national and at a global level. Indeed, a recent Canadian report assessed the changes in emergency department visit volume, care processes, and outcomes for stroke and AMI in the population of Ontario (49). It reported a reduction of 25–40% in emergency department visits for both acute diseases during the initial phase of the pandemic, with a subsequent compensatory increase in the late reopening phase and without a new drop during the second spread, starting in Ontario in September 2020 (49). Conversely, an English analysis comparing the daily incidence of hospital admission with AMI for the pre-COVID-19 period (November 2018 to March 2020) with that of the first and second UK lockdown found the second decline in admissions (by 34%) from the beginning of October 2020 up to November 2020, compared with the pre-COVID-19 period, despite an initial recovery in June 2020 (50).

In conclusion, regardless of the epidemiology of AMI during the COVID-19 outbreak and pandemic, there must be continued efforts through media attention on heart disease and public information to reduce patient fear to go to the hospital, emphasizing the importance of early recognition, and prompt treatment of AMI to ensure that COVID-19 management is no longer at the expense of this time-dependent disease. In this regard, the collateral damage of COVID-19 should not be ignored. Indeed, four different waves of the pandemic have been identified, involving different types of health impacts. After the first wave of immediate response to COVID-19, especially in terms of intensive care unit bed availability, a second non-COVID-19 wave of other urgent health conditions was neglected in the first one and a third wave defined by the result of interrupted care of chronic conditions have been clearly highlighted. Thus, these two waves not directly associated with the infection may have a negative impact on cardiovascular diseases during the following years, with an unprecedented increase in the prevalence of post-ischemic cardiomyopathy and heart failure. Finally, a fourth wave associated with the psychological trauma and economic injury caused by the pandemic can significantly affect the population in the future (51).

THE AMI NETWORK DURING THE COVID-19 OUTBREAK AND PANDEMIC

A relevant indirect consequence of the COVID-19 pandemic is the adverse impact on the efficacy and effectiveness of the network organization required to offer primary patients from PCI to STEMI (and patients from PCI to AMI in general), with the appropriate standards of care, within appropriate time frames, and with dedicated preventive and containment measures against COVID-19 infection. Lombardy is the most densely populated region in Italy, with ∼10 million inhabitants. With regard to the STEMI network, the healthcare system is divided into 8 areas, with an overall availability of 55 catheterization laboratories performing 24/7 primary PCI, and with a well-established STEMI network. However, during the COVID-19 outbreak, most hospitals underwent a sudden and radical transformation:

all deferrable cardiac surgical and interventional procedures were delayed, the number of ICU capacity was exponentially increased, and most departments, such as ICCU, were converted to COVID-19 units (52). Notably, this disruptive effect on cardiovascular disease services has been common to many countries, as confirmed by an ESC survey, in which about 50% of the respondents reported that their cardiovascular wards or departments had undergone a logistical restructuring due to the pandemic (53).

To face the COVID-19 emergency, on March 8, 2020, the Government of Lombardy and local health authorities requested to centralize the regional treatment of cardiovascular time-dependent emergencies in a limited number of centers. Thus, a centralization model based on "macro-hubs" was developed for the treatment of STEMI. One or two macro-hubs were identified in each of the eight areas of the region, according to the estimated transportation time, geographical features, and capacity to admit all the potential patients (52). The following requirements were considered to become a macro-hub: to perform primary PCI to all-incoming STEMI on a 24/7 basis, to guarantee a primary PCI team available in hospital 24/7 and not on-call, and to provide dedicated and separated pathways for STEMI patients with suspected/diagnosed COVID-19 disease from triage, through catheterization laboratory and to isolated ICCU to reduce cross-infection risk. Thus, 13 macro-hubs were identified, with a 63% reduction in the number of the original pre-pandemic hubs. This model of STEMI centralization was established to keep the regional healthcare system from being overwhelmed, and to guarantee, at the same time, standard levels of care to patients with AMI (52). Not only the regional AMI network was modified to face the COVID-19 pandemic but also the in-hospital AMI pathways were changed accordingly. In our hospital, one of the 13 identified macro-hubs, we rapidly developed a local protocol for triage and management of patients with AMI (9). In particular, we attempted to identify a customized pathway to allocate patients to the appropriate hospital ward treat them according to the type and severity of AMI, and to the potential concomitant risk of infection. In patients presenting with STEMI at the emergency department or referred from spoke hospitals, conservative care was not considered an option, and they were immediately transferred to the catheterization laboratory for primary PCI. In particular, the interventional procedure was performed in a catheterization laboratory dedicated to emergencies of potentially infected patients, in whom there was not time to wait for the polymerase chain reaction result of the naso-pharyngeal swab. Patients with a high-risk NSTEMI, as defined by the presence of hemodynamic and/or electrical instability, recurrent or ongoing chest pain refractory to medical treatment, and/or relevant ST-T wave changes, followed the STEMI protocol. Conversely, patients with a low-intermediate risk NSTEMI were evaluated in the emergency department in a dedicated and monitored area and underwent naso-pharyngeal swab immediately after admission. In the case of positive swabs and clinical stability, PCI was deferred. If PCI was clinically indicated, it was performed in a catheterization laboratory dedicated to SARS-CoV-2-positive patients. All patients with AMI, regardless of the treatment modalities, were admitted to different wards according to their naso-pharyngeal swab results. The use of this in-hospital pathway focusing on patients with AMI was implemented in our hospital a few weeks after the beginning of the COVID-19 outbreak. Since June 2020, we have had a new device for rapid analysis of the naso-pharyngeal swab, with results being available within 20 min. This allowed to quickly allocate patients to the proper catheterization laboratory and monitored ward according to the presence or absence of SARS-CoV-2, greatly simplifying their in-hospital pathway (9).

Although firm conclusions on the safety and efficacy of the Lombardy centralization model for AMI management cannot be drawn now, initial experience has been reported in a registry (40). From February 21 to May 7, 2020, 953 patients with AMI were treated. The clinical presentation was STEMI in 58% of the cases and 98% of all patients received coronary angiography, followed by PCI in 84% of the cases. About half of the patients were transported to a macro-hub by the emergency medical service, while a fourth was transferred from the spoke centers. The median time since first medical contact to angiography was 79 min for STEMI and 1,262 min for NSTEMI. Eleven percent of study patients presented a concomitant SARS-CoV-2 infection with pneumonia in 60% of them. Interestingly, STEMI was the clinical presentation in most of these cases, a higher rate compared to that of COVID-19-negative patients (75 vs. 56%). Coronary angiography was performed in 98% of overall patients with COVID-19 and 80% of them underwent PCI. No patient with STEMI was treated with fibrinolysis (40). Thus, during the 2 months with the highest daily increase of COVID-19 cases in Lombardy, nearly all patients received a timely coronary angiography and their treatment time since first medical contact was in line with guidelines recommendations. Although being a preliminary experience, the redefinition of AMI network based on macro-hubs seems to allow physicians to continue with timely AMI management, while reserving a high number of ICU beds for the pandemic. Preliminary data, comparing the second spread (November 2020 to January 2021) to the first one of the pandemic in the same Macro-Hubs in Lombardy, revealed no significant differences in clinical presentation and in the time from symptom onset to first medical contact, with a significant reduction in mortality and time to treatment during the second wave, further supporting the crucial role of centralization model applied in Lombardy (Ferlini et al., submitted).

During the COVID-19 pandemic, the management of patients with acute coronary syndromes has changed worldwide, and several protocols have been developed to guarantee the best treatment while minimizing the virus spread. Chinese physicians of the Sichuan Provincial People's hospital coping with the first wave of the pandemic proposed fibrinolysis as the treatment of choice for stable COVID-19 positive patients with STEMI. Elective PCI was then only considered after patient recovery from COVID-19 pneumonia, regardless of whether the patient was evaluated at a primary PCI center or not (54). Conversely, a primary-PCI strategy for COVID-19 patients with STEMI was the recommended one in a Singapore experience. Moreover, in that center, prophylactic early elective intubation was performed in cases characterized by a likely respiratory deterioration. This

approach allowed to avoid emergency intubation in such frail patients and to reduce the risk of catheterization laboratory staff exposure (55).

Besides these locally developed coping strategies, the main scientific societies have been very active in assisting clinical and interventional cardiologists. A Consensus Statement from the Society for Cardiovascular Angiography and Interventions (SCAI), the American College of Cardiology (ACC), and the American College of Emergency Physicians (ACEP) was published in April 2020 to provide a systematic approach for the care of patients with AMI during the COVID-19 pandemic (56). According to this document, in the case of a STEMI seen at a primary PCI center, the treatment slightly differed whether the patient was a COVID-19-positive/probable or possible (based on an ultra-rapid COVID-19 test). A COVID-19 positive/probable patient with classic clinical symptoms and ECG findings was considered for ultrasound evaluation of cardiac function to assess regional wall motion abnormalities consistent with the ECG findings before undergoing primary PCI. On the contrary, COVID-19 possible patients with classic clinical presentation and ECG finding consistent with a STEMI proceeded directly to primary PCI. In the case of a diagnosis of STEMI in non-PCI-capable hospitals, the primary PCI remained the standard of care for patients in whom reperfusion within 120 min of first medical contact at referral hospital was feasible. Only patients who could not be rapidly moved to the primary PCI center underwent fibrinolysis before transfer. Finally, as regards to patients with NSTEMI, COVID-19-positive or probable patients were initially managed medically and only taken for urgent coronary angiography and possible PCI in the presence of high-risk clinical features. Finally, a document by the ESC was published to help physicians dealing with cardiovascular disease during the COVID-19 pandemic. As in other protocols, a distinction between NSTEMI and STEMI was made. While patients with NSTEMI are suggested to be managed according to risk stratification (very high risk—treated as patients with STEMI, high risk, intermediate risk, and low risk), this indication does not apply to patients with STEMI, to guarantee timely reperfusion. According to the ESC document, all patients with STEMI should be managed as COVID-19 positive, in the absence of previous SARS-CoV-2 testing, to ensure the safety of healthcare personnel (57, 58).

LESSONS LEARNED AND CONCLUSION

The COVID-19 pandemic took the healthcare system worldwide by surprise and "distracted" physician's attention from the management of cardiovascular diseases, particularly time-dependent emergencies, with critical repercussions on the effectiveness of life-saving treatments and patient prognosis. After an initial shock, physicians realized that timely management of cardiac emergencies with appropriate standards of care should be ensured even during major unpredictable events. This can be achieved through a timely adoption of countermeasures against this unprecedented and dramatic emergency aimed at preventing large and long-standing health and social impact. In particular, health authorities should implement protocols that may provide a response to the index emergency and, at the same time, guarantee the best treatment strategy for AMI, based on prompt changes in the hub and spoke interplay. The delay in treatment delivery has also been a matter of serious concern raised during the COVID-19 pandemic, limiting the effectiveness of life-saving therapies for AMI. Indeed, patients have been reluctant to go to the hospital due to the fear of COVID-19, with many patients with AMI not seeking care at all or only late in the course of the acute event. This has contributed to increase the death toll beyond levels directly associated with SARS-CoV-2 infection. Although many questions remain unanswered and further evidence should be collected, we believe that every effort should be made by scientific societies, health authorities, and public media to convince patients not to delay life-saving treatments, even during dynamic crises.

In conclusion, in case the health situation returns to critical emergency levels, the experience gained during the COVID-19 pandemic should be an instructive lesson to help us be better prepared and provide appropriate guidance based on evidence on how to maintain optimal AMI management, even when the healthcare systems are under extreme strain.

AUTHOR CONTRIBUTIONS

GM, NC, and AB: concept and design. OT and JC: drafting the manuscript. GM and AB: study supervision. All authors contributed to the article and approved the submitted version.

REFERENCES

1. The Lancet Infectious Diseases. COVID-19, a pandemic or not? *Lancet Infect Dis*. (2020) 20:383. doi: 10.1016/S1473-3099(20)30180-8
2. Bandyopadhyay D, Akhtar T, Hajra A, Gupta M, Das A, Chakraborty S, et al. COVID-19 pandemic: cardiovascular complications and future implications. *Am J Cardiovasc Drugs*. (2020) 20:311–24. doi: 10.1007/s40256-020-00420-2
3. Boukhris M, Hillani A, Moroni F, Annabi MS, Addad F, Ribeiro MH, et al. Cardiovascular implications of the COVID-19 pandemic: a global perspective. *Can J Cardiol*. (2020) 36:1068–80. doi: 10.1016/j.cjca.2020.05.018
4. Böhm M, Frey N, Giannitsis E, Sliwa K, Zeiher AM. Coronavirus Disease 2019 (COVID-19) and its implications for cardiovascular care: expert document from the German cardiac society and the world heart federation. *Clin Res Cardiol*. (2020) 109:1446–59. doi: 10.1007/s00392-020-01656-3
5. Grasselli G, Pesenti A, Cecconi M. Critical care utilization for the COVID-19 outbreak in Lombardy, Italy: early experience and forecast during an emergency response. *JAMA*. (2020) 323:1545–6. doi: 10.1001/jama.2020.4031
6. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. COVID-19 lombardy ICU network. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy region, Italy. *JAMA*. (2020) 323:1574-81. doi: 10.1001/jama.2020.5394
7. Aldujeli A, Hamadeh A, Briedis K, Tecson KM, Rutland J, Krivickas Z, et al. Delays in presentation in patients with acute myocardial infarction during the COVID-19 pandemic. *Cardiol Res*. (2020) 11:386–91. doi: 10.14740/cr1175
8. Fagiuoli S, Lorini FL, Remuzzi G. Covid-19 Bergamo hospital crisis unit. Adaptations and lessons in the province of Bergamo. *N Engl J Med*. (2020) 382:e71. doi: 10.1056/NEJMc2011599

9. Cosentino N, Assanelli E, Merlino L, Mazza M, Bartorelli AL, Marenzi G. An in-hospital pathway for acute coronary syndrome patients during the COVID-19 outbreak: initial experience under real-world suboptimal conditions. *Can J Cardiol.* (2020) 36:961–4. doi: 10.1016/j.cjca.2020.04.011
10. Cosentino N, Bartorelli AL, Marenzi G. Time to treatment still matters in ST-elevation myocardial infarction: a call to maintain treatment effectiveness during the COVID-19 pandemic. *Eur Heart J Cardiovasc Pharmacother.* (20201) 6:408–9. doi: 10.1093/ehjcvp/pvaa054
11. Nallamothu BK, Bradley EH, Krumholz HM. Time to treatment in primary percutaneous coronary intervention. *N Engl J Med.* (2007) 357:1631–8. doi: 10.1056/NEJMra065985
12. Bonow RO, O'Gara PT, Yancy CW. Cardiology and COVID-19. *JAMA*. (2020) 324:1131–2. doi: 10.1001/jama.2020.15088
13. Libby P. Mechanisms of acute coronary syndromes and their implications for therapy. *N Engl J Med*. (2013) 368:2004–13. doi: 10.1056/NEJMra1216063
14. Schiavone M, Gobbi C, Biondi-Zoccai G, D'Ascenzo F, Palazzuoli A, Gasperetti A, et al. Acute coronary syndromes and Covid-19: exploring the uncertainties. *J Clin Med.* (2020) 9:1683. doi: 10.3390/jcm9061683
15. Cruz Rodriguez JB, Lange RA, Mukherjee D. Gamut of cardiac manifestations and complications of COVID-19: a contemporary review. *J Investig Med.* (2020) 68:1334–40. doi: 10.1136/jim-2020-001592
16. Canzano P, Brambilla M, Porro B, Cosentino N, Tortorici E, Vicini S, et al. Platelet and endothelial activation as potential mechanisms behind the thrombotic complications of COVID-19 patients. *JACC Basic Transl Sci.* (2021) 6:202–18. doi: 10.1016/j.jacbts.2020.12.009
17. Beyrouti R, Adams ME, Benjamin L, Cohen H, Farmer SF, Yen Goh Y, et al. Characteristics of ischaemic stroke associated with COVID-19. *J Neurol Neurosurg Psychiatry*. (2020) 91:889–91. doi: 10.1136/jnnp-2020-323586
18. Zhang L, Feng X, Zhang D, Jiang C, Mei H, Wang J, et al. Deep vein thrombosis in hospitalized patients with COVID-19 in Wuhan, China: prevalence, risk factors, and outcome. *Circulation.* (2020) 142:114–28. doi: 10.1161/CIRCULATIONAHA.120.046702
19. Poissy J, Goutay J, Caplan M, Parmentier E, Duburcq T, Lassalle F, et al. Pulmonary embolism in patients with COVID-19: awareness of an increased prevalence. *Circulation.* (2020) 142:184–6. doi: 10.1161/CIRCULATIONAHA.120.047430
20. Lodigiani C, Iapichino G, Carenzo L, Cecconi M, Ferrazzi P, Sebastian T, et al. Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan, Italy. *Thromb Res.* (2020) 191:9–14. doi: 10.1016/j.thromres.2020.04.024
21. Valtorta NK, Kanaan M, Gilbody S, Ronzi S, Hanratty B. Loneliness and social isolation as risk factors for coronary heart disease and stroke: systematic review and meta-analysis of longitudinal observational studies. *Heart.* (2016) 102:1009–16. doi: 10.1136/heartjnl-2015-308790
22. Pierce M, Hope H, Ford T, Hatch S, Hotopf M, John A, et al. Mental health before and during the COVID-19 pandemic: a longitudinal probability sample survey of the UK population. *The Lancet Psychiatry*. (2020) 7:883–92. doi: 10.1016/S2215-0366(20)30308-4
23. Pessoa-Amorim G, Camm CF, Gajendragadkar P, De Maria GL, Arsac C, Laroche C, et al. Admission of patients with STEMI since the outbreak of the COVID-19 pandemic: a survey by the european society of cardiology. *Eur Hear J - Qual Care Clin Outcomes.* (2020) 6:210–6. doi: 10.1093/ehjqcco/qcaa046
24. Xiang D, Xiang X, Zhang W, Yi S, Zhang J, Gu X, et al. Management and outcomes of patients with STEMI during the COVID-19 pandemic in China. *J Am Coll Cardiol.* (2020) 76:1318–24. doi: 10.1016/j.jacc.2020.06.039
25. De Rosa S, Spaccarotella C, Basso C, Calabrò MP, Curcio A, Perrone Filardi P, et al. Reduction of hospitalizations for myocardial infarction in Italy in the COVID-19 era. *Eur Heart J.* (2020) 41:2083–8. doi: 10.1093/eurheartj/ehaa409
26. Rodríguez-Leor O, Cid-Álvarez B, Ojeda S, Martín-Moreirasf J, Rumoroso JR, López-Palop R, et al. Impacto de la pandemia de COVID-19 sobre la actividad asistencial en cardiología intervencionista en España Impact of the COVID-19 pandemic on interventional cardiology activity in Spain. *REC Interv Cardiol.* (2020) 2:82–9. doi: 10.24875/RECIC.M20000120
27. Garcia S, Albaghdadi MS, Meraj PM, Schmidt C, Garberich R, Jaffer FA, et al. Reduction in ST-Segment elevation cardiac catheterization laboratory activations in the United States during COVID-19 pandemic. *J Am Coll Cardiol.* (2020) 75:2871–2. doi: 10.1016/j.jacc.2020.04.011
28. Mafham MM, Spata E, Goldacre R, Gair D, Curnow P, Bry M, et al. COVID-19 pandemic and admission rates for and management of acute coronary syndromes in England. *Lancet.* (2020) 396:381–9. doi: 10.1016/S0140-6736(20)31356-8
29. Mesnier J, Cottin Y, Coste P, Ferrari E, Schiele F, Lemesle G, et al. Hospital admissions for acute myocardial infarction before and after lockdown according to regional prevalence of COVID-19 and patient profile in France: a registry study. *Lancet Public Heal.* (2020) 5:e536–42. doi: 10.1016/S2468-2667(20)30188-2
30. Papafaklis MI, Katsouras CS, Tsigkas G, Toutouzas K, Davlouros P, Hahalis GN, et al. "Missing" acute coronary syndrome hospitalizations during the COVID-19 era in Greece: medical care avoidance combined with a true reduction in incidence? *Clin Cardiol.* (2020) 43:1142–9. doi: 10.1002/clc.23424
31. Solomon MD, McNulty EJ, Rana JS, Leong TK, Lee C, Sung SH, et al. The Covid-19 pandemic and the incidence of acute myocardial infarction. *N Engl J Med.* (2020) 383:691–3. doi: 10.1056/NEJMc2015630
32. Mohammad MA, Koul S, Olivecrona GK, Götberg M, Tydén P, Rydberg E, et al. Incidence and outcome of myocardial infarction treated with percutaneous coronary intervention during COVID-19 pandemic. *Heart.* (2020) 106:1812–8. doi: 10.1136/heartjnl-2020-317685
33. Gluckman TJ, Wilson MA, Chiu ST, Penny BW, Chepuri VB, Waggoner JW, et al. Case rates, treatment approaches, and outcomes in acute myocardial infarction during the Coronavirus disease 2019 pandemic. *JAMA Cardiol.* (2020) 5:1419–24. doi: 10.1001/jamacardio.2020.3629
34. Wilson SJ, Connolly MJ, Elghamry Z, Cosgrove C, Firoozi S, Lim P, et al. Effect of the COVID-19 pandemic on ST-segment-elevation myocardial infarction presentations and in-hospital outcomes. *Circ Cardiovasc Interv.* (2020) 13:e009438. doi: 10.1161/CIRCINTERVENTIONS.120.009438
35. Garcia S, Stanberry L, Schmidt C, Sharkey S, Megaly M, Albaghdadi MS, et al. Impact of COVID-19 pandemic on STEMI care: an expanded analysis from the United States. *Catheter Cardiovasc Interv.* (2021) 98:217–22. doi: 10.1002/ccd.29154
36. Ball S, Banerjee A, Berry C, Boyle JR, Bray B, Bradlow W, et al. Monitoring indirect impact of COVID-19 pandemic on services for cardiovascular diseases in the UK. *Heart.* (2020) 106:1890–7. doi: 10.1136/heartjnl-2020-317870
37. Lai PH, Lancet EA, Weiden MD, Webber MP, Zeig-Owens R, Hall CB, et al. Characteristics associated with out-of-hospital cardiac arrests and resuscitations during the novel Coronavirus disease 2019 pandemic in New York City. *JAMA Cardiol.* (2020) 5:1154–63. doi: 10.1001/jamacardio.2020.2488
38. Baldi E, Sechi GM, Mare C, Canevari F, Brancaglione A, Primi R, et al. Out-of-hospital cardiac arrest during the Covid-19 outbreak in Italy. *N Engl J Med.* (2020) 383:496–8. doi: 10.1056/NEJMc2010418
39. Rashid Hons M, Gale Hons CP, Curzen Hons N, Ludman Hons P, De Belder Hons M, Timmis Hons A, et al. Impact of COVID19 pandemic on the incidence and management of out of hospital cardiac arrest in patients presenting with acute myocardial infarction in England. *J Am Heart Assoc.* (2020) 9:e018379. doi: 10.1161/JAHA.120.018379
40. Carugo S, Ferlini M, Castini D, Andreassi A, Guagliumi G, Metra M, et al. Management of acute coronary syndromes during the COVID-19 outbreak in Lombardy: the "macro-hub" experience. *IJC Hear Vasc.* (2020) 31:100662. doi: 10.1016/j.ijcha.2020.100662
41. De Filippo O, D'Ascenzo F, Angelini F, Bocchino PP, Conrotto F, Saglietto A, et al. Reduced rate of hospital admissions for ACS during Covid-19 outbreak in northern Italy. *N Engl J Med.* (2020) 383:88–9. doi: 10.1056/NEJMc2009166
42. Primessnig U, Pieske BM, Sherif M. Increased mortality and worse cardiac outcome of acute myocardial infarction during the early COVID-19 pandemic. *ESC Heart Fail.* (2021) 8:333–43. doi: 10.1002/ehf2.13075
43. Gramegna M, Baldetti L, Beneduce A, Pannone L, Falasconi G, Calvo F, et al. ST-segment-elevation myocardial infarction during COVID-19 pandemic: insights from a regional public service healthcare hub. *Circ Cardiovasc Interv.* (2020) 13:e009413. doi: 10.1161/CIRCINTERVENTIONS.120.009413
44. Reinstadler SJ, Reindl M, Lechner I, Holzknecht M, Tiller C, Roithinger FX, et al. Effect of the COVID-19 pandemic on treatment delays in patients with st-segment elevation myocardial infarction. *J Clin Med.* (2020) 9:2183. doi: 10.3390/jcm9072183

45. Popovic B, Varlot J, Metzdorf PA, Jeulin H, Goehringer F, Camenzind E. Changes in characteristics and management among patients with ST-elevation myocardial infarction due to COVID-19 infection. *Catheter Cardiovasc Interv.* (2021) 97:E319–26. doi: 10.1002/ccd.29114
46. Little CD, Kotecha T, Candilio L, Jabbour RJ, Collins GB, Ahmed A, et al. COVID-19 pandemic and STEMI: pathway activation and outcomes from the pan-London heart attack group. *Open Heart.* (2020) 7:e001432. doi: 10.1136/openhrt-2020-001432
47. Tam CCF, Cheung KS, Lam S, Wong A, Yung A, Sze M, et al. Impact of Coronavirus disease 2019 (COVID-19) outbreak on ST-segment-elevation myocardial infarction care in Hong Kong, China. *Circ Cardiovasc Qual Outcomes.* (2020) 13:e006631. doi: 10.1161/CIRCOUTCOMES.120.006631
48. Sofi F, Dinu M, Reboldi G, Stracci F, Pedretti RFE, Valente S, et al. Worldwide differences of hospitalization for ST-segment elevation myocardial infarction during COVID-19: a systematic review and meta-analysis. *Int J Cardiol.* (2021) 347:89–96. doi: 10.1016/j.ijcard.2021.10.156
49. Yu AYX, Lee DS, Vyas MV, Porter J, Rashid M, Fang J, et al. Emergency department visits, care, and outcome after stroke and myocardial infarction during the COVID-19 pandemic phases. *CJC Open*. (2021) 3:1230–7. doi: 10.1016/j.cjco.2021.06.002
50. Wu J, Mamas MA, de Belder MA, Deanfield JE, Gale CP. Second decline in admissions with heart failure and myocardial infarction during the COVID-19 pandemic. *J Am Coll Cardiol.* (2021) 77:1141–3. doi: 10.1016/j.jacc.2020.12.039
51. Bourgeault IL, Maier CB, Dieleman M, Ball J, MacKenzie A, Nancarrow S, et al. The COVID-19 pandemic presents an opportunity to develop more sustainable health workforces. *Hum Resour Health.* (2020) 18:83. doi: 10.1186/s12960-020-00529-0
52. Ferlini M, Andreassi A, Carugo S, Cuccia C, Bianchini B, Castiglioni B, et al. Centralization of the ST elevation myocardial infarction care network in the Lombardy region during the COVID-19 outbreak. *Int J Cardiol.* (2020) 312:24–6. doi: 10.1016/j.ijcard.2020.04.062
53. Roffi M, Capodanno D, Windecker S, Baumbach A, Dudek D. Impact of the COVID-19 pandemic on interventional cardiology practice: results of the EAPCI survey. *EuroIntervention.* (2020) 16:247–50. doi: 10.4244/EIJ-D-20-00528
54. Zeng J, Huang J, Pan L. How to balance acute myocardial infarction and COVID-19: the protocols from Sichuan Provincial People's Hospital. *Intensive Care Med.* (2020) 46:1111–3. doi: 10.1007/s00134-020-05993-9
55. Wong SW, Lee JH, Tan HH, Ling LM, Ho HH. Cardiac catheterisation for ST-Elevation myocardial infarction during COVID-19 in Singapore: protocols and recommendations. *Ann Acad Med Singap.* (2020) 49:600–4. doi: 10.47102/annals-acadmedsg.2020154
56. Mahmud E, Dauerman HL, Welt FGP, Messenger JC, Rao SV, Grines C, et al. Management of acute myocardial infarction during the COVID-19 pandemic: a consensus statement from the Society for Cardiovascular Angiography and Interventions (SCAI), the American College of Cardiology (ACC), and the American College of Emergency Physicians (ACEP). *Catheter Cardiovasc Interv.* (2020) 96:336–45. doi: 10.1002/ccd.28946
57. The Task Force for the management of COVID-19 of the European Society of Cardiology. ESC guidance for the diagnosis and management of cardiovascular disease during the COVID-19 pandemic: part 1 - epidemiology, pathophysiology, and diagnosis. *Eur Heart J.* (2021) ehab696. doi: 10.1093/eurheartj/ehab696
58. The Task Force for the management of COVID-19 of the European Society of Cardiology. ESC guidance for the diagnosis and management of cardiovascular disease during the COVID-19 pandemic: part 2 - care pathways, treatment, and follow-up. *Eur Heart J.* (2021) ehab697. doi: 10.1093/eurheartj/ehab697

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 05 January 2022
doi: 10.3389/fcvm.2021.760651

Frequent Constriction-Like Echocardiographic Findings in Elite Athletes Following Mild COVID-19: A Propensity Score-Matched Analysis

Bálint Károly Lakatos[1], Márton Tokodi[1], Alexandra Fábián[1], Zsuzsanna Ladányi[1], Hajnalka Vágó[1,2], Liliána Szabó[1], Nóra Sydó[1,2], Emese Csulak[1], Orsolya Kiss[1,2], Máté Babity[1], Anna Réka Kiss[1], Zsófia Gregor[1], Andrea Szűcs[1], Béla Merkely[1,2†] and Attila Kovács[1†]*

[1] Heart and Vascular Center, Semmelweis University, Budapest, Hungary, [2] Department of Sports Medicine, Semmelweis University, Budapest, Hungary

OPEN ACCESS

Edited by:
Andrew F. James,
University of Bristol, United Kingdom

Reviewed by:
Edgar Argulian,
Mount Sinai Hospital, United States
Hatem Soliman-Aboumarie,
Royal Brompton & Harefield NHS Foundation Trust, United Kingdom

***Correspondence:**
Bálint Károly Lakatos
lakatos.balint@med.semmelweis-univ.hu

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Cardiovascular Imaging, a section of the journal Frontiers in Cardiovascular Medicine

Received: 18 August 2021
Accepted: 24 November 2021
Published: 05 January 2022

Citation:
Lakatos BK, Tokodi M, Fábián A, Ladányi Z, Vágó H, Szabó L, Sydó N, Csulak E, Kiss O, Babity M, Kiss AR, Gregor Z, Szűcs A, Merkely B and Kovács A (2022) Frequent Constriction-Like Echocardiographic Findings in Elite Athletes Following Mild COVID-19: A Propensity Score-Matched Analysis. Front. Cardiovasc. Med. 8:760651. doi: 10.3389/fcvm.2021.760651

Background: The cardiovascular effects of SARS-CoV-2 in elite athletes are still a matter of debate. Accordingly, we sought to perform a comprehensive echocardiographic characterization of post-COVID athletes by comparing them to a non-COVID athlete cohort.

Methods: 107 elite athletes with COVID-19 were prospectively enrolled (P-CA; 23 ± 6 years, 23% female) 107 healthy athletes were selected as a control group using propensity score matching (N-CA). All athletes underwent 2D and 3D echocardiography. Left (LV) and right ventricular (RV) end-diastolic volumes (EDVi) and ejection fractions (EF) were quantified. To characterize LV longitudinal deformation, 2D global longitudinal strain (GLS) and the ratio of free wall vs. septal longitudinal strain (FWLS/SLS) were also measured. To describe septal flattening (SF—frequently seen in P-CA), LV eccentricity index (EI) was calculated.

Results: P-CA and N-CA athletes had comparable LV and RVEDVi (P-CA vs. N-CA; 77 ± 12 vs. 78 ± 13mL/m2; 79 ± 16 vs. 80 ± 14mL/m2). P-CA had significantly higher LVEF (58 ± 4 vs. 56 ± 4%, $p < 0.001$), while LVGLS values did not differ between P-CA and N-CA (−19.0 ± 1.9 vs. −18.8 ± 2.2%). EI was significantly higher in P-CA (1.13 ± 0.16 vs. 1.01 ± 0.05, $p < 0.001$), which was attributable to a distinct subgroup of P-CA with a prominent SF ($n = 35$, 33%), further provoked by inspiration. In this subgroup, the EI was markedly higher compared to the rest of the P-CA (1.29 ± 0.15 vs. 1.04 ± 0.08, $p < 0.001$), LVEDVi was also significantly higher (80 ± 14 vs. 75 ± 11 mL/m2, $p < 0.001$), while RVEDVi did not differ (82 ± 16 vs. 78 ± 15mL/m2). Moreover, the FWLS/SLS ratio was significantly lower in the SF subgroup (91.7 ± 8.6 vs. 97.3 ± 8.2, $p < 0.01$). P-CA with SF experienced symptoms less frequently (1.4 ± 1.3 vs. 2.1 ± 1.5 symptom during the infection, $p = 0.01$).

Conclusions: Elite athletes following COVID-19 showed distinct morphological and functional cardiac changes compared to a propensity score-matched control athlete group. These results are mainly driven by a subgroup, which presented with some echocardiographic features characteristic of constrictive pericarditis.

Keywords: athlete's heart, COVID-19, speckle-tracking analysis, 3D echocardiography, constrictive pericaditis

INTRODUCTION

The COVID-19 pandemic represents an unprecedented challenge to the healthcare systems worldwide with still increasing patient numbers. While the infection was initially thought to be affecting mainly the respiratory tract, current evidence suggests that the cardiovascular consequences of COVID-19 are not negligible (1). SARS-CoV-2-related myocardial injury is frequently reported as a worrisome manifestation, whereas prior cardiovascular disorders are strong negative prognostic factors for the course of the infection (2, 3).

Fortunately, COVID-19 is often asymptomatic or associated with only mild symptoms, especially in the young (4). Still, the potential cardiac effects of an uncomplicated SARS-CoV-2 infection need to be further explored.

Elite athletes are a distinguished group of young individuals as a relatively high proportion of them underwent (or will undergo) the infection. This is attributable to their high-risk profile: a young community with frequent social interactions; the majority of sport disciplines include direct physical contact; and wearing a mask during training sessions or competitions is rarely a realistic expectation (5). While the vast majority of young athletes experience an uncomplicated disease course, it is important to emphasize that high-intensity training and related cardiac adaptation may even exaggerate the adverse effects of COVID-19, as it does for other cardiac or non-cardiac disorders (6). Initial reports demonstrated that a considerable proportion of athletes may have detectable myocardial damage; however, the lack of proper control groups limited the generalizability of these results (7–10). Recent studies also proposed the possibility of pericardial involvement (10, 11). Nevertheless, all of the aforementioned studies utilized cardiac magnetic resonance (cMR), an imaging modality that can hardly be incorporated into the routine return to play examination protocol. As a potential alternative, the clinical value of state-of-the-art echocardiographic techniques, such as 3D echocardiography and speckle-tracking echocardiography (STE) should be also tested.

Accordingly, we sought to perform a comprehensive echocardiographic characterization of post-COVID athletes and compare them to a propensity score (PS)-matched healthy athlete cohort.

MATERIALS AND METHODS

Patient Characteristics

We consecutively enrolled elite athletes undergoing "return to play" examinations between September and December 2020 at our Center's Sports Cardiology Department (study protocol approved by the National Public Health Center; no: ETT TUKEB IV/10282-1/2020/EKU). The study protocol complies with the Declaration of Helsinki, and participants gave written informed consent to every procedure. SARS-CoV-2 infection was diagnosed by real-time polymerase chain reaction (rt-PCR) or by serum immunoglobulin G (IgG) antibody titer measurement. All athletes were officially released from quarantine defined by having two negative rt-PCR assays of nasopharyngeal swab specimens following the infection and/or passing the appropriate quarantine period (10 or 14 days depending on the time of enrollment). All of the athletes completed a questionnaire regarding the nature and duration of their SARS-CoV-2 infection, based on the recommendation of the National Institute of Health (12). Detailed medical history and training regimen were obtained along with the routine physical examination and 12-lead electrocardiogram. Body surface area (BSA) was calculated using the Mosteller formula (13). Subjects with previously documented uncommon echocardiographic and/or electrocardiographic features or with suboptimal echocardiographic image quality for further analysis ($n = 5$) and athletes who suspended regular training in the preceding 6 months before their SARS-CoV-2 infection ($n = 2$) were excluded.

To enable the appropriate pairwise comparison of COVID vs. non-COVID athletes, PS-matching was performed with the optimal pair matching algorithm (14). Our institutional database comprising 425 elite athletes served as the pool for the matching. First, propensity scores were calculated based on age, BSA, and weekly training hours. Then, each COVID athlete was paired with one non-COVID athlete from our institutional database, targeting the collective optimization of the overall criterion (i.e., minimizing the mean of the within-pair difference in propensity score). Matching was applied in males and females separately to ensure that each COVID athlete is paired with a non-COVID athlete of the same sex. PS-matching was performed in R (version 3.6.3, R Foundation for Statistical Computing, Vienna, Austria) using the MatchIt package (version 3.0.2).

Conventional Echocardiography

Echocardiographic loops were recorded using a Vivid E95 ultrasound system equipped with a 4Vc-D phased-array transducer (GE Vingmed Ultrasound, Horten, Norway). Cardiac chambers were quantified according to current guidelines (15). Left ventricular (LV) wall thicknesses and diameters were measured in the parasternal long-axis view at the level of mitral valve coaptation. Relative wall thickness (RWT) was calculated as 2*posterior wall thickness/LV end-diastolic internal diameter. LV diastolic eccentricity index was measured from parasternal short-axis view at the level of the papillary muscles, defined as the ratio of the distances between the anterior-to-posterior wall and the septal-to-lateral wall in end-diastole. Left- and right atrial volumes were measured using the Simpson method and were indexed to BSA. LV diastolic inflow by pulsed-wave Doppler at the level of the mitral valve coaptation was obtained to determine early (E) and late diastolic (A) peak velocities, their ratio, and E-wave deceleration time. Pulsed-wave tissue Doppler imaging (TDI) was used to measure systolic (s'), early (e'), and late diastolic (a') velocities at the mitral lateral and medial annuli. The ratio of E-wave velocity to averaged e' velocities of the mitral medial and lateral annuli was calculated, serving as an estimate of LV filling pressures. Tricuspid annular plane systolic excursion (TAPSE) was measured by M-mode as the peak longitudinal excursion of the tricuspid annulus on an apical four-chamber view. Inferior vena cava (IVC) diameters estimated right atrial pressure (RAP), pulmonary arterial systolic pressure (PASP), diastolic pressure (PADP), mean pressure (PAMP), and also

pulmonary vascular resistance (PVR) were quantified according to the current echocardiographic recommendations (16). The presence of a visually detectable septal flattening or pericardial effusion was evaluated during postprocessing by a single expert operator (B.L.) blinded to the study groups.

Speckle-Tracking Analysis

ECG-gated, LV-focused apical long axis, four- and two-chamber view loops targeting a frame rate over 50 FPS were obtained for further analysis. STE was performed by a single expert operator (B.L.) blinded to the study groups using dedicated semi-automatic software (EchoPAC v204 AFI, GE). The software automatically detects the myocardial region of interest (ROI) of the given acquisition and tracks its motion throughout the cardiac cycle. If necessary, the ROI was adjusted manually in order to provide adequate tracking. Segments with poor tracking quality (driven by the software's recommendation) were excluded from the analysis; however, subjects with three or more excluded segments were not included in the study (none). The software automatically calculates global longitudinal strain (GLS) and segmental longitudinal strain (LS) values as well. By averaging the segmental data of the free wall (FW—average LS of inferior, posterior, lateral, and anterior segments) and septal (S—average LS of infero- and anteroseptal segments) regions, we have quantified FWLS and SLS, respectively.

3D Echocardiography

LV- and RV-focused ECG-gated full volume 3D datasets were obtained from apical four-chamber view using multi-beat reconstruction from 4 cardiac cycles. Offline analyses of these datasets focused on the LV and RV were performed by the same expert, blinded operator using conventionally available software packages (4D LV Analysis 3 and RV-Function 2, TomTec Imaging Systems GmbH, Unterschleissheim, Germany). The algorithm automatically generates LV and RV endocardial contours, which were manually corrected on multiple short- and long-axis planes throughout the entire cardiac cycle. We determined the LV and RV end-diastolic volume index (EDVi), end-systolic volume index (ESVi), and stroke volume index (SVi) normalized to BSA. To quantify global ventricular function, LV and RV ejection fractions (EF) were also calculated.

Statistical Analysis

All values are expressed as mean ± standard deviation, or median and interquartile range (IQR). The distribution of the variables was assessed by the Shapiro-Wilk normality test. An unpaired two-sided Student's *t*-test, in case of normal distribution, or a Mann-Whitney U test, in case of non-normal distribution, was performed to compare the continuous variables of the study groups. Fisher's exact test was used to compare the incidence of symptoms between groups. A $p < 0.05$ was used as the criterion for statistical significance.

Intra- and interobserver variability of the most relevant parameters were also assessed. The operator of the first measurements (B.L.) and a second expert reader (A.F.), both blinded to the study groups, repeated the measurements in a randomly chosen subset of 5–5 athletes from each group. Lin's concordance correlation coefficient and coefficient of variation were calculated.

RESULTS

One hundred and seven post-COVID athletes (handball $n = 37$, ice hockey $n = 26$, water polo $n = 26$, basketball $n = 12$, speedskating $n = 2$, other $n = 4$) were included in the current analysis. Athletes were asymptomatic at the time of examination with the exception of the loss of taste and/or smell in a handful of cases ($n = 12$), as these symptoms frequently exceed the period of active infection (17). A total of 59 subjects (55%) were completely asymptomatic throughout the disease course. The symptom burden of the study group is summarized in **Supplementary Table 1**. The athletes were symptomatic for a median of 4 [IQR: 1–7] days and presented for the return to play examinations 22 [IQR: 17–25] days following the first rt-PCR or IgG positivity.

The mean age of the post-COVID athletes was 23 ± 6 years. There were no differences in age, BSA and training hours between the post-COVID and the PS-matched non-COVID athletes, indicating successful matching. Systolic and diastolic blood pressures also did not differ between post-COVID and non-COVID athletes, while heart rate was significantly lower in the post-COVID group (**Table 1**).

Basic echocardiographic parameters of the left and right heart are shown in **Table 2**. LV wall thicknesses and RWT were significantly lower in the post-COVID group. Transmitral E/A ratio was higher in the post-COVID group, along with a longer deceleration time. TDI-derived mitral lateral and medial velocities were significantly higher in the post-COVID group resulting in a lower E/e′ ratio; however, e′ lateral/e′ medial ratio was significantly lower. 2D RV, left and right atrial dimensions did not differ between the study groups. Maximal IVC diameter and right atrial pressure were significantly lower in the post-COVID group, whereas other estimated pulmonary artery pressures were comparable between post-COVID athletes and PS-matched non-COVID athletes. TAPSE/PASP ratio was also similar (**Table 2**).

3D echocardiographic and 2D LV STE parameters are summarized in **Table 3**. 3D LV and RV EDVi were comparable between groups, whereas 3D LV ESVi was significantly lower in post-COVID athletes, resulting in elevated LV EF. 2D GLS, SLS, and FWLS were comparable between the post-COVID and non-COVID groups; however, a lower FWLS/SLS ratio was detected in the post-COVID athletes.

Interestingly, LV diastolic eccentricity index was significantly higher in the post-COVID subjects (1.13 ± 0.16 vs. 1.01 ± 0.05, $p < 0.001$). This finding was mainly driven by a subgroup ($n = 35/107$; 33%) of post-COVID athletes, in which an early-diastolic septal flattening (SF) was present consistently throughout the entire echocardiographic examination on multiple views, showing an inspiratory enhancement (**Figure 1**, **Supplementary Video 1**). This phenomenon was not detected in

TABLE 1 | Baseline characteristics of the post-COVID and the non-COVID athlete groups.

	Post-COVID athletes ($n = 107$)	Non-COVID athletes ($n = 107$)	*p*-value
Age (years)	22.9 ± 6.1	22.7 ± 7.0	0.82
Female (n [%])	25 (23%)	25 (23%)	1
Height (cm)	182.9 ± 10.0	181.8 ± 12.0	0.45
Weight (kg)	80.2 ± 15.3	80.6 ± 17.0	0.87
BSA (m²)	2.0 ± 0.2	2.0 ± 0.3	0.93
SBP (mmHg)	130.3 ± 15.1	134.0 ± 15.8	0.09
DBP (mmHg)	79.4 ± 11.3	77.4 ± 9.2	0.16
HR (1/min)	62.9 ± 10.6	66.6 ± 13.3	**<0.05**
Training per week (hours)	13.1 ± 6.0	14.5 ± 6.4	0.08

BSA, body surface area; SBP, systolic blood pressure; DBP, diastolic blood pressure. HR, heart rate. Bold values indicate a $p < 0.05$.

TABLE 2 | Conventional echocardiographic left- and right heart parameters in the post-COVID and the non-COVID athlete groups.

	Post-COVID athletes ($n = 107$)	Non-COVID athletes ($n = 107$)	*p*-value
LVIDd (mm)	51.8 ± 4.4	51.4 ± 5.4	0.56
IVSd (mm)	9.4 ± 1.8	10.4 ± 1.8	**<0.01**
PWd (mm)	8.4 ± 1.3	9.0 ± 1.3	**<0.01**
RWT (%)	0.33 ± 0.05	0.35 ± 0.05	**<0.001**
LAVi (mL/m²)	26.4 ± 6.5	27.9 ± 8.6	0.16
Transmitral E wave (cm/s)	81.7 ± 16.0	82.3 ± 20.6	0.79
Transmitral A wave (cm/s)	50.2 ± 12.3	57.4 ± 15.5	**<0.001**
E/A	1.68 ± 0.40	1.49 ± 0.43	**<0.001**
DT (ms)	192.7 ± 40.8	176.6 ± 39.3	**<0.01**
E/e′ average	4.64 ± 0.88	5.55 ± 1.50	**<0.001**
Mitral lateral s′ (cm/s)	12.8 ± 2.5	12.1 ± 2.3	**<0.05**
Mitral lateral e′ (cm/s)	19.7 ± 3.2	17.7 ± 3.2	**<0.001**
Mitral lateral a′ (cm/s)	8.3 ± 2.0	7.6 ± 1.8	**<0.01**
Mitral medial s′ (cm/s)	10.3 ± 1.5	9.6 ± 1.4	**<0.01**
Mitral medial e′ (cm/s)	15.6 ± 2.7	13.0 ± 2.6	**<0.001**
Mitral medial a′ (cm/s)	8.4 ± 1.4	7.5 ± 1.8	**<0.001**
e′ lateral/e′ septal	1.29 ± 0.21	1.40 ± 0.27	**<0.001**
LV diastolic eccentricity index	1.13 ± 0.16	1.01 ± 0.05	**<0.001**
RV basal diameter (mm)	34.3 ± 4.2	33.7 ± 4.3	0.27
TAPSE (mm)	24.7 ± 3.9	23.6 ± 4.2	0.05
RAVi (mL/m²)	28.0 ± 6.6	28.1 ± 8.1	0.89
PASP (mmHg)	20.7 ± 4.3	20.4 ± 5.2	0.61
PADP (mmHg)	6.9 ± 2.3	7.0 ± 2.8	0.76
PAMP (mmHg)	13.4 ± 4.2	12.3 ± 3.7	0.19
IVC max (mm)	13.2 ± 3.0	16.0 ± 4.1	**<0.001**
IVC min (mm)	11.3 ± 6.0	9.3 ± 6.7	0.39
RAP (mmHg)	3.5 ± 1.8	4.2 ± 2.3	**<0.05**
RVOT VTI (cm)	20.0 ± 3.5	18.8 ± 3.4	**<0.05**
PVR (Wood units)	1.24 ± 0.21	1.21 ± 0.26	0.51
TAPSE/PASP	1.23 ± 0.30	1.24 ± 0.42	0.86
Prevalence of mild pericardial effusion (n [%])	41 (38%)	10 (9%)	**<0.001**

LVIDd, left ventricular end-diastolic diameter; IVSd, interventricular septal thickness; PWd, posterior wall thickness; RWT, relative wall thickness; LAVi, left atrial volume index; DT: deceleration time; LV eccentricity index, left ventricular eccentricity index; RV basal diamater, right ventricular basal diameter; TAPSE, tricuspid annular plane systolic excursion; RAVi, right atrial volume index; PASP, pulmonary arterial systolic pressure; PADP, pulmonary arterial diastolic pressure; PAMP, pulmonary arterial mean pressure; IVC, inferior vena cava; RAP, right atrial pressure; RVOT VTI, right ventricular outflow tract velocity-time integral; PVR, pulmonary vascular resistance. Bold values indicate a $p < 0.05$.

TABLE 3 | Comparison of 3D and speckle-tracking echocardiographic data between the post-COVID and the non-COVID athlete groups.

	Post-COVID athletes ($n = 107$)	Non-COVID athletes ($n = 107$)	p-value
3D LVEDVi (mL/m^2)	76.7 ± 12.2	78.3 ± 13.3	0.39
3D LVESVi (mL/m^2)	32.4 ± 6.3	34.7 ± 7.4	**0.01**
3D LVSVi (mL/m^2)	44.4 ± 7.5	43.5 ± 7.3	0.4
3D LVEF (%)	57.9 ± 4.3	55.8 ± 4.2	**<0.001**
3D RVEDVi (mL/m^2)	78.9 ± 15.5	79.6 ± 14.2	0.72
3D RVESVi (mL/m^2)	35.4 ± 8.4	36.6 ± 8.6	0.32
3D RVSVi (mL/m^2)	43.5 ± 8.5	43.1 ± 7.1	0.72
3D RVEF (%)	55.3 ± 4.5	54.3 ± 4.7	0.14
2D LVGLS (%)	−19.0 ± 1.9	−18.8 ± 2.2	0.51
2D FWLS (%)	−18.6 ± 2.1	−18.6 ± 2.2	0.97
2D SLS (%)	−19.6 ± 2.1	−19.0 ± 2.4	0.06
2D FWLS/SLS (%)	95.5 ± 8.7	98.3 ± 6.8	**<0.01**

LVEDVi, left ventricular end-diastolic index; LVESVi, left ventricular end-systolic volume index; LVSVi, left ventricular stroke volume index; LVEF, left ventricular ejection fraction; RVEDVi, right ventricular end-diastolic volume index; RVESVi, right ventricular end-systolic volume index; RVSVi, right ventricular stroke volume index; RVEF, right ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; FWLS, free wall longitudinal strain; SLS, septal longitudinal strain; FWLS/SLS, free wall to septal longitudinal strain ratio. Bold values indicate a $p < 0.05$.

any athletes of the PS-matched non-COVID group. Therefore, we have also assessed the differences between the athletes with and without SF within the post-COVID group.

Post-COVID athletes with SF were younger; however, they did not differ in other anthropometric or basic hemodynamic measures and in average weekly training hours (**Supplementary Table 2**).

The presence of detectable (trivial) pericardial effusion was more frequent in the SF subgroup of post-COVID athletes compared to the corresponding subset of PS-matched non-COVID athletes (41% vs. 12%, $p < 0.01$). Post-COVID athletes with SF and without SF did not differ in terms of the number of symptomatic days (3 [IQR: 0–7.0] days vs. 5 [IQR: 2.5–8.0] days, $p = 0.09$), the time between the onset of symptoms and the examination (24 [IQR: 17.5–37.5] days vs. 23 [IQR: 18.0–29.0] days, $p = 0.65$), or the time elapsed between the first positive PCR or IgG and the examination (22.5 [IQR: 17.0–25.0] days vs. 21 [IQR: 17.0–25.0] days, $p = 0.70$). The incidence of fever (34 vs. 29%, $p = 0.66$), coughing (9 vs. 7%, $p = 0.71$), headache (29 vs. 44%, $p = 0.15$), and loss of smell and/or taste (47 vs. 52%, $p = 0.54$) were also comparable between the athlete groups. Interestingly, chest pain (0 vs. 15%, $p = 0.01$) and fatigue (17 vs. 34%, $p = 0.04$) were reported more frequently in post-COVID athletes without SF (**Figure 2**). When the symptom burden was summed as a "composite symptom score", athletes with SF generally had fewer symptoms (1.4 ± 1.3 vs. 2.1 ± 1.5 symptom during the infection, $p = 0.01$, **Figure 2**).

Regarding basic echocardiographic measures, post-COVID athletes with SF showed significantly higher E/A ratio, while RAP and PADP were also found to be significantly higher compared to post-COVID athletes without SF (**Supplementary Table 2**). LV diastolic eccentricity index was markedly higher in post-COVID athletes with SF, while it was comparable between post-COVID athletes without SF and their matched non-COVID athletes (1.04 ± 0.08 vs. 1.00 ± 0.04, $p = 0.14$). Regarding 3D echocardiographic measures, post-COVID athletes with SF had significantly higher LV EDVi and LV ESVi compared to post-COVID athletes without SF, while RV morphological measures along with LV and RV EF were similar (**Table 4**). 2D LV GLS did not differ between the post-COVID athlete subgroups; however, the FWLS/SLS ratio was significantly lower in athletes with SF compared to those without (**Figure 3**). During the last phase of the enrollment and already having our awareness at SF and related STE-based alterations, we have referred athletes presented with SF to cMR examination ($n = 5$). Notably, no myopericardial involvement was detected by cMR in these cases. Detailed case reports are presented in **Supplementary Table 3**.

Intra- and interobserver variability of the key echocardiographic parameters showed good intra- and interreader agreements (**Supplementary Table 4**).

DISCUSSION

Our study is the first to investigate a relatively high number of European elite athletes who underwent mild COVID-19, while also comparing them to a PS-matched healthy athlete group using a comprehensive echocardiographic approach. We have shown that post-COVID athletes show distinct changes in cardiac morphology and function compared to matched non-COVID athletes. Of note, the vast majority of these alterations was attributable to a subpopulation of athletes in whom an inspiration-enhanced early diastolic SF could be detected. In these athletes, the E/A ratio of mitral inflow, the 3D echocardiography-derived LV volumes were significantly higher, along with a significantly lower FWLS/SLS ratio.

The earliest reports from China already mentioned the high prevalence of elevated cardiac necroenzymes and the commonly deteriorated LV functional measures in COVID-19 patients (18). With the worldwide expansion of the pandemic, several other studies demonstrated the high frequency of cardiac

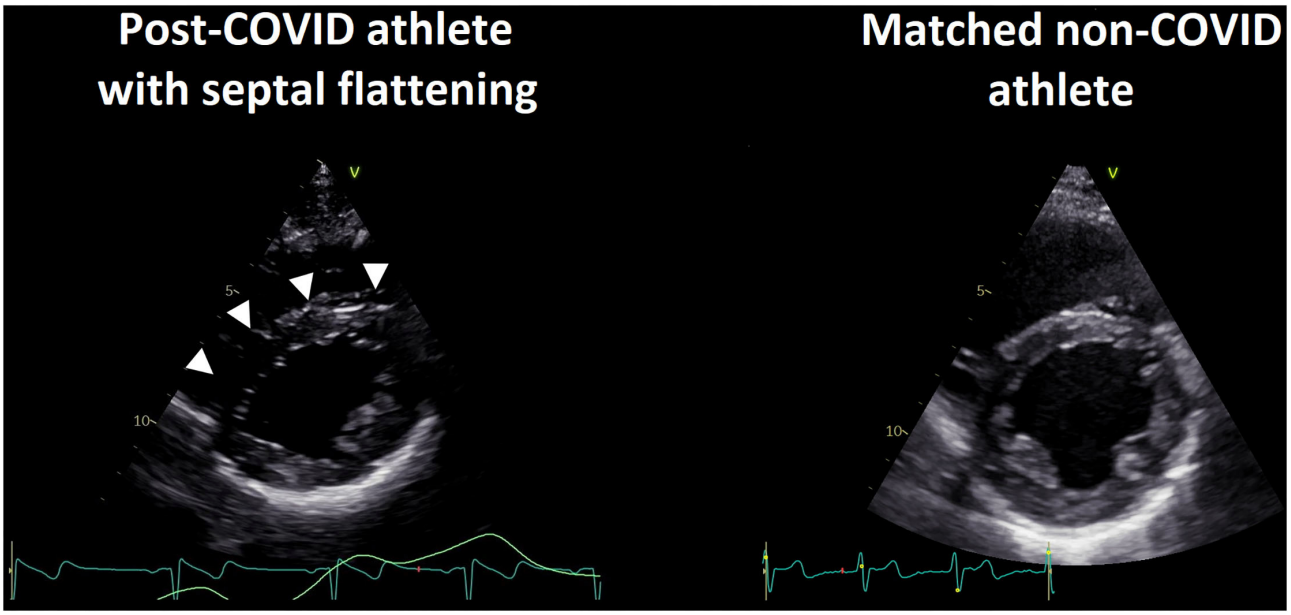


FIGURE 1 | Representative case of the post-COVID septal flattening (SF) in athletes. Parasternal short-axis views at the level of the papillary muscles at mid-diastole in a young athlete underwent asymptomatic SARS-CoV-2 infection and his matched control. In the post-COVID athlete, a prominent SF can be seen with early diastolic dominance and inspiratory enhancement (left, SF shown by arrows), compared to the propensity score-matched control (right).

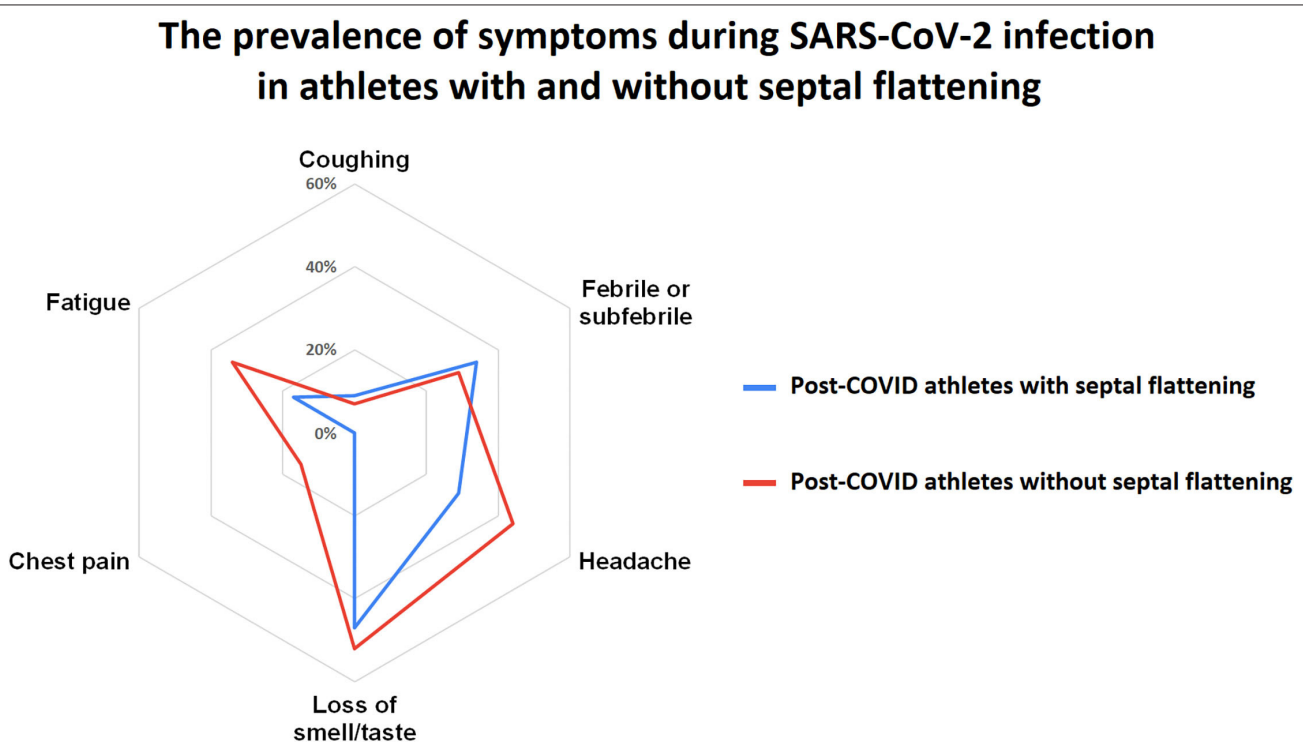


FIGURE 2 | Radar chart comparisons of the most relevant symptoms in post-COVID athletes with our without septal flattening (SF). Athletes with SF (blue line) and without SF (red line) did not differ in the incidence of fever or subfebrility, coughing, headache or the lost of smell and/or taste. On the other hand, chest pain and fatigue were significantly more frequent in athletes without SF. In general, athletes with SF were less symptomatic, as shown by the smaller area of the radar chart compared to athletes without SF (see details in text).

TABLE 4 | Echocardiographic comparison of post-COVID athletes with vs. without septal flattening.

	Post-COVID athletes with SF ($n = 35$)	Post-COVID athletes without SF ($n = 72$)	p-value
LV diastolic eccentricity index	1.29 ± 0.15	1.04 ± 0.08	**<0.001**
3D LVEDVi (mL/m^2)	80.1 ± 14.4	74.9 ± 10.7	**<0.05**
3D LVESVi (mL/m^2)	34.5 ± 8.0	31.3 ± 5.1	**<0.05**
3D LVSVi (mL/m^2)	46.2 ± 8.2	43.5 ± 7.1	0.09
3D LVEF (%)	57.5 ± 4.6	58.1 ± 4.1	0.52
3D RVEDVi (mL/m^2)	82.1 ± 15.9	77.7 ± 15.3	0.3
3D RVESVi (mL/m^2)	36.5 ± 9.8	35.9 ± 7.6	0.37
3D RVSVi (mL/m^2)	44.7 ± 7.8	42.9 ± 8.8	0.31
3D RVEF (%)	55.6 ± 5.5	55.2 ± 4.0	0.68
2D LVGLS (%)	−18.9 ± 1.9	−19.0 ± 2.0	0.70
2D FWLS (%)	−18.3 ± 2.0	−18.8 ± 2.1	0.20
2D SLS (%)	−20.0 ± 2.3	−19.4 ± 2.0	0.16
2D FWLS/SLS (%)	91.7 ± 8.6	97.3 ± 8.2	**<0.001**

SF, septal flattening; LVEDVi, left ventricular end-diastolic index; LVESVi, left ventricular end-systolic volume index; LVSVi, left ventricular stroke volume index; LVEF, left ventricular ejection fraction; RVEDVi, right ventricular end-diastolic volume index; RVESVi, right ventricular end-systolic volume index; RVSVi, right ventricular stroke volume index; RVEF, right ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; FWLS, free wall longitudinal strain; SLS, septal longitudinal strain; FWLS/SLS, free wall to septal longitudinal strain ratio. Bold values indicate a $p < 0.05$.

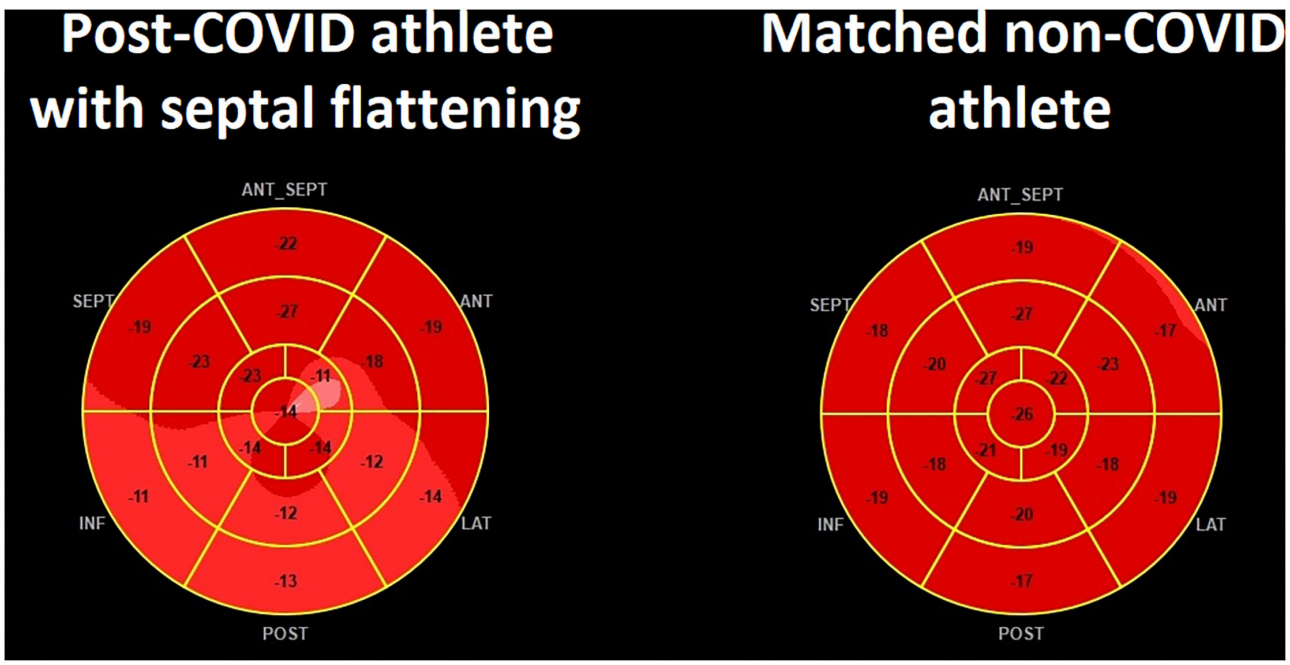


FIGURE 3 | The "Hot Septum Sign" in a post-COVID athlete with septal flattening. While left ventricular global longitudinal strain is preserved, a relative decrease in the free wall segments can be noted (left), suggestive of a characteristic feature of pericardial constriction. In the matched control, segmental strain values of the septum and free wall do not markedly differ (right).

damage; however, the investigations were mainly focused on the severe/critical cases (19). Nowadays, evidence is growing that mild or even asymptomatic disease courses do not exclude myocardial involvement of COVID-19 (20). Special considerations are needed in the case of elite athletes following SARS-CoV-2 infection, even though these young, exceptionally healthy individuals usually undergo COVID-19 with no or very mild symptoms. Robust evidence suggests that even minor cardiac alterations can be exaggerated by high-intensity exercise, and this can worsen the course of various diseases (6). Therefore, the detailed characterization of the athlete's heart following COVID-19 is a relevant clinical demand.

In our post-COVID population, LV wall thicknesses and RWT were significantly lower compared to the matched control athletes. These findings may correspond to the effects of short-term detraining: LV wall thicknesses are known to decrease even after a few weeks of suspended athletic activity along with unaltered ventricular volumes (21, 22). Regarding functional

measures, LV EF was found to be significantly higher in the post-COVID group, with unaltered LV GLS. While data are conflicting regarding the resting LV systolic function of the athlete's heart, low-normal values are commonly reported; therefore, an increase in LV EF following detraining may be expected (23).

In a subpopulation of our post-COVID elite athletes, an early diastolic SF was detected with inspiratory enhancement, commonly resulting in marked LV eccentricity (**Figure 1**, **Supplementary Video 1**). This phenomenon may be attributable to a handful of causes. Previous studies examining sedentary COVID-19 patients and also elite athletes reported alterations of the myocardium with a septal predominance (such as decreased septal LS, or increased native T1 values and/or late gadolinium enhancement of the septum) suggestive of SARS-CoV-2-related myocarditis (7, 11). Nevertheless, in viral myocarditis, segmental or global wall motion abnormalities would be expected rather than a bouncing septal motion with preserved deformation.

COVID-19 is also known to affect the pulmonary vasculature (24). However, in our post-COVID cohort, Doppler-based estimated pulmonary pressures did not markedly differ from matched control athletes, and post-COVID athletes with SF were also comparable to those without SF regarding these measures. Of note, estimated RAP and PADP were significantly higher in athletes with SF, nevertheless, only with a borderline statistical significance. Moreover, a COVID-related imbalance between intrapericardial and intrathoracic pressures should also be considered.

The aforementioned study of Brito et al. reported a surprisingly high prevalence of pericardial involvement in their study enrolling student athletes (11). While acute viral pericarditis does not usually alter myocardial function, previous results suggest that a transient pericardial constriction-like physiology may occur, which could explain the SF (25). As a marker of a possible pericardial inflammation, the prevalence of a detectable (although trivial) pericardial effusion was significantly higher in our post-COVID athletes compared to their matched non-COVID athletes. Furthermore, this constriction-like behavior is also reinforced by the phenomenon that the SF seems to be enhanced by inspiration and becomes the most prominent during early diastole (**Figure 1**) (26). Regarding STE-markers, in our post-COVID athletes with SF, the characteristic "hot septum" sign can be seen as shown by the STE-derived FWLS/SLS ratio (**Figure 3**) (27, 28). Of note, it is important to mention that other, less specific markers of constrictive physiology, such as increased E/A ratio and PADP, can also be measured in this subpopulation. In line with the cMR findings of the post-COVID population of Brito and colleagues, LV volumes were significantly higher in the post-COVID group with SF (11). This may be attributable to the partially similar methodology of cMR and a "multi-beat" 3D echocardiographic acquisition: during expiratory breathhold, the enhanced ventricular interdependence of the constrictive physiology may result in increased LV volumes (26).

Interestingly, SF was more common in athletes with generally fewer symptoms. This corresponds to previous large-scale cMR data demonstrating that all athletes with confirmed inflammatory heart disease were only minimally symptomatic (9). Moreover, in another study, pericardial enhancement was significantly more common in asymptomatic athletes (11). In a large retrospective cardiac surgery registry, post-surgery constrictive pericarditis patients were characterized by more commonly detected postoperative pericardial effusion and a higher LVEF (29). These results may indicate that the main driver of disease progression is the interplay of ongoing serous membrane inflammation and more pronounced friction of the pericardial sac by a hyperdynamic LV. Theoretically, athletes with fewer symptoms are likely to continue training during the infection, potentially creating a similar pathophysiological scenario.

However, it is important to mention that in those cases where cMR was also performed, no signs of pericardial inflammation or constriction were detected. Considering that the main body of data about the reverse remodeling after abrupted training is derived from small sample studies of the early 90's, it is plausible that a temporary change in the pericardial constraint is a benign phenomenon of athletic detraining (HIV). Hemodynamic overload is proved to induce not only myocardial, but pericardial remodeling as well (HIV). Therefore, it is suspected that intense regular exercise may also induce changes of the pericardial structure. While myocardial deconditioning is known to take place over the course of a few weeks of abrupted training regime, the altered characteristics of the pericardium may persist for a longer time, resulting in temporary changes of the pericardial constraint.

The current Sports Cardiology and Exercise Guideline of the European Society of Cardiology recommends at least 30 days of suspended training in the case of pericarditis, however, in the absence of a proven inflammatory process the clinical implications of these findings are hard to judge (HIV).In the case of persistent constriction-like changes in the SF post-COVID group, an impaired peak exercise capacity has high possibility (HIV)., Follow-up of athletes and further research are urged to explain the appearance and the potential clinical consequences of the constriction-like echocardiographic findings in the context of the athlete's heart.

LIMITATIONS

Our study carries limitations that have to be acknowledged for adequate interpretation. First, our case number is limited. Nevertheless, the number of subjects is considered to be relatively high as compared to current COVID-19-related data in athletes. The population has a male predominance; therefore, the study was not powered to examine the role of gender differences. In the post-COVID athlete group, echocardiographic loops prior to the SARS-CoV-2 infection were not available. Therefore, PS-matching was used to provide a matched control athlete group. The observed changes were often subtle and only statistically significant. The most common causes of SF are pulmonary hypertension and constrictive pericarditis, and the gold-standard evaluation method for both these diseases is still right (and left-) heart catheterization (26). For obvious ethical reasons, such invasive procedures were not performed in the

mainly asymptomatic/paucisymptomatic post-COVID athlete group. However, various echocardiographic pressure estimates and other functional parameters were quantified, which may also adequately assess the characteristic features of such diseases. Nevertheless, certain indirect markers of pericardial constriction, such as mitral and/or tricuspid inflow variation, hepatic vein flow and M-mode assessment of the septal motion were not obtained. Computed tomography was not included in this study; therefore, possible pulmonary involvement of the post-COVID athletes was not evaluated. cMR examinations were only performed in a handful of athletes; therefore, the gold-standard measurements of cardiac volumes are not available, and cMR markers of myopericardial inflammation were not assessed in the majority of the subjects. The cMR acquisitions were obtained during breath holding, therefore, free breathing loops confirming the septal flattening were not available. Respirometry was not part of our routine echocardiographic image acquisition protocol (used only in a few cases in the post-COVID group); therefore, the inspiratory enhancement of the SF was not tested consistently. Assessment of the long-term consequences and clinical importance of these findings requires further work and follow-up.

CONCLUSIONS

Our results suggest that even mild SARS-CoV-2 infection may significantly affect cardiac morphology and function in elite athletes. The observed alterations are mainly attributable to a subgroup of athletes, in whom some features of pericardial constriction could be detected, such as pericardial effusion, early diastolic SF with inspiratory enhancement, and STE-derived "hot septum" sign. Interestingly, these athletes seemed to experience fewer symptoms during the course of the infection. Considering that current guidelines usually propose a more thorough return to play examinations in symptomatic athletes only, our data is especially alarming, as many of our athletes presented with SF would not have been eligible for a detailed assessment (30). The pathophysiological background and clinical relevance of these findings are unclear and require further research. Nevertheless, our data support the use of a comprehensive echocardiographic protocol applying advanced techniques in the return to play examination of elite athletes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Public Health Center; No: ETT TUKEB IV/10282-1/2020/EKU. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

BL, MT, AF, MB, and AK contributed to the conception of the study design. BL, HV, LS, NS, EC, OK, MB, AK, ZG, and AS performed the measurents. BL, MT, AF, ZL, LS, and EC managed the database. BL and MT perfomed the statistical analysis. BL, MB and AK wrote the draft of the manuscript. MT, AF, HV, LS, NS, EC, OK, AS, and MB reviewed it. BL, MT, and MB prepared the figures. All authors contributed to the article and approved the submitted version.

FUNDING

Project no. NVKP_16-1-2016-0017 (National Heart Program) has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the NVKP_16 funding scheme. This research was financed by the Thematic Excellence Programme (2020-4.1.1.-TKP2020) of the Ministry for Innovation and Technology in Hungary, within the framework of the Therapeutic Development and Bioimaging Thematic Programmes of the Semmelweis University and also by the New National Excellence Program of the Ministry of Human Capacities (ÚNKP-20-4-I-SE-13 to BL, ÚNKP-20-3-I-SE-41 to MB, and ÚNKP-20-5 to AK). This project was also supported by a grant from the National Research, Development and Innovation Office (NKFIH) of Hungary (K135076 to BM). AK was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. This project was supported by a grant from the National Research, Development and Innovation Office (NKFIH) of Hungary (2020-1.1.6-JÖVŐ-2021-00013).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.760651/full#supplementary-material

Supplementary Video 1 | Post-COVID athlete with septal flattening (SF). An early diastolic dominance of the SF can be seen with inspiratory enhancement (as shown on the parallel ECG and respirometry tracing).

REFERENCES

1. Guzik TJ, Mohiddin SA, Dimarco A, Patel V, Savvatis K, Marelli-Berg FM, et al. COVID-19 and the cardiovascular system: implications for risk assessment, diagnosis, and treatment options. *Cardiovasc Res.* (2020) 116:1666–87. doi: 10.1093/cvr/cvaa106
2. Peng Y, Meng K, He M, Zhu R, Guan H, Ke Z, et al. Clinical characteristics and prognosis of 244 cardiovascular patients suffering from coronavirus disease in Wuhan, China. *J Am Heart Assoc.* (2020) 9:e016796. doi: 10.1161/JAHA.120.016796
3. Bae S, Kim SR, Kim MN, Shim WJ, Park SM. Impact of cardiovascular disease and risk factors on fatal outcomes in patients with COVID-19

according to age: a systematic review and meta-analysis. *Heart.* (2021) 107:373–80. doi: 10.1136/heartjnl-2020-317901
4. Barek MA, Aziz MA, Islam MS. Impact of age, sex, comorbidities and clinical symptoms on the severity of COVID-19 cases: a meta-analysis with 55 studies and 10014 cases. *Heliyon.* (2020) 6:e05684. doi: 10.1016/j.heliyon.2020.e05684
5. Watson A, Haraldsdottir K, Biese K, Goodavish L, Stevens B, McGuine T. The association of COVID-19 incidence with sport and face mask use in United States high school athletes. *medRxiv*. (2001) 2019.21250116. doi: 10.4085/1062-6050-281-21
6. Guasch E, Mont L. Diagnosis, pathophysiology, and management of exercise-induced arrhythmias. *Nat Rev Cardiol.* (2017) 14:88–101. doi: 10.1038/nrcardio.2016.173
7. Rajpal S, Tong MS, Borchers J, Zareba KM, Obarski TP, Simonetti OP, et al. Cardiovascular magnetic resonance findings in competitive athletes recovering from COVID-19 infection. *JAMA Cardiol.* (2020). doi: 10.1001/jamacardio.2020.4916
8. Vago H, Szabo L, Dohy Z, Merkely B. Cardiac magnetic resonance findings in patients recovered from COVID-19: initial experiences in elite athletes. *JACC Cardiovasc Imaging.* (2020) 14:1279–81. doi: 10.1016/j.jcmg.2020.11.014
9. Martinez MW, Tucker AM, Bloom OJ, Green G, Difiori JP, Solomon G, et al. Prevalence of inflammatory heart disease among professional athletes with prior COVID-19 infection who received systematic return-to-play cardiac screening. JAMA Cardiol. (2021) 6:745–52. doi: 10.1001/jamacardio.2021.0565
10. Moulson N, Petek BJ, Drezner JA, Harmon KG, Kliethermes SA, Patel MR, et al. SARS-CoV-2 cardiac involvement in young competitive athletes. *Circulation.* (2021) 144:256–66. doi: 10.1161/CIRCULATIONAHA.121.054824
11. Brito D, Meester S, Yanamala N, Patel HB, Balcik BJ, Casaclang-Verzosa G, et al. High prevalence of pericardial involvement in college student athletes recovering from COVID-19. *JACC Cardiovasc Imaging.* (2020) 14:541–55. doi: 10.1016/j.jcmg.2020.10.023
12. NIH. *Coronavirus Disease 2019 (COVID-19). Treatment Guidelines* (2019:130). Avaliable online at: https://covid19treatmentguidelines.nih.gov/ (accessed May 12, 2021).
13. Mosteller RD. Simplified calculation of body-surface area. *N Engl J Med.* (1987) 317:1098. doi: 10.1056/NEJM198710223171717
14. Gu XS, Rosenbaum PR. Comparison of multivariate matching methods: structures, distances, and algorithms. *J Comput Graph Stat.* (1993) 2:405–20. doi: 10.1080/10618600.1993.10474623
15. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American society of echocardiography and the European association of cardiovascular imaging. *J Am Soc Echocardiogr.* (2015) 28:1–39. doi: 10.1016/j.echo.2014.10.003
16. Rudski LG, Lai WW, Afilalo J, Hua L, Handschumacher MD, Chandrasekaran K, et al. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American society of echocardiography endorsed by the European association of echocardiography, a registered branch of the European society of cardiology, and the canadian society of echocardiography. *J Am Soc Echocardiogr.* (2010) 23:685–713. doi: 10.1016/j.echo.2010.05.010
17. Boscolo-Rizzo P, Borsetto D, Fabbris C, Spinato G, Frezza D, Menegaldo A, et al. Evolution of altered sense of smell or taste in patients with mildly symptomatic COVID-19. *JAMA Otolaryngol Head Neck Surg.* (2020) 146:729–32. doi: 10.1001/jamaoto.2020.1379
18. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* (2020) 5:802–10. doi: 10.1001/jamacardio.2020.0950
19. Parohan M, Yaghoubi S, Seraji A. Cardiac injury is associated with severe outcome and death in patients with coronavirus disease 2019 (COVID-19) infection: a systematic review and meta-analysis of observational studies. *Eur Heart J Acute Cardiovasc Care.* (2020) 9:665–77. doi: 10.1177/2048872620937165
20. Puntmann VO, Carerj ML, Wieters I, Fahim M, Arendt C, Hoffmann J, et al. Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:1265–73. doi: 10.1001/jamacardio.2020.3557
21. Maron BJ, Pelliccia A, Spataro A, Granata M. Reduction in left ventricular wall thickness after deconditioning in highly trained olympic athletes. *Br Heart J.* (1993) 69:125–8. doi: 10.1136/hrt.69.2.125
22. Olah A, Kovacs A, Lux A, Tokodi M, Braun S, Lakatos BK, et al. Characterization of the dynamic changes in left ventricular morphology and function induced by exercise training and detraining. *Int J Cardiol.* (2019) 277:178–85. doi: 10.1016/j.ijcard.2018.10.092
23. Lakatos BK, Molnár AÁ, Kiss O, Sydó N, Tokodi M, Solymossi B, et al. Relationship between cardiac remodeling and exercise capacity in elite athletes: incremental value of left atrial morphology and function assessed by three-dimensional echocardiography. *J Am Soc Echocardiogr.* (2020) 33:101–9. doi: 10.1016/j.echo.2019.07.017
24. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med.* (2020) 383:120–8. doi: 10.1056/NEJMoa2015432
25. Sato K, Ayache A, Kumar A, Cremer PC, Griffin B, Popovic ZB, et al. Improvement in left ventricular mechanics following medical treatment of constrictive pericarditis. Heart. (2021) 107:828–35. doi: 10.1136/heartjnl-2020-317304
26. Syed FF, Schaff HV, Oh JK. Constrictive pericarditis–a curable diastolic heart failure. *Nat Rev Cardiol.* (2014) 11:530–44. doi: 10.1038/nrcardio.2014.100
27. Kusunose K, Dahiya A, Popovic ZB, Motoki H, Alraies MC, Zurick AO, et al. Biventricular mechanics in constrictive pericarditis comparison with restrictive cardiomyopathy and impact of pericardiectomy. *Circ Cardiovasc Imaging.* (2013) 6:399–406. doi: 10.1161/CIRCIMAGING.112.000078
28. Argulian EDH. "Hot septum" sign of constrictive pericarditis. *JACC Case Reports.* (2020) 2:186–90. doi: 10.1016/j.jaccas.2019.12.024
29. Matsuyama K, Matsumoto M, Sugita T, Nishizawa J, Yoshioka T, Tokuda Y, et al. Clinical characteristics of patients with constrictive pericarditis after coronary bypass surgery. *Jpn Circ J.* (2001) 65:480–2. doi: 10.1253/jcj.65.480
30. Wilson MG, Hull JH, Rogers J, Pollock N, Dodd M, Haines J, et al. Cardiorespiratory considerations for return-to-play in elite athletes after COVID-19 infection: a practical guide for sport and exercise medicine physicians. *Br J Sports Med.* (2020) 54:1157–61. doi: 10.1136/bjsports-2020-102710

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

OPINION
published: 17 January 2022
doi: 10.3389/fcvm.2021.830024

Mitochondria, a Missing Link in COVID-19 Heart Failure and Arrest?

Ralph Ryback[1]* *and Alfonso Eirin*[2]

[1] *Mindful Health Foundation, Naples, FL, United States,* [2] *Department of Internal Medicine, Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN, United States*

Keywords: COVID-19, mitochondria, heart failure, cardiovascular disease, ATP

Over the last 2 years, we have all been trying to understand the interrelationships of COVID-19's numerous symptoms, clinical risk factors, and lethality. Autopsy cases of patients with COVID-19 revealed that the virus was present in the heart of more than 60% of patients, associated with evidence of active viral replication, suggesting direct viral cardiac infection (1). Contrarily, a recent study reported that the virus was detected in the heart of only one out of 30 patients who died after a prolonged hospital stay due to Sars-Cov-2 infection., associated with modest histological alterations (2). Macroscopic, histological, and immunohistochemical analysis revealed modest cardiac histological alterations, underscoring the lack of evidence to establish the contribution of a direct effect of SARS-CoV-2 on cardiac lesions. Mehra and Ruschitzka (3) noted in the elderly, especially with cardiovascular disease, mortality was associated with a very significant elevation of natriuretic peptides (NPs) with death attributed to cardiac failure and arrest in almost 25% of cases. They wondered whether cardiac inflammation or dysfunction suggested by elevated NP's might play a role in the respiratory hypoxic failure observed in COVID-19. NPs, hormones secreted from the heart, have many functions including promoting Na^+, excretion by the kidney. In addition, NPs are involved in important mitochondrial mediated processes including Ca^2+ signaling, apoptosis, reactive oxygen species production, biogenesis, and fat oxidation, etc. (4). Cardiac followed by kidney cells have the highest mitochondrial content (high ATP energy needs) and thus their function is directly dependent on mitochondrial health (4). However, cardiac mitochondrial function extends well-beyond energy production and includes modulation of numerous cellular signaling pathways at molecular and biochemical levels (5). Thus, mitochondrial damage has a tremendous impact on overall cardiomyocyte function.

Evidence reveals the virus localizes to mitochondria which it attacks and disrupts (**Figure 1**), thereby taking energy away from the cells' battle with the virus including autophagy (6, 7). In this process, SARS-CoV-2 manipulates mitochondrial function by angiotensin-converting enzyme 2 (ACE2) regulation and open-reading frames (ORFs) to evade host cell immunity and facilitate virus replication. The virus-encoded protein Orf-96 localizes to mitochondria and triggers degradation of mitochondria-related genes, including DRP1, MAVS, TRAF3, and TRAF6 (8). ORFs, such as ORF3a, can target the mitochondrial deubiquitinase USP30, altering mitochondrial homeostasis (biogenesis, fusion, fission, and mitophagy) and function (9). Furthermore, the 3a protein of the virus promotes mitochondrial apoptosis (10). In cellular homeostasis, there is a balance between the BCL-2 family protein, which are neuroprotective and Bax proteins which can be transformed to set off a cell-death cascade. This can occur in response to extracellular stimulation by stress, viral infection, excessive immune cytokines secretions, etc. Bax exist in a relatively stable molecular form, but with viral infection it changes form and moves to the outer membrane of the mitochondria where it inserts itself, causing the release of the cytochrome c, initiating apoptosis (10) with the release of its DNA. In addition, the 3a protein promotes activation of truncated Bid (tBid) which form pores in the mitochondria, favoring the release of apoptogenic factors. The unique DNA of the degraded mitochondria is then released into the blood whose presence at high levels has now been reported (11) to predict poor COVID-19 outcomes.

OPEN ACCESS

Edited by:
Amer Harky, Liverpool Heart and Chest Hospital, United Kingdom

Reviewed by:
Claudio Tinoco Mesquita, Universidade Federal Fluminense, Brazil

***Correspondence:**
Ralph Ryback
rsryback@gmail.com; ralphrybackmd.com

Specialty section:
This article was submitted to Heart Failure and Transplantation, a section of the journal Frontiers in Cardiovascular Medicine

Received: *06 December 2021*
Accepted: *22 December 2021*
Published: *17 January 2022*

Citation:
Ryback R and Eirin A (2022) Mitochondria, a Missing Link in COVID-19 Heart Failure and Arrest? Front. Cardiovasc. Med. 8:830024. doi: 10.3389/fcvm.2021.830024

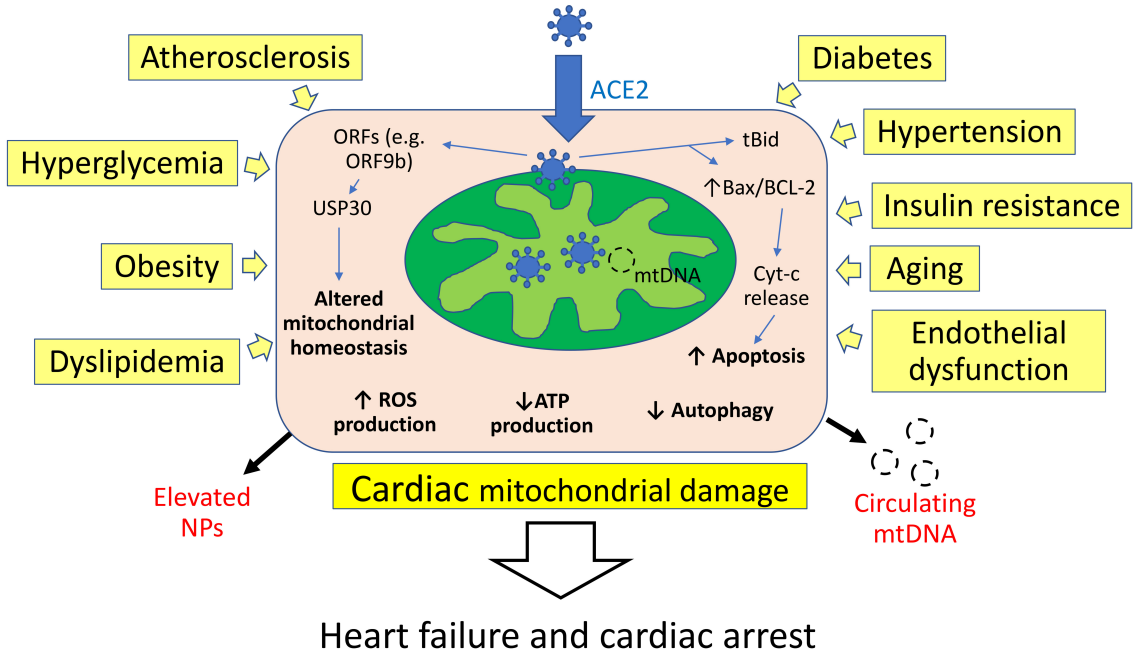


FIGURE 1 | Interplay of mechanisms of cardiac mitochondrial damage in COVID-19. Viral RNA and protein localize to mitochondria and manipulate their function by angiotensin-converting enzyme 2 (ACE2) regulation and open-reading frames (ORFs) to evade host cell immunity and facilitate virus replication. ORFs can target the mitochondrial deubiquitinase USP30, altering mitochondrial homeostasis and function. The virus promotes activation of truncated Bid (tBid) and alters Bax/BCL-2 ratio, favoring the release of apoptogenic factors. Concomitantly, several cardiovascular risk factors can compromise mitochondrial integrity and function by decreasing ATP levels and increasing reactive oxygen species (ROS) production. This in turn leads to cardiac mitochondrial damage and heart failure, associated with elevation of natriuretic peptides (NPs) and circulating mitochondrial DNA (mtDNA).

Importantly, pre-existent mitochondrial damage might exacerbate cardiomyocyte injury and dysfunction. It seems pertinent that all of the clinical risk factors (atherosclerosis, age, obesity, hypertension, and other conditions such as endothelial dysfunction) share impaired mitochondrial respiration, or a decrease in its ability to produce ATP. Hypertension, one of the main risk factors for SARS-COV and SARS-COV-19, is "prominently associated with the loss of cardiolipin" (12), a phospholipid uniquely found in the inner mitochondrial membrane and necessary for its proper formation and function. Furthermore, cardiolipin regulates mitochondrial dynamics and prevents the formation and opening of the mitochondrial permeability transition pore (mPTP), and release of cytochrome C to the cytosol. Hypertension is part of a process whereby a hole is opened in the "armored nuclear power plant" of the mitochondria that can be pierced and destroyed by SARS-COV-19.

Similarly, insulin resistance in skeletal muscle, a major hallmark of type 2 diabetes and obesity, has been linked to decreased muscle mitochondria reproduction and dysfunction (13). Endothelial cells exposed to high glucose concentrations exhibit augmented mitochondrial superoxide generation, which damages lipids, proteins, and mtDNA, and contributes to cellular oxidative stress (14). The uncoupled mitochondrial state is necessary for ATP synthesis. Excessive mitochondrial coupling is a central expression of "dysfunction in obesity that may contribute to the development of metabolic pathologies such as insulin resistance and diabetes" (15). Cardiac mitochondria also deteriorate with age, losing respiratory activity, accumulating damage to their DNA (mtDNA), and producing excessive amounts of reactive oxygen species (ROS), ultimately increasing susceptibility to infections (16). Therefore, results from these studies are consistent with established mitochondrial injury that may aggravate cardiac damage and accelerate COVID-19-related mortality rates in patients with cardiovascular risk factors.

Recent evidence suggests that cardiac troponin I (cTnI), an important structural protein implicated in contraction and relaxation of cardiomyocytes, is a critical biomarker of myocardial injury in COVID-19 and is directly related to survival (17). Interestingly, mitochondrial structure and function are significantly impaired in cardiomyocytes with mutated cTnI, suggesting an important role of this protein in maintaining the structural and functional integrity of myocardial mitochondria (18). Thus, monitoring cTnI may be useful to assess cardiac mitochondrial damage and disease progression in patients with COVID-19.

Beyond mediating damage of infected cardiac cells, mitochondria are emerging as critical components of the innate

immune response. It has been shown that the ATP needed for purinergic signaling (e.g., adenosine and ATP), T-cell regulation, and initial activation of neutrophils comes from mitochondria. ATP production and mitochondrial Ca^{2+} buffering are needed for antigen presentation and processing, and ROS are a part of the signaling pathway that activates inflammatory proteins (19). As mediators of immunity, mitochondria are consequently targeted by several viruses, including the SARS-CoV-2 virus. As noted, Orf-96 localizes on the mitochondrial membrane and suppresses type 1 interferon responses (20). Immune cells (and all cells) cannot function without their multiple healthy mitochondria. With aging, immune T cells don't respond as well to pathogens or vaccines as T cells' mitochondria begin to malfunction. This is reflected in the age related cognitive, cardiovascular, physical, metabolic, etc. changes, experienced and observed. Nevertheless, poor T cell response might not only be the result of aging but may be part of the cause of aging by releasing excessive inflammatory cytokines (the cytokine storm). When T cell mitochondria had been genetically modified (TFAM deficiency) to be energy production inefficient, it forced T cells from ATP into a less efficient mode of energy production. These mice rapidly aged with deterioration in their functions noted previously. "T cell metabolic failure induces the accumulation of circulating cytokines, characteristic of aging ('inflammaging'). This cytokine storm itself acts as a systemic inducer of senescence" (21).

A major immune defense against viral infection necessary for cellular viability, is autophagy. It delivers viral proteins and viruses to lysosomes for degradation. However, lysosomes are impaired by the loss of mitochondrial function (22) such as in SARS-COV, and COV-19 related impairments. "Inflammaging" and decreased autophagy accelerate the metabolic compromised state of people with known risk factors. It is no surprise that our young are more resilient since they usually generate sufficient ATP. However, when elevated blood mtDNA is found even in seemingly younger healthier patients and others, it reflects severe complications that can lead to ICU care and even death (11). All of the 97 adult subjects had COVID-19, but those that died had higher cell free plasma levels of mtDNA and fragments derived from mitochondrial encoded gene cytochrome B (MT-CYTB). MT-CYTB levels were highly correlated with plasma SC5b-9, which is "a marker of complement activation and suggests the formation of a membrane attack complex" (11).

Many questions remain unanswered including, is the appearance of mtDNA just part of an "over exuberant innate immune response?" (11). Would viral infection trigger cellular necrosis if their mitochondria remained more intact? Does viral induced mitochondrial dysfunction underlie the myocardial injury observed? Does the appearance of plasma mtDNA and MT-CYTB fragments portend a possible cascade of negative immunologic responses? Are the extreme elevations in NPs observed in part a hormonal response to protect and stabilize failing cardiac mitochondrial respiration and/or an expression thereof? Given the importance of mitochondria in kidney function, could their failure be expressed as excess deaths from end-stage kidney failure early in the pandemic? (23). Could finding ways to protect cardiac mitochondrial function open a whole new field of prevention or even treatment?

Studies have demonstrated that normalizing tubular cell mitochondrial function and energy balance could be a preventative strategy in kidney disease (24). Moreover, targeting the regulation of mitochondrial biogenesis and/or correcting abnormal electron chain function, can improve renal disease outcome. Could acute IV infusion of beta-hydroxybutyrate, the body's primary ketone body, improve cardiac mitochondrial respiration? This is supported by studies in healthy, and heart failure patients showing improved hemodynamic and cardiac output (25). Undoubtedly, additional studies are needed to establish the exact role of cardiac mitochondrial damage in the setting of COVID-19 and heart failure.

AUTHOR CONTRIBUTIONS

RR and AE conceived the manuscript, revised the drafts, and approved the submitted version. Both authors contributed to the article and approved the submitted version.

FUNDING

Partly supported by the NIH grant DK129240.

REFERENCES

1. Lindner D, Fitzek A, Brauninger H, Aleshcheva G, Edler C, Meissner K, et al. Association of cardiac infection with SARS-CoV-2 in confirmed COVID-19 autopsy cases. *JAMA Cardiol.* (2020) 5:1281–5. doi: 10.1001/jamacardio.2020.3551
2. Ferrer-Gomez A, Pian-Arias H, Carretero-Barrio I, Navarro-Cantero A, Pestana D, de Pablo R, et al. Late cardiac pathology in severe covid-19. A postmortem series of 30 patients. *Front Cardiovasc Med.* (2021) 8:748396. doi: 10.3389/fcvm.2021.748396
3. Mehra MR, Ruschitzka F. COVID-19 illness and heart failure: a missing link? *JACC Heart Fail.* (2020) 8:512–4. doi: 10.1016/j.jchf.2020.03.004
4. Domondon M, Nikiforova AB, DeLeon-Pennell KY, Ilatovskaya DV. Regulation of mitochondria function by natriuretic peptides. *Am J Physiol Renal Physiol.* (2019) 317:F1164–8. doi: 10.1152/ajprenal.00384.2019
5. Marin-Garcia J, Goldenthal MJ. Heart mitochondria signaling pathways: appraisal of an emerging field. *J Mol Med.* (2004) 82:565–78. doi: 10.1007/s00109-004-0567-7
6. Wu KE, Fazal FM, Parker KR, Zou J, Chang HY. RNA-GPS predicts SARS-CoV-2 RNA residency to host mitochondria and nucleolus. *Cell Syst.* (2020) 11:102–8.e3. doi: 10.1016/j.cels.2020.06.008
7. Singh KK, Chaubey G, Chen JY, Suravajhala P. Decoding SARS-CoV-2 hijacking of host mitochondria in COVID-19 pathogenesis. *Am J Physiol Cell Physiol.* (2020) 319:C258–67. doi: 10.1152/ajpcell.00224.2020
8. Lane RK, Hilsabeck T, Rea SL. The role of mitochondrial dysfunction in age-related diseases. *Biochim Biophys Acta.* (2015) 1847:1387–400. doi: 10.1016/j.bbabio.2015.05.021
9. Pasquier C, Robichon A. Computational search of hybrid human/SARS-CoV-2 dsRNA reveals unique viral sequences that diverge from those of other coronavirus strains. *Heliyon.* (2021) 7:e07284. doi: 10.1016/j.heliyon.2021.e07284

10. Padhan K, Minakshi R, Towheed MAB, Jameel S. Severe acute respiratory syndrome coronavirus 3a protein activates the mitochondrial death pathway through p38 MAP kinase activation. *J Gen Virol.* (2008) 89:1960–9. doi: 10.1099/vir.0.83665-0
11. Scozzi D, Cano M, Ma L, Zhou D, Zhu JH, O'Halloran JA, et al. Circulating mitochondrial DNA is an early indicator of severe illness and mortality from COVID-19. *JCI Insight.* (2021) 6:e143299. doi: 10.1172/jci.insight.143299
12. Eirin A, Lerman A, Lerman LO. Mitochondrial injury and dysfunction in hypertension-induced cardiac damage. *Eur Heart J.* (2014) 35:3258–66. doi: 10.1093/eurheartj/ehu436
13. Hojlund K, Mogensen M, Sahlin K, Beck-Nielsen H. Mitochondrial dysfunction in type 2 diabetes and obesity. *Endocrinol Metab Clin North Am.* (2008) 37:713–31, x. doi: 10.1016/j.ecl.2008.06.006
14. Wautier MP, Chappey O, Corda S, Stern DM, Schmidt AM, Wautier JL. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. *Am J Physiol Endocrinol Metab.* (2001) 280:E685–94. doi: 10.1152/ajpendo.2001.280.5.E685
15. Arruda AP, Pers BM, Parlakgul G, Guney E, Inouye K, Hotamisligil GS. Chronic enrichment of hepatic endoplasmic reticulum-mitochondria contact leads to mitochondrial dysfunction in obesity. *Nat Med.* (2014) 20:1427–35. doi: 10.1038/nm.3735
16. Wang Y, Li Y, He C, Gou B, Song M. Mitochondrial regulation of cardiac aging. *Biochim Biophys Acta Mol Basis Dis.* (2019) 1865:1853–64. doi: 10.1016/j.bbadis.2018.12.008
17. Al Abbasi B, Torres P, Ramos-Tuarez F, Dewaswala N, Abdallah A, Chen K, et al. Cardiac troponin-I and COVID-19: a prognostic tool for in-hospital mortality. *Cardiol Res.* (2020) 11:398–404. doi: 10.14740/cr1159
18. Luo J, Zhao W, Gan Y, Pan B, Liu L, Liu Z, et al. Cardiac troponin I R193H mutation is associated with mitochondrial damage in cardiomyocytes. *DNA Cell Biol.* (2021) 40:184–91. doi: 10.1089/dna.2020.5828
19. Angajala A, Lim S, Phillips JB, Kim JH, Yates C, You Z, et al. Diverse roles of mitochondria in immune responses: novel insights into immuno-metabolism. *Front Immunol.* (2018) 9:1605. doi: 10.3389/fimmu.2018.01605
20. Jiang HW, Zhang HN, Meng QF, Xie J, Li Y, Chen H, et al. SARS-CoV-2 Orf9b suppresses type I interferon responses by targeting TOM70. *Cell Mol Immunol.* (2020) 17:998–1000. doi: 10.1038/s41423-020-0514-8
21. Desdin-Mico G, Soto-Heredero G, Aranda JF, Oller J, Carrasco E, Gabande-Rodriguez E, et al. T cells with dysfunctional mitochondria induce multimorbidity and premature senescence. *Science.* (2020) 368:1371–6. doi: 10.1126/science.aax0860
22. Baixauli F, Acin-Perez R, Villarroya-Beltri C, Mazzeo C, Nunez-Andrade N, Gabande-Rodriguez E, et al. Mitochondrial respiration controls lysosomal function during inflammatory T cell responses. *Cell Metab.* (2015) 22:485–98. doi: 10.1016/j.cmet.2015.07.020
23. Kuehn BM. Excess deaths from end-stage kidney disease early in pandemic. *JAMA.* (2021) 326:300. doi: 10.1001/jama.2021.11312
24. Eirin A, Lerman A, Lerman LO. Mitochondria: a pathogenic paradigm in hypertensive renal disease. *Hypertension.* (2015) 65:264–70. doi: 10.1161/HYPERTENSIONAHA.114.04598
25. Stubbs BJ, Koutnik AP, Volek JS, Newman JC. From bedside to battlefield: intersection of ketone body mechanisms in geroscience with military resilience. *Geroscience.* (2021) 43:1071–81. doi: 10.1007/s11357-020-00277-y

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

SYSTEMATIC REVIEW
published: 21 January 2022
doi: 10.3389/fcvm.2021.795750

The Prevalence and Associated Death of Ventricular Arrhythmia and Sudden Cardiac Death in Hospitalized Patients With COVID-19: A Systematic Review and Meta-Analysis

Ziqi Tan[1†], Shan Huang[2†], Kaibo Mei[3], Menglu Liu[4], Jianyong Ma[5], Yuan Jiang[6], Wengen Zhu[7‡], Peng Yu[1‡] and Xiao Liu[8*‡]*

[1] Department of Endocrine, The Second Affiliated Hospital of Nanchang University, Nanchang, China, [2] Department of Psychiatry, The Third People's Hospital of Ganzhou, Ganzhou, China, [3] Department of Anesthesiology, The People's Hospital of Shangrao, Shangrao, China, [4] Department of Cardiology, The Seventh People's Hospital of Zhengzhou, Zhengzhou, China, [5] Department of Pharmacology and Systems Physiology, University of Cincinnati College of Medicine, Cincinnati, OH, United States, [6] Department of Pharmacy, Harbin Medical University, Harbin, China, [7] Department of Cardiology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, [8] Department of Cardiology, The Second Affiliated Hospital of Nanchang University, Nanchang, China

Background: Arrhythmia is a very common complication of coronavirus disease 2019 (COVID-19); however, the prevalence of ventricular arrhythmia and associated outcomes are not well-explored. Here, we conducted a systematic review and meta-analysis to determine the prevalence and associated death of ventricular arrhythmia and sudden cardiac death (SCD) in patients with COVID-19.

Methods: Databases of PubMed, Cochrane Library, Embase, and MdeRxiv were searched. Studies that could calculate the prevalence of ventricular arrhythmia/SCD during hospital admission or associated death in patients with COVID-19 were included. The study was registered with the PROSPERO (CRD42021271328).

Results: A total of 21 studies with 13,790 patients were included. The pooled prevalence of ventricular arrhythmia was 5% (95% CI: 4–6%), with a relatively high-SCD prevalence (1.8% in hospitalized COVID-19 and 10% in deceased cases of COVID-19). Subgroup analysis showed that ventricular arrhythmia was more common in patients with elevated cardiac troponin T [ES (effect size): 10%, 95% CI: −0.2 to 22%] and in European (ES: 20%, 95% CI: 11–29%) populations. Besides, ventricular arrhythmia was independently associated with an increased risk of death in patients with COVID-19 [odds ratio (OR) = 2.83; 95% CI: 1.78–4.51].

Conclusion: Ventricular arrhythmia and SCD resulted as a common occurrence with a high prevalence in patients with COVID-19 admitted to the hospital. Furthermore,

OPEN ACCESS

Edited by:
Hendrik Tevaearai Stahel, Bern University Hospital, Switzerland

Reviewed by:
Nicola Maurea, G. Pascale National Cancer Institute Foundation (IRCCS), Italy
Jose Francisco Islas, Autonomous University of Nuevo León, Mexico

****Correspondence:***
Peng Yu
yupeng_jxndefy@163.com
Xiao Liu
liux587@mail.sysu.edu.cn

†These authors share first authorship
‡These authors share senior authorship

Specialty section:
This article was submitted to Cardiac Rhythmology, a section of the journal Frontiers in Cardiovascular Medicine

Received: *15 October 2021*
Accepted: *07 December 2021*
Published: *21 January 2022*

Citation:
Tan Z, Huang S, Mei K, Liu M, Ma J, Jiang Y, Zhu W, Yu P and Liu X (2022) The Prevalence and Associated Death of Ventricular Arrhythmia and Sudden Cardiac Death in Hospitalized Patients With COVID-19: A Systematic Review and Meta-Analysis. Front. Cardiovasc. Med. 8:795750. doi: 10.3389/fcvm.2021.795750

ventricular arrhythmia significantly contributed to an increased risk of death in hospitalized patients with COVID-19. Clinicians might be vigilant of ventricular arrhythmias for patients with COVID-19, especially for severe cases.

Systematic Review Registration: www.york.ac.uk/inst/crd, identifier: CRD42021271328.

Keywords: arrhythmia, ventricular arrhythmia, coronavirus disease 2019, prevalence, death, prognosis

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a serious life-threatening disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which first occurred in November 2019 (1), and then rapidly spread throughout the rest of the world. As of July 31, 2021, more than 198 million individuals were diagnosed with cases of COVID-19, exceeding 420 thousand deaths. Although COVID-19 is characterized by substantial respiratory pathology, several extrapulmonary manifestations, such as thrombotic complications, myocardial dysfunction, and arrhythmia, acute kidney injury are also commonly found in patients afflicted with the virus (2–4).

Cardiac arrhythmias, such as new-onset atrial fibrillation, heart block, and ventricular arrhythmias, are prevalent in the patients with COVID-19. An early report study on 138 patients from Wuhan, China, showed that 17% of the hospitalized patients suffered from total arrhythmia (5). Our recent meta-analysis also showed that the atrial fibrillation reached 10% and was associated with increased death in COVID-19 (6). Ventricular arrhythmia is still the major leading cause of death from the cardiovascular diseases (7). According to a multicenter cohort from the US, 6% of 4,250 patients with COVID-19 had prolonged QTc interval (corrected QT; >500 ms) at admission (8). This result suggested that COVID-19 might significantly contribute to an increased risk of ventricular tachycardia as QTc prolongation is believed to predispose to the ventricular arrhythmias associated with sudden death in certain cardiac diseases. Although several studies have reported the increased risk of ventricular tachycardia among patients with COVID-19, the exact prevalence of ventricular tachycardia in patients with COVID-19 remains unknown. Moreover, a case series also reported ventricular tachycardia and ventricular fibrillation as the primary cause of death in hospitalized patients with COVID-19 without a prior history of the structural heart disease (9). However, it remains unclear whether COVID-19 associated the ventricular arrhythmias are independently linked to increased death in the patients with COVID-19.

Furthermore, sudden cardiac death (SCD) is the most devastating manifestation of ventricular arrhythmias that has emerged as one of the disturbing concerns associated with the infection of COVID-19. Thus, we conducted a systematic review and meta-analysis to determine the prevalence and associated death of ventricular arrhythmia and SCD in patients with COVID-19.

METHODS

This study has been registered with PROSPERO (International prospective register of systematic reviews. www.york.ac.uk/inst/crd)-registration number-CRD42021271328. Furthermore, we conducted the meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (2020) (**Supplementary Table 1**).

Literature Search

The search was accomplished by two authors independently. PubMed, Embase, the Cochrane Library, and MedRxiv (https://www.medrxiv.org/) databases were searched mainly for the related studies up to July 21, 2021, without language restrictions. The search terms according to PICOS were as follows:

Population:

For COVID-19: "COVID-19" or "COVID-19 Virus Disease" or "COVID-19 Virus Infection" or "2019-nCoV Infection" or "Coronavirus Disease-19" or "2019 Novel Coronavirus Disease" or "2019 Novel Coronavirus Infection" or "2019-nCoV Disease" or "Coronavirus Disease 2019" or "SARS Coronavirus 2 Infection" or "SARS-CoV-2 Infection" or "COVID-19 Pandemic."

Exposure:

For the ventricular arrhythmia: "ventricular arrhythmia" or "premature ventricular beats" or "ventricular ectopic beats" or "ventricular premature complex" or "premature ventricular contractions" or "ventricular tachycardia" or "ventricular tachyarrhythmia" or "ventricular flutter" or "ventricular fibrillation."

For sudden cardiac death: "cardiac sudden death" or "sudden cardiac arrest" or "sudden cardiac death."

Outcomes:

For death: "death" or "mortality."

A detailed search strategy was described in **Supplementary Table 2**.

Study Selection

All the results were organized by EndNote X9 software (Thomson Reuters, New York, NY, USA). After deleting the duplicate literature, the titles and abstracts were checked, and the relevant literature was preliminarily screened. Subsequently, full-texts of the relevant studies were searched to make sure they

met the inclusion criteria. The inclusion criteria included the following: (1) studies that included adult patients diagnosed with COVID-19 based on the polymerase chain reaction tests; (2) studies that could calculate the prevalence of ventricular arrhythmia/SCD during hospital admission or reported the estimated effect between ventricular arrhythmia and death in patients with COVID-19.

If the same population was used in multiple studies, we selected the article with the most informative or the largest sample size. Studies that reported the effect of chloroquine/hydroxychloroquine and azithromycin in patients with COVID-19 were excluded because of the potential drug-induced ventricular arrhythmias risk. Certain publication types without sufficient data (reviews, meta-analysis, cases, editorials, and comments) were also excluded.

Data Collection

The following information was independently abstracted by two researchers: the first author, publication year, country, time, study design, patient characteristics (sample size, age, and sex), number of ventricular arrhythmias, death, odds ratios (ORs), and the corresponding 95% CI and adjustments.

Quality Assessment

For studies that reported the prevalence, the Joanna Briggs Institute (JBI) critical appraisal checklist was used to assess the study quality, where 0 score represented a failure to meet the requirements; 1 represented the lack of detailed description, 2 score represented detailed and comprehensive description. For studies that reported the association between ventricular arrhythmia and death in patients with COVID-19, the Newcastle-Ottawa Scale (NOS) was applied. Studies with scores of NOS ≥ 7 and JBI ≥ 14 were considered as high-quality researches (10).

Statistical Analysis

RevMan software, version 5.3 (The Cochrane Collaboration 2014, Nordic Cochrane Center Copenhagen, Denmark) and Stata software (Version 14.0, Stata Corp LP, College Station, Texas, US) were both applied in our analysis. To explore the prevalence of ventricular arrhythmia and SCD in hospitalized patients with COVID-19, the exact binomial (Clopper–Pearson) method was used to calculate 95% CIs. Freeman–Tukey double arcsine transformation was used for standard estimates. To elucidate the outcome of ventricular arrhythmia and SCD in hospitalized patients with COVID-19, we pooled the ORs for each studies using the inverse variance method. We also estimated the adjusted ORs by calculating the natural logarithm of the OR (log [OR]) and its standard error (SElog [OR]), which is shown with 95% CIs. We evaluated the degree of heterogeneity using the I^2 test (25, 50, and 75% represent low, moderate, and high heterogeneity). We used the random effect model in our study to improve the reliability.

Subgroup analyses were performed to study possible factors influencing our results, including ventricular arrhythmia, region, cardiac injury, and population. To ensure the reliability of study outcomes, we carried out sensitivity analyses by omitting each study in turn. $P < 0.05$ was considered statistically significant.

RESULTS

Study Selection

The flow chart for the study selection process is shown in **Figure 1**. A total of 1,342 publications were identified following initial search (PubMed = 430; the Cochrane Library = 119; Embase = 405; MedRxiv = 388). After deleting 584 duplications and 680 irrelevant studies, the full-text assessment was performed on 78 studies. Subsequently, 57 articles were excluded due to the following reasons: (1) studies without insufficient data ($n = 19$); (2) certain publication types with no data (review = 11; case report = 3); (3) studies without appropriate population or exposure ($n = 11$); (4) studies that did not report target outcome ($n = 13$). Finally, 21 studies were included in the meta-analysis (11–31). All the excluded studies with reasons ($n = 57$) are shown in **Supplementary Table 3**.

Study Characteristics and Quality

The basic characteristics of all the included articles are shown in **Table 1**. Twenty-one studies (11–31), which included 13,790 hospitalized patients with COVID-19 with a mean age ranging from 50 to 70.3 years, and with sample sizes ranging from 54 to 4,526, were published from 2020 to 2021 [eight of them published in 2021 (11, 15, 16, 19, 24–26, 31), others in 2020 (12–14, 17, 18, 20–23, 27–30)]. Nine reports were from Asia [eight from China (13, 16, 18, 21, 22, 28, 29, 31), one from Iran (19)], seven from USA (11, 12, 14, 15, 17, 25, 30), and five from Europe [three from Italy (20, 24, 27), one from Netherland (23), one from Germany (26)]. Besides, 15 of them were retrospective studies (11, 13, 15–19, 21–23, 27–31), 5 were prospective studies (12, 14, 20, 25, 26), and 1 was cross-section study (24).

All studies (11–31) scored between 16 and 20 on the Joanna Briggs Institute Critical Appraisal Checklist, which meant these articles took rigorous methodology. In addition, five studies (16, 19, 21, 25, 27) involved the association between ventricular arrhythmia and death in patients with COVID-19, with NOS scores ≥ 7, thus were regarded as moderate high-quality studies (**Supplementary Table 4**).

The Prevalence of Ventricular Arrhythmia and SCD in Patients With COVID-19

A total of 20 (11–30) studies with 13,509 patients reported the prevalence of ventricular arrhythmia in patients with COVID-19. As shown in **Figure 2**, the pooled prevalence of the ventricular arrhythmia was 5% (95% CI: 4–6%).

Subgroup analysis showed that premature ventricular complex was the most common type in patients with COVID-19 (ES: 13%; 95% CI: 7–19%), followed by ventricular tachycardia (ES: 10%; 95% CI: 6–13%) and ventricular fibrillation (ES: 1%; 95% CI: 1–2%) (**Figure 3A**). According to area subgroup analysis, Europe had the highest prevalence (ES: 20%; 95% CI: 11–29%), followed by the United States (ES: 7%; 95% CI: 1–13%), while the lowest prevalence was found in Asia (ES: 6%; 95% CI: 3–8%) (**Figure 3B**). Furthermore, the prevalence of the ventricular arrhythmia in hospitalized patients with elevated cardiac troponin T was 1.25-fold higher than that

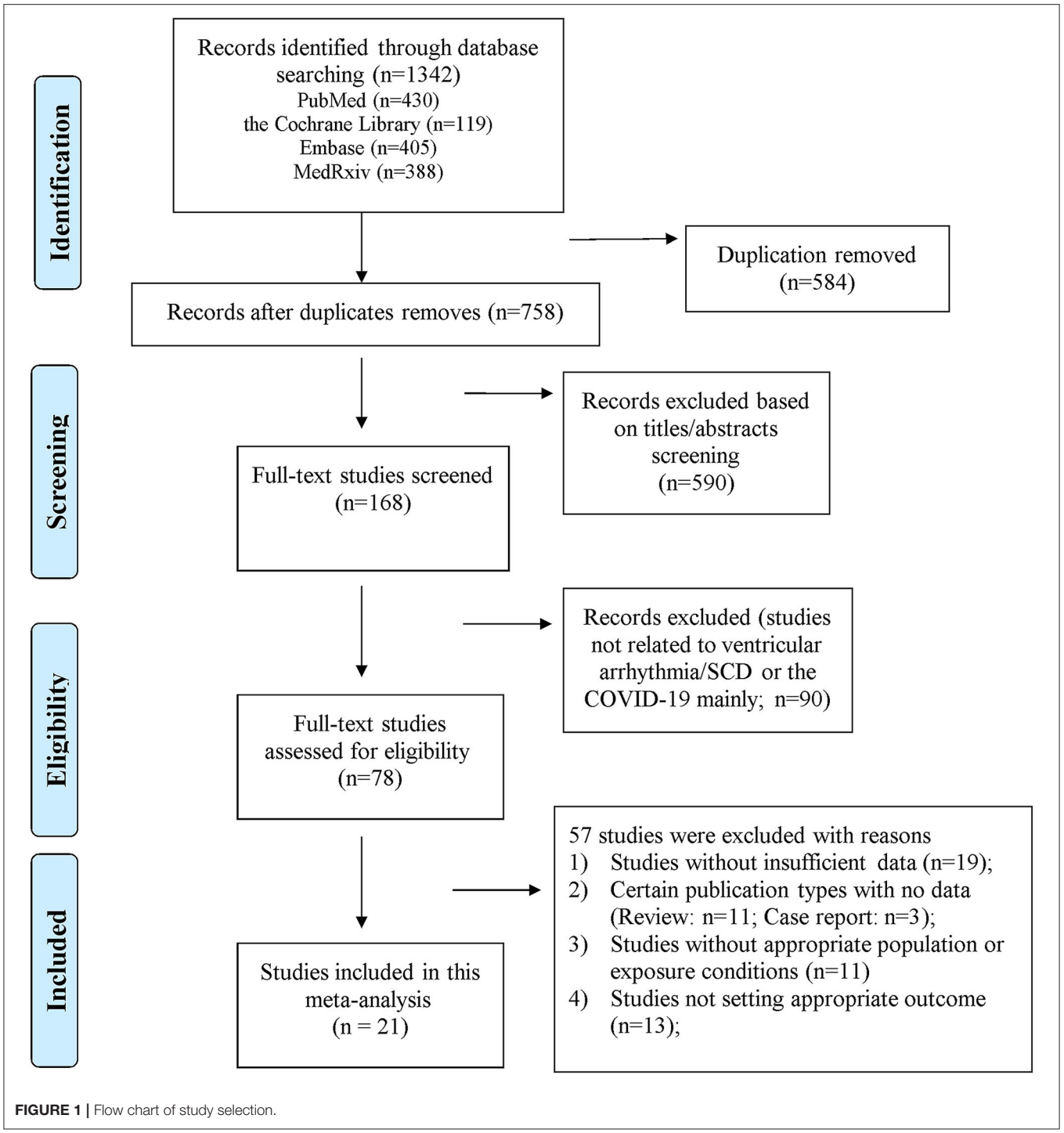


FIGURE 1 | Flow chart of study selection.

without elevated cardiac troponin T (ES: 10 vs. 8%) (**Figure 3C**). The prevalence of ventricular arrhythmia in living and deceased hospitalized patients with COVID-19 was 6 and 12%, respectively (**Figure 3D**).

Two articles reported the prevalence of SCD in hospitalized patients with COVID-19 (28, 31). Article of Shao reported that the prevalence of SCD was 1.8% in the population with COVID-19 (28). Also, according to Yang et al. there was a higher SCD prevalence (10%) in the deceased population with COVID-19 (31).

The Impact of Ventricular Arrhythmia on All-Cause Death With COVID-19

Five multivariable-adjusted publications with 2,568 patients were included in the analysis (16, 19, 21, 25, 27). The results showed a positive association between ventricular arrhythmia and risk

TABLE 1 | Basic characteristics of the articles included in the meta-analysis.

Author, year, country	Study design	Diagnosis	Study populations	Total number	Male, age years	History of cardiovascular disease %	Medication %	Prevalence and endpoint reported	Cases/ Total	Adjusted effector (95% CI) and adjustments
Antwi-Amoabeng et al. (11), USA	RC	ECG	Tertiary Care Hospital	186	53.2%, 60.0	CAD 3.2%; diabetes 37.1%; HF 9.7%; stroke 8.6%; hypertension 43.1%;	QT prolonging medications 57.5%	Premature ventricular complex	10/186	NA
Chen et al. (13), China	RC	ECG	NA	54	66.7%, 57.6	CAD 11.1%; diabetes 46.3%; hypertension 29.6%	NA	Ventricular tachycardia	3/54	NA
Cho et al. (14), USA	PC	Telemetry monitoring	Cedars-Sinai Medical Center	143	61.5%, 70.3	Diabetes 35.0%; CAD 18.9%; hypertension 55.2%; hyperlipidemia 41.3%; AF 12.6%	Azithromycin 59.4%; Tocilizumab 39.2%; Remdesivir 9.1%; HCQ 62.9%; Lopinavir/ritonavir 2.1%;	Premature ventricular complex Ventricular tachycardia Ventricular fibrillation SCD	143/41 24/143 1/143 1/143	NA
Coromilas et al. (15), USA	Case-control	ECG	Across the world for whom data was available	4,526	57.3%, 62.8	Diabetes 34.7%; CHF 16.9%; CAD 13.2%; AF/AFL 9.0%; VT 0.6%; stroke 6.1%; Vascular disease 3.7%; Hypertension 55%;	Azithromycin 49.8%; Antiviral 15.3%; IL-6 inhibitor 9.6%; Anticoagulation 29.4% HCQ 57.6%	Ventricular tachycardia Cardiac damage with ventricular arrhythmia	27/4,526 164/827	NA
Gao et al. (16), China	RC	ECG	Tongji Hospital	79	67.1%, 65.0	Diabetes 22.0%; CHF 3.0%; Cardiovascular disease 14.0%; hypertension 51.0%; stroke 13.0%	Glucocorticoid 77.0%; Antibiotics 67.0%; Anticoagulation 54.0%; Antiviral therapy 71.0%; Intravenous immunoglobulin 75.0%; Beta-blocker 16.0%; Tocilizumab 9.0%	Ventricular tachycardia Death	9/79	3.302 (1.524, 7.154) Clinical characteristics, comorbidities, laboratory indexes or therapies

(Continued)

TABLE 1 | Continued

Author, year, country	Study design	Diagnosis	Study populations	Total number	Male, age years	History of cardiovascular disease %	Medication %	Prevalence and endpoint reported	Cases/ Total	Adjusted effector (95% CI) and adjustments
Haji Aghajani et al. (19), Iran	RC	ECG	Imam-Hossein Hospital	893	55.3%, 61.8	NA	NA	Ventricular arrhythmia Death	28/893	1.854 (1.154, 2.979) Male sex, increase in age, sinus tachycardia, supraventricular arrhythmia, interventricular conduction delay, abnormal R wave progression, abnormal T wave
Lanza et al. (20), Italy	PC	ECG	Universita' Cattolica del Sacro Cuore Hospital	324	66.1%, 65.9	Known heart disease 20.7%; Hypertension 52.2%; diabetes 11.4%	NA	Premature ventricular complex	13/324	NA
Li et al. (21), China	Case-control	ECG	Renmin Hospital of Wuhan University	113	60.2%, 67.3	Hypertension 43.4%; Cardiovascular disease 20.4%; diabetes 18.6%;	Ribavirin 49.6%; Arborol 71.7%; Lopinavir/ritonavir 3.5%; HCQ 15.0%; Interferon α-2b injection 18.6%; Ganciclovir 17.7%; Oseltamivir 30.1%; Glucocorticoid 62.0%; Immunoglobulin 64.6%	Ventricular arrhythmia Premature ventricular complex Ventricular tachycardia Death	8/70 7/70 1/70	2.79 (1.11, 7.04) Age, initial neutrophil count, lactate dehydrogenase, C-reactive protein, immunoglobulin treatment, sinus tachycardia
Li et al. (22), China	Case-control	ICD	Wuhan Seventh People's Hospital	596	47.0%, 58.0	Diabetes 13.3%	Antivirus therapy 78.4%; Antibiotic therapy 74.8%; Glucocorticoid 29.5%; Immunoglobin 9.2%	Ventricular arrhythmia	12/596	NA

(Continued)

TABLE 1 | Continued

Author, year, country	Study design	Diagnosis	Study populations	Total number	Male, age years	History of cardiovascular disease %	Medication %	Prevalence and endpoint reported	Cases/ Total	Adjusted effector (95% CI) and adjustments
Linschoten et al. (23), Netherland	Case-control	ECG	CAPACITY-COVID (www.capacity-covid.eu)	3011	62.8%, 67.0	HF 5.3%; diabetes 23.1%; Hypertension 44.6%; Arrhythmia/conduction disorder 15.1%; CAD 5.3%; Valvular disease 4.3%	NA	Ventricular arrhythmia Cardia damage with ventricular arrhythmia	14/3011 14/349	NA
Malanchini et al. (24), Italy	Cross-sectional	Remote monitoring	Electrophysiology and Cardiac Pacing Unit at ASST Papa Giovanni XXIII Hospital	455	75.8%, 64.9	NA	Beta-blocker 85.9%; Amiodarone 34.2%; Mexiletine 3.5%	Ventricular arrhythmia Ventricular tachycardia Ventricular fibrillation	86/455 77/455 9/455	NA
Pareek et al. (25), USA	PC	ECG	Yale New Haven Hospital	586	47.4%, 67.0	Diabetes 38.5%; CAD 15.7%; Hypertension 58.1%; Cerebrovascular disease 9.3%; HF/cardiomyopathy 14.3%; AF/AFL10.1%; PAD 3.0%; Ventricular arrhythmia 1.4%	Beta blocker 27.4%; ACE inhibitor/ARB 31.9%; Aspirin 29.0%; Anticoagulant 11.1%; Antiarrhythmic 2.6%; CCB 24.0%;	Ventricular arrhythmia SCD Death	12/586 21/586	18.97 (3.68, 97.88) Age, sex, history of heart failure, history of ventricular arrhythmias, P2Y12 inhibitors, oxygen therapy at admission, and respiratory rates. CRP, albumin, and troponin T
Parwani et al. (26), Germany	PC	ECG	University Hospital Center at the Charité Berlin	113	73.5%, 64.1	CAD 18.6%; arrhythmias 15.9%; congestive HF 11.5%; hypertension 61.1%; AF/AFL 14.2%; ventricular tachycardia 0.9%	Beta blocker 27.4%; calcium antagonists 15.9%; ACEi/ARB/ARNI 34.5%; platelet inhibitor 23.9%;	Ventricular arrhythmia Premature ventricular complex Ventricular tachycardia Ventricular fibrillation	64/113 28/113 34/113 2/113	NA

(Continued)

TABLE 1 | Continued

Author, year, country	Study design	Diagnosis	Study populations	Total number	Male, age years	History of cardiovascular disease %	Medication %	Prevalence and endpoint reported	Cases/ Total	Adjusted effector (95% CI) and adjustments
Russo et al. (27), Italy	Case-control	ECG	Emergency Department of 10 Italian Hospitals	414	61.1%, 66.9	Diabetes 25.6%; AF 17.4%; HF 11.1%; stroke 8.4%; Hypertension 63.5%	ACEi/ARB 41.1%; beta-blocker 14.0%; Ca^{2+} antagonist 24.2%	Ventricular tachycardia Death	14/414	2.55 (1.5, 3.35) Male, age, hypertension, heart failure, chronic kidney disease, coronary artery disease
Shao et al. (28), China	Case-control	ECG	West Campus of Union Hospital in Wuhan	136	66.2%, 69.0	Hypertension 30.2%; diabetes 19.9%; Coronary heart disease 11.0%; cerebrovascular disease 3.7%	NA	Ventricular arrhythmia SCD	8/136 151/761	NA
Bhatla et al. (12), USA	PC	NA	Hospital of the University of Pennsylvania	700	45.0%; 50.0	Coronary heart disease 11.0%; hypertension 50.0%; HF 13.0%; diabetes 26.0% AF 6.0%	Hydroxychloroquine 25.0%; remdesivir 8.0%	Ventricular tachycardia SCD	10/700 9/700	NA
Gopinathannair et al. (17), USA	Case-control	NA	The Heart Rhythm Society (HRS) study	683	NA; NA	NA	Hydroxychloroquine/ chloroquine 33.5%; HCQ/chloroquine + azithromycin 31.0%	Ventricular tachycardia Premature ventricular complex Ventricular arrhythmia	93/683 60/683 33/683	NA
Guo et al. (18), China	Case-control	ECG	The Seventh Hospital of Wuhan City	187	48.7%; 58.5	Hypertension 32.6%; coronary heart disease 11.2%; cardiomyopathy 4.3%; diabetes 15.0%	Antivirus 88.8%; antibiotic 97.9%; glucocorticoid 56.7%; immune globulin 11.2%	Ventricular arrhythmia	11/187	NA
Si et al. (29), China	RC	ECG	Tongji Hospital in Wuhan	170	54.7%; 61.5	Hypertension 55.9%; diabetes 21.8%;stroke 3.5%	Antiviral 97.6%; antibiotic 95.9%; QT-prolonging medication 74.7%	Ventricular arrhythmia	1/170	NA

(Continued)

TABLE 1 | Continued

Author, year, country	Study design	Diagnosis	Study populations	Total number	Male, age years	History of cardiovascular disease %	Medication %	Prevalence and endpoint reported	Cases/ Total	Adjusted effector (95% CI) and adjustments
Turagam et al. (30), USA	RC	ECG/telemetry	Mount Sinai Hospital	140	72.9%; 61.0	Diabetes 39.0%; CAD 25.0%; Hypertension 61.0%; congestive HF 16.0%; Ventricular arrhythmias 1.0%; Atrial arrhythmia 14.0%;	Azithromycin 44.0%; remdesivir 1.0%; sorolumab 5.0%; tocilizumab 8.0%; glucocorticoid 5.0%; anticoagulation 18.0%; hydroxychloroquine 76.0%; antiarrhythmics 11.0%	Ventricular arrhythmia	7/140	NA
Yang et al. (31), China	RC	NA	Tongji Hospital	281	68.0%; 69.0	Hypertension 38.8%; diabetes 14.2%; CHD 11.4%	Antiviral 44.8%; antibiotic 96.8%; corticosteroid 89.7%; immune globulin 58.0%	SCD	28/281	NA

ECG, electrocardiogram; CAD, coronary artery disease; HF, heart failure; AF, atrial fibrillation; AFL, atrial flutter; CHF, congestive heart failure; SCD, sudden cardiac death; VT, ventricular tachycardia; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; NA, not application; AHA COVID-19 CVD, the American Heart Association COVID-19 cardiovascular disease; CCB, calcium channel blocker; CRP, C reactive protein; RC, retrospective cohort; PC, prospective cohort; COVID-19, coronavirus disease 19.

of death in hospitalized patients with COVID-19 (OR = 2.83; 95% CI: 1.78–4.51%; $I^2 = 50\%$) (**Figure 4**), revealing a moderate heterogeneity. These results were stable when excluding Pareek et al. (25) with no evidence of heterogeneity (OR = 2.35; 95% CI: 1.74–2.39%; $I^2 = 0\%$). After deleting each study in turn, sensitivity analyses indicated that our results were stable, with a range from 2.35 (95% CI: 1.74–3.19%) to 3.41 (95% CI: 1.94–6.00%) (**Supplementary Figure 1**).

Publication Bias

The potential publication bias for death was not performed due to the limited number of studies ($N < 10$).

DISCUSSION

In this study, we pooled data from 21 studies with 13,790 hospitalized patients with COVID-19, finding that: (i) the prevalence of ventricular arrhythmia in hospitalized patients with COVID-19 was 5%. Specifically, the premature ventricular complex, ventricular tachycardia, and ventricular fibrillation occurred in 13, 10, 1% in the hospitalized patients, respectively. (ii) Ventricular arrhythmia was independently related to an increased risk of death in hospitalized patients with COVID-19. Overall, the ventricular arrhythmia and SCD were not uncommon and were associated with adverse outcomes in the hospitalized patients. To the best of our knowledge, this is the first meta-analysis that reported the prevalence of ventricular arrhythmia, SCD, and associated prognosis in the hospitalized patients with COVID-19.

Cardiac arrhythmia was identified as one of the major complications of SARS-COV during the outbreak in China in 2003 (32). Tachycardia was the most common type of arrhythmia among the patients infected with SARS-COV and was independent of fever (33, 34). Similarly, in hospitalized patients with COVID, arrhythmic events are not uncommon among the COVID-19 related cardiovascular complications. As we previously reported, atrial fibrillation is the most prevalent arrhythmia in hospitalized patients (6). Regarding ventricular arrhythmia, evidence from earliest cohorts from Wuhan, China, showed that ventricular arrhythmia occurred in 7% of patients (13/187) and that the rate of ventricular arrhythmia was almost doubled in patients with elevated troponin T levels on ICU admission (18), which is consistent with our results (**Figure 3**). However, the exact pathophysiology underlying ventricular arrhythmia in COVID-19 may be multifactorial and remains elusive.

First, as we previously described, cardiac injury or myocarditis commonly occurs due to the inhibited activity of angiotensin-converting enzyme 2 (ACE2) by SARS-COV2, which was found in 19% of hospitalized patients with COVID-19, and has been estimated to double among those with pre-existing cardiovascular and non-cardiovascular diseases (e.g., diabetes, hypertension, and cancers). These comorbidities might make their cardiomyocytes more vulnerable to be attacked by SARS-COV2 and thus causing a higher incidence of cardiac injury (35, 36). This cardiac injury might contribute to the abnormalities in cardiac electrophysiology, eventually inducing ventricular

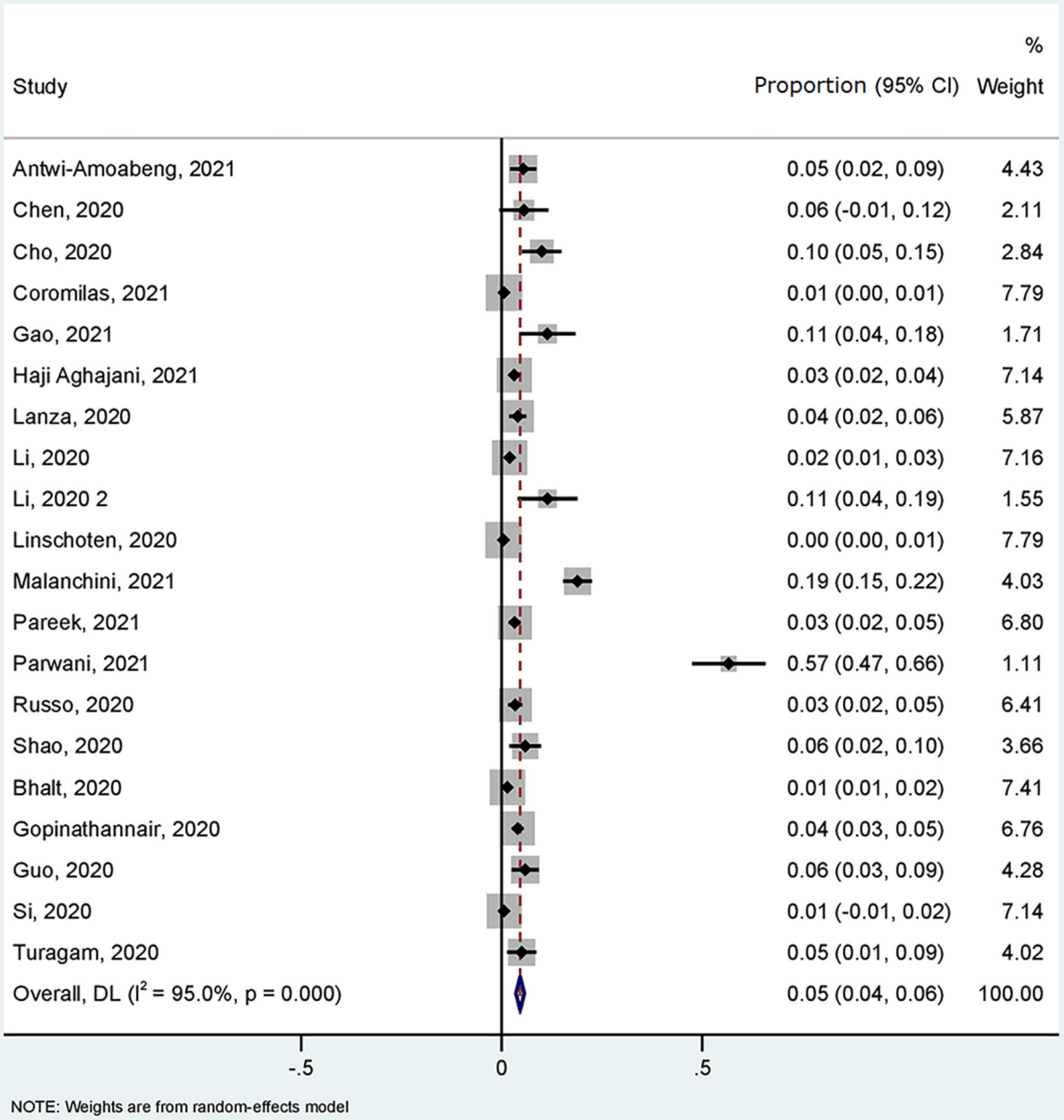


FIGURE 2 | Forest plot for the prevalence of the ventricular arrhythmia in patients with COVID-19.

arrhythmia. This supposition is consistent with our subgroup analysis, which showed that the incident rate of ventricular arrhythmia increased with cardiac injury. Secondly, it is well-known that COVID-19 is characterized by the dysregulated immune response and cytokine release syndrome. Numerous studies have shown an elevation of serum inflammatory markers, such as C-reactive protein, ferritin, erythrocyte sedimentation rate (37). It has also been identified as a predictor of severity and death in patients with COVID-19. On the other hand, various pro-inflammatory factors, such as C-reactive protein and tumor necrosis factor α, have also been shown to promote ventricular arrhythmia significantly (38). Third, thrombotic complications are the main extrapulmonary manifestations of COVID-19 (37). For example, autopsies performed at a single academic medical center revealed deep venous thrombosis in 7 out of 12 patients (58%) who were not suspected of venous thromboembolism

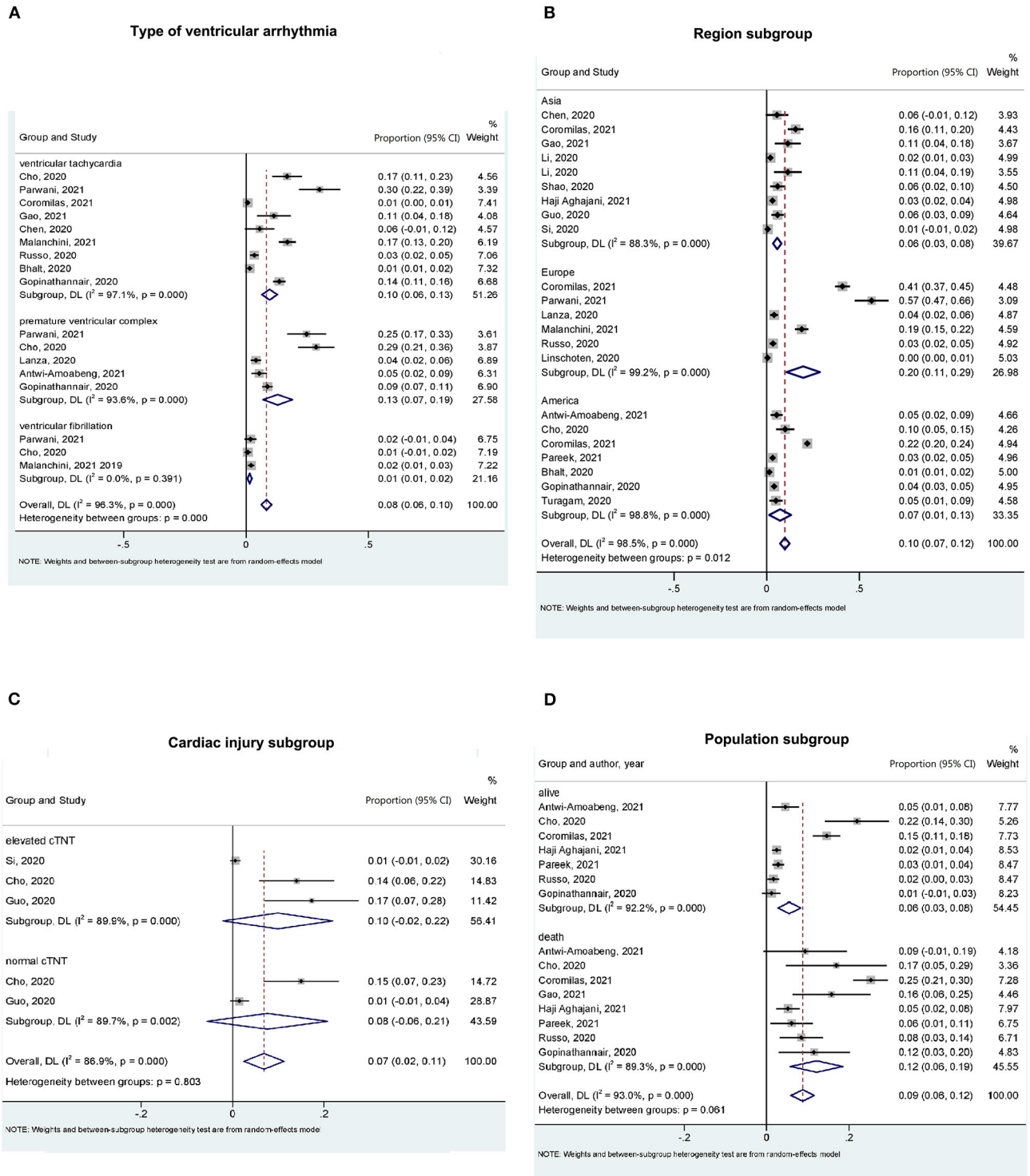


FIGURE 3 | Subgroup analysis for the prevalence of ventricular arrhythmia in patients with COVID-19. **(A)** Ventricular arrhythmia type subgroup. According to ventricular arrhythmia type, ventricular tachycardia, premature ventricular complex, and ventricular fibrillation group were divided. **(B)** Region subgroup. According to region, Asia, Europe, and the United States group were divided. **(C)** Cardiac injury (cardiac troponin T level) subgroup. All the researches were divided into elevated cTNT and normal cTNT group according to cTNT level. **(D)** Population (alive and dead) subgroup. Alive and death population were divided.

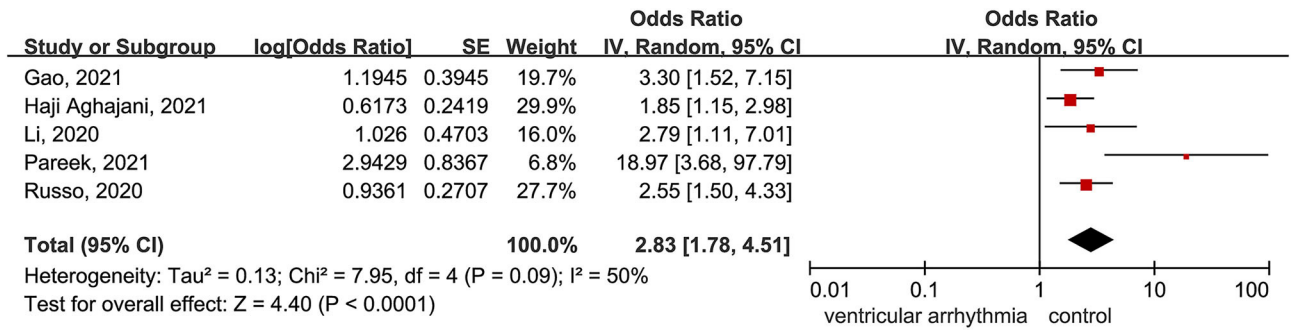


FIGURE 4 | Forest plot for the association between the ventricular arrhythmia and death in patients with COVID-19.

before death. Pulmonary embolism was the direct cause of death in four of these patients (39).

Limited studies reported the incidence of SCD in patients with COVID-19, our review showed it to be 1.8% in the all-hospitalized population with COVID-19 and 10% in the decreased patients. This incident rate is strikingly higher than that reported in the US in patients without COVID-19 (40). One study reported that the incidence of SCD was 14.9–110.8 per 100,000 in population with a non-COVID-19 in different regions (41). Nonetheless, the above results were consistent with several reports. For example, a cohort from Pennsylvania reported a 1.3% incidence of cardiac arrest amongst 700 urban patients admitted for COVID-19 (12). Furthermore, a multicenter cohort study in the US showed that 2.2% of non-ICU (intensive care unit) patients developed in-hospital sudden arrest (42). In the context of COVID-19, Acharya et al. showed the incidence of in-hospital sudden arrest for ICU patients was 15.4% in hospital patients (42). Yet, only ∼7% of the patients with COVID-19 survived to discharge after experiencing in-hospital sudden arrest according to their report (42). Currently, the reason for this high SCD or cardiac arrest rate is not fully evident, and both the cardiac (e.g., undetected ventricular fibrillation) or non-cardiac (e.g., missed pulmonary embolisms) factors might be responsible for this condition.

Our results also showed that ventricular arrhythmia was more likely to occur in American and European patients compared to Asian patients. This should be interpreted considering the limited sample size and differences in baseline characteristics. Notably, the hospitalized American and European patients were mostly older compared with Chinese patients (**Table 1**). Additionally, the prevalence of common comorbidities, such as obesity and diabetes, was also higher in American and European populations than in Asia. All the aforementioned risk factors might be contributing to a higher incidence rate of cardiac injury or severity of COVID-19 cases in American and European populations. Therefore, the regional difference should be validated by further studies.

Compassion With the Previous Study

Previous studies have proved that COVID-19 can significantly affect the cardiovascular system of the patient, leading to serious cardiovascular diseases (3, 4, 43). Also, the previous studies had revealed a positive relationship between arrhythmia and COVID-19 (4, 24, 43, 44). Two meta-analyses studied the relationship between COVID-19 and ventricular arrhythmia. However, one of them focused on the patients after chloroquine or hydroxychloroquine treatment (45), while another explored the effect of COVID-19 on QTd, Tp-e/QTc ratio, and Tp-e interval (46). In addition, two meta-analyses described the relationship between COVID-19 and all types of arrhythmias (47, 48). Nevertheless, most of the included studies reported atrial fibrillation. Our meta-analysis extended the previous study and quantified'prevalence of ventricular arrhythmias and is associated with the clinical outcomes in hospitalized patients with COVID-19.

Clinical Implication

Considering the prevalence of the ventricular arrhythmias in patients with COVID-19, clinicians should be vigilant of ventricular arrhythmias in patients with COVID-19. Screening of high-risk groups for ventricular arrhythmias should be performed at admission. ECG monitoring at admission is suggested for those hospitalized patients who might be at higher risk for the cardiac arrhythmias, such as those with the cardiac injury, palpitations, dizziness, unexplained syncope, and prolonged QTc. In addition, although it is still being debated whether hydroxychloroquine and azithromycin are linked to increased risk of ventricular arrhythmia (49), hydroxychloroquine, and azithromycin can significantly prolong QT interval, which might lead to the ventricular arrhythmia (50, 51). The non-pharmacological treatment (e.g., nutrition support) might also benefit (51). Therefore, these aforementioned treatments might be more carefully evaluated before application or avoided for patients with COVID-19 who were susceptible to ventricular arrhythmias.

Limitation

Our study has several limitations. First, a high degree of heterogeneity was observed in our results, which might be due to study design of the patients and baseline characteristics. For example, ventricular arrhythmia was monitored by a telemetry monitor in the study of Cho (14), while other studies used regular ECG or electrocardiography monitoring. Second, all the studies

included hospitalized patients, which may overestimate the prevalence of the ventricular arrhythmia and its clinical impact on patients with COVID-19 compared with the community patients. Third, due to data restrictions, we could not explore the sex or age differences in the association between the ventricular arrhythmia and death. Third, as shown in **Table 1**, a number of patients receiving drugs tend to prolong the QT interval, which might overestimate the prevalence of the ventricular arrhythmia or SCD. Finally, considering the limited sample size, the incident rate related to the regional differences still needs to be validated by further studies.

CONCLUSION

Ventricular arrhythmia and SCD resulted as a common occurrence with a high prevalence in the hospitalized patients with COVID-19. Furthermore, the ventricular arrhythmia significantly contributed to an increased risk of death in hospitalized patients with COVID-19. Clinicians might be vigilant of ventricular arrhythmias for patients with COVID-19, especially for the severe cases.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

XL and PY were responsible for the entire project and revised the draft. ZT, KM, and ML performed the data extraction, statistical analysis, drafted the first version of the manuscript, and interpreting the data. All authors participated in the interpretation of the results and prepared the final version of the manuscript.

FUNDING

This work was supported in part by the National Natural Science Foundation of China (XL: 82100347, PY: 81760050 and 81760048), China Postdoctoral Science Foundation (XL: 2021M703724), and the Jiangxi Provincial Natural Science Foundation for Youth Scientific Research (PY: 20192ACBL21037).

ACKNOWLEDGMENTS

We acknowledge all people who fought against COVID-19.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.795750/full#supplementary-material

REFERENCES

1. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol.* (2021) 19:141–54. doi: 10.1038/s41579-020-00459-7
2. Babapoor-Farrokhran S, Rasekhi RT, Gill D, Babapoor S, Amanullah A. Arrhythmia in COVID-19. SN comprehensive. *Clin Med.* (2020) 2:1430–5. doi: 10.1007/s42399-020-00454-2
3. Stasi C, Fallani S, Voller F, Silvestri C. Treatment for COVID-19: An overview. *Eur J Pharmacol.* (2020) 889:173644. doi: 10.1016/j.ejphar.2020.173644
4. Chang WT, Toh HS, Liao CT Yu WL. Cardiac involvement of COVID-19: a comprehensive review. *Am J Med Sci.* (2021) 361:14–22. doi: 10.1016/j.amjms.2020.10.002
5. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA.* (2020) 323:1061–9. doi: 10.1001/jama.2020.1585
6. Li Z, Shao W, Zhang J, Ma J, Huang S, Yu P, et al. Prevalence of atrial fibrillation and associated mortality among hospitalized patients with COVID-19: a systematic review and meta-analysis. *Front Cardiovasc Med.* (2021) 8:720129. doi: 10.3389/fcvm.2021.720129
7. Weiss JN, Nademanee K, Stevenson WG, Singh B. Ventricular arrhythmias in ischemic heart disease. *Ann Intern Med.* (1991) 114:784–97. doi: 10.7326/0003-4819-114-9-784
8. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City Area. *JAMA.* (2020) 323:2052–9. doi: 10.1001/jama.2020.6775
9. Abrams MP, Coromilas EJ, Wan EY, Rubin GA, Garan H, Dizon JM. Malignant ventricular arrhythmias in patients with severe acute respiratory distress syndrome due to COVID-19 without significant structural heart disease. *HeartRhythm Case Rep.* (2020) 6:858–62. doi: 10.1016/j.hrcr.2020.08.017
10. Lo CK, Mertz D, Loeb M. Newcastle-Ottawa Scale: comparing reviewers' to authors' assessments. *BMC Med Res Methodol.* (2014) 14:45. doi: 10.1186/1471-2288-14-45
11. Antwi-Amoabeng D, Beutler BD, Singh S, Taha M, Ghuman J, Hanfy A, et al. Association between electrocardiographic features and mortality in COVID-19 patients. *Ann Noninvasive Electrocardiol.* (2021) 26:e12833. doi: 10.1111/anec.12833
12. Bhatla A, Mayer MM, Adusumalli S, Hyman MC, Oh E, Tierney A, et al. COVID-19 and cardiac arrhythmias. *Heart Rhythm.* (2020) 17:1439–44. doi: 10.1016/j.hrthm.2020.06.016
13. Chen Q, Xu L, Dai Y, Ling Y, Mao J, Qian J, et al. Cardiovascular manifestations in severe and critical patients with COVID-19. *Clin Cardiol.* (2020) 43:796–802. doi: 10.1002/clc.23384
14. Cho JH, Namazi A, Shelton R, Ramireddy A, Ehdaie A, Shehata M, et al. Cardiac arrhythmias in hospitalized patients with COVID-19: a prospective observational study in the western United States. *PLoS ONE.* (2020) 15:e0244533. doi: 10.1371/journal.pone.0244533
15. Coromilas EJ, Kochav S, Goldenthal I, Biviano A, Garan H, Goldbarg S, et al. Worldwide survey of COVID-19-associated arrhythmias. *Circ Arrhythm Electrophysiol.* (2021) 14:e009458. doi: 10.1161/CIRCEP.120.009458
16. Gao P, Wu W, Tian R, Yan X, Qian H, Guo F, et al. Association between tachyarrhythmia and mortality in a cohort of critically ill patients with coronavirus disease 2019 (COVID-19). *Ann Transl Med.* (2021) 9:2282. doi: 10.21037/atm-21-2282
17. Gopinathannair R, Merchant FM, Lakkireddy DR, Etheridge SP, Feigofsky S, Han JK, et al. COVID-19 and cardiac arrhythmias: a global perspective on arrhythmia characteristics and management strategies. *J*

Interv Card Electrophysiol. (2020) 59:329–36. doi: 10.1007/s10840-020-00789-9
18. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017
19. Haji Aghajani M, Toloui A, Aghamohammadi M, Pourhoseingholi A, Taherpour N, Sistanizad M, et al. Electrocardiographic findings and in-hospital mortality of covid-19 patients; a retrospective cohort study. *Arch Acad Emerg Med.* (2021) 9:e45. doi: 10.22037/aaem.v9i1.1250
20. Lanza GA, De Vita A, Ravenna SE, D'Aiello A, Covino M, Franceschi F, et al. Electrocardiographic findings at presentation and clinical outcome in patients with SARS-CoV-2 infection. *Europace.* (2021) 23:123–9. doi: 10.1093/europace/euaa245
21. Li J, Guo T, Dong D, Zhang X, Chen X, Feng Y, et al. Defining heart disease risk for death in COVID-19 infection. *QJM.* (2020) 113:876–82. doi: 10.1093/qjmed/hcaa246
22. Li L, Zhang S, He B, Chen X, Wang S, Zhao Q. Risk factors and electrocardiogram characteristics for mortality in critical inpatients with COVID-19. *Clin Cardiol.* (2020) 43:1624–30. doi: 10.1002/clc.23492
23. Linschoten M, Peters S, van Smeden M, Jewbali LS, Schaap J, Siebelink HM, et al. Cardiac complications in patients hospitalised with COVID-19. *Euro Heart J.* (2020) 9:817–23. doi: 10.1177/2048872620974605
24. Malanchini G, Ferrari P, Leidi C, Ferrari G, Racheli M, Senni M, et al. Ventricular arrhythmias among patients with implantable cardioverter-defibrillator during the COVID-19 pandemic. *J Arrhythmia.* (2021) 37:407–13. doi: 10.1002/joa3.12518
25. Pareek M, Singh A, Vadlamani L, Eder M, Pacor J, Park J, et al. Relation of cardiovascular risk factors to mortality and cardiovascular events in hospitalized patients with coronavirus disease 2019 (from the Yale COVID-19 cardiovascular registry). *Am J Cardiol.* (2021) 146:99–106. doi: 10.1016/j.amjcard.2021.01.029
26. Parwani AS, Haug M, Keller T, Guthof T, Blaschke F, Tscholl V, et al. Cardiac arrhythmias in patients with COVID-19: Lessons from 2300 telemetric monitoring days on the intensive care unit. *J Electrocardiol.* (2021) 66:102–7. doi: 10.1016/j.jelectrocard.2021.04.001
27. Russo V, Di Maio M, Mottola FF, Pagnano G, Attena E, Verde N, et al. Clinical characteristics and prognosis of hospitalized COVID-19 patients with incident sustained tachyarrhythmias: a multicenter observational study. *Eur J Clin Invest.* (2020) 50:e13387. doi: 10.1111/eci.13387
28. Shao F, Xu S, Ma X, Xu Z, Lyu J, Ng M, et al. In-hospital cardiac arrest outcomes among patients with COVID-19 pneumonia in Wuhan, China. *Resuscitation.* (2020) 151:18–23. doi: 10.1016/j.resuscitation.2020.04.005
29. Si D, Du B, Ni L, Yang B, Sun H, Jiang N, et al. Death, discharge and arrhythmias among patients with COVID-19 and cardiac injury. *Cmaj.* (2020) 192:E791–8. doi: 10.1503/cmaj.200879
30. Turagam MK, Musikantow D, Goldman ME, Bassily-Marcus A, Chu E, Shivamurthy P, et al. Malignant arrhythmias in patients with COVID-19: incidence, mechanisms, and outcomes. *Circ Arrhythm Electrophysiol.* (2020) 13:e008920. doi: 10.1161/CIRCEP.120.008920
31. Yang N, Tian K, Jin M, Zhang X, Zhang F, Shi X, et al. Sudden death of COVID-19 patients in Wuhan, China: a retrospective cohort study. *J Glob Health.* (2021) 11:05006. doi: 10.7189/jogh.11.05006
32. Lin L, Xu Y, He D, Han Y, Tang G-H, Yang Z-M, et al. A retrospective study on clinical features of and treatment methods for 77 severe cases of SARS. *Am J Chin Med.* (2003) 31:821–39. doi: 10.1142/S0192415X03001521
33. Yu CM, Wong RS, Wu EB, Kong SL, Wong J, Yip GW, et al. Cardiovascular complications of severe acute respiratory syndrome. *Postgrad Med J.* (2006) 82:140–4. doi: 10.1136/pgmj.2005.037515
34. Lau ST, Yu WC, Mok NS, Tsui PT, Tong WL, Cheng SW. Tachycardia amongst subjects recovering from severe acute respiratory syndrome (SARS). *Int J Cardiol.* (2005) 100:167–9. doi: 10.1016/j.ijcard.2004.06.022
35. Quagliariello V, Bonelli A, Caronna A, Conforti G, Iovine M, Carbone A, et al. SARS-CoV-2 infection and cardioncology: from cardiometabolic risk factors to outcomes in cancer patients. *Cancers.* (2020) 12:3316. doi: 10.3390/cancers12113316
36. de Lucena TMC, da Silva Santos AF, de Lima BR, de Albuquerque Borborema ME, de Azevêdo Silva J. Mechanism of inflammatory response in associated comorbidities in COVID-19. *Diab Metab Syndrome.* (2020) 14:597–600. doi: 10.1016/j.dsx.2020.05.025
37. Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, et al. Extrapulmonary manifestations of COVID-19. *Nat Med.* (2020) 26:1017–32. doi: 10.1038/s41591-020-0968-3
38. Chung MK, Martin DO, Sprecher D, Wazni O, Kanderian A, Carnes CA, et al. C-reactive protein elevation in patients with atrial arrhythmias: inflammatory mechanisms and persistence of atrial fibrillation. *Circulation.* (2001) 104:2886–91. doi: 10.1161/hc4901.101760
39. Wichmann D, Sperhake JP, Lutgehetmann M, Steurer S, Edler C, Heinemann A, et al. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. *Ann Intern Med.* (2020) 173:268–77. doi: 10.7326/L20-1206
40. Perman SM, Stanton E, Soar J, Berg RA, Donnino MW, Mikkelsen ME, et al. Location of in-hospital cardiac arrest in the United States-variability in event rate and outcomes. *J Am Heart Assoc.* (2016) 5:e003638. doi: 10.1161/JAHA.116.003638
41. Wong CX, Brown A, Lau DH, Chugh SS, Albert CM, Kalman JM, et al. Epidemiology of sudden cardiac death: global and regional perspectives. *Heart Lung Circ.* (2019) 28:6–14. doi: 10.1016/j.hlc.2018.08.026
42. Acharya P, Ranka S, Sethi P, Bharati R, Hu J, Noheria A, et al. Incidence predictors, and outcomes of in-hospital cardiac arrest in COVID-19 patients admitted to intensive and non-intensive care units: insights from the AHA COVID-19 CVD registry. *J Am Heart Assoc.* (2021) 10:e021204. doi: 10.1161/JAHA.120.021204
43. Salian VS, Wright JA, Vedell PT, Nair S, Li C, Kandimalla M, et al. COVID-19 transmission, current treatment, and future therapeutic strategies. *Mol Pharm.* (2021) 18:754–71. doi: 10.1021/acs.molpharmaceut.0c00608
44. Rav-Acha M, Orlev A, Itzhaki I, Zimmerman SF, Fteiha B, Bohm D, et al. Cardiac arrhythmias amongst hospitalised Coronavirus 2019 (COVID-19) patients: prevalence, characterisation, and clinical algorithm to classify arrhythmic risk. *Int J Clin Pract.* (2021) 75:13788. doi: 10.1111/ijcp.13788
45. Tleyjeh IM, Kashour Z, AlDosary O, Riaz M, Tlayjeh H, Garbati MA, et al. Cardiac toxicity of chloroquine or hydroxychloroquine in patients with COVID-19: a systematic review and meta-regression analysis. *Mayo Clin Proc.* (2021) 5:137–50. doi: 10.1016/j.mayocpiqo.2020.10.005
46. Tondas AE, Mulawarman R, Trifitriana M, Nurmaini S, Irfannuddin I. Arrhythmia risk profile and ventricular repolarization indices in COVID-19 patients: a systematic review and meta-analysis. *J Infect Dev Ctries.* (2021) 15:224–9. doi: 10.3855/jidc.13922
47. Liao SC, Shao SC, Cheng CW, Chen YC, Hung MJ. Incidence rate and clinical impacts of arrhythmia following COVID-19: a systematic review and meta-analysis of 17,435 patients. *Crit Care.* (2020) 24:690. doi: 10.1186/s13054-020-03368-6
48. Pellicori P, Doolub G, Wong CM, Lee KS, Mangion K, Ahmad M, et al. COVID-19 and its cardiovascular effects: a systematic review of prevalence studies. *Cochrane Database Syst Rev.* (2021) 3:Cd013879. doi: 10.1002/14651858.CD013879
49. Abrams MP, Wan EY, Waase MP, Morrow JP, Dizon JM, Yarmohammadi H, et al. Clinical and cardiac characteristics of COVID-19 mortalities in a diverse New York City Cohort. *J Cardiovasc Electrophysiol.* (2020) 31:3086–96. doi: 10.1111/jce.14772
50. Carron J, Sharif Z, Hussein H, Kennedy M, McAdam B, Sheahan R. Clinical guidance for navigating the QTc-prolonging and arrhythmogenic potential of pharmacotherapy during the COVID-19 pandemic. *Ir J Med Sci.* (2021) 190:403–9. doi: 10.1007/s11845-020-02291-7

51. Manolis AS, Manolis AA, Manolis TA, Apostolopoulos EJ, Papatheou D, Melita H. COVID-19 infection and cardiac arrhythmias. *Trends Cardiovasc Med.* (2020) 30:451–60. doi: 10.1016/j.tcm.2020.08.002

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 16 February 2022
doi: 10.3389/fcvm.2022.737592

Using a Systems Approach to Explore the Mechanisms of Interaction Between Severe Covid-19 and Its Coronary Heart Disease Complications

Albertus A. Meyer[1], *Edward H. Mathews*[1,2,3]*, *Andries G. S. Gous*[3] *and Marc J. Mathews*[3]

[1] Centre for Research in Continued Engineering Development (CRCED), North-West University, Potchefstroom, South Africa, [2] Department of Physiology, Medical School, University of Pretoria, Pretoria, South Africa, [3] Department of Industrial Engineering, Stellenbosch University, Stellenbosch, South Africa

Frontiers requested research on how a systems approach can explore the mechanisms of cardiovascular complications in Covid-19. The focus of this paper will thus be on these detailed mechanisms. It will elucidate the integrated pathogenic pathways based on an extensive review of literature. Many severe Covid-19 cases and deaths occur in patients with chronic cardiovascular comorbidities. To help understand all the mechanisms of this interaction, Covid-19 complications were integrated into a pre-existing systems-based coronary heart disease (CHD) model. Such a complete model could not be found in literature. A fully integrative view could be valuable in identifying new *pharmaceutical interventions*, help understand how *health factors* influence Covid-19 severity and give a fully integrated explanation for the Covid-19 *death spiral* phenomenon seen in some patients. Covid-19 data showed that CHD hallmarks namely, *Hypercoagulability, Hypercholesterolemia, Hyperglycemia/Hyperinsulinemia, Inflammation* and *Hypertension* have an important effect on disease severity. The pathogenic pathways that Covid-19 activate in CHD were integrated into the CHD model. This fully integrated model presents a visual explanation of the mechanism of interaction between CHD and Covid-19 complications. This includes a detailed integrated explanation of the death spiral as a result of interactions between *Inflammation*, endothelial cell injury, *Hypercoagulability* and hypoxia. Additionally, the model presents the aggravation of this *death spiral* through the other CHD hallmarks namely, *Hyperglycemia/Hyperinsulinemia, Hypercholesterolemia,* and/or *Hypertension.* The resulting model further suggests systematically how the pathogenesis of nine *health factors* (stress, exercise, smoking, etc.) and seven *pharmaceutical interventions* (statins, salicylates, thrombin inhibitors, etc.) may either aggravate or suppress Covid-19 severity. A strong association between CHD and Covid-19 for all the investigated *health factors* and *pharmaceutical interventions*, except for β-blockers, was found. It is further discussed how the proposed model can be extended in future to do computational analysis to help assess the risk of Covid-19 in cardiovascular disease. With insight gained from this study, recommendations are made

OPEN ACCESS

Edited by:
Eric Sijbrands,
Erasmus Medical Center, Netherlands

Reviewed by:
Eric Sijbrands,
Erasmus Medical Center, Netherlands
Vladimir Uspenskiy,
Almazov National Medical Research Centre, Russia

***Correspondence:**
Edward H. Mathews
ehmathews@researchtoolbox.com

Specialty section:
This article was submitted to Cardiovascular Epidemiology and Prevention, a section of the journal Frontiers in Cardiovascular Medicine

Received: *07 July 2021*
Accepted: *24 January 2022*
Published: *16 February 2022*

Citation:
Meyer AA, Mathews EH, Gous AGS and Mathews MJ (2022) Using a Systems Approach to Explore the Mechanisms of Interaction Between Severe Covid-19 and Its Coronary Heart Disease Complications. Front. Cardiovasc. Med. 9:737592. doi: 10.3389/fcvm.2022.737592

for future research in potential new pharmacotherapeutics. These recommendations could also be beneficial for cardiovascular disease, which killed five times more people in the past year than Covid-19.

Keywords: COVID-19, SARS-CoV-2, coronary heart disease, cardiovascular comorbidities, systems-approaches

INTRODUCTION

The coronavirus disease of 2019 (Covid-19) is caused by the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which first emerged in December 2019 in Wuhan, China (1). In March 2020, the World Health Organization declared this disease a pandemic (2). As of 8 August 2021, the total number of confirmed global deaths were 4,285,421 (3).

It is widely accepted that Covid-19 severity is increased by respiratory complications such as hypoxia (4, 5). Critically ill patients developing hypoxia requires supplemental oxygen and/or mechanical ventilation (4, 5). Although this condition is respiratory related, this hypoxia is fueled by vascular complications which are documented in numerous autopsies (4, 6–9). Moreover, pre-existing cardiovascular related comorbidities are known risk factors that increase Covid-19 severity. These comorbidities include, among others, *Hypertension*, *Hyperglycemia/Hyperinsulinemia*, obesity and/or chronic cardiac disease (10–14).

Furthermore, hospitalized critically ill Covid-19 patients experience cardiovascular complications such as cardiac injury, thrombosis, arrhythmia, heart failure and myocardial dysfunction (15–19). This is again substantiated by autopsies that present various findings of vasculature damage that leads to a state of *Hypercoagulability* in deceased Covid-19 patients (4, 6–9).

Most severe Covid-19 patients also experience a chronic heightened *Inflammatory state*, especially within the alveoli and pulmonary capillaries (20–22). This may be as a result of the dysregulated hyperimmune response (20) and/or direct viral infection mediating inflammatory cell infiltration (11, 22).

Therefore, the prevailing viewpoints in literature are that most severe cases of Covid-19 (i) result in cardiovascular complications (4, 6–9) and/or (ii) are seen in patients with pre-existing cardiovascular comorbidities (10–14). A need therefore exists to further investigate the underlying mechanisms/pathogenesis between cardiovascular disease and Covid-19.

To fully investigate this, the pathogenesis of cardiovascular disease and Covid-19 needs to be integrated. Fortunately, most of the above mentioned vascular Covid-19 effects are included in an existing model of coronary heart disease (CHD) (**Figure 1**) (23, 24). These effects are depicted in **Figure 1** as the following CHD hallmarks (yellow boxes): *(A) Hypercoagulability, (B) Hypercholesterolemia, (C) Hyperglycemia/Hyperinsulinemia, (D) Inflammatory state* and *(E) Hypertension.*

Hypercholesterolemia is a common CHD risk factor, known to aggravate vascular cell dysfunction, aggravate coagulation and upregulate inflammation (29–31). *Hypercholesterolemia (B),* depicted in **Figure 1**, has only been partially linked to Covid-19 through high circulating cholesterol levels that may make a person more susceptible to infection (32). Although this might still be controversial, a recent molecular study showed that SARS-CoV-2 requires cholesterol for viral entry (33). Subsequently, another molecular study (yet unpublished) showed how cholesterol optimally positions furin for priming SARS-CoV-2 (34). In other words, cholesterol improves binding to ACE2 receptor, while producing a more infectious virion (34).

We envisage another association between increased Covid-19 severity and *Hypercholesterolemia,* through vascular complications that arise from high cholesterol levels. Since both *Hypercoagulability* and *Inflammation* are known risk factors for Covid-19 and *Hypercholesterolemia* influences both these hallmarks (23), we also included *Hypercholesterolemia* in our *integrated CHD/Covid-19 model* (more detailed discussions are given in sections Severe Covid-19 Patients With Existing Chronic Hypercholesterolemia and Effects of Different CHD *Pharmaceutical Interventions* on Covid-19 Severity).

All of the CHD Hallmarks identified in the CHD model (**Figure 1**) play a significant role in Covid-19 severity. The question is, will it be possible to use this CHD model and integrate the pathogenesis of Covid-19 with it?

In this paper we will attempt to integrate the CHD pathogenic pathways with those of severe Covid-19 complications, using a systems-based approach. This CHD/Covid-19 integration should provide insight into the following questions, some of which were requested by *Frontiers*:

1. Why do some patients with severe Covid-19 experience sudden death? (Section The Death Spiral: Inflammation, EC Injury, Coagulation, Vascular Leakage and Hypoxia)
2. How do CHD comorbidities influence this *death spiral*? (Section Covid-19 Aggravation in Patients With Pre-existing CHD Comorbidities)
3. How can an individual reduce the risk of developing severe Covid-19 from a cardiovascular point of view? (Sections Effects of Different *Health Factors* on Covid-19 Severity and Effects of Different CHD *Pharmaceutical Interventions* on Covid-19 Severity)
4. How can computational analysis help to assess the risk of COVID-19 in cardiovascular disease? (Section How Can Computational Analysis Help to Assess the Risk of Severity in Covid-19 in Cardiovascular Disease?)
5. Are there other opportunities in cardiovascular disease that can be derived from this paper and the Covid-19 crisis? (Section Are There Other Opportunities in Cardiovascular Disease That Can Be Derived From This Paper and the Covid-19 Crisis?).

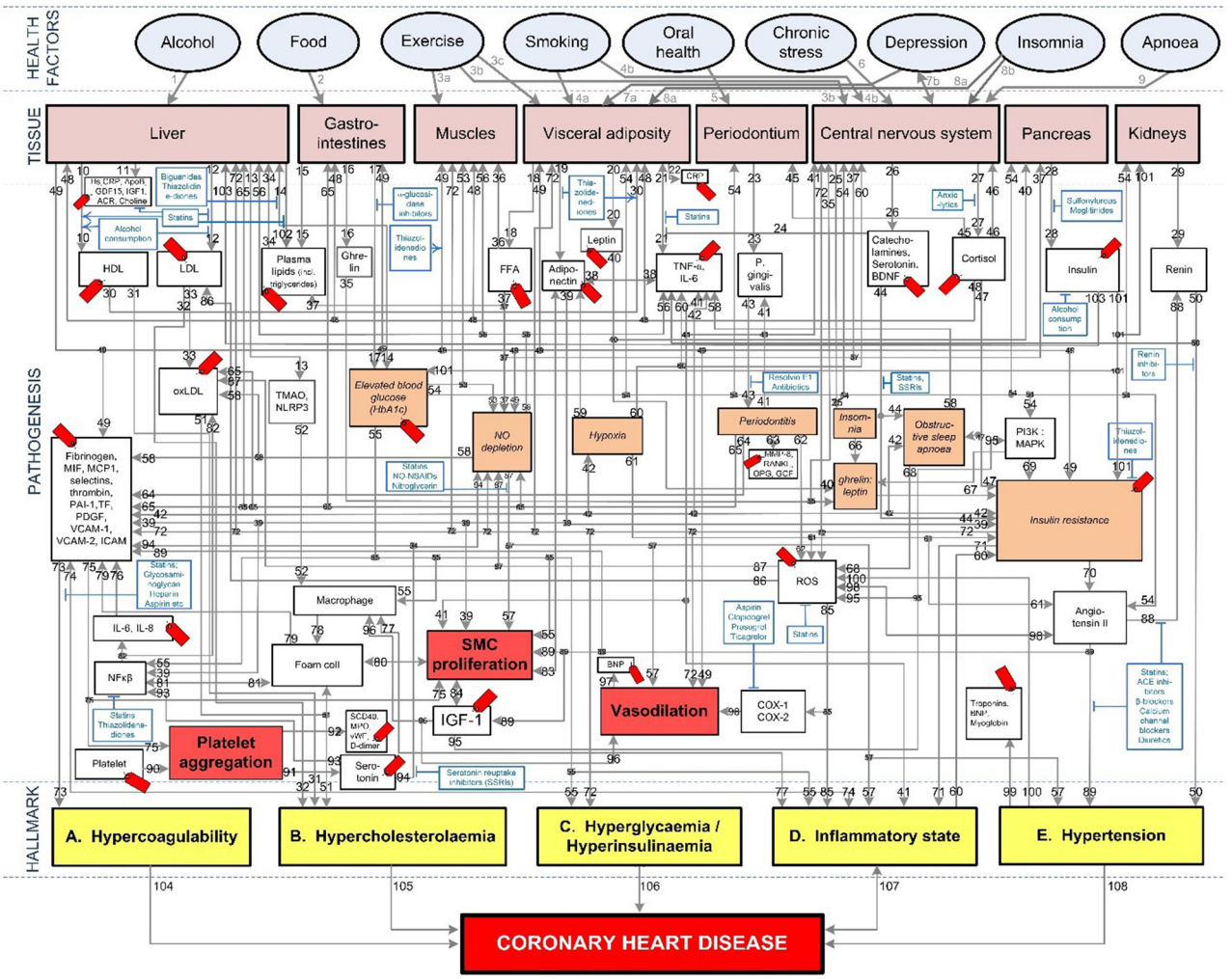


FIGURE 1 | Existing model showing the mechanisms of coronary heart disease (23–28). The affective pathway of pharmaceuticals, blue boxes, is shown in Figure and salient serological biomarkers are indicated by the red tags (). The blunted blue arrows denote antagonize or inhibit and pointed blue arrows denote up-regulate or facilitate. ACE, angiotensin-converting-enzyme; β-blocker, beta-adrenergic antagonists; BDNF, brain-derived neurotrophic factor; BNP, B-type natriuretic peptide; OX, cyclooxygenase; CRP, C-reactive protein; D-dimer, fibrin degradation product D; FFA, free fatty acids; GCF, gingival crevicular fluid; HbA1c, glycated hemoglobin A1c; HDL, high-density lipoprotein; Hs, homocysteine; ICAM, intracellular adhesion molecule; IGF-1, insulin-like growth factor-1; IL, interleukin; LDL, low-density lipoprotein; MAPK, mitogen-activated protein (MAP) kinase; MCP, monocyte chemoattractant protein; MIF, macrophage migration inhibitory factor; MMP, matrix metalloproteinase; MPO, myeloperoxidase; NFκβ, nuclear factor-κβ; NLRP3, Inflammasome responsible for activation of inflammatory processes as well as epithelial cell regeneration and microflora; NO, nitric oxide; NO-NSAIDs, combinational NO-non-steroidal anti-inflammatory drug; OPG, osteoprotegerin; oxLDL, oxidized LDL; PAI, plasminogen activator inhibitor; PDGF, platelet-derived growth factor; P. gingivalis, Porphyromonas gingivalis; PI3K, phosphatidylinositol 3-kinase; RANKL, receptor activator of nuclear factor kappa-beta ligand; ROS, reactive oxygen species; SCD-40, recombinant human sCD40 ligand; SMC, smooth muscle cell; SSRI, serotonin reuptake inhibitors; TF, tissue factor; TMAO, an oxidation product of trimethylamine (TMA); TNF-α, tumor necrosis factor-α; VCAM, vascular cell adhesion molecule; vWF, von Willebrand factor.

We envisage that the proposed *integrated CHD/Covid-19 model* may help answer some of these questions, thereby potentially enhancing the future management of both Covid-19 and CHD.

METHOD

The methodology to develop the pathogenic pathways for the *integrated CHD/Covid-19 model* is divided into three parts namely the following:

1. Section Description of Existing CHD Model discribes the existing CHD model (23).
2. Section Systems-Based Integration of Covid-19 Factors Into the CHD Model discusses the systems-based method for integration (**Figure 2**) of Covid-19 factors into the CHD model (**Figure 1**). The outcome of this method is depicted in **Figures 3**–**6**, **8**–**10**. Its implications are discussed in the Results sections Integrated Covid-19/CHD Model and Covid-19 Aggravation in Patients With Pre-existing CHD Comorbidities.

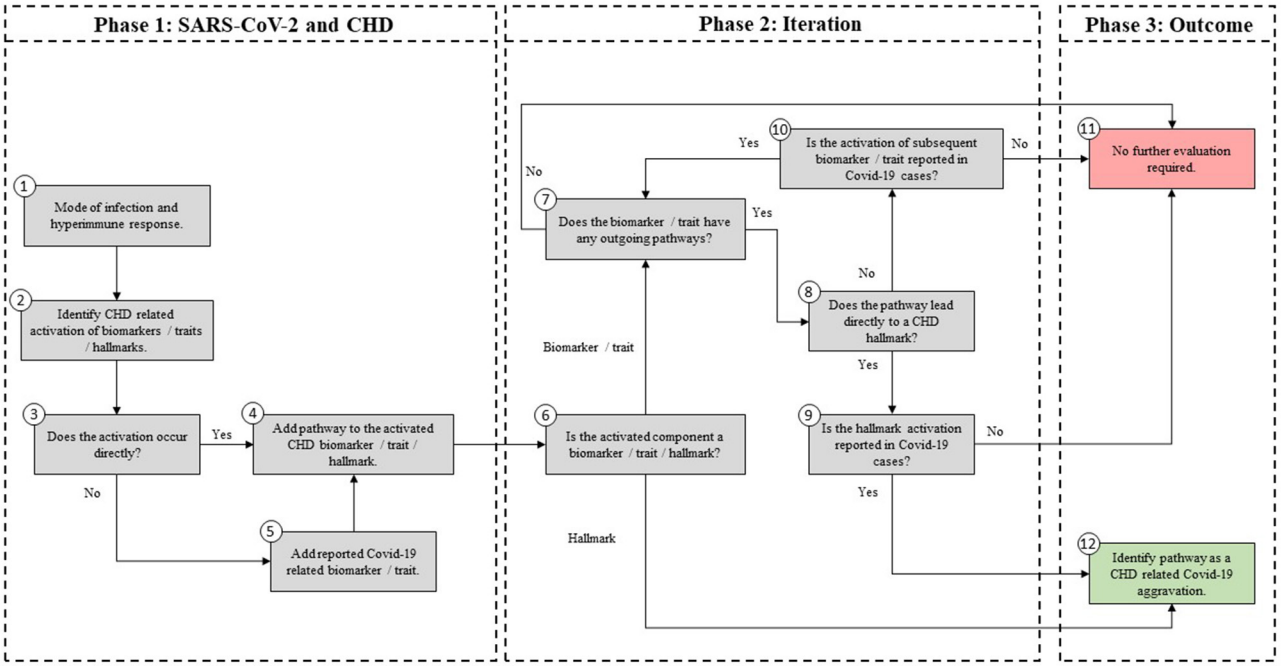


FIGURE 2 | Methodology used to develop an Integrated Covid-19/CHD model.

3. Section Evaluation of *Health Factors* and *Pharmaceutical Interventions* describes the method to evaluate the effects on Covid-19 severity of *health factors* (blue ovals) and *pharmaceutical interventions* (blue boxes) as depicted in **Figure 1**. The relevant pathogenic pathways that are activated are discussed in more detail in the Results sections Effects of Different *Health Factors* on Covid-19 Severity and Effects of Different CHD *Pharmaceutical Interventions* on Covid-19 Severity.

Description of Existing CHD Model

The existing CHD model (**Figure 1**) was developed as a PhD study and extensively described in (23). The model is available online from the university (23). Some results and implications of the model were published (24–28). Hence, we will only discuss the relevant salient elements here. The model defined CHD as the incidence of atherosclerosis, coronary artery disease, or myocardial infarction (23). Subsequently, where results were given for cardiovascular disease these were interpreted as CHD only in scenarios where the effect of stroke could be accounted for (23).

Although cerebrovascular disease is also a component of cardiovascular disease it was not addressed here. Our proposed *integrated CHD/Covid-19 model* is therefore based primarily on CHD attributes, with focus on vascular complications induced by the SARS-CoV-2 virus. We acknowledge that other pathogenic pathways may exist such as the cerebrovascular ones (35), which should warrant further research in an extended model.

The CHD model presented in **Figure 1**, was developed by analyzing the effect of different *health factors* (blue ovals) on *body tissues* (pink boxes) and investigating the respective *pathogenesis* (gray lines with numbers), *traits* (orange boxes) and activated *biomarkers* (white boxes) related to an increased risk of CHD (23–28).

Each gray line and respective number in the CHD model correspond to a certain pathogenesis pathway that could typically be present in a CHD patient. These pathways are visual representations of previously published literature, which link the effects of *health factors* (blue ovals) to the relevant *tissues* (pink boxes) and subsequently to the hallmarks of CHD (yellow boxes) (23–28).

The traits are represented in the lightly shaded orange boxes. Biomarkers are indicated as white boxes, with those that are typically measured, denoted with red tags (). The *pharmaceutical interventions*, acting on the respective pathways are indicated as blue boxes, where blunted blue arrows () denote antagonize or inhibit and pointed blue arrows () denote up-regulate or facilitate (23–28).

Systems-Based Integration of Covid-19 Factors Into the CHD Model

The systems-based integration methodology dicussed here is depicted as three phases in a flow chart in **Figure 2**. The iterative approach followed here is to ensure that only pathways discussed in literature, with substantial evidence, are included. We will use **Figure 3** to show the Covid-19 pathways in green, with all other pathways from the original model in **Figure 1** made transparent.

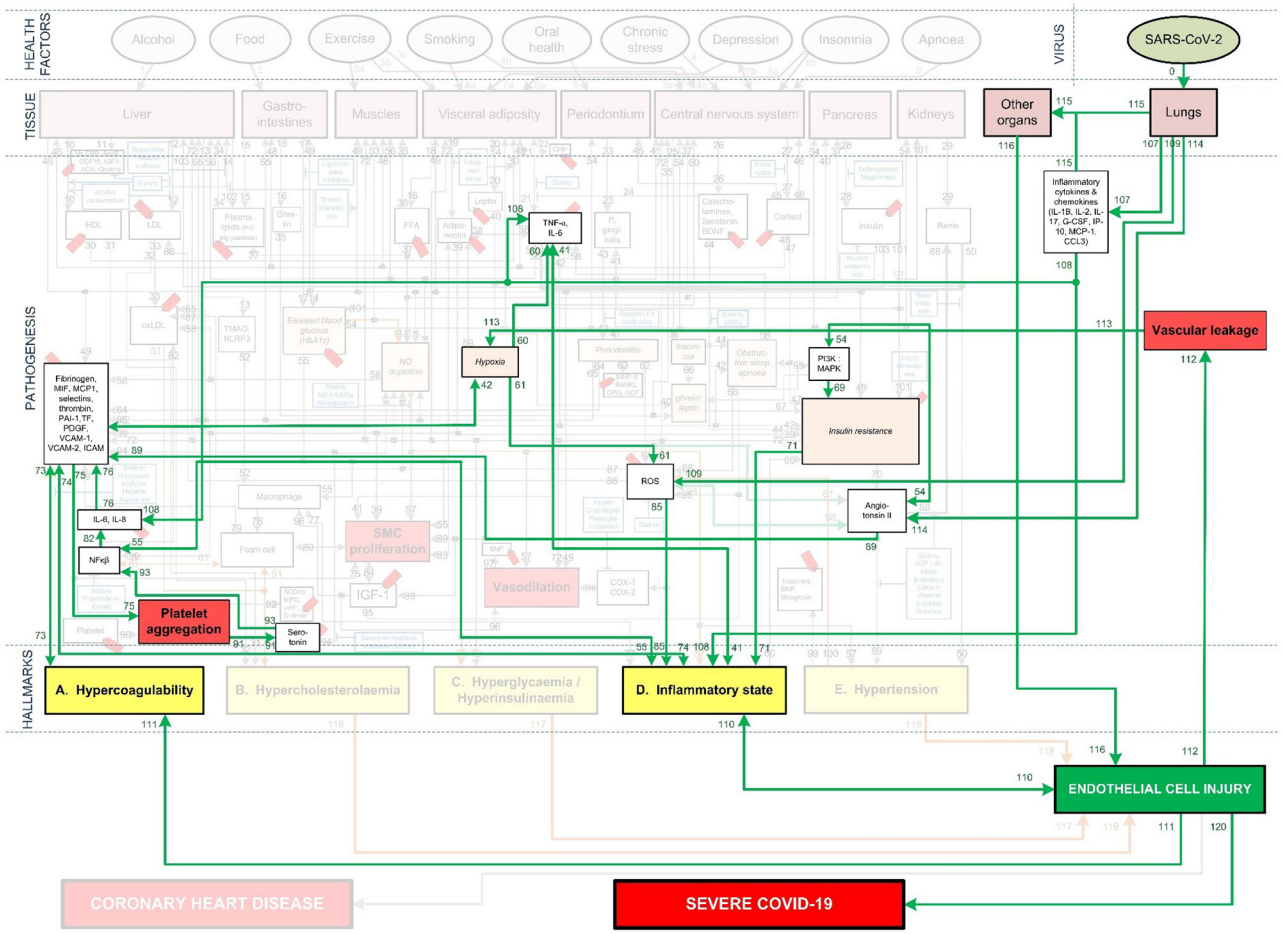


FIGURE 3 | Integration of Covid-19 pathogenic pathways into the pathways of CHD.

Phase 1: SARS-CoV-2 and CHD

SARS-CoV-2, which causes Covid-19, was incorporated into the existing CHD model by investigating pathogenic pathways and biomarkers reported in literature. These biomarkers and pathways were either included or excluded based on the following five steps, presented in **Figure 2** (Phase 1).

Step (1): Firstly, the relevant tissue (denoted as pink boxes in the right-hand corner of **Figure 3**) through which the SARS-CoV-2 virus (green oval in **Figure 3**) enters the body was evaluated. Although EC injury was discussed as the critical element in CHD in (23), it was not shown in **Figure 1**. Here we added EC injury as a green box between pathways 110, 111, 112, and 116 at the bottom of **Figure 3**.

Step (2): The activated CHD related biomarkers, traits or hallmarks, reported in severe Covid-19 patients were then identified from literature. These are, respectively, denoted as white, orange and yellow boxes in **Figure 3**.

Step (3): In this step we evaluated the identified CHD biomarkers, traits or hallmarks found in literature, in order to determine whether the activation of these occurs directly or indirectly as a result of the SARS-CoV-2 virus. Steps (4) and (5) describe the two possible outcomes of the identification process.

Step (4): If the activation occurs directly, as determined in step (3), then a new (green) pathway that led from the virus to the respective CHD biomarker, trait or hallmark was added to the integrated model as shown in **Figure 3**.

Step (5): If the activation occurs indirectly, as determined in step (3), then a new biomarker or trait was added to the model e.g., the inflammatory cytokines in the top, right-hand white box between pathways 107, 108, and 115 in **Figure 3**. A biomarker or trait was only added if its respective pathway eventually led to the activation of a CHD hallmark.

Phase 2: Iteration

For the iteration process in Phase 2, the following steps were conducted:

Step (6): The activated component (CHD hallmark, biomarker or trait) from step (4) to which the green pathway from step (4) leads was further evaluated based on literature. If this component is a biomarker or trait then step (7) was

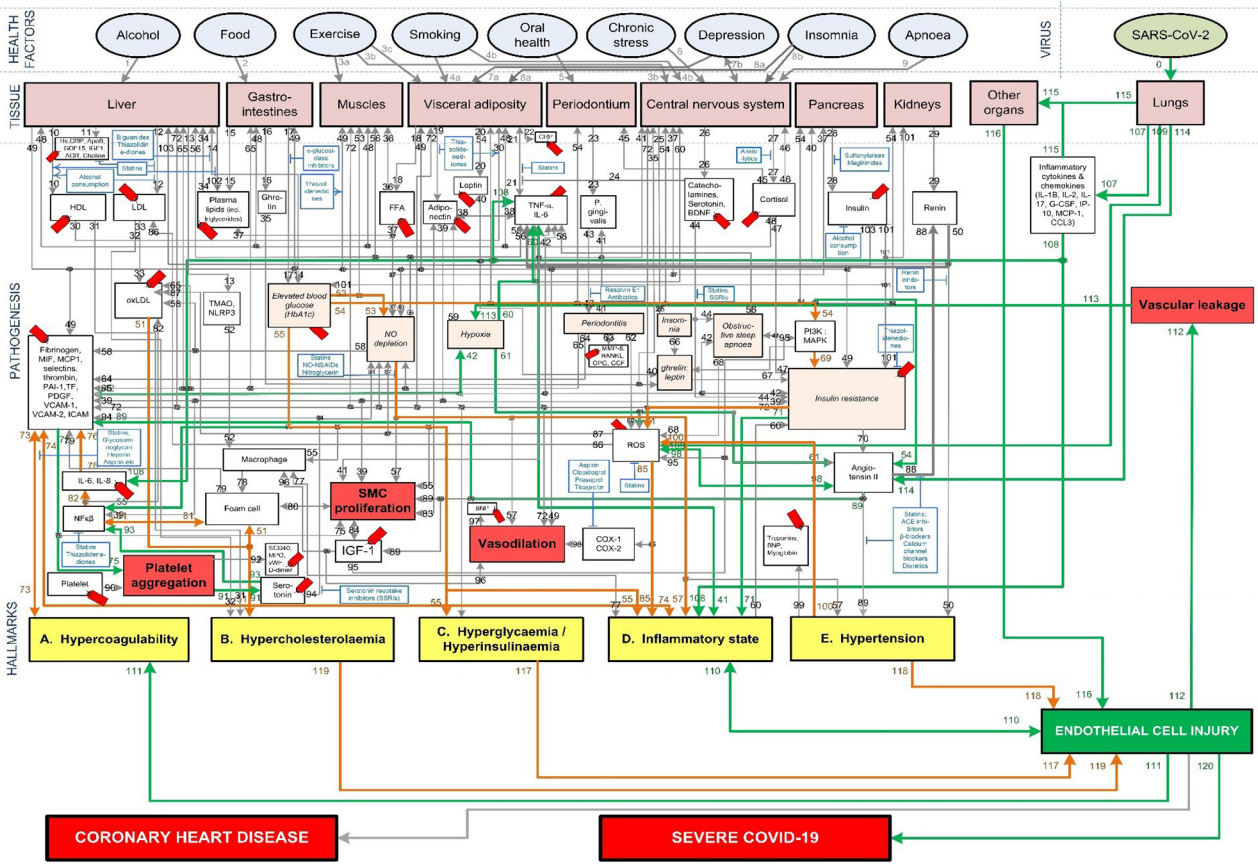


FIGURE 4 | Proposed integrated Covid-19/CHD model.

followed. If this component is rather a CHD hallmark, then step (12) was followed.

Step (7): In this step it was determined whether the CHD biomarker or trait has any outgoing (gray) CHD pathways. Most biomarkers and traits have outgoing CHD pathways. These gray CHD pathways were further assessed in Step (8). For the biomarkers and traits with no outgoing gray pathways (e.g., troponin for pathway 99 in **Figure 3**) step (11) was followed.

Step (8): In this step it was determined whether the gray CHD pathway leads directly or indirectly to a CHD hallmark (yellow boxes in **Figure 3**). If the gray CHD pathway leads directly to a CHD hallmark then step (9) was followed, otherwise step (10) was followed.

Step (9): The CHD hallmark was further investigated to ensure its activation due to SARS-CoV-2 was relevant to severe Covid-19 patients. If it was reported in literature to be aggravated in severe Covid-19 patients then step (12) was followed (changing the gray pathway to a green pathway) otherwise step (11) was followed (keeping the pathway gray). These steps are explained in more detail in phase 3.

Step (10): As determined in step (8), the relevance to Covid-19 severity of the subsequent CHD biomarker or trait to which the gray CHD pathway led to was investigated. If relevance was found, then this CHD biomarker or trait was re-evaluated by following the same approach as in step (7).

Phase 3: Outcome

This phase presents the two outcomes that were reached after integration and iteration of the identified biomarkers, traits, CHD hallmarks and their relevant pathways.

Step (11): This step was followed if the activated CHD biomarkers or traits had, (i) no other outgoing CHD pathway or (ii) the outgoing pathway led to another biomarker or trait that had no relevance to severe Covid-19 patients. If one of these two conditions were met then the biomarker, trait and the subsequent pathway was not evaluated further.

These biomarkers, traits and respective pathways e.g., oxidized low-density lipoprotein (oxLDL), nitric oxide (NO) depletion and cortisol were made transparent, as shown in **Figure 3**. Although these biomarkers or traits do not have a direct link to Covid-19 patients, they may influence Covid-19 severity indirectly by affecting one of the CHD hallmarks. This idea is discussed in more detail in section Covid-19 Aggravation in Patients With Pre-existing CHD Comorbidities.

Step (12): Step (12) was followed if the investigated biomarker, trait, CHD hallmark and respective pathways were relevant

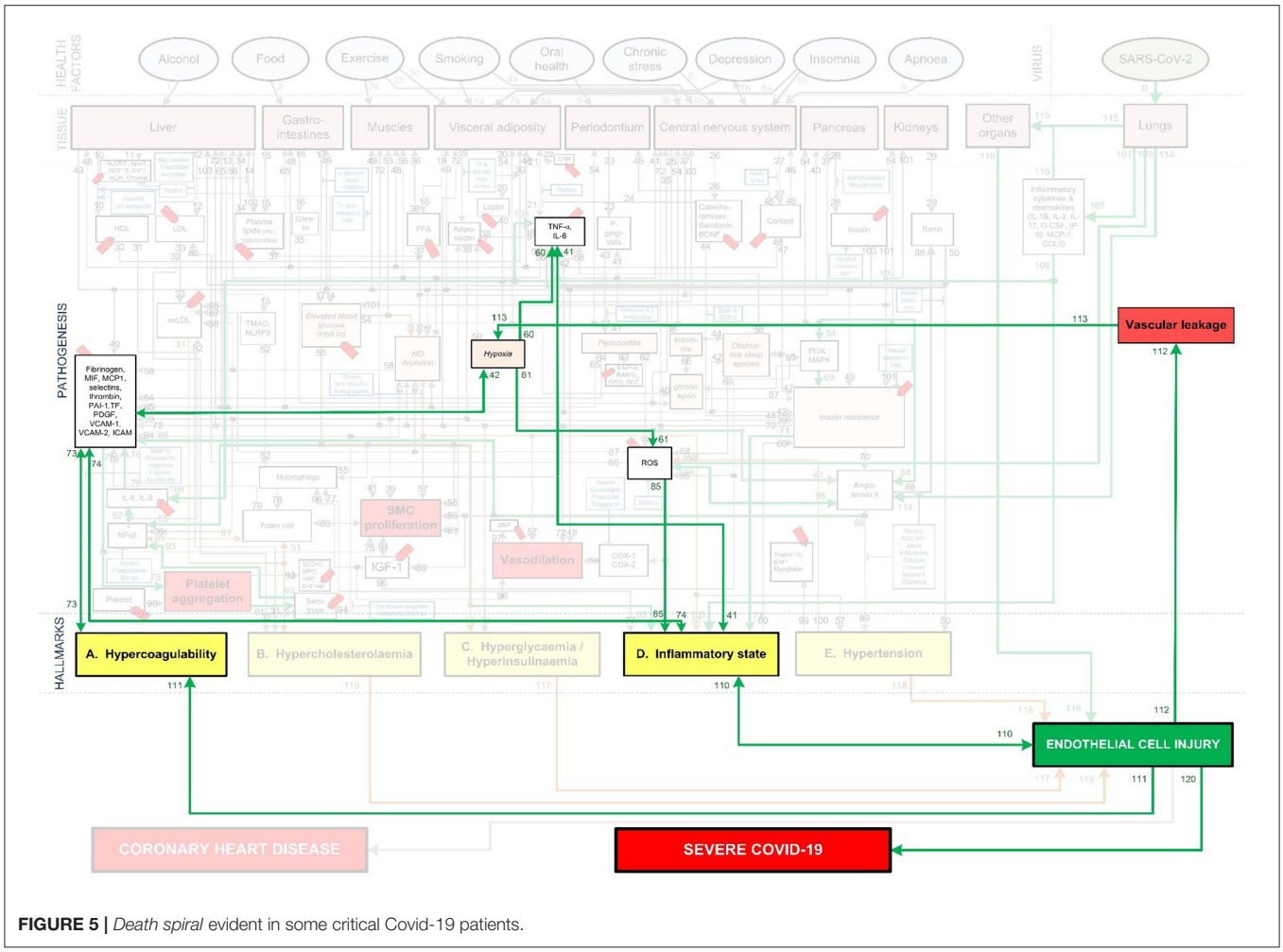


FIGURE 5 | *Death spiral* evident in some critical Covid-19 patients.

in most Covid-19 patients with severe disease and these are therefore prominently shown as green lines in **Figure 3**.

The Covid-19 pathways were described in this section and shown as green lines in **Figure 3**. The final step is to show all the CHD pathways together with the Covid-19 pathways. The complete *integrated CHD/Covid-19 model* is given in **Figure 4**.

Evaluation of *Health Factors* and *Pharmaceutical Interventions*

The mechanisms of interaction between CHD and Covid-19 (**Figure 4**) can help to compare the few factors a patient can control namely, *health factors* (before infection with SARS-CoV-2) and *pharmaceutical interventions* (after infection). Only the *health factors* and *pharmaceutical interventions* investigated in (23) for CHD risk are investigated here for Covid-19.

The *health factors* (blue ovals) in **Figure 4** were defined as the following (23):

- Alcohol use: Indicates moderate alcohol consumption (20–30 g alcohol (ethanol) per day for men and half of that for women).
- Food: High glycemic diets (HGD) (glycemic load > 142).
- Exercise: Regular moderate exercise (550–3,000 kcal/week).
- Smoking: Current smoker.
- Oral Health: oor oral health in the form of periodontal disease.
- Stress: Chronic-level stress at work or home.
- Depression: Self-diagnosed, physician diagnosed or use of antidepressant medication.
- Insomnia: Inability to fall asleep or to maintain sleep or the perception of disturbed sleep.
- Apnoea: Obstructive sleep apnoea or hypopnoea (apnoea-hypopnea index>5/h).

We will discuss in Results section Effects of Different *Health Factors* on Covid-19 Severity to what extent a healthy vascular "baseline," as a result of a healthy lifestyle, will influence Covid-19 severity.

The *pharmaceutical interventions* that were investigated were limited to those investigated in the original CHD model (23). These include statins, salicylates (aspirin), indirect thrombin inhibitors (heparin), direct thrombin inhibitors (angiomax), Angiotensin-Converting-Enzyme (ACE) inhibitors, angiotensin-renin inhibitors, β-blockers, calcium channel blockers, diuretics,

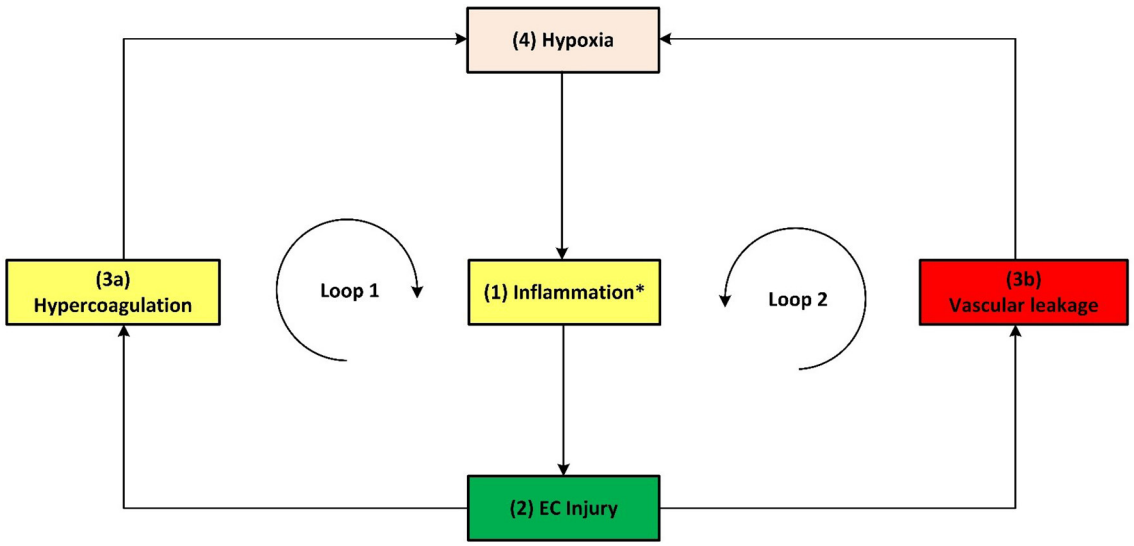


FIGURE 6 | Simplified schematic of the *death spiral* evident in some critical Covid-19 patients. The *death spiral* can be summarized as follows: Increased (1) inflammation at the lungs causes (2) EC injury, which can result in activation of the (3a) coagulation cascade and/or (3b) vascular leakage at the lungs, thereby causing (4) hypoxia which further increases inflammation, creating two closed positive feedback loops and causing severe Covid-19 through a *death spiral*. *As described in the text, this inflammation is initiated by various factors, primarily by a hyperimmune response to infection of SARS-CoV-2 (cytokine storm) but also other factors such as hyperinsulinaemia/hyperglycaemia or hypercholesterolaemia.

biguanides (metformin) and antidepressants. They are indicated in **Figure 1** as blue boxes, where blunted blue arrows (⊣) denote antagonize or inhibit and pointed blue arrows (→) denote up-regulate or facilitate.

Although larger studies of how the *health factors* and *pharmaceutical interventions* influence a person's risk for CHD are usually available, Covid-19 data are often limited. Nevertheless, several studies exist that evaluated the effect of many *health factors* and *pharmaceutical interventions* on Covid-19 severity. Limitations of these studies are that they vary in study size and design i.e., some studies are case-control studies hence only reporting odds ratio (OR), whereas others are cohort studies or clinical trials that report on relative risks (RR) or hazard ratios (HR).

Unfortunately, RR, HR and OR are not the same and should only be compared in cases where the event being assessed is rare in the control group. In other words, the baseline risk of the control group should approximately be zero. However, at present it is the best information we have. Until better data becomes available, these studies were used as an initial indicative comparison between the effect what *health factors* and *pharmaceutical interventions* have on CHD risk and Covid-19 severity. This also applies to the data used to compare the risk between coagulation and Covid-19 severity in section The Death Spiral: Inflammation, EC Injury, Coagulation, Vascular Leakage and Hypoxia.

In this paper the comparison of the data between CHD risk and Covid-19 severity was graphically reported using a non-traditional method (23–28). The risks that indicate an increase in disease severity are displayed as reported, whereas the risk values that show a decrease in severity are presented as the inverse of the reported value.

This method presents a better visual illustration when comparing an increase and decreased risk. For example, a conventional RR = 3 constitutes to a 3-fold increase in risk while a RR = 0.33 constitutes to a 3-fold decrease in risk (1/0.33 = 3). The method has also been used in previous papers (24–28).

RESULTS

Section Integrated Covid-19/CHD Model discusses **Figures 3**, **5**, **6** in detail illustrating the detrimental interplay between inflammation, EC injury, coagulation and hypoxia. This visually explains the *death spiral* seen in some Covid-19 patients.

Section Covid-19 Aggravation in Patients With Pre-existing CHD Comorbidities discusses how each pre-existing CHD comorbidity/hallmark could further aggravate this *death spiral*. *Five* figures are provided (**Figures 5**, **6**, **8–10**). These figures illustrate how patients with pre-existing *Hypercholesterolemia* (**Figure 8**), *Hyperglycemia/Hyperinsulinemia* (**Figure 9**) or *Hypertension* (**Figure 10**) could aggravate this *death spiral*. Note that **Figures 3**, **5**, **6**, **8–10** are simplified versions of **Figure 4** (the fully *integrated CHD/Covid-19 model*). Only the prominent pathways, which are needed to explain a specific phenomenon, are shown in these Figures.

In sections Effects of Different *Health Factors* on Covid-19 Severity and Effects of Different CHD *Pharmaceutical Interventions* on Covid-19 Severity the effects that *health factors*

and *pharmaceutical interventions* have on developing severe Covid-19 are discussed with reference to the model in **Figure 4**.

Integrated Covid-19/CHD Model

EC Injury From SARS-CoV-2 Viral Infection

Cell entry and pathologic effects of the SARS-CoV-2 virus mostly occur through two pathways namely, (i) the mucous membranes (primarily infecting the nasal epithelia) or (ii) the respiratory tract (infecting respiratory epithelial cells) (36). This infection typically occurs *via* ACE2 (36), which partially decreases ACE2 function. This leads to an upregulation of angiotensin II effects, including among others an enhanced *Inflammatory* response (17, 36), increased EC injury (37) and state of *Hypercoagulability* seen in severe Covid-19 patients (4, 6–9).

These effects are illustrated in **Figure 3** by following the relevant pathways (green lines with numbers) from SARS-CoV-2 (green oval) to the respective biomarkers (white boxes) or traits (orange boxes) and/or hallmarks (yellow boxes). The model will be interpreted in the following way for the rest of this paper:

a) Evidence from literature describing the pathogenesis with the respective (references).
b) These relevant pathways in **Figures 3–6**, **8**, **9** are then given to illustrate the pathogenesis. Each pathway starts with the relevant tissue, biomarker or trait.
c) The relevant pathway (pw) numbers (#) are denoted as (pw#) e.g., pathway 112 (pw112) links EC injury with vascular leakage.
d) The upwards arrow (↑) represents an upregulation of the respective biomarker/trait/hallmark while the downwards arrow (↓) represents a downregulation.

Figure 3 illustrates how viral infection from SARS-CoV-2 may lead to an activation of a pro-inflammatory state, which causes EC injury *via* the following process:

- Angiotensin II can downregulate phosphoinositide 3-kinase (PI3K) pathway, which increases insulin resistance that directly effects inflammatory state (38). The relevant pathways in **Figure 3** are: SARS-CoV-2 viral infection within the lungs *via* (pw0), which through (pw114) upregulates angiotensin II. This follows a downregulation of biomarker PI3K *via* (pw54) that increases insulin resistance through (pw69). This leads to a pro-inflammatory state *via* (pw71), which, through (pw110), results in EC injury. The notation for this pathway and the rest of the paper will be as follows: *SARS-CoV-2-(pw0)-Lungs-(pw114)-↑angiotensin II-(pw54)-↓PI3K-(pw69)-↑insulin resistance-(pw71)-↑inflammatory state-(pw110)-↑EC injury.*
- Angiotensin II can also upregulate various reactive oxygen species (ROS) at the site of infection, which causes a heightened inflammatory response (38). See **Figure 3** pathways: *SARS-CoV-2-(pw0)-Lungs-(pw114)-↑angiotensin II-(pw98)-↑ROS-(pw85)-↑inflammatory state-(pw110)-↑EC injury.*
- An upregulation of angiotensin II may increase platelet factors, which increases the risk for coagulability (38, 39). Since hypercoagulation and inflammation are interrelated, an inflammatory state may be enhanced (39). See **Figure 3** pathways: *SARS-CoV-2-(pw0)-Lungs-(pw114)-↑angiotensin II-(pw89)-↑platelet factors-(pw73)-↑Hypercoagulability-(pw73)-(pw74)-↑inflammatory state-(pw110)-↑EC injury.*
- Furthermore, an increase in platelet factors can also upregulate platelet aggregation (38). This could increase the inflammatory mediator nuclear factor-kappa-beta (NFκβ), aggregating inflammation (38). See **Figure 3** pathways: *SARS-CoV-2-(pw0)-Lungs-(pw114)-↑angiotensin II-(pw89)-↑platelet factors-(pw75)-↑platelet aggregation-(pw91)-serotonin-(pw93)-↑NFkβ-(pw55)-↑inflammatory state-(pw110)-↑EC injury.*

In addition to this pro-inflammatory state that causes EC injury, the virus can also directly cause EC injury in other organs. This could happen if the virus enters the bloodstream and binds to ACE2 receptors located in other organs (9). Considerable evidence shows that the lungs of patients who died from Covid-19, have severe EC injury (endothelialitis) associated with the presence of intracellular viral infection (4). The presence of viral particles were also found in the ECs of the liver, kidneys and heart (9, 40). This could then lead to inflammation and EC damage at the infected organ. See **Figure 3** pathways: *SARS-CoV-2-(pw0)-Lungs-(pw115)-infect other organs via blood-(pw116)-↑EC injury.*

EC Injury From a Hyperimmune Response to Infection

Infection from SARS-CoV-2 causes damage-associated molecular patterns to occur, which can trigger a hyperimmune response. Most severe cases of patients with Covid-19 display a defective hyperinflammatory state with significantly increased serum levels of pro-inflammatory cytokines and chemokines (41–44).

This overproduction of pro-inflammatory cytokines and chemokines can damage lung infrastructure and further induce EC injury of pulmonary blood vessels (17, 20, 45), see **Figure 3** pathways: *SARS-CoV-2-(pw0)-Lungs-(pw107)-↑pro-inflammatory cytokines & chemokines-(pw108)-↑inflammatory state-(pw110)-↑EC injury.*

Most critical cases show increased levels of, among others, the pro-inflammatory cytokines interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α) (41–43). These pro-inflammatory cytokines directly cause an upregulation of inflammation (45). See **Figure 3** pathways: *SARS-CoV-2-(pw0)-Lungs-(pw107)-↑pro-inflammatory cytokines-(pw108)-↑TNF-α, IL-6-(pw41)-↑inflammatory state-(pw110)-↑EC injury.*

These cytokines can also indirectly upregulate inflammation through dysregulation of platelet factors (46). See **Figure 3** pathways: *SARS-CoV-2-(pw0)-Lungs-(pw107)-↑pro-inflammatory cytokines-(pw108)-↑IL-6, IL-8-(pw76)-↑platelet factors-(pw74)-↑inflammatory state-(pw110)-↑EC injury.*

Furthermore, a hyperinflammatory state induced by an unmodulated immune response can also cause EC injury. This happens when neutrophils activate pathways that elevate reactive oxygen species (ROS) (22, 47). See **Figure 3** pathways: *SARS-CoV-2-(pw0)-Lungs-(pw109)-↑ROS-(pw85)-↑inflammatory state-(pw110)-↑EC injury.*

A hyperinflammatory response of cytokines can circulate to other organs. This could lead to acute inflammation such as

septic shock and/or multiple organ damage, which may further cause EC injury (48). See **Figure 3** pathways: *SARS-CoV-2-(pw0)-Lungs-(pw107)-pro-inflammatory cytokines & chemokines-(pw115)-other organs-(pw116)-↑EC injury*.

The Death Spiral: Inflammation, EC Injury, Coagulation, Vascular Leakage and Hypoxia

Note that *hypoxia* shown in **Figures 1**, **3**–**6** includes hypoxemia. Although hypoxia might be respiratory related, vascular related EC injury could be one of the main factors fueling this hypoxia (45, 49, 50). This vascular related hypoxia may result from either hypercoagulation or vascular leakage, both stemming from EC injury (17, 22, 45, 46, 49, 50).

Vascular leakage from EC injury leads to an increase in leucocytes and platelets as well as vascular permeability (50). This results in fluid from the blood to enter the alveoli, filling the alveolar space. In turn it decreases the efficiency of gas exchange in the lungs (50). This prevents the body from taking in sufficient oxygen, leading to different severity levels of hypoxia (50). These pathways are denoted in **Figure 5** as: *EC injury-(pw112)-↑vascular leakage-(pw113)-↑hypoxia*.

On the other hand, coagulation stemming from EC injury articulates glycoproteins that are involved in hemostasis, to which platelets bind. This consequently upregulates the expression of platelet tissue factors, which are the prime activators of a coagulation cascade (22, 51). This leads to a high possibility of disseminated intravascular coagulation, congestion of the small capillaries by inflammatory cells and thrombosis in larger vessels (45).

Congestion or clogging of pulmonary blood vessels could increase hypoxemia *via* ventilation/perfusion mismatch and low level of mixed venous blood oxygen (49). This build-up of blood clots in blood vessels within the lungs are commonly found in critically ill and non-surviving Covid-19 patients (6, 21, 52). These pathways are denoted in **Figure 5** as: *EC injury-(pw111)-↑Hypercoagulability-(pw73)-↑platelet factors-(pw42)-↑hypoxia*. Hypoxia also results in further upregulation of inflammation by activating IL-6 & TNF-α (53) or increasing ROS leading to further EC injury (54). See **Figure 5** pathways: *Hypoxia-(pw60)-↑TNF-α, IL-6-(pw41)-↑inflammatory state* or *Hypoxia-(pw61)-↑ROS-(pw85)-↑inflammatory state-(pw110)- EC injury*.

With the aforementioned knowledge a summary of the main pathogeneses describing the *death spiral* are given. Note that inflammation has two different outgoing pathways (loops) that can lead to increased hypoxia. Both pathways are denoted in **Figure 5** as follows:

1. **Hypercoagulability (positive feedback loop 1):** Inflammation from Covid-19 results in EC injury which may activate the coagulation cascade, forming microthrombi in the blood vessels near the alveoli (4, 6–9). This reduces oxygenation efficiency, see pathways: *↑inflammatory state –(pw110)-EC injury-(pw111)-↑Hypercoagulability-(pw73)-↑platelet factors-(pw42)-↑hypoxia-(pw60)-↑TNF-α, IL-6-(pw41) AND/OR (pw61)-↑ROS-(pw85)-↑inflammatory state-(pw110)-Loop repeated-(pw120)-Severe Covid-19*.
2. **Vascular leakage (positive feedback loop 2):** Inflammation from Covid-19 results in EC injury. EC injury in blood vessels near the alveoli can lead to vascular leakage (22). This causes fluid build-up within the alveoli (50), subsequently reducing oxygenation efficiency, see pathways: *↑inflammatory state –(pw110)-EC injury-(pw112)-↑vascular leakage-(pw113)-↑hypoxia-(pw60)-↑TNF-α, IL-6-(pw41) AND/OR (pw61)-↑ROS-(pw85)-↑inflammatory state-(pw110)-Loop repeated-(pw120)-Severe Covid-19*.

A simplified schematic of the *death spiral* is illustrated in **Figure 6**, which shows the two closed positive feedback loops leading to hypoxia. If a Covid-19 patient becomes hypoxic, it is important to break these loops by administering supplemental oxygen. This is currently done in practice where supplemental oxygen reduces disease severity in hypoxic Covid-19 patients (55).

To reduce the risk of developing hypoxia one should focus on reducing inflammation that leads to the downstream effects namely EC injury, coagulation and vascular leakage. This is also seen in practice where various pharmaceutical interventions that treat inflammation have shown promising results e.g., corticosteroid dexamethasone in later stage of illness (56) and anti-inflammatory drugs [Celebrex (57) and aspirin (58)].

If we focus on loop 1 it is expected that people who have a higher risk of developing blood clots (coagulation) should have a higher risk of developing severe Covid-19. There are several uncontrollable factors that are known to increase a person's risk of developing blood clots namely, gender, age, ethnicity, blood type and pregnancy.

Although this does not help the patient, it is of interest to help understand Covid-19 severity in these individuals. The data for the risk of coagulation (blood clots) and Covid-19 severity for these individuals are given in **Table 1**. A qualitative graphical comparison between the data for coagulation and Covid-19 severity from **Table 1** is given in **Figure 7**.

Age

Age is an independent risk factor of coagulation, with thrombotic incidences increasing rapidly in people older than 70 years (59). The odds of venous thromboembolism in a person older than 70 years is three times higher than a person young than 70 years, OR of 3.1 (59).

If we investigate Covid-19 mortality data, a similar trend is seen with age. Risk of mortality due to Covid-19 is much higher in older patients with a RR of 3.61 in patients older than 70 years (60), see **Figure 7**. The increased risk of coagulation due to older age could be one reason for this increased Covid-19 mortality.

Gender

A 25-year population-based study showed that males have a higher risk to coagulate than females (68). At younger ages (<45 years) females have a higher risk of coagulation than males, for various reproductive reasons (61). However, since an increase in Covid-19 severity and mortality is typically seen in older patients (> 45 years) we only focused on these older patients. Men have a 1.9-fold higher risk of developing venous thrombosis than women (61).

TABLE 1 | Data for the qualitative comparison of risk factors between coagulation and Covid-19 severity.

Uncontrollable factor	Risk for coagulation					Risk of covid-19 severity				
	Study size (n = no. of participants, N = no. of studies)	RR/OR	Value	95% CI	References	Study size (n = no. of participants, N = no. of studies)	RR/OR	Value	95% CI	References
Age > 70 years	$n = 607$, $N = 1$	OR	3.10	1.3–7.5	(59)	$n = 36\,470$, $N = 59$	RR	3.61	2.70–4.84	(60)
Male vs. Female	$n = 11\,253$, $N = 1$	RR	1.90	1.9–2.4	(61)	$n = 36\,470$, $N = 59$	RR	1.50	1.18–1.91	(60)
Black vs. Caucasian	#	RR	1.50	#	(62)	$n = 505\,992$, $N = 1$	OR	1.60	1.2–2.0	(63)
Blood Type A vs. Type O	$n = 406\,755$, $N = 1$	HR	1.44	1.39–1.50	(64)	$n = 31\,100$, $N = 4$	OR	1.41	*	(65)
Blood Type B vs. Type O	$n = 406\,755$, $N = 1$	HR	1.45	1.37–1.54	(64)	$n = 31\,100$, $N = 4$	OR	1.69	*	(65)
Pregnant vs. Non-pregnant	$n = 1\,142$, $N = 1$	OR	4.60	2.7–7.8	(66)	$n = 22\,493$, $N = 1$	OR	2.35	1.48–3.74	(67)

1. CI, Confidence Interval; HR, Hazard Ratio; OR, Odds Ratio; RR, Relative Risk.

2. (#) Denotes that the study did not provide this data.

3. (*) Study (65) only provides the 95% CI for each Blood Type separately and not the Blood Type vs. Blood Type O. These individual 95% CI's for Blood Type A, B, and O were (1.11–1.40), (0.99–1.21), and (0.63–0.77), respectively. These data were not included in the table since the OR's for each Blood Type were reported separately. Here we normalized the OR's of Blood Type A vs. O and Blood Type B vs. O.

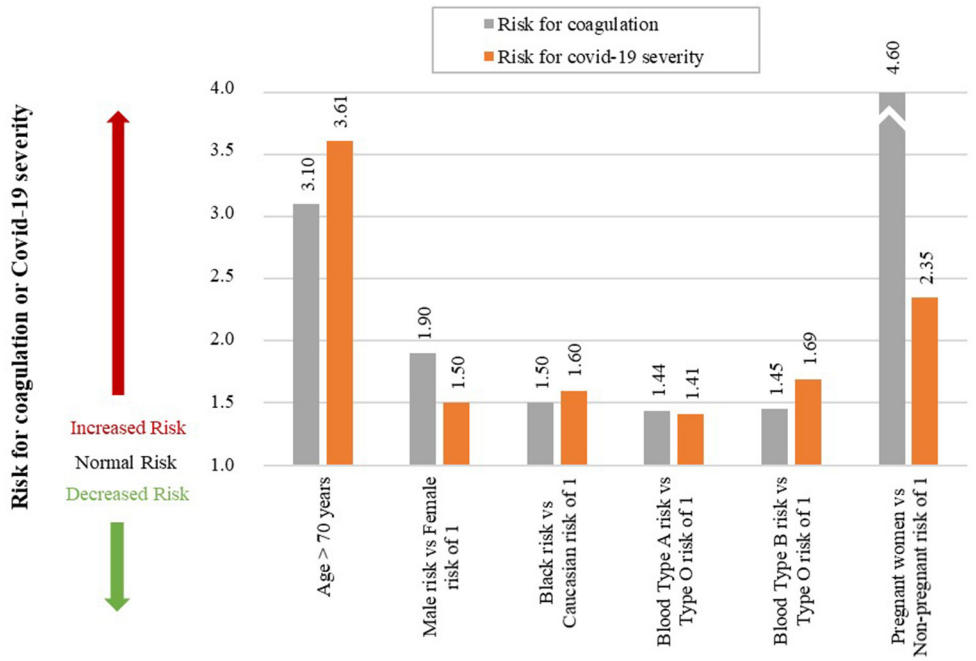


FIGURE 7 | Qualitative comparison of risk factors between coagulation and Covid-19 severity. (An accurate quantitative comparison is not possible, mostly due to differences in study design and size).

Covid-19 data also indicate that males have a higher risk of Covid-19 mortality than females, with a RR of 1.50 (60), see **Figure 7**. The increased risk of coagulation due to gender for individuals older than 45 years could be one reason for this increased Covid-19 mortality.

Ethnicity

Ethnicity has also shown to be an independent risk factor for coagulation. The highest risk of thrombosis being in African Americans, with a RR of 1.5 compared to Europeans (62). This is also seen in Covid-19 mortality data, which shows that African American's have a higher odds of death than Europeans, with an OR of 1.6 (63), see **Figure 7**. The increased risk of coagulation due to ethnicity could be one reason for this increased Covid-19 mortality.

Blood Type

Another risk factor that seems to influence the odds of developing a thromboembolic event is a person's blood type. A single cohort study showed that blood types A&B vs. O have higher risk of developing a thromboembolic event, with the following HRs: A vs. O of 1.44, and B vs. O of 1.45 (64), see **Figure 7**.

A similar trend is seen in the effect of different blood types on Covid-19 severity, with the following ORs: A of 1.06, B of 1.27, O of 0.75 (65). If these values are normalized with respect to blood type O the ORs are the following: A vs. O of 1.41, and B vs. O of 1.69, see **Figure 7**.

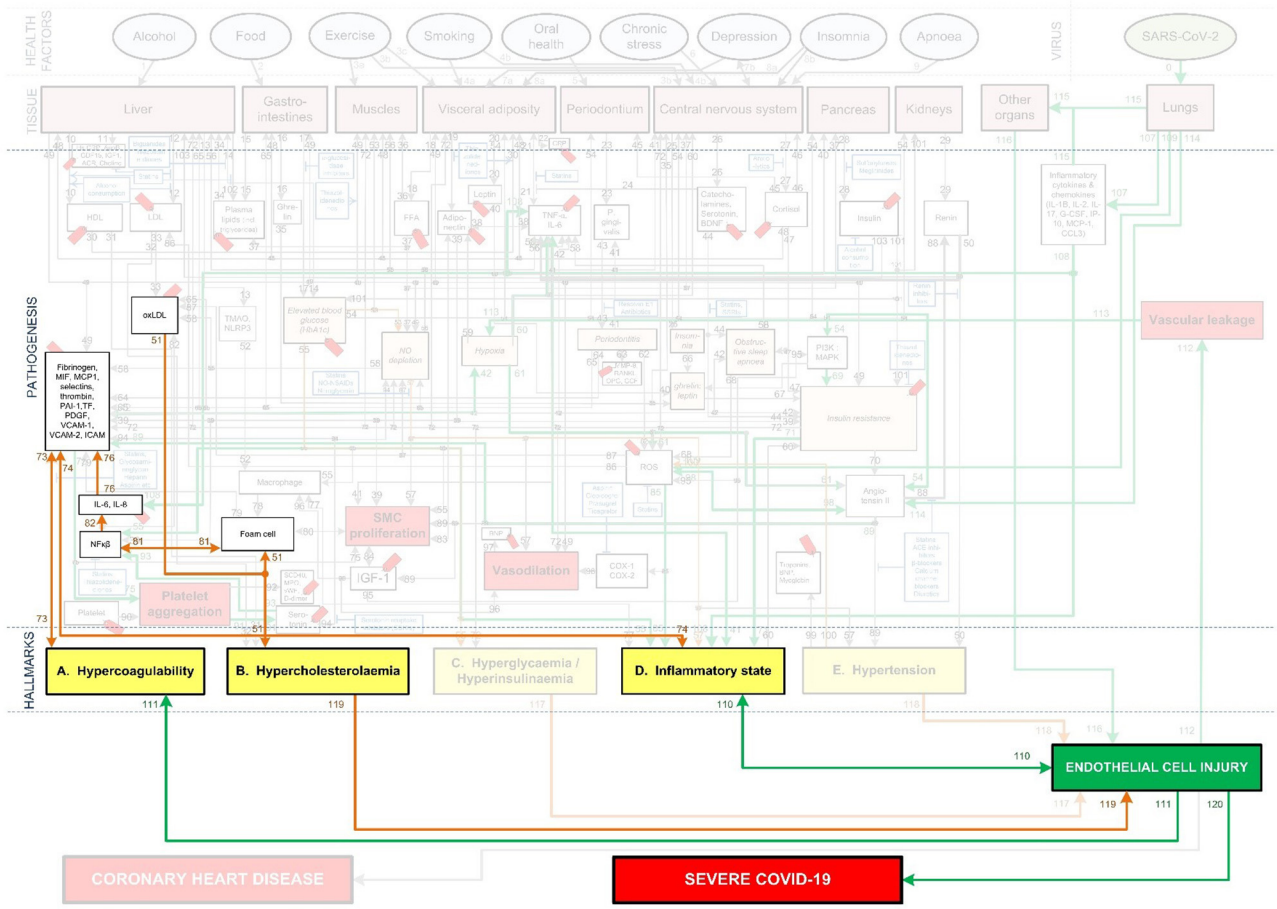


FIGURE 8 | CHD related aggravation of severe Covid-19 in patients with high cholesterol.

None of the blood group values for Covid-19 severity were statistically significant (65). It is however interesting that this limited study shows that patients with blood type O have lower odds of developing severe Covid-19 than blood types A and B. There is however still controversy regarding correlation between blood type and Covid-19 severity (69).

Pregnancy

Pregnancy is not necessarily an uncontrollable factor, but for the duration of being pregnant it is. During pregnancy the risk of venous thrombosis is much higher than for non-pregnant women, with an OR of 4.6 (66), see **Figure 7**.

Pregnant women are also at a higher risk of developing more severe Covid-19 complications than non-pregnant women, with an OR of 2.35 (67). Fortunately, no significant association between pregnant and non-pregnant women was found for Covid-19 mortality risk (67). This may be due to pregnant women seeking medical attention earlier than non-pregnant women. The higher severity risk could partially be due to the higher risk for coagulation during pregnancy. More research is however needed to validate this.

The above mentioned uncontrollable factors may contribute to the coagulation loop 1 of the *death spiral*. This could help explain why some patients experience accelerated disease severity. However, better studies for Covid-19 in especially different blood groups are needed.

The high mortality statistics in patients with pre-existing CHD comorbidities (10–12, 70) are discussed in more detail in the next Section with reference to **Figures 8**–**10**. We will show why a patient with a worse cardiovascular "baseline" before Covid-19 could potentially have a worse outcome than a patient with a healthy cardiovascular "baseline."

Covid-19 Aggravation in Patients With Pre-existing CHD Comorbidities

Severe Covid-19 Patients With Existing Chronic Hypercholesterolemia

One of the risk factors for CHD is *Hypercholesterolemia*. Chronic *Hypercholesterolemia* may fuel the Covid-19 *death spiral* by increasing the risk of EC injury *via* an inflammatory state or plaque buildup. For EC injury induced by an inflammatory state see **Figure 8** pathways: *↑oxLDL-(pw51)-Hypercholesterolemia-(pw51)-↑foam cell-(pw81)-↑NFkβ-(pw82)-↑IL-6, IL-8-(pw76)-↑platelet factors-(pw74)-↑inflammatory state-(pw110)-↑EC injury*. For EC injury induced by

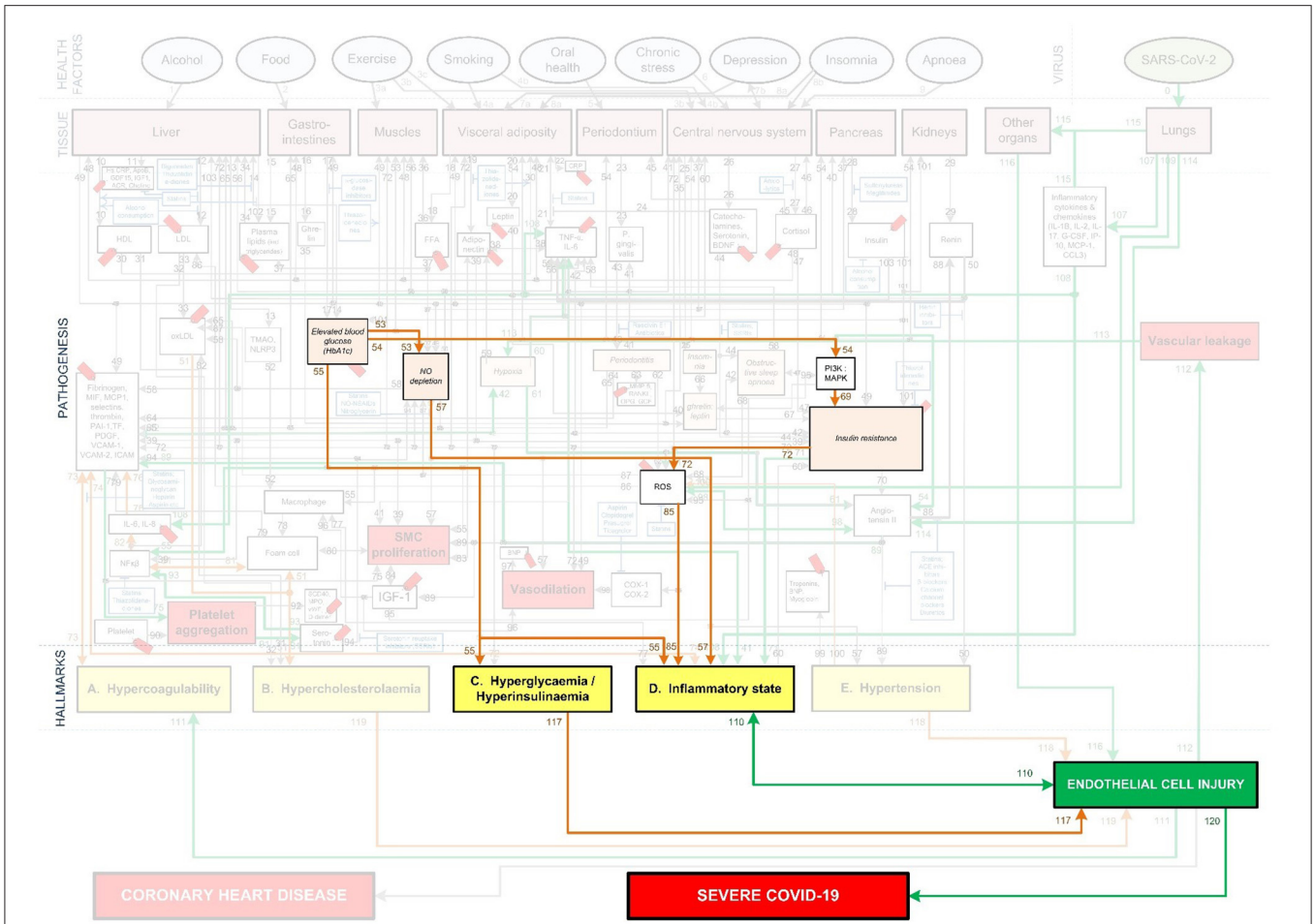


FIGURE 9 | CHD related aggravation of severe Covid-19 in patients with high blood glucose levels.

plaque buildup see **Figure 8** pathways: *↑oxLDL-(pw51)-Hypercholesterolemia-(pw119)-↑EC injury*.

Hypercholesterolemia could also have an impact on the severity of Covid-19 by increasing coagulation. This could happen by increased foam cell production and increased thrombin generation (29). In turn increasing the platelet forming factors and reducing breakdown processes like fibrinolysis, increases coagulation (30). See **Figure 8** pathways: *↑oxLDL-(pw51)-Hypercholesterolemia-(pw51)-↑foam cell-(pw81)-↑NFkβ-(pw82)-↑IL-6, IL-8-(pw76)-↑platelet factors-(pw73)-↑Hypercoagulability*.

The increased coagulation could aggravate thrombi within the lungs and lead to possible hypoxemia (49), potentially cascading the symptoms already experienced by a Covid-19 patient.

The above discussion partially explains why many patients with obesity have a high risk of developing severe Covid-19 complications (71) as obesity is associated with *Hypercholesterolemia* (72, 73).

Interestingly it was also found that free cholesterol, as well as high-and low-density lipoprotein levels are lower in end-stage Covid-19 patients than in patients with less severe Covid-19 (70, 74). Why would cholesterol levels be lower in patients with more severe disease? Could this be explained by the ability of SARS-CoV-2 to use ("consume") serum cholesterol for its entry into host cells (32).

If this is the case, then high cholesterol levels before infection might enhance viral infection *via* increased availability of serum cholesterol levels but as the virus "consumes" cholesterol the levels would decrease. These facts are however still controversial and further studies are warranted.

Severe Covid-19 Patients With Existing Chronic Hyperglycemia or Hyperinsulinemia

Elevated blood glucose aggravates Covid-19 severity and mortality risk irrespective of diabetes (75, 76). One possible reason for this could be the indirect ability of blood glucose to induce EC injury.

Since glucose is the main energy source for cells, any change to its levels could have a direct effect on the cell's metabolism. Changes in blood glucose can cause ECs to undergo apoptosis (cell death or "suicide"), causing the ECs to detach and enter the bloodstream (77). See **Figure 9** pathways:

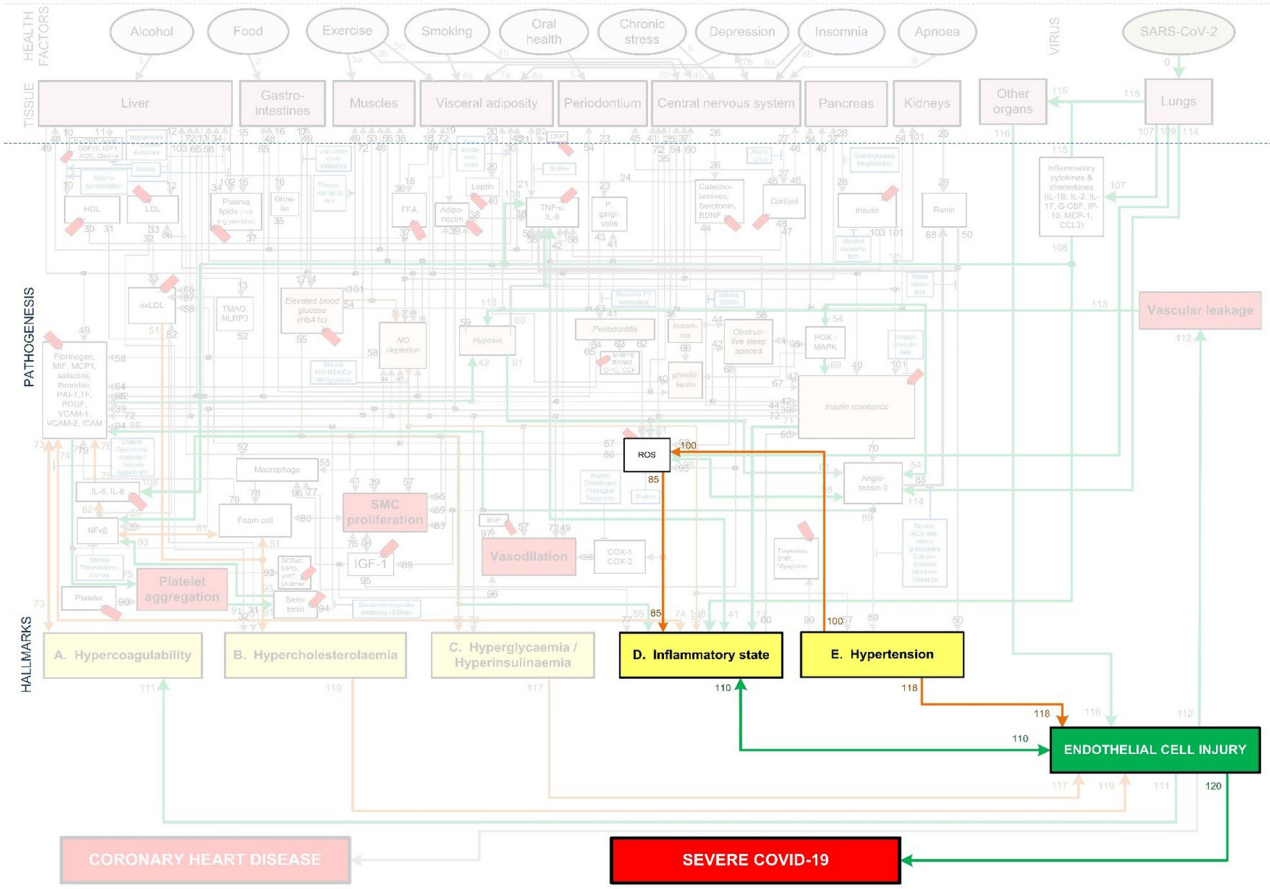


FIGURE 10 | CHD related aggravation of severe Covid-19 in patients with chronic hypertension.

Elevated blood glucose (HbA1c)-(pw55)-Hyperglycemia-(pw117)-↑EC injury. This further leaves behind eroded arteries which activate processes that lead to atherosclerosis, such as smooth cell proliferation (77).

Another pathway through which elevated blood glucose levels contribute to EC injury is through aggravated inflammation. This inflammation is caused by activating the insulin resistance and ROS producing pathways and impaired EC turnover. See **Figure 9** pathways: *Elevated blood glucose (HbA1c)-(pw54)-PI3K:MAPK-(pw69)-↑Insulin resistance-(pw72)-↑ROS-(pw85)-↑inflammatory state-(pw110)-↑EC injury*. EC turnover is possibly impaired due to accelerated aging or reduced renewal of cells (78, 79). This is most prominent in the microvascular and arterial ECs (80), which may be due to the differences in glucose uptake of cells.

A similar pathway also leads to increased inflammation due to a dysregulation of NO, which plays an important role in controlling the vascular tone and arterial pressure. A decrease in NO prevents ECs from responding to increased glucose stress, which may further accelerate cellular deterioration (79). See **Figure 9** pathways: *Elevated blood glucose (HbA1c)-(pw55)-Hyperglycemia-(pw55)-↑inflammatory state-(pw110)-EC injury*.

These indirect impacts on EC injury could potentially explain why *Hyperglycemia* is a significant co-morbidity and risk factor for severe Covid-19 patients (70). It highlights the importance of ensuring that the glucose level of a diabetic patient remains within normal ranges. It may also be advantageous to reduce blood glucose levels in non-diabetic patients as elevated glucose in non-diabetic patients also increased Covid-19 severity (75, 76).

Severe Covid-19 Patients With Existing Chronic Hypertension

Hypertension is another common co-morbidity in Covid-19 related mortality (81). This could be due to its indirect ability to increase inflammation or the direct injury caused to ECs (82, 83).

The indirect impact occurs through hypertension that increases the amount of ROS, especially from the oxidation of endothelial NO synthesis (83). ROS can impact the inflammatory state and the ECs in several ways. It can, among others, cause EC death and increase the adhesion of inflammatory cells to the normally inert endothelium surface (83). This could potentially exacerbate the response and symptoms related to EC injury. See **Figure 10** pathways: *Hypertension-(pw100)-↑ROS-(pw85)-↑Inflammatory state-(pw110)-↑EC injury*.

Chronic hypertension can also directly cause damage to the microvascular ECs (82). High blood pressure strains the ECs and could potentially cause ruptures in plaques that are adhered to the artery wall (82). See **Figure 10** pathways: *Hypertension-(pw118)-EC injury*. This creates additional areas that require attention and would probably also increase the inflammatory response.

Existing chronic hypertension can therefore possibly cause injury to the ECs through either the indirect or direct pathways. This injury could potentially contribute to the rapid worsening of health in Covid-19 patients with chronic hypertension (81).

Effects of Different *Health Factors* on Covid-19 Severity

We discuss the comparison between CHD and severe Covid-19 for different *health factors* with reference to **Figure 4**. The definition of each *health factor* was given in section Evaluation of *Health Factors* and *Pharmaceutical Interventions*.

Different *health factors* (pink ovals in **Figure 4**) were originally analyzed in terms of their effects on CHD risk (23). These *health factors* were either associated with an increase or decrease in risk for CHD (23–28). The same *health factors* were investigated for Covid-19 severity. We will show to what extent a healthy CHD "baseline," as a result of a healthy lifestyle, will influence Covid-19 severity.

Table 2 summarizes the CHD and Covid-19 data extracted from literature namely, study size (N), number of participants (n), risk type (RR/HR/OR), respective risk value, 95% confidence interval (CI), fold change (as calculated *via* the non-traditional method) and the respective references. Data not statistically significant are indicated with an (*) in **Figure 11**.

Where data were unavailable a hash (#) was inserted in **Table 2** e.g., for the two *health factors*, alcohol use and food intake (high glycemic diets). These *health factors* have not yet been fully investigated in Covid-19 patients. Despite no risk values being available for these *health factors,* their probable effects on Covid-19 severity are discussed in this section.

The *health factors* that increase/decrease a person's risk for CHD similarly increase/decrease a person's risk (RR/HR/OR) for developing severe Covid-19 (**Figure 11**). In the rest of this section we will discuss, in more detail, the effects each *health factor* has on the CHD hallmarks, and hypothesize how this could affect Covid-19 severity.

Moderate Exercise

Based on the CHD model (**Figure 1**) our research group has published a detailed description of the mechanism by which moderate exercise may reduce CHD risk (28). Only the salient features of the mechanism will be described here.

Regular moderate exercise is universally accepted to reduce the risk of CHD (23, 28, 84) (the definition of moderate exercise was given in section Evaluation of *Health Factors* and *Pharmaceutical Interventions*). **Table 2** shows a decrease risk (RR) of 0.75 (n = 645 087, N = 33) (84). This translates to a 1.33-fold decrease in CHD risk (23, 28) as illustrated in **Figure 11**.

The effect of moderate exercise on Covid-19 was analyzed in a small cross-sectional study (n = 260) (85). The authors concluded that moderate physical activity before onset of Covid-19 decreases the odds of developing severe Covid-19 (OR of 0.28) by 3.57 times (85), see **Table 2** and **Figure 11**. Although this is only a small study, a larger study (n = 48 440) substantiates the benefit of regular moderate exercise (118).

This larger study's results are not presented in **Table 2** or **Figure 11** since the study reported on inactivity. However, since being active helps reduce the odds of developing severe Covid-19, inactivity is expected to have an opposite effect. This is indeed the case as the study showed that patients who are consistently inactivate are 2.49 (OR) times more likely to die from Covid-19 (118).

Therefore, moderate exercise before the onset of disease decreases both the risk for CHD and Covid-19 severity. This could most likely be explained by the effect of moderate exercise on several CHD hallmarks. Moderate exercise largely influences, among others, glucose, cortisol and inflammatory mediator levels (23, 28), therefore reducing the risk of *Hyperglycemia/Hyperinsulinemia* and a heightened *Inflammatory state* (23, 28).

Regular exercise also reduces the accumulation of visceral fat, which reduces the risk of increased Low-Density Lipoprotein (LDL) levels thus decreasing the risk for *Hypercholesterolemia* (23, 28). A decrease of visceral fat also reduces the risk of insulin resistance, which lowers one's risk for increased platelet factors and the potential for *Hypercoagulability* (23, 28).

The potential decrease of these CHD hallmarks could partially explain the benefit of moderate exercise on the reduced risk of Covid-19 severity. The respective CHD hallmark downregulated by exercise and the activated pathways are denoted in **Figure 4** as follows:

- **Hyperglycemia/Hyperinsulinemia:** *Moderate exercise-(pw3a)-muscles-(pw53)-↓blood glucose-(pw54)-↓PI3K:MAPK-(pw69)-↓insulin resistance-(pw72)-Hyperglycemia/ Hyperinsulinemia.*
- **Inflammatory state:** *Moderate exercise-(pw3b)-central nervous system-(pw27)-↓cortisol-(pw47)-↓insulin resistance-(pw70)-↓angiotensin II-(pw89)-↓hypertension-(pw100)-↓ROS-(pw85)-↓COX1/2-(pw85)-↓Inflammatory state.*
- **Hypercholesterolemia:** *Moderate exercise-(pw3c)-visceral adiposity-(pw18)-↓FFA-(pw37)-↓plasma lipids-(pw34)-liver-(pw12)-↓LDL-(pw33)-↓oxLDL-(pw51)-↓Hypercholesterolemia.*
- **Hypercoagulability**: *Moderate exercise-(pw3a)-muscles-(pw53)-↓blood glucose-(pw54)-↓PI3K:MAPK-(pw69)-↓insulin resistance-(pw72)-↓platelet factors-(pw73)-↓Hypercoagulability.*

The potential decrease in four of the five CHD hallmarks due to moderate exercise before onset of Covid-19 (creating a healthier vascular system "baseline") could partially explain the decreased risk of Covid-19 severity. These beneficial effects of exercise are based on moderate exertion and not heavy exertion. Heavy exertion exercise has the following detrimental effects: transient

TABLE 2 | The effect which different health factors and pharmaceuticals have on CHD risk and Covid-19 severity.

	Risk for CHD					References	Risk for increased COVID-19 severity					References
	Study size (*n* = no. of participants, *N* = no. of studies)	**RR, HR or OR**	**Value**	**95% CI**	**Fold change as per our definition**		**Study size (*n* = no. of participants, *N* = no. of studies)**	**RR, HR or OR**	**Value**	**95% CI**	**Fold change as per our definition**	
HEALTH FACTORS												
Moderate exercise	*n* = 645 087, *N* = 33	RR	0.75	(0.71–0.79)	−1.33	(23, 84)	*n* = 260, *N* = 1	OR	0.28	#	−3.57	(85)
Smoking	*n* = 1 010 000, *N* = 141	RR	1.72	(1.62–1.83)	1.74	(23, 86)	*n* = 32 849, *N* = 47	RR	1.98	(1.16–3.38)	1.98	(87)
Oral health	*n* = 147 821, *N* = 7	RR	1.34	(1.27–1.42)	1.34	(23, 27, 88)	*n* = 568, *N* = 1	OR	8.81	(1.00–77.70)	8.81	(89)
Stress	*n* = 24 767, *N* = 1	OR	2.17	(1.84–2.55)	2.17	(23, 90)	*n* = 535, *N* = 1	HR	1.40	(1.11–1.75)	1.40	(91)
Depression	*n* = 124 509, *N* = 21	RR	1.90	(1.49–2.42)	1.90	(23, 92)	*n* = 421 014, *N* = 1	OR	2.68	(2.03–3.54)	2.68	(93)
Apnoea	*n* = 1 436, *N* = 1	HR	2.06	(1.10–3.86)	2.06	(23, 94)	*n* = 15 835, *N* = 4	OR	2.37	(1.14–4.95)	2.37	(95)
Insomnia	*n* = 122 501, *N* = 13	RR	1.45	(1.29–1.62)	1.45	(23, 96)	*n* = 568, *N* = 1	OR	1.09	(0.44–2.71)	1.09	(97)
Moderate alcohol	*n* = 504 651, *N* = 29	RR	0.71	(0.66–0.77)	−1.41	(23, 26, 98)	#	#	#	#	#	#
Food (HGD)	*n* = 220 050, *N* = 8	RR	1.36	(1.13–1.63)	1.36	(23, 25, 99)	#	#	#	#	#	#
PHARMACEUTICALS												
Statins	*n* = 169 138, *N* = 26	RR	0.78	(0.76–0.80)	−1.28	(23, 100)	*n* = 13 981, *N* = 1	HR	0.58	(0.43–0.80)	−1.72	(101)
Salicylates (Aspirin)	*n* = 112 000, *N* = 6	RR	0.82	(0.75–0.90)	−1.22	(23, 102)	*n* = 412, *N* = 1	HR	0.53	(0.31–0.90)	−1.89	(58)
Indirect thrombin inhibitors (Heparin)	*n* = 31 402, *N* = 6	OR	0.91	(0.84–0.98)	−1.10	(23, 103)	*n* = 449, *N* = 1	OR	0.37	(0.15–0.90)	−2.70	(104)
Direct thrombin inhibitors (Angiomax)	*n* = 1 883, *N* = 1	HR	0.76	(0.59–0.98)	−1.32	(23, 105)	*n* = 103 703, *N* = 1	HR	0.90	(0.71–1.15)	−1.11	(106)
ACE inhibitors	*n* = 19 141, *N* = 8	OR	0.79	(0.71–0.88)	−1.27	(23, 107)	*n* = 19 486, *N* = 1	HR	0.89	(0.75–1.06)	−1.12	(108)
Angiotensin-renin inhibitors	*n* = 108 212, *N* = 26	OR	0.92	(0.87–0.97)	−1.09	(23, 109)	*n* = 2,877, *N* = 1	RR	0.65	(0.45–0.94)	−1.54	(110)
β-blockers	*n* = 12 825, *N* = 9	RR	0.69	(0.59–0.82)	−1.45	(23, 111)	*n* = 101 141, *N* = 8	OR	1.23	(0.74–2.04)	1.23	(112)
Calcium channel blockers	*n* = 10 136, *N* = 8	OR	0.83	(0.67–1.03)	−1.20	(23, 107)	*n* = 106 566, *N* = 8	OR	0.94	(0.8–1.10)	−1.06	(112)
Diuretics	*n* = 192 478, *N* = 42	RR	0.79	(0.69–0.92)	−1.27	(23, 113)	*n* = 99 669, *N* = 5	OR	0.96	(0.81–1.15)	−1.04	(112)
Biguanides (Metformin)	*n* = 11 385, *N* = 6	OR	0.74	(0.62–0.89)	−1.35	(23, 114)	*n* = 1 800 005, *N* = 1	HR	0.77	(0.73–0.81)	−1.30	(115)
Antidepressants	*n* = 93 653, *N* = 1	HR	0.48	(0.44–0.52)	−2.08	(23, 116)	#	#	#	#	#	#

CHD, Coronary Heart Disease; CI, Confidence Interval; Covid-19, Coronavirus Disease of 2019; HGD, High Glycemic Diets; HR, Hazard Ratio; OR, Odds Ratio; RR, Relative Risk. A minus sign shows a reduction in risk. (#) denotes that the respective study did not provide this data. A small preliminary study (117) on the effect of the SSRI antidepressant, fluvoxamine, on Covid-19 has shown positive effects. Risk data were not given.

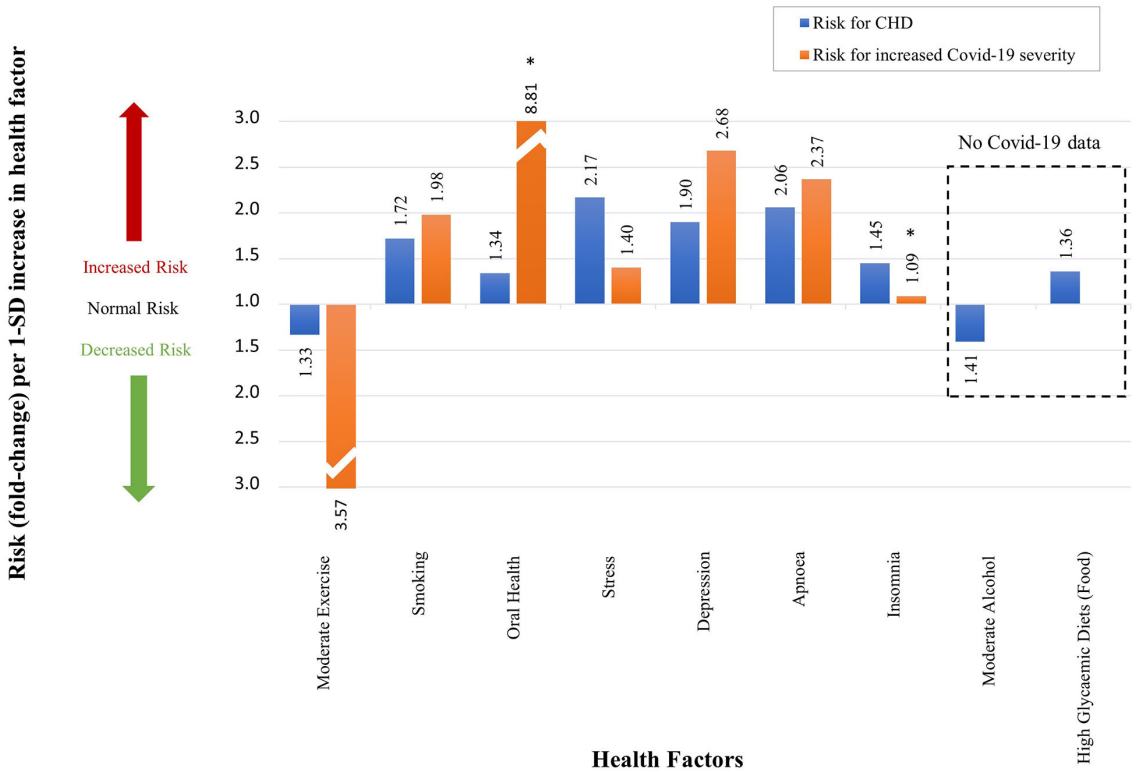


FIGURE 11 | The qualitative effect which different health factors have on CHD risk and Covid-19 severity. (An accurate quantitative comparison is not possible, mostly due to differences in study design and size).

immune dysfunction, elevated inflammatory biomarkers, and increased risk of upper respiratory tract infections (119). Therefore, exercise exertion is an important factor to consider during the Covid-19 pandemic.

Smoking

Smoking is a risk factor for CHD with a RR of 1.72 (86). A recent systematic review and meta-analysis of 47 studies (32 849 hospitalized Covid-19 patients) showed that current smokers have an increased risk of developing severe or critical Covid-19, RR of 1.98 (87).

Most smokers develop insulin resistance and/or Hyperinsulinemia as compared to non-smokers (120, 121). This association may either be due to the lower adiponectin levels or higher cortisol secretion levels seen in current smokers compared to non-smokers (122, 123). This increases a smoker's risk for *Hyperglycemia/Hyperinsulinemia.*

Moreover, most smokers also have higher plasma triglyceride and lower High-Density Lipoprotein (HDL) cholesterol concentrations than non-smokers (121, 124). This increases a smoker's risk of *Hypercholesterolemia.*

Another CHD hallmark that is upregulated in smokers is a heightened *Inflammatory state*. This is due to an upregulation of several inflammatory markers and cytokines such as TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF) and monocyte chemoattractant protein (MCP-1) (125).

Smoking also induces an imbalance between various hemostatic molecules in the blood thereby increasing the state of *Hypercoagulability* (126). This may be due to functional changes in clotting factors such as fibrinogen (126).

The associated pathways and respective CHD hallmarks increased by smoking are shown in **Figure 4** as the following:

- **Hyperglycemia/Hyperinsulinemia:** *Smoking-(pw4a)-visceral adiposity-(pw19)-↓adiponectin-(pw39)-↑insulin resistance-(pw72)- ↑Hyperglycemia/Hyperinsulinemia. Smoking-(pw4b)-central nervous system-(pw27)-↑cortisol-(pw47)-↑insulin resistance-(pw72)- ↑Hyperglycemia/Hyperinsulinemia.*
- **Hypercholesterolemia:** *Smoking-(pw4a)-visceral adiposity-(pw30)-↓HDL-(pw31)-↑ Hypercholesterolemia.*
- **Inflammatory state:** *Smoking-(pw4b)-central nervous system-(pw41)-↑TNF-α-(pw41)-↑Inflammatory state.*
- **Hypercoagulability:** *Smoking-(pw4a)-visceral adiposity-(pw49)-↑Fibrinogen-(pw73)-↑Hypercoagulability.*

The activation of these pathways and respective CHD hallmarks may explain some of the increased risk of smokers developing severe Covid-19 compared to non-smokers (87).

Oral Health

Using **Figure 1**, we published a detailed analysis of the mechanism by which oral health (in the form of periodontal disease) can influence CHD (27). Important elements relevant to this study are discussed below.

Oral health in the form of periodontal disease is known to increase the risk of CHD by 1.34-fold (88) (**Figure 11** and **Table 2**). Covid-19 patients with periodontitis have a much

higher risk of mortality, OR of 8.81 (**Figure 11** and **Table 2**) (89). This value is quite large and could be overestimated. There are several reasons for potential overestimation namely, the small study size ($n = 568$), the data is widely spread (95% CI of 1.00–77.7) and the data is not statistically significant [this statistical insignificance is illustrated on **Figure 11** with an (*)] (89).

Nevertheless, the increased risk of Covid-19 severity due to periodontitis could partially be explained by the increase in several CHD hallmarks namely, *Inflammatory state, Hypercoagulability and Hypercholesterolemia* (23, 27).

An increased risk of *Hypercoagulability* and *Inflammation* in these patients is through a common periodontitis associated bacteria, porphyromonas gingivalis (p.gingivalis) (127). This bacteria invades endothelial cells which concomitantly increases platelet activity and stimulates proinflammatory mediators/cytokines (CRP, TNF-α, and IL-6) (127).

Inflammation can also be increased *via* reactive oxygen species (ROS) which is associated with periodontal disease (23, 27). Subsequently, this also affects oxidized LDL levels pertaining to an increase in the risk for *Hypercholesterolemia* (23, 27).

The associated pathways and respective CHD hallmarks increased by oral health in the form of periodontitis are shown in **Figure 4** as the following:

- **Hypercoagulability:** *Oral health-(pw5)-periodontium-(pw23)-↑P. gingivalis-(pw43)-↑periodontitis-(pw64)-↑platelet factors-(pw73)-↑Hypercoagulability.*
- **Inflammatory state**: *Oral health-(pw5)-periodontium-(pw23)-↑P. gingivalis-(pw43)-↑periodontitis-(pw41)-↑TNFα/IL6-(pw41)-↑inflammatory state. Oral health-(pw5)-periodontium-(pw23)-↑P. gingivalis-(pw43)-↑periodontitis-(pw62)-↑ROS-(pw85)-↑inflammatory state.*
- **Hypercholesterolemia:** *Oral health-(pw5)-periodontium-(pw23)-↑P. gingivalis-(pw43)-↑periodontitis-(pw65)-↑oxLDL-(pw51)-↑Hypercholesterolemia.*

The potential increase of these CHD hallmarks due to periodontitis could partially explain the increased risk of Covid-19 severity.

Chronic Stress

Chronic stress (definition in section Evaluation of *Health Factors* and *Pharmaceutical Interventions*) is also a common factor linked to an increased risk for CHD, with an OR of 2.17 (90), presented in **Figure 11** and **Table 2**. Covid-19 severity is also increased by chronic stress with a HR of 1.4 (91), see **Figure 11**.

Chronic stress is known to elevate secretion of glucocorticoids in the form of cortisol. These high cortisol levels due to stress may elevate biomarkers such as blood glucose, TNF-α and insulin resistance (23). These stress related biomarkers are also upregulated in severe Covid-19 patients (23, 75, 76, 128–131).

The respective CHD hallmarks and activated pathways activated by chronic stress are denoted in **Figure 4** as:

- **Hypercoagulability:** *Chronic stress-(pw6)-central nervous system-(pw27)-↑cortisol-(pw48)-liver-(pw14)-↑blood glucose-(pw54)-PI3K:MAPK-(pw69)-↑insulin resistance-(pw72)-↑platelet factors-(pw73)-↑Hypercoagulability.*
- **Hypercholesterolemia:** *Chronic stress-(pw6)-central nervous system-(pw27)-↑cortisol-(pw48)-liver-(pw12)-↑LDL-(pw33)-↑oxLDL-(pw51)-↑Hypercholesterolemia.*
- **Hyperglycemia/Hyperinsulinemia:** *Chronic stress-(pw6)-central nervous system-(pw27)-↑cortisol-(pw48)-liver-(pw14)-↑blood glucose-(pw54)-PI3K:MAPK-(pw69)-↑insulin resistance-(pw72)-↑Hyperglycemia/Hyperinsulinemia.*
- **Inflammatory state:** *Chronic stress-(pw6)-central nervous system-(pw27)-↑cortisol-(pw48)-liver-(pw14)-↑blood glucose-(pw54)-PI3K:MAPK-(pw69)-↑insulin resistance-(pw70)-↑angiotensin II-(pw88)-renin-(pw50)-↑TNFα-(pw41)-↑Inflammatory state.*
- **Hypertension:** *Chronic stress-(pw6)-central nervous system-(pw27)-↑cortisol-(pw48)-liver-(pw14)-↑blood glucose-(pw54)-PI3K:MAPK-(pw69)-↑insulin resistance-(pw70)-↑angiotensin II-(pw89)- ↑Hypertension.*

Although the Covid-19 study is small ($n = 535$, see **Table 2**) stress affects all five CHD hallmarks. Future larger clinical studies are expected to emphasize the importance of stress management in patients with Covid-19.

Depression

The effect of depression on CHD, using the CHD model in **Figure 1**, was described in detail in a previous paper (24). A summary of the potential effects of depression on Covid-19 are given in the rest of this section.

Depression increases one's risk for CHD by 1.90-fold (RR) (92), shown in **Figure 11** and **Table 2**. This is also the case for Covid-19, where the odds of developing more severe disease in a person with pre-pandemic depression is 2.68-fold (OR) higher that without depression (93), see **Figure 11.**

Depression is thought to mediate, among others, over stimulation of the hypothalamic-pituitary-adrenocortical (HPA) axis induced by elevated levels of corticotropin-releasing factor and adrenocorticotropic hormone (23, 24). Chronic dysregulation of the hypothalamic-pituitary-adrenal axis can lead to increased serum levels of cortisol. Similar to chronic stress, elevated cortisol levels can increase the risk of upregulating four CHD hallmarks namely, *Inflammatory state, Hypercholesterolemia, Hypertension* and *Hyperglycemia/Hyperinsulinemia* (23, 24).

In addition to increased cortisol levels, the overstimulation of the hypothalamic-pituitary-adrenal axis may augment sympathoadrenal hyperactivity *via* central regulatory pathways. This results in increased plasma catecholamines (23, 24). An increase of catecholamines can lead to abnormalities in insulin and platelet factors thus also increasing another CHD hallmark namely, *Hypercoagulability* (23, 24).

The respective CHD hallmarks and activated pathways induced by depression are denoted in **Figure 4** as the following:

- **Hypercholesterolemia:** *Depression-(pw7b)-central nervous system-(pw27)-↑cortisol-(pw48)-liver-(pw12)-↑LDL-(pw33)-↑oxLDL-(pw51)-↑Hypercholesterolemia.*
- **Inflammatory state:** *Depression-(pw7b)-central nervous system-(pw27)-↑cortisol-(pw48)-liver-(pw14)-↑blood*

glucose-(pw54)-PI3K:MAPK-(pw69)-↑insulin resistance-(pw70)-↑angiotensin II-(pw88)-renin-(pw50)-↑TNFα-(pw41)-↑Inflammatory state.

- **Hypertension:** *Depression-(pw7b)-central nervous system-(pw27)-↑cortisol-(pw48)-liver-(pw14)-↑blood glucose-(pw54)-PI3K:MAPK-(pw69)-↑insulin resistance-(pw70)-↑angiotensin II-(pw89)- ↑Hypertension.*
- **Hyperglycemia/Hyperinsulinemia:** *Depression-(pw7b)-central nervous system-(pw26)-↑catecholamines / ↓serotonin / ↓BDNF-(pw44)-↑insulin resistance-(pw72)- ↑Hyperglycemia / Hyperinsulinemia.*
- **Hypercoagulability:** *Depression-(pw7b)-central nervous system-(pw26)-↑catecholamines / ↓serotonin / ↓BDNF-(pw44)-↑insulin resistance-(pw72)-↑platelet factors-(pw73)-↑Hypercoagulability.*

Since depression can upregulate all five CHD hallmarks (23, 24), it may play a more important role in Covid-19 severity than expected.

Apnoea

Figure 11 and **Table 2** show that obstructive sleep apnoea (OSA) is associated with an increased risk for CHD with a HR of 2.06 (94). Among 15 835 Covid-19 patients, those with OSA have a 2.37-fold (OR) increased odds of developing severe Covid-19 (95).

Similar to depression, the effects of OSA may also include alterations of the hypothalamic-pituitary-adrenal axis and sympathetic nervous activity. This results in changes of catecholamine and cortisol secretion levels, which concomitantly serve to up-regulate two CHD hallmarks namely, *Inflammatory state* and *Hypertension* (23). Subsequently, increased cortisol levels also increases the risk for elevated LDL and platelet factors, which influence the risk for two more CHD hallmarks namely, *Hypercholesterolemia* and *Hypercoagulability* (23).

The respective CHD hallmarks and activated pathways induced by OSA are denoted in **Figure 4** as the following:

- **Inflammatory state:** *Apnoea-(pw9)-central nervous system-(pw27)-↑cortisol-OSA-(pw42)-hypoxia-(pw61)-↑ROS-(pw85)-↑Inflammatory state.*
- **Hypertension:** *Apnoea-(pw9)-central nervous system-(pw27)-↑cortisol-OSA-(pw42)-↑hypoxia-(pw42)-↑)-oxia-(pSA-(pus systw70)-↑angiotensin II-(pw89)-↑Hypertension.*
- **Hypercholesterolemia:** *Apnoea-(pw9)-central nervous system-(pw27)-↑cortisol-(pw48)-visceral adiposity-(pw21)-↑TNF-α/IL-6-(pw56)-liver-(pw12)-↑LDL-(pw33)-↑oxLDL-(pw51)-↑Hypercholesterolemia.*
- **Hypercoagulability:** *Apnoea-(pw9)-central nervous system-(pw27)-↑cortisol-(pw47)-↑insulin resistance-(pw42)-↑platelet factors-(pw73)-↑Hypercoagulability.*

The activation of proinflammatory mediators namely, TNF-α, IL-6 and CRP induced by OSA are also elevated in severe Covid-19 patients without OSA (20–22). Therefore, OSA could aggravate these mediators, leading to an increased risk of Covid-19 severity.

Insomnia

Insomnia is another *health factor* that increases a person's risk for CHD with a RR of 1.45 (96), see **Figure 11** and **Table 2**. The effect of insomnia on increased Covid-19 severity seems negligible with an OR of 1.09 (97), see **Figure 11**. Unfortunately the study is small ($n = 568$) and the data are statistically insignificant (95% CI of 0.44–2.71) (97), see **Table 2** and **Figure 11**.

Nevertheless, insomnia affects several pathogenic pathways that may play an important role in Covid-19 severity (23). Insomnia has shown to directly affect the levels of leptin (decreases) and ghrelin (increases), which are important hormones that regulate appetite. This could cause an increase in caloric consumption which, if left untreated, could negatively impact blood glucose levels and insulin sensitivity (23). This would therefore result in an increased risk for *Hyperglycemia/Hyperinsulinemia* (23).

Subsequently, insulin resistance stemming from excessive caloric intake can stimulate proinflammatory mediators and cytokines such as TNF-α, IL-6 and CRP. This could result in a heightened *Inflammatory state*, which is common in severe Covid-19 patients (20–23). Another CHD hallmark upregulated by insulin resistance through the regulation of platelet homeostasis is *Hypercoagulability* (23). Coagulation is also a common risk factor in severe Covid-19 patients (4, 6–9, 23).

The respective CHD hallmarks and activated pathways induced by insomnia are denoted in **Figure 4** as the following:

- **Hyperglycemia/Hyperinsulinemia:** *Insomnia-(pw8b)-central nervous system-(pw25)-(pw66)-↑ghrelin:leptin-(pw67)-↑insulin resistance-(pw72)-liver-(pw14)-↑blood glucose-(pw55)-↑Hyperglycemia/Hyperinsulinemia.*
- **Inflammatory state:** *Insomnia-(pw8b)-central nervous system-(pw25)-(pw66)-↑ghrelin:leptin-(pw67)-↑insulin resistance-(pw70)-↑angiotensin II-(pw88)-renin-(pw50)-↑TNF-α, IL-6-(pw41)-↑Inflammatory state.*
- **Hypercoagulability:** *Insomnia-(pw8b)-central nervous system-(pw25)-(pw66)-↑ghrelin:leptin-(pw67)-↑insulin resistance-(pw72)-↑platelet factors-(pw73)-↑Hypercoagulability.*

Unfortunately, the clinical data on insomnia and its effect on Covid-19 severity are small. Its effect may be underestimated.

Moderate Alcohol Use

The mechanism by which moderate alcohol consumption may influence CHD was described in detail in our previous paper (26). Moderate alcohol consumption is accepted to reduce the risk of CHD (23, 26, 98). **Table 2** shows a decrease risk (RR) of 0.75 ($n = 645\,087$, $N = 33$) (98). This translates to a 1.41-fold decrease in CHD risk (23, 26), illustrated in **Figure 11**. This decrease in CHD risk may be due to several pathways that decrease the risk for CHD hallmarks.

Moderate alcohol consumption may reduce fibrinogen levels, clotting factors, and platelet aggregation. Downregulation of these biomarkers reduces a state of *Hypercoagulability* (26). In addition, it can also upregulate HDL and downregulate LDL, which decrease *Hypercholesterolemia* (26).

Moreover, moderate alcohol consumption can reduce hepatic gluconeogenesis and concomitantly decrease plasma glucose levels, which decreases the incidence of *Hyperglycemia* and *Hyperinsulinemia* (26). Lastly, it can serve

to reduce chronic *Inflammation* through regulation of insulin resistance (26).

These respective CHD hallmarks and pathogenic pathways activated by moderate alcohol consumption (26), are denoted in **Figure 4** as:

- **Hypercoagulability:** *Alcohol-(pw1)-Liver-(pw49)-↓fibrinogen/clotting factors-(pw73)- ↓Hypercoagulability* and *Alcohol-(pw1)-Liver-(pw49)-↑fibrinogen/clotting factors-(pw75)- ↓platelet aggregation.*
- **Hypercholesterolemia:** *Alcohol-(pw1)-Liver-(pw10)-↑HDL-(pw31)-↓Hypercholesterolemia* and *Alcohol-(pw1)-Liver-(pw12)-↓LDL-(pw33)- oxLDL-(pw51)-↓Hypercholesterolemia.*
- **Hyperglycemia/Hyperinsulinemia:** *Alcohol-(pw1)-Liver-(pw14)-↓blood glucose-(pw55)-↓Hyperglycemia/Hyperinsulinemia.*
- **Inflammation:** *Alcohol-(pw1)-Liver-(pw14)-↓blood glucose-(pw54)-PI3K:MAPK-(pw69)-insulin resistance-(pw70)-Angiotensin II-(pw89)-↓Hypertension-(pw100)-↓ROS-(pw85)-↓Inflammatory state.*

These pathways demonstrate an important role moderate alcohol consumption plays in four of the five CHD hallmarks. The argument whether moderate alcohol consumption before infection decreases or increases Covid-19 severity has not yet been thoroughly explored.

However, the prevailing point of view is that alcohol consumption during Covid-19 could increase Covid-19 severity (132). This is due to alcohol increasing the risk of acute respiratory distress syndrome and admission to intensive care unit in patients with pneumonia (132, 133). These are common risk factors in critical Covid-19 patients (132, 133).

Increased hypercoagulability, Hyperglycemia and inflammation are common in severe Covid-19 patients (4, 6–9, 11, 13, 14). Therefore, the reduction of these CHD hallmarks by moderate alcohol consumption before infection of SARS-CoV-2 could be advantageous. It seems to create a better vascular "baseline" and could thus potentially reduce the risk of developing severe Covid-19 complications. These effects should however be studied in well-designed clinical trials.

Food Intake (High Glycemic Diets)

We have previously explained, with reference to **Figure 1**, how high glycemic diets (HGDs) affect CHD (25). Only a summary of the elements relevant to Covid-19 are given below.

A high glycemic diet (HGD) increases the risk for CHD with a RR of 1.36 (99), see **Figure 11** and **Table 2**. These diets could play an important role in Covid-19 severity through regulation of all five CHD hallmarks (23, 25).

HGDs influences glycemic control by raising blood glucose levels *via* carbohydrate consumption. This may result in *Hyperglycemia* (23, 25). *Hyperglycemia* resulting from HGDs can increase the risk of insulin resistance by upregulating the Phosphatidylinositol 3-kinase : Mitogen-activated protein kinase (PI3K:MAPK) ratio (23, 25). Subsequently, an increased insulin resistance has been associated with increased levels of platelet factors that upregulate the potential for *Hypercoagulation* (23, 25).

Excessive intake of HGDs can result in increased adipose tissue, which enhances pro-inflammatory mediators such as CRP and TNF-α (23, 25). These mediators are, among others, important to consider since they are upregulated in critical Covid-19 patients (129, 130, 134–140). Macrophages, residing in adipose tissue, are also one of the most active secretory cells in the body that mediate activities of adipocytes and release a vast array of inflammatory mediators (23, 25). This increases the risk for an *Inflammatory state.*

Moreover, excessive intake of HGDs can also increase visceral fat build up and reduce clearance of triglycerides, which leads to increased LDL and decreased HDL levels (23, 25). This constitutes to a potential risk of *Hypercholesterolemia* (23, 25). Consequently, HGDs pertaining to visceral fat build up also increases the risk of *Hypertension*. This happens through build-up of excess adipose tissue, which increases the expression of angiotensinogen thus leading to activation of the renin-angiotensin system (23, 25).

These respective CHD hallmarks and pathogenic pathways activated by HGD are denoted in **Figure 4** as the following:

- **Hyperglycemia:** *Food-(pw2)-gastro-intestines-(pw17)-↑blood glucose-(pw55)-↑Hyperglycemia.*
- **Hypercoagulability:** *Food-(pw2)-gastro-intestines-(pw17)-↑blood glucose-(pw54)-↑PI3K:MAPK-(pw69)-↑insulin resistance-(pw72)-↑platelet factors-(pw73)-↑Hypercoagulability.*
- **Inflammatory state:** *Food-(pw2)-gastro-intestines-(pw15)-plasma lipids-(pw34)-liver-(pw13)-TMAO/NLRP3-(pw52)-macrophage-(pw77)-↑Inflammatory state.*
- **Hypercholesterolemia:** *Food-(pw2)-gastro-intestines-(pw15)-plasma lipids-(pw34)-liver-(pw12)-↑LDL-(pw33)-oxLDL-(pw51)-↑Hypercholesterolemia.*
- **Hypertension:** *Food-(pw2)-gastro-intestines-(pw14)-blood glucose-(pw54)-↑angiotensin II-(pw89)-↑Hypertension.*

These pathways demonstrate the detrimental effect HGDs may have on an individual's "baseline" vascular system before infection from SARS-CoV-2. It could potentially increase the risk of developing severe Covid-19 complications.

Effects of Different CHD *Pharmaceutical Interventions* on Covid-19 Severity

The *integrated CHD/Covid-19 model* shows that similar outcomes for different *health factors* are seen in CHD and Covid-19. The next question is: Since we know that various *pharmaceutical interventions* decreases one's risk for CHD, will they also work for Covid-19? If they do then this will further show validity of the proposed *integrated CHD/Covid-19 model.*

The *pharmaceutical interventions* are shown in **Figure 4** as blue boxes, where blunted blue arrows (⊣) denote antagonize or inhibit and pointed blue arrows (→) denote up-regulate or facilitate. The question is whether these pharmaceuticals would also decrease one's risk for severe Covid-19. This was investigated, despite the limited clinical data available for Covid-19. The data were extracted from literature and are summarized in **Table 2**.

No risk value was available for antidepressants' effect on Covid-19 severity. However, its effect on Covid-19 severity is still discussed in this section as depression was shown to increase the odds of developing severe Covid-19 complications by 2.68 (Section Effects of Different CHD *Pharmaceutical Interventions* on Covid-19 Severity, **Table 2**). It is thus hypothesized that certain anti-depressants should have an important influence on Covid-19 severity.

In the rest of this section we will discuss, in more detail, the effects each *pharmaceutical intervention* has on the CHD hallmarks, and how this could affect Covid-19 severity.

Statins

The use of statins decreases the risk of CHD with a RR of 0.78 (100). This translates to a 1.28-fold decrease in CHD risk (23), illustrated in **Table 2** and **Figure 12**. Statins also decrease Covid-19 severity, with a HR of 0.58 ($n = 13\,981$) (101) (**Table 2**). This translates to a decrease of Covid-19 severity by 1.72-fold as shown in **Figure 12**. We evaluated the effects statins has on all of the CHD hallmarks, which may partially explain the large reduction in Covid-19 severity with the use of statins.

Firstly, statins cholesterol lowering effect inhibits the following pathways in **Figure 4**: (pw11) and (pw12). Besides these cholesterol lowering effects, it also has an anti-inflammatory effect (23, 101). The anti-inflammatory biomarkers and pathways on which inhibition is observed are denoted in **Figure 4** as: NFκβ, ROS and (pw21), (pw57), (pw74).

In addition to their beneficial effects on cholesterol and inflammation, statins also have antihypertensive effects by reducing systolic, diastolic and mean arterial blood pressure (141). The hypertensive pathways on which its actions are observed are denoted in **Figure 4** as (pw88) and (pw89).

Salicylates

Salicylates such as aspirin is a common anti-inflammatory (142) and anti-thrombotic (143) medication that decreases the risk for CHD with a RR of 0.82 (102), see **Table 2**. This translates to a 1.22-fold decrease in CHD risk (23), illustrated in **Figure 12**. Its use in Covid-19 patients also showed a decrease in severity with HR of 0.53 (58). This is shown in **Figure 12** as a 1.89-fold decrease in Covid-19 severity (58).

This reduction in risk could be expected because of the detrimental effect of inflammation and coagulation seen in most severe Covid-19 patients (4, 6–9, 20–22). The pathways on which aspirin's actions are observed are denoted in **Figure 4** as (pw73) and (pw74).

Indirect Thrombin Inhibitors

Indirect thrombin inhibitors such as heparin is used as an anticoagulant, which decreases the odds of CHD with OR 0.91 (103), see **Table 2**. This translates to a 1.10-fold decrease in CHD risk (23) as shown in **Figure 12**. Since many severe cases of Covid-19 present venous thromboembolisms and microthrombi (4, 6–9), indirect thrombin inhibitors should be of benefit to such cases.

Heparin was thus expected to reduce these thrombi and reduce Covid-19 severity. A small retrospective analysis ($n = 449$) investigated heparin's effect in Covid-19 patients (104). The study found an OR of 0.37 in Covid-19 mortality (104), see **Table 2**. This is illustrated in **Figure 12** as a 2.7-fold decrease in odds of developing severe Covid-19 (104). The coagulation pathway on which heparin's action is observed is shown in **Figure 4** as (pw73).

Heparin also seems to have an anti-inflammatory effect (144), which is presented in **Figure 4** as pathway (pw74). This effect is however only seen at much higher concentrations which could increase the risk of bleeding (144). Therefore, heparin's anti-thrombotic effect would predominantly be the reason for lower Covid-19 severity.

Direct Thrombin Inhibitors

Direct thrombin inhibitors have shown to decrease the risk of CHD with HR of 0.76 (105), see **Table 2**. This translates to a 1.32-fold decrease in CHD risk (23), illustrated in **Figure 12**. These pharmaceuticals' actions are also observed on the coagulation pathway (pw74) (23), see **Figure 4**.

For Covid-19, direct thrombin inhibitors are shown to slightly reduce the risk of developing severe disease with a HR of 0.90 (106), see **Table 2**. This translates to a 1.11-fold reduction in risk (106), illustrated in **Figure 12**. However, as shown in **Figure 12** by an (*), this value is not statistically significant with the 95% CI of 0.71–1.15 presented in **Table 2**.

Antihypertensive Pharmaceuticals

The antihypertensive *pharmaceutical interventions* in **Figure 4** are: ACE inhibitors, angiotensin-renin inhibitors, β-blockers, calcium channel blockers and diuretics. The pathways on which their actions are observed are shown in **Figure 4** as (pw88), (pw89), and (pw50) (23).

The respective reduction of CHD risks for each pharmaceutical (23, 107, 109, 111, 113) is given in **Figure 12** and **Table 2** as the following:

- Angiotensin-renin inhibitors: 1.09 (OR of 0.92)
- Calcium channel blockers: 1.20 (OR of 0.83)
- ACE inhibitors: 1.27 (OR of 0.79)
- β-blockers: 1.46 (RR of 0.69)
- Diuretics: 1.27 (RR of 0.79)

The reduction in CHD risk is small for angiotensin-renin inhibitors with an OR close to one (0.92) (109). However, angiotensin-renin inhibitors seem to be more beneficial for Covid-19 severity with a reduction in 1.54-fold (110), see **Figure 12**.

The risk data for calcium channel blockers (OR of 0.94) (112), ACE inhibitors (HR of 0.89) (108) and diuretics (OR of 0.96) (112) are currently not associated with Covid-19 severity. All risk values are close to one and the respective 95% CI's all show statistically insignificance (containing 1.0), see **Table 2**. This statistically insignificance is illustrated in **Figure 12** with an (*).

The most interesting of the antihypertensive *pharmaceutical interventions* is β-blockers, which reduced the risk of CHD (RR of 0.69). However, its use increases ones odds of developing severe Covid-19 (OR of 1.23) (112), see **Figure 12**. The reason for this is unclear and further studies are warranted to investigate the

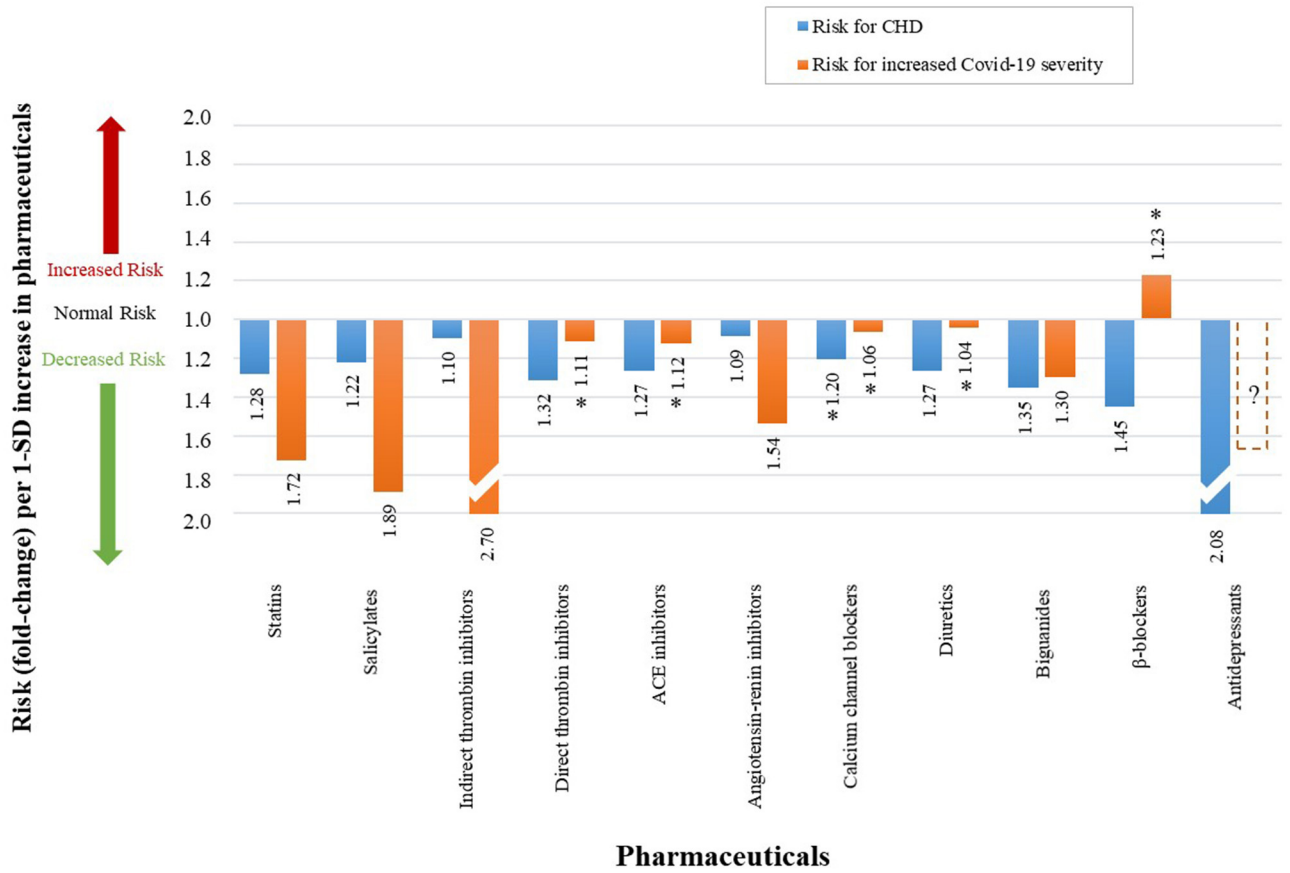


FIGURE 12 | The qualitative effect that different pharmaceuticals have on CHD risk and Covid-19 severity. (An accurate quantitative comparison is not possible, mostly due to differences in study design and size).

mechanism of action involved. However, one explanation for this difference could be that the data is insignificant for Covid-19, with a 95% CI of 0.74-2.04 (112), see **Table 2**.

Biguanides

Biguanides such as metformin has been used for many decades to treat type 2 diabetes and its use decreases the odds of developing CHD, with a OR of 0.74 (114), see **Table 2**. This translates to a 1.35-fold decrease in CHD risk (23), illustrated in **Figure 12**. Metformin's inhibition is observed on pathway (pw14) (23), see **Figure 4**.

Elevated blood glucose levels at admission is an independent predictor of Covid-19 severity irrespective of diabetes (75). Therefore, glucose lowering agents are expected to reduce Covid-19 mortality. This is indeed the case since a large observational cohort study of type-2 diabetics (n = 1 800 005) showed that the use of metformin decreased Covid-19-related mortality by 1.30-fold (HR of 0.77) (115), see **Figure 12** and **Table 2**.

Antidepressants (SSRIs)

We have done a detailed study of the mechanisms by which SSRI antidepressants may reduce CHD risk (24). We showed that SSRIs can influence most of the CHD hallmarks (24). A summary, relevant to Covid-19, is given below.

Selective serotonin uptake inhibitors (SSRIs) such as sertraline has shown to decrease the risk of CHD, with a HR of 0.48 (116), see **Table 2**. This translates to a 2.08-fold decrease in CHD risk (23), illustrated in **Figure 12**. Sertraline's actions are observed on the anti-inflammatory pathway (pw94), as shown in **Figure 4**.

A similar SSRI antidepressant, fluvoxamine's effect on Covid-19 severity is currently being investigated in a clinical trial (NCT04727424). This study was initiated by results from a small double-blind, randomized clinical trial of 152 Covid-19 positive patients treated with fluvoxamine (117). The outcomes of this study showed that patients treated with fluvoxamine, compared with a placebo, had a lower likelihood of clinical deterioration (0% vs. 8.3%) (117).

The study did not report any risk data. For this reason, the data could not be added to **Figure 12** and **Table 2**. Nevertheless, since a therapeutic effect is seen in the small Covid-19 study, a dotted bar was added to **Figure 12**. We hypothesize, based on our previous studies (24), that most SSRIs will be beneficial.

DISCUSSION AND FUTURE RESEARCH

The aim of this paper was to use a systems approach to explore the mechanisms between severe Covid-19 and its cardiovascular complications, as requested by *Frontiers*. The resulting *integrated*

CHD/Covid-19 model may provide insight into the various research questions, some also requested by *Frontiers*.

Why Do Some Patients With Severe Covid-19 Experience Sudden Death?

Although aspects of this has been proposed elsewhere (19, 22, 138, 145–148), here its integrated mechanism is systematically and visually shown with the relevant pathogenetic pathways with reference to CHD. This model further elucidates other underlying pathogenesis that may influence this *death spiral* before infection of SARS-CoV-2.

The *death spiral* was summarized as follows: Increased inflammation at the lungs causes EC injury, which can result in vascular leakage and/or activation of the coagulation cascade at the lungs, thereby causing hypoxia which can further increase inflammation, creating two closed positive feedback loops and causing severe Covid-19 through a *death spiral* (**Figure 5**).

How Do CHD Comorbidities Influence This *Death Spiral*?

It is widely accepted that patients with pre-existing CHD comorbidities (thus a poor initial vascular "baseline") have a high risk of developing severe Covid-19 (11–14). The detailed mechanisms of how these comorbidities may influence the *death spiral* was not fully integrated before.

This question was answered in this paper by visually (**Figures 8–10**) detailing the mechanisms of how three CHD comorbidities namely *Hypercholesterolemia, Hyperglycemia/Hyperinsulinemia* and *Hypertension* can fuel the *death spiral.*

How Can an Individual Reduce the Risk of Developing Severe Covid-19 From a Cardiovascular Point of View?

In literature different *health factors* (85, 87, 89, 91, 93, 95, 97) and CHD related *pharmaceuticals* (58, 101, 104, 106, 108, 110, 112, 115) present either a reduction or aggravation of Covid-19 severity. In this paper we provide the pathogenesis detailing the effect these *health factors* and *pharmaceuticals* may have on this *death spiral,* especially for those with an increased risk for CHD.

We have shown that severe Covid-19 and CHD have similarities in underlying pathogenesis. Therefore, following a lifestyle that would decrease one's risk for CHD before onset of Covid-19 should also decrease the chances of developing severe Covid-19.

The remaining two research questions [(4) and (5)] have partially been answered by the model but future research is still needed. These are discussed in more detail in the following two Sections.

How Can Computational Analysis Help to Assess the Risk of Severity in Covid-19 in Cardiovascular Disease?

One of the research questions posed by *Frontiers* in their request for papers was the following: *"How can computational analysis help to assess the risk of COVID-19 in cardiovascular disease?"* We speculate that to achieve such an outcome, at least the following must be done:

Step 1: Development of a fully integrated network model for the disease, accounting for all effects including cross linking.

Step 2: Characterization of each interaction (typically at the nodes of the network in Step 1) is needed to solve the network.

A first attempt at Step 1 for severe Covid-19 in cardiovascular disease was done in this paper (**Figure 4**). The next step is characterization of the network using the following equation:

$$Out_{1\to n} = f_{1\to n} In_{1\to n} \tag{1}$$

where $In_{1\to n}$ are the inputs (1 to n) to a node and $out_{1\to n}$ are the outputs (1 to n) from the node and f (1 to n) are the resulting transfer functions. The inputs and outputs are typically measured. More detail of this process is given in (23).

Using this process we have developed, over the past four decades, simulation software to solve complex engineering networks e.g., in deep mines and industrial complexes (149). Fortunately, in engineering it is easy to develop transfer functions (Equation 1) as it is relatively easy to do the required measurements. The challenge for medical networks is the measurements of all the relevant pathways in **Figure 4**.

A typical deep level mine simulation model (A) and a CHD simulation model (B) proposed in (23) are shown in **Figure 13**. The following has to be investigated in the future: if all the biomarkers can be measured for the proposed *integrated CHD/Covid-19 model*, will it be possible to individualize the network (**Figure 4**), thereby making it patient specific? This could be similar to us individualizing our engineering simulations to a specific mine.

The question is: can the risk for Covid-19 severity in CHD then be established for any specific individual by inputting the measured biomarkers of that person into the simulation model based on **Figure 4**? We have already attempted Step 1. However, for Step 2 much work and data are still needed.

Fortunately, there are numerous clinical trials currently underway that focus on treatments for Covid-19 with respect to the CHD hallmarks, namely the following:

(A) *Hypercoagulation* (122 trials)
(B) *Hypercholesterolemia* (23 trials)
(C) *Hyperglycemia /Hyperinsulinemia (Diabetes)* (84 trials)
(D) *Inflammation* (265 trials)
(E) *Hypertension* (68 trials)

The clinical trial numbers and respective treatments are available as **Supplementary Data**. The total number of registered clinical trials on CHD hallmarks effect on Covid-19 are 562. This also includes 94 duplicate studies that focus on more than one CHD hallmark.

If we can successfully develop a simulation model, could the best control strategy (pharmaceuticals) be calculated for each individual Covid-19 or CHD patient? This would be similar to identifying the optimum control strategies, which we

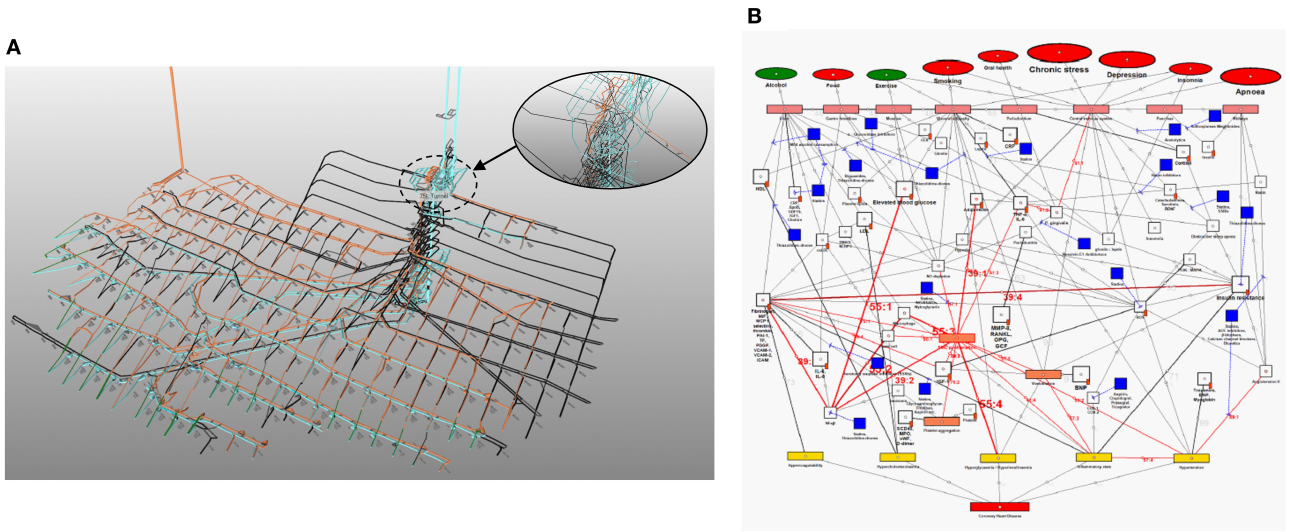


FIGURE 13 | Schematics of typical simulation networks for **(A)** engineering and **(B)** CHD (23). **(A)** provides a small section of computer model of a relatively complex deep mine. **(B)** shows the initial computer model (23) developed from the existing CHD model in **Figure 1** (23) using the simulation software developed for **(A)**. The CHD computer model includes all the known interactions for *health factors,* measured elements (salient biomarkers) and controls (*pharmaceutical interventions).* Remember it is the measured biomarkers that individualize a patient.

routinely calculate in engineering, for each individual mine or industrial complex.

However, we acknowledge that there are many assumptions and restrictions relevant to a CHD/Covid-19 computational analysis, which is completely speculative at present. For example, research is needed to investigate how individualized predictions will be feasible. The full details on the research question of computational analysis will be the purpose of future papers.

Are There Other Opportunities in Cardiovascular Disease That Can Be Derived From This Paper and the Covid-19 Crisis?

We have shown in this paper that the late-stage consequences of severe Covid-19 is often accelerated cardiovascular disease. We have also shown that most *pharmaceutical interventions* which mediate CHD also mediate the effects of Covid-19.

The question arises if the reverse is true. Are there any reported *pharmaceutical interventions* that reduced Covid-19 severity which could potentially be of value for vascular disease? This is an important question as approximately five times more people died during the past year from cardiovascular disease than from Covid-19.

Such a repurposed drug should preferably treat most of the hallmarks of cardiovascular disease. We investigated such a drug namely, ivermectin (150–155). Although ivermectin use is still controversial as a drug against Covid-19, studies over nearly three decades before Covid-19, has shown to reduce four of the five hallmarks of cardiovascular disease. These results and the publication dates are given below:

(A) *Hypercoagulability:* (1992) by increasing prothrombin time in 6.7% (ivermectin group) vs. 1.4% (control group) of participants *in vivo* (humans) (156).
(B) *Hypercholesterolemia:* cholesterol (2013) decreased by 1.5-fold *in vivo* (mice) (157).
(C) *Hyperglycemia:* fasting blood glucose (2013) decreased by 1.4-fold *in vivo* (mice) (157).
(D) *Hyperinsulinemia:* fasting insulin (2013) decreased by 2.0-fold *in vivo* (mice) (157).
(E) *Inflammation:* (2004) decreased IL-1β and TNF-α by 1.27-fold *in vitro* (158).

Except for the CHD hallmark *Inflammation* (159) the focus of ivermectin's proposed mechanism of action (MOA) for Covid-19 is currently on its anti-viral effect (150–153). However, if ivermectin really shows promise for Covid-19 treatment, could the full vascular MOA for ivermectin be as important or even more important than its anti-viral effects?

This research question can only be answered fully by clinical trials, which measure the relevant vascular biomarkers for each CHD hallmark before and after ivermectin use. Side effects of chronic use such as mild elevation of serum aminotransferases should also be investigated (160).

The MOA of ivermectin for prevention of Covid-19, reportedly seen in small studies (161), is also not clear to the authors. Why would the anti-viral MOA of ivermectin have a preventable effect if the patient has not been infected yet?

If ivermectin really helps for prevention of Covid-19, could it rather help create a healthier vascular system ("baseline") before the virus strikes, especially in vascular compromised individuals, rather than only help *via* its proposed anti-viral effect? Therefore,

could ivermectin's effect on the vascular system during severe Covid-19 be its most important MOA?

If well-designed clinical trials show that ivermectin could be a potential cardiovascular drug, could it be an ideal, inexpensive, drug for low- and middle-income countries where a high percentage of global cardiovascular related deaths occur (162)?

Other Research Questions Emanating From This Study

Other research questions that should be investigated in future research are the following:

1. Why would β -blockers have an opposite effect on Covid-19 severity than on CHD?
2. There exists an anomaly between statin's cholesterol lowering effect and low cholesterol levels seen in end-stage Covid-19. How can this drug help decrease Covid-19 severity while it further decreases cholesterol? Can statin's anti-inflammatory effect be so large that it overrides its cholesterol lowering effect? Would it then be better to drop statins and rather only use anti-inflammatory medication? Or does it depend on the stage of the disease, beneficial at first but not in the end stage?
3. Does a high correlation of most CHD related *pharmaceutical interventions* and Covid-19 mean that other CHD pharmaceuticals not investigated in detail for Covid-19 could also help reduce Covid-19 severity?
4. Are there pathways shown in the proposed model (**Figure 4**) that do not have pharmaceuticals to regulate them? Could this be the focus of new drug discovery for Covid-19 and cardiovascular disease?
5. Could the model be extended to include cerebrovascular disease and other cardiac diseases such as heart failure, valvular heart disease and peripheral artery disease?

CONCLUSION

Covid-19 data show that disease severity mostly occurs in patients with pre-existing cardiovascular comorbidities i.e., in patients with poor initial vascular "baselines." *Frontiers* therefore requested papers on how a systems approach can explore the mechanisms of cardiovascular complications in Covid-19.

This study attempted to fulfill this request by integrating pathways for severe Covid-19 into an existing coronary heart disease (CHD) model. The resulting *integrated CHD/Covid-19 model,* depicted in **Figure 4**, gives insights into the following issues, some also raised in the *Frontiers* request for research:

- The integrated CHD/Covid-19 pathogenesis of the *death spiral* seen in some critical Covid-19 patients.
- The comprehensive mechanisms of how underlying CHD comorbidities namely, *Hyperglycemia/Hyperinsulinemia, Hypercholesterolemia* and/or *Hypertension* may fuel the *death spiral.*
- The detailed pathogeneses of different *health factors,* which effect CHD risk and Covid-19 severity.
- The mechanisms of how chronic CHD *pharmaceutical interventions* may influence Covid-19 severity.
- The proposed model shows many pathways that currently do not have pharmaceuticals which influence them. This information can be the focus of future drug discovery.
- The proposed model can be further developed as a computational tool not only for Covid-19 application but also for cardiovascular disease.
- Insights into the hallmarks of CHD, shown in the *integrated CHD/Covid-19 model,* also led to various research questions that can form the basis for future research. This includes potential repurposing of an existing drug for cardiovascular disease.

Although the details in this study are complex the message is simple. Studies such as this one not only highlight the value of a cardiovascular healthy lifestyle in general but also specifically for Covid-19. With the sharp focus on Covid-19 we hope that this "healthy living" message will be intensified, thus help to reduce cardiovascular deaths, the prime killer of man.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

AM developed the first draft (of more than 30) of the manuscript and compiled and analyzed the Covid-19 risk factor data. EM was the principal investigator. During level 5 Lockdown in May 2020 he initiated the original ideas and research including the *death spiral* and potential use of ivermectin for CHD based on MM's Ph.D. AM and AG developed the integrated CHD/Covid-19 model from literature. MM developed the CHD-based model and provided expert opinion for the integration of the model with Covid-19. All authors have assisted in revisions and have approved the final manuscript.

FUNDING

The research was funded by EM and HumanSim (Pty) Ltd.

ACKNOWLEDGMENTS

Kristy Nell compiled the clinical trial data presented in the manuscript as **Supplementary Information**. C. Mathews helped with the initial CHD Covid-19 link and provided funding *via* HumanSim (Pty) Ltd. We thank her for her continuous inputs. The initial angel investor was Dr. Arnold van Dyk.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.737592/full#supplementary-material

REFERENCES

1. Liu YC, Kuo RL, Shih SR. COVID-19: the first documented coronavirus pandemic in history. *Biomed J.* (2020) 43:328–33. doi: 10.1016/j.bj.2020.04.007
2. Valencia DN. Brief review on COVID-19: the 2020 pandemic caused by SARS-CoV-2. *Cureus.* (2020) 12:e7386. doi: 10.7759/cureus.7386
3. World Health Organization. *Weekly Epidemiological Update on COVID-19 - 10 August 2021* (2021). Available online at: https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19 (accessed August 10, 2021).
4. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med.* (2020) 383:120–8. doi: 10.1056/NEJMoa2015432
5. Martins-Filho P, Santos C, Tavares S, Antos V. Factors associated with mortality in patients with COVID-19. A quantitative evidence synthesis of clinical and laboratory data. *Eur J Intern Med.* (2020) 76:97–9. doi: 10.1016/j.ejim.2020.04.043
6. Wichmann D, Sperhake J-P, Lütgehetmann M, Steurer S, Edler C, Heinemann A, et al. Autopsy findings and venous thromboembolism in patients with COVID-19. *Ann Intern Med.* (2020) 173:1030. doi: 10.7326/L20-1206
7. Fox SE, Akmatbekov A, Harbert JL, Li G, Quincy Brown J, Vander Heide RS. Pulmonary and cardiac pathology in African American patients with COVID-19: an autopsy series from New Orleans. *Lancet Respir Med.* (2020) 8:681–6. doi: 10.1016/S2213-2600(20)30243-5
8. Lax SF, Skok K, Zechner P, Kessler HH, Kaufmann N, Koelblinger C, et al. Pulmonary arterial thrombosis in COVID-19 with fatal outcome: results from a prospective, single-center, clinicopathologic case series. *Ann Intern Med.* (2020) 173:330–61. doi: 10.2139/ssrn.3586685
9. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet.* (2020) 395:1417–8. doi: 10.1016/S0140-6736(20)30937-5
10. Clark A, Jit M, Warren-gash C, Guthrie B, Wang HHX, Mercer SW, et al. Global, regional, and national estimates of the population at increased risk of severe COVID-19 due to underlying health conditions in 2020: a modelling study. *Lancet Glob Heal.* (2020) 8:e1003–17. doi: 10.1016/S2214-109X(20)30264-3
11. Zheng Z, Peng F, Xu B, Zhao J, Liu H, Peng J, et al. Risk factors of critical & mortal COVID-19 cases: a systematic literature review and meta-analysis. *J Infect.* (2020) 81:e16–25. doi: 10.1016/j.jinf.2020.04.021
12. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3
13. Ssentongo P, Ssentongo AE, Heilbrunn ES, Ba DM, Chinchilli VM. Association of cardiovascular disease and 10 other pre-existing comorbidities with COVID-19 mortality: a systematic review and meta-analysis. *PLoS ONE.* (2020) 15:1–16. doi: 10.1371/journal.pone.0238215
14. Luo L, Fu M, Li Y, Hu S, Luo J, Chen Z, et al. The potential association between common comorbidities and severity and mortality of coronavirus disease 2019: a pooled analysis. *Clin Cardiol.* (2020) 43:1478–93. doi: 10.1002/clc.23465
15. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol.* (2020) 17:259–60. doi: 10.1038/s41569-020-0360-5
16. Long B, Brady WJ, Koyfman A, Gottlieb M. Cardiovascular complications in COVID-19. *Am J Emerg Med.* (2020) 38:1504–7. doi: 10.1016/j.ajem.2020.04.048
17. Liu PP, Blet A, Smyth D, Li H. The science underlying COVID-19: implications for the cardiovascular system. *Circulation.* (2020) 142:68–78. doi: 10.1161/CIRCULATIONAHA.120.047549
18. Libby P. The heart in COVID-19: primary target or secondary bystander? *JACC Basic Transl Sci.* (2020) 5:537–42. doi: 10.1016/j.jacbts.2020.04.001
19. Atri D, Siddiqi HK, Lang JP, Nauffal V, Morrow DA, Bohula EA. COVID-19 for the cardiologist: basic virology, epidemiology, cardiac manifestations, and potential therapeutic strategies. *JACC Basic to Transl Sci.* (2020) 5:518–36. doi: 10.1016/j.jacbts.2020.04.002
20. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol.* (2020) 20:363–74. doi: 10.1038/s41577-020-0311-8
21. Barton LM, Duval EJ, Stroberg E, Ghosh S, Mukhopadhyay S. COVID-19 autopsies, Oklahoma, USA. *Am J Clin Pathol.* (2020) 153:725–33. doi: 10.1093/ajcp/aqaa062
22. Teuwen LA, Geldhof V, Pasut A, Carmeliet P. COVID-19: the vasculature unleashed. *Nat Rev Immunol.* (2020) 20:389–91. doi: 10.1038/s41577-020-0343-0
23. Mathews MJ. A systems engineering approach to coronary heart disease. *Philos Mech Eng North West Univ South Africa*. (2015). Available online at: https://repository.nwu.ac.za/handle/10394/18953
24. Mathews MJ, Mathews EH, Liebenberg L. The mechanisms by which antidepressants may reduce coronary heart disease risk. *BMC Cardiovasc Disord.* (2015) 15:1–12. doi: 10.1186/s12872-015-0074-5
25. Mathews MJ, Liebenberg L, Mathews EH. How do high glycemic load diets influence coronary heart disease? *Nutr Metab.* (2015) 12:1–15. doi: 10.1186/s12986-015-0001-x
26. Mathews MJ, Liebenberg L, Mathews EH. The mechanism by which moderate alcohol consumption influences coronary heart disease. *Nutr J.* (2015) 14:1–12. doi: 10.1186/s12937-015-0011-6
27. Mathews MJ, Mathews EH, Mathews GE. Oral health and coronary heart disease. *BMC Oral Health.* (2016) 16:1–10. doi: 10.1186/s12903-016-0316-7
28. Mathews MJ, Mathews EH, Mathews GE. The integrated effect of moderate exercise on coronary heart disease. *Cardiovasc J Afr.* (2017) 28:125–33. doi: 10.5830/CVJA-2016-058
29. Puccetti L, Bruni F, Di Renzo M, Bova G, Cercignani M, Iadanza A, et al. Hypercoagulable state in hypercholesterolemic subjects assessed by platelet-dependent thrombin generation: *in vitro* effect of Cerivastatin. *Eur Rev Med Pharmacol Sci.* (1999) 3:197–204.
30. Andersen P. Hypercoagulability and reduced fibrinolysis in hyperlipidemia: relationship to the metabolic cardiovascular syndrome. *J Cardiovasc Pharmacol.* (1992) 20:S29–31. doi: 10.1097/00005344-199200208-00007
31. Tjaden K, Pardali E, Waltenberger J. Hypercholesterolemia induces vascular cell dysfunction: molecular basis for atherosclerosis. *Austin J Vasc Med.* (2015) 2:1–9. Available online at: https://austinpublishinggroup.com/vascular-medicine/fulltext/ajvm-v2-id1011.php
32. Radenkovic D, Chawla S, Pirro M, Sahebkar A, Banach M. Cholesterol in relation to COVID-19: should we care about it? *J Clin Med.* (2020) 9:1909. doi: 10.3390/jcm9061909
33. Sanders DW, Jumper CC, Ackerman PJ, Bracha D, Donlic A, Kim H, et al. Sars-cov-2 requires cholesterol for viral entry and pathological syncytia formation. *Elife.* (2021) 10:1–47. doi: 10.7554/eLife.65962
34. Wang H, Yuan Z, Pavel MA, Hansen SB. The role of high cholesterol in age-related COVID19 lethality. *bioRxiv [Preprint].* (2020) 1–17. doi: 10.1101/2020.05.09.086249
35. Pranata R, Huang I, Lim MA, Wahjoepramono EJ, July J. Impact of cerebrovascular and cardiovascular diseases on mortality and severity of COVID-19–systematic review, meta-analysis, and meta-regression. *J Stroke Cerebrovasc Dis.* (2020) 29:104949. doi: 10.1016/j.jstrokecerebrovasdis.2020.104949
36. Bourgonje AR, Abdulle AE, Timens W, Hillebrands J-L, Navis GJ, Gordijn SJ, et al. Angiotensin-converting enzyme 2 (ACE2), SARS-CoV−2 and the pathophysiology of coronavirus disease 2019 (COVID-19). *J Pathol.* (2020) 251:228–48. doi: 10.1002/path.5471
37. Huertas A, Montani D, Savale L, Pichon J, Tu L, Parent F, et al. Endothelial cell dysfunction: a major player in SARS-CoV-2 infection (COVID-19)? *Eur Respir J.* (2020) 56:2001634. doi: 10.1183/13993003.01634-2020
38. Dandona P, Dhindsa S, Ghanim H, Chaudhuri A. Angiotensin II and inflammation: the effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockade. *J Hum Hypertens.* (2007) 21:20–27. doi: 10.1038/sj.jhh.1002101
39. Esmon CT. The interactions between inflammation and coagulation. *Br J Haematol.* (2005) 131:417–30. doi: 10.1111/j.1365-2141.2005.05753.x
40. Lindner D, Fitzek A, Bräuninger H, Aleshcheva G, Edler C, Meissner K, et al. Association of cardiac infection with SARS-CoV-2 in confirmed COVID-19 autopsy cases. *JAMA Cardiol.* (2020) 5:1281–5. doi: 10.1001/jamacardio.2020.3551

41. Catanzaro M, Fagiani F, Racchi M, Corsini E, Govoni S, Lanni C. Immune response in COVID-19: addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. *Signal Transduct Target Ther.* (2020) 5:1–10. doi: 10.1038/s41392-020-0191-1
42. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clin Infect Dis.* (2020) 71:762–8. doi: 10.1093/cid/ciaa248
43. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med.* (2020) 46:846–8. doi: 10.1007/s00134-020-05991-x
44. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, Akinosoglou K, Antoniadou A, Antonakos N, et al. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. *Cell Host Microbe.* (2020) 27:992–1000.e3. doi: 10.1016/j.chom.2020.04.009
45. Zhang C. The role of inflammatory cytokines in endothelial dysfunction. *Basic Res Cardiol.* (2008) 103:398–406. doi: 10.1007/s00395-008-0733-0
46. Leo N, Alexander L, Sophia B, Rainer K, Tobias W, Michael W, et al. Immunothrombotic dysregulation in COVID-19 pneumonia is associated with respiratory failure and coagulopathy. *Circulation*. (2020) 142:1176–89. doi: 10.1161/CIRCULATIONAHA.120.048488
47. Winterbourn CC, Kettle AJ, Hampton MB. Reactive oxygen species and neutrophil function. *Annu Rev Biochem.* (2016) 85:765–92. doi: 10.1146/annurev-biochem-060815-014442
48. Chaudhry H, Zhou J, Zhong Y, Ali MM, Mcguire F, Nagarkatti PS, et al. Role of cytokines as a double-edged sword in sepsis. *In Vivo.* (2015) 27:669–84.
49. Sarkar M, Niranjan N, Banyal PK. Mechanisms of hypoxemia. *Lung India.* (2017) 34:47–60. doi: 10.4103/0970-2113.197116
50. Millar FR, Summers C, Griffiths MJ, Toshner MR, Proudfoot AG. The pulmonary endothelium in acute respiratory distress syndrome: insights and therapeutic opportunities. *Thorax.* (2016) 71:462–73. doi: 10.1136/thoraxjnl-2015-207461
51. Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. *Nat Rev Immunol.* (2007) 7:803–15. doi: 10.1038/nri2171
52. Bikdeli B, Madhavan M V., Jimenez D, Chuich T, Dreyfus I, Driggin E, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. *J Am Coll Cardiol.* (2020) 75:2950–73. doi: 10.1016/j.jacc.2020.04.031
53. Eltzschig HK, Carmeliet P. Hypoxia and inflammation. *N Engl J Med.* (2011) 364:656–65. doi: 10.1056/NEJMra0910283
54. Görlach A, Dimova EY, Petry A, Martínez-Ruiz A, Hernansanz-Agustín P, Rolo AP, et al. Reactive oxygen species, nutrition, hypoxia and diseases: problems solved? *Redox Biol.* (2015) 6:372–85. doi: 10.1016/j.redox.2015.08.016
55. Mellado-Artigas R, Ferreyro BL, Angriman F, Hernández-Sanz M, Arruti E, Torres A, et al. High-flow nasal oxygen in patients with COVID-19-associated acute respiratory failure. *Crit Care.* (2021) 25:1–10. doi: 10.1186/s13054-021-03469-w
56. The RECOVERY Collaborative Group. Dexamethasone in hospitalized patients with Covid-19 — preliminary report. *N Engl J Med.* (2020) 384:693–704. doi: 10.1056/NEJMoa2021436
57. Hong W, Chen Y, You K, Tan S, Wu F, Tao J, et al. Celebrex adjuvant therapy on coronavirus disease 2019: an experimental study. *Front Pharmacol.* (2020) 11:1–9. doi: 10.3389/fphar.2020.561674
58. Chow JH, Khanna AK, Kethireddy S, Yamane D, Levine A, Jackson AM, et al. Aspirin use is associated with decreased mechanical ventilation, ICU admission, and in-hospital mortality in hospitalized patients with COVID-19. *Anesth Analg.* (2021) 132:930–41. doi: 10.1213/ANE.0000000000005292
59. Bizien N, Noel-Savina E, Tromeur C, Delluc A, Mottier D, Leroyer C, et al. Age is a major risk factor of venous thromboembolism (VTE). *Eur Respir J.* (2011) 38:3936. doi: 10.1097/01.mcp.0000174246.15386.69
60. Pijls BG, Jolani S, Atherley A, Derckx RT, Dijkstra JIR, Franssen GHL, et al. Demographic risk factors for COVID-19 infection, severity, ICU admission and death: a meta-analysis of 59 studies. *BMJ Open.* (2021) 11:e044640. doi: 10.1136/bmjopen-2020-044640
61. Roach REJ, Cannegieter SC, Lijfering WM. Differential risks in men and women for first and recurrent venous thrombosis: the role of genes and environment. *J Thromb Haemost.* (2014) 12:1593–600. doi: 10.1111/jth.12678
62. Zakai NA, Mcclure LA. Racial differences in venous thromboembolism. *J Thromb Haemost.* (2011) 9:1877–82. doi: 10.1111/j.1538-7836.2011.04443.x
63. Golestaneh L, Neugarten J, Fisher M, Billett HH, Gil MR, Johns T, et al. The association of race and COVID-19 mortality. *EClinicalMedicine.* (2020) 25:100455. doi: 10.1016/j.eclinm.2020.100455
64. Groot HE, Sierra LEV, Said MA, Lipsic E, Karper JC, Van Der Harst P. Genetically determined ABO blood group and its associations with health and disease. *Arterioscler Thromb Vasc Biol.* (2020) 40:830–8. doi: 10.1161/ATVBAHA.119.313658
65. Wu B, Gu D, Yu J, Yang J, Shen W. Association between ABO blood groups and COVID-19 infection, severity and demise: a systematic review and meta-analysis. *Infect Genet Evol.* (2020) 84:104485. doi: 10.1016/j.meegid.2020.104485
66. Pomp ER, Lenselink AM, Rosendaal FR, Doggen CJM. Pregnancy, the postpartum period and prothrombotic defects: risk of venous thrombosis in the MEGA study. *J Thromb Haemost.* (2008) 6:632–37. doi: 10.1111/j.1538-7836.2008.02921.x
67. Qeadan F, Mensah NA, Tingey B, Stanford JB. The risk of clinical complications and death among pregnant women with COVID-19 in the Cerner COVID-19 cohort: a retrospective analysis. *BMC Pregn Childbirth.* (2021) 21:1–14. doi: 10.1186/s12884-021-03772-y
68. Silverstein MD, Heit JA, Mohr DN, Petterson TM, O'Fallon WM, Melton J. Trends in the incidence of deep vein thrombosis and pulmonary embolism. *Arch Intern Med.* (1998) 158:585–593. doi: 10.1001/archinte.158.6.585
69. Boudin L, Dutasta F. Relationship between ABO blood groups and coronavirus disease 2019: study design matters. *Clin Infect Dis.* (2021) 72:e927–8. doi: 10.1093/cid/ciaa1473
70. Zaki N, Alashwal H, Ibrahim S. Association of hypertension, diabetes, stroke, cancer, kidney disease, and high-cholesterol with COVID-19 disease severity and fatality: a systematic review. *Diabetes Metab Syndr Clin Res Rev.* (2020) 14:1133–42. doi: 10.1016/j.dsx.2020.07.005
71. Chu Y, Yang J, Shi J, Zhang P, Wang X. Obesity is associated with increased severity of disease in COVID-19 pneumonia: a systematic review and meta-analysis. *Eur J Med Res.* (2020) 25:1–15. doi: 10.1186/s40001-020-00464-9
72. Veghari G, Sedaghat M, Joshghani H, Banihashem S, Moharloei P, Angizeh A, et al. Obesity and risk of hypercholesterolemia in Iranian northern adults. *ARYA Atheroscler.* (2013) 9:2–6.
73. Veghari G, Sedaghat M, Maghsodlo S, Banihashem S, Moharloei P, Angizeh A, et al. The association between abdominal obesity and serum cholesterol level. *Int J Appl Basic Med Res.* (2015) 5:83. doi: 10.4103/2229-516X.157150
74. Wei X, Zeng W, Su J, Wan H, Yu X, Cao X, et al. Hypolipidemia is associated with the severity of COVID-19. *J Clin Lipidol.* (2020) 14:297–304. doi: 10.1016/j.jacl.2020.04.008
75. Wang S, Ma P, Zhang S, Song S, Wang Z, Ma Y, et al. Fasting blood glucose at admission is an independent predictor for 28-day mortality in patients with COVID-19 without previous diagnosis of diabetes: a multi-centre retrospective study. *Diabetologia.* (2020) 63:2102–11. doi: 10.1007/s00125-020-05209-1
76. Wu J, Huang J, Zhu G, Wang Q, Lv Q, Huang Y, et al. Elevation of blood glucose level predicts worse outcomes in hospitalized patients with COVID-19: a retrospective cohort study. *BMJ Open Diabetes Res Care.* (2020) 8:1–7. doi: 10.1136/bmjdrc-2020-001476
77. Avogaro A, Albiero M, Menegazzo L, De Kreutzenberg S, Fadini GP. Endothelial dysfunction in diabetes: the role of reparatory mechanisms. *Diabetes Care.* (2011) 34:S285–S90. doi: 10.2337/dc11-s239
78. Loomans CJM, De Koning EJP, Staal FJT, Rookmaaker MB, Verseyden C, De Boer HC, et al. Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. *Diabetes.* (2004) 53:195–9. doi: 10.2337/diabetes.53.1.195
79. Rogers SC, Zhang X, Azhar G, Luo S, Wei JY. Exposure to high or low glucose levels accelerates the appearance of markers of endothelial cell senescence and induces dysregulation of nitric oxide synthase. *J Gerontol Ser A Biol Sci Med Sci.* (2013) 68:1469–81. doi: 10.1093/gerona/glt033
80. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes.* (2005) 54:1615–25. doi: 10.2337/diabetes.54.6.1615

81. Zuin M, Rigatelli G, Zuliani G, Rigatelli A, Mazza A, Roncon L. Arterial hypertension and risk of death in patients with COVID-19 infection: systematic review and meta-analysis Dear. *J Infect.* (2020) 81:e84–6. doi: 10.1016/j.jinf.2020.03.059
82. Bleakley C, Hamilton PK, Pumb R, Harbinson M, Mcveigh GE. Endothelial function in hypertension: victim or culprit? *J Clin Hypertens.* (2015) 17:651–4. doi: 10.1111/jch.12546
83. Taniyama Y, Griendling KK. Reactive oxygen species in the vasculature: molecular and cellular mechanisms. *Hypertension.* (2003) 42:1075–81. doi: 10.1161/01.HYP.0000100443.09293.4F
84. Sattelmair J, Pertman J, Ding EL, Kohl HW, Haskell W, Lee I-M. Dose-response between physical activity and risk of coronary heart disease: a meta-analysis. *Circulation.* (2011) 124:789–95. doi: 10.1161/CIRCULATIONAHA.110.010710
85. Tavakol Z, Ghannadi S, Tabesh MR, Halabchi F, Noormohammadpour P, Akbarpour S, et al. Relationship between physical activity, healthy lifestyle and COVID-19 disease severity; a cross-sectional study. *Z Gesundh Wiss.* (2021) 1–9. doi: 10.1007/s10389-020-01468-9
86. Hackshaw A, Morris JK, Boniface S, Tang JL, Milenkovi D. Low cigarette consumption and risk of coronary heart disease and stroke: meta-analysis of 141 cohort studies in 55 study reports. *BMJ.* (2018) 360:j5855. doi: 10.1136/bmj.j5855
87. Reddy RK, Charles WN, Sklavounos A, Dutt A, Seed PT, Khajuria A. The effect of smoking on COVID-19 symptom severity: systematic review and meta-analysis. *J Med Virol.* (2020) 93:1045–56. doi: 10.1002/jmv.26389
88. Blaizot A, Vergnes J-N, Nuwwareh S, Amar J, Sixou M. Periodontal diseases and cardiovascular events: meta-analysis of observational studies. *Int Dent J.* (2009) 59:197–209.
89. Marouf N, Cai W, Said KN, Daas H, Diab H, Chinta VR, et al. Association between periodontitis and severity of COVID-19 infection: a case–control study. *J Clin Periodontol.* (2021) 48:483–91. doi: 10.1111/jcpe.13435
90. Rosengren A, Hawken S, Ôunpuu S, Sliwa PK, Zubaid M, Almahmeed WA, Ngu Blackett K, et al. Association of psychosocial risk factors with risk of acute myocardial infarction in 11 119 cases and 13 648 controls from 52 countries (the INTERHEART study): case-control study. *Lancet.* (2004) 364:953–62. doi: 10.1016/S0140-6736(04)17019-0
91. Tant T, Khoot B, Mills EG, Phylactou M, Patel B, Eng PC, et al. Association between high serum total cortisol concentrations and mortality from COVID-19. *Lancet Diabetes Endocrinol.* (2020) 8:659–60. doi: 10.1016/S2213-8587(20)30216-3
92. Nicholson A, Kuper H, Hemingway H. Depression as an aetiologic and prognostic factor in coronary heart disease: a meta-analysis of 6362 events among 146 538 participants in 54 observational studies. *Eur Heart J.* (2006) 27:2763–74. doi: 10.1093/eurheartj/ehl338
93. Yang H, Chen W, Hu Y, Chen Y, Zeng Y, Sun Y, et al. Pre-pandemic psychiatric disorders and risk of COVID-19: a UK Biobank cohort analysis. *Lancet Heal Longev.* (2020) 1:e69–79. doi: 10.1016/S2666-7568(20)30013-1
94. Shah NA, Yaggi HK, Concato J, Mohsenin V. Obstructive sleep apnea as a risk factor for coronary events or cardiovascular death. *Sleep Breath.* (2010) 14:131–6. doi: 10.1007/s11325-009-0298-7
95. Strausz S, Kiiskinen T, Broberg M, Ruotsalainen S, Koskela J, Bachour A, et al. Sleep apnoea is a risk factor for severe COVID-19. *BMJ Open Respir Res.* (2021) 8:6–11. doi: 10.1136/bmjresp-2020-000845
96. Sofi F, Cesari F, Casini A, Macchi C, Abbate R, Gensini GF. Insomnia and risk of cardiovascular disease: a meta-analysis. *Eur J Prev Cardiol.* (2014) 21:57–64. doi: 10.1177/2047487312460020
97. Kim H, Hegde S, Lafiura C, Raghavan M, Luong E, Cheng S, et al. COVID-19 illness in relation to sleep and burnout. *BMJ Nutr Prev Heal.* (2021) 4:132–9. doi: 10.1136/bmjnph-2021-000228
98. Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali WA. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. *BMJ.* (2011) 342:d671. doi: 10.1136/bmj.d671
99. Dong JY, Zhang YH, Wang P, Qin LQ. Meta-analysis of dietary glycemic load and glycemic index in relation to risk of coronary heart disease. *Am J Cardiol.* (2012) 109:1608–13. doi: 10.1016/j.amjcard.2012.01.385
100. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170 000 participants in 26 randomised trials. *Lancet.* (2010) 376:1670–81. doi: 10.1016/S0140-6736(10)61350-5
101. Zhang XJ, Qin JJ, Cheng X, Shen L, Zhao YC, Yuan Y, et al. In-hospital use of statins is associated with a reduced risk of mortality among individuals with COVID-19. *Cell Metab.* (2020) 32:1–12. doi: 10.1016/j.cmet.2020.06.015
102. Collins R, Peto R, Hennekens C, Doll R, Bubes V, Buring J, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. *Lancet.* (2009) 373:1849–60. doi: 10.1016/S0140-6736(09)60503-1
103. Boersma E, Harrington RA, Moliterno DJ, White H, Théroux P, Van De Werf F, et al. Platelet glycoprotein IIb/IIIa inhibitors in acute coronary syndromes: a meta-analysis of all major randomised clinical trials. *Lancet.* (2002) 359:189–98. doi: 10.1016/S0140-6736(02)07442-1
104. Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost.* (2020) 18:1094–9. doi: 10.1111/jth.14817
105. Wallentin L, Wilcox RG, Weaver WD, Emanuelsson H, Goodvin A, Nyström P, et al. Oral ximelagatran for secondary prophylaxis after myocardial infarction: the ESTEEM randomised controlled trial. *Lancet.* (2003) 362:789–97. doi: 10.1016/S0140-6736(03)14287-0
106. Flam B, Wintzell V, Ludvigsson JF, Mårtensson J, Pasternak B. Direct oral anticoagulant use and risk of severe COVID-19. *J Intern Med.* (2021) 289:411–9. doi: 10.1111/joim.13205
107. Verdecchia P, Reboldi G, Angeli F, Gattobigio R, Bentivoglio M, Thijs L, et al. Angiotensin-converting enzyme inhibitors and calcium channel blockers for coronary heart disease and stroke prevention. *Hypertension.* (2005) 46:386–92. doi: 10.1161/01.HYP.0000174591.42889.a2
108. Hippisley-Cox J, Young D, Coupland C, Channon KM, Tan PS, Harrison DA, et al. Risk of severe COVID-19 disease with ACE inhibitors and angiotensin receptor blockers: cohort study including 8.3 million people. *Heart.* (2020) 106:1503–11. doi: 10.1136/heartjnl-2020-317393
109. Savarese G, Costanzo P, Cleland JGF, Vassallo E, Ruggiero D, Rosano G, et al. A meta-analysis reporting effects of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in patients without heart failure. *J Am Coll Cardiol.* (2013) 61:131–42. doi: 10.1016/j.jacc.2012.10.011
110. Gao C, Cai Y, Zhang K, Zhou L, Zhang Y, Zhang X, et al. Association of hypertension and antihypertensive treatment with COVID-19 mortality: a retrospective observational study. *Eur Heart J.* (2020) 41:2058–66. doi: 10.1093/eurheartj/ehaa433
111. De Peuter OR, Lussana F, Peters RJG, Büller HR, Kamphuisen PW. A systematic review of selective and non-selective beta blockers for prevention of vascular events in patients with acute coronary syndrome or heart failure. *Neth J Med.* (2009) 67:284–94.
112. Ren L, Yu S, Xu W, Overton JL, Chiamvimonvat N, Thai PN. Lack of association of antihypertensive drugs with the risk and severity of COVID-19: a meta-analysis. *J Cardiol.* (2021) 77:482–91. doi: 10.1016/j.jjcc.2020.10.015
113. Psaty BM, Lumley T, Furberg CD, Schellenbaum G, Pahor M, Alderman MH, et al. Health Outcomes associated with various antihypertensive therapies used as first-line agents: a network meta-analysis. *J Am Med Assoc.* (2003) 289:2534–44. doi: 10.1001/jama.289.19.2534
114. Selvin E, Bolen S, Yeh HC, Wiley C, Wilson LM, Marinopoulos SS, et al. Cardiovascular outcomes in trials of oral diabetes medications: a systematic review. *Arch Intern Med.* (2008) 168:2070–80. doi: 10.1001/archinte.168.19.2070
115. Khunti K, Knighton P, Zaccardi F, Bakhai C, Barron E, Holman N, et al. Prescription of glucose-lowering therapies and risk of COVID-19 mortality in people with type 2 diabetes: a nationwide observational study in England. *Lancet Diabetes Endocrinol.* (2021) 8587:1–11. doi: 10.1016/S2213-8587(21)00050-4
116. Scherrer JF, Garfield LD, Lustman PJ, Hauptman PJ, Chrusciel T, Zeringue A, et al. Antidepressant drug compliance: reduced risk of MI and mortality in depressed patients. *Am J Med.* (2011) 124:318–24. doi: 10.1016/j.amjmed.2010.11.015
117. Lenze EJ, Mattar C, Zorumski CF, Stevens A, Schweiger J, Nicol GE, et al. Fluvoxamine vs placebo and clinical deterioration in outpatients

with symptomatic COVID-19: a randomized clinical Trial. *JAMA*. (2020) 324:2292–300. doi: 10.1001/jama.2020.22760
118. Sallis R, Young DR, Tartof SY, Sallis JF, Sall J, Li Q, et al. Physical inactivity is associated with a higher risk for severe COVID-19 outcomes: a study in 48 440 adult patients. *Br J Sports Med.* (2021) 55:1099–105. doi: 10.1136/bjsports-2021-104080
119. Nieman DC, Wentz LM. The compelling link between physical activity and the body's defense system. *J Sport Heal Sci.* (2019) 8:201–17. doi: 10.1016/j.jshs.2018.09.009
120. Facchini FS, Hollenbeck CB, Jeppesen J, Ida Chen YD, Reaven GM. Insulin resistance and cigarette smoking. *Lancet.* (1992) 339:1128–30. doi: 10.1016/0140-6736(92)90730-Q
121. Reaven G, Tsao PS. Insulin resistance and compensatory hyperinsulinemia: the key player between cigarette smoking and cardiovascular disease? *J Am Coll Cardiol.* (2003) 41:1044–7. doi: 10.1016/S0735-1097(02)02982-0
122. Takefuji S, Yatsuya H, Tamakoshi K, Otsuka R, Wada K, Matsushita K, et al. Smoking status and adiponectin in healthy Japanese men and women. *Prev Med.* (2007) 45:471–5. doi: 10.1016/j.ypmed.2007.07.001
123. Badrick E, Kirschbaum C, Kumari M. The relationship between smoking status and cortisol secretion. *J Clin Endocrinol Metab.* (2007) 92:819–24. doi: 10.1210/jc.2006-2155
124. Craig WY, Palomaki GE, Haddow JE. Cigarette smoking and serum lipid and lipoprotein concentrations: an analysis of published data. *BMJ.* (1989) 298:784–8. doi: 10.1136/bmj.298.6676.784
125. Lee J, Taneja V, Vassallo R. Cigarette smoking and inflammation: cellular and molecular mechanisms. *J Dent Res.* (2012) 91:142–9. doi: 10.1177/0022034511421200
126. Barua RS, Sy F, Srikanth S, Huang G, Javed U, Buhari C, et al. Effects of cigarette smoke exposure on clot dynamics and fibrin structure: an *ex vivo* investigation. *Arterioscler Thromb Vasc Biol.* (2010) 30:75–9. doi: 10.1161/ATVBAHA.109.195024
127. Deshpande RG, Khan MB, Genco CA. Invasion of aortic and heart endothelial cells by Porphyromonas gingivalis. *Infect Immun.* (1998) 66:5337–43. doi: 10.1128/IAI.66.11.5337-5343.1998
128. Fan X, Yin C, Wang J, Yang M, Ma H, Jin G, et al. Pre-diagnostic circulating concentrations of insulin-like growth factor-1 and risk of COVID-19 mortality: results from UK Biobank. *Eur J Epidemiol.* (2021) 36:311–8. doi: 10.1101/2020.07.09.20149369
129. Kong Y, Han J, Wu X, Zeng H, Liu J, Zhang H. VEGF-D: a novel biomarker for detection of COVID-19 progression. *Crit Care.* (2020) 24:1–4. doi: 10.1186/s13054-020-03079-y
130. Chen Y, Wang J, Liu C, Su L, Zhang D, Fan J, et al. IP-10 and MCP-1 as biomarkers associated with disease severity of COVID-19. *Mol Med.* (2020) 26:1–12. doi: 10.1186/s10020-020-00230-x
131. Merzon E, Green I, Shpigelman M, Vinker S, Raz I, Golan-Cohen A, et al. Haemoglobin A1c is a predictor of COVID-19 severity in patients with diabetes. *Diabetes Metab Res Rev.* (2020) 2019:1–6. doi: 10.1002/dmrr.3398
132. Saengow U, Assanangkornchai S, Casswell S. Alcohol: a probable risk factor of COVID-19 severity. *Addiction.* (2021) 116:202–8. doi: 10.1111/add.15194
133. Simou E, Leonardi-Bee J, Britton J. The effect of alcohol consumption on the risk of ARDS: a systematic review and meta-analysis. *Chest.* (2018) 154:58–68. doi: 10.1016/j.chest.2017.11.041
134. Keddie S, Ziff O, Chou MKL, Taylor RL, Heslegrave A, Garr E, et al. Laboratory biomarkers associated with COVID-19 severity and management. *Clin Immunol.* (2020) 221:1–5. doi: 10.1016/j.clim.2020.108614
135. Malik P, Patel U, Mehta D, Patel N, Kelkar R, Akrmah M, et al. Biomarkers and outcomes of COVID-19 hospitalisations: systematic review and meta-analysis. *BMJ Evidence-Based Med.* (2020) 26:107–8. doi: 10.1136/bmjebm-2020-111536
136. Jalali Nadoushan M, Ahmadi S, Jalali Nadoushan P, Azzi L, Carcano G, Gianfagna F, et al. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clin Chem Lab Med.* (2020) 58:1021–8. doi: 10.1515/cclm-2020-0369
137. Ji P, Zhu J, Zhong Z, Li H, Pang J, Li B, et al. Association of elevated inflammatory markers and severe COVID-19: a meta-analysis. *Medicine.* (2020) 99:e23315. doi: 10.1097/MD.0000000000023315
138. Akhmerov A, Marbán E. COVID-19 and the Heart. *Circ Res.* (2020) 126:1443–55. doi: 10.1161/CIRCRESAHA.120.317055
139. Mcelvaney OJ, Mcevoy NL, Carroll P, Murphy MP, Dunlea DM, Orna N, et al. Characterization of the Inflammatory Response to Severe COVID-19 Illness. *Am J Respir Crit Care Med.* (2020) 202:812–21. doi: 10.1164/rccm.202005-1583OC
140. Luis García de Guadiana R, Mulero MDR, Olivo MH, Rojas CR, Arenas VR, Morales MG, et al. Circulating levels of GDF-15 and calprotectin for prediction of in-hospital mortality in COVID-19 patients: a case series. *J Infect.* (2020) 82:e40–42. doi: 10.1016/j.jinf.2020.08.010
141. Milionis HJ, Liberopoulos EN, Achimastos A, Elisaf MS, Mikhailidis DP. Statins: another class of antihypertensive agents? *J Hum Hypertens.* (2006) 20:320–35. doi: 10.1038/sj.jhh.1002001
142. Amann R, Peskar BA. Anti-inflammatory effects of aspirin and sodium salicylate. *Eur J Pharmacol.* (2002) 447:1–9. doi: 10.1016/S0014-2999(02)01828-9
143. Undas A, Brummel-Ziedins KE, Mann KG. Antithrombotic properties of aspirin and resistance to aspirin: beyond strictly antiplatelet actions. *Blood.* (2007) 109:2285–92. doi: 10.1182/blood-2006-01-010645
144. Young E. The anti-inflammatory effects of heparin and related compounds. *Thromb Res.* (2008) 122:743–52. doi: 10.1016/j.thromres.2006.10.026
145. Siddiqi HK, Libby P, Ridker PM. COVID-19 –a vascular disease. *Trends Cardiovasc Med.* (2021) 31:1–5. doi: 10.1016/j.tcm.2020.10.005
146. Marchetti M. COVID-19-driven endothelial damage: complement, HIF-1, and ABL2 are potential pathways of damage and targets for cure. *Ann Hematol.* (2020) 99:1701–7. doi: 10.1007/s00277-020-04138-8
147. Danta CC. SARS-CoV-2, hypoxia, and calcium signaling: the consequences and therapeutic options. *ACS Pharmacol Transl Sci.* (2021) 4:400–2. doi: 10.1021/acsptsci.0c00219
148. Jahani M, Dokaneheifard S, Mansouri K. Hypoxia: a key feature of COVID-19 launching activation of HIF-1 and cytokine storm. *J Inflamm.* (2020) 17:1–10. doi: 10.1186/s12950-020-00263-3
149. Mathews I, Mathews EH, van Laar JH, Hamer W, Kleingeld M. A simulation-based prediction model for coal-fired power plant condenser maintenance. *Appl Therm Eng.* (2020) 174:115294. doi: 10.1016/j.applthermaleng.2020.115294
150. Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 *in vitro*. *Antiviral Res.* (2020) 178:3–6. doi: 10.1016/j.antiviral.2020.104787
151. Mody V, Ho J, Wills S, Mawri A, Lawson L, Ebert MCCJC, et al. Identification of 3-chymotrypsin like protease (3CLPro) inhibitors as potential anti-SARS-CoV-2 agents. *Commun Biol.* (2021) 4:1–10. doi: 10.1038/s42003-020-01577-x
152. Lehrer S, Rheinstein PH. Ivermectin docks to the SARS-CoV-2 spike receptor-binding domain attached to ACE2. *In Vivo.* (2020) 34:3023–26. doi: 10.21873/invivo.12134
153. Swargiary A. Ivermectin as a promising RNA-dependent RNA polymerase inhibitor and a therapeutic drug against SARS-CoV2: evidence from *in silico* studies. *Res Sq. [Preprint]* (2020). doi: 10.21203/rs.3.rs-73308/v1
154. Kory P, Meduri GU, Varon J, Iglesias J, Marik PE. Review of the emerging evidence demonstrating the efficacy of ivermectin in the prophylaxis and treatment of COVID-19. *Am J Ther.* (2021) 28:e299–318. doi: 10.1097/MJT.0000000000001377
155. Bryant A, Lawrie T, Fordham E, Scott M, Hill S, Tham T. Ivermectin for prevention and treatment of COVID-19 infection: a systematic review and meta-analysis. *Am J Ther.* (2021) 27:e1–27. doi: 10.31219/osf.io/k37ft
156. Whitworth JAG, Hay CRM, Nicholas AM, Morgan D, Maude GH, Taylor DW. Coagulation abnormalities and ivermectin. *Ann Trop Med Parasitol.* (1992) 86:301–5. doi: 10.1080/00034983.1992.11812667

157. Jin L, Feng X, Rong H, Pan Z, Inaba Y, Qiu L, et al. The antiparasitic drug ivermectin is a novel FXR ligand that regulates metabolism. *Nat Commun.* (2013) 4:1–8. doi: 10.1038/ncomms2924
158. Ci X, Li H, Yu Q, Zhang X, Yu L, Chen N, et al. Avermectin exerts anti-inflammatory effect by downregulating the nuclear transcription factor kappa-B and mitogen-activated protein kinase activation pathway. *Fundam Clin Pharmacol.* (2009) 23:449–55. doi: 10.1111/j.1472-8206.2009.00684.x
159. DiNicolantonio JJ, Arranda JB-, McCarty M. Ivermectin may be a clinically useful anti-inflammatory agent for late-stage COVID-19. *Open Hear.* (2020) 7:e001350. doi: 10.1136/openhrt-2020-001350
160. Gracia-Ramos AE, Jaquez-Quintana JO, Contreras-Omaña R, Auron M. Liver dysfunction and SARS-CoV-2 infection. *World J Gastroenterol.* (2021) 27:3951–70. doi: 10.3748/wjg.v27.i26.3951
161. Kory P, Meduri GU, Iglesias J, Varon J, Berkowitz K, Kornfeld H, et al. Review of the emerging evidence demonstrating the efficacy of ivermectin in the prophylaxis and treatment of COVID-19. *Front Pharmacol.* (2021) 1–32. doi: 10.31219/osf.io/wx3zn
162. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. *J Am Coll Cardiol.* (2017) 70:1–25. doi: 10.1016/j.jacc.2017.04.052

Conflict of Interest: This study received funding from HumanSim (Pty) Ltd. The funder was not involved in the study design, collection, analysis,interpretation of data, the writing of this article or the decision to submit it for publication.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

NOMENCLATURE

ACE, Angiotensin-converting-enzyme
β-blocker, Beta-adrenergic antagonists
BDNF, Brain-derived neurotrophic factor
BNP, B-type natriuretic peptide
Covid-19, Coronavirus disease of 2019
COX, Cyclooxygenase
CRP, C-reactive protein
CHD, Coronary heart disease
D-dimer, Fibrin degradation product D
EC, Endothelial cell
FFA, Free fatty acids
GCF, Gingival crevicular fluid
GM-CSF, Granulocyte-Macrophage Colony-Stimulating Factor
HbA1c, Glycated hemoglobin A1c
HDL, High-density lipoprotein
HGD, High Glycemic Diet
HR, Hazard Ratio
Hs, Homocysteine
ICAM, Intracellular adhesion molecule
IGF-1, Insulin-like growth factor-1
IL, Interleukin
LDL, Low-density lipoprotein
MAPK, Mitogen-activated protein kinase
MCP, Monocyte chemoattractant protein
MIF, Macrophage migration inhibitory factor
MMP, Matrix metalloproteinase
MOA, Mechanism Of Action
MPO, Myeloperoxidase
NFκβ, Nuclear factor-kappa-beta
NLRP3, Nod-like receptor family pyrin domain containing 3
NO, Nitric oxide
NO-NSAID, Nitric oxide-non-steroidal anti-inflammatory drug
OPG, Osteoprotegerin
OR, Odds Ratio
oxLDL, Oxidized LDL
PAI, Plasminogen activator inhibitor
PDGF, Platelet-derived growth factor
P. gingivalis, Porphyromonas gingivalis
PI3K, Phosphatidylinositol 3-kinase
RANKL, Receptor activator of nuclear factor kappa-beta ligand
ROS, Reactive oxygen species
RR, Relative Risk
SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2
SCD-40, Recombinant human sCD40 ligand
SMC, Smooth muscle cell
SSRI, Serotonin reuptake inhibitors
TF, Tissue factor
TMAO, Oxidation product of trimethylamine
TNF-α, Tumor necrosis factor-alpha
VCAM, Vascular cell adhesion molecule
vWF, von Willebrand factor.

frontiers in Cardiovascular Medicine

SYSTEMATIC REVIEW
published: 14 March 2022
doi: 10.3389/fcvm.2022.831143

Comparison of the Characteristics, Management, and Outcomes of STEMI Patients Presenting With vs. Those of Patients Presenting Without COVID-19 Infection: A Systematic Review and Meta-Analysis

Yanjiao Wang[1,2†], *Linlin Kang*[1,2†], *Ching-Wen Chien*[2], *Jiawen Xu*[2], *Peng You*[2], *Sizhong Xing*[1*] and *Tao-Hsin Tung*[3*]

[1] Shenzhen Bao'an District Traditional Chinese Medicine Hospital, Shenzhen, China, [2] Institute for Hospital Management, Tsing Hua University, Shenzhen, China, [3] Evidence-Based Medicine Center, Taizhou Hospital of Zhejiang Province Affiliated to Wenzhou Medical University, Linhai, China

Objectives: This study aimed to investigate the differences in the characteristics, management, and clinical outcomes of patients with and that of those without coronavirus disease 2019 (COVID-19) infection who had ST-segment elevation myocardial infarction (STEMI).

Methods: Databases including Web of Science, PubMed, Cochrane Library, and Embase were searched up to July 2021. Observational studies that reported on the characteristics, management, or clinical outcomes and those published as full-text articles were included. The Newcastle-Ottawa Scale (NOS) was used to assess the quality of all included studies.

Results: A total of 27,742 patients from 13 studies were included in this meta-analysis. Significant delay in symptom onset to first medical contact (SO-to-FMC) time (mean difference = 23.42 min; 95% CI: 5.85–40.99 min; $p = 0.009$) and door-to-balloon (D2B) time (mean difference = 12.27 min; 95% CI: 5.77–18.78 min; $p = 0.0002$) was observed in COVID-19 patients. Compared to COVID-19 negative patients, those who are positive patients had significantly higher levels of C-reactive protein, D-dimer, and thrombus grade ($p < 0.05$) and showed more frequent use of thrombus aspiration and glycoprotein IIbIIIa (Gp2b3a) inhibitor ($p < 0.05$). COVID-19 positive patients also had higher rates of in-hospital mortality (OR = 5.98, 95% CI: 4.78–7.48, $p < 0.0001$), cardiogenic shock (OR = 2.75, 95% CI: 2.02–3.76, $p < 0.0001$), and stent thrombosis (OR = 5.65, 95% CI: 2.41–13.23, $p < 0.0001$). They were also more likely to be admitted to the intensive care unit (ICU) (OR = 4.26, 95% CI: 2.51–7.22, $p < 0.0001$) and had a longer length of stay (mean difference = 4.63 days; 95% CI: 2.56–6.69 days; $p < 0.0001$).

OPEN ACCESS

Edited by:
Nicola Mumoli, ASST Ovest Milanese, Italy

Reviewed by:
Antonia Anna Lukito, University of Pelita Harapan, Indonesia
Wenyang Jiang, Chinese Academy of Medical Sciences and Peking Union Medical College, China
Efstratios Karagiannidis, Aristotle University of Thessaloniki, Greece
Vinod Krishnappa, University of North Carolina Health Southeastern, United States

***Correspondence:**
Tao-Hsin Tung
ch2876@gmail.com
Sizhong Xing
xsz7220@163.com

†These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Cardiovascular Epidemiology and Prevention, a section of the journal Frontiers in Cardiovascular Medicine

Received: *09 December 2021*
Accepted: *07 February 2022*
Published: *14 March 2022*

Citation:
Wang Y, Kang L, Chien C-W, Xu J, You P, Xing S and Tung T-H (2022) Comparison of the Characteristics, Management, and Outcomes of STEMI Patients Presenting With vs. Those of Patients Presenting Without COVID-19 Infection: A Systematic Review and Meta-Analysis. Front. Cardiovasc. Med. 9:831143. doi: 10.3389/fcvm.2022.831143

Conclusions: This study revealed that COVID-19 infection had an impact on the time of initial medical intervention for patients with STEMI after symptom onset and showed that COVID-19 patients with STEMI were more likely to have thrombosis and had poorer outcomes.

Keywords: COVID-19, SARS-CoV-2, mortality, ST-segment elevation myocardial infarction, STEMI

INTRODUCTION

An eventual pandemic brought by the coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulted in plenty of deaths and has had a strong impact on the world's healthcare system (1–3). Although the disease is predominantly characterized by respiratory symptoms, including pneumonia, dyspnea, and cough (4), various extrapulmonary features, such as myocardial damage, arrhythmia, thrombotic events, and renal injury have also been observed (5, 6).

A type of heart attack called ST-segment elevation myocardial infarction (STEMI) is usually caused by thrombotic occlusion at the site of a ruptured plaque in the coronary artery (7). Although the survival rates of STEMI patients have improved, it is still associated with high morbidity and mortality worldwide with a 1-year mortality rate of up to 10% (8–10). The COVID-19 pandemic may lead to a decrease in the number of STEMI admissions and could have a significant impact on the reperfusion strategy for patients with STEMI (11, 12). The tendency of patients with COVID-19 to be predisposed to cardiac arrest and coronary thrombosis due to increased inflammation, platelet activation, endothelial dysfunction, and SARS-CoV-2 invasion of cardiomyocytes has been reported (13–15). Moreover, data regarding the characteristics, management strategies, and clinical outcomes including in-hospital mortality and cardiogenic shock in patients presenting with STEMI concurrent with COVID-19 infection are limited (16). Accordingly, we aimed to conduct a systematic review and meta-analysis to compare the characteristics, management, and clinical outcomes between the COVID-19 and non-COVID-19 patients concomitant STEMI.

METHODS

Literature Search

We performed a literature search using databases including Web of Science (Beijing), PubMed (Bethesda), Cochrane Library (UK), and Embase (Amsterdam) for relevant papers without language limitation on July 31, 2021. The search strategy included a mix of MeSH and free-text terms relevant to the critical concept of "STEMI" and "COVID-19" (**Table 1**). The protocol for this meta-analysis was registered at PROSPERO under the number CRD42021283880.

Study Selection

Studies were included if they met the following inclusion criteria: (i) studies involving STEMI patients; (ii) the exposure group included patients diagnosed with COVID-19 using PCR test or had a high index of clinical suspicion, and the control group included patients without COVID-19; (iii) studies that reported at least one of the following information: characteristics, management strategy, or clinical outcomes; (iv) relevant cohort studies, cross-sectional studies, case series, and case-control studies. Two independent authors screened the titles and abstracts of all relevant studies and identified whether they met the inclusion criteria by reviewing the full text of each potential study. Any discrepancy was resolved through consensus with a third author.

Data Extraction and Quality Assessment

Relevant data from all included studies were extracted by two authors independently, and any disagreement was resolved by discussion with a third author. The following data were extracted: authors, publication year, country, study design, study subject, sample size, mean age of patients/subjects, sex, comparison period, participant characteristics, management strategies, and clinical outcomes. The Newcastle–Ottawa Scale (NOS), which includes participant selection, comparability, and outcome, was used to assess the quality of the included studies. Likewise, all included studies were rated by two authors independently, and any discrepancy was adjudicated by consensus.

Statistical Analysis

We used Review Manager 5.4 (The Nordic Cochrane Center, Cochrane Collaboration, 2020, Denmark) to perform the statistical analysis. If studies only reported median values and interquartile ranges (IQR), means and *SD*s were calculated according to the Box-Cox method (17). Categorical variables were presented as odds ratios (ORs), including 95% CIs, and continuous variables were presented as the mean difference (MD) or standardized mean difference (SMD), including 95% CI. Heterogeneity was assessed using the I^2 statistic and the *p*-value of the chi-square test. The I^2 statistic $> 50\%$ indicates significant heterogeneity. The choice between the fixed and random effects models depended on the comparability among the studies. A two-tailed *p*-value of < 0.05 was interpreted to be statistically significant. The risk of publication bias was evaluated using the funnel plots.

RESULTS

Characteristics of Included Studies

A total of 2,702 articles were retrieved through electronic database searches, of which 1,371 were duplicates. After screening the titles and abstracts, 24 potential articles were assessed for eligibility after a full-text review, and 13 articles (18–30) with a total of 27,742 patients were finally included

TABLE 1 | Search strategy.

Database	Searching key words	
PubMed	(1) "ST Segment Elevation Myocardial Infarction": 9451	(10) SARS-CoV-2: 106826
	(2) "ST Elevated Myocardial Infarction": 317	(11) "Coronavirus disease 19": 1603
	(3) STEMI: 28060	(12) "Severe Acute Respiratory Syndrome Coronavirus 2": 16865
	(4) "Acute myocardial infarction": 61630	(13) "novel coronavirus": 9766
	(5) AMI: 25165	(14) "2019 novel coronavirus": 1550
	(6) "Acute coronary syndromes": 13188	(15) #1 or #2 or #3 or #4 or #5 or #6 or #7: 208085
	(7) ACS: 116546	(16) #8 or #9 or #10 or #11 or #12 or #13 or #14: 169136
	(8) "SARSCoV-2 pandemic": 120	(17) #15 and #16: 1340
	(9) COVID-19: 168784	
Web of science	(1) "ST Segment Elevation Myocardial Infarction": 17531	(10) SARS-CoV-2: 127748
	(2) "ST Elevated Myocardial Infarction": 1899	(11) "Coronavirus disease 19": 3460
	(3) STEMI: 23388	(12) "Severe Acute Respiratory Syndrome Coronavirus 2": 58794
	(4) "Acute myocardial infarction": 145384	(13) "novel coronavirus": 14678
	(5) AMI: 44201	(14) "2019 novel coronavirus": 2224
	(6) "Acute coronary syndromes": 27560	(15) #1 or #2 or #3 or #4 or #5 or #6 or #7: 248982
	(7) ACS: 58425	(16) #8 or #9 or #10 or #11 or #12 or #13 or #14: 262441
	(8) "SARSCoV-2 pandemic": 25	(17) #15 and #16: 1098
	(9) COVID-19: 248069	
Cochrane library	(1) "ST Segment Elevation Myocardial Infarction": 4031	(10) SARS-CoV-2: 322
	(2) "ST Elevated Myocardial Infarction": 156	(11) "Coronavirus disease 19": 43
	(3) STEMI: 3616	(12) "Severe Acute Respiratory Syndrome Coronavirus 2": 631
	(4) "Acute myocardial infarction": 9325	(13) "novel coronavirus": 497
	(5) AMI: 3603	(14) "2019 novel coronavirus": 55
	(6) "Acute coronary syndromes": 2562	(15) #1 or #2 or #3 or #4 or #5 or #6 or #7: 19050
	(7) ACS: 4853	(16) #8 or #9 or #10 or #11 or #12 or #13 or #14: 6784
	(8) "SARSCoV-2 pandemic": 52	(17) #15 and #16: 31
	(9) COVID-19: 6666	
Embase	('acute myocardial infarction':ti,ab,kw OR ami:ti,ab,kw OR 'acute coronary syndromes':ti,ab,kw OR acs:ti,ab,kw OR 'st segment elevation myocardial infarction':ti,ab,kw OR 'st elevated myocardial infarction':ti,ab,kw OR stemi:ti,ab,kw) AND ('sarscov-2 pandemicor COVID-19':ti,ab,kw OR 'sars cov 2':ti,ab,kw OR 'coronavirus disease 19':ti,ab,kw OR 'novel coronavirus':ti,ab, kw OR 'severe acute respiratory syndrome coronavirus 2':ti,ab,kw) AND [1-1-1900]/sd NOT [1-8-2021]/sd; result = 233	

(**Figure 1**). A summary of the main characteristics of these 13 studies and the baseline characteristics of all study subjects is presented in **Tables 2A**,**B**. One study originated from Poland (19), two each from the United Kingdom (24, 28), France (18, 21), Turkey (20, 30), Italy (25, 26), and Spain (27, 29), and the remaining two studies (22, 23) were international studies. The NOS score for all included studies varied from 5 to 8 points.

Delays

The symptom onset to first medical contact (SO-to-FMC) time among STEMI, which was reported in four studies (19, 20, 27, 30), was significantly different between the COVID-19 group and the non-COVID-19 group (MD = 23.42 min, 95% CI: 5.85 to 40.99 min, $p = 0.009$; **Figure 2A**). Furthermore, seven studies (18, 22–25, 28, 30) reported the time from door to balloon (D2B) and found that D2B was significantly longer in the COVID-19 group (MD = 12.27 min, 95% CI: 5.77 to 18.78 min, $p = 0.0002$; **Figure 2B**) than in the non-COVID-19 group. *3.3 Laboratory values.*

The meta-analysis showed that compared to the non-COVID-19 group, the COVID-19 group had significantly higher levels of C-reactive protein (CRP), white blood cell count (WBC), and D-dimer (SMD = 0.76, 95% CI: 0.38 to 1.13, $p < 0.0001$; SMD = 0.39, 95% CI: 0.1 to 0.69, $p = 0.009$; SMD = 0.79, 95% CI: 0.36 to 1.22, $p = 0.0003$, respectively, **Figures 3A–C**), and had significantly lower level of lymphocyte count (SMD = −0.52, 95% CI: −0.69, −0.36, $p < 0.0001$, **Figure 3D**).

Management and Procedural Characteristic

There was no significant difference in the rate of primary angioplasty between the two groups (OR = 0.28, 95% CI: 0.08 to 1.01, $p = 0.05$; **Figure 4A**). Myocardial infarction with no obstructive coronary atherosclerosis (MINOCA) was more frequently observed, and the rate of stent implantation was lower in patients with COVID-19 infection (OR = 9.57, 95% CI: 2.14 to 42.83, $p = 0.003$; OR = 0.28, 95% CI: 0.11 to 0.71, $p = 0.008$, respectively, **Figures 4B,C**). Baseline thrombus grade > 3 and modified thrombus grade > 3 were significantly higher in the COVID-19 group than in the non-COVID-19 group (OR = 3.09, 95% CI: 1.83 to 5.23, $p < 0.0001$; OR = 5.84, 95% CI: 1.36 to 25.06, $p = 0.02$, respectively; **Figures 4D,E**). Intracoronary thrombus was angiographically identified and scored in 0–5 grades as previously described (31). In patients initially presenting with grade 5, thrombus grade will be reclassified into one of the other categories after flow achievement (32). After reclassification and based on clinical outcomes, the thrombus burden can be divided into 2 categories: low thrombus grade for thrombus < grade 4, and high thrombus grade for thrombus grade 4 (32). Consistent with this, the COVID-19 group showed a higher use of thrombus aspiration and glycoprotein IIbIIIa (Gp2b3a) inhibitor (OR = 1.68, 95% CI: 1.25 to 2.26, $p = 0.0007$; OR = 2.86, 95% CI: 1.78 to 4.62, $p < 0.0001$, respectively; **Figures 4F,G**). Moreover, thrombolysis in myocardial infarction (TIMI)-3 flow post-procedure was less common in the COVID-19 group than in the non-COVID-19 group (OR = 0.6, 95% CI: 0.42 to 0.84, $p = 0.003$, **Figure 4H**).

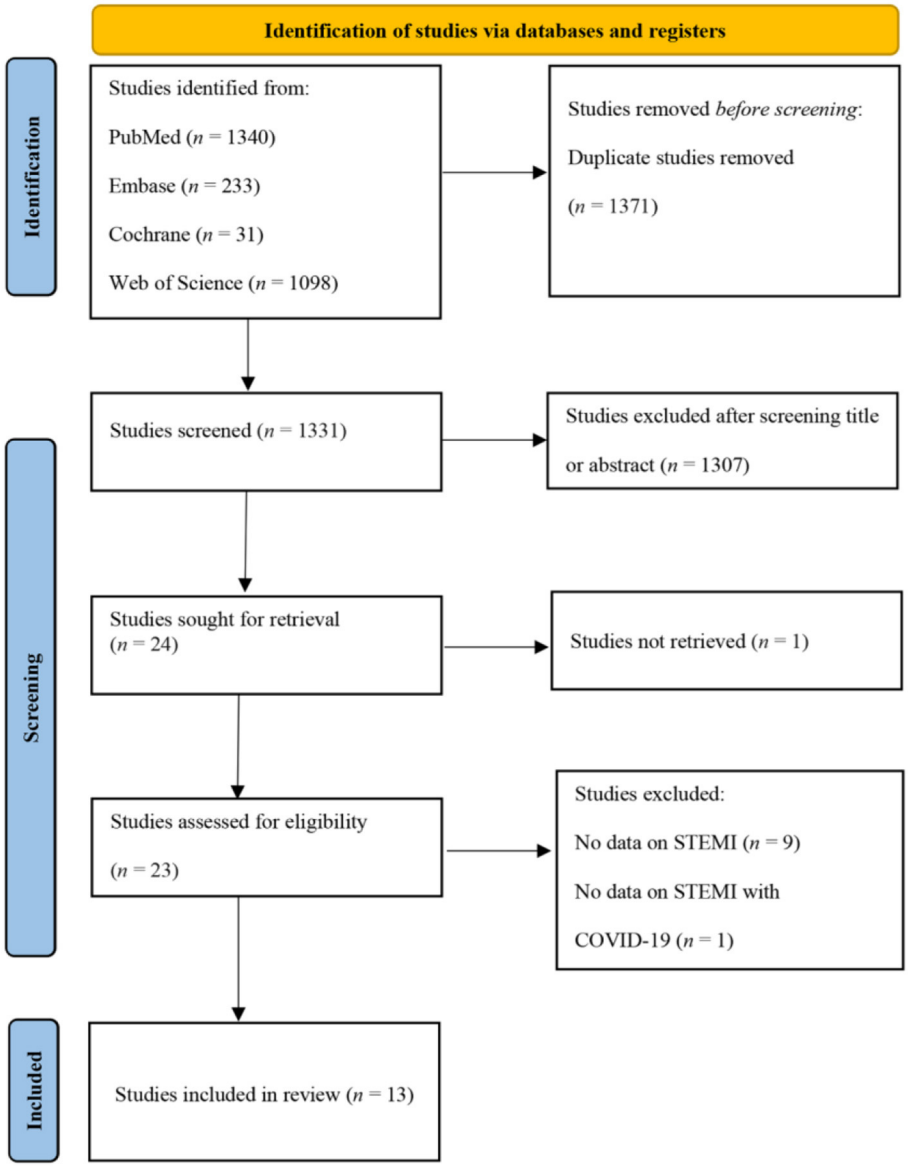


FIGURE 1 | PRISMA flow diagram.

In-Hospital Outcomes

In-hospital mortality among patients with COVID-19 was significantly higher than that in patients without COVID-19 (OR = 5.98, 95% CI: 4.78 to 7.48, $p < 0.0001$, **Figure 5A**). The rates of cardiogenic shock as well as stent thrombosis were also higher in the COVID-19 group than in the non-COVID-19 group (OR = 2.75, 95% CI: 2.02 to 3.76, $p < 0.0001$; OR = 5.65, 95% CI: 2.41 to 13.23, $p < 0.0001$, respectively; **Figures 5B,C**). Although bleeding was more common in STEMI patients with COVID-19, there was no significant difference between the two groups (OR = 2.82, 95% CI: 0.88 to 9.05, $p = 0.08$, **Figure 5D**). In addition, patients with COVID-19 were more likely to be admitted to the intensive care unit (ICU) and had a longer length of hospital stay (OR = 4.26, 95% CI: 2.51 to 7.22, $p < 0.0001$; MD = 4.63 days, 95% CI: 2.56 to 6.69 days, $p < 0.0001$, respectively, **Figures 5E,F**).

Grade Summary of Findings

The GRADE summary of findings tool was used to evaluate the quality of evidence, and the assessment for each outcome is presented in **Table 3**. In addition to in-hospital mortality, which moderates the quality of evidence, other outcomes had low or very low quality of evidence because all included studies were observational.

Sensitivity Analysis and Publication Bias

The leave-one-out approach was applied for sensitivity analysis to evaluate the impact of a single study on

TABLE 2A | Characteristics of included studies.

References	Country	Study design	Study group	Participants characteristics	Comparison period	COVID-19 diagnosis approach/time to diagnosis	Major findings
Popovic et al. (18)	France	Monocentric cohort study	COVID-19 STEMI	$n = 11$, age 63.6 ± 17.4 years, 63.9% males	26/2/2020–10/5/2020	RT-PCR or typical clinical features plus CT results/NA	D2B time, Laboratory values, Primary angioplasty, MINOCA, Stent implantation, Gp2b3a inhibitor use, TIMI status, In-hospital mortality
			Non-COVID-19 STEMI	$n = 72$, age 62.5 ± 12.6 years, 73.6% males	26/2/2020–10/5/2020		
Siudak et al. (19)	Poland	Multicentric cohort study	COVID-19 STEMI	$n = 145$, age 63.19 ± 12.55 years, 71.33% males	13/3/2020–13/5/2020	Swabs for molecular RT-PCR testing/NA	SO-to-FMC time
			Non-COVID-19 STEMI	$n = 2276$, age 65.43 ± 12.23 years, 67.65% males	13/3/2020–13/5/2020		
Kiris et al. (20)	Turkey	Multicentric cross-sectional study	COVID-19 STEMI	$n = 65$, age 66.8 ± 12.0 years, 68% males	11/3/2020–15/5/2020	Nasal/pharyngeal swabs or semptoms plus radiological imaging/NA	SO-to-FMC time, Laboratory values, Primary angioplasty, Thrombus aspiration, Gp2b3a inhibitor use, Baseline thrombus grade, Modified thrombus grade, TIMI status, In-hospital mortality, Bleeding, Stent thrombosis, Cardiogenic shock
			Non-COVID-19 STEMI	$n = 668$, age 60.0 ± 12.3 years, 78% males	11/3/2020–15/5/2020		
Koutsoukis et al. (21)	France	Multicentric cross-sectional study	COVID-19 STEMI	$n = 17$, age 63.4 ± 13.2 years, 70% males	1/4/2020–22/4/2020	RT-PCR on nasopharyngeal samples/NA	Laboratory values, Primary angioplasty, Thrombus aspiration, MINOCA, Stent implantation, Gp2b3a inhibitor use, In-hospital mortality
			Non-COVID-19 STEMI	$n = 99$, age 63.8 ± 13.9 years, 67% males	1/4/2020–22/4/2020		
Garcia et al. (22)	USA & Canada	Multicentric cohort study	COVID-19 STEMI	$n = 230$, 71% males	1/1/2020–6/12/2020	Comfirmed COVID+ by any commercially available test/NA	D2B time, Primary angioplasty, MINOCA, In-hospital mortality, LOS
			Non-COVID-19 STEMI	$n = 460$, 68% males	1/2015–12/2019		
Kite et al. (23)	Data from 55 international centers	Multicentric corhort study	COVID-19 STEMI	$n = 144$, age 63.1 ± 12.6 years, 77.8% males	1/3/2020–31/7/2020	RT-PCR or clinical status plus CXR or CT findings/NA	D2B time, Laboratory values, Thrombus aspiration, In-hospital mortality, Bleeding, Cardiogenic shock, LOS
			Non-COVID-19 STEMI	$n = 24961$, age 65.6 ± 13.4 years, 72.2% males	2018–2019		
Little et al. (24)	UK	Multicentric cohort study	COVID-19 STEMI	$n = 46$, age 61.80 ± 7.95 years, 80.4% males	1/3/2020–30/4/2020	RT-PCR on oro/nasopharyngeal throat swabs or typical symptoms plus radiographic appearances and characteristic blood test/NA	D2B time, Laboratory values, Thrombus aspiration, Gp2b3a inhibitor use, TIMI status, In-hospital mortality, Cardiogenic shock, ICU admission, LOS

(Continued)

TABLE 2A | Continued

References	Country	Study design	Study group	Participants characteristics	Comparison period	COVID-19 diagnosis approach/time to diagnosis	Major findings
			Non-COVID-19 STEMI	$n = 302$, age 64.18 ± 13.41 years, 79.8% males	1/3/2020–30/4/2020		
Marfella et al. (25)	Italy	Multicentric cohort study	COVID-19 STEMI	$n = 46$, age 56.13 ± 6.21 years, 67.4% males	2/2020–11/2020	RT-PCR on nasal/pharyngeal swabs/NA	D2B time, Laboratory values, Gp2b3a inhibitor use, Modified thrombus grade, TIMI status, In-hospital mortality, LOS, ICU admission, Cardiogenic shock
			Non-COVID-19 STEMI	$n = 130$, age 68.43 ± 6.46 years, 66.2% males	2/2020–11/2020		
Pellegrini et al. (26)	Italy	Monocentric cohort study	COVID-19 STEMI	$n = 24$, age 69.63 ± 11.00 years, 83.3% males	8/3/2020–20/4/2020	RT-PCR on nasal swab or endotracheal aspirate/3–6 h	Thrombus aspiration, MINOCA, Stent implantation, Gp2b3a inhibitor use, In-hospital mortality, Cardiogenic shock, Bleeding
			Non-COVID-19 STEMI	$n = 26$, age 64.65 ± 13.04 years, 84.6% males	8/3/2020–20/4/2020		
Rodriguez-Leor et al. (27)	Spain	Multicentric cohort study	COVID-19 STEMI	$n = 91$, age 64.8 ± 11.8 years, 84.4% males	14/3/2020–30/4/2020	PCR assay/NA	SO-to-FMC time, Primary angioplasty, Thrombus aspiration, MINOCA, Stent implantation, Gp2b3a inhibitor use, TIMI status, In-hospital mortality, Cardiogenic shock, Stent thrombosis, bleeding
			Non-COVID-19 STEMI	$n = 919$, age 62.5 ± 13.1 years, 78.4% males	14/3/2020–30/4/2020		
Choudry et al. (28)	UK	Monocentric cohort study	COVID-19 STEMI	$n = 39$, age 61.7 ± 11.0 years, 84.6% males	1/3/2020–20/5/2020	PT-PCR on nasal/pharyngeal swabs/NA	D2B time, Laboratory values, Primary angioplasty, Thrombus aspiration, Gp2b3a inhibitor use, Baseline thrombus grade, Modified thrombus grade, TIMI status, In-hospital mortality, Stent thrombosis
			Non-COVID-19 STEMI	$n = 76$, age 61.7 ± 12.6 years, 75% males	1/3/2020–20/5/2020		
Blasco et al. (29)	Spain	Monocentric cross-sectional study	COVID-19 STEMI	$n = 5$, age 62 ± 14 years, 80% males	23/3/2020–11/4/2020	RT-PCR on nasopharyngeal and throat swab samples/NA	Laboratory values
			Non-COVID-19 STEMI	$n = 50$, age 58 ± 12 years, 88% males	7/2015–12/2015		
Güler et al. (30)	Turkey	Monocentric cross-sectional study	COVID-19 STEMI	$n = 62$, age 60.2 ± 9.5 years, 66.1% males	11/3/2020–10/1/2021	RT-PCR on nasopharyngeal swabs / NA	SO-to-FMC time, D2B time, Laboratory values, Thrombus aspiration, Gp2b3a inhibitor use, Baseline thrombus grade, TIMI status, In-hospital mortality, ICU admission, LOS
			Non-COVID-19 STEMI	$n = 64$, age 63 ± 8 years, 70.3% males	11/3/2020–10/1/2021		

UK, United Kingdom; NOS, Newcastle-Ottawa Scale; D2B, door to balloon; MINOCA, myocardial infarction with non-obstructive coronary arteries; TIMI, thrombolysis in myocardial infarction; SO-to-FMC, symptom onset to first medical contact; LOS, length of stay; ICU, intensive care unit; RT-PCR, reverse transcriptase-polymerase chain reaction; CT, computed tomography; CXR, chest x-ray.

TABLE 2B | Baseline characteristics of study subjects.

References	Study group	Total subjects (n)	Age (years) (mean ± SD)	Male (%)	Body mass index (kg/m^2)	Diabetes mellitus (%)	Hypertension (%)	Dyslipidemia (%)	Smoking (%)	Multivessel desease (%)	Previous myocardial infarction (%)
Popovic et al. (18)	COVID-19 STEMI	11	63.6 ± 17.4	63.9	25.1 ± 8.1	18.2	45.5	27.3	36.4	0	NA
	Non-COVID-19 STEMI	72	62.5 ± 12.6	73.6	27.02 ± 4.8	19.4	43.1	38.9	55.6	12.5	NA
Siudak et al. (19)	COVID-19 STEMI	145	63.19 ± 12.55	71.33	NA	14.48	46.21	NA	37.24	NA	12.41
	Non-COVID-19 STEMI	2,276	65.43 ± 12.23	67.65	NA	16.86	57.55	NA	31.08	NA	15.94
Kiris et al. (20)	COVID-19 STEMI	65	66.8 ± 12.0	68	NA	26	48	NA	34	44	NA
	Non-COVID-19 STEMI	668	60.0 ± 12.3	78	NA	29	42	NA	33	40	NA
Koutsoukis et al. (21)	COVID-19 STEMI	17	63.4 ± 13.2	70	NA	NA	NA	NA	NA	30.7	NA
	Non-COVID-19 STEMI	99	63.8 ± 13.9	67	NA	NA	NA	NA	NA	61.2	NA
Garcia et al. (22)	COVID-19 STEMI	230	18–55 yrs: 23%; 55–65 yrs: 32%; 66–75 yrs: 28%; >75 yrs: 17%	71	29.3 ± 7.6	46	73	46	44	0	13
	Non-COVID-19 STEMI	460	18–55 yrs: 26%; 55–65 yrs: 30%; 66–75 yrs: 27%; >75 yrs: 17%	68	29.5 ± 6.4	28	69	60	59	16	24
Kite et al. (23)	COVID-19 STEMI	144	63.1 ± 12.6	77.8	27.3 ± 4.5	34	64.8	46	31.7	NA	16.4
	Non-COVID-19 STEMI	24,961	65.6 ± 13.4	72.2	27.8 ± 5.5	20.9	44.8	28.9	33.7	NA	13
Little et al. (24)	COVID-19 STEMI	46	61.80 ± 7.95	80.4	NA	32.6	54	52.2	41.3	NA	10.9
	Non-COVID-19 STEMI	302	64.18 ± 13.41	79.8	NA	23.5	50.7	33.1	41.7	NA	12.6
Marfella et al. (25)	COVID-19 STEMI	46	56.13 ± 6.21	67.4	27.09 ± 1.81	17.4	39.1	15.2	6.5	NA	NA
	Non-COVID-19 STEMI	130	68.43 ± 6.46	66.2	29.55 ± 1.97	29.2	55.4	23.7	29.2	NA	NA
Pellegrini et al. (26)	COVID-19 STEMI	24	69.63 ± 11.00	83.3	26.60 ± 3.36	41.7	70.8	62.5	29.2	45.8	29.2
	Non-COVID-19 STEMI	26	64.65 ± 13.04	84.6	26.11 ± 3.43	15.4	53.9	65.4	38.5	28.6	19.2
Rodriguez-Leor et al. (27)	COVID-19 STEMI	91	64.8 ± 11.8	84.4	NA	23.1	51.7	48.4	18.7	37.4	NA
	Non-COVID-19 STEMI	919	62.5 ± 13.1	78.4	NA	20.9	53.3	46.9	45.5	37.1	NA
Choudry et al. (28)	COVID-19 STEMI	39	61.7 ± 11.0	84.6	26.7 (24.8–30.7)	46.2	71.8	61.6	61.6	NA	15.4
	Non-COVID-19 STEMI	76	61.7 ± 12.6	75	26.7 (24.8–30.7)	46.2	42.1	36.8	46.1	NA	3.9
Blasco et al. (29)	COVID-19 STEMI	5	62 ± 14	80	28.0 (27.3–30.1)	0	80	0	40	NA	NA
	Non-COVID-19 STEMI	50	58 ± 12	88	27.6 (24.9–30.3)	8	42	52	78	NA	NA
Güler et al. (30)	COVID-19 STEMI	62	60.2 ± 9.5	66.1	NA	48.4	59.7	43.5	51.6	NA	9.7
	Non-COVID-19 STEMI	64	63 ± 8	70.3	NA	54.7	57.8	34.3	56.3	NA	28.1

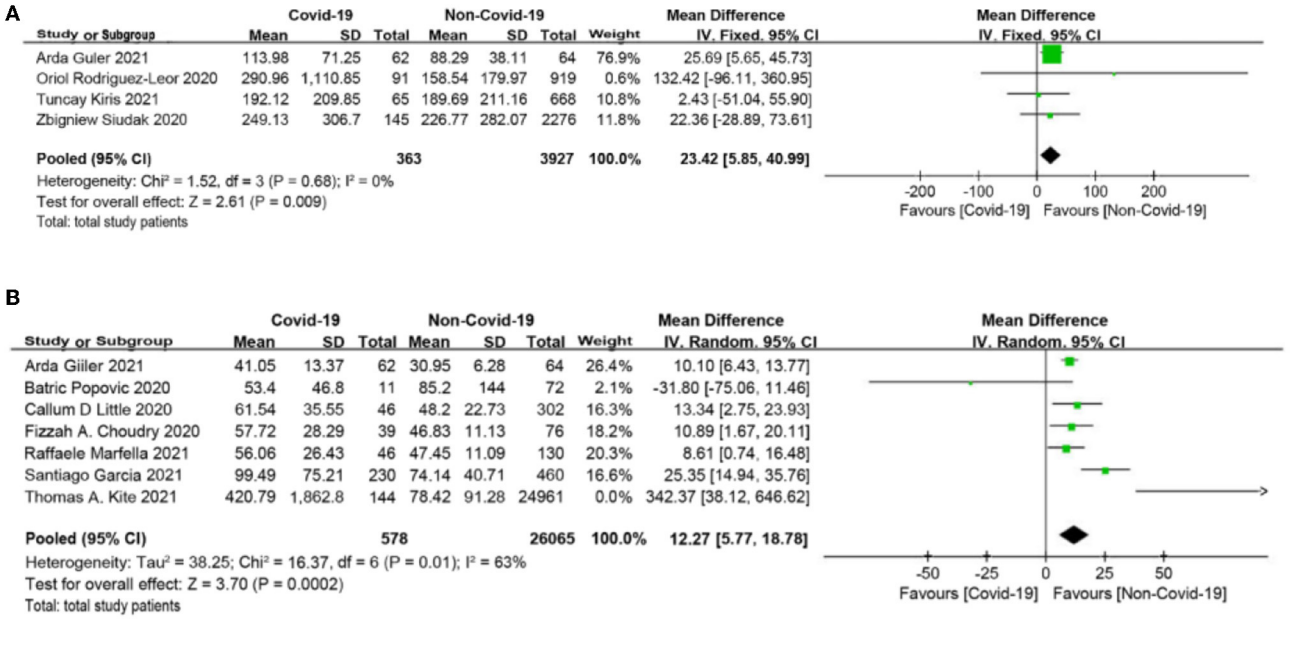


FIGURE 2 | (A) Symptom onset to first medical contact (SO-to-FMC) time forest plot (minutes). **(B)** Door to balloon (D2B) time forest plot (minutes).

outcomes with a high degree of heterogeneity. As shown in **Table 4**, the overall results were relatively robust and not influenced by a single study, except for primary angioplasty, stent implantation, and modified thrombus grade. An asymmetrical plot was observed in some funnel plots, suggesting that publication bias may exist (**Figures 6A–9F**).

DISCUSSION

Clinical Implications

This is the first meta-analysis to compare the characteristics, management, and clinical outcomes of patients with STEMI presenting with COVID-19 infection and that of those patients without COVID-19 infection. Compared to the non-COVID-19 group, the COVID-19 group had significant delays in SO-to-FMC and D2B times. Among the two groups, laboratory values, such as CRP, WBC, and D-dimer, were elevated in the COVID-19 group, while lymphocyte count was found to be lower compared to the non-COVID-19 group. In addition, STEMI concomitant with COVID-19 infection was characterized by a higher rate of MINOCA, lower rate of stent implantation, and higher thrombus grade, and associated higher use of thrombus aspiration and Gp2b3a inhibitors. Furthermore, we found that the COVID-19 group had an increased rate of in-hospital mortality, cardiogenic shock, stent thrombosis, ICU admission, longer length of hospital stays, and decreased TIMI flow post-procedure.

The COVID-19 pandemic started in late 2019 and has caused severe delays in the treatment of patients with STEMI compared to the pre-COVID-19 era, and this is mostly explained by the limited access to emergency medical services (EMS) and the lack of effective organization of healthcare systems (33, 34). Several studies reported that the time from SO-to-FMC and D2B was longer in STEMI patients with COVID-19 than in those without COVID-19, which may be related to the following factors: a higher rate of respiratory symptoms without chest pain as a clinical manifestation in COVID-19 patients may result in an unclear diagnosis of heart attack and lead to a delay in seeking medical service (35), Furthermore, interventional procedures may be more complex in COVID-19 patients than in non-COVID-19 patients (24).

The reperfusion strategy for patients with STEMI during the COVID-19 pandemic remains controversial. The Chinese Cardiac Society and the Canadian Association of Interventional Cardiology recommend thrombolysis as the preferred reperfusion strategy for patients with STEMI (36, 37). In contrast, the American College of Cardiology (ACC) and the Society for Cardiovascular Angiography and Interventions (SCAI) still suggested the use of primary percutaneous coronary intervention (PPCI) as the main treatment for all patients with STEMI during the COVID-19 crisis (1, 2). Rashid et al. reported that STEMI patients with COVID-19 were less likely to receive PPCI than STEMI patients without COVID-19 (38). However, in this study, we did not find a significant difference in the rate of primary angioplasty between both groups. Moreover, we found that the COVID-19 group had a lower rate of stent implantation, which may be associated with a higher rate of MINOCA.

Previous studies have shown that COVID-19 may lead to a prothrombotic state and that a high thrombus burden is more common in STEMI patients with COVID-19 (39–42). SARS-CoV-2 causes a systemic inflammatory response, resulting

TABLE 3 | GRADE summary of findings.

Effects of COVID-19 in STEMI patients						
Patient or population: STEMI Patients **Setting:** Europe, Asian, North America **Intervention:** COVID-19 **Comparison:** Non-COVID-19						
Outcomes	**Anticipated absolute effects* (95% CI)**		**Relative effect (95% CI)**	**No of participants (studies)**	**Certainty of the evidence (GRADE)**	**Comments**
	Risk with Non-COVID-19	**Risk with COVID-19**				
Symptom-to-FMC time	The mean symptom-to-FMC time was 0	MD 23.42 higher (5.85 higher to 40.99 higher)	–	4,290 (4 observational studies)	⊕◯◯◯ Very low	NA
D2B time	The mean D2B time was 0	MD 12.27 higher (5.77 higher to 18.78 higher)	–	26,643 (7 observational studies)	⊕◯◯◯ Very low	NA
CRP	–	SMD 0.76 higher (0.38 higher to 1.13 higher)	–	1,576 (7 observational studies)	⊕◯◯◯ Very low	NA
WBC	–	SMD 0.39 higher (0.1 higher to 0.69 higher)	–	1,205 (5 observational studies)	⊕◯◯◯ Very low	NA
D–Dimer	–	SMD 0.79 higher (0.36 higher to 1.22 higher)	–	324 (3 observational studies)	⊕◯◯◯ Very low	NA
Lymphocyte count	–	SMD 0.52 lower (0.69 lower to 0.36 lower)	–	848 (5 observational studies)	⊕⊕◯◯ Low	NA
Primary angioplasty	942 per 1,000	820 per 1,000 (566 to 943)	OR 0.28 (0.08 to 1.01)	2,796 (7 observational studies)	⊕◯◯◯ Very low	NA
MINOCA	55 per 1,000	356 per 1,000 (110 to 712)	OR 9.57 (2.14 to 42.83)	1,949 (5 observational studies)	⊕◯◯◯ Very low	NA
Stent implantation	895 per 1,000	704 per 1,000 (483 to 858)	OR 0.28 (0.11 to 0.71)	1,264 (4 observational studies)	⊕◯◯◯ Very low	NA
Baseline thrombus grade > 3	677 per 1,000	866 per 1,000 (793 to 916)	OR 3.09 (1.83 to 5.23)	974 (3 observational studies)	⊕◯◯◯ Very low	NA
Modified thrombus grade > 3	350 per 1,000	759 per 1,000 (423 to 931)	OR 5.84 (1.36 to 25.06)	1,024 (3 observational studies)	⊕◯◯◯ Very low	NA
Thrombus aspiration	204 per 1,000	301 per 1,000 (243 to 367)	OR 1.68 (1.25 to 2.26)	2,498 (7 observational studies)	⊕⊕◯◯ Low	NA
Gp2b3a inhibitor	176 per 1,000	379 per 1,000 (275 to 496)	OR 2.86 (1.78 to 4.62)	2,757 (9 observational studies)	⊕◯◯◯ Very low	NA
TIMI-3 Flow	892 per 1,000	832 per 1,000 (776 to 874)	OR 0.60 (0.42 to 0.84)	2,572 (7 observational studies)	⊕⊕◯◯ Low	NA
In- hospital mortality	57 per 1,000	265 per 1,000 (224 to 311)	OR 5.98 (4.78 to 7.48)	25,266 (11 observational studies)	⊕⊕⊕◯ Moderate	NA
Cardiogenic shock	84 per 1,000	201 per 1,000 (156 to 256)	OR 2.75 (2.02 to 3.76)	24,085 (5 observational studies)	⊕⊕◯◯ Low	NA
Stent thrombosis	10 per 1,000	52 per 1,000 (23 to 114)	OR 5.65 (2.41 to 13.23)	1,858 (3 observational studies)	⊕⊕◯◯ Low	NA
Bleeding	5 per 1,000	13 per 1,000 (4 to 39)	OR 2.82 (0.88 to 9.05)	15,850 (4 observational studies)	⊕◯◯◯ Very low	NA
ICU admission	83 per 1,000	277 per 1,000 (184 to 394)	OR 4.26 (2.51 to 7.22)	650 (3 observational studies)	⊕◯◯◯ Very low	NA
Length of stay	The mean length of stay was 0	MD 4.63 higher (2.56 higher to 6.69 higher)	-	26,445 (5 observational studies)	⊕◯◯◯ Very low	NA

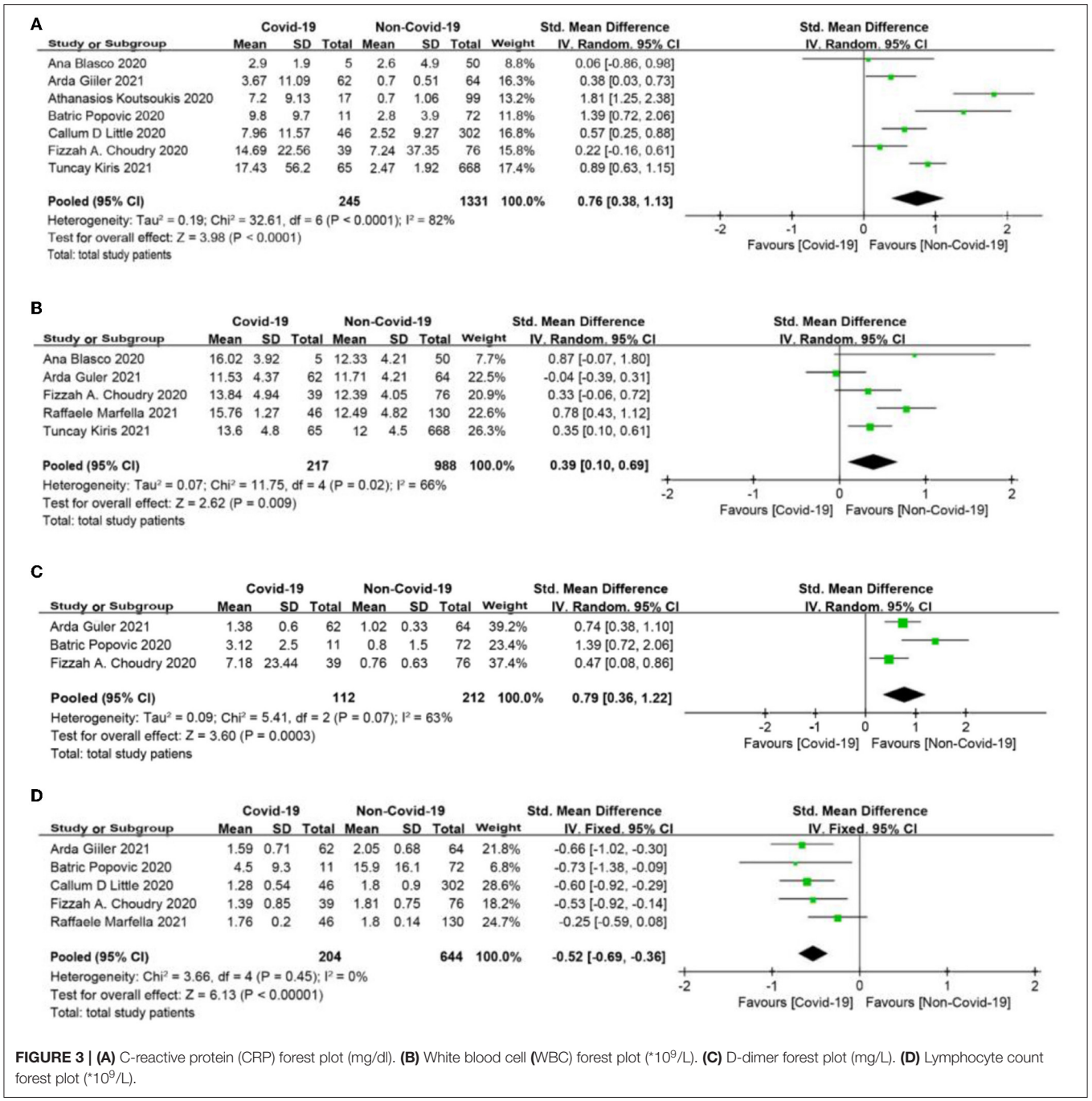
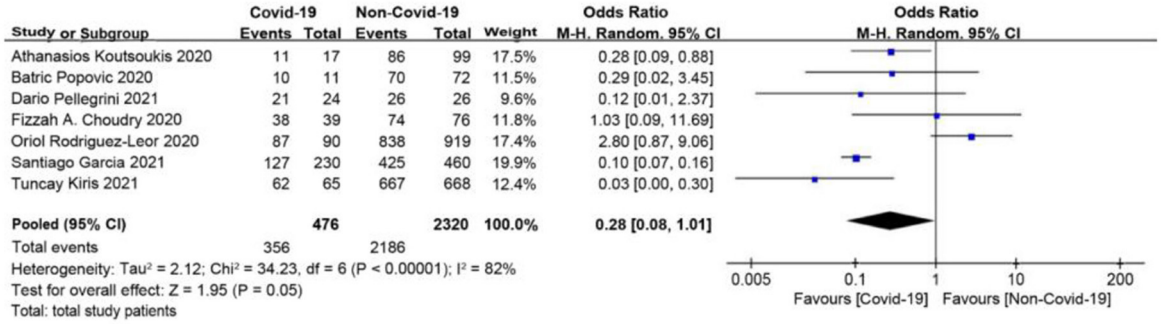


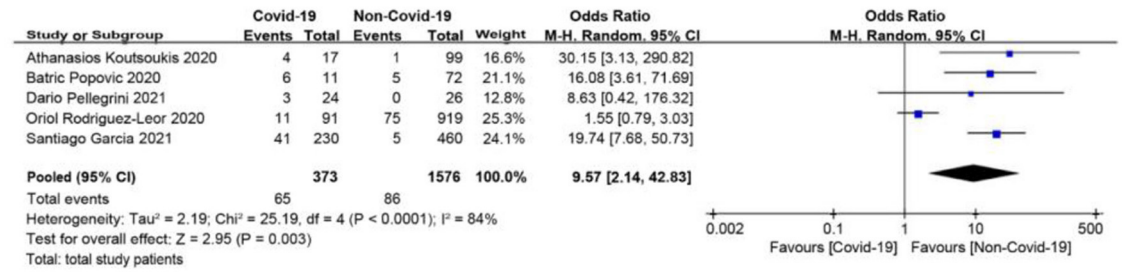
FIGURE 3 | (A) C-reactive protein (CRP) forest plot (mg/dl). **(B)** White blood cell **(**WBC) forest plot (*10^9/L). **(C)** D-dimer forest plot (mg/L). **(D)** Lymphocyte count forest plot (*10^9/L).

in endothelial and hemostatic activation, which involves the activation of platelets and the coagulation cascade (43). In addition, our study found that the time from SO-to-FMC and D2B was longer in STEMI patients with COVID-19 than in those without COVID-19. The studies of Duman et al. (44) and Ge et al. (45) reported that the delay in SO-to-FMC and D2B would prolong the time for opening infarct-related vessels which may account for a higher thrombus burden. Therefore, in the COVID era, it is of great significance that novel technologies should be developed so as to achieve more efficient thrombus aspiration in patients with very high intracoronary thrombus burden such as patients with STEMI and coexistent COVID-19 infection (46). Furthermore, strategies to reduce reperfusion delay times such as educating the public about the recognition and diversity of coronary symptoms and optimizing interventional procedures are essential. In keeping with the high thrombus burden, the COVID-19 group had elevated CRP, WBC, and D-dimer levels and a lower lymphocyte count compared to the non-COVID-19 group. High thrombus grade, reduced TIMI flow, high rate of MINOCA, and stent

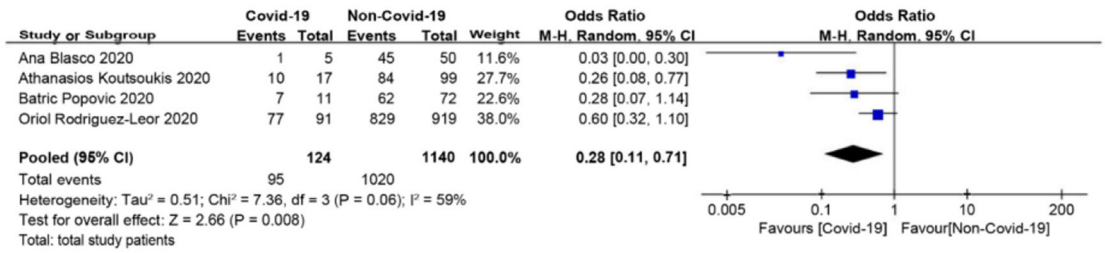
A



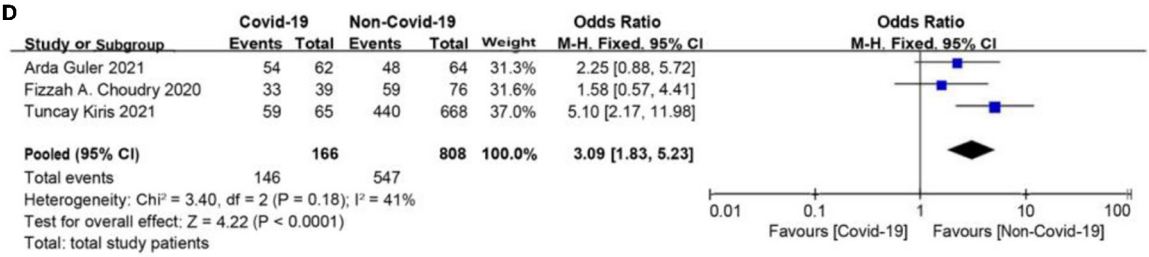
B



C



D



E

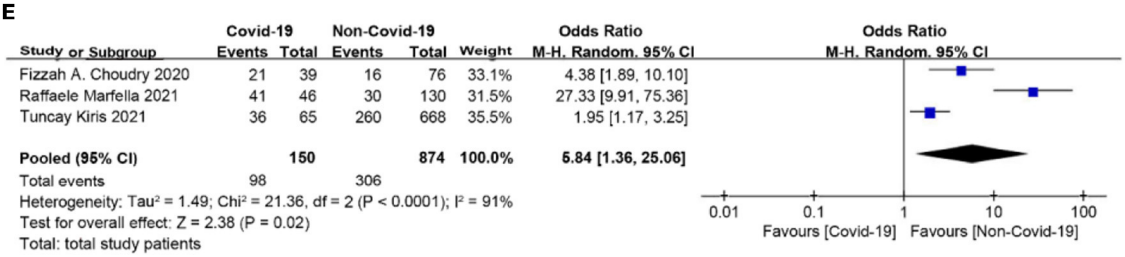


FIGURE 4 | Continued

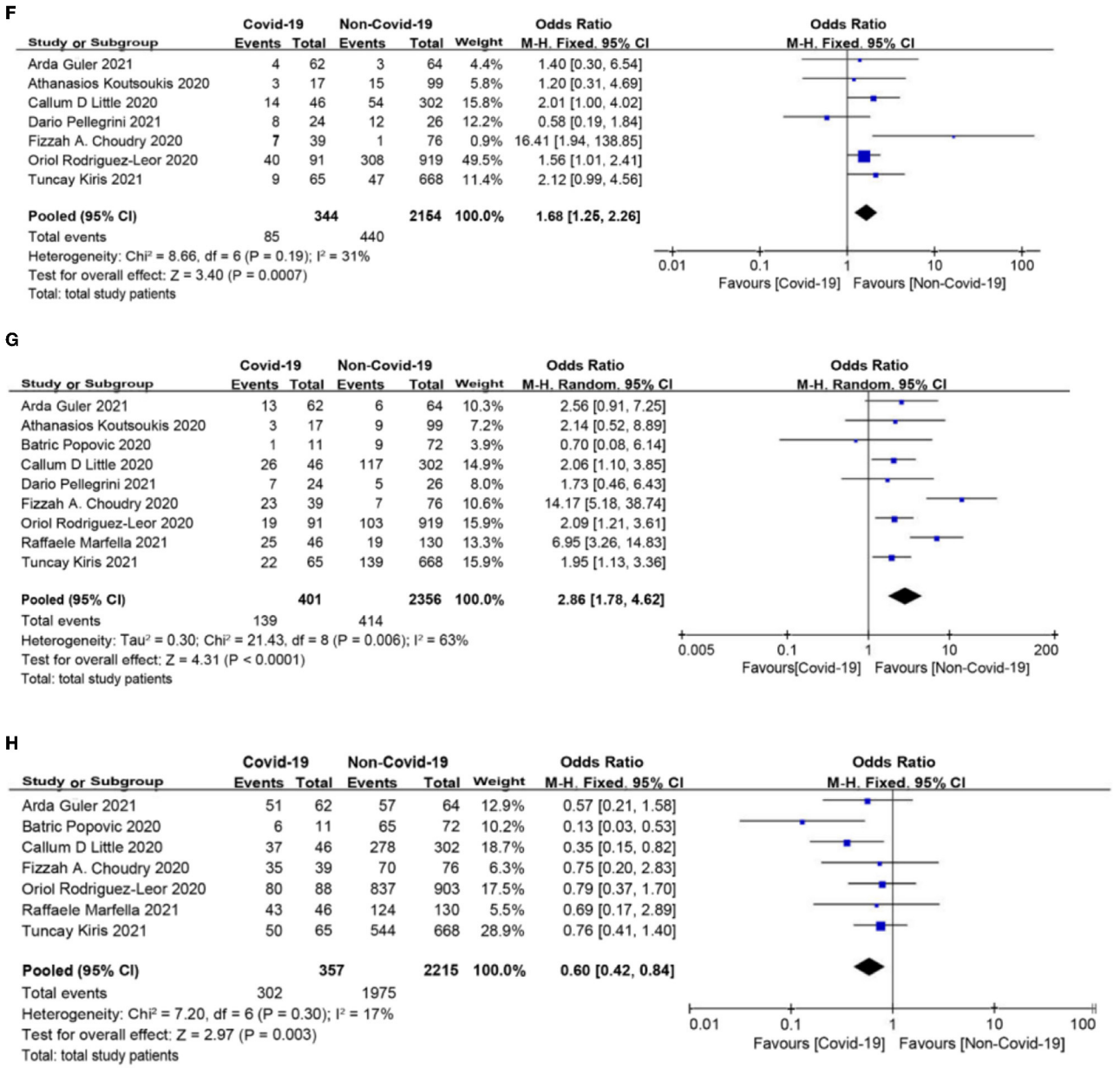


FIGURE 4 | (A) Primary angioplasty forest plot. **(B)** Myocardial infarction with no obstructive coronary atherosclerosis (MINOCA) forest plot. **(C)** Stent implantation forest plot. **(D)** Baseline thrombus grade forest plot. **(E)** Modified thrombus grade forest plot. **(F)** Thrombus aspiration forest plot. **(G)** Glycoprotein IIbIIIa (Gp2b3a) inhibitor use forest plot. **(H)** Thrombolysis in myocardial infarction (TIMI)-3 flow forest plot.

thrombosis may be the result of the intense inflammatory and heightened thrombus burden observed in COVID-19 patients (18, 27, 28, 34). Consistently, the data presented here demonstrated a more aggressive use of thrombus aspiration and a Gp2b3a inhibitor in STEMI patients with concomitant SARS-CoV-2 infection. The use of a Gp2b3a inhibitor may also increase the risk of bleeding (47), but this study showed no significant difference between the two groups in terms of bleeding.

Hospital-mortality was dramatically higher in STEMI patients who presented with COVID-19 than in those without COVID-19. Longer ischemia time, higher thrombus burden, and increased rate of adverse cardiovascular events, including cardiogenic shock, may also be contributory (48, 49). Current studies (50, 51) have reported that STEMI patients with concomitant COVID-19 have higher ICU admission rates and longer lengths of stay, and the results of this meta-analysis support this finding. An

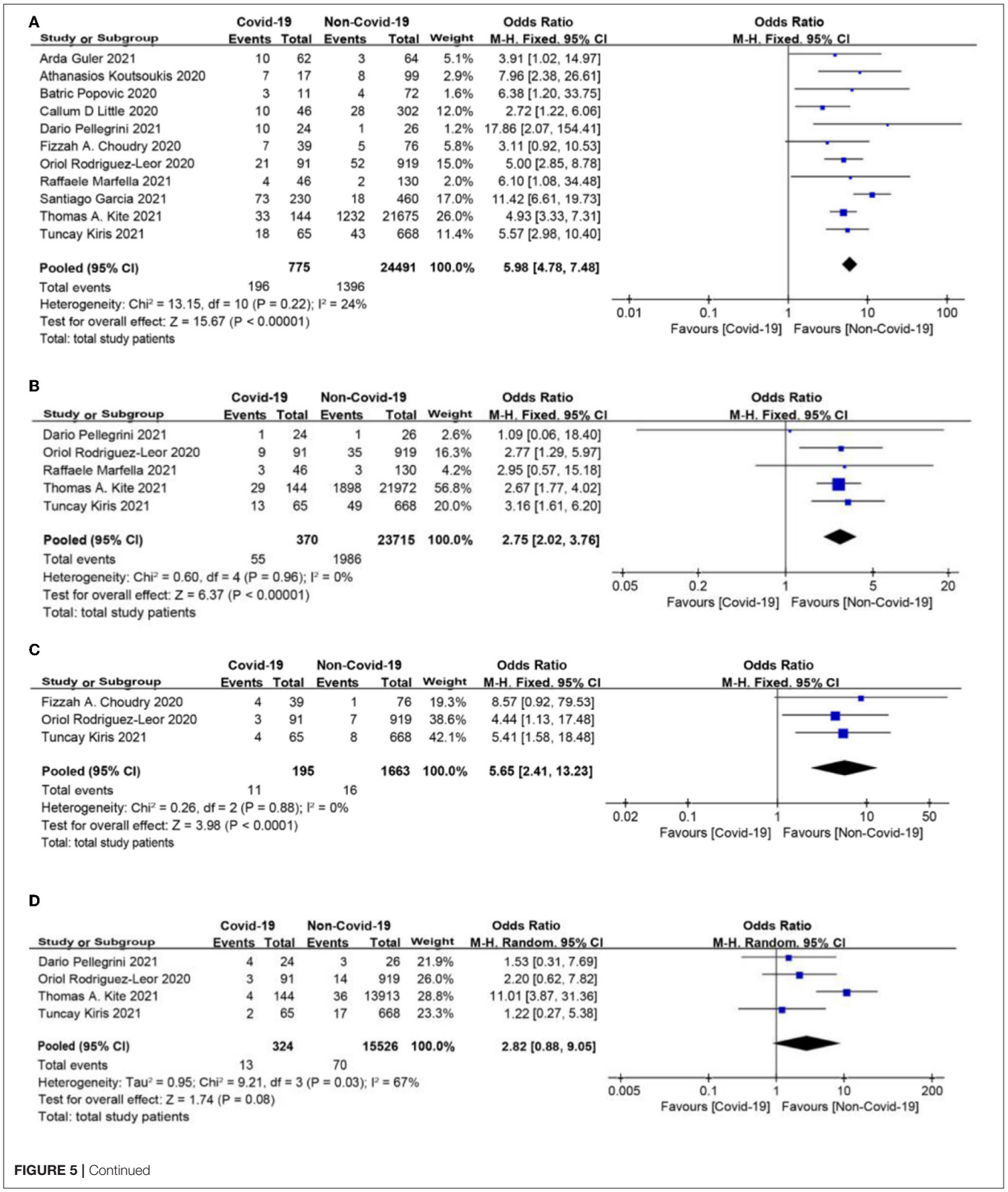


FIGURE 5 | Continued

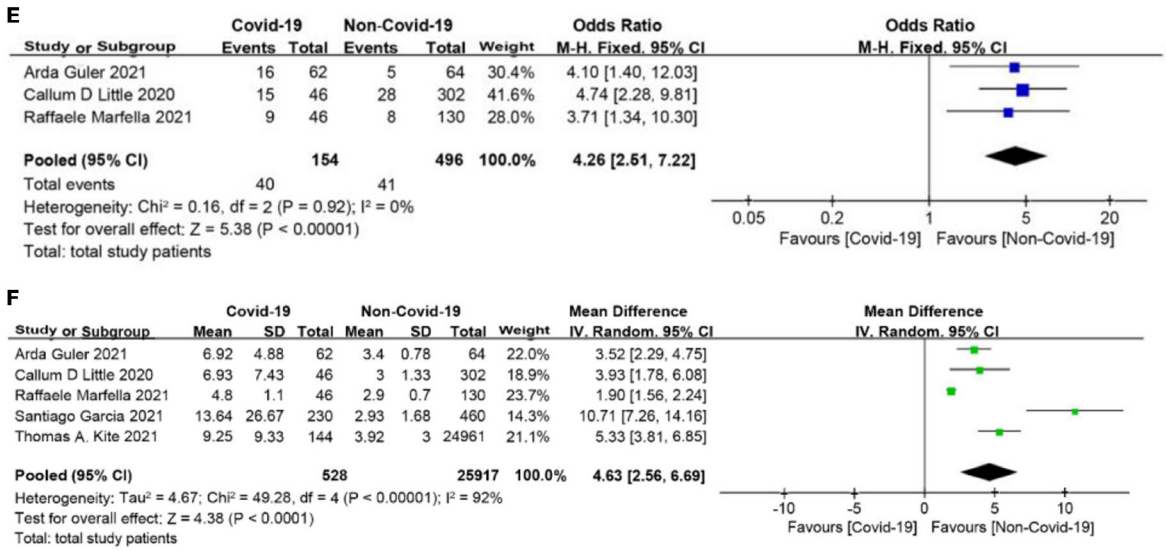


FIGURE 5 | (A) In-hospital mortality forest plot. **(B)** Cardiogenic shock forest plot. **(C)** Stent thrombosis forest plot. **(D)** Bleeding forest plot. **(E)** Intensive care unit (ICU) admission rate forest plot. **(F)** Length of stay forest plot (days).

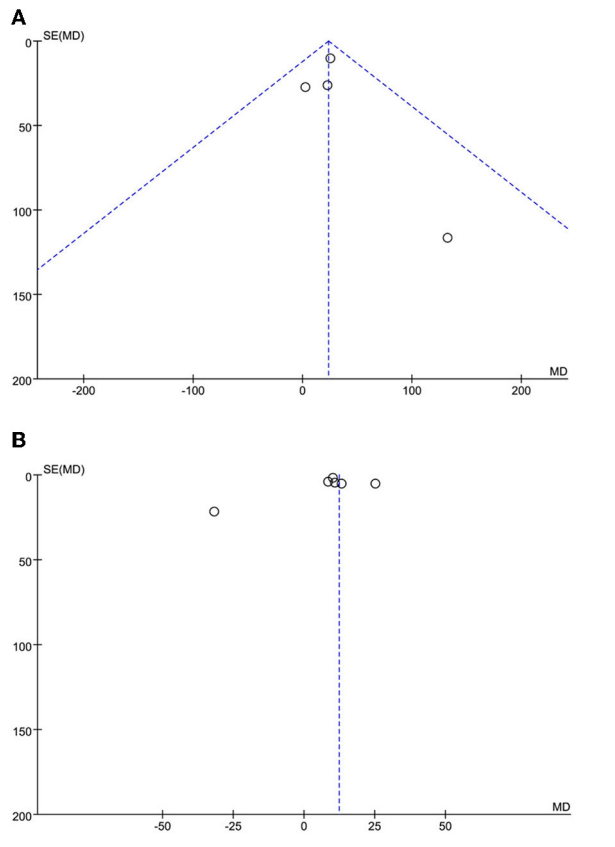


FIGURE 6 | (A) SO-to-FMC time funnel plot. **(B)** D2B time funnel plot.

increased ICU admission rate and length of stay may have a significant impact on hospital resources. Taken together, COVID-19 status may have great implications on the characteristics, management, and outcomes of patients with STEMI.

Heterogeneity of Meta-Analysis

In a meta-analysis, heterogeneity may exist while the sample estimates for the population risk were of different magnitudes (52). The I^2 statistic means the percentage of total variation across effect size estimates that is due to heterogeneity rather than chance. In our study, there are significant and high degrees of heterogeneity for some outcomes. The existing heterogeneity can partly result from different sample sizes, study designs, study times, study scope (nation and region), diagnostic methods, the severity of the disease. We aggregate studies that are different methodologies, but the heterogeneity in the results is still inevitable.

Methodological Considerations

To our knowledge, this is the first meta-analysis that summarizes the comparison of clinical information on STEMI patients presenting with vs. those presenting without COVID-19 infection. We included multiple studies that were conducted in Asia, Europe, and North America, so that our findings can provide a broad overview of COVID-19 infection in patients with STEMI. However, our study has several limitations. First, the delay time, laboratory values, and length of stay were reported in terms of median values and IQR in many studies, which have been adjusted to means and SDs using the Box-Cox method. Nevertheless, using this method to calculate SDs may entail inaccuracy and make the SDs greater than the mean in some cases, which is an inherent feature of the method (17). Second, the disparity in study size may affect the weighting of the

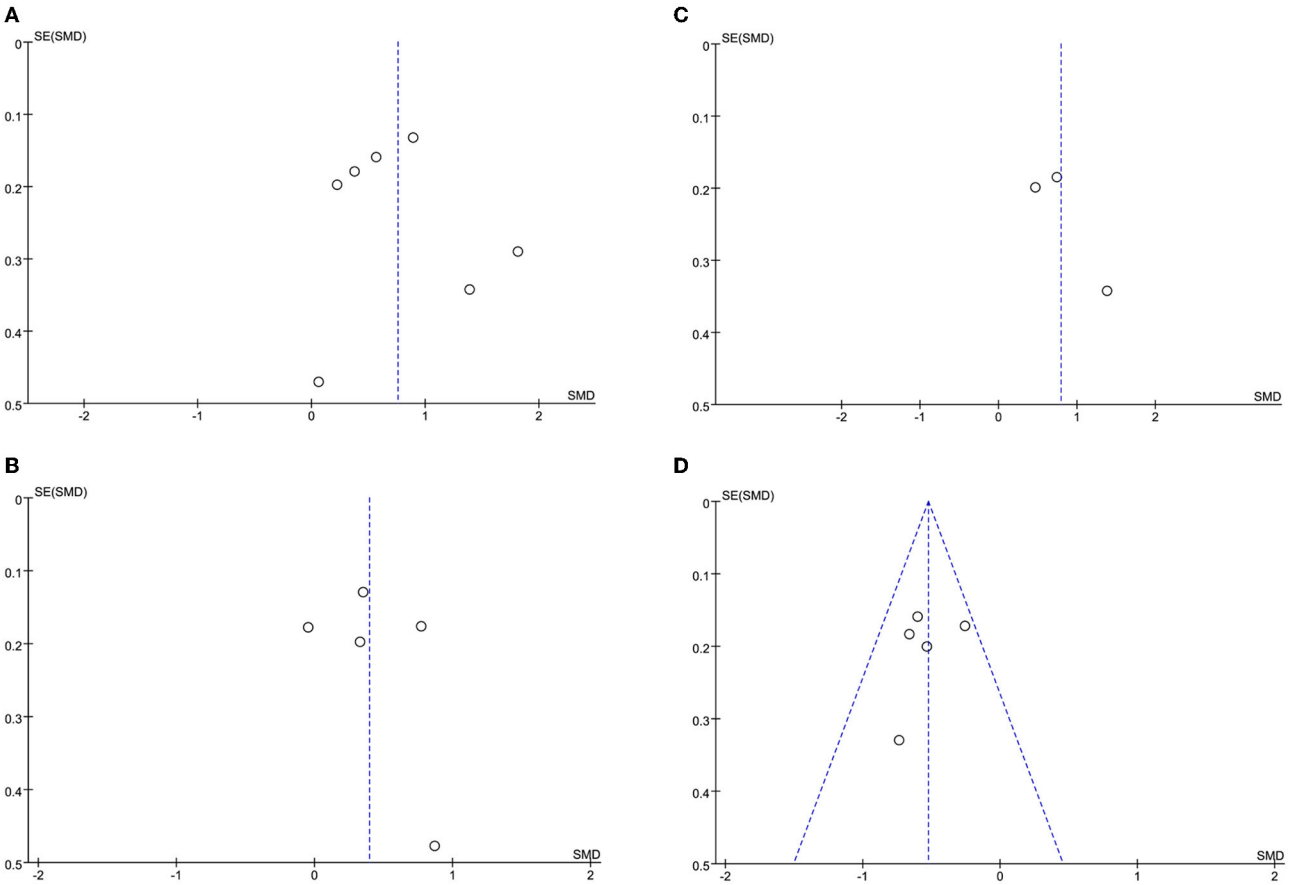


FIGURE 7 | (A) CRP funnel plot**. (B)** WBC funnel plot. **(C)** D-dimer funnel plot. **(D)** Lymphocyte count funnel plot.

studies and the pooled effect size, which is innate to meta-analyses (53, 54). Third, a high degree of heterogeneity was observed in some outcomes. Due to inadequate information for the included studies, it is difficult to conduct a subgroup analysis to explain the heterogeneity. We performed a sensitivity analysis to assess the reliability of our findings and used the random-effects model when I^2 statistics were more than 50%. Fourth, we were unable to compare the rate of thrombosis and elective PCI, and the revascularization rate of patients undergoing primary angioplasty between the two groups due to a lack of sufficient data. Future studies are needed to further investigate these outcomes. Finally, our data were limited to in-hospital outcomes. Long-term follow-up is required to explore the association between SARS-CoV-2 infection and poor outcomes in patients with STEMI.

CONCLUSION

In patients with STEMI, COVID-19 has had a deep impact on their therapeutic management and clinical outcomes. A longer time from SO-to-FMC and D2B was observed in STEMI patients with COVID-19 in our study. Moreover, patients with STEMI who also had COVID-19 had more severe thrombotic events adverse outcomes. Further studies are required to explore the mechanism of coronary thrombus burden and the optimal treatment for patients with STEMI and COVID-19.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

YW, LK, C-WC, SX, and T-HT: conception. YW, LK, C-WC, JX, PY, SX, and T-HT: methodology. YW, LK, JX, PY, and T-HT: analysis. YW, LK, JX, and PY: interpretation and writing. C-WC, SX, and T-HT: supervision. All authors have read and agreed to the published version of the manuscript.

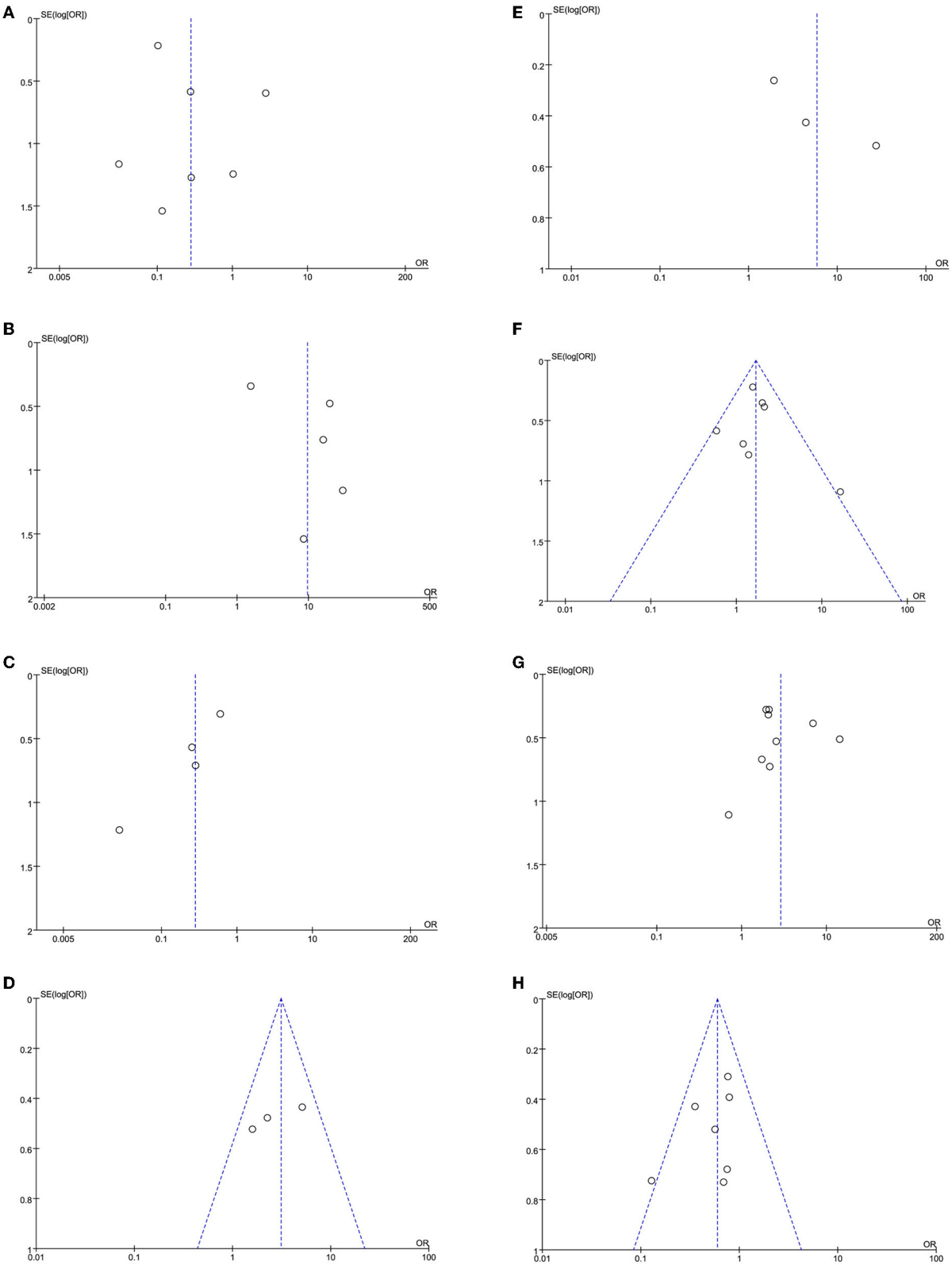


FIGURE 8 | (A) Primary angioplasty funnel plot. **(B)** MINOCA funnel plot. **(C)** Stent implantation funnel plot. **(D)** Baseline thrombus grade funnel plot. **(E)** Modified thrombus grade funnel plot. **(F)** Thrombus aspiration funnel plot. **(G)** Gp2b3a inhibitor use funnel plot. **(H)** TIMI-3 flow funnel plot.

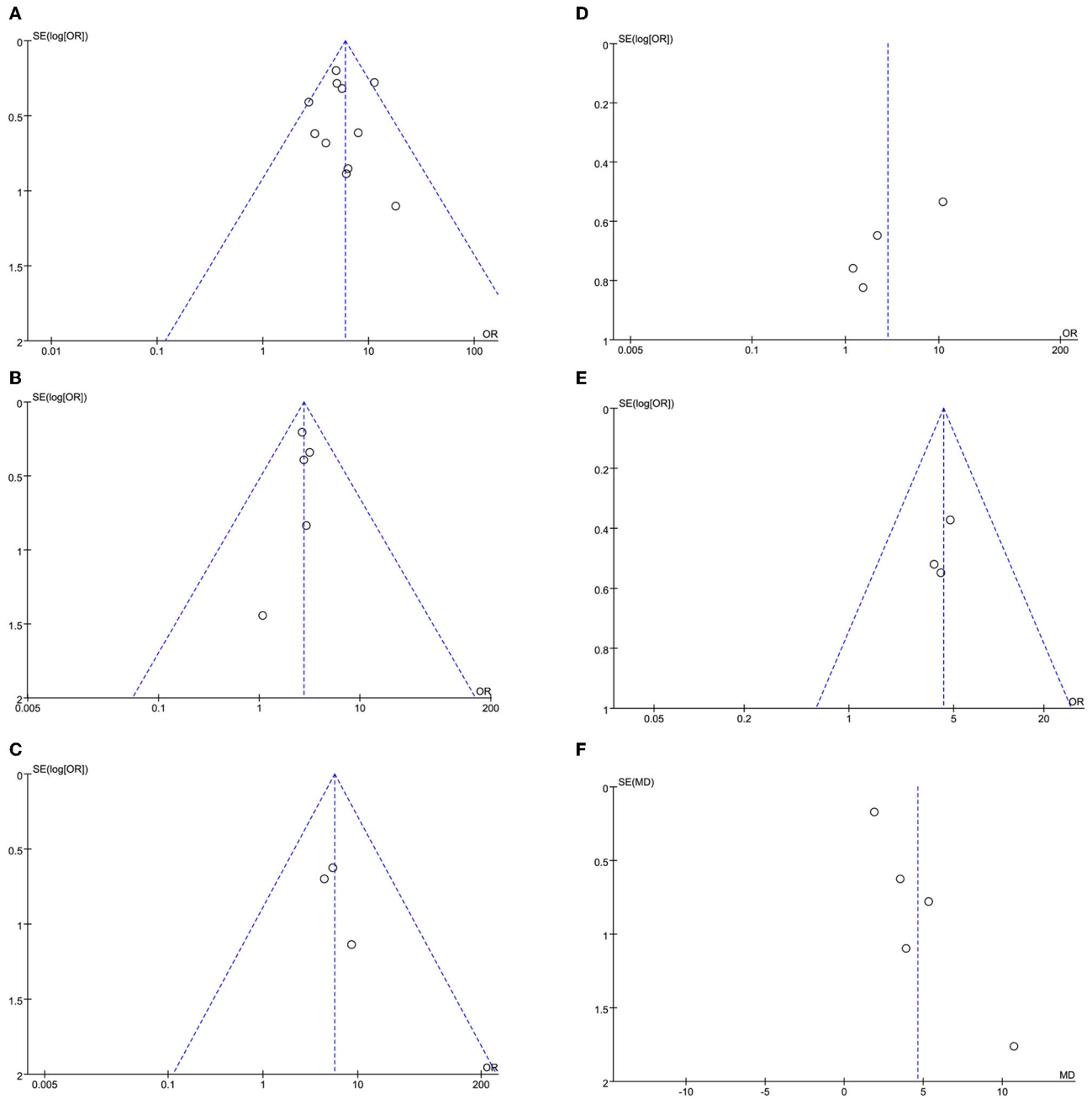


FIGURE 9 | (A) In-hospital mortality funnel plot. **(B)** Cardiogenic shock funnel plot. **(C)** Stent thrombosis funnel plot. **(D)** Bleeding funnel plot. **(E)** ICU admission rate funnel plot. **(F)** Length of stay funnel plot.

TABLE 4 | Leave-one-out analysis.

Study name	Statistics with study excluded		
	Odds ratio or SMD	95% CI	*P*-value
D2B time			
Güler et al. (30)	12.66	2.96 to 22.35	0.01
Popovic et al. (18)	13.06	7.13 to 18.99	<0.0001
Little et al. (24)	12.01	4.16 to 19.86	0.003
Choudry et al. (28)	12.52	4.35 to 20.68	0.003
Marfella et al. (25)	13.1	4.66 to 21.54	0.002
Garcia et al. (22)	9.92	4.47 to 15.35	0.0004
Kite et al. (23)	12.15	6.47 to 17.82	<0.0001
CRP			
Blasco et al. (29)	0.82	0.43 to 1.21	<0.0001
Güler et al. (30)	0.83	0.40 to 1.26	0.0002
Koutsoukis et al. (21)	0.59	0.29 to 0.90	0.0001
Popovic et al. (18)	0.67	0.28 to 1.06	0.0007
Little et al. (24)	0.8	0.33 to 1.26	0.0007
Choudry et al. (28)	0.86	0.45 to 1.26	<0.0001
Kiris et al. (20)	0.73	0.27 to 1.20	0.002
WBC			
Blasco et al. (29)	0.35	0.04 to 0.67	0.03
Güler et al. (30)	0.5	0.25 to 0.76	<0.0001
Choudry et al. (28)	0.42	0.04 to 0.81	0.03
Marfella et al. (25)	0.26	0.08 to 0.44	0.004
Kiris et al. (20)	0.038	0.18 to 0.59	0.0002
D-Dimer			
Güler et al. (30)	0.89	0.01 to 1.78	0.05
Popovic et al. (18)	0.62	0.35 to 0.88	<0.0001
Choudry et al. (28)	1.00	0.38 to 1.62	0.002
Primary Angioplasty			
Koutsoukis et al. (21)	0.27	0.05 to 1.43	0.12
Popovic et al. (18)	0.28	0.07 to 1.15	0.08
Pellegrini et al. (26)	0.31	0.01 to 1.24	0.10
Choudry et al. (28)	0.23	0.06 to 0.94	0.04
Rodriguez-Leor et al. (27)	0.12	0.08 to 0.17	<0.0001
Garcia et al. (22)	0.36	0.09 to 1.49	0.16
Kiris et al. (20)	0.21	0.16 to 0.29	<0.0001
MINOCA			
Koutsoukis et al. (21)	7.63	1.44 to 40.43	0.02
Popovic et al. (18)	8.49	1.37 to 52.74	0.02
Pellegrini et al. (26)	9.81	1.84 to 52.38	0.01
Rodriguez-Leor (27)	18.62	8.73 to 39.72	<0.0001
Garcia et al. (22)	7.56	1.38 to 41.37	0.02
Stent Implantation			
Blasco et al. (29)	0.46	0.28 to 0.75	0.002
Koutsoukis et al. (21)	0.25	0.06 to 1.01	0.05
Popovic et al. (18)	0.25	0.07 to 0.90	0.03
Rodriguez-Leor et al. (27)	0.20	0.09 to 0.43	<0.0001
Modified Thrombus Grade			
Choudry et al. (28)	7.03	0.52 to 96.03	0.14
Marfella et al. (25)	2.72	1.25 to 5.94	0.01
Kiris et al. (20)	10.69	1.75 to 65.11	0.01
Gp2b3a inhibitor use			
Güler et al. (30)	2.90	1.70 to 4.93	<0.0001
Koutsoukis et al. (21)	2.93	1.75 to 4.90	<0.0001
Popovic et al. (18)	3.03	1.87 to 4.93	<0.0001
Little et al. (24)	3.02	1.72 to 5.30	0.0001
Pellegrini et al. (26)	2.99	1.79 to 5.01	<0.0001
Choudry et al. (28)	2.37	1.81 to 3.11	<0.0001
Rodriguez-Leor et al. (27)	2.93	2.19 to 3.92	<0.0001
Marfella et al. (25)	2.41	1.83 to 3.17	<0.0001
Kiris et al. (20)	3.01	2.25 to 4.03	<0.0001
Bleeding			
Pellegrini et al. (26)	3.30	0.77 to 14.07	0.11
Rodriguez-Leor et al. (27)	2.95	0.55 to 15.73	0.21
Kite et al. (23)	1.62	0.71 to 3.73	0.25
Kiris et al. (20)	3.62	0.92 to 14.23	0.07
Length of Stay			
Güler et al. (30)	5.11	2.17 to 8.06	0.0007
Little et al. (24)	4.84	2.41 to 7.27	< 0.0001
Marfella et al. (25)	5.42	3.24 to 7.26	< 0.0001
Garcia et al. (22)	3.56	1.85 to 5.27	< 0.0001
Kite et al. (23)	4.41	2.14 to 6.69	0.0001

REFERENCES

1. Mahmud E, Dauerman HL, Welt FGP, Messenger JC, Rao SV, Grines C, et al. Management of Acute myocardial infarction during the COVID-19 pandemic: a position statement from the society for cardiovascular angiography and interventions (SCAI), the American college of cardiology (acc), and the American college of emergency physicians (ACEP). *J Am Coll Cardiol.* (2020) 76:1375–84. doi: 10.1016/j.jacc.2020.04.039
2. Reed GW, Rossi JE, Cannon CP. Acute myocardial infarction. *Lancet.* (2017) 389:197–210. doi: 10.1016/S0140-6736(16)30677-8
3. Gulati A, Pomeranz C, Qamar Z, Thomas S, Frisch D, George G, et al. A comprehensive review of manifestations of novel coronaviruses in the context of deadly COVID-19 global pandemic. *Am J Med Sci.* (2020) 360:5–34. doi: 10.1016/j.amjms.2020.05.006
4. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019. Novel Coronavirus-Infected Pneumonia in Wuhan, China. *Jama.* (2020) 323:1061–9. doi: 10.1001/jama.2020.1585
5. Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, et al. Extrapulmonary manifestations of COVID-19. *Nat Med.* (2020) 26:1017–32. doi: 10.1038/s41591-020-0968-3
6. Behzad S, Aghaghazvini L, Radmard AR, Gholamrezanezhad A. Extrapulmonary manifestations of COVID-19: Radiologic and clinical overview. *Clin Imaging.* (2020) 66:35–41. doi: 10.1016/j.clinimag.2020.05.013
7. Choudhury T, West NE, El-Omar M. ST elevation myocardial infarction. *Clin Med (Lond).* (2016) 16:277–82. doi: 10.7861/clinmedicine.16-3-277
8. Gale CP, Allan V, Cattle BA, Hall AS, West RM, Timmis A, et al. Trends in hospital treatments, including revascularisation, following acute myocardial infarction, 2003-2010: a multilevel and relative survival analysis for the National Institute for Cardiovascular Outcomes Research (NICOR). *Heart.* (2014) 100:582–9. doi: 10.1136/heartjnl-2013-304517
9. Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M. Cardiovascular disease in Europe: epidemiological update 2016. *Eur Heart J.* (2016) 37:3232–45. doi: 10.1093/eurheartj/ehw334
10. Pedersen F, Butrymovich V, Kelbæk H, Wachtell K, Helqvist S, Kastrup J, et al. Short- and long-term cause of death in patients treated with primary PCI for STEMI. *J Am Coll Cardiol.* (2014) 64:2101–8. doi: 10.1016/j.jacc.2014.08.037
11. Baumhardt M, Dreyhaupt J, Winsauer C, Stuhler L, Thiessen K, Stephan T, et al. The effect of the lockdown on patients with myocardial infarction during the COVID-19 pandemic. *Dtsch Arztebl Int.* (2021) 118:447–53. doi: 10.3238/arztebl.m2021.0253
12. Xiang D, Xiang X, Zhang W, Yi S, Zhang J, Gu X, et al. Management and outcomes of patients with STEMI during the COVID-19 pandemic in China. *J Am Coll Cardiol.* (2020) 76:1318–24. doi: 10.1016/j.jacc.2020.06.039
13. Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. *J Am Coll Cardiol.* (2020) 75:2950–73. doi: 10.1016/j.jacc.2020.04.031
14. Pérez-Bermejo JA, Kang S, Rockwood SJ, Simoneau CR, Joy DA, Ramadoss GN, et al. SARS-CoV-2 infection of human iPSC-derived cardiac cells predicts novel cytopathic features in hearts of COVID-19 patients. *bioRxiv.* (2020) doi: 10.1101/2020.08.25.265561
15. Hayek SS, Brenner SK, Azam TU, Shadid HR, Anderson E, Berlin H, et al. In-hospital cardiac arrest in critically ill patients with covid-19: multicenter cohort study. *Bmj.* (2020) 371:m3513. doi: 10.1136/bmj.m3513
16. Bonow RO, Fonarow GC, O'Gara PT, Yancy CW. Association of coronavirus disease (2019). (COVID-19) with myocardial injury and mortality. *JAMA Cardiol.* (2020) 5:751–3. doi: 10.1001/jamacardio.2020.1105
17. McGrath S, Zhao XF, Steele R, Thombs BD, Benedetti A, Levis B, et al. Estimating the sample mean and standard deviation from commonly reported quantiles in meta-analysis. *Statistic Methods Med Res.* (2020) 29:2520–37. doi: 10.1177/0962280219889080
18. Popovic B, Varlot J, Metzdorf PA, Jeulin H, Goehringer F, Camenzind E. Changes in characteristics and management among patients with ST-elevation myocardial infarction due to COVID-19 infection. *Catheter Cardiovasc Interv.* (2021) 97:E319–e26. doi: 10.1002/ccd.29114
19. Siudak Z, Grygier M, Wojakowski W, Malinowski KP, Witkowski A, Gasior M, et al. Clinical and procedural characteristics of COVID-19 patients treated with percutaneous coronary interventions. *Catheter Cardiovasc Interv.* (2020) 96:E568–e75. doi: 10.1002/ccd.29134
20. Kiris T, Avci E, Ekin T, Akgün DE, Tiryaki M, Yidirim A, et al. Impact of COVID-19 outbreak on patients with ST-segment elevation myocardial infarction (STEMI) in Turkey: results from TURSER study (TURKISH St-segment elevation myocardial infarction registry). *J Thromb Thrombolysis.* (2021) 2021:1–14. doi: 10.1007/s11239-021-02487-3
21. Koutsoukis A, Delmas C, Roubille F, Bonello L, Schurtz G, Manzo-Silberman S, et al. Acute coronary syndrome in the era of sars-cov-2 infection: a registry of the french group of acute cardiac care. *CJC Open.* (2021) 3:311–7. doi: 10.1016/j.cjco.2020.11.003
22. Garcia S, Dehghani P, Grines C, Davidson L, Nayak KR, Saw J, et al. Initial findings from the North American COVID-19 myocardial infarction registry. *J Am Coll Cardiol.* (2021) 77:1994–2003. doi: 10.1016/j.jacc.2021.02.055
23. Kite TA, Ludman PF, Gale CP, Wu J, Caixeta A, Mansourati J, et al. International prospective registry of acute coronary syndromes in patients with COVID-19. *J Am Coll Cardiol.* (2021) 77:2466–76. doi: 10.1016/j.jacc.2021.03.309
24. Little CD, Kotecha T, Candilio L, Jabbour RJ, Collins GB, Ahmed A, et al. COVID-19 pandemic and STEMI: pathway activation and outcomes from the pan-London heart attack group. *Open Heart.* (2020) 7:2. doi: 10.1136/openhrt-2020-001432
25. Marfella R, Paolisso P, Sardu C, Palomba L, D'Onofrio N, Cesaro A, et al. SARS-COV-2 colonizes coronary thrombus and impairs heart microcirculation bed in asymptomatic SARS-CoV-2 positive subjects with acute myocardial infarction. *Crit Care.* (2021) 25:217. doi: 10.1186/s13054-021-03643-0
26. Pellegrini D, Fiocca L, Pescetelli I, Canova P, Vassileva A, Faggi L, et al. Effect of respiratory impairment on the outcomes of primary percutaneous coronary intervention in patients with ST-segment elevation myocardial infarction and coronavirus disease-2019 (COVID-19). *Circ J.* (2021) 85:1701–7. doi: 10.1253/circj.CJ-20-1166
27. Rodriguez-Leor O, Cid Alvarez AB, Pérez de Prado A, Rossello X, Ojeda S, Serrador A, et al. In-hospital outcomes of COVID-19 ST-elevation myocardial infarction patients. *EuroIntervention.* (2021) 16:1426–33. doi: 10.4244/EIJ-D-20-00935
28. Choudry FA, Hamshere SM, Rathod KS, Akhtar MM, Archbold RA, Guttmann OP, et al. High thrombus burden in patients with COVID-19 presenting with st-segment elevation myocardial infarction. *J Am Coll Cardiol.* (2020) 76:1168–76. doi: 10.1016/j.jacc.2020.07.022
29. Blasco A, Coronado MJ, Hernández-Terciado F, Martín P, Royuela A, Ramil E, et al. Assessment of neutrophil extracellular traps in coronary thrombus of a case series of patients with COVID-19 and myocardial infarction. *JAMA Cardiol.* (2020) 6:1–6. doi: 10.1001/jamacardio.2020.7308
30. Güler A, Gürbak I, Panç C, Güner A, Ertürk M. Frequency and predictors of no-reflow phenomenon in patients with COVID-19 presenting with ST-segment elevation myocardial infarction. *Acta Cardiol.* (2021) 2021:1–9. doi: 10.1080/00015385.2021.1931638
31. Gibson CM, de Lemos JA, Murphy SA, Marble SJ, McCabe CH, Cannon CP, et al. Combination therapy with abciximab reduces angiographically evident thrombus in acute myocardial infarction: a TIMI 14 substudy. *Circulation.* (2001) 103:2550–4. doi: 10.1161/01.CIR.103.21.2550
32. Sianos G, Papafakli MI, Daemen J, Vaina S, van Mieghem CA, van Domburg RT, et al. Angiographic stent thrombosis after routine use of drug-eluting stents in ST-segment elevation myocardial infarction: the importance of thrombus burden. *J Am Coll Cardiol.* (2007) 50:573–83. doi: 10.1016/j.jacc.2007.04.059
33. Ferlini M, Andreassi A, Carugo S, Cuccia C, Bianchini B, Castiglioni B, et al. Centralization of the ST elevation myocardial infarction care network in the Lombardy region during the COVID-19 outbreak. *Int J Cardiol.* (2020) 312:24–6. doi: 10.1016/j.ijcard.2020.04.062
34. Tam CF, Cheung KS, Lam S, Wong A, Yung A, Sze M, et al. Impact of coronavirus disease (COVID-19) outbreak on st-segment-elevation myocardial infarction care in Hong Kong, China. *Circ Cardiovasc Qual Outcomes.* (2020) 13:e006631. doi: 10.1161/CIRCOUTCOMES.120.006631
35. Carugo S, Ferlini M, Castini D, Andreassi A, Guagliumi G, Metra M, et al. Management of acute coronary syndromes during the COVID-19 outbreak

in Lombardy: The "macro-hub" experience. *Int J Cardiol Heart Vasc.* (2020) 31:100662. doi: 10.1016/j.ijcha.2020.100662
36. Han Y, Zeng H, Jiang H, Yang Y, Yuan Z, Cheng X, et al. CSC Expert consensus on principles of clinical management of patients with severe emergent cardiovascular diseases during the COVID-19 epidemic. *Circulation.* (2020) 141:e810–e6. doi: 10.1161/CIRCULATIONAHA.120.047011
37. Wood DA, Sathananthan J, Gin K, Mansour S, Ly HQ, Quraishi AU, et al. Precautions and procedures for coronary and structural cardiac interventions during the COVID-19 pandemic: guidance from canadian association of interventional cardiology. *Can J Cardiol.* (2020) 36:780–3. doi: 10.1016/j.cjca.2020.03.027
38. Rashid M, Wu J, Timmis A, Curzen N, Clarke S, Zaman A, et al. Outcomes of COVID-19-positive acute coronary syndrome patients: a multisource electronic healthcare records study from England. *J Intern Med.* (2021) 290:88–100. doi: 10.1111/joim.13246
39. Fan BE, Chong VCL, Chan SSW, Lim GH, Lim KGE, Tan GB, et al. Hematologic parameters in patients with COVID-19 infection. *Am J Hematol.* (2020) 95:E131–e4. doi: 10.1002/ajh.25774
40. Klok FA, Kruip M, van der Meer NJM, Arbous MS, Gommers D, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res.* (2020) 191:145–7. doi: 10.1016/j.thromres.2020.04.013
41. Beyrouti R, Adams ME, Benjamin L, Cohen H, Farmer SF, Goh YY, et al. Characteristics of ischaemic stroke associated with COVID-19. *J Neurol Neurosurg Psychiatry.* (2020) 91:889–91. doi: 10.1136/jnnp-2020-323586
42. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med.* (2020) 46:846–8. doi: 10.1007/s00134-020-05991-x
43. Masi P, Hékimian G, Lejeune M, Chommeloux J, Desnos C, Pineton De Chambrun M, et al. Systemic inflammatory response syndrome is a major contributor to COVID-19-associated coagulopathy: insights from a prospective, single-center cohort study. *Circulation.* (2020) 142:611–4. doi: 10.1161/CIRCULATIONAHA.120.048925
44. Duman H, Çetin M, Durakoglugil ME, Degirmenci H, Hamur H, Bostan M, et al. Relation of angiographic thrombus burden with severity of coronary artery disease in patients with ST segment elevation myocardial infarction. *Med Sci Monit.* (2015) 21:3540–6. doi: 10.12659/MSM.895157
45. Ge J, Li J, Dong B, Ning X, Hou B. Determinants of angiographic thrombus burden and impact of thrombus aspiration on outcome in young patients with ST-segment elevation myocardial infarction. *Catheter Cardiovasc Interv.* (2019) 93:E269–e76. doi: 10.1002/ccd.27944
46. Karagiannidis E, Papazoglou AS, Sofidis G, Chatzinikolaou E, Keklikoglou K, Panteris E, et al. Micro-CT-Based quantification of extracted thrombus burden characteristics and association with angiographic outcomes in patients with ST-elevation myocardial infarction: the QUEST-STEMI study. *Front Cardiovasc Med.* (2021) 8:646064. doi: 10.3389/fcvm.2021.646064
47. Elian D, Guetta V. Glycoprotein 2b3a inhibitors for acute coronary syndromes: what the trials tell us. *Harefuah.* (2003) 142:350–498.
48. Scholz KH, Maier SKG, Maier LS, Lengenfelder B, Jacobshagen C, Jung J, et al. Impact of treatment delay on mortality in ST-segment elevation myocardial infarction (STEMI) patients presenting with and without haemodynamic instability: results from the German prospective, multicentre FITT-STEMI trial. *Eur Heart J.* (2018) 39:1065–74. doi: 10.1093/eurheartj/ehy004
49. Singh M, Berger PB, Ting HH, Rihal CS, Wilson SH, Lennon RJ, et al. Influence of coronary thrombus on outcome of percutaneous coronary angioplasty in the current era (the Mayo Clinic experience). *Am J Cardiol.* (2001) 88:1091–6. doi: 10.1016/S0002-9149(01)02040-9
50. Solano-López J, Zamorano JL, Pardo Sanz A, Amat-Santos I, Sarnago F, Gutiérrez Ibañes E, et al. Risk factors for in-hospital mortality in patients with acute myocardial infarction during the COVID-19 outbreak. *Rev Esp Cardiol (Engl Ed).* (2020) 73:985–93. doi: 10.1016/j.recesp.2020.07.023
51. Matsushita K, Hess S, Marchandot B, Sato C, Truong DP, Kim NT, et al. Clinical features of patients with acute coronary syndrome during the COVID-19 pandemic. *J Thromb Thrombolysis.* (2021) 52:95–104. doi: 10.1007/s11239-020-02340-z
52. Sedgwick P. Meta-analyses: what is heterogeneity? *Bmj-Br Med J.* (2015) 15:350. doi: 10.1136/bmj.h1435
53. Rattka M, Dreyhaupt J, Winsauer C, Stuhler L, Baumhardt M, Thiessen K, et al. Effect of the COVID-19 pandemic on mortality of patients with STEMI: a systematic review and meta-analysis. *Heart.* (2020) 20:360. doi: 10.1136/heartjnl-2020-318360
54. Kamarullah W, Sabrina AP, Rocky MA, Gozali DR. Investigating the implications of COVID-19 outbreak on systems of care and outcomes of STEMI patients: a systematic review and meta-analysis. *Indian Heart J.* (2021) 73:404–12. doi: 10.1016/j.ihj.2021.06.009

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

CASE REPORT
published: 18 March 2022
doi: 10.3389/fcvm.2022.837759

Case Report: Myocarditis Associated With COVID-19 mRNA Vaccination Following Myocarditis Associated With *Campylobacter Jejuni*

Nobuko Kojima[1], Hayato Tada[1], Hirofumi Okada[1], Shohei Yoshida[1], Kenji Sakata[1], Soichiro Usui[1], Hiroko Ikeda[2], Masaki Okajima[3], Masa-aki Kawashiri[1] and Masayuki Takamura[1]*

[1] Department of Cardiovascular Medicine, Kanazawa University Graduate School of Medical Sciences, Kanazawa, Japan, [2] Diagnostic Pathology, Kanazawa University Hospital, Kanazawa, Japan, [3] Department of Emergency Medicine, Kanazawa University Hospital, Kanazawa, Japan

OPEN ACCESS

Edited by:
Shuyang Zhang, Peking Union Medical College Hospital (CAMS), China

Reviewed by:
Bernhard Maisch, University of Marburg, Germany
Katelyn Ann Bruno, Mayo Clinic Florida, United States

***Correspondence:**
Hayato Tada
ht240z@sa3.so-net.ne.jp

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *17 December 2021*
Accepted: *21 February 2022*
Published: *18 March 2022*

Citation:
Kojima N, Tada H, Okada H, Yoshida S, Sakata K, Usui S, Ikeda H, Okajima M, Kawashiri M-a and Takamura M (2022) Case Report: Myocarditis Associated With COVID-19 mRNA Vaccination Following Myocarditis Associated With Campylobacter Jejuni. Front. Cardiovasc. Med. 9:837759. doi: 10.3389/fcvm.2022.837759

We herein present our experience with a case involving a 17-year-old Japanese boy suffering from acute myocarditis after his second coronavirus disease-2019 (COVID-19) messenger RNA (mRNA) vaccine shot. The patients had a history of myocarditis associated with *Campylobacter jejuni* 3 years prior. This has been the first-ever documented case of myocarditis associated with COVID-19 mRNA vaccination in a patient with a history of myocarditis. We present a series of images and blood biomarkers for different types of myocarditis that developed in this single patient.

Keywords: cardiomyopathy, left ventricle, chest pain, myocarditis, COVID-19

LEARNING OBJECTIVE

History of myocarditis may be a risk factor for COVID-19 mRNA vaccine-associated myocarditis. Thus, vigilance is required for patients with such a history when considering indications for COVID-19 mRNA vaccination, especially among young boys.

INTRODUCTION

Myocarditis, the main cause of which is viral infections such as coronavirus disease 2019 (COVID-19), is a rare condition, wherein signs of inflammation can be observed in the myocardium (1, 2). Studies have shown that other conditions such as nonviral infections, autoimmune syndromes, and vaccines can also cause myocarditis (1). Soon after the introduction of COVID-19 mRNA vaccination, many case reports exhibiting acute myocarditis associated with the vaccination had emerged (3–5). Accumulated data appear to suggest that the occurrence of myocarditis is more frequent among young adult and adolescent males (6–8). However, it remains unclear whether other risk factors, particularly a history of myocarditis, are present for this condition. Given the current global situation caused by the COVID-19 pandemic, additional data regarding this issue, especially among younger individuals, need to be accumulated. We herein present the first-ever documented case of acute myocarditis associated with COVID-19 mRNA vaccination in a patient who had a history of myocarditis.

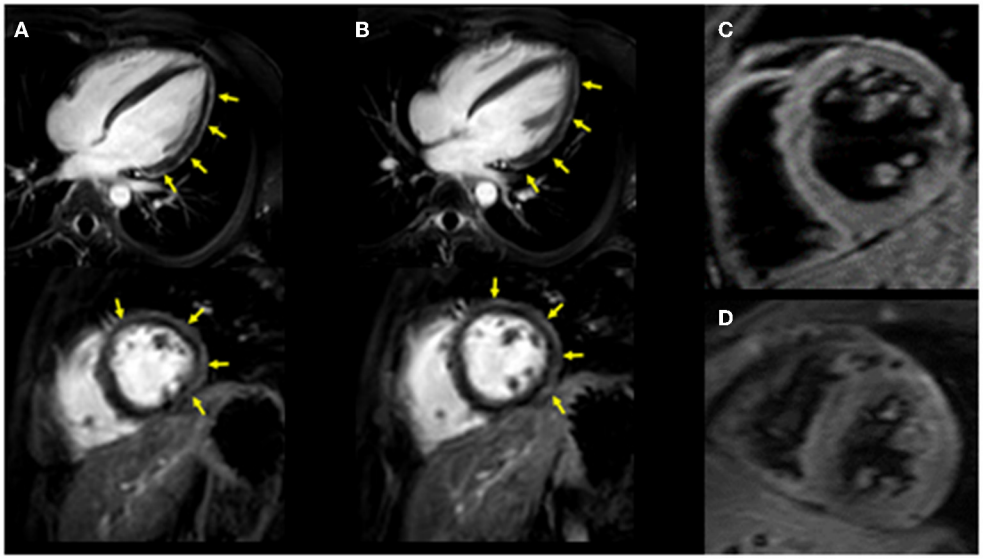


FIGURE 1 | Cardiac MRI imaging. Diffuse late gadolinium enhancement at the epicardium was observed in both images. **(A)** Images obtained 3 years ago when he suffered from his previous myocarditis (top: long-axis view, bottom: short-axis view). **(B)** Images obtained during the current myocarditis episode associated with coronavirus disease-2019 (COVID-19) messenger RNA (mRNA) vaccination (top: long-axis view, bottom: short-axis view). T2-weighted MR images. **(C)** Image obtained 3 years ago when he suffered from his previous myocarditis episode. **(D)** Image obtained during the current myocarditis episode associated with COVID-19 mRNA vaccination.

CASE DESCRIPTION

History of Presentation

A 17-year-old Japanese boy, with chest pain occurring 2 days after his second COVID-19 mRNA vaccination (BNT 162b2, manufactured by Pfizer and BioNTech), was presented to a previous hospital. Electrocardiography showed ST elevation in V2 to V5 leads (**Supplemental Material**). Moreover, his serum cardiac enzymes, including cardiac troponin T (1.605 ng/ml, normal range ≤0.014 ng/ml) and creatinine kinase (CK, 462 IU/L, normal range 62–287 IU/L) were elevated. He was then referred to Kanazawa University Hospital for further investigation and treatment of his chest symptom.

Medical History

The patient had a history of myocarditis (causative bacteria was *Campylobacter*) when he was 13 years old, which was treated with intravenous immunoglobulin (IVIG). His initial symptoms included fever, chest pain, and diarrhea. His maximum CK was 1,682 IU/L. Cardiac MRI revealed diffuse late gadolinium enhancement at the epicardium (**Figure 1A**). A cardiac biopsy was not performed. After the introduction of IVIG, his symptoms improved, for which he was discharged from the hospital without any apparent cardiac dysfunction assessed by echocardiography and myocardial scintigraphy. Enalapril 5 mg/day was introduced and was discontinued 1 year after this episode. He received regular follow-up at our institute, during which, his serum cardiac enzymes were assessed, and electrocardiography and echocardiography were performed. No signs of recurrence had been observed until his last visit 6 days before his second COVID-19 mRNA vaccination. Echocardiography revealed a normal left ventricular ejection fraction (LVEF = 75%), without other dilatations in any chambers, and his cardiac troponin T level was within the normal range (0.006 ng/ml) in his last visit (6 days before his second COVID-19 mRNA vaccination).

Abbreviations: CK, creatinine kinase; IVIG, intravenous immunoglobulin; MRI, magnetic resonance imaging; LVEF, left ventricular ejection fraction.

Differential Diagnosis

Acute coronary syndrome and acute systolic heart failure of any cause were considered as differential diagnoses.

Diagnostic Assessment

Upon admission to our hospital, the patient had blood pressure, heart rate, and body temperature of 135/65 mmHg, 97 bpm, and 37.3°C, respectively. Chest radiography showed no signs of cardiomegaly or pulmonary congestion. Blood tests revealed an elevation in white blood cells (9,560/μl) and C-reactive protein (4.44 mg/dl, normal range ≤0.3 mg/dl), together with elevations in cardiac enzymes, including CK (818 IU/L, normal range 62–287 IU/L), CK-MB (59 IU/L, normal range 2–21 IU/L), and cardiac troponin T (1.41 ng/ml, normal range ≤0.014 ng/ml). The N-terminal pro-brain natriuretic peptide (NT-pro BNP) level was also elevated (221.2 pg/ml, normal range ≤ 125 pg/ml). Electrocardiography revealed ST elevations in V2–V5 leads, whereas echocardiography revealed systolic dysfunction (LVEF = 55%) associated with left ventricular dilatation (LVDd, 55 mm) without any pericardial effusion. Coronary CT showed no signs of coronary atherosclerosis. A myocardial specimen obtained from the septum of the right

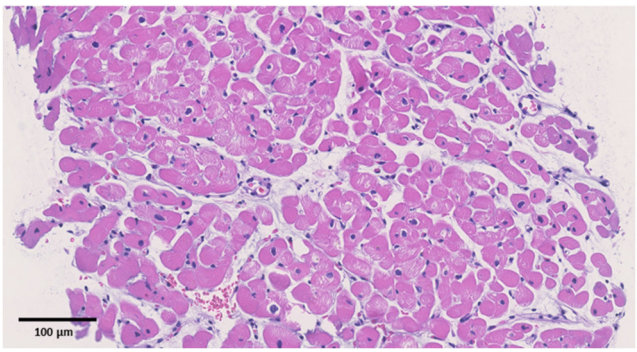


FIGURE 2 | Pathological specimens. Hematoxylin and eosin staining (original magnification ×200). The black bar indicates 100 μm. No apparent signs of inflammation were observed.

ventricle showed no apparent signs of myocardial destruction or inflammation (**Figure 2**). Hemodynamic evaluation by Swan–Ganz catheterization revealed a pulmonary artery pressure of 27/11 (19) mmHg, pulmonary capillary wedge pressure of 13 mmHg, and cardiac output of 6.62 L/min. Cardiac MRI revealed diffuse late gadolinium enhancement at the epicardium (**Figure 1B**) that was similar but somewhat different from the images observed 3 years prior when he suffered from his previous myocarditis (**Figure 1A**). A T2-weighted MRI revealed diffuse high-intensity areas, suggesting edematous changes in the left ventricle during his previous bout of myocarditis, as well as during the current myocarditis (**Figures 1C,D**). Enzyme-linked immunosorbent assays of sera were all negative for potential causes of viral myocarditis (Coxsackie, echo, influenza A and B, cytomegalovirus, and Epstein-Barr virus (EBV)]. Negative T waves were observed in V3 to V6 leads following electrocardiography on day 5 (**Supplemental Material**). All the aforementioned results, except for pathological findings from the myocardial specimen, were consistent with a diagnosis of COVID-19 mRNA vaccination-related myocarditis. We ruled out acute coronary syndrome given the absence of cardiac asynergy and cardiac MRI findings. We also ruled out acute systolic heart failure of any cause based on the hemodynamic evaluation findings by Swan–Ganz catheterization.

Management

The patient was started on IVIG treatment (5 g/day × 3 days), colchicine (0.5 g/day × 14 days), and aspirin (300 mg/day × 14 days) (**Figure 3**). The Japanese guideline (9) utilized by our institute has no clear first-choice therapy for this situation. Among several potential medical therapies, we opted to use IVIG to avoid complications when using immunosuppressive agents. The CK, CK–MB, cardiac troponin T, and NT-proBNP levels gradually returned to normal, and follow-up echocardiography showed normal cardiac function (LVEF = 68%). After being hospitalized for a total of 23 days, the patient was discharged without any symptoms.

DISCUSSION

Currently, myocarditis is being recognized as one of the complications of COVID-19 mRNA vaccination (1–3). Albeit rare, the prognosis of this condition seems to be quite good. Nonetheless, more information on risk factors for this unfavorable phenomenon needs to be collected (6–8). So far, epidemiological studies have suggested that this condition is more frequently observed among young adult and adolescent males (6–8). However, it is unclear whether a history of other types of myocarditis can be considered a risk factor. In this report, we present the first-ever documented case of myocarditis associated with COVID-19 mRNA vaccination in a patient who had a history of myocarditis (**Supplemental Material**). Based on a series of investigations, including cardiac enzymes, electrocardiogram, echocardiography, and cardiac MRI, we found similarities between COVID-19 mRNA vaccination-related myocarditis and myocarditis associated with *Campylobacter jejuni*. We observed unique yet similar patterns on cardiac MRI wherein diffuse late gadolinium enhancement was located mainly at the epicardium during both the current COVID-19 mRNA vaccination-related myocarditis and the previous myocarditis episode associated with *Campylobacter jejuni*. Cardiac MRI has been considered a useful modality for diagnosing acute myocarditis (10, 11) given its great potential for not only diagnosis but also understanding of the pathophysiological mechanism of COVID-19 mRNA vaccination-related myocarditis (12–14). There are several limitations to be considered. First, we obtained three specimens at the time of endomyocardial biopsy. Although the patient had no apparent signs of myocardial destruction or inflammation from the endomyocardial biopsy, a diagnosis of myocarditis was established because of his elevated cardiac troponin T, elevated creatinine kinase, reduced EF, changes in the electrocardiogram, and MRI findings. Second, we could not determine the causal association between the history of myocarditis and the current vaccination-associated myocarditis. Third, we did not compare the cardiac MR images between the previous and current myocarditis episodes. Thus, the diffuse late gadolinium enhancement at the epicardium observed during the current myocarditis episode may not have represented acute myocarditis. However, we observed edematous changes in the myocardium using T2-weighted MR images. In addition, the area of late gadolinium enhancement at the epicardium observed in the current myocarditis episode was somewhat different from that of the previous one. These facts support the notion that late gadolinium enhancement at the epicardium observed in the current episode represents acute myocarditis. Lastly, we were unable to perform the suggested immunohistochemical testing on our biopsy specimens to investigate whether there were any autoantibodies against the myocardium. The second episode might, indeed, be associated with post-infectious autoimmune syndrome; however, this situation has been described as a chronic condition rather than an acute one with complications in multiple organs (1). Of note is that the mechanism of myocarditis induced by mRNA vaccination remains unclear. In most cases without a history of previous myocarditis,

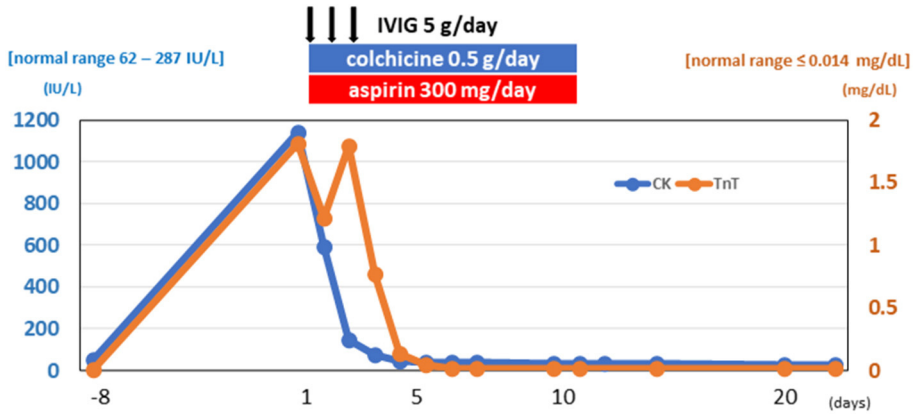


FIGURE 3 | Clinical course. Elevated cardiac enzymes decreased during his clinical course. CK, creatinine kinase; TnT, cardiac troponin T; IVIG, intravenous immunoglobulin.

molecular mimicry between the spike protein of virus and self-antigens, trigger of pre-existing dysregulated immune pathways in certain individuals, immune response to mRNA, activation of immunologic pathways, and dysregulated cytokine expression have been proposed (8). However, in this case with a history of myocarditis, there may be something more in addition to these common mechanisms, although observations from a single case cannot produce any concrete evidence.

In conclusion, special attention may be needed when introducing COVID-19 mRNA vaccination to individuals who have a history of myocarditis. Cardiac MRI can be useful for diagnosing COVID-19 mRNA vaccination-related myocarditis.

PATIENT PERSPECTIVE

We suggest that this episode would not have any serious impact on his cardiac function. However, we will advise the patient to avoid the booster COVID-19 mRNA vaccine because of this episode.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because patients data are not available except for the requests based on legal measures. Requests to access the datasets should be directed to HT, ht240z@sa3.so-net.ne.jp.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

NK, HT, HO, SY, KS, SU, HI, MO, M-aK, and MT contributed to the patient's care and contributed to the preparation of the manuscript. All authors approved the final version of the manuscript.

ACKNOWLEDGMENTS

We thank Ms. Kazuko Honda and Sachio Yamamoto for their technical assistance.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.837759/full#supplementary-material

REFERENCES

1. Heymans S, Eriksson U, Lehtonen J, Cooper LT Jr. The quest for new approaches in myocarditis and inflammatory cardiomyopathy. *J Am Coll Cardiol.* (2016) 68:2348–64. doi: 10.1016/j.jacc.2016.09.937
2. Siripanthong B, Nazarian S, Muser D, Deo R, Santangeli P, Khanji MY, et al. Recognizing COVID-19-related myocarditis: the possible pathophysiology and proposed guideline for diagnosis and management. *Heart Rhythm.* (2020) 17:1463–71. doi: 10.1016/j.hrthm.2020.05.001
3. Abu Mouch S, Roguin A, Hellou E, Ishai A, Shoshan U, Mahamid L, et al. Myocarditis following COVID-19 mRNA vaccination. *Vaccine.* (2021) 39:3790–3. doi: 10.1016/j.vaccine.2021.05.087
4. Mansour J, Short RG, Bhalla S, Woodard PK, Verma A, Robinson X, et al. Acute myocarditis after a second dose of the mRNA COVID-19 vaccine: a report of two cases. *Clin Imaging.* (2021) 78:247–9. doi: 10.1016/j.clinimag.2021.06.019

5. Dickey JB, Albert E, Badr M, Laraja KM, Sena LM, Gerson DS, et al. A series of patients with myocarditis following SARS-CoV-2 vaccination with mRNA-1279 and BNT162b2. *JACC Cardiovasc Imaging.* (2021) 14:1862–3. doi: 10.1016/j.jcmg.2021.06.003
6. Barda N, Dagan N, Ben-Shlomo Y, Kepten E, Waxman J, Ohana R, et al. Safety of the BNT162b2 mRNA Covid-19 vaccine in a nationwide setting. *N Engl J Med.* (2021) 385:1078–90. doi: 10.1056/NEJMoa2110475
7. Dionne A, Sperotto F, Chamberlain S, Baker AL, Powell AJ, Prakash A, et al. Association of myocarditis with BNT162b2 messenger RNA COVID-19 vaccine in a case series of children. *JAMA Cardiol.* (2021) 6:1446–50. doi: 10.1001/jamacardio.2021.3471
8. Bozkurt B, Kamat I, Hotez PJ. Myocarditis with COVID-19 mRNA vaccines. *Circulation.* (2021) 144:471–84. doi: 10.1161/CIRCULATIONAHA.121.056135
9. JCS Joint Working Group. Guidelines for diagnosis and treatment of myocarditis (JCS 2009): digest version. *Circ J.* (2011) 75:734–43. doi: 10.1253/circj.CJ-88-0008
10. Liguori C, Farina D, Vaccher F, Ferrandino G, Bellini D, Carbone I. Myocarditis: imaging up to date. *Radiol Med.* (2020) 125:1124–34. doi: 10.1007/s11547-020-01279-8
11. Ammirati E, Veronese G, Bottiroli M, Wang DW, Cipriani M, Garascia A, et al. Update on acute myocarditis. *Trends Cardiovasc Med.* (2021) 31:370–9. doi: 10.1016/j.tcm.2020.05.008
12. Isaak A, Feisst A, Luetkens JA. Myocarditis following COVID-19 vaccination. *Radiology.* (2021) 301:E378–9. doi: 10.1148/radiol.2021211766
13. Maurus S, Weckbach LT, Marschner C, Kunz WG, Ricke J, Kazmierczak PM, et al. Differences in cardiac magnetic resonance imaging markers between patients with COVID-19-associated myocardial injury and patients with clinically suspected myocarditis. *J Thorac Imaging.* (2021) 36:279–85. doi: 10.1097/RTI.0000000000000599
14. Tanacli R, Doeblin P, Götze C, Zieschang V, Faragli A, Stehning C, et al. COVID-19 vs. classical myocarditis associated myocardial injury evaluated by cardiac magnetic resonance and endomyocardial biopsy. *Front Cardiovasc Med.* (2021) 8:737257. doi: 10.3389/fcvm.2021.737257

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 24 March 2022
doi: 10.3389/fcvm.2022.842641

In silico Drug Screening Approach Using L1000-Based Connectivity Map and Its Application to COVID-19

Takaharu Asano[1], *Sarvesh Chelvanambi*[1], *Julius L. Decano*[1], *Mary C. Whelan*[1], *Elena Aikawa*[1,2,3] *and Masanori Aikawa*[1,2,3,4]*

[1] Center for Interdisciplinary Cardiovascular Sciences, Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, United States, [2] Center for Excellence in Vascular Biology, Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, United States, [3] Department of Human Pathology, I.M. Sechenov First Moscow State Medical University of the Ministry of Health, Moscow, Russia, [4] Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, United States

Conventional drug screening methods search for a limited number of small molecules that directly interact with the target protein. This process can be slow, cumbersome and has driven the need for developing new drug screening approaches to counter rapidly emerging diseases such as COVID-19. We propose a pipeline for drug repurposing combining *in silico* drug candidate identification followed by *in vitro* characterization of these candidates. We first identified a gene target of interest, the entry receptor for the SARS-CoV-2 virus, angiotensin converting enzyme 2 (ACE2). Next, we employed a gene expression profile database, L1000-based Connectivity Map to query gene expression patterns in lung epithelial cells, which act as the primary site of SARS-CoV-2 infection. Using gene expression profiles from 5 different lung epithelial cell lines, we computationally identified 17 small molecules that were predicted to decrease ACE2 expression. We further performed a streamlined validation in the normal human epithelial cell line BEAS-2B to demonstrate that these compounds can indeed decrease ACE2 surface expression and to profile cell health and viability upon drug treatment. This proposed pipeline combining *in silico* drug compound identification and *in vitro* expression and viability characterization in relevant cell types can aid in the repurposing of FDA-approved drugs to combat rapidly emerging diseases.

Keywords: L1000, connectivity map (CMap), ACE2, COVID-19, drug repurposing, lung epithelial cell

OPEN ACCESS

Edited by:
Ryuichi Morishita,
Osaka University, Japan

Reviewed by:
Hirinori Nakagami,
Osaka University, Japan
Sivareddy Kotla,
University of Texas MD Anderson Cancer Center, United States

***Correspondence:**
Masanori Aikawa
maikawa@bwh.harvard.edu

Specialty section:
This article was submitted to Atherosclerosis and Vascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *23 December 2021*
Accepted: *14 February 2022*
Published: *24 March 2022*

Citation:
Asano T, Chelvanambi S, Decano JL, Whelan MC, Aikawa E and Aikawa M (2022) In silico Drug Screening Approach Using L1000-Based Connectivity Map and Its Application to COVID-19. Front. Cardiovasc. Med. 9:842641. doi: 10.3389/fcvm.2022.842641

INTRODUCTION

Coronavirus disease 2019 (COVID-19), which is caused by the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), broke out in December 2019. The World Health Organization designated COVID-19 as a global pandemic in March of 2020. Since then, multiple variants have emerged, spread globally, and continue to hit the health, life, and economy of people worldwide even with the advent of various vaccines designed to provide immunity against the virus. Intensive research efforts have revealed that morbidity, severity, and mortality from COVID-19 are strongly associated with various cardiovascular comorbidities (1). Since COVID-19 infection of lung epithelium could directly signal toward an increased risk for cardiovascular diseases (2),

reducing productive infection of these cells becomes an important strategy to not only counter acute infection but prevent progression to cardiovascular diseases. Acute infections of viruses such as influenza virus (3), HIV (4), and SARS-CoV-2 (5) have been shown to have a direct impact on cardiovascular health and act as the initial insult that increases the incidence of cardiovascular disease in these patients. However, due to the massive number of patients globally infected by the virus, increase in cardiovascular disease prevalence due to infection with SARS-CoV-2 virus could severely burden the cardiovascular healthcare system in the future. Therefore, repurposing FDA approved drugs to contain SARS-CoV-2 infection can be an effective strategy to limit the risk of developing cardiovascular diseases in these patients.

The first step of SARS-CoV-2 invasion into human host cells is implemented by the SARS-CoV-2 spike protein binding to a host cell receptor, angiotensin-converting enzyme 2 (ACE2) (6). Inhibiting the spike protein and ACE2 interaction is, therefore, one of the promising drug targets for combating COVID-19 (7). Most current studies aim to inhibit the interaction by drugs that binds to the spike protein or ACE2 protein (7, 8).

In case drug targets are known, target-based drug discovery, in which a specific drug target that associates a target disease is identified and then hit compounds that interact with the target are searched for, is a proven strategy to generate new drugs (9). Conventional drug screening methods used in this strategy such as high-throughput screening (HTS), however, search for a limited number of small molecules that directly interact with the target protein. Moreover, this process can be slow, cumbersome and has driven the need for developing new drug screening approaches to counter rapidly emerging diseases like COVID-19.

The Connectivity Map (CMap) is a database of gene expression profiles induced by exposing a variety of cell types to various perturbagens including small molecules and has been expanded to have over one million gene expression profiles using over 20,000 small molecules through the introduction of L1000 assay technology (10, 11). L1000-based CMap has been widely used for rapid drug repurposing and the core idea is to identify small molecules that induce a gene expression profile canceling or mimicking the differential gene expression caused by diseases (12, 13). This approach is a kind of phenotypic screening, which is a counter approach to the target-based drug discovery and identifies small molecules that provide nice phenotypes (e.g., gene expression) to cells or animals first and then investigates the mechanism. Phenotypic screening has attracted attention recently because it was shown to be the most successful approach for first-in-class drugs (9, 14). As described above, although the conventional L1000-based CMap approach is an attractive way to find drugs that the conventional methods could overlook, it hardly has been applied to target-based drug discovery because this approach requires decreased and/or increased gene set, not a single target gene.

In this study, we propose a pipeline for drug repurposing that applies the L1000-based CMap to a single gene target, ACE2 which is the entry receptor for the SARS-CoV-2 virus. Using gene expression profiles from 5 different lung epithelial cell lines which act as the primary site of SARS-CoV-2 infection, we computationally identify small molecules that were predicted to decrease ACE2 expression. We further perform a streamlined validation in the normal human epithelial cell line BEAS-2B to identify the potential of these compounds to decrease ACE2 surface expression as well as profile cell health and viability upon drug treatment. This proposed pipeline combining *in silico* drug compound identification and *in vitro* expression and viability characterization in relevant cell types can aid in the repurposing of FDA-approved drugs to combat rapidly emerging diseases.

MATERIALS AND METHODS

L1000-Based CMap Dataset

Level 5 gene expression profiles of L1000-based CMap were downloaded from GSE92742 and GSE70138. This dataset has the gene expression profiles in a total of 591,697 conditions consisting of various combinations of perturbagens, cell types, doses, and time points. The profile values mean mRNA expression levels compared to control (the background of the plate). Each gene expression profile comprises 12,328 genes, 978 of which are measured directly (called landmark genes). Of the remaining genes, 9,196 are well-inferred genes the expression levels of which correlate to the actual measured levels with p-values $\leq$0.05, and the other 2,154 less-well inferred genes. ACE2 is in the well-inferred genes.

Cell Culture and Reagents

BEAS-2B normal human epithelial cell line was purchased from ATCC (Catalog number: CRL-9609) and cultured according to vendor instructions using BEGM kit from LONZA (Catalog number: CC-3170). Cells were cultured on 96-well black μ-plate from ibidi (Catalog Number: 89626) for imaging studies. Tanespimycin (abcam ab141433), Acetylcysteine (Cayman, 20261), Amifostine (Cayman 14398), Bortezomib (Ayman 10008822), FK-866 (Cayman 13287), Gemcitabine (Cayman 11690), Idarubicin (Cayman 14176), NVP-AUY922 (Cayman 10012698), NVP-BEZ235 (Cayman 10565), PIK-75 (Cayman 10009210), SN-38 (Cayman 15362), Tretinoin (Cayman 11017), YM-155 (Cayman 11490), Ingenol (Cayman 14031), Sulforaphane (LKT S8044), CD-437 (Sigma C5865), and Parbendazole (Sigma 1498706) were dissolved in DMSO. 1000x concentration working solution was used for downstream experimentation.

Immuno-Fluorescent Staining With High Content Imaging (HCI) for Quantifying ACE2 Expression

BEAS-2B cells were treated overnight with indicated drugs at indicated doses. Cells were then washed twice with PBS and fixed in 4% Paraformaldehyde for 15 min at room temperature. Cells were then stained with primary anti-human ACE2 antibody (Abcam ab239924) or isotype control for 1 h at 4°C with gentle shaking. Cells were then washed thrice with PBS and stained using AF555 labeled secondary antibody. Hoescht 33342 was used as nuclear counterstain.

Sixteen images were captured per well using 20x objective of an Image Express Pico (Molecular Devices) and analyzed using 2

color cell scoring system. Isotype control stained well was used to identify threshold for detecting ACE2 positivity in cells.

Cell Viability Measurement

BEAS-2B cells were plated in 96 well plates at 70% confluency and treated for 48 h with each drug at indicated doses. Cell mitochondrial activity was profiled using CyQuant MTT Cell Viability Assay (Thermo Fisher, Catalog Number V13154) following manufacturer instructions. The absorbance at 590 nm was quantified using Spectramax i3 (Molecular Devices). Cytotoxicity was quantified using CyQuant LDH Cytotoxicity Assay (Thermo Fisher Catalog Number C20301) following manufacturer instructions. Briefly, 50 μl of media supernatant from each well was used to quantify cell toxicity and was normalized to cells lysed with 10x cell lysis buffer as 100% cell death. Absorbance was measured at 490 nm and 680 nm with the 680 nm absorbance used to determine background plate absorbance. Mitochondrial Super Oxide production was quantified using MitoSOX™ Red Mitochondrial Superoxide Indicator (Thermo Fisher Catalog Number M36008) using manufacturer instructions. Hoescht 33342 was used as a nuclear counterstain. Sixteen images were captured per well using 20x objective of the Image Express Pico and analyzed using 2 color cell scoring system to determine average Mitochondrial Superoxide Intensity per cell.

FACS Staining

BEAS-2B cells were treated with indicated doses of drugs overnight and cells detached using accutase. Cells were resuspended in Stain Buffer with FBS (BD Biosciences) and stained with Fixed Viability Stain (FVS-780 BD Biosciences) followed by staining with ACE2-AF647 antibody (Biolegend). Cells stained with isotype AF647 antibody (BD Biosciences) were used to draw gates for ACE2 positivity. Cells were acquired on Cytek Aurora and data analyzed using Flowjo 10.8.

Statistical Methods

ACE2 expression and cell viability data were analyzed using a Python library, SciPy. FACS data was analyzed using Graphpad Prism 9.0. Student's *t*-test with Welch's correction was used to compare the effect of each treatment to control (vehicle) treated samples. Comparisons between the effects of each candidates were not performed.

RESULTS

Preprocessing for Drug Screening

The following filters were applied to the L1000-based CMap dataset to identify small molecules that are effective to COVID-19 therapy before searching for small molecules that decrease ACE2 expression (**Figure 1**).

Perturbagen Selection

A perturbagen is a reagent used to treat cells and measure the resulting biological response includes CRISPR/Cas9 constructs, short hairpin RNA (shRNA), open reading frames (ORFs), biological agents, small molecules, and so forth. The goal of this study was to identify small molecules suitable for rapid drug repurposing. Drug Repurposing Hub is a curated and annotated collection of FDA-approved drugs, clinical trial drugs, and pre-clinical tool compounds with a companion information resource, and the mechanisms of action of 6,232 drugs are explicitly stated in it (drug information version: 3/24/2020) (15). L1000-based CMap dataset contains 20,547 small molecules, and 2,760 small molecules out of them overlap with these 6,232 drugs. We therefore extracted the gene expression profiles in conditions treated with these 2,760 small molecules, resulting 160,003 profiles induced by exposing 82 cell types to 2,760 small molecules with up to 177.6 μM for 3, 6, 24, or 48 h.

Dose Selection

Each small molecule was measured at various dosages within the database. To compare these dosages and to increase ease of handling, doses from a similar range were converted to a single value as indicated in **Supplementary Table 1**. Among available dosages, 0.0001–100 μM, the most used dosage was 10 μM and the profiles using over 1 μM dosage dominate 68.5% of the total. These dosages over 1 μM were eliminated due to concerns about cytotoxicity and the need to identify drugs that would work at low doses and minimal side-effects to reduce gene expression of ACE2.

Time-Point Selection

Drugs for COVID-19 need to work within hours to reduce ACE2 expression since that would be the ideal window for drug intervention of a patient testing positive for SARS-CoV-2. The available time-points were 3, 6, and 24 h after the dose selection. We removed the timepoints over 24 h to identify fast-acting drug candidates. This step provided 5,411 profiles induced by exposing 67 cell types to 300 small molecules with 0.0003–0.3162 μM for 3 or 6 h.

Cell Type Selection

After the time-point selection, 67 cell types derived from 14 organs such as large intestine, lung, breast, etc. were available. Each cell type shows a different gene expression profile even though the same small molecule is applied. It is advisable to use the gene expression profiles in a specific cell type of interest. However, human lung epithelial cells such as BEAS-2B that are suitable for model host cells in COVID-19 study are not included in L1000-based CMap. We thus selected 5 cell types (A549, CORL23, H1299, NCIH596, and SKLU1) derived from lung in the dataset. Of note, all five cell types were from lung epithelial cell lines derived from various tumors. The gene expression profiles in conditions using these 5 cell types were extracted, resulting 588 profiles induced by exposing 5 cell types to 80 small molecules with 0.0003–0.3162 μM for 6 h.

The 588 profiles came from 485 unique conditions. Finally, the gene expression levels were averaged over the same conditions, resulting the preprocessed dataset that has 485 profiles induced by exposing 5 cell types to 80 small molecules with 0.0003–0.3162 μM for 6 h.

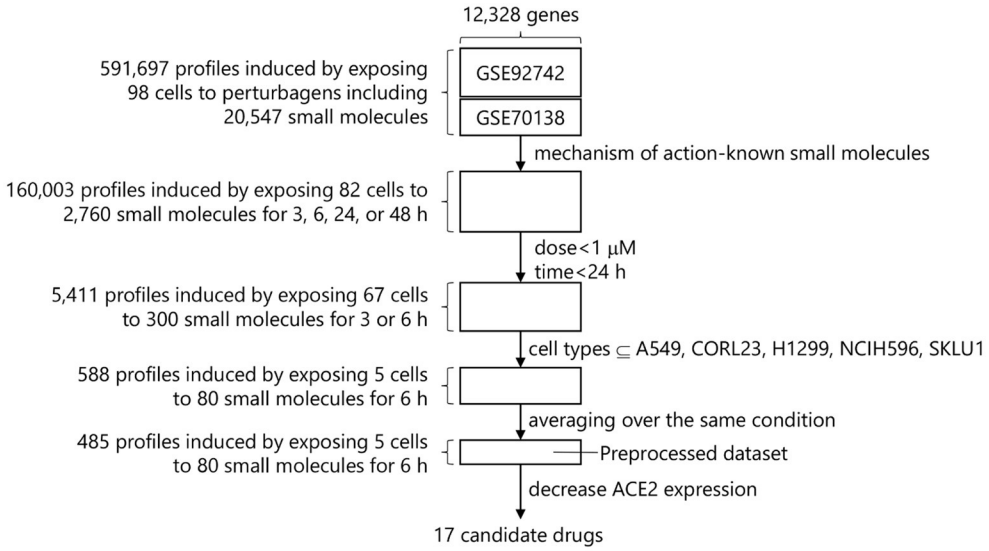


FIGURE 1 | Drug screening process in L1000-based CMap.

Identification of Small Molecules That Decrease ACE2 Expression

We focused on the expression levels of ACE2 and extracted the combinations of the small molecules and their dosages that decrease ACE2 expression (i.e., show the negative ACE2 expression levels) in each cell type. As for A549, since 214 combinations were identified, the top 10 combinations with the lower ACE2 expression levels were selected. In the other 4 cell types, 4, 4, 6, and 5 combinations were identified in CORL23, H1299, NCIH596, and SKLU1, respectively. Out of these 29, 19 combinations were unique. We removed 0.0032 μM veliparib which was commercially unavailable and 0.01 μM idarubicin, while keeping the larger dose, 0.1 μM idarubicin. As a result, 17 small molecules and their optimal dosages in 6 h were obtained as the drug repurposing candidates. The ACE2 expression levels in each cell type treated with these 17 small molecules with their optimal dosages are shown in **Table 1**.

NVP-AUY922 and tanespimycin are heat shock protein (HSP) inhibitors. NVP-BEZ235 and PIK-75 are PI3K inhibitors. CD-437 and tretinoin are retinoid receptor agonists. SN-38 and idarubicin are topoisomerase inhibitors. The other 9 small molecules have different mechanisms of action. The candidates cover a wide variety of mechanisms of action. On the other hand, most small molecules have been developed for cancer drugs except for acetylcysteine, ingenol, and sulforaphane.

For 9 among 17 small molecules, ACE2 expression levels were available in all 5 cell types (**Table 1**). The ACE2 expression levels were quite different in each cell even though the same small molecules are applied with the same doses, suggesting the other 8 small molecules whose ACE2 levels were available only in A549 also have different ACE2 expression levels depending on the cell types. On the other hand, these small molecules show negative ACE2 expression levels in at least 1 cell type, suggesting that these small molecules have a potential to decrease ACE2 expression in human lung epithelial cells.

For 13 among 17 small molecules, ACE2 expression levels at 6 dose points in A549 were available in the preprocessed dataset. The dose-response of ACE2 expression levels in each small molecule are shown in **Figure 2**. The top 6 small molecules with lower ACE2 levels in A549 in **Table 1** (acetylcysteine, CD-437, NVP-BEZ235, amifostine, ingenol, and NVP-AUY922) decreased ACE2 expression almost dose-dependently within the ranges up to their optimal doses. These small molecules are expected to decrease ACE2 expression in A549, human adenocarcinoma alveolar basal epithelial cells.

In-vitro Pipeline for Evaluation of Predicted Drug Repurposing Candidates That Reduce ACE2 Expression in Human Lung Epithelial Cells

Evaluation of Cytotoxicity Profile of Drug Repurposing Candidates in Normal Human Immortalized Bronchial Epithelial Cells

The first *in vitro* step in our drug repurposing validation pipeline was to evaluate the effect of the various predicted small molecules in impacting cellular health and viability. As listed in **Table 1**, these compounds have a wide range of mechanisms of action. Stringent characterization of the effect of a treating relevant cell type with these compounds was therefore performed.

The five lung epithelial cell lines utilized in the L1000 were tumor-derived lung epithelial cell lines. Evaluating the effectiveness of these predicted drug repurposing candidates in reducing ACE2 expression in COVID-19 infected patients, however, required analysis in a non-tumor lung epithelial cell

TABLE 1 | The identified 17 small molecules, their optimal doses, mechanisms of action, and ACE2 expression levels in each cell type.

Drug name (uM)	Mechanism of action	Cell type				
		A549	CORL23	H1299	NCIH596	SKLU1
Acetylcysteine (0.01)	Mucolytic agent	−2.29				
CD-437 (0.1)	Retinoid receptor agonist	−1.74				
NVP-BEZ235 (0.0316)	mTOR inhibitor, PI3K inhibitor	−1.47				
Amifostine (0.3162)	Reducing agent	−1.47				
Ingenol (0.01)	PKC activator	−1.45		No data		
NVP-AUY922 (0.1)	HSP inhibitor	−1.41				
Tretinoin (0.3162)	Retinoid receptor agonist, retinoid receptor ligand	−1.40				
Sulforaphane (0.001)	Anticancer agent, aryl hydrocarbon receptor antagonist	−1.32				
Bortezomib (0.0316)	NFkB pathway inhibitor, proteasome inhibitor	−0.54	0.13	0.13	−0.80	−0.96
Parbendazole (0.3162)	Tubulin polymerization inhibitor	−0.53	0.01	0.80	−0.80	−0.09
Idarubicin (0.1)	Topoisomerase inhibitor	−0.47	0.70	−1.44	0.00	0.46
Tanespimycin (0.3162)	HSP inhibitor	−0.23	0.00	0.09	0.39	0.07
SN-38 (0.3162)	Topoisomerase inhibitor	−0.19	−0.37	−0.55	−0.38	0.20
FK-866 (0.1)	Niacinamide phosphoribosyltransferase inhibitor	0.06	−0.79	0.41	−0.50	0.10
Gemcitabine (0.1)	Ribonucleotide reductase inhibitor	0.09	0.63	0.94	0.50	−1.28
YM-155 (0.3162)	Survivin inhibitor	0.36	0.00	−0.82	−0.57	−0.17
PIK-75 (0.1)	DNA protein kinase inhibitor, PI3K inhibitor	2.32	0.45	−1.04	0.54	−0.37

The small molecules are sorted based on the ACE2 expression levels in A549. The numbers in brackets beside drug names are their optimal dosages (μM). Gray area means no data in the preprocessed dataset.

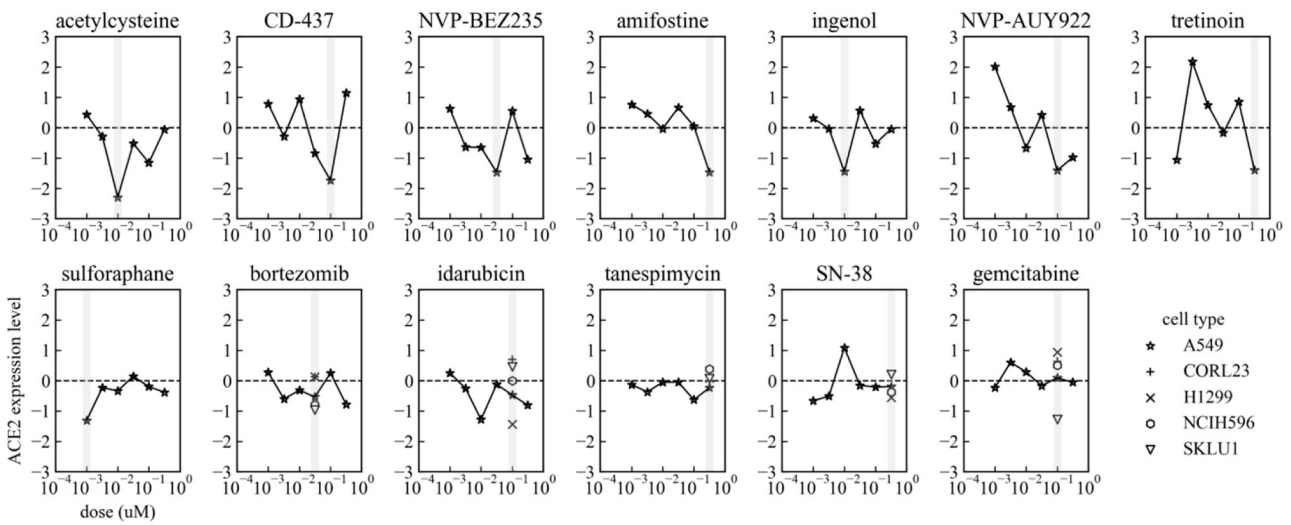


FIGURE 2 | Dose-response of ACE2 expression levels in each small molecule in A549. Gray vertical bars mean the optimal dosages in each small molecule indicated in **Table 1**. ACE2 expression levels in CORL23, H1299, NCIH596, and SKLU1 are also shown in Bortezomib, Idarubicin, Tanespimycin, SN-38, and Gemcitabine.

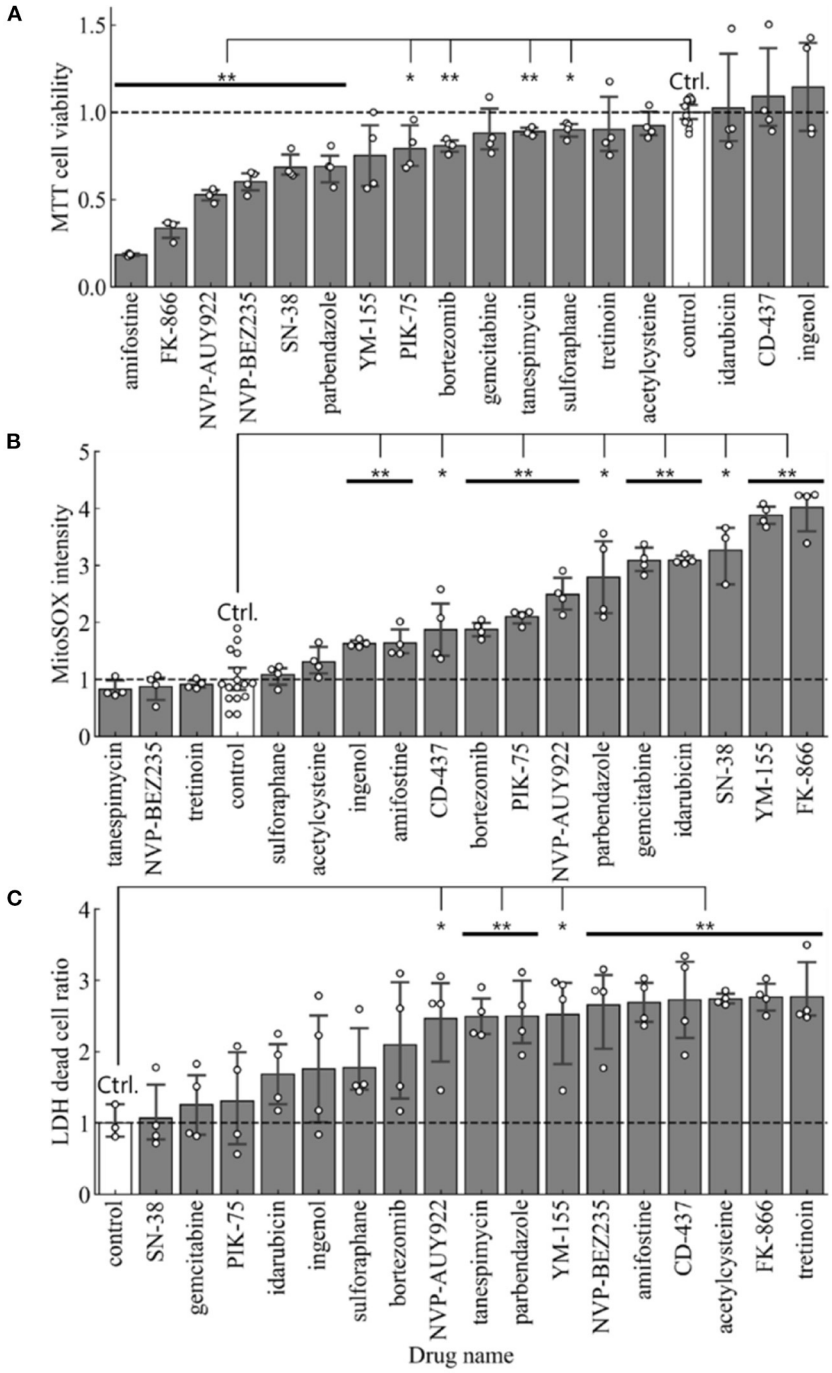


FIGURE 3 | Cell toxicity assay in BEAS-2B treated with each of 17 small molecules and the control. **(A)** Cell viability measured by MTT assay ($n = 4$ in samples and $n = 12$ in control), **(B)** Mitochondrial Super Oxide production measured by MitoSOX assay ($n = 4$ in samples and $n = 16$ in control), and **(C)** Cytotoxicity measured by LDH assay ($n = 4$). Error bars mean 95% confidence interval. $^{*}p < 0.05$, $^{**}p < 0.01$.

setting. BEAS-2B is a normal human immortalized bronchial epithelial cell line, which has been extensively used to study cellular and molecular mechanisms involved in lung. This study therefore used BEAS-2B as a host cell model that could be infected by SARS-CoV-2.

For cell toxicity studies, the BEAS-2B cells were treated with indicated amounts of each drug repurposing candidate, and cell health was evaluated using multiple orthogonal measures. MTT assay measured the amount viable cells in each well by quantifying the amount of MTT converted to formazan crystals.

The average absorbance in cells receiving vehicle were used to normalize the effect of each drug repurposing candidate. Therefore, a ratio <1.0 indicates treatments which exerted a negative effect on cell viability when compared to control cells. In this analysis, all compounds other than CD-437, Idarubicin and Ingenol had a ratio <1.0 (**Figure 3A**). Comparison of cell viability using Student's *t*-test ($p < 0.05$) showed that 10 out of 17 drug repurposing candidates had a statistically significant lower viability as indicated by MTT assay.

To further characterize whether mitochondrial stress was present upon treatment with these drug re-purposing candidates, we evaluated mitochondrial specific superoxide species production using MitoSOX. We used an HCI strategy to quantify the intensity of mitochondrial superoxide species and compared the average intensity of mitochondrial superoxide per cell in each treatment. A ratio >1.0, therefore, indicated higher levels of mitochondrial reactive oxygen species production upon treatment with the respective drug-repurposing candidates as described in **Figure 3B**. NVP-BEZ235, Tanespimycin and Tretinoin were the three compounds with ratio <1.0. All other compounds had elevated mitochondrial superoxide species when compared to control treatment. Comparison of MitoSOX intensity using Student's *t*-test ($p < 0.05$) showed that 12 out of 17 drug repurposing candidates had a statistically significant increase in mitochondrial stress as indicated by MitoSOX staining.

Finally, we used a third method to characterize the effect of these drug repurposing candidates in cell cytotoxicity using Lactate Dehydrogenase (LDH) assay. This assay measures the amount of LDH released out into the supernatant from dead cells with leaky plasma membrane. All readouts were normalized to wells where 100% of cells were lysed using a cell lysis buffer. Compared to this number, the average percentage of dead cells in each well was calculated. Again, percent cell death was normalized to control wells receiving vehicle treatment (**Figure 3C**). Therefore, a ratio >1.0 indicates elevated levels of cytotoxicity. All drug repurposing candidates showcased a ratio >1.0 indicated increased cell death upon treatment. Comparison of cell death using Student's *t*-test ($p < 0.05$) showed that 10 out of 17 drug repurposing candidates had a statistically significant increased cell death as indicated by LDH cytotoxicity assay.

Consensus Ranking of Cytotoxicity in Normal Human Lung Epithelial Cell Lines

To compile the three formats used to evaluate cytotoxicity in BEAS-2B cells treated with our drug repurposing candidates, we built a consensus ranking table comprising of MTT, MitoSOX and LDH cytotoxicity assays. **Table 2** depicts the consensus ranking based on these three assays and ranks small molecules based on low cytotoxicity across all three formats. In this consensus ranking analysis, the following seven candidates, Ingenol, Sulforaphane, Tanespimycin, Idarubicin, CD-437, PIK-75, and Gemcitabine showed a consistently low cytotoxic profile at indicated doses in BEAS-2B lung epithelial cells. This identification of small molecules with favorable safety profile in target cell type is required for our drug repurposing pipeline in order to find suitable candidates for treatment where low cytotoxic side effects are crucial for applicability of identified small molecules.

TABLE 2 | Consensus ranking comprising of MTT, MitoSOX, and LDH cytotoxicity assay.

Drug name	Cyto toxicity ranking			
	MTT	MitoSOX	LDH	Ave.
Control	4	4	1	3.0
Ingenol	1	7	6	4.7
Sulforaphane	7	5	7	6.3
Tanespimycin	8	1	10	6.3
Idarubicin	3	15	5	7.7
CD-437	2	9	15	8.7
PIK-75	11	11	4	8.7
Gemcitabine	9	14	3	8.7
Acetylcysteine	5	6	16	9.0
Tretinoin	6	3	18	9.0
Bortezomib	10	10	8	9.3
NVP-BEZ235	15	2	13	10.0
SN-38	14	16	2	10.7
NVP-AUY922	16	12	9	12.3
Parbendazole	13	13	11	12.3
Amifostine	18	8	14	13.3
YM-155	12	17	12	13.7
FK-866	17	18	17	17.3

Each value means ranking of low toxicity (i.e., high number means high toxicity). Drugs are sorted based on the average ranking over 3 assays.

Evaluation of ACE2 Surface Expression in Human Lung Epithelial Cells With Predicted Candidates for Drug Repurposing

The next step in our drug repurposing pipeline was to determine the effect of these predicted drug repurposing candidates to reduce surface expression of ACE2. For this study, ACE2 surface expression serves as the most important determinant since we were evaluating the capacity of these small molecules to reduce the prevalence of surface receptors to which the SARS-CoV-2 spike protein can bind to and subsequently infect target cells. We thus used immuno-fluorescent staining to quantify surface expression of human ACE2 expression using HCI followed by a cell scoring system using red staining intensity for ACE2 and cell calling using Hoescht 33342 nuclear counterstain. A representative image for ACE2 staining in BEAS-2B is shown in **Figure 4A**. Thresholds for determining ACE2 expression were established using cells stained with isotype primary antibody followed by secondary antibody staining. Cell calling was performed using nuclear counter stain and establishing appropriate cell diameter to detect all cells within 4 different fields of view. Using these parameters, we observed cells that were high for ACE2 expression and others that expressed ACE2 at low levels (**Figure 4B**). Of note, even when looking with the normal immortalized cell line, we observed heterogeneity in ACE2 expression across cells. Using this categorization strategy, we will

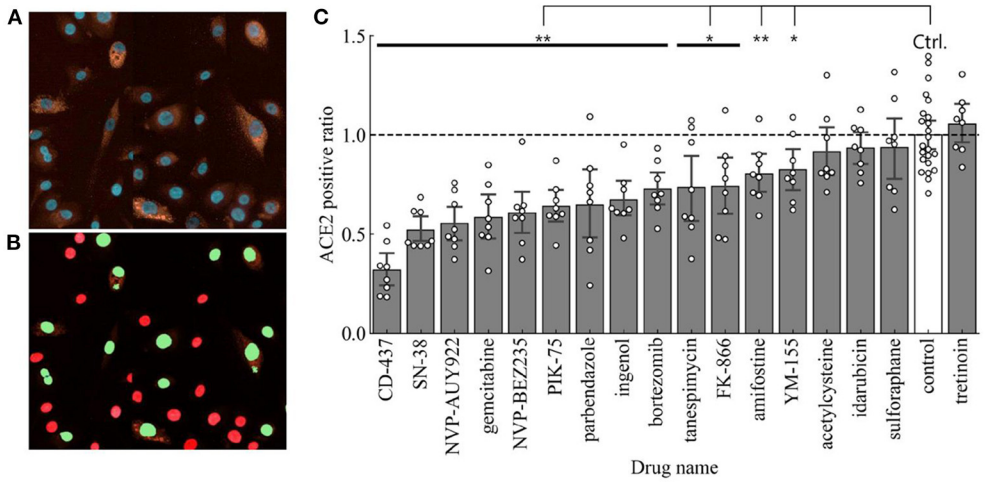


FIGURE 4 | (A) Representative image showing ACE2 staining in BEAS-2B. ACE2-high expressed (ACE2 positive) cells are detected and then ACE2 positive ratio is calculated. **(B)** Representative cell scoring masks indicating ACE2 expressing cells (Green) and ACE2 negative cells (Red). **(C)** ACE2 positive ratios in each drug treatment compared to the control ($n = 8$ in samples and $n = 23$ in control). Error bars mean 95% confidence interval. $^{*}p < 0.05$, $^{**}p < 0.01$.

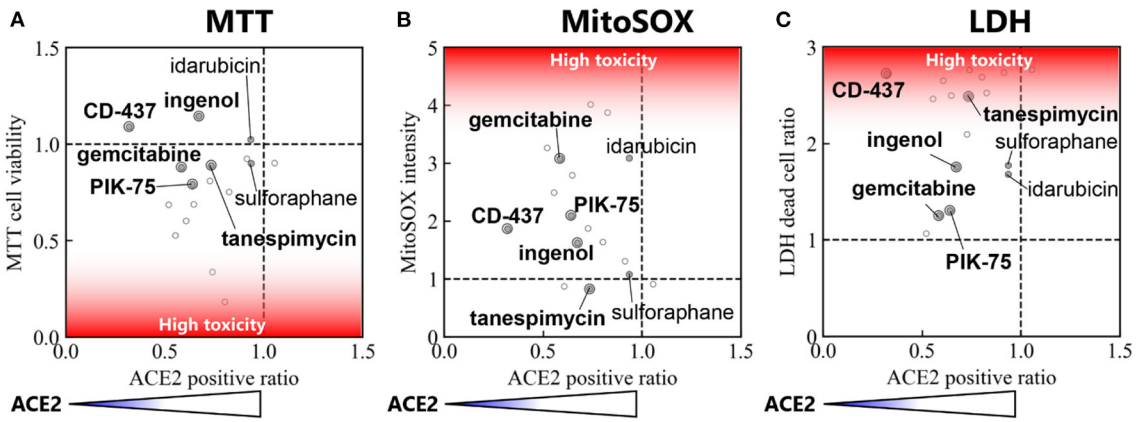


FIGURE 5 | Correlation between ACE2 positive ratio vs. cell toxicity. Correlation between ACE2 positive ratio and **(A)** cell viability measured by MTT assay, **(B)** MitoSOX Assay, and **(C)** LDH assay. The locations of the top 7 small molecules with reduced cytotoxicity in **Table 2** are labeled.

use the nomenclature of ACE2 high- or low-expressed cells to determine the effect of our various treatments in modifying ACE2 expression in a lung epithelial cell population. The number ratios of high-expressed cells in the total cell number were calculated as ACE2 positive ratios in each small molecule treatment and were normalized with the ACE2 positive ratio of the control (no treatment).

The ACE2 positive ratios after our candidate treatments are shown in **Figure 4C**. Using Student's *t*-test ($p < 0.05$) to compare to control treated BEAS-2B cells, 13 out of 17 treatments showed a statistically significant reduction in surface ACE2 expression. In this regard, the top 3 small molecules with the higher decreasing level of ACE2 positive ratio are CD-437, SN-38, and NVP-AUY922.

Integration of ACE2 Expression and Cell Viability Data to Identify Top Drug Repurposing Candidates

This pipeline follows the L1000 powered identification of small molecule candidates for drug repurposing by evaluating the effect of these compounds in affecting both cell health and surface ACE2 expression. Therefore, correlation analysis was performed in order to narrow down the candidates and identify small molecules that could be used to reduce ACE2 expression without inducing high levels of cell death specifically in our target cell type BEAS-2B (**Figure 5**). Three correlation matrices were constructed to compare the effect of each candidate in affecting cell health as well as the potency with which they reduced ACE2 expression. Using this matrix, Ingenol, CD-437, Tanespimycin, PIK-75, and Gemcitabine were five compounds that consistently

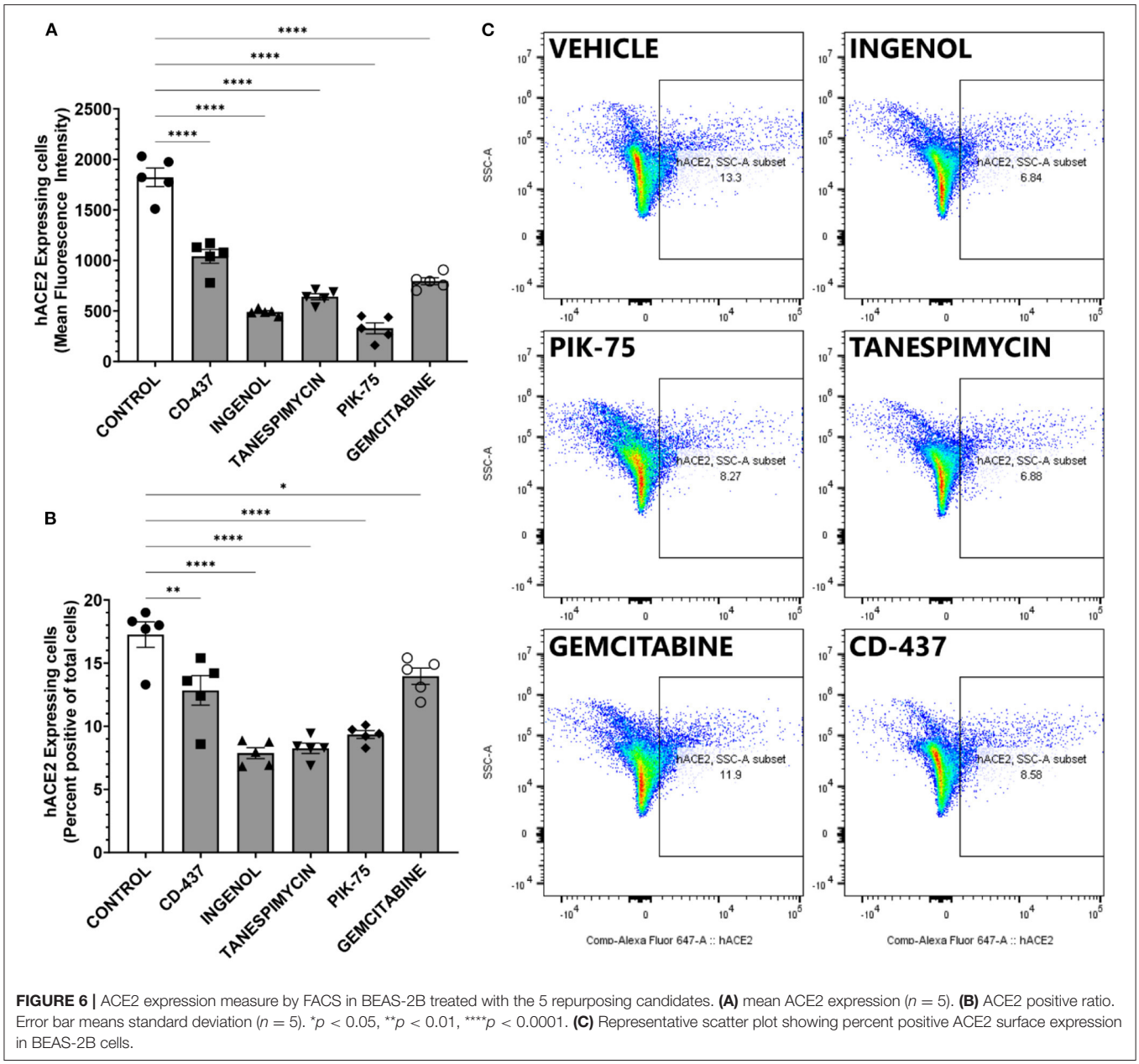


FIGURE 6 | ACE2 expression measure by FACS in BEAS-2B treated with the 5 repurposing candidates. **(A)** mean ACE2 expression ($n = 5$). **(B)** ACE2 positive ratio. Error bar means standard deviation ($n = 5$). *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$. **(C)** Representative scatter plot showing percent positive ACE2 surface expression in BEAS-2B cells.

showed low cytotoxicity and effective downregulation of surface ACE2 expression. This data suggests that these five candidates identified using our novel drug repurposing pipeline can be evaluated for large scale studies in preventing SARS-CoV-2 infection by targeting ACE2 expression levels.

Validation of Identified Top Candidates Combining Viability and ACE2 Expression

The top five candidates with low toxicity and potent decrease in ACE2 expression were further validated using an orthogonal platform, Fluorescence Assisted Cell Sorting. This platform allows for the quantification of surface ACE2 expression in viable, living cells. Furthermore, quantitative analysis can be performed in an unbiased fashion in a large number of cells. BEAS-2B cells were treated with indicated doses of drugs and then percent positive cells for surface ACE2 were determined using control cells treated with isotype antibody (**Supplementary Figure 1**). All five candidates produced statistically significant reductions in both the overall amount of surface ACE2 receptors and the mean fluorescent intensity of ACE2 staining in viable BEAS-2B cells (**Figure 6A**). Furthermore, the fraction of cells determined to be positive for ACE2 expression (**Figures 6B,C**) also showed statistically significant reductions with all five compounds identified using our pipeline. Taken together, our data suggest that intervention with these five small molecule compounds can decrease the number of viable cells susceptible to SARS-CoV-2 infection by altering surface ACE2 expression.

DISCUSSION

We identified 17 candidate small molecules that possibly decrease ACE2 expression by processing the L1000-based CMap dataset with focusing on the drug repurposing for COVID-19 (**Table 1** and **Figure 1**). These candidates decreased ACE2 mRNA levels within 6 h in the lung epithelial cell lines in the preprocessed dataset (**Table 1** and **Figure 2**). This suggests that the decrease in ACE2 mRNA levels likely led to reduced ACE2 surface expression in the target cells BEAS-2B derived from the same organ. Indeed, most candidates decreased ACE2 expression on the surface of BEAS-2B (**Figure 4**). These results indicate that our L1000-powered drug screening effectively identifies small molecules that modulate a single drug target. Further investigation, however, is required to address the mechanism of action for ACE2 suppression by these compounds as well as the effects on ACE2 expression in other cell types.

On the other hand, the identified small molecules were mostly drugs that have been developed for cancer treatment, therefore, their cytotoxic effects in BEAS-2B were evaluated using MTT, MitoSOX, and LDH assays. Over half candidates showed significant cytotoxicity or cell viability reduction in each assay (**Figure 3**). To identify small molecules that consistently show a low cytotoxic profile, we built a consensus ranking table (**Table 2**). The top 2 small molecules with the lowest toxicity were Ingenol and Sulforaphane. Ingenol is an FDA-approved drug for keratosis. Sulforaphane is a naturally occurring isothiocyanate found in cruciferous vegetables such as broccoli. These facts support the validity of this table.

Our goal was to obtain the drug repurposing candidates that could be used to reduce surface ACE2 expression without inducing high cell toxicity. Therefore, correlation analysis was performed to narrow down the candidates (**Figure 5**). In this correlation matrix, Ingenol, CD-437, Tanespimycin, PIK-75, and Gemcitabine consistently showed low cytotoxicity and effective downregulation of surface ACE2 expression. These were five compounds, out of the top 7 with low toxicity in **Table 2**, that show significant ACE2 reduction in **Figure 4**. Moreover, additional validation experiments demonstrated that all these five candidates decreased the surface ACE2 expression in living BEAS-2B (**Figure 6**). These results suggest that these five candidates can be evaluated for large scale studies in preventing SARS-CoV-2 infection by targeting ACE2 expression levels.

Our proposed pipeline consists of L1000-powered drug screening and the further narrowing of the drug repurposing candidates based on their cytotoxic effect. As described above, the L1000 screening can be applied to a single target, allowing us to apply this method to target-based drug discovery which is a gold standard strategy. The small molecules identified by this method are different from those by conventional screenings like HTS because our approach focuses on the target gene mRNA level instead of the target protein. In addition, conventional L1000-CMap approaches have no applicability to target-based drug discovery because it requires a gene set rather than a single gene. Furthermore, our pipeline can be adapted to a wide range of cell types including endothelial cells and monocytes. This is of interest since viruses including HIV and SARS-CoV-2 have been shown to specifically induce pulmonary endothelial cell activation (16, 17). Our cell types of interest in the present study were epithelial cells. We thus used the identified compounds in this cell type to perform a series of assays to evaluate cell health and stress. COVID-19, however, affects other cell types. Application of our pipeline to other cell types could include various additional functional assays depending on the cell type of interest, for example, angiogenesis assays in endothelial cells, or measuring pro-inflammatory chemokine secretion in monocytes. Thus, our pipeline provides a novel screening method that is different from both HTS and conventional L1000-CMap approaches, contributing to the repurposing of FDA approved drugs to combat rapidly emerging diseases as well as other diseases like vascular calcification that conventional approaches have yet found the therapeutic options.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE92742; https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE70138.

AUTHOR CONTRIBUTIONS

TA and SC: conception and design, collection of data, data analysis and interpretation, manuscript writing, and final approval of the manuscript. JD: conception and design and final approval of the manuscript. MW: collection of data, data analysis and interpretation, and final approval of the manuscript. EA: financial support, administrative support, and final approval of the manuscript. MA: conception and design, financial support, administrative support, data interpretation, manuscript editing, and final approval of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

The authors declare that this study received funding from Kowa Company, Ltd, Nagoya, Japan (research grant A11014 to MA). The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

ACKNOWLEDGMENTS

The authors thank John Tigges and Brandy Pinckney from Flow Cytometry Core Facility of Beth Israel Deaconess Medical Center (MA, USA) for helping us with setting-up of the flow cytometry analysis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.842641/full#supplementary-material

REFERENCES

1. Brandão SCS, Ramos J, de OX, Dompieri LT, Godoi, E. T. A. M., et al. Is Toll-like receptor 4 involved in the severity of COVID-19 pathology in patients with cardiometabolic comorbidities? *Cytok Growth Factor Rev.* (2021) 58:102–10. doi: 10.1016/j.cytogfr.2020.09.002
2. Jha PK, Vijay A, Halu A, Uchida S, Aikawa M. Gene expression profiling reveals the shared and distinct transcriptional signatures in human lung epithelial cells infected with SARS-CoV-2, MERS-CoV, or SARS-CoV: potential implications in cardiovascular complications of COVID-19. *Front Cardiovasc Med.* (2021) 7:1–15. doi: 10.3389/fcvm.2020.623012
3. Lu H, Chelvanambi S, Poirier C, Saliba J, March KL, Clauss M, et al. EMAPII monoclonal antibody ameliorates influenza A virus-induced lung injury. *Mol Ther.* (2018) 26:2060–9. doi: 10.1016/j.ymthe.2018.05.017
4. Chelvanambi S, Gupta SK, Chen X, Ellis BW, Maier BF, Colbert TM, et al. HIV-Nef protein transfer to endothelial cells requires Rac1 activation and leads to endothelial dysfunction: implications for statin treatment in HIV patients. *Circ Res.* (2019) 125:805–20. doi: 10.1161/CIRCRESAHA.119.315082
5. Nishiga M, Wang DW, Han Y, Lewis DB, Wu JC. COVID-19 and cardiovascular disease: from basic mechanisms to clinical perspectives. *Nat Rev Cardiol.* (2020) 17:543–58. doi: 10.1038/s41569-020-0413-9
6. Rabi FA, Al Zoubi MS, Al-Nasser AD, Kasasbeh GA, Salameh DM. Sars-cov-2 and coronavirus disease 2019: what we know so far. *Pathogens.* (2020) 9:231. doi: 10.3390/pathogens9030231
7. Gil C, Ginex T, Maestro I, Nozal V, Barrado-Gil L, Cuesta-Geijo MÁ, et al. COVID-19: drug targets and potential treatments. *J Med Chem.* (2020) 63:12359–86. doi: 10.1021/acs.jmedchem.0c00606
8. Kifle ZD, Ayele AG, Enyew EF. Drug repurposing approach, potential drugs, and novel drug targets for COVID-19 treatment. *J Environ Public Health.* (2021) 2021:6631721. doi: 10.1155/2021/6631721
9. Swinney DC, Anthony J. How were new medicines discovered? *Nat Rev Drug Discov.* (2011) 10:507–19. doi: 10.1038/nrd3480
10. Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, Wrobel MJ, et al. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. *Science.* (2006) 313:1929–35. doi: 10.1126/science.1132939
11. Subramanian A, Narayan R, Corsello SM, Peck DD, Natoli TE, Lu X, et al. A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. *Cell.* (2017) 171:1437–52.e17. doi: 10.1016/j.cell.2017.10.049
12. Duan Q, Reid SP, Clark NR, Wang Z, Fernandez NF, Rouillard AD, et al. L1000CDS2: LINCS L1000 characteristic direction signatures search engine. *Npj Syst Biol Appl.* (2016) 2:1–12. doi: 10.1038/npjsba.2016.15
13. Musa A, Ghoraie LS, Zhang SD, Glazko G, Yli-Harja O, Dehmer M, et al. A review of connectivity map and computational approaches in pharmacogenomics. *Brief Bioinform.* (2018) 19:506–23. doi: 10.1093/bib/bbw112
14. Swinney DC. Phenotypic vs. Target-based drug discovery for first-in-class medicines. *Clin Pharmacol Ther.* (2013) 93:299–301. doi: 10.1038/clpt.2012.236
15. Corsello SM, Bittker JA, Liu Z, Gould J, McCarren P, Hirschman JE, et al. The drug repurposing hub: a next-generation drug library and information resource. *Nat Med.* (2017) 23:405–8. doi: 10.1038/nm.4306
16. Chelvanambi S, Bogatcheva NV, Bednorz M, Agarwal S, Maier B, Alves NJ, et al. HIV-Nef protein persists in the lungs of aviremic patients with HIV and induces endothelial cell death. *Am J Respir Cell Mol Biol.* (2019) 60:357–66. doi: 10.1165/rcmb.2018-0089OC
17. Clauss M, Chelvanambi S, Cook C, Elmergawy R, Dhillon N. Viral bad news sent by evail. *Viruses.* (2021) 13:1–13. doi: 10.3390/v13061168

Conflict of Interest: TA is an employee of Kowa Company, Ltd and was a visiting scientist at Brigham and Women's Hospital when experiments demonstrated in this study were performed.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 25 April 2022
doi: 10.3389/fcvm.2022.809997

Biomarkers of Cardiac Injury, Renal Injury, and Inflammation Are Strong Mediators of Sex-Associated Death in COVID-19

Heidi S. Lumish[1], Eunyoung Kim[1], Caitlin Selvaggi[2,3,4], Tingyi Cao[2,3,4], Aakriti Gupta[5], Andrea S. Foulkes[2,3,4†] and Muredach P. Reilly[1,6*†]*

[1] Division of Cardiology, Columbia University, New York, NY, United States, [2] Biostatistics Center, Massachusetts General Hospital, Boston, MA, United States, [3] Department of Medicine, Harvard Medical School, Boston, MA, United States, [4] Department of Biostatistics, Harvard T. H. Chan School of Public Health, Boston, MA, United States, [5] Division of Interventional Cardiology, Cedars-Sinai Medical Center, Los Angeles, CA, United States, [6] Irving Institute for Clinical and Translational Research, Columbia University, New York, NY, United States

OPEN ACCESS

Edited by:
Hester Den Ruijter, University Medical Center Utrecht, Netherlands

Reviewed by:
Stanislovas S. Jankauskas, Albert Einstein College of Medicine, United States
Ines Falcão-Pires, University of Porto, Portugal

***Correspondence:**
Heidi S. Lumish
hl2738@cumc.columbia.edu
Muredach P. Reilly
mpr2144@cumc.columbia.edu

† These authors have contributed equally to this work and share senior authorship

Specialty section:
This article was submitted to Sex and Gender in Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *05 November 2021*
Accepted: *29 March 2022*
Published: *25 April 2022*

Citation:
Lumish HS, Kim E, Selvaggi C, Cao T, Gupta A, Foulkes AS and Reilly MP (2022) Biomarkers of Cardiac Injury, Renal Injury, and Inflammation Are Strong Mediators of Sex-Associated Death in COVID-19. Front. Cardiovasc. Med. 9:809997. doi: 10.3389/fcvm.2022.809997

Background: Studies examining outcomes among individuals with COronaVIrus Disease 2019 (COVID-19) have consistently demonstrated that men have worse outcomes than women, with a higher incidence of myocardial injury, respiratory failure, and death. However, mechanisms of higher morbidity and mortality among men remain poorly understood. We aimed to identify mediators of the relationship between sex and COVID-19-associated mortality.

Methods: Patients hospitalized at two quaternary care facilities, New York Presbyterian Hospital (CUIMC/NYPH) and Massachusetts General Hospital (MGH), for SARS-CoV-2 infection between February and May 2020 were included. Five independent biomarkers were identified as mediators of sex effects, including high-sensitivity cardiac troponin T (hs-cTNT), high sensitivity C-reactive protein (hs-CRP), ferritin, D-dimer, and creatinine.

Results: In the CUIMC/NYPH cohort ($n = 2{,}626$, 43% female), male sex was associated with significantly greater mortality (26 vs. 21%, $p = 0.0146$) and higher peak hs-cTNT, hs-CRP, ferritin, D-dimer, and creatinine ($p < 0.001$). The effect of male sex on the primary outcome of death was partially mediated by peak values of all five biomarkers, suggesting that each pathophysiological pathway may contribute to increased risk of death in men. Hs-cTnT, creatinine, and hs-CRP were the strongest mediators. Findings were highly consistent in the MGH cohort with the exception of D-dimer.

Conclusions: This study suggests that the effect of sex on COVID-19 outcomes is mediated by cardiac and kidney injury, as well as underlying differences in inflammation and iron metabolism. Exploration of these specific pathways may facilitate sex-directed diagnostic and therapeutic strategies for patients with COVID-19 and provides a framework for the study of sex differences in other complex diseases.

Keywords: biomarkers, myocardial injury, SARS-CoV-2, sex differences, inflammation

INTRODUCTION

Across numerous studies of COronaVIrus Disease 2019 (COVID-19), men have had consistently worse rates of severe outcomes than women, with higher rates of cardiac injury, respiratory failure, shock, intensive care unit (ICU) admission, and death (1–3). This sex-related difference in outcomes has been confirmed in cohorts from China (4), Italy (5), and the United States (2, 6). There are multiple mechanisms hypothesized to contribute to this sex difference in COVID-19 outcomes – for example, sex-related factors that affect disease susceptibility, including differences in smoking and drinking habits, rates of handwashing, and social obligations (7). Men are also known to have higher rates of baseline co-morbidities, including hypertension and cardiovascular disease (8), and they may be more susceptible to the effects of age and co-morbidities than women (9). However, prior studies have demonstrated that the sex-related difference in outcomes is not entirely accounted for by a difference in baseline co-morbidities (10). Hormonal and genetic factors are also thought to play a role in disease pathogenesis. Sex hormones have been shown to modify inflammatory pathways and affect the regulation of angiotensin converting enzyme 2 (ACE2), which mediates Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) entry into cells (11–13). Genes conferring immunity are located on the X chromosome, some of which escape X-inactivation leading to a dose-related difference in the gene effect between men and women (11, 12). There are likely other important pathophysiological determinants of sex differences that are incompletely understood.

Downstream of social, clinical, hormonal and genetic processes, there are multiple biological pathways driving COVID-19 outcomes that may be mechanistically important and therapeutically tractable in the described sex differences. Several studies have demonstrated a sex difference in the relationship between various biomarkers and COVID-19 outcomes. Candidate biomarkers are implicated in immune response, inflammatory pathways, and coagulation pathways, as well as end organ dysfunction (9, 11, 14, 15). Specifically, men have been shown to have higher incidence of myocardial injury, as measured by troponin elevation (3). However, to date there has been no systematic examination of biomarkers of these pathophysiological processes and their relative effects as mediators of the sex difference in COVID-19 outcomes. Mediation analysis, our novel focus in this paper, aims to inform whether a biomarker (of a pathologic process) is in the causal pathway between an exposure (e.g., sex) and outcome (e.g., death) whereas the assessment of modification, a focus of prior studies (15–17), examines whether the exposure interacts with a biomarker in its association with the outcome.

In this study, we investigated potential pathophysiological biomarker mediators of the effect of sex on COVID-19 outcomes, to better understand the potential mechanisms and therapeutic implications for increased risk of poor outcomes in men. We evaluated 15 candidate blood biomarkers of biological pathways perturbed in COVID-19 as potential mediators of sex differences in COVID-19 outcomes, including markers of cardiac injury, inflammation, iron metabolism and coagulation, as well as renal and liver injury.

METHODS

Study Populations

The study population included a total of 4,017 patients hospitalized for SARS-CoV-2 infection between February and May 2020 at two independent quaternary care facilities, Columbia University Irving Medical Center/New York Presbyterian Hospital (CUIMC/NYPH) and Massachusetts General Hospital (MGH).

CUIMC/NYPH COVID-19 Cohort

The CUIMC/NYPH COVID-19 cohort includes 2,626 adult patients (≥18 years of age) who were hospitalized at CUIMC and the Allen Hospital sites of NYPH between February 1 and May 12, 2020, with positive SARS-CoV-2 reverse transcriptase-polymerase chain reaction testing of nasopharyngeal or oropharyngeal specimens (18). Patients who were admitted for <24 h were excluded from the analysis. Patients were followed until discharge, death, or the end of study follow-up on June 11, 2020. Patient data were identified in the electronic health record (EHR) by using the institution's clinical data warehouse, which included information on individuals who receive care at CUIMC/NYPH. Analysis was based on index hospitalization. Clinical comorbidities including hypertension, diabetes, coronary artery disease (CAD), heart failure, stroke or transient ischemic attack, atrial arrhythmias (atrial fibrillation, atrial flutter, and supraventricular tachycardia), chronic lung disease, chronic kidney disease, and chronic liver disease, were identified using ICD-10 medical billing codes (**Supplementary Table S1**). Cancer was defined by an automated search of the EHR for the terms "cancer," "carcinoma," "malignancy," "malignant," "neoplasm," "-noma," or "blastoma," excluding those with the terms "screen" or "hypertension." Obesity was defined as body mass index (BMI) ≥30 at the time of index hospitalization. The primary outcome was in-hospital mortality within 30 days of admission. Peak biomarker values were defined over the duration of hospitalization.

MGH COVID-19 Patient Registry

A replication analysis was performed using retrospective data on 1,391 individuals from the MGH COVID-19 Patient Registry (19, 20). All patients were hospitalized between March 11, 2020 and May 31, 2020 and tested positive for SARS-CoV2. Demographic information, comorbid conditions, medications, laboratory tests, and clinical outcomes at index hospitalization were manually extracted from electronic health records. The primary outcome was death within 28 days of presentation to care, defined as first contact with a health care provider due to COVID-19-related symptoms. Peak biomarker values were defined over the duration of hospitalization within 28 days of index date.

Statistical Methods

Baseline characteristics were summarized for the overall cohort and stratified by sex for both the CUIMC/NYP and MGH

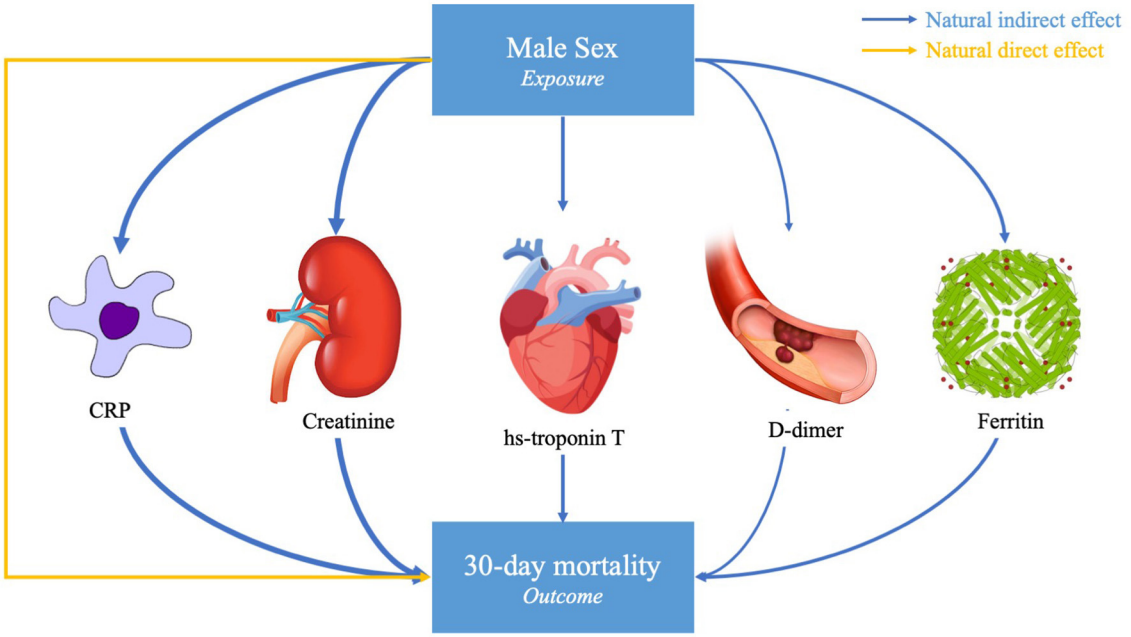


FIGURE 1 | Causal mediation model. This study assessed the degree to which peak serum biomarkers mediated the association between male sex and death due to SARS-CoV-2 infection in hospitalized patients. Of the fifteen biomarkers tested, five were significant mediators of the association between male sex and 30-day mortality, with varying proportion mediated as represented by the arrow thickness. Potential confounders accounted for in the primary analysis included age, sex, race/ethnicity, and number of biomarker measurements. The proportion mediated is given by the Natural Indirect Effect (NIE) divided by the Total Effect [NIE + Natural Direct Effect (NDE)].

registries. Unadjusted two-sided tests of proportions (or means) were used to compare baseline characteristics for male and female patients.

In the CUIMC/NYPH data, we evaluated 15 biomarkers of pathways perturbed in COVID-19 as potential mediators of sex differences in COVID-19 outcomes. Of these, two (IL-6, lactate) were excluded because of >50% missing data, and four (albumin, ALC, ESR, platelets) were excluded because their peak values were not associated with sex. Although associated with sex, WBC and automated lymphocytes were not reported as main findings because they were correlated and redundant with hs-CRP, which had stronger mediation effects (**Supplementary Table S2**). Hepatic injury markers (AST and ALT) were excluded because of race/ethnicity interactions in their association with death, limiting statistical power for race/ethnicity-specific mediation effects (**Supplementary Table S2**). Details on all additional biomarkers that were screened but not presented as primary findings are given in **Supplementary Table S2**. Thus, five biomarkers representing distinct biological pathways – hs-cTNT, hs-CRP, ferritin, creatinine, and D-dimer – were analyzed and presented here for their mediation of sex effects on death during index hospitalization. Each of these had peak values that were significantly associated with sex, had observed data in >50% of individuals, and represented distinct pathophysiological processes. Peak biomarker values were natural log transformed and standardized prior to inclusion in models.

We applied the causal mediation analysis approach described by Imai et al. (21) and as we applied in Foulkes et al. (22) which uses the results of three models to determine the proportion of the association between sex and severe outcomes that is mediated by the biomarker: (1) A Total Effect Model using a logit link with death (Y) as the outcome and sex (T) as a predictor variable, where the biomarker (M) is not included in the model; (2) A Mediator Model using an identify link with peak biomarker as the outcome and sex as a predictor; and (3) An Outcome Model using a logit link with death as the outcome and both sex and peak biomarker as predictor variables. All models were initially conditioned on age, sex, obesity, race/ethnicity and number of biomarker measurements. The reported odds ratios (ORs) are computed in the same way as using standard statistical model fitting procedures.

The average proportion mediated and corresponding *p*-value were reported for each biomarker. Models were fitted overall based on data from adult patients (≥18 years of age) using the peak biomarker value for each patient. Analysis used a complete case analysis for each biomarker separately assuming data were missing completely at random. Summary level data on characteristics of patients with and without missing data are provided (**Supplementary Table S3**). Primary analysis was based on data derived from the CUIMC/NYP cohort. Replication analysis was based on the MGH cohort. To explore mediation effects that might differ by menopausal status in women, we performed secondary analyses of age strata (≥ 50 vs. <50 years of age) designed as a surrogate for pre- and post-menopausal status in women. Additional sensitivity analyses in the CUIMC/NYP cohort were performed to check the

consistency of our conclusions when (1) adjusting for additional potential confounders (coronary artery disease, chronic kidney disease, lung disease, hypertension, type 2 diabetes mellitus, cancer, heart failure, and stroke) in the multivariable models, (2) restricting to individuals with complete data for all variables ($N =$ 1,688). Analyses were completed using R version 3.5.0. Mediation analysis was performed using the R package "mediation."

Human Subjects and IRB Approvals

The CUIMC IRB approved this study (#AAAS9835) and waived the requirement for obtaining informed consent. The Partners HealthCare Institutional Review Board (IRB) (#2020P000829) approved collection of curated data based on comprehensive manual chart reviews and data extractions from EHRs on patients who receive care through the Mass General Brigham (MGB, formerly Partners) system.

RESULTS

Baseline Characteristics of the CUIMC/NYP COVID-19 Cohort

Baseline clinical, demographic, and laboratory findings, overall and stratified by sex, are shown in **Table 1**. Median age was 66 (IQR 54, 77) years. Women accounted for 43% of the study cohort, and women were older (69 [57, 80] vs. 64 [53, 75], $p < 0.001$) and more obese (BMI 29.0 [24.9, 34.1] vs. 27.5 [24.4, 31.5], $p < 0.001$) than men. There were no significant differences in baseline statin and angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blocker (ARB) use between men and women. Men had a trend toward higher rates of baseline CAD, though this was not statistically significant, and women had significantly higher rates of hypertension (58 vs. 52%, $p =$ 0.0047) and lung disease (23 vs. 14%, $p < 0.001$). There were no sex differences in the baseline proportions of patients with diabetes mellitus, chronic kidney disease (CKD), cancer, heart failure or stroke (**Table 1**). On admission, compared to women, men had significantly higher hs-CRP, creatinine, hs-cTnT levels and especially ferritin. There was no sex difference in D-dimer levels at the time of admission (**Table 1**).

Unadjusted Analyses of Biomarkers and Outcome by Sex in the CUIMC/NYP COVID-19 Cohort

Peak values for all five biomarkers were significantly higher in men than in women, as shown in **Table 2**. The most notable sex difference was in median peak ferritin level, which was 1184.5 (IQR 621, 2,269) in men as compared to 615.5 (IQR 295, 1,307) in women. Mortality at 30 days was significantly higher in men than in women (26 vs. 21%, $p = 0.0146$, **Table 2**).

Mediation Effects in the CUIMC/NYP COVID-19 Cohort

In adjusted models, male sex was a significant predictor of death at 30 days (OR ∼2.0 depending on the specific sample of patients with available biomarker data, $p < 0.001$, **Table 3**). Male sex was also a significant predictor of the peak hs-CRP, ferritin, D-dimer, hs-cTnT, and creatinine ($p < 0.001$, **Table 3**). The effect of sex on the primary outcome of death was partially attenuated after adjustment for each of the five peak biomarker values, suggesting a potential mediation effect for each. The proportion mediated was significantly different than 0 for all of the biomarkers. The estimated proportion mediated was greatest for hs-cTnT (0.45, $p < 0.001$), hs-CRP (0.42, $p < 0.001$), and creatinine (0.35, $p < 0.001$) and lowest for D-dimer (0.22, $p < 0.001$, **Table 3**, **Figure 1**).

There were interaction effects with biomarkers in the outcome model for hs-CRP and ferritin with obesity and for D-dimer with age, and therefore stratified analyses are included in these cases. In the obesity (BMI >30) stratified analyses for hs-CRP, the estimated proportion mediated remained the same for obese and non-obese patients (0.42, $p < 0.001$ for each). While ferritin remained a significant mediator of the effect of sex on COVID-19 outcomes after stratifying by obesity, the estimated proportion mediated was larger among obese patients (0.51, $p = 0.010$) as compared to non-obese patients (0.24, $p < 0.001$). For D-dimer, the estimated proportion mediated was slightly lower in the older strata (0.18 for >65 years vs. 0.25 for age <65 years, **Table 3**). In secondary age-stratified analyses ($\geq$ or $<$ age 50), a surrogate for menopausal status in women, the mediation effects of each biomarker were largely consistent across younger vs. older age categories. However, hs-CRP had a greater proportion mediated for age <50 years (0.59) as compared to those with age $\geq$ 50 years (0.35), and ferritin had a trend toward a greater proportion mediated in those aged <50 (0.57 vs. 0.42, **Supplementary Table S4**).

Replication Analyses in the MGH COVID-19 Patient Registry

Replication analysis was performed in the MGH registry. The clinical characteristics, laboratory values, and outcomes overall and by sex in the MGH cohort are shown in **Supplementary Tables S5, S6**. Similar to the CUIMC/NYP cohort, women were more obese than men (BMI 29.8 [25.6, 35.0] vs. 28.6 [25.2, 32.9], $p = 0.016$) and had significantly more lung disease (33 vs. 27%, $p = 0.030$). While in the CUIMC/NYP cohort men had a trend toward higher rates of CAD, in the MGH cohort men had significantly more CAD and were also more likely to be smokers. In unadjusted analysis, peak values for all five biomarkers were significantly higher in men than in women, consistent with the CUIMC/NYP cohort. Also in unadjusted analysis and similar to the CUIMC/NYP cohort, men in the MGH cohort had significantly higher rates of the primary endpoint of death.

The results of the mediation analysis of the five candidate biomarkers in the MGH cohort are shown in **Table 4**. CRP, ferritin, creatinine, and hs-cTnT were all significant mediators of the effect of sex on COVID-19-related mortality. Stratified analyses by obesity for CRP and ferritin had similar patterns to the CUIMC/NYP cohort with no difference in proportion mediated between obese and non-obese for CRP but a greater proportion mediated for obese with ferritin (0.51 vs. 0.24). While

TABLE 1 | Clinical characteristics and admission labs overall and by sex in the CUIMC/NYP COVID-19 cohort.

	Overall (*N* = 2,626)	Male (*N* = 1,497)	Female (*N* = 1,129)	*P*-value*
Presentation to care				
Age in years (median [IQR])	66 (54, 77)	64 (53, 75)	69 (57, 80)	<0.001
Age ≥65 years	1,420/2,626 (0.54)	748/1,497 (0.50)	672/1,129 (0.60)	<0.001
White/non-Hispanic	237/2,626 (0.09)	139/1,497 (0.09)	98/1,129 (0.09)	0.6406
Black/non-Hispanic	320/2,626 (0.12)	180/1,497 (0.12)	140/1,129 (0.12)	0.8169
Hispanic	1,314/2,626 (0.50)	747/1,497 (0.50)	567/1,129 (0.50)	0.9015
Other	755/2,626 (0.29)	431/1,497 (0.29)	324/1,129 (0.29)	0.9932
Fever	604/2,624 (0.23)	371/1,497 (0.25)	233/1,127 (0.21)	0.0152
Body mass index (Median [IQR])	28.02 (24.60, 32.66)	27.46 (24.36, 31.46)	28.96 (24.89, 34.13)	<0.001
On statins	951/2,626 (0.36)	519/1,497 (0.35)	432/1,129 (0.38)	0.0634
On ACEi or ARBs[†]	442/2,626 (0.17)	238/1,497 (0.16)	204/1,129 (0.18)	0.1559
Co-morbidities				
Obesity[‡]	794/2,113 (0.38)	393/1,219 (0.32)	401/894 (0.45)	<0.001
Coronary artery disease	329/2,626 (0.13)	204/1,497 (0.14)	125/1,129 (0.11)	0.0576
Hypertension	1,430/2,626 (0.54)	779/1,497 (0.52)	651/1,129 (0.58)	0.0047
Diabetes mellitus type 2	968/2,626 (0.37)	553/1,497 (0.37)	415/1,129 (0.37)	0.9561
Chronic kidney disease	370/2,626 (0.14)	219/1,497 (0.15)	151/1,129 (0.13)	0.3908
Lung disease	463/2,626 (0.18)	207/1,497 (0.14)	256/1,129 (0.23)	<0.001
Cancer	261/2,626 (0.10)	155/1,497 (0.10)	106/1,129 (0.09)	0.4517
Heart failure	275/2,626 (0.10)	149/1,497 (0.10)	126/1,129 (0.11)	0.3494
Stroke	225/2,626 (0.09)	130/1,497 (0.09)	95/1,129 (0.08)	0.8620
Admission labs (median [IQR])[§]				
hs-CRP (mg/L; *n* = 2,414)	118.46 (56.79, 205.18)	130.48 (63.66, 215.36)	105.79 (45.40, 184.04)	<0.001
D-Dimer (ng/mL; *n* = 2,179)	1,510 (830, 3,290)	1,490 (800, 3,440)	1,520 (873, 3,170)	0.6767
Ferritin (ng/mL; *n* = 2,391)	702.6 (345.40, 1,293)	870.4 (457.80, 1584.50)	479.4 (238.80, 929.60)	<0.001
Creatinine (mg/dL; *n* = 2,609)	1.07 (0.81, 1.64)	1.17 (0.91, 1.75)	0.92 (0.70, 1.48)	<0.001
hs-cTnT (ng/L; *n* = 2,402)	17 (8, 42)	19 (9, 43)	16 (8, 39)	0.0028

**P-values correspond to a two-sample test of proportions (for categorical variables) or Wilcoxon rank sum tests (for numeric variables) comparing corresponding characteristics of male vs. female patients; [†] ACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; [‡] Obesity is defined as BMI ≥30 and is missing for 513 patients; $Admission labs - recorded within +/-3 days of hospital admission.*
IQR, interquartile range; hs-CRP, high sensitivity C-reactive protein; hs-cTNT, high sensitivity cardiac Troponin T.

TABLE 2 | Peak laboratory values and outcomes overall and by sex in the CUIMC/NYP COVID-19 cohort.

	Overall (*N* = 2,626)	Male (*N* = 1,497)	Female (*N* = 1,129)	*P*-value*
Peak labs (median [IQR])[†]				
hs-CRP (mg/L; *n* = 2,416)	167.12 (83.80, 281.45)	185.11 (101.11, 293.01)	143.99 (65.80, 261.38)	<0.001
D-Dimer (ng/ml; *n* = 2,180)	2,565 (1060, 9805)	2,790 (1060, 12260)	2,230 (1050, 7463)	0.0016
Ferritin (ng/ml; *n* = 2,393)	931.40 (437.90, 1,934)	1,184.5 (620.70, 2,269)	615.5 (295.40, 1,307)	<0.001
Creatinine (mg/dL; *n* = 2,609)	1.34 (0.92, 2.71)	1.49 (1.05, 3.07)	1.11 (0.80, 2.28)	<0.001
hs-cTnT (ng/L; *n* = 2,402)	26 (10, 79)	29 (11, 84)	24 (9, 67)	<0.001
Follow-up (30 day) outcomes				
Ventilator or death	908/2,626 (0.35)	555/1,497 (0.37)	353/1,129 (0.31)	0.0022
Ventilator[‡]	559/2,626 (0.21)	365/1,497 (0.24)	194/1,129 (0.17)	<0.001
Death	623/2,626 (0.24)	382/1,497 (0.26)	241/1,129 (0.21)	0.0146

**P-values correspond to a two-sample test of proportions (for categorical variables) or Wilcoxon rank sum tests (for numeric variables) comparing corresponding characteristics of male vs. female patients; [†] Peak labs – high sensitivity C-reactive protein (hs-CRP), high sensitivity cardiac Troponin T (hs-cTnT) [‡] 349 patients died without being on ventilator.*
IQR, interquartile range; hs-CRP, high sensitivity C-reactive protein; hs-cTNT, high sensitivity cardiac Troponin T.

TABLE 3 | Primary mediation analyses of peak values* of biomarkers in CUIMC/NYP COVID-19 cohort.

	Total effect model† outcome: death	Mediator model† outcome: biomarker	Outcome model† outcome: death		Proportion mediated
	OR (sex)	Estimate (sex)	OR (sex)	OR (biomarker)	
hs-CRP					
All ($n = 1,978$)	2.00 ($p < 0.001$)	0.285 ($p < 0.001$)	1.71 ($p < 0.001$)	-	0.42 ($p < 0.001$)
Obese ($n = 748$)	2.00 ($p = 0.001$)	0.204 ($p = 0.002$)	1.87 ($p = 0.005$)	8.24 ($p < 0.001$)	0.42 ($p < 0.001$)
Non-obese ($n = 1,230$)	2.00 ($p < 0.001$)	0.329 ($p < 0.001$)	1.62 ($p = 0.003$)	2.80 ($p < 0.001$)	0.42 ($p < 0.001$)
Ferritin					
All ($n = 1,070$)	2.33 ($p < 0.001$)	0.474 ($p < 0.001$)	2.04 ($p = 0.002$)	-‡	0.33 ($p < 0.001$)
Obese ($n = 483$)	1.96 ($p = 0.046$)	0.510 ($p < 0.001$)	1.57 ($p = 0.204$)	2.62 ($p < 0.001$)	0.51 ($p = 0.010$)
Non-obese ($n = 587$)	2.64 ($p = 0.001$)	0.456 ($p < 0.001$)	2.47 ($p = 0.003$)	1.92 ($p < 0.001$)	0.24 ($p < 0.001$)
D-dimer					
All ($n = 1,814$)	2.03 ($p < 0.001$)	0.207 ($p < 0.001$)	1.84 ($p < 0.001$)	-‡	0.22 ($p < 0.001$)
$\geq$ *65 yrs* ($n = 945$)	1.88 ($p < 0.001$)	0.165 ($p = 0.010$)	1.76 ($p < 0.001$)	2.13 ($p < 0.001$)	0.18 ($p = 0.008$)
$<$ *65 yrs* ($n = 869$)	2.44 ($p = 0.001$)	0.205 ($p = 0.005$)	2.17 ($p = 0.007$)	3.35 ($p < 0.001$)	0.25 ($p = 0.006$)
Creatinine					
All ($n = 2,106$)	1.98 ($p < 0.001$)	0.430 ($p < 0.001$)	1.52 (p=0.001)	2.24 ($p < 0.001$)	0.45 ($p < 0.001$)
hs-Troponin T					
All ($n = 1,954$)	2.05 ($p < 0.001$)	0.301 ($p < 0.001$)	1.70 ($p < 0.001$)	2.55 ($p < 0.001$)	0.35 ($p < 0.001$)

**Peak biomarker level was determined based on all measurements. All values were natural log transformed and standardized for analysis; † All models included terms for sex and were adjusted for age, obesity, race/ethnicity, and the number of biomarker measurements. The outcome model included both sex and the biomarker as predictor variables; ‡ The outcome model included a biomarker by obesity/age interaction and therefore the main effect of the biomarker was not reported here.*
OR, odds ratio; hs-CRP, high sensitivity C-reactive protein; hs-Troponin T, high sensitivity cardiac Troponin T.

D-dimer was a significant mediator in the CUIMC/NYP cohort, it was not a significant mediator in the MGH registry data.

Sensitivity Analyses

Additional analyses in the CUIMC/NYP COVID-19 Cohort tested the robustness of findings. In mediation analyses that adjusted for additional covariates and potential confounders (CAD, CKD, lung disease, hypertension, type 2 diabetes mellitus, cancer, heart failure, and stroke), the findings for each biomarker were highly consistent with the primary findings **(Supplementary Table S7)**. Similarly, in models that restricted data to patients that had complete data for all biomarkers and covariates ($N = 1,688$), mediation findings were also consistent with the main analyses **(Supplementary Table S8)**.

DISCUSSION

In this study of 4,017 patients with COVID-19 at two tertiary care centers, we confirm that compared to female sex, male sex was associated with higher mortality at 30 days. Further, we report the novel finding that specific biomarkers of pathophysiological processes mediate the effect of sex on COVID-19 outcomes. These include hs-cTnT, hs-CRP, D-dimer, ferritin and creatinine – with proportion mediated estimated to be greatest for cardiac injury (hs-cTnT), intermediate for inflammation (hs-CRP) and kidney function (creatinine), and least for thrombosis (D-dimer). Additional evaluated biomarkers were excluded because their peak values were not associated with sex (albumin, ALC, ESR, platelets), they were correlated and redundant with hs-CRP (WBC and automated lymphocytes) or because of excess missing data (IL-6 and lactate). Our findings suggest that biological pathways of inflammation, iron metabolism, and coagulation, as well as cardiac and kidney injury that may be downstream of these pathways, are implicated in the strong sex-related difference in COVID-19 outcomes.

Several prior small studies have looked at patterns of biomarker elevation by sex in association with COVID-19 outcomes, though none have performed rigorous analyses of these biomarkers as mediators. A study of 776 patients hospitalized in New Orleans with COVID-19 found that troponin and D-dimer were predictors of worse outcomes in men, while ferritin was associated with death only in women (16). In a retrospective review of 168 patients hospitalized with COVID-19 in Wuhan, China, there were five biomarkers identified that were higher among men who died than among women who died (NLR, CRP, AST, LDH, and creatinine) (9). Importantly, ours is the first study to report which biomarkers and pathways are potential causal mediators of sex effects. Our findings are robust even after adjusting for multiple baseline comorbidities, and our work is rigorous in providing replication of key findings at two independent major academic medical centers with large numbers of complex and severe COVID-19 cases.

TABLE 4 | Replication mediation analyses of peak values* of biomarkers in MGH cohort.

	Total effect model† outcome: death	Mediator model† outcome: biomarker	Outcome model† outcome: death		Proportion mediated
	OR (sex)	Estimate (sex)	OR (sex)	OR (biomarker)	
hs-CRP					
All ($n = 1,088$)	2.24 ($p < 0.001$)	0.102 ($p = 0.058$)	2.09 ($p = 0.002$)	-‡	0.20 ($p = 0.048$)
Obese ($n = 491$)	1.92 ($p = 0.051$)	0.080 ($p = 0.277$)	1.78 ($p = 0.123$)	9.07 (p<0.001)	0.22 ($p = 0.284$)
Non-obese ($n = 597$)	2.44 ($p = 0.002$)	0.144 ($p = 0.065$)	2.18 ($p = 0.012$)	4.64 ($p < 0.001$)	0.22 ($p = 0.062$)
Ferritin					
All ($n = 1,070$)	2.33 ($p < 0.001$)	0.474 (p<0.001)	2.04 ($p = 0.002$)	-‡	0.33 ($p < 0.001$)
Obese ($n = 483$)	1.96 ($p = 0.046$)	0.510 ($p < 0.001$)	1.57 ($p = 0.204$)	2.62 (p<0.001)	0.51 ($p = 0.010$)
Non-obese ($n = 587$)	2.64 ($p = 0.001$)	0.456 (p<0.001)	2.47 ($p = 0.003$)	1.92 ($p < 0.001$)	0.24 ($p < 0.001$)
D-dimer					
All ($n = 1,050$)	2.17 ($p < 0.001$)	0.022 ($p = 0.676$)	2.27 (p<0.001)	-‡	0.02 ($p = 0.700$)
≥ *65 yrs* ($n = 421$)	2.00 ($p = 0.005$)	0.025 ($p = 0.762$)	2.13 ($p = 0.003$)	1.99 ($p < 0.001$)	0.02 ($p = 0.732$)
< *65 yrs* ($n = 629$)	3.21 ($p = 0.041$)	0.025 ($p = 0.718$)	3.29 ($p = 0.044$)	2.97 ($p < 0.001$)	0.02 (p=0.710)
Creatinine					
All ($n = 1,084$)	2.25 ($p < 0.001$)	0.568 ($p < 0.001$)	1.47 ($p = 0.104$)	2.44 ($p < 0.001$)	0.57 (p<0.001)
hs-Troponin T					
All ($n = 1,026$)	2.25 ($p < 0.001$)	0.194 ($p < 0.001$)	1.99 ($p = 0.003$)	2.26 ($p < 0.001$)	0.19 ($p = 0.002$)

**Peak biomarker level was determined based on all measurements. All values were natural log transformed and standardized for analysis; † All models included terms for sex and were adjusted for age, obesity, race/ethnicity, and the number of biomarker measurements. The outcome model included both sex and the biomarker as predictor variables; ‡ The outcome model included a biomarker by obesity interaction and therefore the main effect of the biomarker was not reported here.*
OR, odds ratio; hs-CRP, high sensitivity C-reactive protein; hs-Troponin T, high sensitivity cardiac Troponin T.

Sex differences in inflammatory responses, as reflected by peak hs-CRP and ferritin, may amplify sex differences in cardiac and renal injury and thus contribute to our finding that biomarkers of cardiac and kidney end-organ damage mediate a substantial proportion of the effect of sex on COVID-19 outcomes. Although previous studies have identified sex differences in the rate of COVID-19-related myocardial injury (3) and an association between acute cardiac injury and death in COVID-19 (23) ours is the first to address cardiac injury as a mediator of sex effects on COVID-19 outcomes. Similarly, prior studies have demonstrated a sex difference in COVID-19-related kidney disease and acute kidney injury (24), yet biomarkers of renal function have never been tested as mediators. Further studies are required to determine the extent to which the effects of inflammation and cardiac and renal damage are independent contributors to sex-mediation of poor COVID-19 outcomes.

Of several markers (including WBC and ESR, see **Supplementary Table S2**), we focused on hs-CRP as representative of systemic inflammation and broad activation of innate and adaptive immunity in COVID-19. Prior studies, including our own (22), have demonstrated an association between elevated inflammatory markers and death or ICU admission, with a stronger association in men as compared to women (9, 15, 25). However, our study is the first to suggest that inflammation directly mediates the effect of sex on COVID-19-related mortality. This mediation could be due to established sex differences in both the innate and adaptive immune pathways (26). Many of the genes involved in innate and adaptive immunity are located on the X chromosome. Some of them may escape X inactivation, leading to a more comprehensive immune response in women as compared to men (26, 27). Men and women also differ in the production of cytokines and chemokines by innate immune cells. During inflammatory stress, men have higher levels of pro-inflammatory cytokine production than females (26, 28), which could lead to a more severe cytokine storm associated with worse COVID-19 outcomes. Apart from genetics, there may be hormonal factors contributing to sex differences in the immune response to infection (29, 30). Testosterone is known to have an immunosuppressive effect (11, 26). At the same time, estradiol is thought to enhance cell-mediated and humoral immune responses, and progesterone has anti-inflammatory effects and may also contribute to differences in T cell populations (26). In secondary analyses, we found a trend toward a greater mediation effect of hs-CRP in patients age <50 years, which might support a role for the greater hormonal differences at pre-menopausal age in the contribution to sex differences in the inflammatory milieu and COVID-19.

Ferritin was a less potent mediator than hs-CRP of the effect of sex on COVID-19 outcomes, though the mediation effect was statistically significant and may be clinically important. In studies of patients with COVID-19, ferritin has been shown to correlate with disease severity in both men and women (31–33). In one study and in contrast to our findings, ferritin levels were found to be independently associated with death in women but not in men (16). Our larger CUIMC/NYPH data is the first to suggest that ferritin mediates the effect of male sex on worse outcomes in COVID-19 and this finding was highly consistent in the independent MGH registry. There are multiple

possible explanations for the role of ferritin in mediating sex-related outcomes. Ferritin synthesis may increase as a result of the COVID-19 cytokine storm or inflammation may stimulate leakage of intracellular ferritin (33). This would suggest that peak ferritin levels, similar to hs-CRP, reflect systemic inflammation in mediating the effect of sex on outcomes. Alternatively, ferritin may play an independent causal role in the inflammatory cascade, acting as a mediator of immune dysregulation (33). Given that women of all ages have lower ferritin levels than men (34), lower baseline levels of ferritin could in fact be protective in women. Interestingly, when stratified by obesity, more of the effect of male sex on the primary outcome of death was explained by peak ferritin among obese patients as compared to non-obese patients. This finding may relate to differences in significance of ferritin levels in non-obese vs. obese patients. While in lean individuals ferritin may predominantly be a marker of iron stores, in obesity ferritin may correlate more with inflammation (35).

In the primary CUIMC/NYP cohort, we found that peak D-dimer levels were also mediators of the effect of sex on COVID-19 outcomes. In contrast, D-dimer levels were not found to be a mediator of the association between sex and COVID-19 outcomes in the MGH cohort. One possible explanation is that this might reflect differences in anticoagulation practice patterns for patients with COVID-19 across institutions, particularly early in the pandemic (36). Published work does suggest that microvascular dysfunction and thrombosis play a role in the pathogenesis of COVID-19, and studies have demonstrated an increased risk of severe outcomes and death in patients with elevated D-dimer (37). While there have been insufficient data to demonstrate a sex difference in the association between D-dimer and outcomes, sex differences in endothelial dysfunction have been well-established (38). Increased coagulation disorders may also cause more myocardial injury in men than in women, leading to worse outcomes (3). Prior studies have suggested that menopause is a risk factor for worse outcomes in women with COVID-19 (13, 39). In our age-stratified secondary analyses ($\geq$ 50 vs. <50 years of age) designed as a surrogate for pre- and post-menopausal status in women, the proportion mediated by D-dimer was similar across younger vs. older age strata suggesting that thromboembolic mechanisms that drive higher risk in men are unlikely to reflect hormonal differences found in pre- or post-menopausal women. Future studies will need to focus more specifically on sex-related COVID-19 risks, including thromboembolic, in pre- and post-menopausal women.

Methodological strengths of this work include the novel application of mediation analysis to sex-related COVID-19 outcomes as well as a robust replication sample in which findings were highly consistent. Additional strengths include the sample size, the inclusion of a large percentage of under-represented minorities and the evaluation of 15 distinct biomarkers as potential mediators of sex effects on COVID-19 outcomes. There are several limitations of our study. First, the study used observational data extracted from the EHR in which missing data and measurement error are inherent and can result in biased findings (40). Second, the causal mediation framework assumes no unmeasured confounding (21). Third, despite robust replication across CUIMC/NYP and MGH cohorts, these two studies have differences in design and regional clinical contexts. Fourth, biomarkers are inherently limited as a markers of causal mechanisms and in therapeutic targeting, as they are surrogates for the underlying causal pathway. Indeed, serum creatinine has significant limitations as a measure of kidney function or as a surrogate for kidney injury, but data were missing for calculation of more reliable measures of acute kidney injury (e.g., using KDIGO recommendations) (41). Further, all validated equations for eGFR incorporate sex rendering invalid analyses of sex mediation through such a derived biomarker. However, ease in clinical use of these biomarkers means our findings are immediately translatable to clinical practice. There are also redundancies between clinically available biomarkers, e.g., we selected hsCRP over WBC and ESR because hsCRP had stronger sex-mediation effects than the other inflammatory markers (**Supplementary Table S2**). Moreover, given the complex relationships among sex, mediators and other factors in severe COVID-19 outcomes, future work on additional pathophysiological pathways is needed. Our analyses were limited to the 15 clinically available candidate blood biomarkers selected for our study, and therefore we cannot exclude other biomarkers and organs as mediators. Specifically, our initial analyses suggest that larger sample sizes than ours are required to study the effect of biomarkers of hepatic injury within racial and ethnic strata. Finally, future studies are needed to define optimal clinical translation including use of mediators in clinical trials stratifying for high-risk patients.

In summary, we identified several distinct biomarkers of pathophysiological processes, including cardiac injury, that are reproducible mediators of the effect of sex on COVID-19 outcomes. Each of these pathways is a downstream manifestation of genetic, hormonal, and socio-demographic differences between men and women. And each offers a unique opportunity for better risk stratification, resource utilization, and targeted clinical trials toward personalized interventions and therapies for subgroups of patients at highest risk for poor COVID-19 outcomes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the CUIMC Institutional Review Board (#AAAS9835) and the Partners HealthCare Institutional Review Board (#2020P000829). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HL: literature search and writing—original draft preparation. EK and CS: database organization and statistical analysis.

HL, AF, and MR: conceptualization and writing—draft revision. All authors contributed to manuscript revision, provided comments, and agreed to the published version of the manuscript.

FUNDING

Support for the design, conduct, and reporting of this research was provided by NIH R01 GM127862 to AF and R01 HL132561 and R01 HL113147 to MR.

ACKNOWLEDGMENTS

Support for the creation of the MGH Patient Registry was provided by the MGH Division of Clinical Research and the Department of Medicine.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.809997/full#supplementary-material

REFERENCES

1. Galbadage T, Peterson BM, Awada J, Buck AS, Ramirez DA, Wilson J, et al. Systematic review and meta-analysis of sex-specific COVID-19 clinical outcomes. *Front Med.* (2020) 7:348. doi: 10.3389/fmed.2020.00348
2. Palaiodimos L, Kokkinidis DG Li W, Karamanis D, Ognibene J, Arora S, et al. Severe obesity, increasing age and male sex are independently associated with worse in-hospital outcomes, and higher in-hospital mortality, in a cohort of patients with COVID-19 in the Bronx, New York. *Metabolism.* (2020) 108:154262. doi: 10.1016/j.metabol.2020.154262
3. Cheng R, Liu C, Yang J, Yang Y, Chen R, Ding X, et al. Sex Differences in the incidence and risk factors of myocardial injury in covid-19 patients: a retrospective cohort study. *Front Physiol.* (2021) 12:632123. doi: 10.3389/fphys.2021.632123
4. Guan WJ Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med.* (2020) 382:1708–20. doi: 10.1056/NEJMoa2002032
5. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. Baseline characteristics and outcomes of 1,591 patients infected with sars-Cov-2 admitted to icus of the lombardy region, Italy. *JAMA.* (2020) 323:1574–81. doi: 10.1001/jama.2020.5394
6. Rapp JL, Lieberman-Cribbin W, Tuminello S, Taioli E. Male sex, severe obesity, older age, and chronic kidney disease are associated with COVID-19 severity and mortality in New York City. *Chest.* (2021) 159:112–5. doi: 10.1016/j.chest.2020.08.2065
7. Gebhard C, Regitz-Zagrosek V, Neuhauser HK, Morgan R, Klein SL. Impact of sex and gender on COVID-19 outcomes in Europe. *Biol Sex Differ.* (2020) 11:29. doi: 10.1186/s13293-020-00304-9
8. Medzikovic L, Cunningham CM Li M, Amjedi M, Hong J, Ruffenach G, et al. Sex differences underlying preexisting cardiovascular disease and cardiovascular injury in COVID-19. *J Mol Cell Cardiol.* (2020) 148:25–33. doi: 10.1016/j.yjmcc.2020.08.007
9. Meng Y, Wu P, Lu W, Liu K, Ma K, Huang L, et al. Sex-specific clinical characteristics and prognosis of coronavirus disease-19 infection in Wuhan, China: a retrospective study of 168 severe patients. *PLoS Pathog.* (2020) 16:e1008520. doi: 10.1371/journal.ppat.1008520
10. Alkhouli M, Nanjundappa A, Annie F, Bates MC, Bhatt DL. Sex differences in case fatality rate of COVID-19: insights from a multinational registry. *Mayo Clin Proc.* (2020) 95:1613–20. doi: 10.1016/j.mayocp.2020.05.014
11. Haitao T, Vermunt JV, Abeykoon J, Ghamrawi R, Gunaratne M, Jayachandran M, et al. COVID-19 and sex differences: mechanisms and biomarkers. *Mayo Clin Proc.* (2020) 95:2189–203. doi: 10.1016/j.mayocp.2020.07.024
12. Viveiros A, Rasmuson J, Vu J, Mulvagh SL, Yip CYY, Norris CM, et al. Sex differences in COVID-19: candidate pathways, genetics of ACE2, and sex hormones. *Am J Physiol Heart Circ Physiol.* (2021) 320:H296–304. doi: 10.1152/ajpheart.00755.2020
13. Ding T, Zhang J, Wang T, Cui P, Chen Z, Jiang J, et al. Potential influence of menstrual status and sex hormones on female severe acute respiratory syndrome coronavirus 2 infection: a cross-sectional multicenter study in Wuhan, China. *Clin Infect Dis.* (2021) 72:e240–e8. doi: 10.1093/cid/ciaa1022
14. Ten-Caten F, Gonzalez-Dias P, Castro I, Ogava RLT, Giddaluru J, Silva JCS, et al. In-depth analysis of laboratory parameters reveals the interplay between sex, age, and systemic inflammation in individuals with COVID-19. *Int J Infect Dis.* (2021) 105:579–87. doi: 10.1016/j.ijid.2021.03.016
15. Lau ES, McNeill JN, Paniagua SM, Liu EE, Wang JK, Bassett IV, et al. Sex differences in inflammatory markers in patients hospitalized with COVID-19 infection: insights from the MGH COVID-19 patient registry. *PLoS ONE.* (2021) 16:e0250774. doi: 10.1371/journal.pone.0250774
16. Yoshida Y, Gillet SA, Brown MI, Zu Y, Wilson SM, Ahmed SJ, et al. Clinical characteristics and outcomes in women and men hospitalized for coronavirus disease 2019 in New Orleans. *Biol Sex Differ.* (2021) 12:20. doi: 10.1186/s13293-021-00359-2
17. Marik PE, DePerrior SE, Ahmad Q, Dodani S. Gender-based disparities in COVID-19 patient outcomes. *J Investig Med.* (2021). doi: 10.1136/jim-2020-001641
18. Gupta A, Madhavan MV, Poterucha TJ, DeFilippis EM, Hennessey JA, Redfors B, et al. Association between antecedent statin use and decreased mortality in hospitalized patients with COVID-19. *Nat Commun.* (2021) 12:1325. doi: 10.1038/s41467-021-21553-1
19. Bassett IV, Triant VA, Bunda BA, Selvaggi CA, Shinnick DJ, He W, et al. Massachusetts general hospital Covid-19 registry reveals two distinct populations of hospitalized patients by race and ethnicity. *PLoS ONE.* (2020) 15:e0244270. doi: 10.1371/journal.pone.0244270
20. Seiglie J, Platt J, Cromer SJ, Bunda B, Foulkes AS, Bassett IV, et al. Diabetes as a risk factor for poor early outcomes in patients hospitalized with COVID-19. *Diabetes Care.* (2020) 43:2938–44. doi: 10.2337/dc20-1506
21. Imai K, Keele L, Yamamoto T. Identification, inference and sensitivity analysis for causal mediation effects. Statistical Science (2010) 25:51–71. doi: 10.1214/10-STS321
22. Foulkes AS, Selvaggi C, Shinnick D, Lumish H, Kim E, Cao T, et al. Understanding the link between obesity and severe COVID-19 outcomes: causal mediation by systemic inflammatory response. *J Clin Endocrinol Metab.* (2021) 107:e698-e707. doi: 10.1210/clinem/dgab629
23. Li JW, Han TW, Woodward M, Anderson CS, Zhou H, Chen YD, et al. The impact of 2019 novel coronavirus on heart injury: a Systematic review and meta-analysis. *Prog Cardiovasc Dis.* (2020) 63:518–24. doi: 10.1016/j.pcad.2020.04.008
24. Cheng Y, Luo R, Wang K, Zhang M, Wang Z, Dong L, et al. Kidney disease is associated with in-hospital death of patients with COVID-19. *Kidney Int.* (2020) 97:829–38. doi: 10.1016/j.kint.2020.03.005
25. Qin L, Li X, Shi J, Yu M, Wang K, Tao Y, et al. Gendered effects on inflammation reaction and outcome of COVID-19 patients in Wuhan. *J Med Virol.* (2020) 92:2684–92. doi: 10.1002/jmv.26137
26. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol.* (2016) 16:626–38. doi: 10.1038/nri.2016.90
27. Libert C, Dejager L, Pinheiro I. The X chromosome in immune functions: when a chromosome makes the difference. *Nat Rev Immunol.* (2010) 10:594–604. doi: 10.1038/nri2815
28. Ferguson JF, Patel PN, Shah RY, Mulvey CK, Gadi R, Nijjar PS, et al. Race and gender variation in response to evoked inflammation. *J Transl Med.* (2013) 11:63. doi: 10.1186/1479-5876-11-63
29. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev.* (2007) 28:521–74. doi: 10.1210/er.2007-0001

30. Taneja V. Sex hormones determine immune response. *Front Immunol.* (2018) 9:1931. doi: 10.3389/fimmu.2018.01931
31. Gandini O, Criniti A, Ballesio L, Giglio S, Galardo G, Gianni W, et al. Serum ferritin is an independent risk factor for acute respiratory distress syndrome in COVID-19. *J Infect.* (2020) 81:979–97. doi: 10.1016/j.jinf.2020.09.006
32. Gandini O, Criniti A, Gagliardi MC, Ballesio L, Giglio S, Balena A, et al. Sex-disaggregated data confirm serum ferritin as an independent predictor of disease severity both in male and female COVID-19 patients. *J Infect.* (2021) 82:414–51. doi: 10.1016/j.jinf.2020.10.012
33. Lin Z, Long F, Yang Y, Chen X, Xu L, Yang M. Serum ferritin as an independent risk factor for severity in COVID-19 patients. *J Infect.* (2020) 81:647–79. doi: 10.1016/j.jinf.2020.06.053
34. Ellidag HY, Eren E, Akdag M, Giray O, Kiraz K, Yilmaz N. The relationship between serum ferritin levels and serum lipids and HDL function with respect to age and gender. *Ukr Biochem J.* (2016) 88:76–86. doi: 10.15407/ubj88.06.076
35. Khan A, Khan WM, Ayub M, Humayun M, Haroon M. Ferritin is a marker of inflammation rather than iron deficiency in overweight and obese people. *J Obes.* (2016) 2016:1937320. doi: 10.1155/2016/1937320
36. Rosovsky RP, Sanfilippo KM, Wang TF, Rajan SK, Shah S, Martin KA, et al. Anticoagulation practice patterns in COVID-19: a global survey. *Res Pract Thromb Haemost*. (2020). doi: 10.1002/rth2.12414
37. Shah S, Shah K, Patel SB, Patel FS, Osman M, Velagapudi P, et al. Elevated D-dimer levels are associated with increased risk of mortality in coronavirus disease 2019: a systematic review and meta-analysis. *Cardiol Rev.* (2020) 28:295–302. doi: 10.1097/CRD.0000000000000330
38. Stanhewicz AE, Wenner MM, Stachenfeld NS. Sex differences in endothelial function important to vascular health and overall cardiovascular disease risk across the lifespan. *Am J Physiol Heart Circ Physiol.* (2018) 315:H1569–H88. doi: 10.1152/ajpheart.00396.2018
39. Wang XW, Hu H, Xu ZY, Zhang GK Yu QH, Yang HL, et al. Association of menopausal status with COVID-19 outcomes: a propensity score matching analysis. *Biol Sex Differ.* (2021) 12:16. doi: 10.1186/s13293-021-00363-6
40. Weber GM, Adams WG, Bernstam EV, Bickel JP, Fox KP, Marsolo K, et al. Biases introduced by filtering electronic health records for patients with "complete data". *J Am Med Inform Assoc.* (2017) 24:1134–41. doi: 10.1093/jamia/ocx071
41. KDIGO. Clinical practice guideline for acute kidney injury. *Kidney Internat Suppl.* (2012) 2:8–12. doi: 10.1038/kisup.2012.1

Conflict of Interest: AF receives sponsored research support from Bristol Myers Squibb/Pfizer and Fitbit and has received expert witness testimonial fees from Round Table Group.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 26 April 2022
doi: 10.3389/fcvm.2022.845439

Smoking in Patients With Chronic Cardiovascular Disease During COVID-19 Lockdown

Frédéric Chagué[1,2], Mathieu Boulin[3], Jean-Christophe Eicher[1], Florence Bichat[1], Maïlis Saint-Jalmes[1], Amélie Cransac[3], Agnès Soudry[4], Nicolas Danchin[5], Gabriel Laurent[1], Yves Cottin[1] and Marianne Zeller[2,6]*

[1] Service de Cardiologie, Centre Hospitalier Universitaire, Dijon, France, [2] Réseau Français d'Excellence de Recherche sur le tabac, la nicotine et les produits connexes, Paris, France, [3] Département de Pharmacie, Centre Hospitalier Universitaire, Dijon, France, [4] Département de Recherche Clinique, Centre Hospitalier Universitaire, Dijon, France, [5] Service de Cardiologie, Hôpital Européen Georges Pompidou, Paris, France, [6] PEC2, EA 7460, Université Bourgogne Franche-Comté, Dijon, France

Objectives: This cross-sectional study aims to investigate health-related behaviors including tobacco consumption among patients with cardiovascular diseases (CVD), during the first COVID-19-related lockdown.

Methods: After 5 weeks of COVID-19 lockdown, 220 patients with chronic coronary syndromes (CCS) and 124 with congestive heart failure (CHF) answered a phone questionnaire.

Results: Among these 344 patients, 43 (12.5%) were current smokers, and none had quit during the lockdown. When compared with non-smokers, smokers were 15 years younger, more often diabetic, more likely to live in an urban than a rural lockdown location, and more often in the CCS cohort ($p = 0.011$). Smokers described greater psychological impairment, but their rates of decrease in physical activity and of increase in screen time were similar to non-smokers. More than one-third (13/43) increased their tobacco consumption, which was mainly related to stress or boredom, but not driven by media messages on a protective effect of nicotine.

Conclusions: During the first COVID-19 lockdown, we found a decrease in favorable lifestyle behaviors among patients with CVD. Strikingly, one-third of smokers with CCS or CHF increased their tobacco consumption. Given the major impact of persistent smoking in patients with CVD, this highlights the need for targeted prevention strategies, in particular during such periods.

Keywords: smoking, COVID-19, lockdown, chronic coronary syndrome, congestive heart failure (CHF)

OPEN ACCESS

Edited by:
Man Kit Lei,
University of Georgia, United States

Reviewed by:
Oxana Rotar,
Almazov National Medical Research Centre, Russia
Angelo Di Vincenzo,
University of Padua, Italy

****Correspondence:***
Frédéric Chagué
frederic.chague@gmail.com

Specialty section:
This article was submitted to Cardiovascular Epidemiology and Prevention, a section of the journal Frontiers in Cardiovascular Medicine

Received: *29 December 2021*
Accepted: *14 March 2022*
Published: *26 April 2022*

Citation:
Chagué F, Boulin M, Eicher J-C, Bichat F, Saint-Jalmes M, Cransac A, Soudry A, Danchin N, Laurent G, Cottin Y and Zeller M (2022) Smoking in Patients With Chronic Cardiovascular Disease During COVID-19 Lockdown. Front. Cardiovasc. Med. 9:845439. doi: 10.3389/fcvm.2022.845439

INTRODUCTION

Cardiovascular disease (CVD), including congestive heart failure (CHF) and chronic coronary syndrome (CCS), and smoking are among the factors that can dramatically worsen prognosis in patients hospitalized for COVID-19 (1). Tobacco smoking is a major reversible risk factor for CVD, and cessation is a major target for prevention. Unfortunately, patients often do not quit smoking after an acute CVD event (2, 3). Although considered as less harmful than smoking, the cardiovascular impact of vaping is still debated (1).

Since the start of the current pandemic, the fear of severe acute respiratory syndrome coronavirus-2 (SARS-CoV2) infection and the strict lockdowns may have generated anxiety and stress, delayed access to care, and favored unhealthy behaviors, such as smoking increase, start or relapse. All of these factors can worsen a CVD patient's long-term prognosis (1, 3–5). On the other hand, the pandemic-related lockdowns were particular situations that may also have potentially favored smoking cessation through fear of illness, the lifting of social barriers, and enabling patients to focus on the health benefits of a healthier lifestyle (6, 7). At the same time, some media outlets spread the unconfirmed information that nicotine could confer a protective effect against COVID-19, thus potentially encouraging patients to smoke (6, 8). While the subject of smoking during the COVID-19 lockdowns has been addressed, investigations in CVD patients are paradoxically very scarce. We hypothesized that smoking rate and related health behaviors could have been modified in patients with CVD during the 2020 lockdown.

METHODS

CLEO-CD (COVID-19 Lockdown Effect On Chronic Diseases) is a cross-sectional study including more than 1200 outpatients with chronic disease from our university hospital in Dijon, France. Among them, 250 CCS subjects were randomly selected from the RICO (observatoire des Infarctus du myocarde de Côte d'Or) survey, which prospectively includes all patients hospitalized for acute myocardial infarction (AMI) in the coronary care unit of our hospital, as previously described (9). Only patients hospitalized for AMI in 2018 and 2019 were selected for inclusion. In addition, 150 CHF outpatients were randomly selected from the Heart Failure Clinic (10). This questionnaire was previously tested on 10 subjects (members of our research unit) as an internal procedure in order to assess compliance (understanding, coherence, reliability), leading to changes in the questions regarding medications and tobacco consumption. Then the questionnaire was tested by phone on eight CCS outpatients and eight CHF outpatients, all non-included in the randomly-selected patients and no changes were found to be necessary. A translated version of the questionnaire addressing tobacco consumption is available in **Supplementary File 1 - Questionnaire**. A smoker and a vaper were defined as a current tobacco smoker or electronic cigarette user (daily or occasional) at the time of the interview, and an ex-smoker and ex-vaper as having quit any time before the interview. Psychological distress was assessed by the Kessler 6 (K6) score (11). Residence during the lockdown was defined as rural when patients were living in areas with <2,000 inhabitants, and urban when in areas with 2,000 inhabitants or more, in agreement with French demographical definition (https://www.insee.fr/fr/metadonnees/definition/c1501) and as previously described (9). Informed consent was obtained from all of individual participants included in the study.

Because of the nature of the survey, patients were invited to participate and had to give their oral consent before the beginning of the interview.

TABLE 1 | Population characteristics according to cardiovascular disease.

		CCS	CHF	*P*-value
Population		**220 (64.0)**	**124 (36.0)**	
Female		66 (29.6)	49 (39.5)	0.08
Age (y)		67 (58–75)	70 (64–82)	0.01
Diagnosis ≥6 months		215 (97.7)	120 (96.8)	0.73
CCS				
History of revascularisation		184 (83.6)		
Medications	Antiplatelets agents	200 (91.7)		
	Betablockers	188 (87.0)		
	ACEI or ARB	181 (84.5)		
	Statins	188 (87.0)		
CHF				
Type of CHF	HFrEF		87 (70.2)	
	HFmrEF		12 (9.7)	
	HFpEF		25 (20.2)	
Etiology	DCM		50 (40.3)	
	Ischemic		23 (18.5)	
	Others		51 (41.2)	
NYHA Class	I		39 (31.5)	
	II		48 (38.7)	
	III		28 (22.6)	
	IV		9 (7 .3)	

CCS, chronic coronary syndrome; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; CHF, congestive heart failure; IQR, interquartile range; HFrEF, heart failure with reduced ejection fraction; HFmrEF, heart failure with mildly-reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; DCM, dilated cardiomyopathy; NYHA, New York Heart Association.
n (%) or median (IQR).

The present study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the Dijon University Hospital (NCT04390126).

Statistical Analysis

Continuous variables were expressed as medians and interquartile ranges (IQR) and dichotomous variables as *n* (%). Student *t*-tests or Mann-Whitney tests were used to compare continuous variables, and Pearson's Chi² or Fisher's tests to compare dichotomous data, as appropriate. Current smokers were compared with non-smokers.

RESULTS

Among the 400 selected patients, 56 declined the interview or were lost to follow-up and 344 questionnaires were finally analyzed, including 220 CCS and 124 CHF; patients with CCS were 3 years younger than those with CHF ($p = 0.01$). The rate of smoking was high ($n = 43$, 12.5%), and smokers were 15 years younger than non-smokers ($p < 0.001$). Population characteristics are summarized in **Tables 1**, **2**. Prevalence of smoking was higher in the CCS than in the CHF group ($p = 0.011$). Smokers were more frequently diabetic, single or divorced, and unemployed than non-smokers, and they were

TABLE 2 | Patient characteristics according to smoking status.

	Total *N* = 344	Non-smoker *N* = 301	Smoker *N* = 43	*P*-value*
Risk factors				
Age, years	70 (59–78)	71 (62–79)	56 (52–65)	<0.001
Men	229 (66.6)	197 (65.4)	32 (74.4)	0.243
Diabetes	81 (23.7) {342}	68 (22.7) {299}	13 (30.2)	0.028
BMI, kg/m²	27 (24-30) {319}	27 (24-30) {279}	28 (25-31) {40}	0.202
BMI ≥ 25 kg/m²	231 (72.4) {319}	200 (71.7) {279}	31 (77.5) {40}	0.442
BMI ≥ 30 kg/m²	86 (24.5) {319}	73 (26.2)	13 (32.5)	0.398
Type of CVD	{344}	{301}	{43}	0.011
CCS	220 (64.0)	185 (61.5)	35 (81.4)	
CHF	124 (36.0)	116 (38.5)	8 (18.6)	
History of depression	54 (16.0) {338}	46 (15.5) {297}	8 (19.5) {41}	0.510
COVID-19 screening (RT-PCR)	11 (3.2) {342}	7 (2.3) {299}	4 (9.3)	**0.037**
Socio-economic status				
Marital status	{336}	{296}	{40}	0.004
Single	35 (10.4)	26 (8.8)	9 (22.5)	
Divorced	28 (8.3)	22 (7.4)	6 (15.0)	
Married	225 (67.0)	203 (68.6)	22 (55)	
Widower	48 (14.3)	45 (15.2)	3 (7.5)	
Professional activity	{341}	{299}	{42}	<0.001
Current	69 (20.2)	55 (18.4)	14 (33.3)	
Retired	238 (69.8)	222 (74.2)	16 (38.1)	
Unemployed	13 (3.8)	7 (2.3)	6 (14.3)	
Other	21 (6.2)	15 (5.0)	6 (14.3)	
Education	{342}	{300}	{42}	0.112
≥High school diploma	106 (32.1)	97 (33.7)	9 (21.4)	0.133
Lockdown place				
Residence area				0.066
Urban	163 (45.3)	137 (45.5)	26 (60.5)	
Rural	181 (54.7)	164 (54.5)	17 (39.5)	
Type of accommodation				0.086
Flat without terrace/garden	47 (12.4)	37 (12.4)	10 (23.3)	
Flat with terrace/garden	66 (18.8)	56 (18.8)	10 (23.3)	
House with garden	228 (68.8)	205 (68.8)	23 (63.5)	
Alone in accommodation	83 (24.4) {340}	68 (22.9) {297}	15 (34.9)	0.087
Number of cohabitants	{340}	{297}	{43}	
Median (IQR)	1 (1–2)	1 (1–2)	1 (0–2)	0.864
Minimum/maximum	0/6	0/6	0/5	

IQR, interquartile range; BMI, Body Mass Index; CVD, cardiovascular disease; CCS, chronic coronary syndrome; CHF, congestive heart failure; RT-PCR, nasal Reverse Transcriptase-Polymerase Chain Reaction detection for SARS-CoV-2.

**p value comparison between smokers and non-smokers.*

n (%) or median (IQR), {Number of answers}.

more often screened for COVID-19. In addition, smokers' place of residence during the lockdown tended to be more often urban and they were more likely to be living alone. Feeling and lifestyle behavior of patients according to their smoking status is summarized in **Table 3**. Among psychological factors, smokers were three times more likely to feel cramped and the psychological distress level (K6 ≥ 8) tended to be higher. Among the 43 current smokers, a high rate [$n = 10$ (30%)] increased their tobacco consumption during the lockdown period. Moreover, during this period, one started to smoke and two had relapsed. Only six patients were vapers, and none was a dual user. Among the ex-smokers, none had quitted since the beginning of the lockdown.

Stress was the most commonly cited cause of smoking, followed by boredom. Lifestyle changes, including physical activity, alcohol consumption, and increase in screen time were similar for the two groups. In contrast, smokers had a much higher rate of weight variations, either for increase or for

TABLE 3 | Patients feeling and behavior according to smoking status during lockdown.

	Total *N* = 344	Non-smoker *N* = 301	Smoker *N* = 43	*P*-value*
Psychological factors				
Lockdown rules compliance	335 [97.7) {343}	294 [98.0) {300}	41 [95.3)	0.264
Feeling cramped	19 [5.6) {337}	13 [4.4) {294}	6 [14)	0.023
Sleep quality/duration change	83 [24.3) {342}	68 [22.7) {300}	15 [35.7) {42}	0.158
Currently feeling:	{342}	{300}	{42}	0.427
Bad	21 [6.1)	16 [5.3)	5 [11.9)	
Fairly good	75 (21.9)	67 (22.3)	8 (19.0)	
Well	175 (51.2)	154 (51.3)	21 (50.0)	
Very well	71 (20.8)	63 (21.0)	8 (19)	
Feeling less well (compared to before lockdown)	75 (21.9) {342}	65 (21.7) {300}	10 (32) {42}	0.743
Kessler score	2 (0–4) {337}	2 (0–4) {294}	2 (0–4)	0.633
K6 $\geq$ 8	37 (11.0)	29 (9.9)	8 (18.6)	0.079
Health behavior change				
Physical activity	{341}	{298}		0.466
Same	171 (50.1)	153 (51.3)	18 (41.9)	
Decreased	147 (43.1)	125 (41.9)	22 (51.2)	
Increased	23 (6.7)	20 (6.7)	3 (7.0)	
Alcohol intake	284	245	39	0.341
Same	242 (85.2)	210 (85.7)	32 (82.1)	
Decreased	27 (9.5)	24 (9.8)	3 (7.7)	
Increased	15 (6.7)	11 (4.5)	4 (10.3)	
Screen time increase	155 (45.3) {342}	131 (43.7) {300}	24 (57.1) {42}	0.10
Weight	{343}	{301}	{42}	0.01
Same	223 (65.0)	204 (67.8)	19 (45.2)	
Decreased	43 (12.5)	33 (11.0)	10 (23.8)	
Increased	77 (22.4)	64 (21.3)	13 (31)	
Tobacco consumption				
Same			21 (48.8)	
Decreased			9 (20.9)	
Increased (or started)			13 (30.2)	
Cause of increase/start smoking			{12}	
Stress	–	–	7 (58.3)	
Boredom	–	–	3 (25.0)	
Other			2 (16.7)	
Electronic cigarette	6 (1.8) {333}	6 (2.1) {290}	0 (0.0)	1
With nicotine	1 (20) {5}	1 (20) {5}	0	

IQR, interquartile range.
**p-value comparison between smokers and non-smokers.*
n (%) or median (IQR), {Number of answers}.

decrease, when compared with non-smokers. At the time of the interview, 29 patients reported the use of telemedicine, 16 in the CCS group (7.3%) and 13 in CHF group (10.5%); the difference was non-significant (p = 0.317). As tobacco quitting may have been encouraged during these sessions, we assume that such advice may have been given in the same way in both groups.

Among the 344 patients, three patients developed conditions highly suggesting a COVID-19 (anosmia and/or ageusia associated with fever and cough) and underwent PCR testing (unknown timing according to the symptoms), of whom only one was positive. Eight other patients underwent PCR testing, of whom all were negative. Among them, four had no symptoms, neither contact with any COVID-19 patient.

Among the patients with CCS, 13 declared an increase of symptoms of angina, of whom two were smokers. One of them did not report a change in smoking behavior, the other declared a reduction in tobacco consumption.

The subgroup analysis among smokers showed that the decrease in physical activity and the increase in screen time were more common in urban than in rural areas (61.5 vs. 35.3%, $p = 0.092$ and 69.2 vs. 37.5%, $p = 0.044$, respectively). Although not significant, tobacco consumption increased less

frequently among rural vs. urban patients (17.6 and 38.5%, $p = 0.187$).

DISCUSSION

Smoking cessation is associated with major health benefits and some studies even suggest a favorable effect on biological age (12). Although smoking cessation is one of the key targets for secondary prevention in CVD, we found a high rate of current smokers (12.5%) among French CVD patients interviewed during the first lockdown (March-May 2020), consistent with smoking prevalence in CAD patients from contemporary European surveys (2, 13).

Relations between tobacco smoking and COVID-19 are controversial. Comorbidities including tobacco-induced diseases are associated with severe forms of COVID-19 and smokers are at higher risk of poor outcomes when infected (14, 15). Moreover, tobacco smoking up-regulates angiotensin-converting enzyme 2 (ACE2), receptor, binding site of Sars-Cov2 on membrane, promoting cell-invasion (16). The initial lower prevalence of smokers among patients with COVID-19 in early publications were not confirmed and could be related to selection bias, inadequate tobacco smoking definition and other confounding factors such as social habits (7, 16).

As expected, younger age and unemployment were more prevalent among smokers, which could interfere with other findings such as occupational characteristics.

Smokers also reported a higher rate of COVID-19 screening, which could be a result of respiratory symptoms mimicking COVID-19 symptoms, thus justifying the request for testing. In our population, diabetes was more prevalent among smokers than non-smokers, and the association of these factors exacerbates CV risk. This underlines the importance of implementing strategies for tobacco cessation in smokers with comorbidities (7, 17).

Although the lockdown period provided a potential opportunity for smoking cessation, none of our participants had quit, a third of patients had increased their tobacco consumption, one patient started smoking, and two patients relapsed (7). Psychological distress induced by social isolation and fear of the disease may have created conditions for smoking increase during the lockdown (1, 4). In addition, weight variations were more common among smokers than non-smokers. Whether it could relate to the influence of lockdown on mental health or to other factors such as variations in physical activity, or any confounding factors including socio-economic status is only speculative (6).

In a web-survey conducted in US dual users, 28.3 and 24.9% decreased their smoking and vaping consumption, but more subjects, 30.3 and 29.1%, respectively, had increased consumption since the beginning of the COVID-19 outbreak, and there was a positive correlation between the two products (18). In England, an analysis of monthly cross-sectional surveys demonstrated the stability of smoking prevalence and found an increase quitting since the lockdown, but they could not exclude an increase in uptake or relapse (19). In a german survey, almost 10% of smokers quit and 50% increased their tobacco consumption. The increase was associated with COVID-19-related stress and living alone (20). To the best of our knowledge, our work is the first to specifically address smoking in CVD outpatients, who constitute a high-risk population.

In France during the first COVID-19-related lockdown, a nationwide web-based survey was conducted in 1,454 respondents aged 25–64 years, including some with CVD (21). When compared with our findings, they found a similar rate of smokers who decreased their tobacco consumption (22.6 vs. 20.9% respectively), but a higher rate of increased consumption (40.4 vs. 30.2%, respectively). A cross-sectional study in smokers from the general French population covering the same lockdown period yielded similar variations, including decreased tobacco consumption in 18.6% of and increased consumption in 26.7% (6). In this online survey, smoking increase was closely related with anxiety and overcrowded housing.

A large nationwide cross-sectional survey was conducted in USA smokers and e-cigarettes users during 2020 August; 21% of smokers had decreased their tobacco consumption in the 6 last months. Although they were aware of the amplified risk of COVID-19 related to tobacco smoking, 33% of smokers had increased their consumption; one the main reasons was stress; results were similar between only cigarettes users and dual-users. Moreover, 15% of the subjects who had quitted during the last 6 months relapsed. Conversely, 23% of vapers increased their e-cigarette consumption. However, 26% of smokers reported trying to quit, and this was associated with an increase risk perception of COVID-19 related to tobacco smoking (22). In California, an online survey did not find an increase in the number of smokers but tobacco consumption was higher among smokers likely related to a shift in time spent in smoke free places toward time spent at home (20). In an on-line survey in Pennsylvania, stress, more time to smoke and boredom were the main reasons to smoking increase (23).

A link between stress and unhealthy behaviors has been found in Australian subjects, of whom more than 50% suffered from chronic disease, mostly driven by a decrease in physical activity (almost 50%) (24).

In Netherlands, Van der Werf et al. observed some change in lifestyle behaviors among 1,004 adults who answered an online questionnaire after the first 3 months of COVID-19 pandemics, of whom 153 (15.2%) were smokers (25). A greater number of subjects declared healthier than unhealthier lifestyle behaviors (19.3 vs. 12.2%, respectively). Unhealthier lifestyle was associated to stress and was similar among smokers and non-smokers. Most of smokers did not change their tobacco consumption; however, 8.3% declared a decrease in tobacco consumption and only 3.7% an increase which is very different from findings from other surveys including our present study (18–24, 26). In Netherlands the lockdown rules were much less strict than in other countries including France, thus potentially influencing such findings.

Altogether, these data suggest that tobacco-smoking patterns evolution during lockdown were quite similar whatever CV health status. Although smoking has been associated with increased COVID-19 severity, studies have suggested that nicotine could be protective against SARS-COv2 infection (1, 8,

19). Our data suggest that smoking increase was not related to medical or media messages.

Smoking during lockdown was characterized by living alone, feeling cramped and urban environment. Both living alone and overcrowded housing have been associated with increased smoking, even if other socio-economic factors can interfere (6, 20). Living in a rural location during lockdown was associated with less tobacco use when compared with an urban area. Green spaces have been associated with better CV health, through reduced stress, and increased physical activity (27). However, socioeconomic factors may also influence these findings by selecting subjects with a psychological profile more prone to healthy lifestyle. An Irish study reported that increasing smoking was associated with increased alcohol intake and stress, but was not influenced by the type of residence (28). In a recent French survey, a rural residence was protective against increased screen time but not smoking (29).

Unfortunately, we did not evaluate the motivation of our patients to reduce their tobacco consumption or to quit. Among the patients with CCS, 13 declared an increase of symptoms of angina of whom two were smokers. One of them did not report a change in smoking behavior, the other declared a reduction of tobacco consumption; unfortunately, we did not assess if this reduction was related to worsening angina. These motivations have been studied among 659 smokers living in Hong-Kong. In this phone-call survey performed during the COVID-19 pandemic (while no stay-at-home orders were displayed), perceived susceptibility to COVID-19 and perceived severity of COVID-19 due to smoking were associated with likelihood of quit attempts; the authors suggested that the lower rate of perceived susceptibility than severity could be explained by medias misinformation (30, 31). Data addressing patients are however very scarce. Although not detailing their health conditions, Rigotti et al. conducted a survey enrolling post-hospitalized smokers wishing to quit; among these patients, 32% have increased their tobacco consumption since the beginning of the pandemic (mainly because of stress) and 31% have decreased or stopped; these latter behaviors were associated with increase in perceived risk of COVID-19 or developing severe infections (32). Interestingly, Gold et al. have evaluated motivations to reduce or quit smoking through an online survey. Among the 103 daily smokers, 88.3% declared one or more comorbidities - including cardiovascular diseases, known to be associated with severe COVID-19 patterns. The main reasons of reducing their tobacco consumption (68.9% of the subjects) were health concerns (33).

We acknowledge some limitations in our study. Our study was conducted at the beginning of the pandemic and thus the design was only exploratory and hypothesis-free, given the uniqueness and the previously unknown magnitude of the subsequent lockdown. However, given the consistency of our data, in agreement with current literature, we think our works provide contributory findings on this high health impact topic.

The present data were obtained by self-reporting, so we cannot exclude a reporting bias for the declaration of behaviors such as smoking and alcohol consumption, screentime, physical activity or weight. Some randomly-selected patients could not be included because they declined to participate in the study, could not be reached by phone or because of language barriers. However, the participation rate was high (86%) and the characteristics of the study population, consistent with contemporary data (13) suggest the representativeness of the study population.

Because of the small sample of smokers in our cohort, an extrapolation of our results to other population is only speculative. However, our findings are consistent with larger French general populations covering the same lockdown period, thus strongly suggesting the representativeness of our study population (6, 21).

As cardiovascular risk gradually increases with daily tobacco consumption even for one cigarette, we did not perform a quantitative evaluation of cigarette consumption (34).

Our study did not assess cardiovascular outcomes, which was out of our scope, thus we were not able to analyse the prognosis in subjects who increased their tobacco consumption.

In conclusion, CVD patients had a high rate of smoking during the 1st COVID-19 related lockdown; their behaviors were characterized by a triad of factors: psychological, socio-demographic and living environment. Moreover, the frequent increase in smoking (30%), mainly driven by stress, was particularly alarming in patients with diabetes, suggesting that more aggressive lifestyle management is needed. A longitudinal extension of this cross-sectional survey could provide relevant information regarding the duration of the behaviors described herein and their longer-term health consequences. If confirmed by large sample or experiment design, our findings may help to target tailored preventive strategies in this high-risk population.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The present study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the Dijon University Hospital (NCT04390126). The patients/participants provided their oral informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FC, MB, J-CE, ND, YC, and MZ: conceptualization. MS-J, AS, and GL: methodology. YC: funding acquisition. FC, FB, and MS-J: data acquisition. J-CE, FB, AC, and AS: analysis. MB, AC, ND, and YC: project administration. FC and MZ: writing draft. FC, MB, ND, YC, and MZ: writing, review, and editing. All authors has approved the submitted version and agrees to be personally accountable for its own contribution.

FUNDING

This work was supported by the Dijon Football Côte d'Or, Dijon University Hospital, the French Federation of Cardiology, the

Association de Cardiology de Bourgogne, and by grants from the Agence Régionale de Santé (ARS) de Bourgogne-Franche-Comté and from the Regional Council of Burgundy-Franche-Comté.

ACKNOWLEDGMENTS

The authors thank Maud Maza for statistical assistance, Michèle Vourc'h for technical assistance, Lucie Vadot and the research assistant team for their involvementin the survey, Sylvie Mazencieux-Agobert and Jessica Massenot for editing assistance, and Suzanne Rankin for English reviewing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.845439/full#supplementary-material

REFERENCES

1. Münzel T, Hahad O, Kuntic M, Keaney JF, Deanfield JE, Daiber A. Effects of tobacco cigarettes, e-cigarettes, and waterpipe smoking on endothelial function and clinical outcomes. *Eur Heart J.* (2020) 41:4057–70. doi: 10.1093/eurheartj/ehaa460
2. Piepoli MF, Abreu A, Albus C, Ambrosetti M, Brotons C, Catapano AL, et al. Update on cardiovascular prevention in clinical practice: a position paper of the European Association of Preventive Cardiology of the European Society of Cardiology. *Eur J Prev Cardiol.* (2020) 27:181–205. doi: 10.1177/2047487319893035
3. Gaalema D, Pericot-Valverde I, Bunn JY, Villanti AC, Cepeda-Benito A, Doogan NJ, et al. Tobacco use in cardiac patients: perceptions, use, and changes after a recent myocardial infarction among US adults in the PATH study [2013-2015]. *Prev Med.* (2018) 117:76–82. doi: 10.1016/j.ypmed.2018.05.004
4. Duffy EY, Cainzos-Achirica M, Michos ED. Primary and secondary prevention of cardiovascular disease in the era of the coronavirus pandemic. *Circulation.* (2020) 141:1943–5. doi: 10.1161/CIRCULATIONAHA.120.047194
5. Lavie CJ, Ozemek C, Kachur S. Promoting physical activity in primary and secondary prevention. *Eur Heart J.* (2019) 40:3556–8. doi: 10.1093/eurheartj/ehz697
6. Guignard R, Andler R, Quatremère G, Pasquereau A, du Roscoät E, Arwidson P, et al. Changes in smoking and alcohol consumption during COVID-19-related lockdown: # cross-sectional study in France. *Eur J Public Health.* (2021) 31:ckab054. doi: 10.1093/eurpub/ckab054
7. Berlin I, Thomas D, Le Faou AL, Cornuz J. COVID-19 and smoking. *Nicotine Tob Res.* (2020) 22:1650–2. doi: 10.1093/ntr/ntaa059
8. Dautzenberg B, Levi A, Adler M, Gaillard R. Transdermal nicotine in non-smokers: a systematic review to design COVID-19 clinical trials. *Respir Med Res.* (2021) 80:100844. doi: 10.1016/j.resmer.2021.100844
9. Cransac-Miet A, Zeller M, Chagué F, Faure AS, Bichat F, Danchin N, et al. Impact of COVID-19 lockdown on lifestyle adherence in stay-at-home patients with chronic coronary syndromes: towards a time bomb. *Int J Cardiol.* (2021) 323:285–7. doi: 10.1016/j.ijcard.2020.08.094
10. Chagué F, Boulin M, Eicher JC, Bichat F, Saint Jalmes M, Cransac-Miet A, et al. Impact of lockdown on patients with congestive heart failure during the coronavirus disease 2019 pandemic. *ESC Heart Fail.* (2020) 7:4420–3. doi: 10.1002/ehf2.13016
11. Breslau J, Finucane ML, Locker AR, Baird MD, Roth EA, Collins RL. A longitudinal study of psychological distress in the United States before and during the COVID-19 pandemic. *Prev Med.* (2021) 143:106362. doi: 10.1016/j.ypmed.2020.106362
12. Lei MK, Beach SR, Dogan MV, Philibert RA. A pilot investigation of the impact of smoking cessation on biological age. *Am J Addict.* (2017) 26:129–35. doi: 10.1111/ajad.12502
13. De Bacquer D, Astin F, Kotseva K, Pogosova N, De Smedt D, De Backer G, et al. Poor adherence to lifestyle recommendations in patients with coronary heart disease: results from the EUROASPIRE surveys. *Eur J Prev Cardiol.* (2021) 55:zwab115. doi: 10.1093/eurjpc/zwab115
14. Guan WJ, Liang WH, Zhao Y, Liang HR, Chen ZS, Li YM, et al. Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. *Eur Respir J.* (2020) 55:2000547. doi: 10.1183/13993003.00547-2020
15. Reddy RK, Charles WN, Sklavounos A, Dutt A, Seed PT, Khajuria A, et al. The effect of smoking on COVID-19 severity: a systematic review and meta-analysis. *J Med Virol.* (2021) 93:1045–56. doi: 10.1002/jmv.26389
16. Alla F, Berlin I, Nguyen-Thanh V, Guignard R, Pasquereau A, Quelet S, et al. Tobacco and COVID-19: a crisis within a crisis? *Can J Public Health.* (2020) 111:995–9. doi: 10.17269/s41997-020-00427-x
17. Campagna D, Alamo A, Di Pino A, Russo C, Calogero A.E, Purrello F, et al. Smoking and diabetes: dangerous liaisons and confusing relationships. *Diabetol Metab Syndr.* (2019) 11:85. doi: 10.1186/s13098-019-0482-2
18. Klemperer EM, West JC, Peasley-Miklus C, Villanti AC. Change in tobacco and electronic cigarette use and motivation to quit in response to COVID-19. *Nicotine Tob Res.* (2020) 22:1662–3. doi: 10.1093/ntr/ntaa072
19. Jackson SE, Garnett C, Shahab L, Oldham M, Brown J. Association of the COVID-19 lockdown with smoking, drinking and attempts to quit in England: an analysis of 2019-20 data. *Addiction.* (2021) 116:1233–44. doi: 10.1111/add.15295
20. Koopman A, Georgiadou E, Reinhard I, Müller A, Lemenager T, Kiefer F, et al. The effects of lockdown during the COVID-19 pandemic on alcohol and tobacco consumption behavior in Germany**.** *Eur Addict Res.* (2021) 27:242–56. doi: 10.1159/000515438
21. Rossinot H, Fantin R, Venne J. Behavioral changes during COVID-19 confinement in France: a web-based study. *Int J Env Res Public Health.* (2020) 17:8444. doi: 10.3390/ijerph17228444
22. Kalkhoran SM, Levy DE, Rigotti NA. Smoking and e-cigarette use among U.S. adults during the COVID-19 pandemic. *Am J Prev Med.* (2021) 6:2582. doi: 10.1101/2021.03.18.21253902
23. Yingst JM, Krebs NM, Bordner CR, Hobkirk AL, Allen SI, Foulds J. Tobacco use changes and perceived health risk among current tobacco users during the COVID-19 pandemic. *Int J Environ Res Public Health.* (2021) 18:1975. doi: 10.3390/ijerph18041795
24. Stanton R, To QG, Khalesi S, Williams SL, Alley SJ, Thwaite TL, et al. Depression, anxiety and stress during COVID-19: associations with changes in physical activity, sleep, tobacco and alcohol use in Australian adults. *Int J Environ Res Public Health.* (2020) 17:4065. doi: 10.3390/ijerph17114065
25. Van der Werf ET, Busch M, Jong MC, Rogier Hoenders HJ. Lifestyle changes during the first wave of the COVID-19 pandemic: a cross-sectional survey in the Netherlands. *BMC Public Health.* (2021) 21:1226. doi: 10.1186/s12889-021-11264-z
26. Gonzalez M, Epperson AE, Halpern-Felsher B, Halliday DM, Song AV. Smokers are more likely to smoke more after the COVID-19 California lockdown order. *Int J Environ Res Public Health.* (2021) 18:2582. doi: 10.3390/ijerph18052582
27. Yeager RA, Smith TR, Bhatnagar A. Green environments and cardiovascular health. *Trends Cardiovasc Med.* (2020) 30:241–6. doi: 10.1016/j.tcm.2019.06.005
28. Reynolds C, Purdy J, Rodriguez L, McAvoy H. Factors associated with changes in consumption among smokers and alcohol drinkers during the COVID-19 'lockdown' period. *Eur J Public Health*. (2021) 6:ckab050. doi: 10.1093/eurpub/ckab050
29. Rolland B, Haesebaert F, Zante E, Benyamina A, Haesebaert J, Franck N. Global changes and factors of increase in caloric/salty food intake, screen use, and substance use during the early COVID-19 containment phase in the general population in France: Survey study. *JMIR Public Health Surveill.* (2020) 6:e19630. doi: 10.2196/19630

30. Li Y, Luk TT, Wu Y, Cheung DYT, Li WHC, Tong HSC, et al. High perceived susceptibility to and severity of COVID-19 in smokers are associated with quitting-related behaviors. *Int J Environ Res Public Health*. (2021) 18:10894. doi: 10.3390/ijerph182010894
31. Luk TT, Zhao S, Weng X, Wong JY, Wu YS, Ho SY, et al. Exposure to health misinformation about COVID-19 and increased tobacco and alcohol use: a population-based survey in Hong Kong. *Tob Control*. (2021) 30:696–9. doi: 10.1136/tobaccocontrol-2020-055960
32. Rigotti NA, Chang Y, Regan S, Lee S, Kelley JHK, Davis E, et al. Cigarette smoking and risk perceptions during the COVID-19 pandemic reported by recently hospitalized participants in a smoking cessation trial. *J Gen Intern Med*. (2021) 36:3786–93. doi: 10.1007/s11606-021-06913-3
33. Gold AK, Hoyt DL, Milligan M, Hiserodt ML, Samora J, Leyro TM, et al. The role of fear of COVID-19 in motivation to quit smoking and reductions in cigarette smoking: a preliminary investigation of at-risk cigarette smokers. *Cogn Behav Ther*. (2021) 50:295–304. doi: 10.1080/16506073.2021.1877340
34. Teo KK, Ounpuu S, Hawken S, Pandey MR, Valentin V, Hunt D, et al. Tobacco use and risk of myocardial infarction in 52 countries in the INTERHEART study: a case-control study. *Lancet*. (2006) 368:647–58. doi: 10.1016/S0140-6736(06)69249-0

Conflict of Interest: FC reports having received non-financial support and speaking fees from Amgen, MSD, Novartis and Pfizer. MB reports having Consulting or Advisory Role from Sanofi Aventis, BMS, Pfizer, Pierre Fabre, Grünenthal, Celgene. ND reports having received grants, speaking fees, consulting fees, or non-financial support from: Amgen, AstraZeneca, Bayer, BMS, Boehringer-Ingelheim, Intercept, MSD, Novo-Nordisk, Pfizer, Sanofi, Servier, UCB and Vifor. YC reports having received grants, consulting fees, honoraria and/or delivering lectures for Servier, Novartis, Boehringer, Pfizer, MSD, and Bayer. MZ received research grants from Amarin Corp.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Cardiovascular Medicine

CASE REPORT
published: 27 April 2022
doi: 10.3389/fcvm.2022.844626

Case Report: Importance of MRI Examination in the Diagnosis and Evaluation of COVID-19 mRNA Vaccination Induced Myocarditis: Our Experience and Literature Review

Keita Watanabe[1,2]*, *Takashi Ashikaga*[1], *Yasuhiro Maejima*[2], *Susumu Tao*[2], *Mao Terui*[1], *Tetsuya Kishigami*[1], *Masakazu Kaneko*[1], *Ryota Nakajima*[1], *Shinichiro Okata*[1], *Tetsumin Lee*[1], *Tomoki Horie*[1], *Masashi Nagase*[1], *Giichi Nitta*[1], *Ryoichi Miyazaki*[1], *Sho Nagamine*[1], *Yasutoshi Nagata*[1], *Toshihiro Nozato*[1], *Masahiko Goya*[2] and *Tetsuo Sasano*[2]

[1] Department of Cardiology, Japanese Red Cross Musashino Hospital, Tokyo, Japan, [2] Department of Cardiology, Tokyo Medical and Dental University, Tokyo, Japan

Acute myocarditis is a rare but serious complication associated with mRNA-based coronavirus disease 2019 (COVID-19) vaccination. In this article, four COVID-19 mRNA vaccination induced myocarditis cases managed at our tertiary Medical Center have been discussed. Three patients had typical myocarditis. One patient suffered from atrioventricular block and heart failure, which required more intensive treatment, but eventually improved. Additionally, a review of cardiac magnetic resonance imaging (MRI) features related to the diagnosis of myocarditis showed that COVID-19 mRNA vaccine-associated myocarditis tend to have more late-gadolinium enhancement (LGE) accumulation in the inferior lateral wall direction. According to a report by the U.S. Centers for Disease Control and Prevention (CDC), the diagnosis of COVID-19 mRNA vaccine-associated myocarditis is based on clinical symptoms, altered myocardial enzymes, cardiac MRI finding, or histopathology. Cardiac MRI is relatively less invasive than myocardial biopsy and plays an important role in the diagnosis of myocarditis. This review may aid in the diagnosis of COVID-19 mRNA vaccine-associated myocarditis.

Keywords: cardiac MRI, COVID-19, mRNA vaccination, myocarditis, case series, review, case report

OPEN ACCESS

Edited by:
Masanori Aikawa, Brigham and Women's Hospital and Harvard Medical School, United States

Reviewed by:
Hendrik Tevaearai Stahel, Bern University Hospital, Switzerland
Tetsuro Miyazaki, Juntendo University Urayasu Hospital, Japan

***Correspondence:**
Keita Watanabe
keita.w.tmdu@gmail.com

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *28 December 2021*
Accepted: *28 March 2022*
Published: *27 April 2022*

Citation:
Watanabe K, Ashikaga T, Maejima Y, Tao S, Terui M, Kishigami T, Kaneko M, Nakajima R, Okata S, Lee T, Horie T, Nagase M, Nitta G, Miyazaki R, Nagamine S, Nagata Y, Nozato T, Goya M and Sasano T (2022) Case Report: Importance of MRI Examination in the Diagnosis and Evaluation of COVID-19 mRNA Vaccination Induced Myocarditis: Our Experience and Literature Review. Front. Cardiovasc. Med. 9:844626. doi: 10.3389/fcvm.2022.844626

INTRODUCTION

The Ministry of Health, Labor and Welfare Japan approved a range of coronavirus disease 2019 (COVID-19) vaccines in February 2021, and vaccination has been since then widely promoted by the government through various health education campaigns and initiatives. By the end of November 2021, 79.2% of the Japanese population had received their first dose of the COVID-19 vaccine, and 77.3% had received their second dose (1).

As the younger population started receiving vaccines, adverse events different from those commonly seen in older adults began to occur, including myocarditis. In general, myopericarditis is a very rare adverse event associated with vaccination and has been reported particularly after administration of the smallpox vaccine (2, 3). To the best of our knowledge, in the case of COVID-19, as this is the first time that mRNA vaccines have been used clinically, the current occurrence of post-vaccination myocarditis is of particular concern.

In this study, several cases of myocarditis that were suspected to be associated with mRNA-based COVID-19 vaccination were reviewed, and a literature review has been presented regarding the efficacy and utility of late-gadolinium enhancement (LGE) on cardiac magnetic resonance imaging (cMRI) in the diagnosis of myocarditis.

CASE PRESENTATION

From February to October 2021, four patients with COVID-19 mRNA vaccine-associated myocarditis were admitted to our hospital (**Table 1**). The diagnosis was based on the definition reported by the U.S. Centers for Disease Control and Prevention (CDC) (4). All patients fulfilled the Lake Louise Criteria (LLC) (5), which is considered as a diagnostic criterion for myocarditis on cMRI.

The study was approved by the institutional review board of the Japanese Red Cross Musashino Hospital and was conducted in accordance with the ethical principles of the Declaration of Helsinki as well as with the Japanese Ethical Guidelines for Medical and Health Research Involving Human Subjects. All participants provided their written informed consent for the anonymized publication of these findings and were provided information disclosure documents on our website. The participants were free to opt out of participation at any time without any adverse consequences or the loss of benefits to which they were otherwise entitled.

Case 1, Case 2, and Case 3

These three cases are relatively similar. Male patients under 30 years of age had developed myocarditis after their second dose of vaccination. They experienced some kind of chest symptoms such as chest pain and chest pressure within a few days after vaccine administration. Electrocardiography changes (ST elevation) were observed only in Case2. Particularly, negative T waves appeared after ST elevation; these negative T waves improved over time. High-sensitivity troponin-I levels were elevated in all cases, and creatine kinase-myocardial band (CK-MB) was also elevated to above the reference level in Case 1 and Case 2. There was no elevation of white blood cell (WBC) and B-type natriuretic peptide (BNP), but C-reactive protein (CRP) levels were elevated in all patients. Cardiac MRI was performed in all patients. LGE was observed in each case with varying localization, and was more common in the sub-epicardial wall. In Cases 1 and Case 3, T2 high signal intensity and LGE were observed simultaneously in the same segment. In Case 2, an examination performed 6 days after vaccination showed only T2 high signal intensity at the sub-epicardial wall of the basal inferior left ventricle. However, an examination performed 47 days later showed LGE in the same area and likewise demonstrated that the T2 high signal intensity had disappeared. Ibuprofen was administered to all patients due to its anti-inflammatory effects. An angiotensin-converting enzyme inhibitor (ACE-I) was administered as well to prevent remodeling.

Case 4

Case 4 had a relatively different course compared to the previous three cases. The patient developed fever the day after first dose vaccination, and was referred to the hospital 5 days after vaccination with syncope as the chief complaint. An electrocardiogram revealed paroxysmal atrioventricular block, which was thought to be the cause of syncope. Although a pacemaker lead had to be temporarily inserted for AV block, the paroxysmal AV block resolved 2 days after admission. On echocardiography, marked myocardial hypertrophy and decreased left ventricular contractility were observed. Improvements in hypertrophy and contraction were observed on subsequent echocardiography. In laboratory findings, compared to the previous three cases, BNP levels were elevated, and high-sensitivity troponin-I and CK-MB levels were relatively high. On cardiac MRI, T2 high signal intensity and LGE were observed simultaneously in the mid-wall of basal inferior and sub-epicardial wall of mid-septum and infero-septum left ventricular. Only this patient had an accumulation of LGE and high T2 signal on the left ventricular septum side (**Figure 1**). Catecholamines (i.e., dobutamine) and diuretics were administered during hospitalization as a treatment for heart failure. Diuretic, ibuprofen, and ACE-I were discontinued following confirmation of negative troponin levels in the outpatient clinic, with no apparent adverse events.

DISCUSSION

COVID-19 mRNA Vaccination-Associated Myocarditis

The CDC has recently reported diagnostic criteria for post-vaccination myocarditis (4). The criteria for diagnosis include specific clinical symptoms following vaccination, as well as cMRI findings consistent with myocarditis in the presence of troponin levels above the upper limit of normal and/or histopathologic confirmation of myocarditis. The diagnosis of myocarditis on cMRI is based on the implementation of either the original or the revised LLC (5, 6). We note that cMRI is less invasive than myocardial biopsy and is considered an important diagnostic tool for evaluating vaccine-associated myocarditis.

In this case series, cMRI was performed in all the cases. In Cases 1, 3, and 4, T2 high signals and LGE were observed simultaneously in the same segment, which was considered to fulfill the LLC. In Case 2, T2 high signal intensity was seen on initial examination. Later examination demonstrated the appearance of LGE in the same region. The initial examination showed inflammatory findings, and the LGE observed on the second examination was thought to be the result of fibrosis occurring due to these inflammatory findings. During the observation period, there were cases in which the MRI showed

TABLE 1 | Patient demographic and medicals characteristics and associated health outcomes.

	Case 1	Case 2		Case 3	Case 4
Definition	**Confirmed**	**Confirmed**		**Confirmed**	**Confirmed**
Age, y	19	20		29	48
Sex	Male	Male		Male	Male
Race/ethnicity	Caucasian	Again		Again	Again
Vaccine type					
Types of mRNA vaccines	mRNA-1273-Moderna	mRNA-1273-Moderna		mRNA-1273-Moderna	BNT162b2 mRNA-Pfizer-BioNTech
Number of vaccinations	2	2		2	1
History of previous COVID-19 infection	Denied/ negative antigen	Denied/ negative antigen		Denied/ negative PCR	Denied/ negative PCR
Symptoms					
Day 1 post-vaccination	Chest discomfort	Fever		Fever	No symptom
Day 2 post-vaccination	Chest pain, pain with breathing, hospital admission	Chest pressure, nausea		Chest pain, hospital admission	Fever, tiredness, diarrhea
Day 3 post-vaccination		Hospital admission			Tiredness
Day 4 post-vaccination					Tiredness
Day 5 post-vaccination					Syncope, tiredness, hospital admission
Vital signs at presentation					
Temperature, °C	36.9	39.1		36.2	35.4
Heart rate, bpm	100	106		73	80
Blood pressure, mm Hg	109/58	120/57		117/69	85/57
Respirations, per min	18	20		18	20
Chest x-ray findings	No acute pulmonary disease	No acute pulmonary disease		No acute pulmonary disease	enlarged cardiac shadow
Cardiothoracic ratio (CTR)	48.4%	48.4%		43.4%	54.5%
ECG findings					
ST changes	No	ST elevation in V3–6		No	Negative T wave in V4–6
Rhythm	Normal sinus rhythm	Normal sinus rhythm		Normal sinus rhythm	Paroxysmal atrioventricular block
Echocardiogram					
Number of days after vaccination	3 days	5 days		3 days	5 days
LV ejection fraction	52	62		58	30
LV end-diastolic internal dimension	48	51		40	47
LV end-systolic internal dimension	35	36		28	36
Intraventricular septal diastolic thickness	9	10		9	14
LV posterior wall thickness	12	10		12	15
E/A	2.09	1.7		1.13	0.63
E/e'	3.68	7.15		4.65	9.02
Regional wall motion abnormalities	None	None		Non	Diffuse hypokinesis
Diastolic function	Normal	Normal		Normal	
Cardiac magnetic resonance imaging (cMRI)					
Number of days between last vaccination and cMRI	5 days	6 days (first time)	45 days (second time)	12 days	12 days
LGE	Sub-epicardial wall of basal-mid infero-lateral LV	Na	mid wall of basal inferior LV	mid wall of anterior, and inferior LV	Mid-wall of basal inferior, Sub-epicardial wall of mid- and infero-septum LV

(Continued)

TABLE 1 | Continued

	Case 1	Case 2		Case 3	Case 4
Definition	**Confirmed**	**Confirmed**		**Confirmed**	**Confirmed**
T2WBB high signal	Sub-epicardial wall of basal-mid infero-lateral LV	mid wall of basal inferior LV	Na	mid wall of anterior, and inferior LV	Mid-wall of basal inferior, Sub-epicardial wall of mid- and infero-septum LV
Laboratory findings					
Cardiac troponin I pg/mL					
Presentation	1,801.7		1,885.6	4,419.6	17,888.7
Peak	5,321.9		5,749	4,419.6	17,888.7
Postdischarge	<10		<10	<10	15.5
CK U/L peak	415		324	154	765
CK-MB U/L peak	30.8		15.3	9	64
WBC	6,500		5,900	6,100	5,000
BNP, pg/mL	9.1		12.1	9	111
CRP, mg/dL	5.63		8.78	1.16	11.32
Coronary angiography findings	ND	MRI negative		CCT negative	CAG no stenosis
Clinical course					
Hospitalization duration	5		8	5	11
Treatment(s)	Ibuprofen, ACE-I	Ibuprofen, ACE-I		Ibuprofen, ACE-I	dobutamine, Diuretic, Ibuprofen, ACE-I

BNP, B-type natriuretic peptide; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; E/A, transmitral Doppler early (E-wave) to late (A-wave) ventricular filling velocities; E/e', E-wave to tissue Doppler early diastolic mitral annular velocity; LGE, late gadolinium enhancement; LV, left ventricular; T2WBB, T2 Weighed black blood; WBC, white blood cell.

only LGE and no T2 findings, so the LLC could not be fulfilled, and the diagnosis could not be confirmed. More specifically, though these cases had clinical presentations consistent with myocarditis, there are two reasons they were not classified as confirmed cases. First, the quality of MRI was a limiting factor. Namely, the quality of cMRI at our hospital was unacceptable; specifically, the myocardial early gadolinium enhancement ratio could not be evaluated and used parametric mapping techniques with the currently available technology. Thus, findings of sufficient quality might not have been obtained; and may thus, have failed to confirm true myocarditis cases. The second limiting factor is the accuracy of the LLC. Though the LLC present a widely used diagnostic classification system for myocarditis, previous studies have reported that the sensitivity of diagnosing myocarditis when two out of the three main criteria were fulfilled was only ∼78% (7). Thus, the accuracy of these criteria alone may not be sufficient to accurately diagnose myocarditis with acceptable sensitivity and specificity. However, studies to date indicate that the modified version of the LLC may provide more accuracy if parametric mapping techniques can be applied (6).

Cardiac MRI for Myocarditis

Numerous cases of COVID-19 mRNA vaccine-associated myocarditis have been reported till date. cMRI plays an important role in the diagnosis of any form of myocarditis. The American Heart Association (AHA) scientific statement on the management of myocarditis (8) as well as the current European Society of Cardiology (ESC) position statement (9) consider cMRI useful for the evaluation of suspected myocarditis. Japanese guidelines likewise suggest its usefulness (10). More specifically, cMRI provides a non-invasive, biopsy-like approach in order to verify the pathognomonic imaging features associated with and plays a role in the exclusion of myocardial inflammation. The current ESC guidelines on acute and chronic heart failure include a Class I indication for the efficacy of cMRI in the assessment of myocarditis (11). cMRI characteristics of myocardial inflammation may not only aid in the diagnosis of myocarditis but may also provide important and accurate information on prognoses. In acute cases, myocardial edema presenting without LGE on cMRI has been associated with improved recovery and outcomes (12). The relationship between the localization of LGE on cMRI and mortality in myocarditis has been reported as well (13). Thus, cMRI has evolved to become a key evaluation tool in patients with suspected myocardial inflammation.

Significance of LGE in Myocarditis

LGE is not an essential finding in the original or revised LLC for the diagnosis of myocarditis. However, LGE is the most established technique for detecting myocardial damage (14). The presence of LGE seems to be a good predictor of adverse outcomes in patients with biopsy-proven myocarditis, and has been shown to be superior to other variables in this regard (15). Some recent reports suggest that the location, pattern, extent, and distribution of LGE can stratify the risk for patients with suspected myocarditis. For example, Gräni et al. reported that septal and mid-wall LGE were most strongly associated with major cardiovascular events, including all-cause mortality,

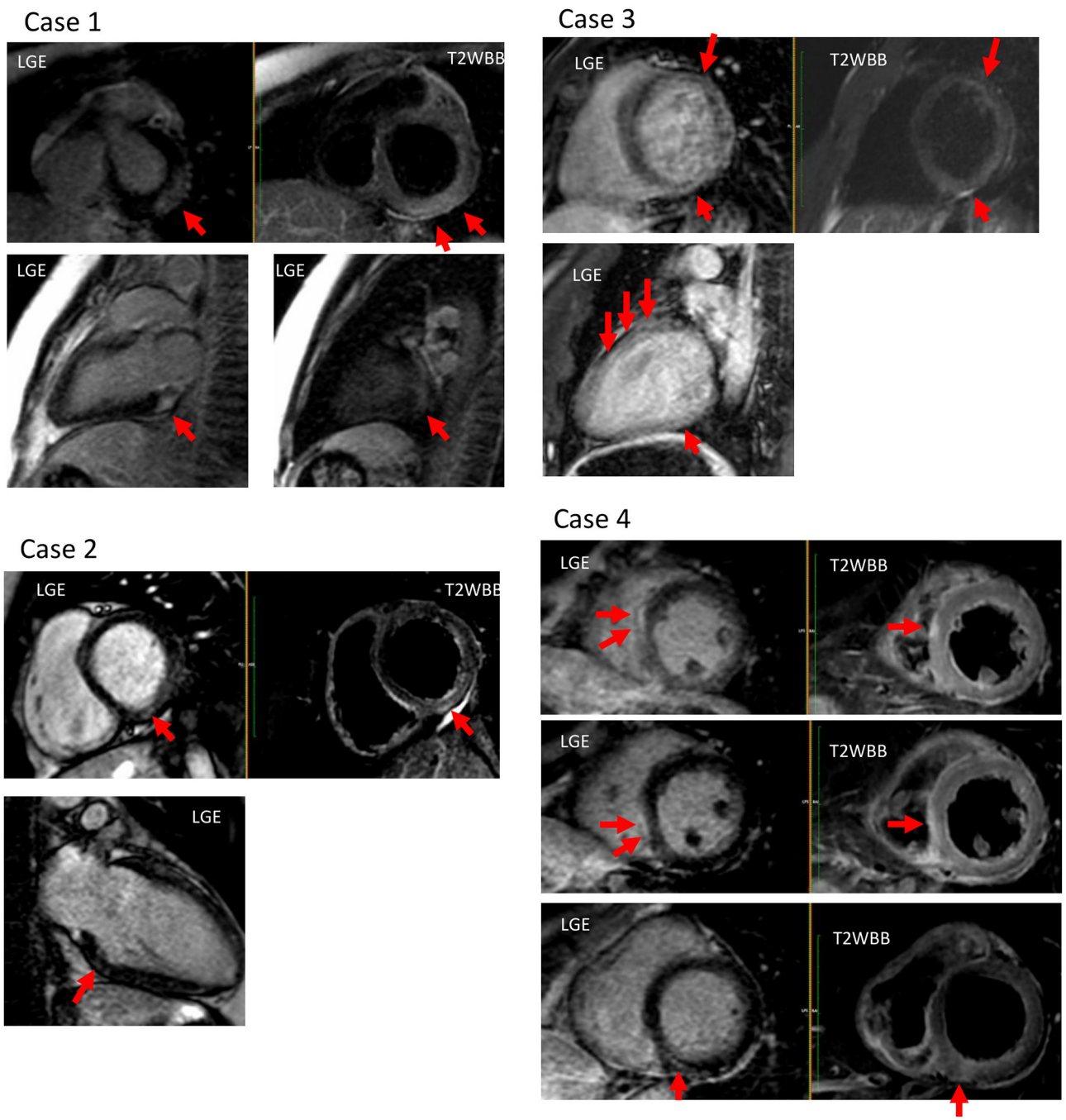


FIGURE 1 | Cardiac magnetic resonance imaging (MRI) of all profiled cases. Case 1: T2 high signal intensity and late-gadolinium enhancement (LGE) were observed at the sub-epicardial wall of the basal-mid inferolateral left ventricular (LV). Case 2: T2 high signal intensity and LGE were observed at the mid wall of the basal inferior LV. Case 3: T2 high signal intensity and LGE were observed at the mid wall of the anterior, and at the inferior LV. Case 4: T2 high signal intensity and LGE were observed at the mid-wall of basal inferior, and at the sub-epicardial wall of the mid- and inferoseptum LV.

worsening heart failure, heart transplantation, and ventricular arrhythmias (16). Aquaro et al. showed that patients with anteroseptal LGE have a worse prognosis than those with LGE at other sites (17). Greulich et al. demonstrated that the presence of mid-wall LGE in the septal segments was associated with a higher long-term mortality rate as compared with the absence of LGE or other LGE patterns in patients with biopsy-proven viral myocarditis (13). One reported mechanism potentially mediating these effects is that the septal LGE might involve the conduction system, thus yielding the substrate for malignant

TABLE 2 | Published case reports and case series regarding COVID-19 vaccine-associated myocarditis that describe LGE on cardiac magnetic resonance imaging (MRI).

		Age	Sex	Vaccine types	Number of vaccinations	Time from last vaccination to cardiac MRI	LGE: layer	LGE: segment
Marshall et al.	Case 1	16	Male	Pfizer	2nd	NA	Subepicardia	Apical and midchamber lateral wall
	Case 2	19	Male	Pfizer	2nd	NA	Mid wall	Basal inferolateral wall
	Case 3	17	Male	Pfizer	2nd	NA	Subepicardial	Basal anterolateral segment, basal to midventricular inferolateral segments
	Case 5	17	Male	Pfizer	2nd	NA	Epicardia	Anterior and lateral LV
	Case 7	14	Male	Pfizer	2nd	5 days	Subepicardial	Mid and apical left ventricle free wall
Rosner et al.	Case 2	39	Male	Pfizer	2nd	11 days	Subepicardial	Along the anterior and lateral walls
	Case 3	39	Male	Modelna	2nd	5 days	Subepicardial and midmyocardial	Anterior wall
	Case 4	24	Male	Pfizer	1st	7 days	Midmyocardial	Septal and inferior walls
			Male				Subepicardial	Anterior, lateral, and inferior walls
	Case 5	19	Male	Pfizer	2nd	3 days	Multifocal patchy subepicardial and midmyocardial	Lateral and inferolateral walls
	Case 6	20	Male	Pfizer	2nd	6 days	Subepicardial	Lateral, inferolateral, anterolateral walls, apex
	Case 7	23	Male	Pfizer	2nd	3 days	Mid wall	Basal anteroseptal
Mouch et al.	Case 1	24	Male	Pfizer	2nd	NA	Subepicardial	Basal septum
							Mid myocardial	Inferolateral
	Case 2	20	Male	Pfizer	2nd	NA	Subepicardial	Basal and middle anterolateral and inferolateral walls
	Case 3	29	Male	Pfizer	2nd	NA	Diffuse	Basal, inferolateral, anterolateral and anteroseptal walls
	Case 4	45	Male	Pfizer	1st	NA	Subepicardial	Middle anterolateral, inferolateral and apical anterior walls
	Case 5	16	Male	Pfizer	2nd	NA	Midmyocardial	Basal inferolateral
			Male				Subepicardial	Middle anterolateral
	Case 6	17	Male	Pfizer	2nd	NA	Subepicardial	Basal inferolateral, middle inferolateral and infero-septal and apical lateral, anterior and inferior walls
							Mid-myocardial	Middle inferolateral and anterolateral and apical anterior and lateral walls
Kim et al.	Case 1	36	Male	Moderna	2nd	3 days	Epicardial	Apical lateral
	Case 4	24	Male	Pfizer	2nd	3 days	Epicardial, patchy	Lateral
Ammirati et al.		56	Male	Pfizer	2nd	NA	Subepicardial-intramyocardial regions	Basal and apical segments of the infero-lateral wall
Angelo et al.		30	Male	Pfizer	2nd	6 days	Subepicardial	Sparing of the basal and mid-septal segments
Albert et al.		24	Male	Moderna	2nd	5 days	Mid-myocardial and epicardial	Lateral, anterolateral and inferolateral segments
Muthukumar et al.		52	Male	Moderna	2nd	6 days	Midmyocardial and subepicardial	Infero-septal, inferolateral, anterolateral, and apical walls
							Subepicardial	Inferior basal and mesocardial midventricular region
Minocha et al.		17	Male	Pfizer	2nd	NA	Subepicardial	Mid-lateral and apical

(Continued)

TABLE 2 | Continued

		Age	Sex	Vaccine types	Number of vaccinations	Time from last vaccination to cardiac MRI	LGE: layer	LGE: segment
Mansour et al.	Case 1	25	Male	Moderna	2nd	6 days	Subepicardial	Anterolateral wall of the mid and apical left ventricle
	Case 2	21	Female	Moderna	2nd	4 days	Subepicardial	Inferolateral wall at the base
Habib et al.		37	Male	Pfizer	2nd	NA	Subepicardial	Basal lateral wall
Cereda et al.		21	Male	Pfizer	2nd	NA	Patchy epicardial	The posterior, anterior, inferior, and lateral walls
Vidula et al.	Case 1	19	Male	Pfizer	2nd	NA	Subepicardial	Basal to mid lateral wall
	Case 2	18	Male	Moderna	2nd	NA	Subepicardial	Mid lateral wall
Williams et al.		34	Male	Moderna	2nd	7 days	Subepicardial	Anterolateral and inferolateral segments
Isaak et al.		15	Male	Pfizer	2nd	NA	Subepicardial	Inferolateral wall
Hasnie et al.		22	Male	Moderna	1st	NA	Subepicardial	Lateral wall and inferior segments at the midventricular and apical LV
Patrignani et al.		56	Male	Pfizer	1st	11 days	Sub-epicardial	Basal and middle segments of the infero-lateral wall
Kim et al.		24	Male	Pfizer	2nd	NA	Sub-epicardial	Basal inferior and inferolateral segment
Ehrlich et al.		40	Male	Pfizer	1st	12 days	Diffuse	Basal and mid anteroseptal and inferoseptal segments as well as in the apical septal segment
Patel et al.	Case 1	22	Male	Pfizer	1st	NA	Subepicardial	Basal inferior, basal inferolateral, and apical lateral
	Case 2	19	Male	Pfizer	2nd	NA	Subepicardial	Basal inferolateral
	Case 3	25	Male	Moderna	2nd	NA	Subepicardial	Lateral
	Case 4	37	Male	Pfizer	2nd	NA	Subepicardial	Basal anteroseptal segment
	Case 5	20	Male	Pfizer	2nd	NA	Subepicardial and mid-myocardial	Basal, mid, and apical lateral segments
Tailor et al.		44	Male	Moderna	2nd	5 days	Mid-myocardial	Mid-septum, infero-septum, and inferior walls at the base to midventricle
							Sub-epicardial and mid-myocardial	Lateral wall at the mid-ventricle and apical lateral wall
Nguyen et al.		20	Male	Moderna	1st	NA	Subepicardial	Mid and basal inferolateral segments
Onderko et al.	Case 2	28	Male	Pfizer	2nd	NA	Epicardium	Apical lateral wall, midanterolateral segments
	Case 3	36	Male	Moderna	2nd	NA	Epicardial	Mid- to distal inferolateral and lateral walls
Shiyovich et al.	Case 1	41	Male	Pfizer	2nd	107 days	Mid-wall	Inferolateral (basal)
	Case 2	24	Male	Pfizer	2nd	103 days	Mid-wall and epicardia	Inferolateral (basal)
	Case 3	17	Male	Pfizer	2nd	7 days	Epicardial	Inferolateral, anterolateral, (basal to apical)
	Case 4	37	Male	Pfizer	1st	48 days	Epicardial	Inferolateral (basal, mid)
	Case 5	39	Male	Pfizer	2nd	8 days	Mid-wall and epicardia	Inferoseptal, anteroseptal (basal), inferolateral, anterolateral (basal), Inferolateral (med), septum, lateral (apical)
	Case 7	19	Male	Pfizer	2nd	43 days	Mid-wall	Inferior (apicalbasal), Inferolateral (mid, basal), anterior (basal, mid), septum, lateral (apical)
	Case 8	28	Male	Pfizer	2nd	139 days	Mid-wall	Inferolateral, anterolateral (basal)

(Continued)

TABLE 2 | Continued

		Age	Sex	Vaccine types	Number of vaccinations	Time from last vaccination to cardiac MRI	LGE: layer	LGE: segment
	Case 10	17	Male	Pfizer	2nd	17 days	Epicardial	Inferior, inferolateral (basal), inferior, inferoseptal, inferolateral (mid)
	Case 11	36	Male	Pfizer	1st	63 days	Mid-wall	Lateral (apical)
	Case 12	27	Male	Pfizer	2nd	105 days	Epicardial	Inferolateral (basal)
	Case 13	42	Male	Pfizer	1st	53 days	Epicardial	Inferolateral (apical, basal), anterolateral (basal)
	Case 14	76	Male	Pfizer	2nd	117 days	Mid-wall	Inferolateral (basal)
	Case 15	32	Male	Pfizer	2nd	83 days	Mid-wall	Inferior (basal), inferolateral (basal)
Our Cases	Case 1	19	Male	Moderna	2nd	5 days	Sub-epicardial	Infero-lateral (basal-mid)
	Case 2	20	Male	Moderna	2nd	45 days	Mid-wall	Inferior (basal)
	Case 3	29	Male	Moderna	2nd	12 days	mid-wall	Anterior, inferior wall
	Case 4	48	Male	Pfizer	1st	12 days	Mid-wall	Inferior walls at the base to midventricle
							Subepicardial	Mid-septum, and infero-septum of left ventricular Wall

LGE, late-gadolinium enhancement; LV, left ventricular.

arrhythmias (13). Among the cases presented in this report, only Case 4 revealed LGE in the septal segment. Specifically, Case 4 showed paroxysmal atrioventricular block as a disturbance of the conduction system; this case presentation was relatively severe as compared to the other three cases and thus necessitated intensive treatment. The importance of attaining a greater comprehension of LGE characteristics on cMRI of patients with myocarditis has been emphasized.

LGE in COVID-19 mRNA Vaccination-Associated Myocarditis

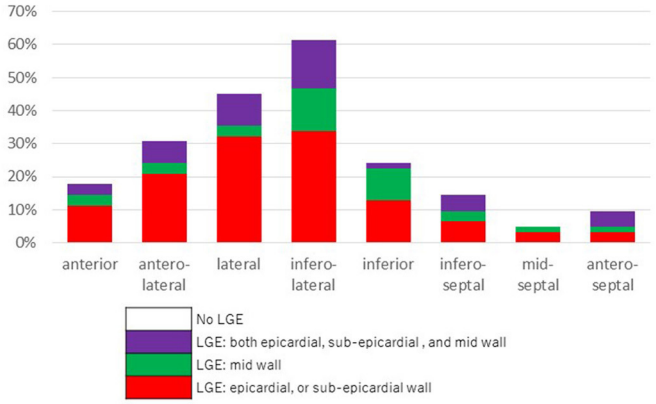
There have been numerous reports of COVID-19 mRNA vaccination-associated myocarditis in recent months; however, adverse reactions are relatively rare and comprehensive reports are limited. Shiyovich et al. reported 15 cases of vaccine-associated myocarditis; and, to the best of our knowledge, it is the largest case series published till date (18). In that report, LGE was observed in 13/15 patients with vaccine-associated myocarditis, and was more common in the inferolateral region.

"PubMed" was mainly accessed and reports were extracted using the keywords "myocarditis," "mRNA vaccination," and "COVID-19." Cases, articles, and review articles without detailed MRI descriptions were excluded. Finally, numerous cases of COVID-19 mRNA vaccination-associated myocarditis presented within 24 publications were reviewed, all of which described the localization of LGE. A total of 62 cases (four cases evaluated by the study authors in the current report, and 58 cases evaluated within previously published case reports and case series) were reviewed in terms of diagnostic imaging, with a focus on LGE findings of cMRI (18–41). The localization was classified as anterior, anterolateral, lateral, inferolateral, inferior, inferoseptal, mid-septal, and anteroseptal. The layers of the myocardium were classified as epicardial or sub-epicardial, mid, and endocardial or sub-endocardial wall. Vertical localization (basal, mid, apical) was not described in many of the cases (i.e., only transverse localization was evaluated). In the present review, the examined cases comprised 61 males and one female with an average age of 29 (±12.4) years. Forty-one patients (66.1%) were under the age of 30 years. Forty-six patients (74.2%) had been vaccinated with the Pfizer mRNA-based vaccine and 16 patients (25.8%) had been vaccinated with Moderna mRNA-based vaccine. **Table 2** and **Figure 2** summarize the LGE features within cMRI. We found that LGE occurred more frequently on the free wall side (i.e., mainly in the inferolateral region) and occurred relatively less frequently on the septal side. LGE was mostly detected on the epicardia or sub-epicardia; no case with LGE on the left ventricular endocardia was identified.

Generally, myocarditic infiltrations due to viral infection occur in a peculiar pattern (i.e., predominantly in the lateral free wall, originating from the epicardial quartile of the ventricular wall in myocarditis patients) (42). The patterns of LGE occurring in general viral myocarditis as well as in COVID-19 mRNA vaccination-associated myocarditis appear to be similar.

Possible Mechanism of COVID-19 mRNA Vaccination-Associated Myocarditis

The mechanisms mediating COVID-19 mRNA vaccination-associated myocarditis have not been elucidated in detail till date. We suspect that the mechanisms mediating COVID-19 mRNA vaccination-associated myocarditis may be similar to those underlying viral myocarditis. Viral myocarditis is mainly due to direct



		anterior	antero-lateral	lateral	infero-lateral	inferior	infero-septal	mid-septal	antero-septal
Marshall et al.	Case 1								
	Case 2								
	Case 3								
	Case 5								
	Case 7								
Rosner et al.	Case 2								
	Case 3								
	Case 4								
	Case 5								
	Case 6								
	Case 7								
Mouch et al.	Case 1								
	Case 2								
	Case 3								
	Case 4								
	Case 5								
	Case 6								
Kim et al.	Case 1								
	Case 4								
Ammirati et al.									
Angelo et al.									
Albert et al.									
Muthukumar et al.									
Minocha et al.									
Mansour et al.	Case 1								
	Case 2								
Habib et al.									
Cereda et al.									
Vidula et al.	Case 1								
	Case 2								
Williams et al.									
Isaak et al.									
Hasnie et al.									
Patrignani et al.									
Kim et al.									
Ehrlich et al.									
Patel et al.	Case 1								
	Case 2								
	Case 3								
	Case 4								
	Case 5								
Tailor et al.									
Nguyen et al.									
Onderko et al.	Case 2								
	Case 3								
Shiyovich et al.	Case 1								
	Case 2								
	Case 3								
	Case 4								
	Case 5								
	Case 7								
	Case 8								
	Case 10								
	Case 11								
	Case 12								
	Case 13								
	Case 14								
	Case 15								
Our Cases	Case 1								
	Case 2								
	Case 3								
	Case 4								

FIGURE 2 | A summary of results regarding late-gadolinium enhancement (LGE) on cardiac magnetic resonance imaging (MRI) for the diagnosis of myocarditis in published cases reports and case series. The localizations were classified as anterior, anterolateral, lateral, inferolateral, inferior, inferoseptal, mid-septal, and anteroseptal. The layers of the myocardium were classified as epicardial or sub-epicardial, mid, and endocardial or sub-endocardial wall.

viral damage and the subsequent IL-6-mediated immune response (43).

The mRNA vaccine mainly elicits a local immune response after being injected intramuscularly. However, this immune response is also present systematically, including in the liver, pancreas, and lymph nodes. In experiments among animal models, lipid nanoparticle-modified mRNA influenza vaccines were distributed mainly in the above mentioned organs, but were also detected to a lesser extent in the heart (44). mRNA vaccines do not cause COVID-19, as the mRNA breaks down rapidly in the cell and the vaccine encodes only a portion of the complete virion. Due to the structural design of the mRNA vaccine, it is uncertain whether distribution of vaccine components to the heart could cause direct damage.

The possibility of immunological mechanisms mediating the development of myocarditis following mRNA vaccination needs to be considered. For example, naive T lymphocytes may be primed by autologous proteins released from damaged cardiomyocytes via antigen-presenting cells. In rare cases, it has been reported that this can cause the migration of primed T lymphocytes into cardiovascular tissue, as well as cell-mediated cytotoxicity and lymphocytic myocarditis (45). Pro-inflammatory cytokines are released, increasing T lymphocyte activation and contributing to myocardial damage (46). In various published cases of vaccine-associated myocarditis, myocarditis was found to develop after the second vaccination. We suspect that T lymphocytes primed by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) proteins primed by the first vaccination may cause myocarditis. We note that the mRNA-based COVID-19 vaccine is a new vaccine that has not been used previously. More comprehensive elucidation of its pathogenesis is desirable to ensure its safety.

Report From the Ministry of Health, Labour and Welfare, Japan

In Japan, suspected myocarditis-associated events, including myocarditis and pericarditis, have been reported more frequently among males in the age groups of 10–19 and 20–29 years (3.69 and 9.62 cases per million, respectively, for combined first and second vaccinations for the Pfizer mRNA-based vaccine; and 28.83 and 25.65 cases per million, respectively, for combined first and second vaccinations for the Moderna mRNA- based vaccine) (47). Based on reports of suspected adverse drug reactions in Japan and overseas, the Japanese Ministry of Health, Labour and Welfare had decided to revise the medical package inserts for mRNA vaccines. More specifically, the Ministry considered issuing an alert in light of the high frequency of myocarditis-associated events in young males. The recommendation of the Pfizer-BioNTech mRNA-based vaccine is being considered for males in their teens and twenties, as the frequency of reports of suspected myocarditis-associated adverse events following vaccination with the Moderna mRNA-based vaccine were clearly higher than the frequency of events following vaccination with the Pfizer-BioNTech mRNA-based vaccine. Those who had received the Modena vaccine in the past would be able to choose the Pfizer-BioNTech vaccine later on.

Clinical Perspective

COVID-19 vaccine-associated cardiomyopathy is frequently reported to develop within 2–3 days following vaccination and presents as chest pain symptomology (44). Elevated myocardial devitalizing enzymes are found in all cases, whereas changes on electrocardiography as well as decreased contraction (left ventricular ejection fraction $<$50%) on echocardiography have been reported in only 87 and 15% of patients, respectively (44). If chest symptoms are observed following vaccination, it is advisable to consider the possibility of myocarditis and to perform appropriate blood tests and cMRI scans. In the aforementioned CDC report, myocardial biopsy is included as one of the diagnostic criteria for vaccine-associated myocarditis. However, there are few reports regarding relevant pathology findings, likely because the infiltration of inflammatory cells is reduced compared with that in ordinary acute myocarditis; hence, these findings may not lead directly to a definitive diagnosis. There have been many reports on the characteristics of cardiac MRI in evaluating COVID-19 myocarditis, including the current report, and these reports may be more useful and informative in guiding diagnostics and effective clinical decision-making.

In this review, LGE on cardiac MRI was found to be more common on the inferior lateral wall of the left ventricle and relatively less common on the septal side. This finding is similar to existing reports on viral acute myocarditis (14). In viral myocarditis, cases of LGE on the septal side are considered to have a poor prognosis because of its effect on the conduction system of stimulation to the myocardium. In the current review, Case 4, with LGE on the septal side, showed affected atrioventricular conduction and required relatively intensive treatment, and may have the same tendency in COVID-19 vaccine-associated cardiomyopathy. Hence, cardiac MRI may be useful not only for the diagnosis itself, but also with respect to risk stratification. Although most cases occur in young males and the severity of vaccine-associated myocarditis is relatively low in these age groups, cases of cardiac failure have been reported. Thus, based on existing reports, risk stratification should be performed, hospitalization should be considered in some cases, and careful follow-up is always necessary.

CONCLUSIONS

Herein, a report on the detailed features of COVID-19 vaccine-associated myocarditis has been presented. It was found that cMRI is minimally invasive and may aid in the diagnosis of myocarditis. LGE on cMRI tends to occur more frequently on the free wall side and relatively less frequently on the septal side, as in viral myocarditis. These findings can guide future epidemiologic research on this topic of immediate public health importance,

directly inform medical guidelines, and help in effective clinical decision-making.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of the Japanese Red Cross Musashino Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KW, TA, and YM contributed principally to writing the manuscript. ST, MT, TK, MK, RN, SO, TL, TH, MN, GN, RM, SN, YN, TN, MG, and TS revised the manuscript. ST selected the cardiac MRI images and drafted an explanation for the images. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Mathieu E, Ritchie H, Ortiz-Ospina E, Roser M, Hasell J, Appel C, et al. A global database of COVID-19 vaccinations. *Nat Hum Behav.* (2021) 5:947–53. doi: 10.1038/s41562-021-01122-8
2. Su JR, McNeil MM, Welsh KJ, Marquez PL, Ng C, Yan M, et al. Myopericarditis after vaccination, vaccine adverse event reporting system (VAERS), 1990–2018. *Vaccine.* (2021) 39:839–45. doi: 10.1016/j.vaccine.2020.12.046
3. Cassimatis DC, Atwood JE, Engler RM, Linz PE, Grabenstein JD, Vernalis MN. Smallpox vaccination and myopericarditis: a clinical review. *J Am Coll Cardiol.* (2004) 43:1503–10. doi: 10.1016/j.jacc.2003.11.053
4. Gargano JW, Wallace M, Hadler SC, Langley G, Su JR, Oster ME, et al. Use of mRNA COVID-19 vaccine after reports of myocarditis among vaccine recipients: update from the advisory committee on immunization practices — United States, June 2021. *Morb Mortal Wkly Rep.* (2021) 70:977–82. doi: 10.15585/mmwr.mm7027e2
5. Friedrich MG, Sechtem U, Schulz-Menger J, Holmvang G, Alakija P, Cooper LT, et al. Cardiovascular magnetic resonance in myocarditis: a JACC white paper. *J Am Coll Cardiol.* (2009) 53:1475–87. doi: 10.1016/j.jacc.2009.02.007
6. Ferreira VM, Schulz-Menger J, Holmvang G, Kramer CM, Carbone I, Sechtem U, et al. Cardiovascular magnetic resonance in nonischemic myocardial inflammation: expert recommendations. *J Am Coll Cardiol.* (2018) 72:3158–76. doi: 10.1016/j.jacc.2018.09.072
7. Luetkens JA, Faron A, Isaak A, Dabir D, Kuetting D, Feisst A, et al. Comparison of original and 2018 lake louise criteria for diagnosis of acute myocarditis: results of a validation cohort. *Radiol Cardiothorac Imaging.* (2019) 1:e190010. doi: 10.1148/ryct.2019190010
8. Bozkurt B, Colvin M, Cook J, Cooper LT, Deswal A, Fonarow GC, et al. Current diagnostic and treatment strategies for specific dilated cardiomyopathies: A Scientific Statement From the American Heart Association. *Circulation.* (2016) 134:e579–646. doi: 10.1161/CIR.0000000000000455
9. Caforio ALP, Pankuweit S, Arbustini E, Basso C, Gimeno-Blanes J, Felix SB, et al. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J.* (2013) 34:2636–48. doi: 10.1093/eurheartj/eht210
10. Group JJW. Guidelines for diagnosis and treatment of myocarditis (JCS 2009) – digest version. *Circ J.* (2011) 75:734–43. doi: 10.1253/circj.CJ-88-0008
11. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J.* (2021) 42:3599–726. doi: 10.1093/eurheartj/ehab368
12. Vermes E, Childs H, Faris P, Friedrich MG. Predictive value of CMR criteria for LV functional improvement in patients with acute myocarditis. *Eur Heart J Cardiovasc Imaging.* (2014) 15:1140–4. doi: 10.1093/ehjci/jeu099
13. Greulich S, Seitz A, Müller KAL, Grün S, Ong P, Ebadi N, et al. Predictors of mortality in patients with biopsy-proven viral myocarditis: 10-year outcome data. *J Am Heart Assoc.* (2020) 9:e015351. doi: 10.1161/JAHA.119.015351
14. Greulich S, Ferreira VM, Dall'Armellina E, Mahrholdt H. Myocardial inflammation-are we there yet? *Curr Cardiovasc Imaging Rep.* (2015) 8:6. doi: 10.1007/s12410-015-9320-6
15. Grn S, Schumm J, Greulich S, Wagner A, Schneider S, Bruder O, et al. Long-term follow-up of biopsy-proven viral myocarditis: predictors of mortality and incomplete recovery. *J Am Coll Cardiol.* (2012) 59:1604–15. doi: 10.1016/j.jacc.2012.01.007
16. Gräni C, Eichhorn C, Bière L, Murthy VL, Agarwal V, Kaneko K, et al. Prognostic value of cardiac magnetic resonance tissue characterization in risk stratifying patients with suspected myocarditis. *J Am Coll Cardiol.* (2017) 70:1964–76. doi: 10.1016/j.jacc.2017.08.050
17. Aquaro GD, Perfetti M, Camastra G, Monti L, Dellegrottaglie S, Moro C, et al. Cardiac MR with late gadolinium enhancement in acute myocarditis with preserved systolic function: ITAMY Study. *J Am Coll Cardiol.* (2017) 70:1977–87. doi: 10.1016/j.jacc.2017.08.044
18. Shiyovich A, Witberg G, Aviv Y, Eisen A, Orvin K, Wiessman M, et al. Myocarditis following COVID-19 vaccination: magnetic resonance imaging study. *Eur Hear J Cardiovasc Imaging.* (2021) jeab230. doi: 10.1093/ehjci/jeab230
19. Marshall M, Ferguson ID, Lewis P, Jaggi P, Gagliardo C, Collins JS, et al. Symptomatic acute myocarditis in 7 adolescents after pfizer-biontech covid-19 vaccination. *Pediatrics.* (2021) 148:e2021052478. doi: 10.1542/peds.2021-052478
20. Rosner CM, Genovese L, Tehrani BN, Atkins M, Bakhshi H, Chaudhri S, et al. Myocarditis temporally associated with COVID-19 vaccination. *Circulation.* (2021) 144:502–5. doi: 10.1161/CIRCULATIONAHA.121.055891
21. Abu Mouch S, Roguin A, Hellou E, Ishai A, Shoshan U, Mahamid L, et al. Myocarditis following COVID-19 mRNA vaccination. *Vaccine.* (2021) 39:3790–3. doi: 10.1016/j.vaccine.2021.05.087
22. Kim HW, Jenista ER, Wendell DC, Azevedo CF, Campbell MJ, Darty SN, et al. Patients with acute myocarditis following mRNA COVID-19 vaccination. *JAMA Cardiol.* (2021) 6:1196–201. doi: 10.1001/jamacardio.2021.2828
23. Ammirati E, Cavalotti C, Milazzo A, Pedrotti P, Soriano F, Schroeder JW, et al. Temporal relation between second dose BNT162b2 mRNA Covid-19 vaccine and cardiac involvement in a patient with previous SARS-COV-2 infection. *IJC Hear Vasc.* (2021) 34:1–4. doi: 10.1016/j.ijcha.2021.100774
24. D'Angelo T, Cattafi A, Carerj ML, Booz C, Ascenti G, Cicero G, et al. Myocarditis after SARS-CoV-2 vaccination: a vaccine-induced reaction? *Can J Cardiol.* (2021) 37:1665–7. doi: 10.1016/j.cjca.2021.05.010
25. Albert E, Aurigemma G, Saucedo J, Gerson DS. Myocarditis following COVID-19 vaccination. *Radiol Case Rep.* (2021) 16:2142–5. doi: 10.1016/j.radcr.2021.05.033
26. Muthukumar A, Narasimhan M, Li QZ, Mahimainathan L, Hitto I, Fuda F, et al. In-depth evaluation of a case of presumed myocarditis after the second dose of COVID-19 mRNA vaccine. *Circulation.* (2021) 144:487–98. doi: 10.1161/CIRCULATIONAHA.121.056038
27. Minocha PK, Better D, Singh RK, Hoque T. Recurrence of acute myocarditis temporally associated with receipt of the mRNA coronavirus disease 2019 (COVID-19) vaccine in a male adolescent. *J Pediatr.* (2021) 238:321–3. doi: 10.1016/j.jpeds.2021.06.035

28. Mansour J, Short RG, Bhalla S, Woodard PK, Verma A, Robinson X, et al. Cardiothoracic imaging acute myocarditis after a second dose of the mRNA COVID-19 vaccine: a report of two cases. *Clin Imaging.* (2021) 78:247–9. doi: 10.1016/j.clinimag.2021.06.019
29. Habib MB, Hamamyh T, Elyas A, Altermanini M, Elhassan M. Acute myocarditis following administration of BNT162b2 vaccine. *IDCases.* (2021) 25:e01197. doi: 10.1016/j.idcr.2021.e01197
30. Cereda A, Conca C, Barbieri L, Ferrante G, Tumminello G, Lucreziotti S, et al. Acute myocarditis after the second dose of SARS-CoV-2 vaccine: serendipity or atypical causal relationship? *Anatol J Cardiol.* (2021) 25:522–3. doi: 10.5152/AnatolJCardiol.2021.99
31. Vidula MK, Ambrose M, Glassberg H, Chokshi N, Chen T, Ferrari VA, et al. Myocarditis and other cardiovascular complications of the mRNA-based COVID-19 vaccines. *Cureus.* (2021) 13:1–6. doi: 10.7759/cureus.15576
32. Williams CB, Choi J, Hosseini F, Roberts J, Ramanathan K, Ong K. Acute myocarditis following mRNA-1273 SARS-CoV-2 vaccination. *CJC Open.* (2021) 3:1410–2. doi: 10.1016/j.cjco.2021.07.008
33. Isaak A, Feisst A, Luetkens JA. Myocarditis following COVID-19 vaccination. *Radiology.* (2021) 301:E378–9. doi: 10.1148/radiol.2021211766
34. Hasnie AA, Hasnie UA, Patel N, Aziz MU, Xie M, Lloyd SG, et al. Perimyocarditis following first dose of the mRNA-1273 SARS-CoV-2 (Moderna) vaccine in a healthy young male: a case report. *BMC Cardiovasc Disord.* (2021) 21:1–6. doi: 10.1186/s12872-021-02183-3
35. Patrignani A, Schicchi N, Calcagnoli F, Falchetti E, Ciampani N, Argalia G, et al. Acute myocarditis following comirnaty vaccination in a healthy man with previous SARS-CoV-2 infection. *Radiol Case Rep.* (2021) 16:3321–5. doi: 10.1016/j.radcr.2021.07.082
36. Kim IC, Kim H, Lee HJ, Kim JY, Kim JY. Cardiac imaging of acute myocarditis following COVID-19 mRNA vaccination. *J Korean Med Sci.* (2021) 36:1–6. doi: 10.3346/jkms.2021.36.e229
37. Ehrlich P, Klingel K, Ohlmann-Knafo S, Hüttinger S, Sood N, Pickuth D, et al. Biopsy-proven lymphocytic myocarditis following first mRNA COVID-19 vaccination in a 40-year-old male: case report. *Clin Res Cardiol.* (2021) 110:1855–9. doi: 10.1007/s00392-021-01936-6
38. Patel YR, Louis DW, Atalay M, Agarwal S, Shah NR. Cardiovascular magnetic resonance findings in young adult patients with acute myocarditis following mRNA COVID-19 vaccination: a case series. *J Cardiovasc Magn Reson.* (2021) 23:1–8. doi: 10.1186/s12968-021-00795-4
39. Tailor PD, Feighery AM, El-Sabawi B, Prasad A. Case report: acute myocarditis following the second dose of mRNA-1273 SARS-CoV-2 vaccine. *Eur Heart J Case Reports.* (2021) 5:1–6. doi: 10.1093/ehjcr/ytab319
40. Nguyen TD, Mall G, Westphal JG, Weingärtner O, Möbius-Winkler S, Schulze PC. Acute myocarditis after COVID-19 vaccination with mRNA-1273 in a patient with former SARS-CoV-2 infection. *ESC Hear Fail.* (2021) 8:4710–4. doi: 10.1002/ehf2.13613
41. Onderko L, Starobin B, Riviere AE, Hohl PK, Phillips CT, Morgan RB, et al. Myocarditis in the setting of recent COVID-19 vaccination. *Case Reports Cardiol.* (2021) 2021:1–5. doi: 10.1155/2021/6806500
42. Mahrholdt H, Goedecke C, Wagner A, Meinhardt G, Athanasiadis A, Vogelsberg H, et al. Cardiovascular magnetic resonance assessment of human myocarditis: a comparison to histology and molecular pathology. *Circulation.* (2004) 109:1250–8. doi: 10.1161/01.CIR.0000118493.13323.81
43. Esfandiarei M, McManus BM. Molecular biology and pathogenesis of viral myocarditis. *Annu Rev Pathol Mech Dis.* (2008) 3:127–55. doi: 10.1146/annurev.pathmechdis.3.121806.151534
44. Bahl K, Senn JJ, Yuzhakov O, Bulychev A, Brito LA, Hassett KJ, et al. Preclinical and clinical demonstration of immunogenicity by mRNA vaccines against H10N8 and H7N9 influenza viruses. *Mol Ther.* (2017) 25:1316–27. doi: 10.1016/j.ymthe.2017.03.035
45. Basso C, Leone O, Rizzo S, De Gaspari M, Van Der Wal AC, Aubry MC, et al. Pathological features of COVID-19-associated myocardial injury: a multicentre cardiovascular pathology study. *Eur Heart J.* (2020) 41:3827–35. doi: 10.1093/eurheartj/ehaa664
46. Chen L, Li X, Chen M, Feng Y, Xiong C. The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. *Cardiovasc Res.* (2020) 116:1097–100. doi: 10.1093/cvr/cvaa078
47. Ministry of Health, Labour and Welfare, Japan. *Status Report of Suspected Myocarditis-Related Events*. Available from: https://www.mhlw.go.jp/content/10601000/000844075.pdf (accessed December 28, 2021).

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 24 May 2022
doi: 10.3389/fcvm.2022.844441

Transcriptional Effects of Candidate COVID-19 Treatments on Cardiac Myocytes

Tobias Jakobi[1], Julia Groß[2,3], Lukas Cyganek[4,5] and Shirin Doroudgar[1]**

[1] Department of Internal Medicine and the Translational Cardiovascular Research Center, University of Arizona – College of Medicine – Phoenix, Phoenix, AZ, United States, [2] Department of Cardiology, Angiology, and Pneumology, Heidelberg University Hospital, Heidelberg, Germany, [3] DZHK (German Centre for Cardiovascular Research), Partner Site Heidelberg/Mannheim, Heidelberg, Germany, [4] Stem Cell Unit, Department of Cardiology and Pneumology, University Medical Center Göttingen, Göttingen, Germany, [5] DZHK (German Centre for Cardiovascular Research), Partner Site Göttingen, Göttingen, Germany

Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease (COVID-19) has emerged as a major cause of morbidity and mortality worldwide, placing unprecedented pressure on healthcare. Cardiomyopathy is described in patients with severe COVID-19 and increasing evidence suggests that cardiovascular involvement portends a high mortality. To facilitate fast development of antiviral interventions, drugs initially developed to treat other diseases are currently being repurposed as COVID-19 treatments. While it has been shown that SARS-CoV-2 invades cells through the angiotensin-converting enzyme 2 receptor (ACE2), the effect of drugs currently repurposed to treat COVID-19 on the heart requires further investigation.

Methods: Human induced pluripotent stem cell-derived cardiac myocytes (hiPSC-CMs) were treated with five repurposed drugs (remdesivir, lopinavir/ritonavir, lopinavir/ritonavir/interferon beta (INF-β), hydroxychloroquine, and chloroquine) and compared with DMSO controls. Transcriptional profiling was performed to identify global changes in gene expression programs.

Results: RNA sequencing of hiPSC-CMs revealed significant changes in gene programs related to calcium handling and the endoplasmic reticulum stress response, most prominently for lopinavir/ritonavir and lopinavir/ritonavir/interferon-beta. The results of the differential gene expression analysis are available for interactive access at https://covid19drugs.jakobilab.org.

Conclusion: Transcriptional profiling in hiPSC-CMs treated with COVID-19 drugs identified unfavorable changes with lopinavir/ritonavir and lopinavir/ritonavir/INF-β in key cardiac gene programs that may negatively affect heart function.

Keywords: COVID-19, SARS-CoV-2, chloroquine, hydroxychloroquine, remdesevir, ritonavir, lopinavir, cardiac myocytes

OPEN ACCESS

Edited by:
Hendrik Tevaearai Stahel, Bern University Hospital, Switzerland

Reviewed by:
Christian Zuppinger, Bern University Hospital, Switzerland
Dongchao Lu, Hannover Medical School, Germany

****Correspondence:***
Tobias Jakobi
tjakobi@arizona.edu
Shirin Doroudgar
sdoroudgar@arizona.edu

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *28 December 2021*
Accepted: *03 March 2022*
Published: *24 May 2022*

Citation:
Jakobi T, Groß J, Cyganek L and Doroudgar S (2022) Transcriptional Effects of Candidate COVID-19 Treatments on Cardiac Myocytes. Front. Cardiovasc. Med. 9:844441. doi: 10.3389/fcvm.2022.844441

INTRODUCTION

The current COVID-19 pandemic has resulted in more than 271 million confirmed cases as of December 2021 with more than five million deaths reported to be directly linked to the SARS-CoV-2 infection (1). To address the need of treatment options for COVID-19, several different drugs are currently being investigated as possible options. Remdesivir (Rem) is a broad-spectrum antiviral drug initially developed to treat hepatitis C infections. While Rem did not yield the expected results against hepatitis C, it was later tested as treatment option for the Ebola virus (2). Although less efficient against Ebola than monoclonal antibody-based treatments, further trials during an Ebola outbreak from 2013 to 2016 were able to demonstrate its safety (3). With the rise of COVID-19 infections in early 2020, a new study showed effectiveness against severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) in animal models (4), thus making it a promising treatment option against SARS-CoV-2. Lopinavir/ritonavir (LR), a combination of lopinavir and ritonavir was specifically developed to treat and prevent the human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) (5). Since *in vitro* studies with SARS and MERS yielded promising results (6, 7), the treatment is being studied either in its original combination LR or as LRI, with the addition of interferon-β (INF-β), since LR and INF-β were earlier shown to improve outcome in a non-human primate model of MERS (8). Chloroquine (CQ) has immunomodulatory effects and is widely used to treat several forms of malaria (9). Hydroxychloroquine (HCQ) was found to be less toxic compared to CQ (10) and is also commonly used for prevention and treatment of malaria as well as other conditions such as lupus or post-Lyme arthritis. Its immunomodulatory effect was proposed to be valuable during the cytokine storm in severely ill patients (9) and recent studies found *in vivo* antiviral activity against SARS-CoV-2 (9, 11).

While the investigations of the repurposed drugs provide rapid insights into current clinical questions, knowledge of how specific cell types react to treatment with the candidate drugs is still scarce. The infection with SARS-CoV-2 is facilitated by binding to the human angiotensin-converting enzyme 2 (ACE2) using the viral surface proteins (12). While ACE2 receptors are highly abundant in lung cells, the receptor is also expressed in the heart (13). Moreover, cardiomyopathy is described in patients with severe COVID-19, and increasing evidence suggests that cardiovascular involvement portends a high mortality (14, 15). Emerging evidence suggests that patients with COVID-19 present with cardiac abnormalities, including new myocardial infarction, myocarditis, and takotsubo cardiomyopathy (16). Moreover, a recent prospective study suggests that even after recovery from a COVID-19 infection, 60% of the patients suffer from ongoing myocardial inflammation independent of preexisting conditions (17), indicating yet-unknown long-term effects of COVID-19 on the cardiovascular system. In this novel global, unbiased study, we integrated molecular, biomedical, and bioinformatics approaches to examine the effects of candidate COVID-19 treatments on human induced pluripotent stem cell-derived cardiac myocytes (hiPSC-CMs). Our findings shed light on the effects of new candidate treatments on molecular pathways and help to assess potential effects and side effects of the treatments.

MATERIALS AND METHODS

Maintenance of Human Induced Pluripotent Stem Cells

Experiments were performed using the human induced pluripotent stem cell (hiPSC) line UMGi014-C clone 14 (isWT1.14) provided by LC (Stem Cell Unit, University Medical Center Göttingen). The iPSC generation and characterization for pluripotency and genomic stability was described previously (18). The line was reprogrammed from somatic cells of a healthy 35-year-old Caucasian male individual. Human iPSC cultures were maintained in Stem MACS iPS Brew XF Medium (Miltenyi Biotec; #130-104-368) in 17 $\mu g/cm^2$ Growth Factor Reduced Matrigel-coated (Corning; #354230) 6-well dishes in a humidified normoxic incubator (37°C, 5% CO_2). Cultures were routinely passaged in colonies at a ratio of 1:16 every 4–5 days after dissociation using Versene Solution [0.48 mM ethylenediamine tetraacetic acid (EDTA)] (Thermo Fisher; #15040033). Cells were plated in Stem MACS iPS Brew XF Medium and 1 μl/ml of 2 mM Thiazovivin (Millipore; #420220) in DMSO (final concentration 2 μM) for the first 24 h. The culture medium was changed daily with 2 ml per well Stem MACS iPS Brew XF Medium.

Directed Differentiation of Human Induced Pluripotent Stem Cells Into Ventricular Cardiac Myocytes

HiPSCs were differentiated along the ventricular lineage *via* the modulation of the WNT signaling pathway as previously described (19). Briefly, single cells were harvested using Versene Solution and plated on 6-well dishes at 120,000–160,000 per well into a final volume of 2 ml per well. Differentiation was started when the hiPSC cultures reached a confluency of 80–95% using 3 ml per well Cardio Differentiation Medium [RPMI 1640 (with GlutaMAX and HEPES) (Thermo Fisher; #72400021) supplemented with 0.2 mg/ml L-ascorbic acid 2-phosphate (Sigma; #A8960) and 0.5 mg/ml human recombinant albumin (Sigma; A9731)] and freshly added 4 μM CHIR99021 (Millipore; #361559; 0.4 μl/ml of 10 mM stock solution in DMSO). After 24 h, medium was exchanged to Cardio Differentiation Medium. On day 2, medium was changed to 3 ml Cardio Differentiation Medium supplemented with freshly added 5 μM IWP2 (Millipore; #681671; 1 μl/ml of 5 mM stock solution in DMSO) for 2 days and afterward medium was changed to 3 ml Cardio Differentiation Medium for another 2 days and then again medium was changed to 3 ml Cardio Differentiation Medium for another 2 days. From day 8 onward, medium was changed to 2 or 3 ml of Cardio Culture Medium RPMI 1640 (with GlutaMAX and HEPES) supplemented with the final concentration of 1 × B-27 Supplement (from 50×; Thermo Fisher; #17504044) per well every 2 or 3 days, respectively. On day 15, cells were detached using 0.25% trypsin-EDTA solution (Thermo Fisher; #25200056)

and re-plated in 2 ml Cardio Culture Medium supplemented with 20% fetal bovine serum (Thermo Fisher; #10270106, Lot no: 2243865, South American FBS) and 2 μM Thiazovivin at 1×10^6 cells per well in 6-well dishes and medium was changed to Cardio Culture Medium the following day and again on day 18. On day 20, cardiac myocyte selection (20) was performed by changing medium to 2 ml Cardio Selection Medium RPMI 1640 (without glucose and HEPES) with 0.2 mg/ml L-ascorbic acid 2-phosphate and 0.5 mg/ml human recombinant albumin, as well as a final concentration of 4 mM lactate/HEPES (1:250 from 1 M stock) for 2 days. On day 23, medium was replaced with 2 ml Cardio Selection Medium. Starting on day 25, cultures were maintained in Cardio Culture Medium, with regular media changes every 2–3 days. Cells were re-plated after day 30 at 750,000 cells per well in 6-well dishes or at 160,000 cells per well in 24-well dishes for MTT. Experiments were performed on 60-day differentiated cells (hiPSC-CMs).

Drug Treatment of Human Induced Pluripotent Stem Cell-Derived Cardiac Myocytes

Cultures were treated with or without 5 μM chloroquine (Sigma; #C6628) (21), 5 μM hydroxychloroquine (Sigma; # H0915), 5 μM remdesivir (Biosynth Carbosynth; AG170167), 25 μM lopinavir/ritonavir (Sigma; #SML1222-10MG, #SML0491-10MG), and lopinavir/ritonavir/8 U interferon-β (Sigma; #IF014) for 24 h. After treatment, cells were analyzed as described below. Drug concentrations chosen are based on literature data and are below the 50% cytotoxic concentrations (22, 23). Incubation of cardiac myocytes with drugs for 24 h under normal culture conditions did not result in cytotoxicity.

MTT Assay

Cell viability was determined using the MTT assay, which measures the reduction of thiazolyl blue tetrazolium bromide (MTT) into an insoluble formazan product by the mitochondria of viable cells. In brief, hiPSC-CMs were seeded into 24-well plates at a density of 160,000 cells per well. The cells were treated with various concentrations of drugs or DMSO as a vehicle control. After 24 h incubation, 50 μl 0.5 mg/ml MTT solution (Sigma-Aldrich; Merck KGaA) was added to each well, followed by further incubation for 4 h. The medium was then removed, and the formazan crystals were dissolved in 500 μl solubilization buffer (10% SDS in 0.01 M HCl). The absorbance was measured at 570 nm on a plate reader (Perkin Elmer, EnSpire Multimode Plate Reader). The relative cell viability was expressed as a percentage of the control group.

RNA Sequencing

Total RNA was isolated from cultured cells by using QIAzol Lysis Reagent (Qiagen; #79306) according to manufacturer's instructions. RNA sequencing (RNA-seq) was carried out *via* a commercially available long non-coding RNA service (BGI, Shenzhen, China). Briefly, total RNA was fragmented into short fragments and ribosomal RNA was removed. The cDNA synthesis was performed using random priming. Double-stranded cDNA was purified and enriched by PCR amplification, after which the library products were sequenced using BGISEQ-500.

Quantitative Real-Time PCR

Total RNA was isolated from cultured cells by using QIAzol Lysis Reagent (Qiagen; #79306) according to manufacturer's instructions, and reverse-transcribed into complementary DNA (cDNA) by using iScript cDNA Reverse Transcription Kit (Biorad; #1708891). Quantitative real-time PCR was performed using iTAQ SYBR Green PCR Kit (Biorad; #1725124) according to the manufacturer's instructions.

Bioinformatics Analysis

Paired-end rRNA-depleted sequencing data were analyzed in detail. After initial quality assessment, low quality regions and adapter sequences were removed with Flexbar (24) (version 3.5). Residual rRNA reads were removed using Bowtie2 with an rRNA sequence-based index (25). Principal read mapping against the ENSEMBL human reference genome build 100 (hg38) was performed with the STAR RNA-seq aligner (26) (version 2.7.5a). Mapped reads were assigned to genes using the Rsubread package (27) (version 2.2.6). Quality of sequencing data and mapping results was assessed with MultiQC (28) (version 1.9). Differential gene expression was analyzed with edgeR (29) (version 3.30.3). Gene Ontology (GO) analyses were performed using topGO version 2.40.0 with all genes having an RPKM $\geq$ 1 throughout all samples acting as background list. Pathway analyses were performed using the PathVisio software (30) (version 3.3.0) and individual pathways provided by WikiPathways (31). Heatmaps were generated using the ComplexHeatmap package (32) (version 2.5.4). All downstream analyses were performed with R version 3.6.3.

Statistical Analysis

Cell culture experiments were performed in at least three independent experiments with at least three biological replicates per experiment. Statistical analysis was performed using GraphPad Prism 7.0 (Graphpad Software Inc.[1]) or R. Data values are mean ± standard error of the mean (SEM). For statistical analyses, when only two conditions where compared, unpaired two tailed *t*-test was used.

RESULTS

Human Induced Pluripotent Stem Cell-Derived Cardiac Myocytes Treated With COVID-19 Candidate Treatments Show Distinct Gene Expression Patterns

Incubation of 60-day differentiated hiPSC-CMs with a range of concentrations of the drugs chosen based on literature data for 24 h under normal culture conditions did not result in cytotoxicity as assessed by MTT assay (**Figure 1A**). Next, hiPSC-CMs were treated with the drugs at selected, relevant experimental doses, which are most commonly used. Total RNA

[1]www.graphpad.com

was isolated using QIAzol Lysis Reagent and RNA samples (RNA integrity number [RIN] $\geq$ 9.6) were subjected to deep sequencing library preparation and RNA sequencing. Principal component analysis of RNA-seq data showed a clear clustering of samples by treatments (**Figure 1B**). Specifically, samples treated with LR, LRI, and Rem cluster far apart from each other as well as the control samples. Investigation of the differentially expressed genes in each candidate treatment versus vehicle control yielded noticeable differences in the number of differentially expressed genes (**Figure 1C**). The numbers of differentially expressed genes in CQ and HCQ treatments were comparable with 350–400 genes (FDR $\leq$ 0.05, $|\log_2$ fold change$| \geq$ 0.5) differentially expressed with CQ or HCQ compared to vehicle control. The number of differentially expressed genes was higher by one order of magnitude for LR, LRI, and Rem treatment compared to control; moreover, the number of upregulated and downregulated genes was balanced. We continued to specifically investigate differentially expressed genes shared between LR, LRI, and Rem. On the one hand, overall, nearly 900 genes were shared between the three treatments (**Figure 1D**). On the other hand, LR and LRI share more than 3,700 genes, with 2,054 differentially regulated genes that were specific to the LRI treatment. The number of genes specific to LR was one order of magnitude less, while around 1,000 differentially expressed genes were specific to Rem. Further inspection of the top differentially expressed genes for LR and LRI showed a strong downregulation of Caveolin-3 (CAV3) that has been implicated in the biogenesis of t-tubules (33) and moreover been shown to be associated with cardiac hypertrophy and heart failure when expression is decreased (34) (**Figures 1E,F**). On the other side of the spectrum, LR treatment showed a strong upregulation of the transcript of calcitonin gene-related peptide (CALCA), a protein that is secreted from the heart during ischemia or simulated ischemia (35, 36). LRI treatment yielded in upregulation of two genes of the 2′,5′-oligo(A) synthetase family, OAS1 and OAS2 (**Figure 1F**), which are known to be interferon-inducible (37) and thus is in line with the treatment. Interestingly, one of the top upregulated transcripts after Rem treatment is MIR4430 (**Figure 1G**), a hardly characterized microRNA that however was recently associated with other repurposed drugs to combat COVID-19 in an *in silico* study (38). A comprehensive list of significantly differentially expressed genes for each comparison is provided in **Supplementary Table 1**. Moreover, we set up an interactive web portal for visualization of differential gene expression results, which can be accessed *via* https://covid19drugs.jakobilab.org.

To gain insights into the biological basis for the differences in gene expression, we performed GO-Term enrichment analyses for each of the differential gene expression experiments and identified key pathways. While the number of differentially expressed genes is lower in CQ and HCQ when compared to LR, LRI, and Rem, pathway analyses showed significant enrichment and upregulation of GO categories of cholesterol biosynthesis, cholesterol homeostasis, and tricarboxylic acid cycle. In contrast, genes in GO categories for sarcomere organization, muscle filament sliding, and cardiac conduction were downregulated with HCQ and in part with CQ treatment (**Figures 2A,B**). Decrease of gene products in these categories may underlie reduced contractile function. Strikingly, for LR we identified a strong upregulation of the endoplasmic reticulum (ER) unfolded protein response (**Figure 2C**). During LRI treatment we observed an overall downregulation throughout all enriched GO molecular process categories. Specifically, cardiac muscle cell development and homophilic cell adhesion were strongly downregulated (**Figure 2D**). However, for both, LR and LRI the enriched GO categories showed both, up and downregulation to comparable extents, while for Rem we observed a more global trend to downregulation in the enriched GO categories, with the notable exception of the PERK-mediated branch of the unfolded protein response, which was strongly upregulated (**Figure 2E**).

Next, we performed hierarchical clustering based on the RNA-seq data from LR, LRI, and Rem treatments, mapped genes involved in these key pathways onto the heatmap, and observed a clear grouping based on treatments (**Figure 3**). Compellingly, we identified a compact cluster of around 400 genes upregulated in LRI (**Figure 3**), many of which are associated with type I interferon signaling and thus clearly differentiating between the LR and LRI treatments. Furthermore, we recognized an enrichment for upregulated genes involved in the ER stress response for LR and LRI treatments compared to control. In contrast to the ER stress response genes upregulated with LR and LRI treatments, several other key pathways showed large-scale downregulation of genes such as cardiac muscle contraction, regulation of heart contraction, sarcomere organization, and heart development (**Figure 3**). While downregulation of genes involved in these pathways was most pronounced with LRI treatment, LR treatment showed a similar trend, although with lower $\log_2$ fold changes. In comparison to LR and LRI, only few genes of these pathways show downregulation with Rem treatment. Moreover, the $\log_2$ fold changes of those genes are lower compared to LR and LRI, thus indicating a less significant contribution of Rem treatment to potentially unfavorable changes in these gene programs.

Dysregulation of Endoplasmic Reticulum Stress and Key Cardiac Function Pathways in LR and LRI-Treated Human Induced Pluripotent Stem Cell-Derived Cardiac Myocytes

To obtain further insights into the details of the altered pathways, we combined differential gene expression results and pathway structures. We performed the pathway analysis using KEGG/wikipathway due to the ability to directly integrate not only the gene names into pathways, but also directly map expression values into the graphical pathway. This way, we were able provide more information within the representative pathway as a Reactome pathway analysis could have provided. We selected a panel of 11 key ER stress response genes and profiled their expression after LR, LRI, and Rem treatment (**Figure 4A**, FDR $\leq$ 0.05, $\log_2$ fold change $\geq$ 0.5). We observed a strong upregulation of the ER stress response genes for LR and LRI that was less pronounced in Rem-treated samples. Specifically, mesencephalic astrocyte-derived neurotrophic factor (MANF) and heat shock protein family A (Hsp70) member 5 (HSPA5)

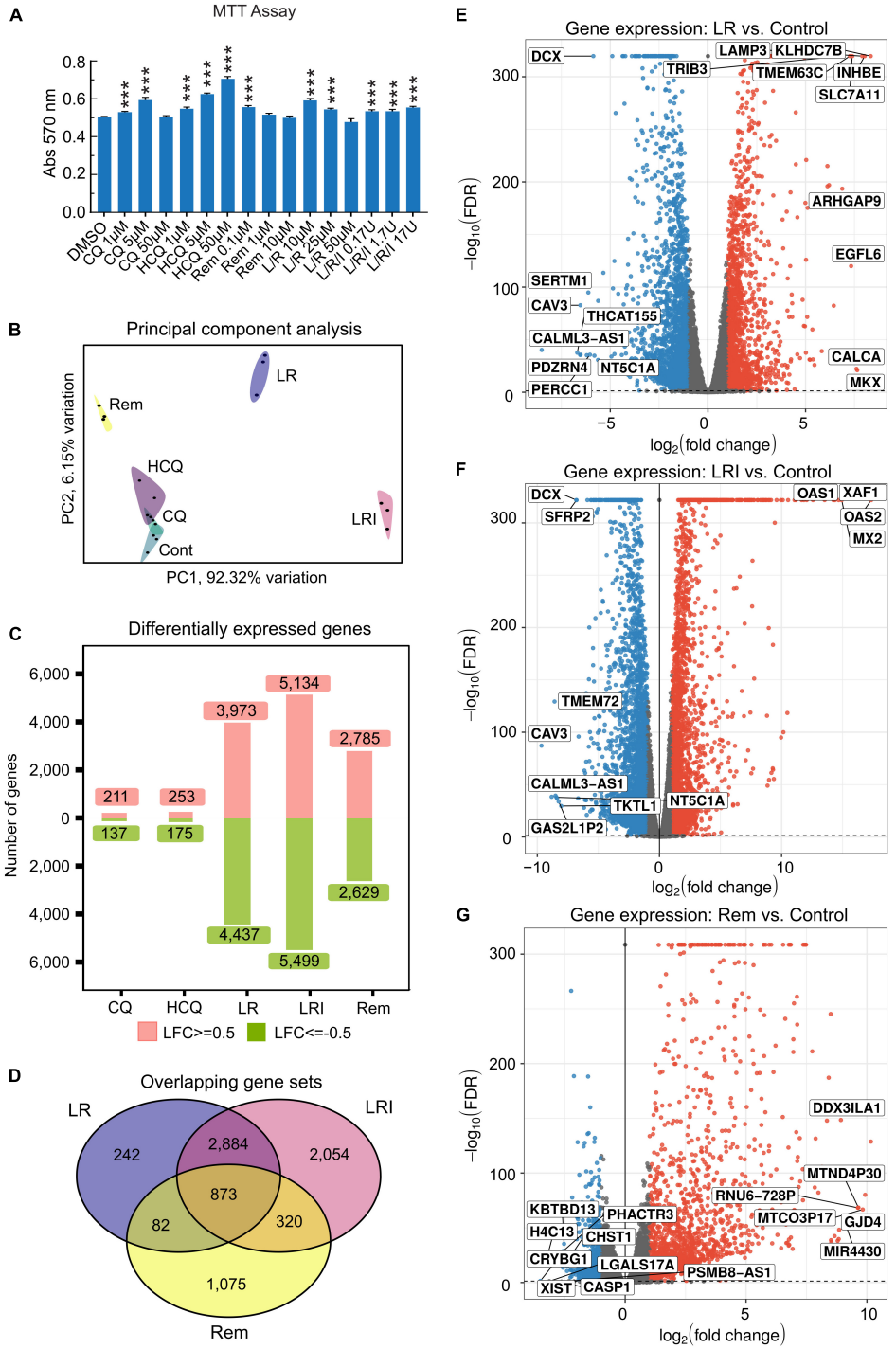


FIGURE 1 | Differences in the response of cardiac myocytes to drug treatments assessed by RNA-seq. **(A)** Various concentrations of each drug were added to hiPSC-CMs for 24 h, and cell viability was determined using the MTT assay. Absorbance at 570 nm was measured using a plate reader. All the cell viability assays were performed at least three times. L/R/I: 25 μm L/R + 0.17, 1.7, or 17U interferon. Two-group comparisons were performed using Student's two-tailed *t*-test. Data are represented as mean with all error bars indicating ± s.e.m. ***$P \leq 0.001$ compared to DMSO control. **(B)** Principal component analysis of RNA-seq data from hiPSC-CMs treated with the five drugs and control samples. CQ (blue): chloroquine; HCQ (light purple): hydroxychloroquine; LR (dark blue): lopinavir/ritonavir; LRI (pink): lopinavir/ritonavir + interferon-β; Rem (yellow): remdesivir. **(C)** Absolute numbers of differentially expressed genes downregulated (green: Log2 fold change ≤ 0.5) and upregulated (red: Log2 fold change ≥ 0.5) after treatments compared to control. **(D)** Shared differentially expressed genes between LR, LRI, and Rem. **(E,F)** Differential expression of genes shown in volcano plots in panel **(E)**, LR vs. control, in panel **(F)**, LRI vs. control, and in panel **(G)**, Rem vs. control. Blue: significantly downregulated, red: significantly upregulated, gray: not significantly expressed, FDR ≤ 0.05.

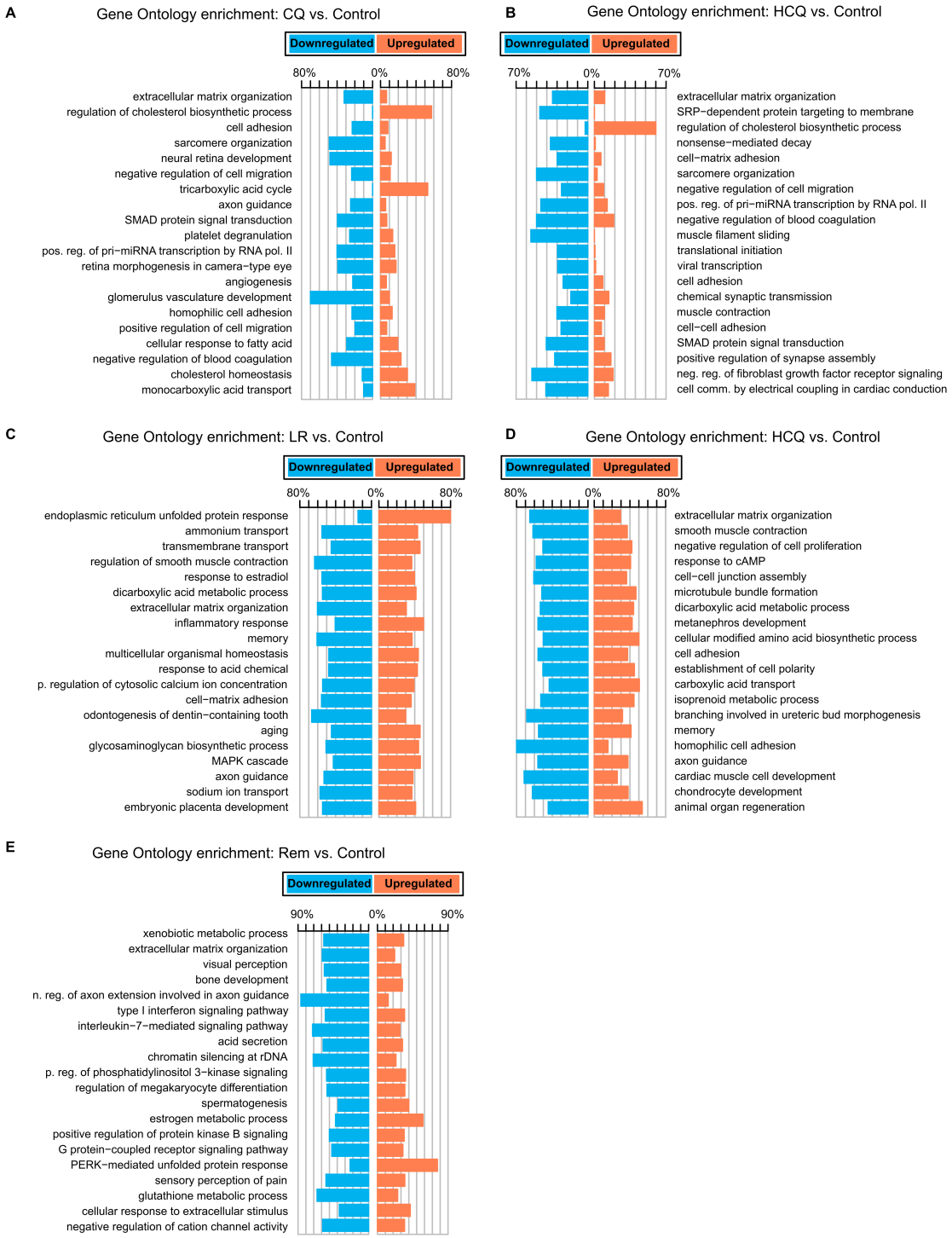


FIGURE 2 | Gene Ontology (GO) functional enrichments for candidate COVID-19 treatments. **(A)** CQ vs. control. **(B)** HCQ vs. control. **(C)** LR vs. control. **(D)** LRI vs. control. **(E)** Rem vs. control. FDR ≤ 0.05. CQ: chloroquine; HCQ: hydroxychloroquine; LR: lopinavir/ritonavir; LRI: lopinavir/ritonavir + interferon-β; Rem: remdesivir.

show significant downregulation after Rem treatment but are upregulated after LR and LRI treatment. To validate our findings, we performed qRT-PCR on a set of eight ER stress response genes and found, in line with the RNA-seq data, upregulation of nodal ER stress response regulators, such as C/EBP homologous protein (CHOP), activating transcription factor 4 (ATF4), X-box binding protein 1 (XBP1), and endoplasmic reticulum to nucleus signaling 1 (ERN1) in LR and LRI compared to control

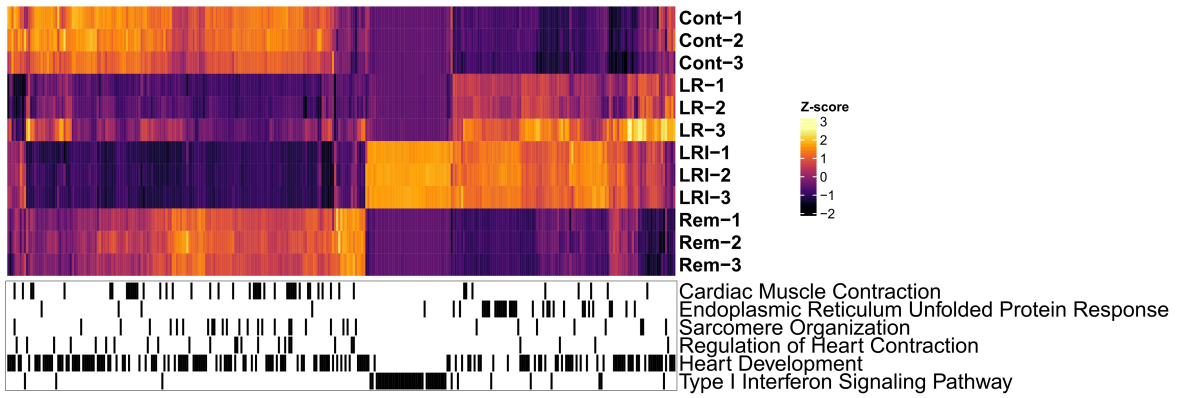


FIGURE 3 | Global map of Gene Ontology (GO) functional enrichments after LR, LRI, and Rem treatment. Heatmap based on TPM-normalized RNA-seq data (orange: high expression, purple: low expression) for differentially expressed genes between control and LR, LRI, or Rem (FDR $\leq$ 0.05, | Log2 fold change $\geq$ 0.5|). Below, black lines indicate genes associated with the respective GO category. TPM: Transcripts Per Kilobase Million. LR: lopinavir/ritonavir; LRI: lopinavir/ritonavir + interferon-β; Rem: remdesivir.

samples (**Figure 4B**). With Rem treatment, only CHOP, ATF4, and XBP1 showed upregulation, while HSPA5 and MANF were downregulated, which is in line with the RNA-seq data. Furthermore, we overlaid gene expression data with a pathway representation of the ER stress pathway and observed an upregulation of ER stress response genes at several key positions in the pathway, such as XBP1, CHOP, HSPA5, ERN1, ATF6, and PERK (**Figure 4C**). These results demonstrate dysregulation of the ER stress response with LR and LRI treatments.

Our initial global pathway analysis (**Figure 3**) showed downregulated genes in LR and LRI treated samples that were associated with cardiac muscle contraction, regulation of heart contraction, and sarcomere organization. Based on these GO term associations, we curated a list of 100 genes, profiled their expression in detail (**Figure 5A**, FDR $\leq$ 0.05, $|\log_2$ fold change$| \geq$ 0.5), and observed that 80% of these genes show decreased expression after treatment with LR and LRI, while only 20% show increased expression. In contrast, only 14 of the 100 selected genes show overall significant differential expression with Rem treatment, including the upregulated troponin T1, slow skeletal type (TNNT1), ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 1 (ATP2A1), and Myozenin-1 (MYOZ1). We validated the RNA-seq data by performing qRT-PCR for two sets of genes, comprising heavy and light myosin chains (**Figure 5B**). We observed a strong decrease in myosin heavy and light chain transcripts for LR and LRI, whereas the heavy chain transcripts were more strikingly downregulated than the light chain transcripts in LR (**Figure 5B**). In LRI-treated cells, we observed a strong decrease in expression of both types of myosin chains. In contrast, Rem treatment significantly decreased MYL3 and MYL4, while MYL2 and MYH7 were only slightly, but significantly decreased, and MYH6 levels were unchanged compared to control (**Figure 5B**).

We subsequently examined a transcript panel of troponins, tropomyosins, and cardiac muscle alpha actin and found a general downregulation after LR and LRI treatment with a striking effect on cardiac actin (ACTC1, **Figure 5C**). In contrast, Rem treatment resulted in upregulation of tropomyosin 1 and 2 (TPM1, TPM2), while TNNC1 (troponin C1, slow skeletal and cardiac type) and TNNT2 (troponin T2, cardiac type) were slightly but significantly downregulated and ACTC1 and troponin I3, cardiac type (TNNI3) were significantly downregulated compared to vehicle control. These findings suggest significant negative effects of LR and LRI on contractile components of cardiac myocytes.

DISCUSSION

In this study we evaluated the effects of the five drugs repurposed to treat COVID-19 on hiPSC-CMs. Currently, little is known about the effect of the treatments on different tissues and cells, including cardiac cells. Although there have been studies addressing the effects of treatment with CQ (39), HCQ (40), and LR (41, 42) *in vitro* in different cell lines, to the best of our knowledge no assessment of the effects of CQ, HCQ, LR, LRI, and Rem on cardiac cells has been performed. Here, we provide a detailed analysis of the transcriptional changes in hiPSC-CMs after treatment with the five treatments compared to controls.

Chloroquine and Hydroxychloroquine Treatments

The anti-malarial activity of CQ is mainly attributed to the diffusion of CQ into lysosomes, which neutralizes the pH and becomes trapped in lysosomes by protonation, thus resulting in the inhibition of normal autophagy activity (43). Moreover, CQ is also used as adjuvant in several cancer therapy trials which employ the autophagy inhibiting properties of CQ. In this context, a recent study revealed a role for cholesterol biosynthesis in maintaining lysosomal integrity under stress; since inhibiting autophagy interferes with processing extracellularly derived cholesterol esters, thus making cells dependent on the cholesterol biosynthesis pathway (44). Moreover, lysosomal membrane cholesterol decreases permeability to water and ions which

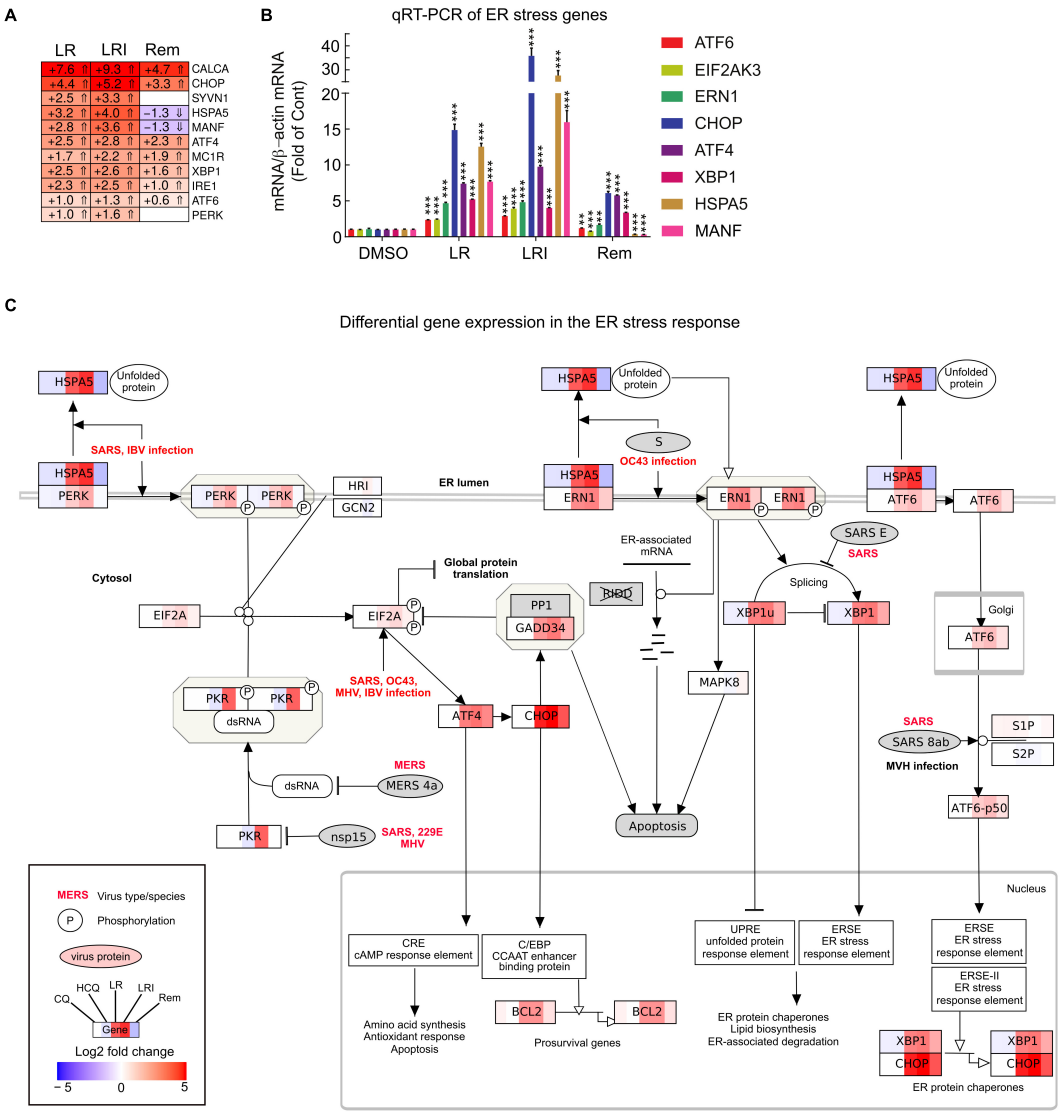


FIGURE 4 | Expression of ER stress associated genes with LR, LRI, and Rem treatment. **(A)** Clustered heatmap of a panel of selected ER stress associated genes showing the calculated Log2 fold change after LR, LRI, and Rem treatment compared to control (FDR ≤ 0.05, | Log2 fold change| ≥ 0.5). LR: lopinavir/ritonavir; LRI: lopinavir/ritonavir + interferon-β; Rem: remdesivir. **(B)** Gene expression was determined by qRT-PCR using RNA from hiPSC-CMs plated at 1×10^6 cells/well on 6-well culture dishes and treated for 24 h with LR, LRI, Rem, or vehicle control (DMSO). **(C)** Mapping of differential gene expression data onto a representation of the ER stress response pathway. Each box contains a color-coded representation of the Log2 fold change of the gene in the following sample order: CQ, HCQ, LR, LRI, Rem. CQ: chloroquine; HCQ: hydroxychloroquine. Two-group comparisons were performed using Student's two-tailed *t*-test. Data are represented as mean with all error bars indicating ± s.e.m. $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$ compared to DMSO control.

suppresses swelling and destabilization under osmotic stress. Intriguingly, in our study, we observed that with CQ and HCQ treatment, most of the enriched pathways are downregulated, except for the regulation of the cholesterol biosynthetic process, where CQ- and HCQ-treated samples show strong upregulation for key genes. We found key enzymes for cholesterol synthesis, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR), hydroxymethylglutaryl-CoA synthase (HMGC1), farnesyl pyrophosphate synthase (FDPS), and farnesyl-diphosphate farnesyltransferase 1 (FDFT1) robustly upregulated with CQ and HCQ treatment. Thus, in line with a previous report (44), our findings may indicate that CQ-treated cells upregulate the cholesterol biosynthesis in order to compensate for reduced processing of cholesterol esters and to counteract CQ-toxicity by adapting the cholesterol biosynthesis pathway. Nevertheless, CQ or HCQ may lead to lower levels of cholesterol, as HCQ was recently found to lower total cholesterol in a large study of patients with systemic lupus erythematosus and rheumatoid arthritis, where CQ and HCQ are common treatment options (45). In contrast, low cholesterol concentrations in COVID-19 patients have recently been linked to more severe outcomes (46, 47). However, due to the short duration of treatment of

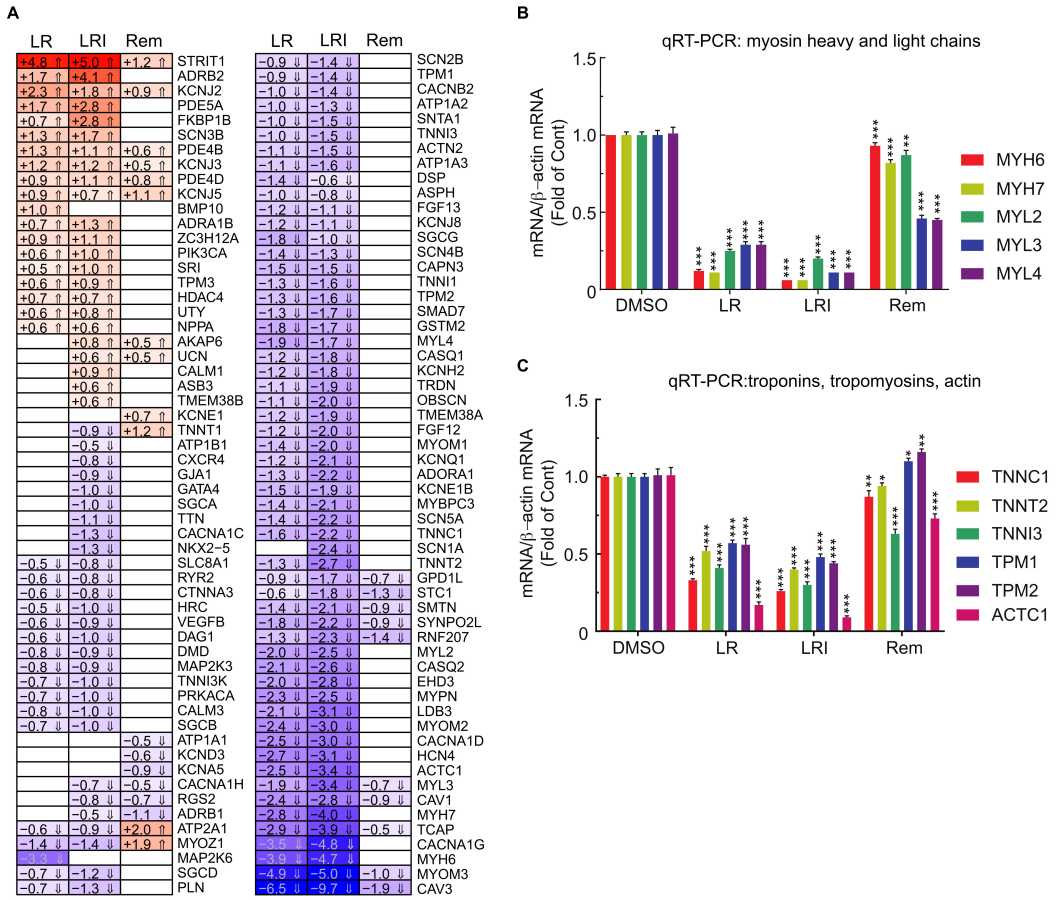


FIGURE 5 | Calcium handling-associated genes and genes associated with sarcomere organization with LR, LRI, and Rem treatment. **(A)** Clustered heatmap of a panel of 100 selected genes associated with calcium handling and sarcomere organization showing the calculated Log2 fold change after LR, LRI, and Rem treatment compared to control (FDR $\leq$ 0.05, |Log2 fold change| $\geq$ 0.5). LR: lopinavir/ritonavir; LRI: lopinavir/ritonavir + interferon-β; Rem: remdesivir. **(B)** Expression of myosin light and heavy chains was determined by qRT-PCR using total RNA from hiPSC-CMs plated at 1 $\times$ 10^6 cells/well on 6-well culture dishes and treated for 24 h with LR, LRI, or Rem. **(C)** Expression of selected troponin, tropomyosin, and actin genes was determined by qRT-PCR using total RNA from hiPSC-CMs plated at 1 $\times$ 10^6 cells/well on 6-well culture dishes and treated for 24 h with LR, LRI, or Rem. Two-group comparisons were performed using Student's two-tailed *t*-test. Data are represented as mean with all error bars indicating $\pm$ s.e.m. $^*P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$ compared to DMSO control.

acute COVID-19 patients compared to long term patients with rheumatoid arthritis treated with CQ or HCQ, the cholesterol-lowering effects might not play a role in progression of COVID-19 in these patients.

The autophagy-inhibiting properties of CQ were found to affect several cellular processes such as bioenergetics (48). Experiments in primary rat cortical neurons showed that the inhibition of autophagy by CQ increased mitochondrial DNA damage and at the same time decreased bioenergetics (48). CQ was found to reduce glycolysis activity, as well as decrease intermediate products of the tricarboxylic acid (TCA) cycle and key components of glutaminolysis (48). In our study, we observed an upregulation of genes enriched for TCA activity with CQ treatment. Indeed, this upregulation may be consistent with an upregulation of glutaminolysis itself as it was previously found that succinate, fumarate, and malate were not affected by CQ-treatment, thus hinting at adaptive changes of the TCA cycle (48). Therefore, our study may contribute to uncovering the transcriptional changes of the TCA cycle components upon treatment with autophagy inhibitors such as CQ or HCQ.

LR and LRI Treatments

Treatment of hiPSC-CMs with LR or LRI showed significant changes in key cardiac gene programs such as Ca^{2+} handling and sarcomere organization. The downregulation of several key genes encoding subunits of cardiac ion channels we observed such as KCNQ1, KCNH2, and CACNA1C, is in line with findings that show QT prolongation and torsade de pointes in patients treated with protease inhibitors (PI) (49), such as lopinavir and ritonavir. Moreover, the strict downregulation of other key categories of cardiac function such as cardiac muscle cell development with LR and LRI is coordinate with a recent study describing risks of bradycardia with LR treatment for COVID-19 patients (50). Similarly, we observed perturbations in the regulation of PP1, a critical regulator of cardiac function that mediates restoration of contractility to basal levels after beta-adrenergic stimulation.

Dysregulation of PP1, in turn, has been suggested to contribute to impaired function of the heart (51), thus highlighting unfavorable effects of LR and LRI treatment on hiPSC-CMs.

Recent guidelines recommend the use of low- to moderate-dose statins for patients with one or more CVD risk factor as preemptive measure (52). However, treatment with statins together with LR or LRI, should be implemented with selected statins such as pitavastatin or pravastatin due to known drug-drug interactions between statins and antiviral PIs (53), thus requiring special attention for COVID-19 patients on statins.

When comparing LR and LRI, we observed specific effects of INF-β as sharply defined enrichment of genes specifically associated in the type I interferon response for LRI. The interferon response employs double-stranded RNA-activated protein kinase (PKR) to reduce viral replication *via* phosphorylation of eIF2α and subsequent reduction of protein synthesis (54). Interestingly, genes induced by INF-β were shown to be associated with ER stress (55) and while in general we observed that the addition of INF-β increased the effects of LR treatment on gene expression, this was especially pronounced for key genes of the ER stress response, such as CHOP, which has been shown to play a role at the interface between ER stress and CVD (56). While the ER stress response exerts an initial protective effect, the strength and duration of activation of the ER stress response will determine if the response will eventually switch to the proapoptotic phase and yield to cell death (56). Alterations in protein folding demand, such as those that occur during cardiac ischemia, hypertrophy, and remodeling, result in homeostatic imbalance in ER, causing activation of the ER stress response and thus induction of translation inhibition and gene expression tailored to stress conditions in the ER (57). We and others have shown that in cardiovascular disease, stresses such as oxidative stress or hypoxia can perturb ER homeostasis and activate the ER stress response, which induces expression of proteins that can function to protect the myocardium (58). Both, the interferon response and ER stress response are known to be activated during viral infection and are linked by the phosphorylation of the α subunit of translation initiation factor eIF-2 (eIF2α) (59, 60), which results in inhibition of synthesis of viral proteins. The upregulation of the ER stress response genes in LR-treated cells, in addition to the ER stress response the viral infection itself, may negatively affect the replication of the virus. This effect might further be enhanced by IFN-β treatment as part of the LRI regime, adding the burden of the activation of the interferon response to the already activated ER stress response. Taken together, our results show elevated expression of key ER stress response genes in LR and even more pronounced in LRI that might result in cell death depending on the duration of LR/LRI treatment.

The activation of the ER stress response caused by PIs was studied in the context of the HIV/AIDS, where PIs are routinely employed as treatment option. While long-term studies for HIV treatment and prevention with LR report general long-term safety (61), cardio-metabolic side effects in the heart have been reported (62). Specifically, lopinavir, which has been shown to induce the highest levels of ER stress amongst PIs (41), may cause adverse effects that have been observed in macrophages (63) and hepatocytes (64). Moreover, clinical studies linked ER stress-associated diseases like metabolic syndrome to patients administered PIs over longer terms (41). Thus, the combination of potential adverse effects and unfavorable dysregulation of key cardiac gene programs after LR and LRI treatment likely outweigh negative effects of LR and LRI on viral infection. Besides dysregulation of critical cardiac gene programs, we observed significant dysregulation of several G protein-coupled receptor-associated (GPCR) genes which in turn might lead to different cardiovascular pathologies, such as hypertrophic and fibrotic remodeling of left and right cardiac ventricles and systemic and pulmonary hypertension (65).

Remdesivir Treatment

In hiPSC-CMs treated with Rem, we detected a slight, but significant upregulation of some of the ER stress response genes, specifically CHOP, ATF4, and XBP1. Interestingly, we found MANF is significantly downregulated in Rem compared to LR, LRI, and even vehicle-treated control, even though ATF6, a known inducer of MANF (66) is significantly upregulated after Rem treatment. Similarly, HSPA5, a key chaperon in the ER stress response is also significantly downregulated in Rem. Interestingly, HSPA5 which encodes the GRP78 protein, has been identified as a potential target for the treatment of Ebola virus (67) and is currently discussed as a potential treatment for COVID-19 (68, 69). Thus, the downregulation of HSPA5 might be one cause for the more promising results of treating COVID-19 with Rem such as the Adaptive COVID-19 Treatment Trial (ACTT-1) found that patients receiving Rem treatment tend to profit from a significantly shorter time to recovery compared to placebo (70). Available data on the effect of Rem on the heart is limited to a single study of 681 patients infected with the Ebola virus, where one of the patients suffered hypotension and subsequent death by cardiac arrest (71), which might be in line with our observation that several genes associates with hypotension show dysregulation, however, the effect of LR and LRI is more pronounced.

CONCLUSION

In summary, the results of this study suggest that all tested repurposed drugs show alterations of the transcriptional profiles of hiPSC-CMs. While the changes to gene programs with CQ and HCQ treatment are less pronounced, we identified widespread unfavorable dysregulation of cardiac gene programs such as calcium handling, sarcomere organization, hypotension and GPCR activity specifically for LR and LRI. The induction of the ER stress response may on the one hand be able to aid in slowing down viral replication, but on the other hand also add adverse effects to the dysregulated gene programs. While treatment of hiPSC-CMs with Rem also induced changes in gene expression, the effect on the cardiac gene programs affected by LR and LRI is significantly more pronounced. Taken together, our results suggest that only Rem displays a fair balance between negative

effects on transcriptional profiles of hiPSC-CMs and potential antiviral activity.

DATA AVAILABILITY STATEMENT

Raw sequencing data are available at the National Center for Biotechnology Information Sequence Read Archive (PRJNA666773). Differential gene expression data is available online in interactive form via https://covid19drugs.jakobilab.org. All other data, methods, and materials that support the findings of this study are available from the corresponding authors on request.

AUTHOR CONTRIBUTIONS

TJ and SD: conceptualization, writing–review and editing, and funding acquisition. TJ: data curation, software, writing–original draft preparation, and visualization. TJ, JG, LC and SD: investigation. All authors have read and agreed to the published version of the manuscript.

FUNDING

This study was supported by the DZHK (German Centre for Cardiovascular Research).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://covid19drugs.jakobilab.org and https://www.frontiersin.org/articles/10.3389/fcvm.2022.844441/full#supplementary-material

Supplementary Table 1 | Differential gene expression results as Excel file. CQ vs. Control, HCQ vs. Control, LR vs. Control, LRI vs. Control, and Rem vs. Control.

REFERENCES

1. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis.* (2020) 20:533–4. doi: 10.1016/S1473-3099(20)30120-1
2. Warren Jordan TK, Jordan R, Lo MK, Ray AS, Mackman RL, Soloveva V, et al. Therapeutic efficacy of the small molecule GS-5734 against ebola virus in rhesus monkeys. *Nature.* (2016) 531:381–5. doi: 10.1038/nature17180
3. Cao Y, Deng Q, Dai S. Remdesivir for severe acute respiratory syndrome coronavirus 2 causing COVID-19: an evaluation of the evidence. *Travel Med Infect Dis.* (2020) 35:101647. doi: 10.1016/j.tmaid.2020.101647
4. de Wit E, Feldmann F, Cronin J, Jordan R, Okumura A, Thomas T, et al. Prophylactic and therapeutic remdesivir (GS-5734) treatment in the rhesus macaque model of MERS-CoV infection. *Proc Natl Acad Sci USA.* (2020) 117:6771–6. doi: 10.1073/pnas.1922083117
5. Sham HL, Kempf DJ, Molla A, Marsh KC, Kumar GN, Chen CM, et al. ABT-378, a highly potent inhibitor of the human immunodeficiency virus protease. *Antimicrob Agents Chemother.* (1998) 42:3218–24. doi: 10.1128/AAC.42.12.3218
6. Chu CM, Cheng VC, Hung IF, Wong MM, Chan KH, Chan KS, et al. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. *Thorax.* (2004):252–6. doi: 10.1136/thorax.2003.012658
7. de Wilde AH, Jochmans D, Posthuma CC, Zevenhoven-Dobbe JC, van Nieuwkoop S, Bestebroer TM, et al. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of middle East respiratory syndrome coronavirus replication in cell culture. *Antimicrob Agents Chemother.* (2014) 58:4875–84. doi: 10.1128/AAC.03011-14
8. Chan Yao JFW, Yao Y, Yeung ML, Deng W, Bao L, Jia L, et al. Treatment with lopinavir/ritonavir or interferon-β1b improves outcome of MERS-CoV infection in a nonhuman primate model of common marmoset. *J Infect Dis.* (2015) 212:1904–13. doi: 10.1093/infdis/jiv392
9. Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, et al. *In vitro* antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *Clin Infect Dis.* (2020) 9:ciaa237. doi: 10.1093/cid/ciaa237
10. McChesney EW. Animal toxicity and pharmacokinetics of hydroxychloroquine sulfate. *Am J Med.* (1983) 75:11–8. doi: 10.1016/0002-9343(83)91265-2
11. Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection *in vitro*. *Cell Discov.* (2020) 6:16. doi: 10.1038/s41421-020-0156-0
12. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell.* (2020)181:271–280.e8. doi: 10.1016/j.cell.2020.02.052
13. Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol.* (2004) 203:631–7. doi: 10.1002/path.1570
14. Clerkin Kevin J, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A, et al. COVID-19 and cardiovascular disease. *Circulation.* (2020) 141:1648–55. doi: 10.1161/CIRCULATIONAHA.120.046941
15. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese center for disease control and prevention. *JAMA.* (2020) 323:1239–42. doi: 10.1001/jama.2020.2648
16. Omidi F, Hajikhani B, Kazemi SN, Tajbakhsh A, Riazi S, et al. COVID-19 and Cardiomyopathy: a Systematic Review. *Front Cardiovasc Med.* (2021) 8:695206. doi: 10.3389/fcvm.2021.695206
17. Puntmann VO, et al. Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:1265–73. doi: 10.1001/jamacardio.2020.3557
18. Rössler U, Hennig AF, Stelzer N, Bose S, Kopp J, Søe K, et al. Efficient generation of osteoclasts from human induced pluripotent stem cells and functional investigations of lethal CLCN7-related osteopetrosis. *J Bone Miner Res.* (2021) 36:1621–35. doi: 10.1002/jbmr.4322
19. Kleinsorge M, Cyganek L. Subtype-directed differentiation of human iPSCs into atrial and ventricular cardiomyocytes. *STAR Protoc.* (2020) 1:100026. doi: 10.1016/j.xpro.2020.100026
20. Tohyama S, Hattori F, Sano M, Hishiki T, Nagahata Y, Matsuura T, et al. distinct metabolic flow enables large-scale purification of mouse and human pluripotent stem cell-derived cardiomyocytes. *Cell Stem Cell.* (2013) 12:127–37. doi: 10.1016/j.stem.2012.09.013
21. Iwai-Kanai E, Yuan H, Huang C, Sayen MR, Perry-Garza CN, Kim L, et al. A method to measure cardiac autophagic flux *in vivo*. *Autophagy.* (2008) 4:322–9. doi: 10.4161/auto.5603
22. Choi SW, Shin JS, Park SJ, Jung E, Park YG, Lee J, et al. Antiviral activity and safety of remdesivir against SARS-CoV-2 infection in human pluripotent stem cell-derived cardiomyocytes. *Antiviral Res.* (2020) 184:104955. doi: 10.1016/j.antiviral.2020.104955
23. Lan X, Kiyota T, Hanamsagar R, Huang Y, Andrews S, Peng H, et al. The effect of HIV protease inhibitors on amyloid-β Peptide degradation and synthesis in human cells and Alzheimer's disease animal model. *J Neuroimmune Pharmacol.* (2012) 7:412. doi: 10.1007/s11481-011-9304-5
24. Roehr JT, Dieterich C, Reinert K. Flexbar 3.0 – SIMD and multicore parallelization. *Bioinformatics.* (2017) 33:2941–2. doi: 10.1093/bioinformatics/btx330

25. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods.* (2012) 9:357–60. doi: 10.1038/nmeth.1923
26. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics.* (2013) 29:15–21. doi: 10.1093/bioinformatics/bts635
27. Liao Y, Smyth GK, Shi W. The subread aligner: fast, accurate and scalable read mapping by seed-and-vote. *Nucleic Acids Res.* (2013) 41:e108. doi: 10.1093/nar/gkt214
28. Ewels P, Magnusson M, Lundin S, Käller M. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics.* (2016) 32:3047–8. doi: 10.1093/bioinformatics/btw354
29. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics.* (2009) 26:139–40. doi: 10.1093/bioinformatics/btp616
30. van Iersel MP, Kelder T, Pico AR, Hanspers K, Coort S, Conklin BR, et al. Presenting and exploring biological pathways with path visio. *BMC Bioinformatics.* (2008) 9:399. doi: 10.1186/1471-2105-9-399
31. Slenter DN, Kutmon M, Hanspers K, Riutta A, Windsor J, Nunes N, et al. WikiPathways: a multifaceted pathway database bridging metabolomics to other omics research. *Nucleic Acids Res.* (2018) 46:D661–7. doi: 10.1093/nar/gkx1064
32. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics.* (2016) 32:2847–9. doi: 10.1093/bioinformatics/btw313
33. Parton RG, Way M, Zorzi N, Stang E. Caveolin-3 associates with developing T-tubules during muscle differentiation. *J Cell Biol.* (1997) 136:137–54. doi: 10.1083/jcb.136.1.137
34. Bryant SM, Kong CHT, Watson JJ, Gadeberg HC, James AF, Cannell MB, et al. Caveolin 3-dependent loss of t-tubular ICa during hypertrophy and heart failure in mice. *Exp Physiol.* (2018) 103:652–65. doi: 10.1113/EP086731
35. Chai W, Mehrotra S, Jan Danser AH, Schoemaker RG. The role of calcitonin gene-related peptide (CGRP) in ischemic preconditioning in isolated rat hearts. *Eur J Pharmacol.* (2006) 531:246–53. doi: 10.1016/j.ejphar.2005.12.039
36. Doroudgar S, Glembotski CC. The cardiokine story unfolds: ischemic stress-induced protein secretion in the heart. *Trends Mol Med.* (2011) 17:207–14. doi: 10.1016/j.molmed.2010.12.003
37. Benech P, Vigneron M, Peretz D, Revel M, Chebath J. Interferon-responsive regulatory elements in the promoter of the human 2',5'-oligo(A) synthetase gene. *Mol Cell Biol.* (1987) 7:4498–504. doi: 10.1128/mcb.7.12.4498-4504.1987
38. Adhami M, Sadeghi B, Rezapour A, Haghdoost AA, MotieGhader H. Repurposing novel therapeutic candidate drugs for coronavirus disease-19 based on protein-protein interaction network analysis. *BMC Biotechnol.* (2021) 21:22. doi: 10.1186/s12896-021-00680-z
39. Blignaut M, Espach Y, van Vuuren M, Dhanabalan K, Huisamen B. Revisiting the cardiotoxic effect of chloroquine. *Cardiovasc Drugs Ther.* (2019) 33:1–11. doi: 10.1007/s10557-018-06847-9
40. Capel RA, Herring N, Kalla M, Yavari A, Mirams GR, Douglas G, et al. Hydroxychloroquine reduces heart rate by modulating the hyperpolarization-activated current If: novel electrophysiological insights and therapeutic potential. *Heart Rhythm.* (2015) 12:2186–94. doi: 10.1016/j.hrthm.2015.05.027
41. Taura M, Kariya R, Kudo E, Goto H, Iwawaki T, Amano M, et al. Comparative analysis of ER stress response into HIV protease inhibitors: lopinavir but not darunavir induces potent ER stress response *via* ROS/JNK pathway. *Free Radic Biol Med.* (2013) 65:778–88. doi: 10.1016/j.freeradbiomed.2013.08.161
42. Wakabayashi Y, Yoshino Y, Seo K, Koga I, Kitazawa T, Ota Y. Inhibition of osteoblast differentiation by ritonavir. *Biomed Rep.* (2018) 9:491–6. doi: 10.3892/br.2018.1154
43. Boya P, Gonzalez-Polo RA, Poncet D, Andreau K, Vieira HL, Roumier T, et al. Mitochondrial membrane permeabilization is a critical step of lysosome-initiated apoptosis induced by hydroxychloroquine. *Oncogene.* (2003) 22:3927–36. doi: 10.1038/sj.onc.1206622
44. King MA, Ganley IG, Flemington V. Inhibition of cholesterol metabolism underlies synergy between mTOR pathway inhibition and chloroquine in bladder cancer cells. *Oncogene.* (2016) 35:4518–28. doi: 10.1038/onc.2015.511
45. Jorge A, Lu N, Choi H, Esdaile JM, Lacaille D, vina-Zubieta JAA. Hydroxychloroquine use and cardiovascular events among patients with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Care Res.* (2021). doi: 10.1002/acr.24850
46. Zinellu A, Paliogiannis P, Fois AG, Solidoro P, Carru C, Mangoni AA. Cholesterol and triglyceride concentrations, COVID-19 Severity, and mortality: a systematic review and meta-analysis with meta-regression. *Front Public Health.* (2021) 9:1210. doi: 10.3389/fpubh.2021.705916
47. Masana L, Correig E, Capdevila E, Gargallo E, González N, Nadal M, et al. Low HDL and high triglycerides predict COVID-19 severity. *Sci Rep.* (2021) 11:7217. doi: 10.1038/s41598-021-86747-5
48. Redmann M, Benavides GA, Berryhill TF, Wani WY, Ouyang X, Johnson MS, et al. Inhibition of autophagy with bafilomycin and chloroquine decreases mitochondrial quality and bioenergetic function in primary neurons. *Redox Biol.* (2017) 11:73–81. doi: 10.1016/j.redox.2016.11.004
49. Anson BD, Weaver JG, Ackerman MJ, Akinsete O, Henry K, January CT, et al. Blockade of HERG channels by HIV protease inhibitors. *Lancet.* (2005) 365:682–6. doi: 10.1016/S0140-6736(05)17950-1
50. Beyls C, Martin N, Hermida A, Abou-Arab O, Mahjoub Y. Lopinavir-ritonavir treatment for COVID-19 infection in intensive care unit. *Circ Arrhythm Electrophysiol.* (2020) 13:e008798. doi: 10.1161/CIRCEP.120.008798
51. Nicolaou P, Kranias EG. Role of PP1 in the regulation of Ca cycling in cardiac physiology and pathophysiology. *Front Biosci.* (2009) 14:3571–85. doi: 10.2741/3472
52. Us Preventive Services Task Force, Bibbins-Domingo K, Grossman DC, Curry SJ, Davidson KW, Epling JW Jr., et al. Statin use for the primary prevention of cardiovascular disease in adults: US preventive services task force recommendation statement. *JAMA.* (2016) 316:1997–2007. doi: 10.1001/jama.2016.15450
53. Chauvin B, Drouot S, Barrail-Tran A, Taburet A-M. Drug–drug interactions between HMG-CoA reductase inhibitors (Statins) and antiviral protease inhibitors. *Clin Pharmacokinet.* (2013) 52:815–31. doi: 10.1007/s40262-013-0075-4
54. Gale M, Katze MG. Molecular mechanisms of interferon resistance mediated by viral-directed inhibition of PKR, the interferon-induced protein kinase. *Pharmacol Ther.* (1998) 78:29–46. doi: 10.1016/S0163-7258(97)00165-4
55. Sprooten J, Garg AD. Type I interferons and endoplasmic reticulum stress in health and disease. *Int Rev Cell Mol Biol.* (2020) 350:63–118. doi: 10.1016/bs.ircmb.2019.10.004
56. Glembotski Christopher C. Endoplasmic reticulum stress in the heart. *Circ Res.* (2007) 101:975–84. doi: 10.1161/CIRCRESAHA.107.161273
57. Doroudgar S, Thuerauf DJ, Marcinko MC, Belmont PJ, Glembotski CC. Ischemia activates the ATF6 branch of the endoplasmic reticulum stress response. *J Biol Chem.* (2009) 284:29735–45. doi: 10.1074/jbc.M109.018036
58. Doroudgar S, Glembotski CC. New concepts of endoplasmic reticulum function in the heart: programmed to conserve. *J Mol Cell Cardiol.* (2013) 55:85–91. doi: 10.1016/j.yjmcc.2012.10.006
59. He B. Viruses, endoplasmic reticulum stress, and interferon responses. *Cell Death Differ.* (2006) 13:393–403. doi: 10.1038/sj.cdd.4401833
60. Fung TS, Liu DX. Human coronavirus: host-pathogen interaction. *Annu Rev Microbiol.* (2019) 73:529–57. doi: 10.1146/annurev-micro-020518-115759
61. Rudin C, Burri M, Shen Y, Rode R, Nadal D, Pediatric Infectious Disease Group of Switzerland, et al., "Long-term safety and effectiveness of ritonavir, nelfinavir, and lopinavir/ritonavir in antiretroviral-experienced HIV-infected children. *Pediatr Infect Dis J.* (2008) 27:431–7. doi: 10.1097/INF.0b013e3181646d5a
62. Reyskens KMSE, Fisher T-L, Schisler JC, O'Connor WGO, Rogers AB, Willis MS, et al. Cardio-metabolic effects of HIV protease inhibitors (lopinavir/ritonavir). *PLoS One.* (2013) 8:e73347. doi: 10.1371/journal.pone.0073347
63. Zhou H, Jarujaron S, Emily C, Gurley EC, Chen L, Ding H, et al. HIV protease inhibitors increase TNF-α and IL-6 expression in macrophages: involvement of the RNA-binding protein HuR. *Atherosclerosis.* (2007) 195:e134–43. doi: 10.1016/j.atherosclerosis.2007.04.008
64. Cao R, Hu Y, Wang Y, Gurley EC, Studer EJ, Wang X, et al. Prevention of HIV protease inhibitor-induced dysregulation of hepatic lipid metabolism by raltegravir *via* endoplasmic reticulum stress signaling pathways. *J Pharmacol Exp Ther.* (2010) 334:530–9. doi: 10.1124/jpet.110.168484
65. Fernandez-Patron C, Filep JG. GPCRs in cardiovascular pathologies. *Drug Discov Today Dis Mech.* (2012) 9:e75–8. doi: 10.1016/j.ddmod.2012.07.001

66. Belmont PJ, et al. Coordination of growth and endoplasmic reticulum stress signaling by regulator of Calcineurin 1 (RCAN1), a novel ATF6-inducible gene. *J Biol Chem*. (2008) 283:14012–21. doi: 10.1074/jbc.M709776200
67. Patrick Reid St, Shurtleff AC, Costantino JA, Tritsch SR, Retterer C, Spurgers KB, et al. HSPA5 is an essential host factor for ebola virus infection. *Antiviral Res.* (2014) 109:171–4. doi: 10.1016/j.antiviral.2014.07.004
68. Ha DP, Van Krieken R, Carlos AJ, Lee AS. The stress-inducible molecular chaperone GRP78 as potential therapeutic target for coronavirus infection. *J Infect.* (2020) 81:452–82. doi: 10.1016/j.jinf.2020.06.017
69. Ibrahim IM, Abdelmalek DH, Elshahat ME, Elfiky AA. COVID-19 spike-host cell receptor GRP78 binding site prediction. *J Infect.* (2020) 80:554–62. doi: 10.1016/j.jinf.2020.02.026
70. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the treatment of covid-19 – final report. *N Engl J Med.* (2020) 383:1813–26. doi: 10.1056/NEJMoa2007764
71. Aggarwal G, Henry BM, Aggarwal S, Bangalore S. Cardiovascular safety of potential drugs for the treatment of coronavirus disease 2019. *Am J Cardiol.* (2020) 128:147–50. doi: 10.1016/j.amjcard.2020.04.054

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 17 June 2022
doi: 10.3389/fcvm.2022.876718

Colchicine for the Treatment of Cardiac Injury in Hospitalized Patients With Coronavirus Disease-19

Amir Rabbani[1], Asim Rafique[1], Xiaoyan Wang[2], Danielle Campbell[1], Daniel Wang[1], Nicholas Brownell[1], Kenia Capdevilla[3], Victoria Garabedian[3], Sandra Chaparro[3], Raul Herrera[3], Rushi V. Parikh[1] and Reza Ardehali[1]*

[1] Division of Cardiology, University of California, Los Angeles, Los Angeles, CA, United States, [2] Division of General Internal Medicine and Health Services Research, University of California, Los Angeles, Los Angeles, CA, United States, [3] Miami Cardiac and Vascular Institute, Baptist Health South Florida, Miami, FL, United States

OPEN ACCESS

Edited by:
Hendrik Tevaearai Stahel, Bern University Hospital, Switzerland

Reviewed by:
Spyridon Deftereos, National and Kapodistrian University of Athens, Greece
Diego Vinicius Santinelli Pestana, Brigham and Women's Hospital and Harvard Medical School, United States

***Correspondence:**
Amir Rabbani
arabbani@mednet.ucla.edu

Specialty section:
This article was submitted to General Cardiovascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

Received: *15 February 2022*
Accepted: *24 May 2022*
Published: *17 June 2022*

Citation:
Rabbani A, Rafique A, Wang X, Campbell D, Wang D, Brownell N, Capdevilla K, Garabedian V, Chaparro S, Herrera R, Parikh RV and Ardehali R (2022) Colchicine for the Treatment of Cardiac Injury in Hospitalized Patients With Coronavirus Disease-19. Front. Cardiovasc. Med. 9:876718. doi: 10.3389/fcvm.2022.876718

Introduction: The impact of colchicine on hospitalized patients with Coronavirus disease-19 (COVID-19) related cardiac injury is unknown.

Materials and Methods: In this multicenter randomized controlled open-label clinical trial, we randomized hospitalized adult patients with documented COVID-19 and evidence of cardiac injury in a 1:1 ratio to either colchicine 0.6 mg po twice daily for 30 days plus standard of care or standard of care alone. Cardiac injury was defined as elevated cardiac biomarkers, new arrhythmia, new/worsened left ventricular dysfunction, or new pericardial effusion. The primary endpoint was the composite of all-cause mortality, need for mechanical ventilation, or need for mechanical circulatory support (MCS) at 90 days. Key secondary endpoints included the individual components of the primary endpoint and change in and at least 2-grade reduction in the World Health Organization (WHO) Ordinal Scale at 30 days. The trial is registered with clinicaltrials.gov (NCT04355143).

Results: We enrolled 93 patients, 48 patients in the colchicine arm and 45 in the control arm. There was no significant difference in the primary outcome between the colchicine and control arms (19 vs. 15%, $p = 0.78$), nor in the individual components of all-cause mortality (17 vs. 15%, $p = 1.0$) and need for mechanical ventilation (8 vs. 5%, $p = 0.68$); no patients in either group required MCS. The change in (-1.8 ± 2.4 vs. -1.2 ± 2.0, $p = 0.12$) and at least 2-grade reduction (75 vs. 75%, $p = 1.0$) in the WHO ordinal scale was also similar between groups.

Conclusion: Patients hospitalized with COVID-19 and evidence of cardiac injury did not benefit from colchicine therapy.

Keywords: COVID-19, colchicine, cardiac injury, myocarditis, inflammasome

INTRODUCTION

Coronavirus disease-19 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has infected over 250 million patients and resulted in over 5 million deaths worldwide since December 2019. Cardiac injury in the setting of COVID-19 as defined by elevated cardiac biomarkers, arrhythmias, and/or structural abnormalities including ventricular dysfunction or pericardial effusion is common and has been reported in 16.1–62.3% of cases (1–7). Among patients with elevated cardiac biomarkers, mortality rates are approximately 30% in those without underlying cardiovascular disease (CVD) and up to 70% in those with underlying CVD (8–15). While several mechanisms have been postulated for how SARS-CoV-2 may damage the heart, it is plausible that the indirect injury from innate, cellular, or humoral immune responses including "cytokine storm" may play a pivotal role (16–20). To date, remdesivir has been approved by the FDA for hospitalized patients with severe pneumonia and several other agents have also been given Emergency Use Authorization. However, there are no FDA approved therapies specifically for the treatment of COVID-19 related cardiac injury (21).

Colchicine is a microtubule polymerization inhibitor and an inhibitor of interleukins 1 and 6, granulocyte macrophage colony stimulating factor (GM-CSF), and the nucleotide-binding oligomerization leucine-rich repeat and pyrin domain (NLRP3) inflammasome, making it a potent anti-inflammatory agent (22, 23). Its benefit in other inflammatory-based cardiovascular conditions has been established, including the treatment of acute and recurrent pericarditis, prevention of post-cardiotomy syndrome, and reduction of major adverse cardiovascular events after acute myocardial infarction (24–28). Although dose adjustments are required for certain comorbidities and concomitant drugs, colchicine is safe, cost-effective, widely available, and orally administered, making it an attractive potential therapeutic option for patients with COVID-19 (29–31).

Considering the prevalence of cardiac injury in COVID-19 patients and the associated high mortality rate among these patients, the need for effective treatment is critical, however, the impact of colchicine specifically among hospitalized COVID-19 patients with evidence of cardiac injury has not been described. In the present multicenter open-label RCT entitled Colchicine for the Treatment of Cardiac Injury in Hospitalized Patients with COVID-19 (COLHEART-19), we sought to determine if colchicine improved clinical outcomes in this key high-risk population.

MATERIALS AND METHODS

Study Design

In this pilot multicenter open-label RCT, hospitalized adult patients with documented COVID-19 and evidence of cardiac injury were randomized in a 1:1 ratio to either colchicine 0.6 mg po twice daily for 30 days plus current standard of care or current standard of care alone. An adaptive trial design allowed for patients in either arm to be co-enrolled in other investigational therapeutic trials for COVID-19. Standard of care was defined as the current background treatment of COVID-19 at each institution, allowing for dynamic changes in therapy based on emerging research and experience during the rapidly evolving pandemic. The primary trial site was the University of California, Los Angeles (UCLA), with the Miami Cardiac and Vascular Institute (MCVI) at Baptist Health South Florida serving as a secondary site. The trial biostatistician (XW) was blinded to patient-level data and provided the randomization sequence and assignment using a permuted block design with varying block sizes between 2, 4, and 6 (SAS Version 9.4). All patients provided informed consent prior to enrollment. The trial protocol was approved by the institutional review boards at UCLA and MCVI; the trial was registered with clinicaltrials.gov (NCT04355143).

Study Population

Adult patients ($\geq$18 years) hospitalized with SARS-CoV-2 infection were eligible for COLHEART-19 if they had any of the following objective markers of cardiac injury: (1) elevated troponin, (2) newly elevated B-type natriuretic peptide (BNP), (3) new ischemic or arrhythmogenic changes on electrocardiogram (ECG) or telemetry, or (4) new reduction in left ventricular ejection fraction (LVEF) or new pericardial effusion on transthoracic echocardiogram (TTE). Exclusion criteria included severe hematologic or neuromuscular disorders, severe renal impairment with concomitant hepatic impairment, co-administration of CYP3A4 and P-glycoprotein transport system inhibitors, concurrent use of strong CYP3A4 or P-glycoprotein transport system inhibitors in patients with renal or hepatic impairment, pregnancy, breastfeeding mothers, or women of childbearing age unable to take adequate contraception. Additionally, patients taking colchicine for other indications (e.g., gout) or those already requiring mechanical ventilation or mechanical circulatory support (MCS) were ineligible for enrollment.

Study Procedures

All patients underwent laboratory testing for cardiac biomarkers (troponin, BNP) and inflammatory biomarkers [C-reactive protein (CRP) and D-Dimer] as well as ECG and TTE on the day of enrollment if not already performed as part of their clinical care. Serial cardiac and inflammatory biomarkers were obtained on days 3 and 7 among those still hospitalized. Of note, to accommodate hospital logistics and workflow, the aforementioned tests were allowed to be collected within $\pm$1 day of the study-assigned collection day.

Patients in both arms of the trial received the current standard of care treatment for COVID-19 per institutional protocols generated by local infectious disease and pulmonary/critical care medicine experts, though the final decision for treatment strategy was at the discretion of the care team. Those patients randomized to the colchicine arm received 0.6 mg twice daily for 30 days. Dose adjustments were made for gastrointestinal intolerance, co-morbidities such as chronic kidney disease, and drug-drug interactions. Additionally, all patients were eligible to be concurrently enrolled in other COVID-19 clinical trials.

Telephone follow-up to evaluate symptoms was performed at 30 days, and telephone as well as electronic medical record follow-up to assess clinical outcomes was completed at 90 days.

Endpoints

The primary endpoint was the composite of all-cause mortality, need for mechanical ventilation, or need for MCS at 90 days. Secondary endpoints included the individual components of the primary endpoint, time to the primary endpoint, change in the World Health Organization (WHO) R&D Blueprint Ordinal Scale at 30 days, and at least 2-grade reduction (i.e., clinical improvement) in the WHO Ordinal Scale at 30 days, re-hospitalization at 90 days, peak and maximum change (delta) in cardiac and inflammatory biomarkers (i.e., troponin, BNP, CRP, D-dimer), and length of hospital stay. Ordinal scales for clinical improvement have been used in prior COVID-19 therapeutic trials and consists of an 8-grade scale ranging from ambulatory without limitation of activities to death (32).

Statistical Analysis

Descriptive statistics such as frequency and percentage were summarized for baseline demographic and clinical characteristics. For each study arm, the proportion of patients achieving the primary composite endpoint at 90 days was calculated, along with the 95% exact confidence interval (CI). Risk difference and the corresponding 95% CI between study arms were also estimated. Fisher's exact test was used to compare the proportion of patients achieving the composite event between two study arms. Similar analyses were performed on the individual components (secondary endpoints) of the primary endpoint. For the secondary endpoint of time to the primary end point, methodologies used for time-to-event data were adopted. Log-rank test was used to compare the event curves between the study arms. Hazard ratios (HR) and the corresponding 95% CIs were obtained *via* a Cox proportional hazards regression model. For each of the other secondary endpoints, summary statistics such as mean, median, standard deviation, minimum, maximum were calculated and reported. Wilcoxon rank rum test was used to compare the two study arms. The analyses for the primary endpoint and the secondary endpoints were performed on the intent-to-treat (ITT) population. All analyses were run using complete cases; in the rare event of missing data, patients were excluded from analyses. All statistical tests were two-sided, with an alpha level of 0.05 as the cut-off for statistical significance. Statistical analyses were carried out using statistical software SAS Version 9.4.

RESULTS

Baseline Characteristics

Between May 5, 2020 and March 11, 2021, 93 hospitalized patients with COVID-19 were enrolled into the COLHEART-19 trial. The majority (85%) of patients were enrolled within the first 3 days of hospitalization and likely all were located on the hospital floor/wards at the time of enrollment [data regarding enrollment location—floor/wards vs. intensive care unit (ICU) was not captured, though mechanical ventilation and MCS which require ICU-level care were exclusion criteria]. Forty-eight patients were randomly assigned to colchicine plus standard of care (colchicine arm), while 45 patients were randomized to the standard of care (control arm) (**Figure 1**).

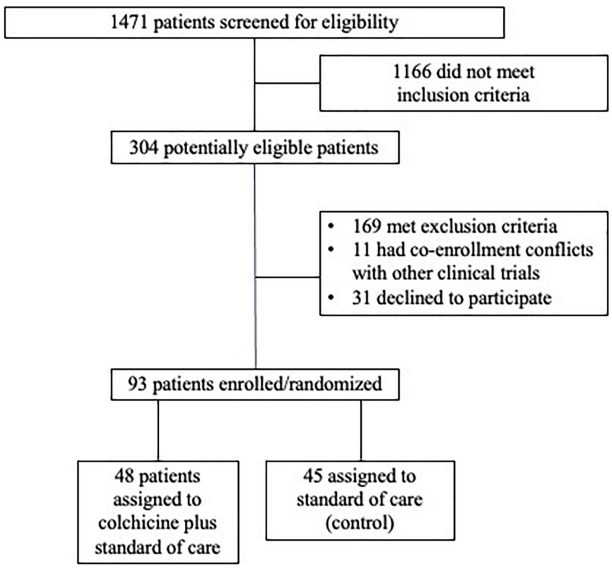


FIGURE 1 | Study design.

Baseline characteristics are summarized in **Table 1**. Of note, despite randomization, patients in the colchicine arm were more likely to be male (81 vs. 53%, $p = 0.007$) and had higher rates of hyperlipidemia (73 vs. 51%, $p = 0.03$) and chronic kidney disease (38 vs. 18%, $p = 0.03$) compared with those in the control arm. Otherwise, the two arms were largely balanced in their baseline demographic and clinical characteristics as well as COVID-19 related medications (**Table 1**).

Colchicine Data

Among the colchicine arm, 28 (58%) patients received the standard dose of 0.6 mg bid, while the remainder had the initial dose adjusted based on comorbidities and/or concomitant pharmacotherapy. Four (8%) patients underwent dose adjustments during the study period. With respect to adverse events related to colchicine, 7 (15%) patients experienced side effects that were classified as mild per protocol; no moderate or serious adverse events occurred. Of note, 4 of these patients terminated their courses of colchicine early due to the side effects. No patients in the control arm reported any side effects.

Outcomes

Primary Endpoint

Overall, 96% of patients completed 90-day follow-up (4 patients in control arm and none in the colchicine arm were lost to follow-up). The proportion of patients who met the primary composite endpoint of all-cause mortality, need for mechanical ventilation, or need for MCS at 90 days was similar between the colchicine

TABLE 1 | Baseline patient characteristics*.

	Colchicine N = 48	Control N = 45	*P-value*
Demographics			
Age (years)	71.2 ± 17	71.5 ± 19.5	0.86
Sex (male)	39 (81)	24 (53)	0.01
Race			0.57
White	28 (58)	21 (47)	
Black	2 (4)	4 (9)	
Other	18 (37)	20 (45)	
Clinical risk factors			
Hypertension	39 (83)	34 (76)	0.35
Hyperlipidemia	35 (73)	23 (51)	0.03
Diabetes	19 (40)	16 (36)	0.69
Tobacco use (current or former)	14 (29)	19 (42)	0.41
Cerebrovascular disease	4 (8)	8 (18)	0.18
Chronic kidney disease	18 (38)	8 (18)	0.03
COPD†	5 (10)	5 (11)	0.91
Other lung disease	5 (10)	5 (11)	0.91
Body mass index	29.6 ± 6.9	28.4 ± 8.0	0.24
COVID-19† therapeutics			
Steroids	24 (56)	34 (72)	0.10
Remdesivir	36 (75)	29 (64)	0.37
Convalescent plasma	6 (13)	7 (16)	0.77
Hydroxychloroquine	1 (2)	0 (0)	1
Leronlimab	7 (15)	2 (4)	0.16
Gimsilumab	1 (2)	2 (4)	0.06
Anticoagulation			0.26
VTE† prophylaxis dose	26 (54)	32 (71)	
Therapeutic dose	18 (38)	10 (22)	

Data are presented as mean ± standard deviation or numbers (percentage) as appropriate.
†COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease-19 (COVID-19); VTE, venous thromboembolism.

and standard of care arms (19 vs. 15%; $p = 0.78$) (**Figure 2**). These data remained statistically non-significant in a sensitivity analysis adjusting for the imbalance of gender, hyperlipidemia, and chronic kidney disease between groups (**Table 2**).

Secondary Endpoints

There were no significant differences between the colchicine and control arms with respect to the individual components of the primary composite endpoint (**Figure 2**). Notably, the rate of mechanical ventilation was low in both groups (8 vs. 5%), and no patients in either group required MCS. The time to primary endpoint was not significantly different between the 2 arms (HR = 1.52, 95% CI: 0.57–4.27, $p = 0.42$) (**Figure 3**). Additionally, hospital length of stay (10.7 vs. 8.8 days, $p = 0.20$) and re-hospitalization rates at 90 days (15 vs. 22%, $p = 0.37$) were similar in the colchicine and control arms.

Patients in the colchicine arm had higher mean WHO ordinal scale scores at baseline compared with those in the control arm (4.2 ± 0.7 vs. 3.9 ± 0.8, $p = 0.07$). At 30 days, however, the change/reduction from baseline was similar between the colchicine and control arms (−1.8 ± 2.4 vs. −1.2 ± 2.0, $p = 0.12$), and there was no between-group difference in at least 2-grade reduction (75 vs. 75%, $p = 1.0$).

We also observed no significant difference in peak or delta troponin and BNP levels between the colchicine and control arms (**Table 3**). Patients in the colchicine arm did have significantly higher peak CRP levels (13.6 ± 8.9 vs. 9.8 ± 7.8, $p = 0.03$), though delta CRP as well as peak and delta D-dimer levels were similar between the 2 arms (**Table 3**).

DISCUSSION

The principal finding of the multicenter randomized controlled open-label COLHEART-19 clinical trial is that colchicine did not reduce the primary endpoint of all-cause mortality, need for mechanical ventilation, or need for MCS at 90 days in hospitalized adult patients with COVID-19 and evidence of cardiac injury. The lack of benefit of colchicine extended across multiple key secondary endpoints including the individual components of the composite primary endpoint, change in and at least 2-grade reduction in the WHO Ordinal Scale at 30 days, and re-hospitalization at 90 days. To the best of our knowledge, COLHEART-19 is the first trial to specifically evaluate the impact of colchicine in hospitalized COVID-19 patients with manifestations of cardiac injury, and in doing so, adds to the growing body of evidence of its limited role in the treatment of COVID-19.

Multiple studies have previously suggested that colchicine may be an attractive therapeutic agent to treat patients presenting with COVID-19 given its unique anti-inflammatory properties, relative lack of serious side effects, and wide availability (30, 33–41). To date, several RCTs assessing the effect of colchicine on outcomes have been conducted in varying COVID-19 populations. Among hospitalized patients with COVID-19, the GRECCO-19 trial did not show a mortality benefit with colchicine administration, although there was a statistically significant decrease in 2-grade reduction in the WHO Ordinal Scale (42). Similarly, the larger RECOVERY trial, which randomized 11,162 hospitalized COVID-19 patients to colchicine vs. standard of care, also did not show a difference in the primary endpoint of 28-day mortality (risk ratio = 1.02, 95% CI: 0.94–1.11, $p = 0.63$) (43). Of note, several meta-analyses have suggested potential benefit for colchicine in hospitalized patients with COVID-19 with signals toward lower mortality, but these included mainly observational studies in addition to RCTs. Among outpatients with COVID-19, the recent COLCORONA trial randomized 4,488 patients to colchicine vs. placebo. COLCORONA failed to demonstrate an improvement in the primary endpoint of mortality or hospital admission (OR = 0.79, 95% CI: 0.61–1.03, $p = 0.81$). Interestingly, the authors made the observation that if only PCR-confirmed COVID-19 patients were included, there was a statistically significant improvement in the primary endpoint (OR = 0.75, 95% CI: 0.57–0.99, $p = 0.042$) (44).

In contrast to these prior RCTs, our COLHEART-19 trial is unique in that we focused on hospitalized COVID-19 patients with evidence of cardiac injury, a high-risk subgroup

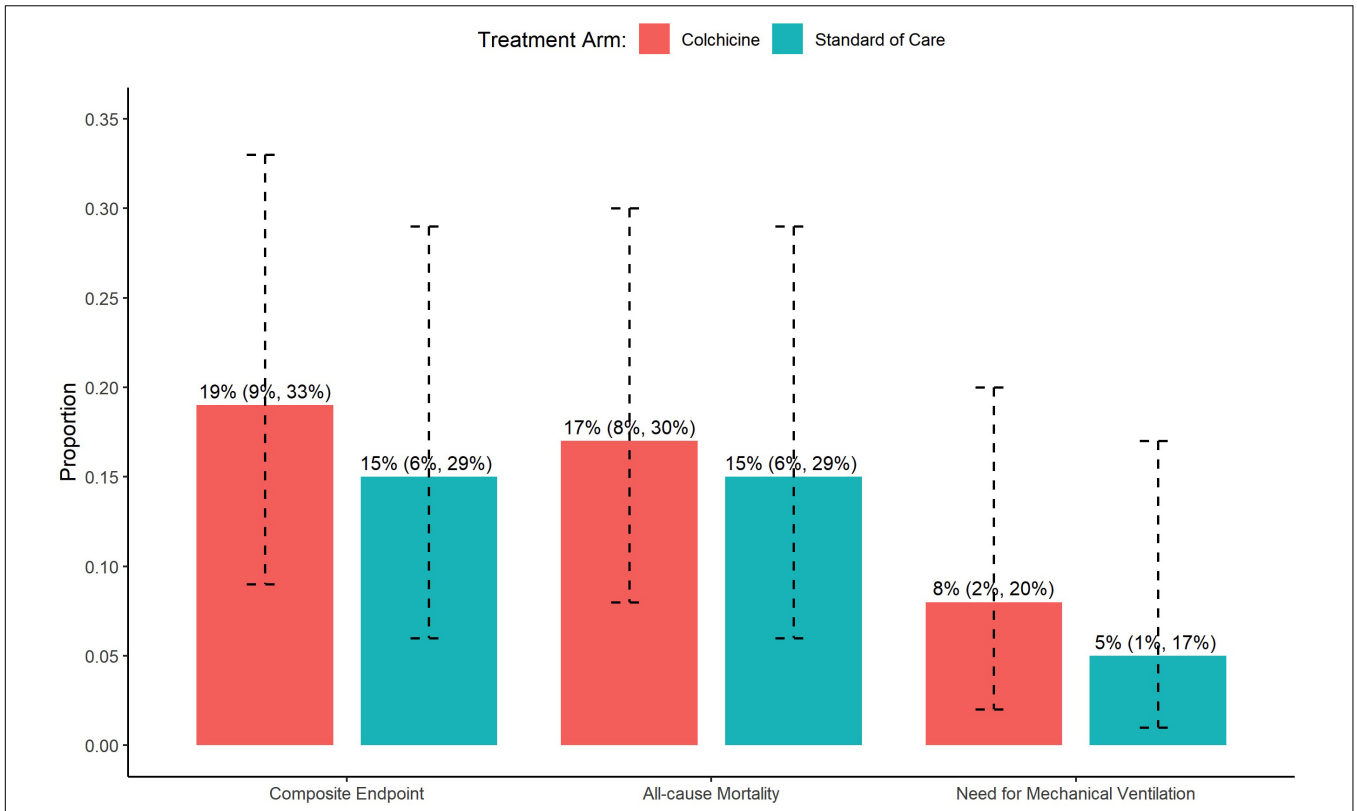


FIGURE 2 | Impact of colchicine on the primary composite endpoint, and individual components of the primary composite endpoint including all-cause mortality and need for mechanical ventilation.

that theoretically would benefit the most from colchicine therapy. We hypothesized that treating these patients early during their hospitalization with colchicine would blunt the cytokine storm and improve short-term clinical outcomes. However, our results mirror those of the negative GRECCO-19, RECOVERY, and COLCORONA trials in that colchicine does not appear to be provide a benefit across multiple clinical and laboratory endpoints in hospitalized patients with COVID-19 and cardiac injury.

TABLE 2 | Adjusted primary and individual component secondary endpoint data*.

	Colchicine $N = 48$	Control $N = 45$	Adjusted proportion difference
Primary endpoint (Composite of all-cause mortality, need for mechanical ventilation, or need for MCS at 90 days)	9 (19)	6 (15)	0%, 95% CI: −17%–18%, $p = 0.96$
All-cause mortality	8 (17)	6 (15)	1%, 95% CI: −18%–16%, $p = 0.91$
Need for mechanical ventilation	4 (8)	2 (5)	5%, 95% CI: −10%–20%, $p = 0.51$
Need for MCS†	0 (0)	0 (0)	0%

Data are presented as numbers (percentage).
†*CI, confidence interval; MCS, mechanical circulatory support.*

Our study had key limitations worth considering. First, our sample size did not reach our pre-specified sample size of 150 patients due to slower than expected enrollment at our institutions and hence is underpowered and precluded meaningful subgroup analyses. However, given the lack of a positive trend in the colchicine arm, it seems unlikely that a larger sample size would have changed the outcome of the trial. Second, the study had an open-label design. This open-label structure was specifically chosen to allow for co-enrollment into additional COVID-19 based therapeutic clinical trials at our institutions. Notably, the colchicine and control arms were balanced with respect to other COVID-19 investigational therapies, and we adjusted for this in our analyses. Third, the standard of care therapy in the control arm had significant variability owing to the rapidly evolving treatment of COVID-19 over the course of the study period as new research data and experience emerged; this introduces unmeasured confounding, though it also reflects real-world practice during a dynamic pandemic. Fourth, we elected for a short 30-day course of colchicine to treat acute COVID-19, though a longer course may have had a more appreciable impact on long-term outcomes. Fifth, we did not obtain follow-up imaging data (echocardiography or cardiac magnetic resonance imaging); although this would have provided additional valuable information, logistical and budgetary concerns precluded its inclusion in the protocol. Sixth, although troponin is an objective marker of cardiac injury, its

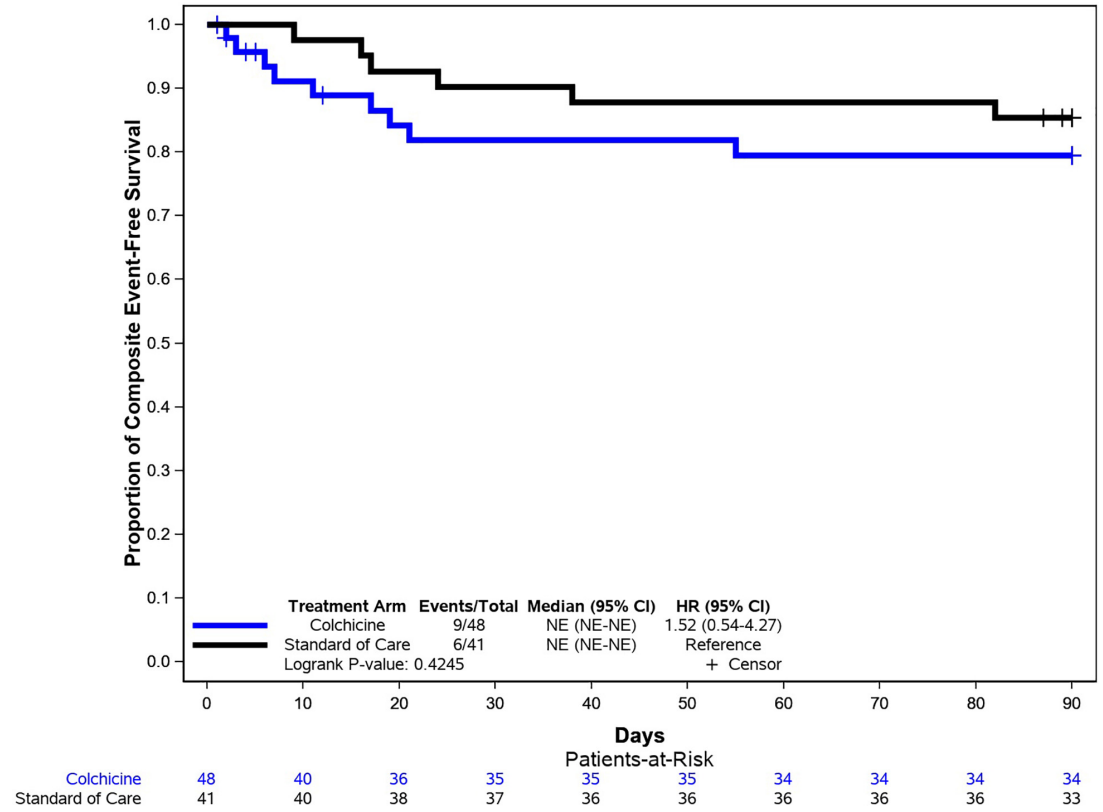


FIGURE 3 | Kaplan–Meier analysis for the primary endpoint. Kaplan–Meier analysis demonstrated similar rates of event-free survival (composite of all-cause mortality, need for mechanical ventilation, or need for mechanical circulatory support) among patients in the colchicine and control arms.

heightened sensitivity may led to inclusion of patients without clinically significant COVID-19 cardiac involvement. Finally, our primary endpoint necessitated excluding hospitalized patients who immediately required intubation or MCS; however, we hypothesized that colchicine would have less of an impact in these critically ill patients whose inflammatory burden was already exceedingly high.

TABLE 3 | Cardiac and inflammatory biomarkers*.

	Colchicine	Control	*p*-value
Troponin (ng/mL)			
Baseline	0.1 ± 0.1	0.10 ± 0.5	0.37
Peak	0.2 ± 0.4	0.1 ± 0.5	0.16
Delta†	0.1 ± 0.3	0	0.06
BNP‡ (pg/mL)			
Baseline	503 ± 765	389 ± 798	0.42
Peak	611 ± 796	514 ± 817	0.42
Delta†	108 ± 218	134 ± 241	0.85
CRP‡ (mg/dL)			
Baseline	9.9 ± 7.3	7.2 ± 5.7	0.09
Peak	13.6 ± 8.9	9.8 ± 7.8	0.03
Delta†	3.9 ± 7.2	2.6 ± 4.1	0.57
D-Dimer (μ g/mL)			
Baseline	493 ± 854	669 ± 1101	0.92
Peak	639 ± 1112	861 ± 1564	0.95
Delta†	145 ± 353	212 ± 604	0.19

Data are presented as mean ± standard deviation.
†*Delta is defined as the difference between peak and baseline values.*
‡*BNP, B-type natriuretic peptide; CRP, C-reactive protein.*

CONCLUSION

In this multicenter open-label RCT, colchicine administration in hospitalized adult patients with COVID-19 and evidence of cardiac injury did not provide a benefit across multiple clinical and laboratory endpoints compared with standard of care. These findings are in agreement with other recent RCTs that similarly have not shown benefit of colchicine in COVID-19.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by UCLA Institutional Review Board (IRB). The

patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AmR, AsR, RP, and RA had full access to all of the data in the study, took responsibility for the integrity of the data and the accuracy of the data analysis, and contributed to administrative, technical, or material support and supervision. AmR, XW, DW, AsR, RP, and RA drafted the manuscript. XW contributed to statistical analysis. All authors contributed to acquisition, analysis, or interpretation of data, contributed to the article, and approved the submitted version.

FUNDING

This work was supported by the UCLA David Geffen School of Medicine–Broad Stem Cell Research Center (BSCRC) COVID-19 Research Award. The funder had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

REFERENCES

1. Lala A, Johnson KW, Januzzi JL, Russak AJ, Paranjpe I, Richter F, et al. Prevalence and impact of myocardial injury in patients hospitalized with COVID-19 infection. *J Am Coll Cardiol.* (2020) 76:533–46. doi: 10.1016/j.jacc.2020.06.007
2. Giustino G, Croft LB, Stefanini GG, Bragato R, Silbiger JJ, Vicenzi M, et al. Characterization of myocardial injury in patients with COVID-19. *J Am Coll Cardiol.* (2020) 76:2043–55. doi: 10.1016/j.jacc.2020.08.069
3. Fu L, Li XY, Fei J, Xiang Y, Xiang HX, Li MD, et al. Myocardial injury at early stage and its association with the risk of death in COVID-19 patients: a hospital-based retrospective cohort study. *Front Cardiovasc Med.* (2020) 7:590688. doi: 10.3389/fcvm.2020.590688
4. Bansal M. Cardiovascular disease and COVID-19. *Diabetes Metab Syndr.* (2020) 14:247–50. doi: 10.1016/j.dsx.2020.03.013
5. Bieber S, Kraechan A, Hellmuth JC, Muenchhoff M, Scherer C, Schroeder I, et al. Left and right ventricular dysfunction in patients with COVID-19-associated myocardial injury. *Infection.* (2021) 49:491–500. doi: 10.1007/s15010-020-01572-8
6. Labbé V, Ederhy S, Lapidus N, Salem JE, Trinh-Duc A, Cohen A, et al. Characterization and outcomes of acute myocardial injury in COVID-19 intensive care patients. *Infection.* (2021) 49:563–6. doi: 10.1007/s15010-020-01560-y
7. Prasitlumkum N, Chokesuwattanaskul R, Thongprayoon C, Bathini T, Vallabhajosyula S, Cheungpasitporn W. Incidence of myocardial injury in covid-19-infected patients: a systematic review and meta-analysis. *Diseases.* (2020) 8:40. doi: 10.3390/diseases8040040
8. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* (2020) 5:802–10. doi: 10.1001/jamacardio.2020.0950
9. Deng Q, Hu B, Zhang Y, Wang H, Zhou X, Hu W, et al. Suspected myocardial injury in patients with COVID-19: evidence from front-line clinical observation in Wuhan, China. *Int J Cardiol.* (2020) 311:116–21. doi: 10.1016/j.ijcard.2020.03.087
10. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest.* (2020) 130:2620–9. doi: 10.1172/JCI137244
11. Arentz M, Yim E, Klaff L, Lokhandwala S, Riedo FX, Chong M, et al. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington State. *JAMA.* (2020) 323:1612–4. doi: 10.1001/jama.2020.4326
12. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA.* (2020) 323:1061–9. doi: 10.1001/jama.2020.1585
13. Uriel N, Sayer G, Clerkin KJ. Myocardial injury in COVID-19 patients: the beginning or the end? *J Am Coll Cardiol.* (2020) 76:547–9. doi: 10.1016/j.jacc.2020.06.045
14. Harmouch F, Shah K, Hippen JT, Kumar A, Goel H. Is it all in the heart? myocardial injury as major predictor of mortality among hospitalized COVID-19 patients. *J Med Virol.* (2021) 93:973–82. doi: 10.1002/jmv.26347
15. Dalia T, Lahan S, Ranka S, Acharya P, Gautam A, Goyal A, et al. Impact of congestive heart failure and role of cardiac biomarkers in COVID-19 patients: a systematic review and meta-analysis. *Indian Heart J.* (2021) 73:91–8. doi: 10.1016/j.ihj.2020.12.002
16. Imazio M, Klingel K, Kindermann I, Brucato A, De Rosa FG, Adler Y, et al. COVID-19 pandemic and troponin: indirect myocardial injury, myocardial inflammation or myocarditis? *Heart.* (2020) 106:1127–31. doi: 10.1136/heartjnl-2020-317186
17. Atri D, Siddiqi HK, Lang JP, Nauffal V, Morrow DA, Bohula EA. COVID-19 for the cardiologist: basic virology, epidemiology, cardiac manifestations, and potential therapeutic strategies. *JACC Basic Transl Sci.* (2020) 5:518–36. doi: 10.1016/j.jacbts.2020.04.002
18. Babapoor-Farrokhran S, Gill D, Walker J, Rasekhi RT, Bozorgnia B, Amanullah A. Myocardial injury and COVID-19: possible mechanisms. *Life Sci.* (2020) 253:117723. doi: 10.1016/j.lfs.2020.117723
19. Cheng R, Leedy D. COVID-19 and acute myocardial injury: the heart of the matter or an innocent bystander? *Heart.* (2020) 106:1122–4. doi: 10.1136/heartjnl-2020-317025
20. Centurión OA, Scavenius KE, García LB, Torales JM, Miño LM. Potential mechanisms of cardiac injury and common pathways of inflammation in patients with COVID-19. *Crit Pathw Cardiol.* (2021) 20:44–52. doi: 10.1097/HPC.0000000000000227
21. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the treatment of COVID-19 – final report. *N Engl J Med.* (2020) 383:1813–26. doi: 10.1056/NEJMoa2007764
22. Leung YY, Yao Hui LL, Kraus VB. Colchicine–update on mechanisms of action and therapeutic uses. *Semin Arthritis Rheum.* (2015) 45:341–50. doi: 10.1016/j.semarthrit.2015.06.013
23. Slobodnick A, Shah B, Pillinger MH, Krasnokutsky S. Colchicine: old and new. *Am J Med.* (2015) 128:461–70. doi: 10.1016/j.amjmed.2014.12.010
24. Imazio M, Cecchi E, Ierna S, Trinchero R, CORP Investigators. Corp (Colchicine for recurrent pericarditis) and Corp-2 trials–two randomized placebo-controlled trials evaluating the clinical benefits of colchicine as adjunct to conventional therapy in the treatment and prevention of recurrent pericarditis: study design and rationale. *J Cardiovasc Med (Hagerstown).* (2007) 8:830–4. doi: 10.2459/JCM.0b013e3280110616
25. Imazio M, Cecchi E, Ierna S, Trinchero R, ICAP Investigators. Investigation on colchicine for acute pericarditis: a multicenter randomized placebo-controlled trial evaluating the clinical benefits of colchicine as adjunct to conventional therapy in the treatment and prevention of pericarditis; study design amd rationale. *J Cardiovasc Med (Hagerstown).* (2007) 8:613–7. doi: 10.2459/01.JCM.0000281702.46359.07
26. Imazio M, Cecchi E, Trinchero R, COPPS Investigators. Colchicine for the prevention of the postpericardiotomy syndrome: the copps trial. *Int J Cardiol.* (2007) 121:198–9. doi: 10.1016/j.ijcard.2006.08.071
27. Imazio M, Bobbio M, Cecchi E, Demarie D, Demichelis B, Pomari F, et al. Colchicine in addition to conventional therapy for acute pericarditis: results of the colchicine for acute pericarditis (COPE) trial. *Circulation.* (2005) 112:2012–6. doi: 10.1161/CIRCULATIONAHA.105.542738

28. Imazio M, Bobbio M, Cecchi E, Demarie D, Pomari F, Moratti M, et al. Colchicine as first-choice therapy for recurrent pericarditis: results of the core (colchicine for recurrent pericarditis) trial. *Arch Intern Med.* (2005) 165:1987–91. doi: 10.1001/archinte.165.17.1987
29. Yeh YT, Liu CW, Wu YW. The benefit from anti-inflammatory properties of colchicine in cardiovascular diseases. *Am J Cardiol.* (2015) 116:493. doi: 10.1016/j.amjcard.2015.05.003
30. Reyes AZ, Hu KA, Teperman J, Wampler Muskardin TL, Tardif JC, Shah B, et al. Anti-inflammatory therapy for COVID-19 infection: the case for colchicine. *Ann Rheum Dis.* (2020) 80:550–7. doi: 10.1136/annrheumdis-2020-219174
31. Papadopoulos C, Patoulias D, Teperikidis E, Mouselimis D, Tsarouchas A, Toumpourleka M, et al. Colchicine as a potential therapeutic agent against cardiovascular complications of COVID-19: an exploratory review. *SN Compr Clin Med.* (2020) 2:1419–29. doi: 10.1007/s42399-020-00421-x
32. World Health Organization [WHO]. *R&D Blueprints*. Geneva: World Health Organization (2020).
33. Anton-Vazquez V, Byrne L, Anderson L, Hamzah L. COVID-19 cardiac injury and the use of colchicine. *BMJ Case Rep.* (2021) 14:e241047. doi: 10.1136/bcr-2020-241047
34. Kevorkian JP, Lopes A, Sène D, Riveline JP, Vandiedonck C, Féron F, et al. Oral corticoid, aspirin, anticoagulant, colchicine, and furosemide to improve the outcome of hospitalized COVID-19 patients - the COCAA-COLA cohort study. *J Infect.* (2021) 82:276–316. doi: 10.1016/j.jinf.2021.02.008
35. Lopes MI, Bonjorno LP, Giannini MC, Amaral NB, Menezes PI, Dib SM, et al. Beneficial effects of colchicine for moderate to severe COVID-19: a randomised, double-blinded, placebo-controlled clinical trial. *RMD Open.* (2021) 7:e001455. doi: 10.1136/rmdopen-2020-001455
36. Manenti L, Maggiore U, Fiaccadori E, Meschi T, Antoni AD, Nouvenne A, et al. Reduced mortality in COVID-19 patients treated with colchicine: results from a retrospective, observational study. *PLoS One.* (2021) 16:e0248276. doi: 10.1371/journal.pone.0248276
37. Sandhu T, Tieng A, Chilimuri S, Franchin GA. Case control study to evaluate the impact of colchicine on patients admitted to the hospital with moderate to severe COVID-19 infection. *Can J Infect Dis Med Microbiol.* (2020) 2020:8865954. doi: 10.1155/2020/8865954
38. Brunetti L, Diawara O, Tsai A, Firestein BL, Nahass RG, Poiani G, et al. Colchicine to weather the cytokine storm in hospitalized patients with COVID-19. *J Clin Med.* (2020) 9:2961. doi: 10.3390/jcm9092961
39. Scarsi M, Piantoni S, Colombo E, Airó P, Richini D, Miclini M, et al. Association between treatment with colchicine and improved survival in a single-centre cohort of adult hospitalised patients with covid-19 pneumonia and acute respiratory distress syndrome. *Ann Rheum Dis.* (2020) 79:1286–9. doi: 10.1136/annrheumdis-2020-217712
40. Mansouri N, Marjani M, Tabarsi P, von Garnier C, Mansouri D. Successful treatment of COVID-19 associated cytokine release syndrome with colchicine. a case report and review of literature. *Immunol Invest.* (2020) 50:884–90. doi: 10.1080/08820139.2020.1789655
41. Pelechas E, Drossou V, Voulgari PV, Drosos AA. COVID-19 in patients with gout on colchicine. *Rheumatol Int.* (2021) 41:1503–7. doi: 10.1007/s00296-021-04902-7
42. Deftereos SG, Giannopoulos G, Vrachatis DA, Siasos GD, Giotaki SG, Gargalianos P, et al. Effect of colchicine vs standard care on cardiac and inflammatory biomarkers and clinical outcomes in patients hospitalized with coronavirus disease 2019: the GRECCO-19 randomized clinical trial. *JAMA Netw Open.* (2020) 3:e2013136. doi: 10.1001/jamanetworkopen.2020.13136
43. RECOVERY Collaborative Group. Colchicine in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet Respir Med.* (2021) 9:1419–26. doi: 10.1016/S2213-2600(21)00435-5
44. Tardif JC, Bouabdallaoui N, L'Allier PL, Gaudet D, Shah B, Pillinger MH, et al. Colchicine for community-treated patients with Covid-19 (COLCORONA): a phase 3, randomised, double-blinded, adaptive, placebo-controlled, multicentre trial. *Lancet Respir Med.* (2021) 9:924–32. doi: 10.1016/S2213-2600(21)00222-8

Conflict of Interest: RP reports unrelated research support from the American Heart Association, Janssen, Infraredx, and Abbott Vascular; consulting fees from Abbott Vascular; serving on advisory board of HeartCloud, DocVocate, and Stallion Cardio.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

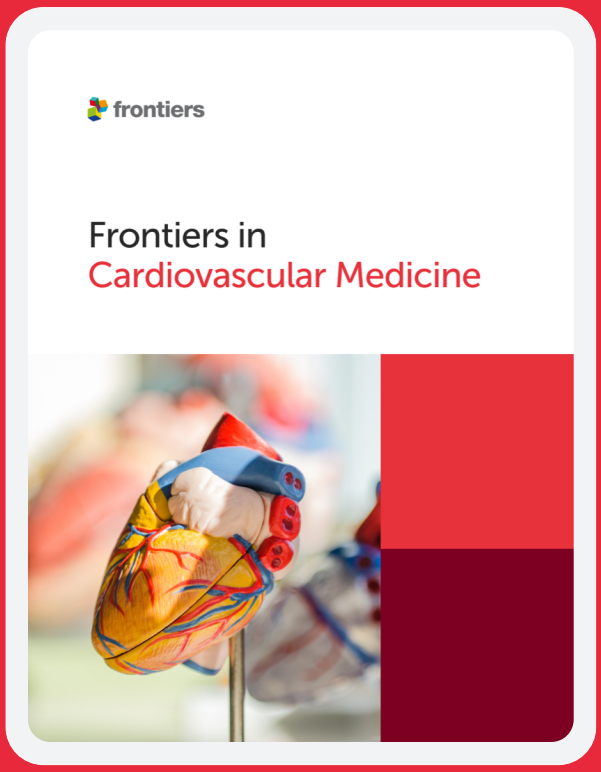
Frontiers in
Cardiovascular Medicine

Innovations and improvements in cardiovascular treatment and practice

Focuses on research that challenges the status quo of cardiovascular care, or facilitates the translation of advances into new therapies and diagnostic tools.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact