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

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

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

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Editorial on the Research Topic
What do we know about COVID-19 implications for cardiovascular disease?

Public health emergencies caused by Coronavirus-19 (COVID-19) continue to exist across the globe. Studies have shown that infection with COVID-19 is more severe in people with preexisting cardiovascular disease (1, 2). Likewise, the findings of previous clinical studies indicate that COVID-19 can lead to a wide range of cardiovascular complications (3). It has been suggested that there may be a bidirectional cause-effect relationship between COVID-19 and cardiovascular disease. Over 2 years after the initial outbreak of SARS-CoV-2, there is strong evidence that people with cardiovascular diseases are both more susceptible to severe COVID-19 and are more likely to experience post-acute sequelae (4). Given this, it's significant and urgent to the underlying mechanisms need to be clarified. The present Research Topic aims to present some of the more recent acquisitions on the integration of clinical observations and experimental findings linking COVID-19 and cardiovascular disease.

With regards to better diagnostic measures, Li et al. demonstrated the prognostic value of echocardiographic parameters in COVID-19 infections with underlying cardiovascular disease; Yu et al. found that the myoglobin level may help assess the prognosis and treatment response of COVID-19 patients.

In terms of research methodology, human induced pluripotent stem cell-derived cardiac myocytes (hiPSC-CMs) may be a practical research vehicle, Jakobi et al. evaluated the effects of five drugs used to treat COVID-19 on hiPSC-CMs, which deepened our understanding of the cardiomyocyte response to drugs. Meyer et al. then developed a systematic integrated approach to summarize the detailed mechanisms of cardiovascular complications in COVID-19. By integrating COVID-19 factors into the existing coronary heart disease (CHD) Model, and evaluating the effects of different health factors and pharmacological interventions on the severity of COVID-19, Meyer et al. explained in detail the mechanisms by which the interactions between inflammation, endothelial cell injury, hypercoagulability, and hypoxia lead

to patient death, elaborating the influence of each factor on the severity of the disease, which has implications for understanding the mechanisms of this disease and guiding the clinical management. Structural biology and computer-assisted drug screening may be an emerging research approach. Al-Moubarak et al. explored the interaction between potential COVID-19 antiviral therapy and hERG potassium channel pores through computer model simulations based on the cryoelectron microscopic structure of hERG, explaining the differences in the ability of different drugs to enter the lateral binding pocket.

COVID-19 affects the cardiovascular system through various mechanisms. The SARS-CoV-2 infection can cause myocardial injury and associated with increased in-hospital mortality (5). Pathologists performed cardiac tissue autopsies on patients with COVID-19, thereby confirming the presence of myocarditis, and noted that most patients exhibited increased cardiac interstitial macrophage infiltration (6). Song et al. suggested that a high inflammatory burden might be a potential cause of myocardial injury in critically ill patients with COVID-19. In addition, COVID-19 also involves the vascular endothelium. Jud et al. suggest that it may promote COVID-19-related endothelial dysfunction and inflammatory vasculopathy by affecting arterial stiffness, capillary morphology, homocysteine metabolism, etc. Meanwhile, Jha et al. analyzed gene expression in patients' lung epithelial cells by transcriptomics and found alterations in genes related to apoptosis, coagulation, and vascular function, suggesting that these may be associated with the development of cardiovascular complications. The human receptor angiotensin-converting enzyme 2 (ACE2) dimer may be an important target for mediating viral damage to the cardiovascular system and indeed the systemic system by binding to the viral trimeric stinger protein (7). A multicenter retrospective cohort study by Huang et al. showed that angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARBs) may alleviate organ damage by reducing pro-inflammatory cytokine levels, but simultaneously prolong viral shedding, so antiviral therapy should be intensified concomitantly and hemodynamic changes should be closely monitored. However, Raisi-Estabragh et al. reviewed the status of 7,099 people on UK Biobank and found that ACE/ARB use was not associated with COVID-19 status. Activation of autoantibodies and systemic inflammation in COVID-19 patients may also have cardiovascular involvement (8). Lumish et al. noted that higher levels of high-sensitivity cardiac troponin T (hs-cTNT), high-sensitivity C-reactive protein (hs-CRP), and creatinine may be associated with higher morbidity and mortality in men. Sun et al. noted that patients with severe COVID-19 have lower levels of HDL-C and apoA-1, and these may be promising predictors of severe disease and in-hospital mortality in COVID-19 patients.

COVID-19 patients with combined cardiovascular complications require particular attention in terms of treatment and prognosis. Myocardial injury with elevated plasma troponin is seen in 8–12% of COVID-19 patients (3). The studies performed by Rubattu et al. have found the potential beneficial role of angiotensin receptor II blocker - neprilysin inhibitor (ARNI) in heart failure patients with COVID-19. Petersen-Uribe et al. found that heart failure and pre-existing cardiovascular disease in COVID-19 patients are associated with serious complications such as acute respiratory distress syndrome (ARDS). Remaining in the field of cardiovascular impairment treatment during COVID-19, Wang et al. validated the key importance of anti-inflammatories to address the cardiac implications of COVID-19, especially among severe cases and critical cases. Given the large scale of the COVID-19 pandemic worldwide, cardiovascular sequelae of COVID-19 are a significant concern to all populations with worse short-term outcomes (9). Hence, physicians must be aware of these diseases and establish treatment as early as possible.

In summary, the present Research Topic indicates that advances in clinical management and mechanistic basis achieved recently with the interaction of SARS-CoV-2 infection and the cardiovascular system. Additionally, the findings discussed herein might promote awareness of these implications and stimulate further research.

Author contributions

ZW, MT, XL, and SZ: concept and design. ZW and MT: drafting of the manuscript. HT, MA, MX, and SG: administrative and supervision. XL and SZ: critical revision of the manuscript for important intellectual content, administrative, and supervision. All authors contributed to the article and approved the submitted version.

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.

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References

1. 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

2. 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

3. 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

4. Maestre-Muñiz MM, Arias Á, Mata-Vázquez E, Martin-Toledano M, Lopez-Larramona G, Ruiz-Chicote AM, et al. Long-term outcomes of patients with coronavirus disease 2019 at one year after hospital discharge. *J Clin Med.* (2021) 10:2945. doi: 10.3390/jcm10132945

5. 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

6. 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

7. 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

8. Wang EY, Mao T, Klein J, Dai Y, Huck JD, Jaycox JR, et al. Diverse functional autoantibodies in patients with COVID-19. *Nature.* (2021) 595:283–8. doi: 10.1038/s41586-021-03631-y

9. Xie Y, Xu E, Bowe B Al-Aly Z. Long-term cardiovascular outcomes of COVID-19. *Nat Med.* (2022) 28:583–90. doi: 10.1038/s41591-022-01689-3

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OPINION
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COVID and the Renin-Angiotensin System: Are Hypertension or Its Treatments Deleterious?

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INTRODUCTION

Since its outbreak in December 2019, Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) has spread worldwide and is considered a pandemic. Coronavirus disease (COVID-19) can lead to acute respiratory distress syndrome (ARDS) or death. Many efforts have been made to identify risk factors predisposing to a severe issue. In the first SARS-CoV epidemic in 2002, hypertension was noted in 9/19 patients who died from SARS-CoV in Toronto (1). In the two largest cohorts of SARS-CoV-2 published, hypertension is the most common comorbidity in patients with severe disease or in those who died or were ventilated (2, 3). Nevertheless, these data are not adjusted for age, although age appears to be a strong predictor of adverse outcome (4) and hypertension is a very common finding in older patients. Finally, cohort studies only show correlation, not causality. In this paper, we hypothesize that the reductions in Angiotensin-Converting Enzyme 2 (ACE-2) observed in hypertension and obesity can explain many abnormalities observed in SARS-CoV-2 and question the role of treatments interfering with ACE2.

ACE2 IN THE CARDIOVASCULAR SYSTEM

Like SARS-CoV, SARS-CoV-2 fuses with human cells after the receptor-binding domain of its S (Spike) protein binds with Angiotensin-Converting Enzyme 2 (ACE-2), an enzyme located on membrane of lung alveolar epithelial cells, renal tubular epithelial cells, enterocytes of the small intestine, and arterial and venous endothelial cells of the kidney (5–10). Cardiomyocytes, fibroblasts, endothelial cells, and pericytes account for the vast majority of cells expressing ACE2 in the heart (10).

ACE-2 is a monocarboxypeptidase homologous to Angiotensin-Converting Enzyme (ACE) whose active site is exposed at the extracellular surface (8, 11). ACE cleaves angiotensin I (ANGI) to generate angiotensin II (ANGII), which binds to and activates Angiotensin Type 1 Receptor (AT1R) to constrict blood vessels and increase salt and fluid retention, thereby elevating blood pressure. ACE2 inactivates ANGII by converting it to angiotensin-(1–7), which has a vasodilator effect when binding to Mas receptor (12) (**Figure 1A**). Moreover, ACE2 cleaves ANGI into angiotensin-(1–9) (albeit with lower affinity than for ANGII), which is further converted into angiotensin-(1–7) by ACE (12). Thus, ACE2 negatively regulates the renin-angiotensin system and modulates the vasoconstriction, fibrosis, and hypertrophy induced by that system (8, 11). In rats, ACE2 deficiency worsens hypertension when ANGII is in excess (8, 13). In human, gene expression and/or ACE2 activity is lower in hypertensive patients than in normotensive ones (13).

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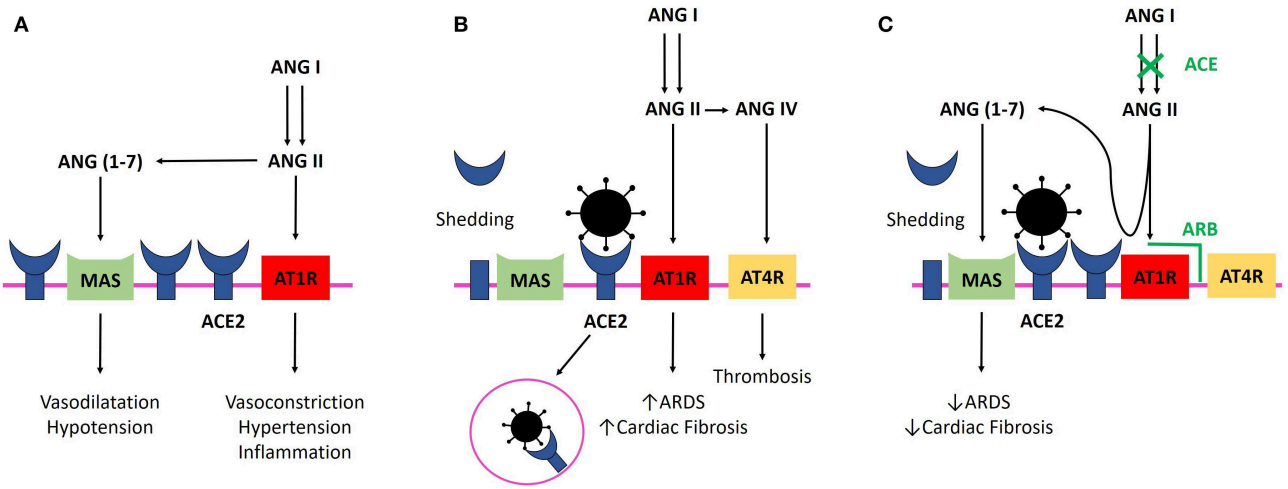

FIGURE 1 | (A) ANGII binding to AT1R elevates blood pressure and promotes inflammation. ACE2 inactivates ANGII by converting it to ang-(1–7) and negatively regulates the renin-angiotensin system, promoting vasodilatation and hypotension. **(B)** SARS-CoV-2 infection. Binding of SARS-CoV-2 with ACE2 leads to their internalization and to ACE2 shedding by ADAM17 (enzyme not shown). Lower availability of ACE2 results in a lower rate of ANGII degradation and excessive stimulation of AT1R, which facilitates ARDS and myocardial injury. Binding of ANGII to AT1R leads to membranous ACE2 internalization, decreasing ACE2 availability even more (not shown). Excessive ANGII is metabolized to ANGIV, which binds to AT4R and promotes thrombosis. Virus replication could also reduce cellular ACE2 expression (not shown). **(C)** SARS-CoV-2 infection and ACEi/ARB treatment. ACEi and ARB upregulate ACE2, and freer ACE2 remains after viral binding. ANGII is still degraded by ACE2 in its beneficial metabolite Ang-(1–7), and AT1R and AT4R are less stimulated. ANGII binding on AT1R prevention with ARB and ANGII synthesis decrease with ACE lead to less AT1R stimulation and persistent interaction with ACE2, avoiding ACE2 internalization. ACE2, Angiotensin Converting Enzyme 2; ACEi, Angiotensin Converting Enzyme Inhibitor; ang-(1–7), Angiotensin-(1–7); ANGII, angiotensin II; ANGIV, angiotensin IV; ARB, Angiotensin Receptor Blocker; ARDS, Acute Respiratory Distress Syndrome; AT1R, Angiotensin II Type 1 Receptor; AT4R, Angiotensin II Type 4 Receptor; SARS-CoV-2, Severe Acute Respiratory Syndrome CoronaVirus 2.

Conversely, ANGII negatively regulates ACE2. AT1R and ACE2 physically interact to form complexes on the cell membrane in the absence of excess Ang II (11). ANGII increase separates AT1R and ACE2 on the cell surface and leads to ACE2 internalization and lysosomal degradation through an AT1R-dependent mechanism (11, 13). Moreover, cellular ACE2 can be cleaved and released (shedding) by the metalloproteinase ADAM17, which is upregulated by ANGII (14). The soluble form of ACE2 circulates in small amounts in the blood, but its physiological role remains elusive, and shedding could be only a mechanism to regulate ACE2 activity on the cell surface (15).

Notably, it has been shown that infection with SARS-CoV can be blocked with soluble ACE2 molecules (6), and some have hypothesized that a soluble recombinant form can be used to overwhelm SARS-CoV-2 to prevent its binding to cellular ACE2 (16). Recombinant human ACE2 has been tested in a phase 2–3 trial in ARDS with interesting results (17), and a pilot trial has recently been launched in COVID-19 (NCT04287686).

ACE inhibitors (ACEi) and AT1R blockers (ARB) are two classes of drugs that are widely used in medicine to treat hypertension or heart failure. ACEi and ARB upregulate ACE2 expression on the cell surface, and ACE2 activity is not prevented by ACEi (8, 11, 18). Accordingly, patients treated with ACEi/ARB could have a higher level of membrane-bound ACE2, providing a more potent binding site to COVID-19 S protein. Nevertheless, in the absence of excess ANGII (either by reduction of ANGII synthesis by ACEi or by AT1R blockade thanks to ARB), AT1R is thought to interact with ACE2 (11). This interaction could reduce the affinity of COVID S protein to ACE2 and then reduce COVID-19 viral entry (11).

In the heart, ACE and ACE2 balance Ang II levels and ACE2 is known to be cardioprotective (8). ACE2 loss leads to a decrease in myocardial function in rodents, likely mediated by ANGII-induced oxidative stress and inflammation through AT1R, but it is unknown whether excess ANGII has a role in an acute setting (8, 19). This decrease is corrected by ARB or ACEi, and these drugs rapidly increase ACE2 activity and mRNA expression in the heart of rats (8, 20). Evidence for such an increase in humans is lacking, but studies checked for variation in the circulating level rather than the tissular level of ACE2 (21). In human failing heart, ACE2 expression is increased, correlating with disease severity, and is thought to be a compensatory mechanism (8, 10).

ROLE OF ACE2 IN SARS-COV-2 INFECTION

SARS-CoV-2 has a 10–20-fold higher affinity for ACE2 than does the 2002 SARS-CoV (22). An increased abundance of cellular ACE2 is associated with a higher susceptibility to SARS-CoV infection in mice (23). However, in both heart and lung, binding of the SARS-CoV to ACE2 leads to the loss of ACE2 by ACE2 internalization with the virus and ACE2 shedding (7, 9, 14). Lower availability of ACE2 results in a

lower rate of ANGII degradation. In rodent lungs, excess ANGII binding to AT1R increases pulmonary vascular permeability and neutrophil accumulation and enhances lung injury (7, 24) (**Figure 1B**). Thus, decreased ACE2 expression promotes increased lung injury and ARB prevents it by limiting ANGII binding to AT1R (7, 8, 24, 25) (**Figure 1C**). This hypothesis is supported *in vivo* by the increased frequency of severe ARDS in patients infected with SARS-CoV with higher levels of ACE determined by genetic predisposition, leading to higher levels of ANGII (26), and by the correlation between viral load, ANGII plasma level, and disease severity in influenza H7N5 (27) and respiratory syncytial virus infection (25). More notably, in a small cohort of patients infected with SARS-CoV-2, viral load was correlated with plasma ANGII level (28). Unfortunately, baseline treatments are unknown in this cohort, and correlation between ARDS severity and plasma ANGII level failed to reach statistical significance, maybe because of the low number of patients.

Moreover, some have suggested that viral replication by itself can reduce cellular ACE2 expression (29). This point is of importance because limitation of ANGII formation by ACEi and binding to AT1R by ARB may yet become the best ways to limit lung injuries if ACE2 is less or not synthetized following viral infection.

SARS-CoV- and SARS-CoV-2-associated cardiac injury contributes significantly to morbidity and mortality and could hit as much as a third of patients with a severe form of the disease (9, 28, 30, 31). SARS-CoV was found in the heart of a third of human autopsy hearts, with a concomitant marked reduction in cellular ACE2 (9). As in lungs, ANGII probably contributes to the deleterious effect of SARS-CoV on the heart and to SARS-associated cardiomyopathy, even if myocardial dysfunction can also be influenced by the strong immune response observed in those patients (9). Inflammatory signals are likely to suppress ACE2 transcription and down-regulate cell-surface expression of ACE2 (8). Thus, inflammatory signals could decrease the cellular susceptibility to SARS-CoV infection but increase the ANGII-mediated tissular injury. Moreover, because pericytes are supposed to play a role in myocardial microcirculation, SARS-CoV-2-induced microcirculation disorder could explain the frequent cardiac marker increase observed in hospitalized patients (2), exacerbated by the reduced oxygen supply caused by lung failure (10).

In summary, a decrease in cellular ACE2 may reduce the susceptibility of cells to SARS CoV-2 but leads to greater activation of AT1R and more severe tissue damage. In contrast, the higher the abundance of ACE2 on the cell membrane, the greater the susceptibility to viral particles but the less the damage, due to less AT1R activation occurring. This latter condition is the one provoked by ACEi/ARB treatment. On the one hand, ACE2 increase under ARB/ACEi treatment could be protective during COVID-19 because some ACE2 remains free to degrade ANGII, but on the other hand, this ACE2 increase could be deleterious by favoring cellular infection by COVID-19, leading to potent myocarditis (**Figure 1C**). The protective or deleterious role of ACEi/ARB in COVID-19 is harder to modelize, as ACE2 is not the only protein required for SARS-COV-2 penetration (5).

ARE ACEI AND ARB DELETERIOUS IN SARS-COV-2 INFECTION?

It has been shown that both ACEi and ARB upregulates ACE2, and a hypothesis was proposed by several authors of a potential deleterious effect of treatment with ARB and ACEi in the course of SARS-CoV-2 infection (32, 33). Since these molecules are widely used to treat hypertension or heart failure, such a fact could be a huge matter of concern.

Obesity seems to be a major determinant of adverse outcome in COVID-19 (34). Besides the altered pulmonary function associated with obesity, it must be noted that obesity is associated with a decrease in membranous ACE2 (35, 36). Moreover, empirical observations are suggestive of an abnormally high prevalence of pulmonary embolism in patients with COVID-19 (37), and prophylactic curative anticoagulation is recommended in severe patients (38). Severe infections are a known precipitant factor for acute venous thrombo-embolism because of epithelial damage and platelet and endothelial cell dysfunction, but does it by itself explain the observed high prevalence of pulmonary embolism in these patients? When ANGII is increased, it can be metabolized to angiotensin IV (ANGIV) by aminopeptidase A and binds to Angiotensin Type 4 Receptor (AT4R) (39). Multiple datasets underline the enhancement of thrombosis development by ANGII and ANGIV (40, 41), and it can be hypothesized that a reduction in ACE2 can increase thrombotic risk.

Despite the many potential cofounders, reduction in membranous ACE2 expression could be an explanation for numerous abnormalities observed in SARS-CoV-2 infection. Thus, even if both ARB and ACEi increase the level of ACE2, more ACE2 could be better rather than worse: more ACE2 remains on the cell surface after virus binding, maintaining ANGII degradation and less stimulation of AT1R. Furthermore, treatment with ARB inhibits AT1R and limits the damage induced by its overstimulation. It is not clear whether continuation or discontinuation of ARB or ACEi is a good option in COVID-19 infection, as there is a lack of clinical data to support an increased risk of contracting a severe form of COVID-19. In addition, we do not even know whether renin angiotensin system inhibitor therapy is beneficial or harmful for virally mediated lesions, and switching to other drugs may worsen the patient's condition, especially for heart failure patients with reduced ejection fraction (42). Clinical trials are ongoing to analyze the beneficial effect of LOSARTAN in COVID-19 (NCT04311177 and NCT04312009), and a trial will start soon to analyze the consequences of discontinuation or continuation of ACEi/ARB (NCT04338009).

ACEi and ARB are not the only treatments for hypertension or heart failure, but other classes only have a limited impact on ACE2. Beta blockers suppress plasma angiotensin II levels by inhibiting prorenin processing to renin and probably do not

interfere with ACE or ACE2 (43). Calcium channel blockers seem to reduce ANGII-induced downregulation of ACE2, but data are limited to those presented in one paper on the effect of nifedipine on fractionated cell extracts (44). In hypertensive rats, neither thiazides nor mineralocorticoid-receptor antagonists (MRAs) improve the spontaneous low ACE2 activity (18, 45), but MRA could decrease ACE expression (18). Conversely, MRAs increase membranous ACE2 activity in patients (46) with heart failure. If the reduction of membranous ACE2 observed in hypertension and obesity plays an important role in the pathophysiology of severe COVID-19, can it be hypothesized that non-ACEi/BRA drugs (beta-blockers, calcium channel blockers, diuretics) are more likely to increase the risk of deleterious outcomes than ACEi/BRA drugs that increase ACE2 and provide theoretical protection? Data on baseline treatments are urgently needed but are lacking to date in published cohorts.

CONCLUSION

The downregulation of ACE2 induced by viral binding, resulting in increased stimulation of AT1R, may be an important element in explaining severe COVID-19. Overall, the ACEi/ARB-mediated increase in ACE2 is not obviously deleterious and may even be protective. Only a well-conducted trial will provide a valid answer to this question. To date, stopping this treatment solely on the basis of presumed considerations does not seem to be a good option.

AUTHOR CONTRIBUTIONS

MR and FZ wrote the manuscript, conceptualized the idea, and made the figure. All authors reviewed and approved the final version of the manuscript.

REFERENCES

1. Farcas GA, Poutanen SM, Mazzulli T, Willey BM, Butany J, Asa SL, et al. Fatal severe acute respiratory syndrome is associated with multiorgan involvement by coronavirus. *J Infect Dis*. (2005) 191:193–7. doi: 10.1086/426870
2. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. (2020). doi: 10.1056/NEJMoa2002032. [Epub ahead of print].
3. 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). doi: 10.1001/jama.2020.5394. [Epub ahead of print].
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. 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). doi: 10.1016/j.cell.2020.02.052. [Epub ahead of print].
6. 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
7. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. 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
8. Kuba K, Imai Y, Ohto-Nakanishi T, Penninger JM. Trilogy of ACE2: a peptidase in the renin–angiotensin system, a SARS receptor, and a partner for amino acid transporters. *Pharmacol Ther*. (2010) 128:119–28. doi: 10.1016/j.pharmthera.2010.06.003
9. Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutanen SM, Penninger JM, et al. SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. *Eur J Clin Invest*. (2009) 39:618–25. doi: 10.1111/j.1365-2362.2009.02153.x
10. 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) cvaa078. doi: 10.1093/cvr/cvaa078
11. Deshotels MR, Xia H, Sriramula S, Lazartigues E, Filipeanu CM. Angiotensin II mediates angiotensin converting enzyme type 2 internalization and degradation through an angiotensin II type I receptor–dependent mechanism. *Hypertension*. (2014) 64:1368–75. doi: 10.1161/HYPERTENSIONAHA.114.03743
12. Hamming I, Cooper M, Haagmans B, Hooper N, Korstanje R, Osterhaus A, et al. The emerging role of ACE2 in physiology and disease. *J Pathol*. (2007) 212:1–11. doi: 10.1002/path.2162
13. Soler MJ, Wysocki J, Batlle D. ACE2 alterations in kidney disease. *Nephrol Dial Transplant*. (2013) 28:2687–97. doi: 10.1093/ndt/gft320
14. Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Pöhlmann S. TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein. *J Virol*. (2014) 88:1293–307. doi: 10.1128/JVI.02202-13
15. Lambert DW, Hooper NM, Turner AJ. Angiotensin-converting enzyme 2 and new insights into the renin–angiotensin system. *Biochem Pharmacol*. (2008) 75:781–6. doi: 10.1016/j.bcp.2007.08.012
16. Batlle D, Wysocki J, Satchell K. Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy? *Clin Sci*. (2020) 134:543–5. doi: 10.1042/CS20200163
17. Khan A, Benthin C, Zeno B, Albertson TE, Boyd J, Christie JD, et al. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit Care*. (2017) 21:234. doi: 10.1186/s13054-017-1823-x
18. Takeda Y, Zhu A, Yoneda T, Usukura M, Takata H, Yamagishi M. Effects of aldosterone and angiotensin II receptor blockade on cardiac angiotensinogen and angiotensin-converting enzyme 2 expression in Dahl salt-sensitive hypertensive rats. *Am J Hypertens*. (2007) 20:1119–24. doi: 10.1016/j.amjhyper.2007.05.008
19. Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*. (2002) 417:822–8. doi: 10.1038/nature00786
20. Burrell LM, Risvanis J, Kubota E, Dean RG, MacDonald PS, Lu S, et al. Myocardial infarction increases ACE2 expression in rat and humans. *Eur Heart J*. (2005) 26:369–75. doi: 10.1093/eurheartj/ehi114
21. Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, et al. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation*. (2005) 111:2605–10. doi: 10.1161/CIRCULATIONAHA.104.510461
22. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh C-L, 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
23. Yang X, Deng W, Tong Z, Liu Y, Zhang L, Zhu H, et al. *Mice Transgenic for Human Angiotensin-Converting Enzyme 2 Provide a Model for SARS Coronavirus Infection*. American Association for Laboratory Animal Science (2007).
24. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*. (2005) 436:112–6. doi: 10.1038/nature03712
25. Gu H, Xie Z, Li T, Zhang S, Lai C, Zhu P, et al. Angiotensin-converting enzyme 2 inhibits lung injury induced by respiratory syncytial virus. *Sci Rep*. (2016) 6:19840. doi: 10.1038/srep19840

26. Itoyama S, Keicho N, Quy T, Phi NC, Long HT, Ha LD, et al. ACE1 polymorphism and progression of SARS. *Biochem Biophys Res Commun*. (2004) 323:1124–9. doi: 10.1016/j.bbrc.2004.08.208
27. Huang F, Guo J, Zou Z, Liu J, Cao B, Zhang S, et al. Angiotensin II plasma levels are linked to disease severity and predict fatal outcomes in H7N9-infected patients. *Nat Commun*. (2014) 5:1–7. doi: 10.1038/ncomms4595
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. Dijkman R, Jebbink MF, Deijs M, Milewska A, Pyrc K, Buelow E, et al. Replication-dependent downregulation of cellular angiotensin-converting enzyme 2 protein expression by human coronavirus NL63. *J Gen Virol*. (2012) 93:1924–9. doi: 10.1099/vir.0.043919-0
30. 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). doi: 10.1001/jama.2020.4326. [Epub ahead of print].
31. 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). doi: 10.1001/jamacardio.2020.0950. [Epub ahead of print].
32. Sommerstein, R, Gräni, C. Re: preventing a covid-19 pandemic: ACE inhibitors as a potential risk factor for fatal Covid-19. *BMJ*. (2020) 368:m810. doi: 10.1136/bmj.m810
33. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir Med*. (2020). doi: 10.1016/S2213-2600(20)30116-8. [Epub ahead of print].
34. Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, et al. High prevalence of obesity in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) requiring invasive mechanical ventilation. *Obesity*. (2020) doi: 10.1002/oby.22831. [Epub ahead of print].
35. Patel VB, Mori J, McLean BA, Basu R, Das SK, Ramprasath T, et al. ACE2 deficiency worsens epicardial adipose tissue inflammation and cardiac dysfunction in response to diet-induced obesity. *Diabetes*. (2016) 65:85–95. doi: 10.2337/dbi15-0037
36. Shoemaker R, Tannock LR, Su W, Gong M, Gurley SB, Thatcher SE, et al. Adipocyte deficiency of ACE2 increases systolic blood pressures of obese female C57BL/6 mice. *Biol Sex Differ*. (2019) 10:45. doi: 10.1186/s13293-019-0260-8
37. Chen J, Wang X, Zhang S, Liu B, Wu X, Wang Y, et al. *Findings of Acute Pulmonary Embolism in COVID-19 Patients*. Rochester, NY: Social Science Research Network. Report No.: ID 3548771 (2020).
38. Société Française d'Anesthésie et de Réanimation. *Traitement Anticoagulant pour la Prévention du Risque Thrombotique chez un Patient Hospitalisé avec Covid-19 et Surveillance de l'Hémostase*. Société Fr D'Anesthésie Réanimation.
39. Wolf G, Wenzel U, Assmann KJM, Stahl RAK. Renal expression of aminopeptidase A in rats with two-kidney, one-clip hypertension. *Nephrol Dial Transplant*. (2000) 15:1935–42. doi: 10.1093/ndt/15.12.1935
40. Mogielnicki A, Chabielska E, Pawlak R, Szemraj J, Buczko W. Angiotensin II enhances thrombosis development in renovascular hypertensive rats. *Thromb Haemost*. (2005) 93:1069–76. doi: 10.1160/TH04-10-0701
41. Senchenkova EY, Russell J, Esmon CT, Granger DN. Roles of coagulation and fibrinolysis in angiotensin II-enhanced microvascular thrombosis. *Microcirculation*. (2014) 21:401–7. doi: 10.1111/micc.12120
42. Halliday BP, Wassall R, Lota AS, Khalique Z, Gregson J, Newsome S, et al. Withdrawal of pharmacological treatment for heart failure in patients with recovered dilated cardiomyopathy (TRED-HF): an open-label, pilot, randomised trial. *Lancet*. (2019) 393:61–73. doi: 10.1016/S0140-6736(18)32484-X
43. Vilas-Boas WW, Ribeiro-Oliveira A Jr., da Cunha Ribeiro R, Vieira RLP, Almeida J, Nadu AP, et al. Effect of propranolol on the splanchnic and peripheral renin angiotensin system in cirrhotic patients. *World J Gastroenterol*. (2008) 14:6824–30. doi: 10.3748/wjg.14.6824
44. Iizuka K, Kusunoki A, Machida T, Hirafuji M. Angiotensin II reduces membranous angiotensin-converting enzyme 2 in pressurized human aortic endothelial cells. *J Renin Angiotensin Aldosterone Syst*. (2009) 10:210–5. doi: 10.1177/1470320309343710
45. Jessup JA, Brosnihan KB, Gallagher PE, Chappell MC, Ferrario CM. Differential effect of low dose thiazides on the renin angiotensin system in genetically hypertensive and normotensive rats. *J Am Soc Hypertens*. (2008) 2:106–15. doi: 10.1016/j.jash.2007.10.005
46. Keidar S, Gamliel-Lazarovich A, Kaplan M, Pavlotzky E, Hamoud S, Hayek T, et al. Mineralocorticoid receptor blocker increases angiotensin-converting enzyme 2 activity in congestive heart failure patients. *Circ Res*. (2005) 97:946–53. doi: 10.1161/01.RES.0000187500.24964.7A

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Cardiovascular Impairment in COVID-19: Learning From Current Options for Cardiovascular Anti-Inflammatory Therapy

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In December 2019, Coronavirus Disease 2019 (COVID-19) caused by SARS-CoV-2, occurred in China and has currently led to a global pandemic. In addition to respiratory involvement, COVID-19 was also associated with significant multiple organ dysfunction syndrome (MODS). Cardiovascular impairment has been observed and is now drawing growing attention. Cardiovascular protective strategies are urgent and of great significance to the overall prognosis of COVID-19 patients. Direct viral infection, cytokine storm, and aggravation of existing cardiovascular diseases were recognized as possible mechanisms of cardiovascular impairment in COVID-19. Hyperactivated inflammation plays an important role in all three mechanisms and is considered to be fundamental in the development of cardiovascular impairment and MODS in COVID-19. Therefore, in addition to conventional cardiovascular treatment, anti-inflammatory therapy is a reasonable strategy for severe cases to further enhance cardiovascular protection and potentially mitigate MODS. We reviewed the inflammatory features and current promising treatments of COVID-19 as well as cardiovascular anti-inflammatory therapies that have been verified in previous clinical trials with positive outcomes. We believe that targeting the central pathway (IL-1β, TNF-α, IL-6), balancing the Th1 and Th2 response, and administering long-term anti-inflammatory therapy might be promising prospects to reduce cardiovascular impairment and even MODS during the acute and recovery phases of COVID-19. The cardiovascular anti-inflammatory therapies might be of great application value to the management of COVID-19 patients and we further propose an algorithm for the selection of anti-inflammatory therapy for COVID-19 patients with or at high risk of cardiovascular impairment. We recommend to take the experiences in cardiovascular anti-inflammatory therapy as references in the management of COVID-19 and conduct related clinical trials, while the clinical translation of novel treatments from preclinical studies or *in vitro* drug screening should proceed with caution due to unguaranteed efficacy and safety profiles.

Keywords: Coronavirus Disease 2019, cardiovascular impairment, inflammation, cardiovascular diseases, cardiovascular anti-inflammatory therapy

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INTRODUCTION

In December 2019, a series of pneumonia cases, now known as Coronavirus Disease 2019 (COVID-19), occurred in Wuhan, Hubei Province, China. A novel coronavirus was later identified as the cause of COVID-19 (1).

By February 11, 2020, a total of 72,314 cases had been reported in mainland China with 44,672 (61.8%) confirmed cases. A total of 1023 patients died, with a case fatality rate of 2.3%, and most of the deaths were in patients over 60 years of age. Among the confirmed cases, severe cases and critical cases accounted for 13.8 and 4.7%, respectively.

The Coronavirus Study Group of the International Committee on Taxonomy of Viruses assessed the novelty of the novel coronavirus and formally recognized it as a sister to severe acute respiratory syndrome coronaviruses (SARS-CoVs), designating it as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) based on phylogeny, taxonomy and established practice (2).

However, unlike the SARS that occurred in 2003, SARS-CoV-2 infection not only leads to pneumonia and acute respiratory distress syndrome (ARDS) but is also associated with significant multiple organ dysfunction syndrome (MODS). The name of COVID-19 was chosen recently by the World Health Organization to cover the diverse clinical manifestations and reflect the complexity of the disease. Common complications among COVID-19 patients include shock, ARDS, arrhythmia, and acute cardiac injury (1). Especially for patients who require ICU care, significant cardiovascular impairment has already been observed, characterized by elevation of cardiac biomarkers, abnormalities in electrocardiography and echocardiography, and eventual circulatory failure. Cardiovascular impairment is now drawing growing attention in clinical practice, and the American College of Cardiology has already released a clinical bulletin on Feb 13, 2020, to address the cardiac implications of COVID-19 (3).

Inflammation plays an important role in the development of cardiovascular impairment and even MODS. As a cardiologist and a member of the high-level expert group appointed by the National Health Commission to fight COVID-19, during the clinical practice, I found that the experiences in cardiovascular anti-inflammatory therapy might be instructive in the management of COVID-19, especially those severe cases. Therefore, in this article, we would like to summarize the related available information and share our perspectives.

MECHANISM OF CARDIOVASCULAR IMPAIRMENT IN COVID-19

The main reasons for cardiovascular impairment in COVID-19 patients can be summarized as follows.

Direct Infection

The virus might directly infect the myocardial tissue and lead to cardiac injury. Cardiac injury has been noted as a protruding clinical feature in COVID-19 patients. In a study of 138 patients, 10 patients were diagnosed with cardiac injury and 8 of them required ICU care, accounting for 22% of all the severe cases. Compared with the non-ICU patients, ICU patients had higher level of hypersensitive troponin I and creatine kinase–MB, indicating that cardiac injury is associated with the disease severity (1). SARS-CoV-2 and SARS-CoV share the same functional host-cell receptor, angiotensin-converting enzyme 2 (ACE2) for cell entry (4), but the affinity of ACE2 for SARS-CoV-2 is approximately 10- to 20-fold higher than that for SARS-CoV (5). ACE2 is highly expressed in both the lung and heart (6), and the SARS-CoV viral RNA has been detected in autopsied heart samples from SARS patients (7). However, large-scale autopsy or biopsy studies are still required to further confirm the myocardial infection in COVID-19 by the tissue viral RNA detection or *in situ* hybridization at heart and endothelium. In addition, it is worth noticing that both blockades of AT1 receptors and inhibition of Ang II synthesis would increase the expression of cardiac ACE2 (8); therefore, for patients with hypertension or congestive heart failure (HF), regular treatment with ACE inhibitors or angiotensin receptor blockers (ARB) could further increase the risk of coronavirus infection (**Figure 1**). However, the causal relationship between ACEI/ARB intake and increased viral load and deleterious outcomes in COVID-19 is still uncertain. Animal studies even showed a protective effect of ARB in lung injury during SARS-CoV infection (9, 10). Considering the solid evidence of the beneficial effect of ACEI/ARB in cardiovascular diseases, it is

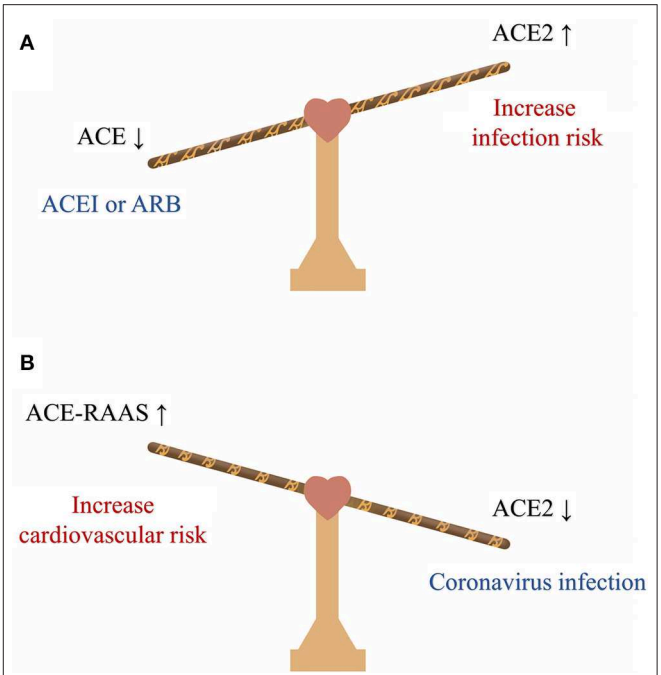

FIGURE 1 | The balance between ACE and ACE2 in COVID-19. **(A)** Treatment with ACEI or ARB increases the expression of cardiac ACE2 and could further increase the risk of coronavirus infection. **(B)** Coronavirus infection can downregulate ACE2, further activate the RAAS system and increase the cardiovascular burden. ACE, angiotensin-converting enzyme; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; RAAS, renin-angiotensin-aldosterone system.

currently not recommended to discontinue the RASS inhibition treatment in COVID-19 (11).

Cytokine Storm

Similar to SARS-CoV and MERS-CoV infection, SARS-CoV-2 infection can also induce excessive and aberrant host immune responses, leading to a cytokine storm (12). Studies have shown increased amounts of cytokines, such as IL-1β, IL-1ra, IL-6, TNF-α, IL-7, IL-8, IL-9, IL-10, FGF basic, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1a, MIP-1b, in the serum of COVID-19 patients, and the cytokine storm was associated with disease severity (1, 13). An autopsy study of a COVID-19 patient also revealed that there were a few interstitial mononuclear inflammatory infiltrates in the heart tissue; besides, the flow cytometric analysis of peripheral blood found that CD4 and CD8 T cells were hyperactivated and the concentration of highly proinflammatory Th17 cells significantly increased (14). Cytokines play an important role in the immune response to defend against viral infections; however, it has also been recognized that dysregulated and excessive immune responses may cause immunopathology. Inflammation after infection can be progressively amplified through positive feedback and eventually form a cytokine storm, leading to systematic self-attack, which is a well-established explanation for MODS during coronavirus infection (15, 16).

Aggravation of Existing Cardiovascular Diseases

SARS-CoV-2 infection is more likely to affect older patients with underlying cardiovascular comorbidities (17). According to a study, 4.2% of the confirmed cases and 22.7% of deaths have cardiovascular comorbidities (18). The fatality rate of patients with comorbidities was much higher than that of patients without comorbidities, and the fatality rate of patients with cardiovascular diseases (10.5%) was the highest (18). Therefore, COVID-19 patients are at risk of acute cardiovascular events. Secondary infection, disorder of sodium and water homeostasis, hypoxia, tissue hypoperfusion, and shock occurring during COVID-19 can all result in the aggravation of existing cardiovascular diseases and trigger severe events, such as acute coronary syndromes or exacerbation of HF. Additionally, a study has demonstrated that SARS-CoV infection can lead to the downregulation of ACE2 and activate the renin-angiotensin-aldosterone system (RASS), which would further increase the cardiovascular burden and contribute to adverse outcomes (7) (**Figure 1**).

In addition to the three mechanisms, the treatment with non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, and anti-viral agents, such as lopinavir/ritonavir (LVP/r), interferon-α (IFN-α), ribavirin, and azithromycin, could further increase the cardiovascular risk of COVID-19 patients and bring additional challenges. The harmful effects of NSAIDs and glucocorticoids on the cardiovascular system have been well-demonstrated by numerous studies that they can increase the risk of all cardiovascular events, myocardial infarction (MI), HF, and cerebral infarction (19, 20). LPV/r can induce cardiac conduction alteration and QTc and/or PR interval prolongation, further leading to atrioventricular block and torsade de pointes. LPV/r may also increase the risk of MI (21). Besides, protease inhibitor therapy has been associated with hyperglycemia, hyperlipidemia, and lipodystrophy (22) and such metabolic disturbances were also verified in patients treated with LPV/r (23). IFN-α is associated with hypertension, hypertriglyceridemia, and various cardiovascular adverse reactions and has been given a US boxed warning for its potential risk of ischemic disorders (24). A statement from the American Heart Association has announced that IFN-α can cause numerous direct cardiotoxicities, including arrhythmias, MI, and cardiomyopathy, and can also exacerbate underlying myocardial dysfunction (25). A US boxed warning has been issued for ribavirin that the hemolytic anemia associated with ribavirin may worsen underlying cardiac disease and lead to fatal and non-fatal MI (26). A recent study has shown that azithromycin could reinforce the anti-viral effect of hydroxychloroquine (27); however, its proven risk of severe QT prolongation should also be considered (28), especially when it is combined with hydroxychloroquine to treat the elderly COVID-19 patients (29).

RATIONALE FOR CARDIOVASCULAR ANTI-INFLAMMATORY THERAPY IN COVID-19

Cardiovascular protective strategies are urgent for the prevention and management of severe adverse cardiovascular events, which is of great significance to the overall prognosis of COVID-19 patients. The clinical bulletin released by the American College of Cardiology has issued several points of clinical guidance regarding cardiac complications (3), and the Chinese Society of Cardiology of Chinese Medical Association also developed an expert consensus on the clinical management of patients with emergent high-risk cardiovascular disease during the epidemic period (30). However, whether the conventional treatment is sufficient to overcome such challenges and whether any additional strategy to further reduce the risk of cardiovascular attack is needed in severe cases of COVID-19 remain unclear.

Excessive inflammation should be considered as a promising target because it plays an important role in all three mechanisms described above. It has already been demonstrated that for myocarditis with or without viral trigger, inflammation is implicated in the development of both acute cardiac injury and subsequent dilated cardiomyopathy (31). There is also abundant evidence that inflammation participates in various cardiovascular diseases, such as coronary artery disease (CAD) and HF. Especially in atherosclerosis, inflammation promotes the formation, destabilization, and rupture of atheromatous plaques and has already been recognized as an independent risk factor and prognostic predictor (32).

Therefore, conventional cardiovascular treatment plus anti-inflammatory therapy is a reasonable enhanced strategy for better management of cardiovascular impairment in severe cases of COVID-19. In addition, as the inflammatory attack on different organs shares numerous similar mechanisms and pathways, such as the inflammatory response under ischemia/reperfusion injury (IRI) of the heart, liver, and kidney (33), suppression of the systematic inflammatory response will

not only exert cardiovascular protection effect but also have potential benefits for MODS. Rheumatologists have also focused on the dysregulated inflammation and suggested that there might be a "window of opportunity" for immunosuppressive strategies used to treat rheumatic diseases to serve as strong allies in the fight against COVID-19 (34, 35).

VERIFIED CARDIOVASCULAR ANTI-INFLAMMATORY THERAPIES

Many clinical trials have been conducted in the past decade to directly test the feasibility of using different anti-inflammatory agents for cardiovascular protection under various conditions, mainly including CAD, myocardial IRI, HF, myocarditis/dilated cardiomyopathy, and rheumatic diseases [rheumatoid arthritis (RA), psoriatic arthritis, etc.]. Accumulating evidence has supported the efficacy of this novel strategy in improving cardiovascular outcomes. Here, we reviewed the currently available cardiovascular anti-inflammatory therapies that have been verified in clinical trials with positive results (36–47). The detailed information of these trials is listed in **Table 1**.

PROMISING PROSPECTS

Based on the above review and summarization, there are several perspectives that we can conclude to possibly guide the selection of anti-inflammatory therapies in COVID-19.

Targeting the Central Pathway (IL-1β, TNF-α, IL-6)

The immune pathway linking IL-1β, TNF-α, and IL-6, known as the central pathway, has long been implicated in atherosclerosis and is considered to play an important role in CAD (48). The significant effect of such a central pathway has also been recognized in many other fields of cardiovascular research (49–52), and a large portion of the clinical trials reviewed above was designed to target this pathway. Activation of the central pathway has already been observed in COVID-19 and should, therefore, be considered as a promising target (13). A multicenter, randomized controlled trial (ChiCTR2000029765) has been registered to evaluate the efficacy and safety of IL-6 blockade using tocilizumab in COVID-19. According to the preliminary treatment results currently released, among the 14 patients recruited (maximum age 82), including 9 severe cases and 2 critical cases, tocilizumab significantly improved the fever symptom and lung function and also accelerated the absorption of lung lesions. In addition to the benefits reviewed above, it is worth mentioning that tocilizumab also has a potential electrophysiological protective effect. Increased IL-6 level has been associated with acquired long QT-syndrome in patients with systemic inflammation, leading to higher risks for arrhythmias such as torsade de pointes (53). In RA patients, tocilizumab treatment led to a rapid and significant QT shortening correlating with the decrease in CRP and cytokine levels, which might benefit the overall mortality (54, 55). Such anti-arrhythmic potential may further support the application of tocilizumab in COVID-19 patients to counteract the risk of adverse QT prolongation and related life-threatening arrhythmias associated with elevated IL-6 and the anti-viral agents. A series of clinical trials on chloroquine in the treatment of COVID-19 are also currently underway and have revealed considerable benefits (27). Chloroquine has now been included in the 6th version of the Diagnosis and Treatment Plan for Novel Coronavirus Pneumonia (56). In addition to its direct antiviral effect (57), chloroquine might also exert anti-inflammatory effects by inhibiting the central pathway (58). Hydroxychloroquine (HCQ) has long been used to reduce inflammation in patients with RA and lupus. In a nationwide cohort study, HCQ use was associated with decreased CAD risk in the RA population (59). Besides, an OXI trial (NCT02648464) is currently underway to study the effect of HCQ on the prevention of recurrent cardiovascular events among MI patients (60). Therefore, we recommend targeting the central pathway by blockade of IL-6 (tocilizumab), IL-1β [canakinumab (IL-1β monoclonal antibody), anakinra (IL-1 receptor antagonist)], and TNF-α (etanercept, infliximab) to control the cytokine storm in the acute phase of COVID-19, thereby reducing cardiovascular impairment and even mitigating MODS.

Balancing the T-helper-1 (Th1) Cell and T-helper-2 (Th2) Cell Response and Promoting the Secretion of Anti-Inflammatory Cytokines

Studies of IRI and myocarditis have revealed that the Th1 response, characterized by the expression of multiple proinflammatory cytokines, is activated in the early disease phase and is associated with acute cardiac injury (61–63), while the Th2 response dominates later, promoting the resolution of acute inflammation and tissue repair. M2 macrophage polarization was found to be a significant change contributing to the transition from the Th1 to Th2 response, and monocyte-derived IL-10 is a well-recognized Th2-related anti-inflammatory cytokine that is highly expressed in the reparative phase and inhibits the Th1 response (64, 65). Early activation of the Th2 response or increased IL-10 expression in IRI and myocarditis could significantly inhibit the secretion of Th1-related proinflammatory cytokines and reduce myocardial necrosis (66–69). Patients with COVID-19 had high amounts of IL-1β, IFN-γ, IP-10, and MCP-1, indicating an activated Th1 response; besides, SARS-CoV-2 could also initiate increased secretion of Th2 cytokines, especially IL-10, which is different from SARS-CoV infection (70). Therefore, the implantation of mesenchymal stromal cells (MSCs) from allogeneic donors with an activated Th2 response or *ex vivo* bone marrow-derived MSCs after M2 macrophage polarization might increase the level of IL-10 in the acute phase of COVID-19 and serve as a possible solution to inflammation-mediated damage. In addition to the application in cardiovascular diseases reviewed above, cellular therapy using MSCs has also shown efficacy in the management of ARDS and is now being evaluated in phase 1/2 trials (12).

TABLE 1 | Clinical trials of anti-inflammatory therapy for cardiovascular protection with positive outcomes.

Clinical trial	Treatment	Target and mechanism	Patients	Outcomes	Reference
Cardiovascular protection in rheumatoid arthritis					
NCT01566201	Anakinra	IL-1R	Rheumatoid arthritis	Improvement in endothelial, coronary aortic function and left ventricular myocardial deformation and twisting after CAD	(33)
ENTRACTE	Etanercept	TNF-α	Rheumatoid arthritis	Rare cardiovascular events in both groups.	Preliminary results (34)
	Tocilizumab	IL-6			
Coronary artery disease					
CANTOS	Canakinumab	IL-1β	Previous MI and hsCRP≥2 mg	Lower rate of nonfatal MI, nonfatal stroke, cardiovascular death, and hospitalized UA leading to urgent revascularization	(35)
LoDoCo	Colchicine	Central pathway*	SCAD	Prevention of ACS, out-of-hospital cardiac arrest, and non-cardioembolic ischemic stroke	(36)
COLCOT	Colchicine	Central pathway*	Within 30 days after a MI	Lower risk of cardiovascular death, resuscitated cardiac arrest, MI, stroke, and hospitalized UA leading to urgent revascularization)	(37)
Myocardial ischemia/reperfusion injury					
NCT01491074	Tocilizumab	IL-6	NSTEMI	Attenuated hsCRP and primarily PCI-related hsTnT release	(38)
Heart failure					
CANTOS	Canakinumab	IL-1β	Previous MI and hsCRP≥2 mg	Dose-dependent reduction in HHF and the composite of HHF or HF–related mortality	(39)
ACCLAIM	Immunomodulation therapy†	Macrophages	NYHA II-IV chronic HF	Reduction in all-cause mortality and cardiovascular admission in patients with no history of MI or with NYHA II HF,	(40)
STAR-heart	Intracoronary bone marrow cell therapy	Resident cardiac macrophages#	Chronic HF due to ischemic cardiomyopathy	Improvement in ventricular performance, quality of life and survival	(41)
ixCELL-DCM	Ixmyelocel-T§	Bone marrow mononuclear cells¶	NYHA III or IV symptomatic HF due to ischemic dilated cardiomyopathy	Improvement in all-cause mortality, cardiac admissions, HF admissions, and left ventricular function	(42)
Chronic myocarditis/dilated cardiomyopathy					
CZECH-ICIT	Steroids and azathioprine	T cells suppression	Dilated cardiomyopathy and increased HLA expression on biopsy specimens	Long-term benefit in LVEF, LVV, LVDd, and NYHA class	(43)
TIMIC	Steroids and azathioprine	T cells suppression	Virus-negative myocarditis with chronic HF	Improvement in LVEF, LVV, LVD, and NYHA class	(44)

* *Central pathway refers to the immune pathway linking IL-1β, TNF-α, and IL-6.*

† *Patients' own blood was stressed to induced cell death, and then the mixture of apoptotic cells was injected intramuscularly into the same patient. Macrophages that phagocytose apoptotic cells downregulate proinflammatory cytokines and upregulate anti-inflammatory cytokines.*

Stem cells were taken up by resident cardiac macrophages which would exert cardioprotective effects.

§ *Intramyocardial injection of expanded bone marrow–derived mesenchymal stem cells with macrophages activated ex vivo.*

¶ *Bone marrow mononuclear cells express the anti-inflammatory cytokine IL-10 to exert protective role by limiting T-cell recruitment.*

IL-1R, interleukin-1 receptor; CAD, coronary artery disease; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; IL-1β, interleukin-1β; MI, myocardial infarction; hsCRP, high-sensitivity C-reactive protein; UA, unstable angina; SCAD, stable coronary artery disease; ACS, acute coronary syndrome; NSTEMI, non-ST segment elevation myocardial infarction; PCI, percutaneous coronary intervention; hsTnT, high-sensitivity troponin T; HHF, hospitalization for heart failure; HF, heart failure; NYHA, New York Heart Association; IL-10, interleukin-10; HLA, human leukocyte antigen; LVEF, left ventricular ejection fraction; LVV, left ventricular volume; LVDd, left ventricular diastolic dimension.

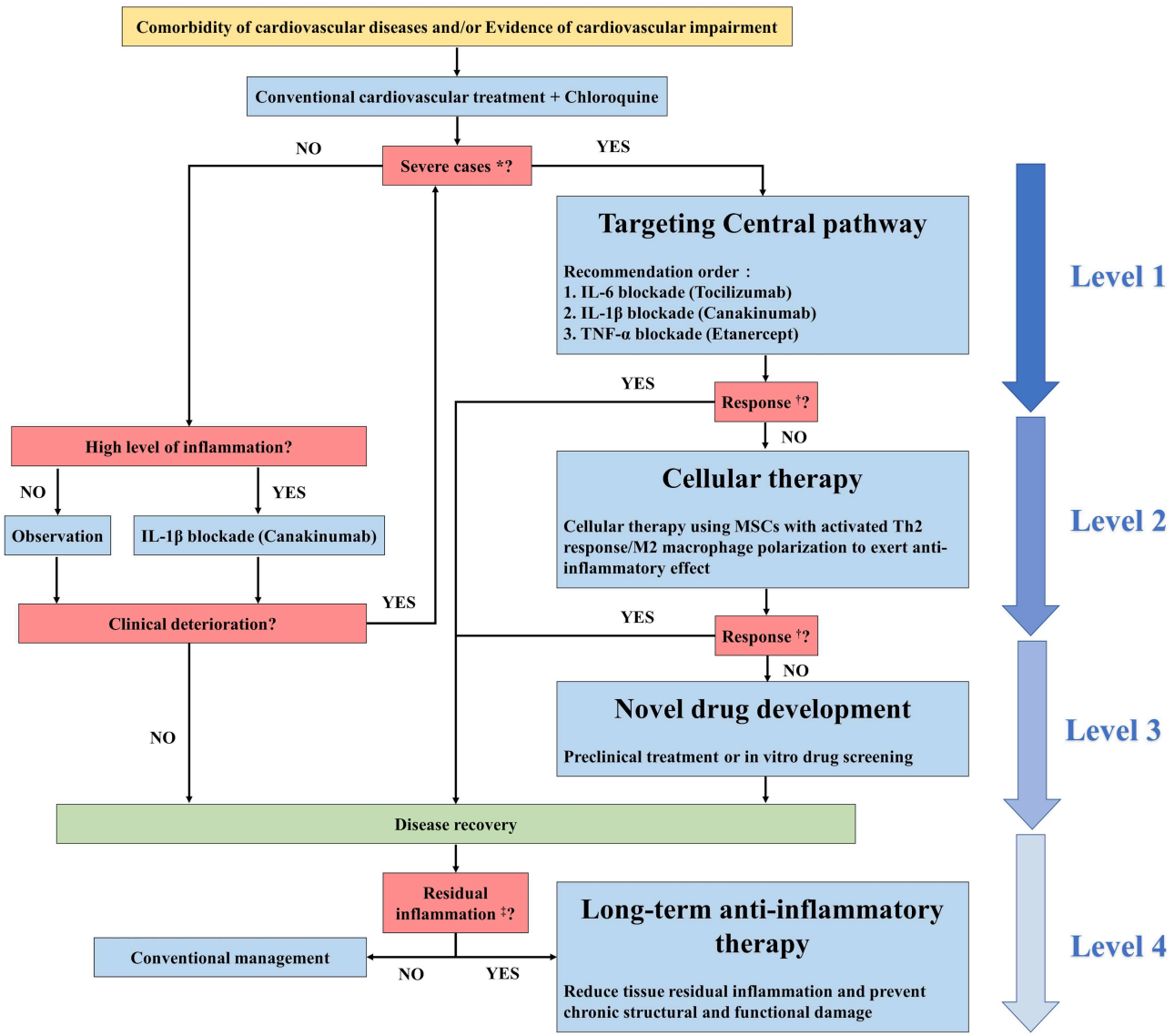

FIGURE 2 | Possible algorithm regarding the selection of anti-inflammatory therapy for COVID-19 patients with cardiovascular impairment. *Severe cases refer to patients with the following conditions according to the 6th version of the Diagnosis and Treatment Plan for Novel Coronavirus Pneumonia: respiratory rate≥30 bpm; SpO2≤30% at rest; PaO2/FiO2≤300 mmHg; progression on chest images>50% within 24 to 48 h; respiratory failure that requires mechanical ventilation; shock; organ dysfunction that requires ICU admission. † Response refers to the response to anti-inflammatory therapy. The criteria include but are not limited to decreased white blood cell counts, decreased hsCRP levels, decreased cytokine levels, significant symptom improvement, and significant improvement in chest images. Residual inflammation refers to evidence of a persistent state of high inflammation characterized by high levels of hsCRP and/or serum cytokines.

Long-Term Anti-Inflammatory Therapy in the Recovery Phase

Inflammation in the acute phase can lead to extensive injury; however, it should also be noted that after the acute damage, chronic residual inflammation that occurs with fibrosis during the reparatory phase can also result in persistent organ dysfunction. Studies of HF due to ischemic cardiomyopathy and chronic myocarditis have found that chronic residual inflammation is associated with myocardial fibrosis and adverse ventricular remodeling (49, 71–73). In addition, the long-term inflammatory status is also a hazard to atherosclerosis (32). During the follow-up of SARS and MERS patients, it was also observed that in those who survive intensive care, residual immune responses could lead to long-term lung damage and fibrosis, causing functional disability and reduced quality of life (74, 75). Therefore, in addition to the control of acute injury, it is also of great significance to conduct long-term follow-up after admission to monitor residual inflammation in COVID-19 patients, especially those with severe clinical manifestations or intense acute inflammatory responses. After excluding potential

contraindications, long-term anti-inflammatory therapy, such as steroids, azathioprine, and canakinumab, should be considered to reduce the residual inflammation and prevent further chronic structural and functional damage.

DISCUSSION

Hyperactivated inflammation is fundamental in the development of cardiovascular impairment and even MODS in COVID-19. In addition to conventional cardiovascular treatment, anti-inflammatory therapy is a reasonable strategy to further enhance cardiovascular protection and potentially mitigate MODS. By reviewing the inflammatory features and current promising treatments of COVID-19 as well as cardiovascular anti-inflammatory therapies that have been verified in clinical trials with positive results, we believed that targeting the central pathway (IL-1β, TNF-α, IL-6), balancing the Th1 and Th2 response, and administering long-term anti-inflammatory therapy should be considered as promising strategies to control cardiovascular impairment or even MODS during the acute and recovery phases of COVID-19. The experiences in cardiovascular anti-inflammatory therapies might be of great value to the management of COVID-19 patients and we recommended to take such experiences as references for clinical practice and conduct related clinical trials. We here propose a possible algorithm regarding the selection of anti-inflammatory therapy for COVID-19 patients with or at high risk of cardiovascular impairment (**Figure 2**).

Despite all the beneficial effects described above, it is also important to pay attention to the potential adverse cardiovascular effects of these drugs. For tocilizumab, hypercholesterolemia and hypertension are both common adverse events, prompting concern about increased cardiovascular risk (76). Especially, tocilizumab was widely noted to induce a proatherogenic lipid profile with increased serum levels of low-density lipoprotein cholesterol and total cholesterol (77–79). However, these changes can be improved by concomitant therapy with statins (80). For TNF inhibitor (TNFi), although not universally acknowledged, it might not be beneficial to HF. Severe HF remains a contraindication to TNFi treatment in RA patients. A clinical trial showed that high-dose infliximab could be harmful to patients with moderate-to-severe HF (81). For RA populations, a cohort study found that TNFi might increase the risk of both first hospitalization and exacerbation of HF in elderly patients with RA (82). Additionally, for hydroxychloroquine, a recent study pointed out that hydroxychloroquine could lead to unwanted QT interval prolongation by blocking the KCNH2-encoded hERG/Kv11.1 potassium channel, thereby increasing the risk of drug-induced torsade de pointes and sudden cardiac death (83). Therefore, biochemical indicators, hemodynamic parameters, and cardiac electrophysiology profiles should be monitored in clinical practice to avoid drug-induced adverse effects on cardiovascular risk factors, cardiac function, or lethal arrhythmias. Besides, corresponding treatment, such as lipid-lowering or antihypertensive medications should be prescribed if necessary.

Currently, a large number of novel therapies from preclinical studies or *in vitro* drug screening have been registered and accelerated into clinical practice. However, the safety profiles of these therapies have always not been well-characterized, especially for elderly COVID-19 patients with hepatic or renal dysfunction. In addition, as many of the therapies were proven to be effective only by *in vitro* experiments or poorly designed small-scale clinical studies, the exact benefits were also not guaranteed. Therefore, clinical translation of novel treatments from preclinical studies or *in vitro* drug screening should proceed with caution due to unguaranteed efficacy and safety profiles. Recently, experts from multiple research institutions in China raised their criticism and announced an urgent call for increasing the scientific rigorousness of clinical trials on COVID-19 (84). Considering timeliness and safety, we suggest prioritizing cardiovascular protective strategies that have been proven by large-scale clinical trials for proof-of-concept studies and clinical application on COVID-19 instead of rushing into new drug research and development.

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.

AUTHOR CONTRIBUTIONS

LW contributes to the literature search, manuscript preparation, and manuscript editing. YZ contributes to concept, design, manuscript preparation, and manuscript editing. SZ contributes to concept, design, definition of intellectual content, and manuscript review.

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REFERENCES

1. 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:e201585. doi: 10.1001/jama.2020.1585

2. Gorbalenya AE, Baker SC, Baric RS, Groot RJd, Drosten C, Gulyaeva AA, et al. Severe acute respiratory syndrome-related coronavirus: The species and its viruses – a statement of the coronavirus study group. *bioRxiv [Preprint]*. (2020). doi: 10.1101/2020.02.07.937862

3. Mohammad Madjid SDS, Vardeny O, Mullen B. *Acc Clinical Bulletin: Cardiac Implications of Novel Wuhan Coronavirus (2019-ncov)*. American College of Cardilogy (2020).
4. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in china. *Nature*. (2020) 579:265–9. doi: 10.1038/s41586-020-2008-3
5. 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: 10.1101/2020.02.11.944462
6. 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
7. Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutanen SM, Penninger JM, et al. Sars-coronavirus modulation of myocardial ace2 expression and inflammation in patients with sars. *Eur J Clin Invest*. (2009) 39:618–25. doi: 10.1111/j.1365-2362.2009.02153.x
8. Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, et al. Effect of angiotensin-converting enzyme inhibition and angiotensin ii receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation*. (2005) 111:2605–10. doi: 10.1161/CIRCULATIONAHA.104.510461
9. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*. (2005) 436(7047):112-6. doi: 10.1038/nature03712
10. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. 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
11. Kuster GM, Pfister O, Burkard T, Zhou Q, Twerenbold R, Haaf P, et al. Sars-cov2: Should inhibitors of the renin-angiotensin system be withdrawn in patients with covid-19? *Eur Heart J*. (2020) 20:ehaa235. doi: 10.1093/eurheartj/ehaa235
12. Zumla A, Hui DS, Azhar EI, Memish ZA, Maeurer M. Reducing mortality from 2019-ncov: host-directed therapies should be an option. *Lancet*. (2020) 395:e35–6. doi: 10.1016/S0140-6736(20)30305-6
13. 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
14. 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
15. Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. Into the eye of the cytokine storm. *Microbiol Mol Biol Rev*. (2012) 76:16–32. doi: 10.1128/MMBR.05015-11
16. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: Causes and consequences of cytokine storm and immunopathology. *Semin Immunopathol*. (2017) 39:529–39. doi: 10.1007/s00281-017-0629-x
17. 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
18. Novel Coronavirus Pneumonia Emergency Response Epidemiology Team. [the epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (covid-19) in china]. *Zhonghua Liu Xing Bing Xue Za Zhi*. (2020) 41:145–51. doi: 10.3760/cma.j.issn.0254-6450.2020.02.003
19. England BR, Thiele GM, Anderson DR, Mikuls TR. Increased cardiovascular risk in rheumatoid arthritis: mechanisms and implications. *BMJ*. (2018) 361:k1036. doi: 10.1136/bmj.k1036
20. Roubille C, Richer V, Starnino T, McCourt C, McFarlane A, Fleming P, et al. The effects of tumour necrosis factor inhibitors, methotrexate, non-steroidal anti-inflammatory drugs and corticosteroids on cardiovascular events in rheumatoid arthritis, psoriasis and psoriatic arthritis: a systematic review and meta-analysis. *Ann Rheum Dis*. (2015) 74:480–9. doi: 10.1136/annrheumdis-2014-206624
21. Lopinavir and ritonavir: *Drug Information*. Available online at: https://wwwuptodatecom/contents/lopinavir-and-ritonavir-drug-information
22. Tsiodras S, Mantzoros C, Hammer S, Samore M. Effects of protease inhibitors on hyperglycemia, hyperlipidemia, and lipodystrophy: a 5-year cohort study. *Arch Intern Med*. (2000) 160:2050–6. doi: 10.1001/archinte.160.13.2050
23. Hill A, Sawyer W, Gazzard B. Effects of first-line use of nucleoside analogues, efavirenz, and ritonavir-boosted protease inhibitors on lipid levels. *HIV Clin Trials*. (2009) 10:1–12. doi: 10.1310/hct1001-1
24. Interferon alfa-2b: *Drug Information*. Available online at: https://wwwuptodatecom/contents/interferon-alfa-2b-drug-information
25. Page RL, 2nd, O'Bryant CL, Cheng D, Dow TJ, Ky B, Stein CM, et al. Drugs that may cause or exacerbate heart failure: a scientific statement from the american heart association. *Circulation*. (2016) 134:e32–69. doi: 10.1161/CIR.0000000000000426
26. Ribavirin (systemic): *Drug Information*. Available online at: https://wwwuptodatecom/contents/ribavirin-systemic-drug-information?source=see_link
27. 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) 105949. doi: 10.1016/j.ijantimicag.2020.105949
28. Gibson PG, Yang IA, Upham JW, Reynolds PN, Hodge S, James AL, et al. Effect of azithromycin on asthma exacerbations and quality of life in adults with persistent uncontrolled asthma (amazes): a randomised, double-blind, placebo-controlled trial. *Lancet*. (2017) 390:659–68. doi: 10.1016/S0140-6736(17)31281-3
29. Choi Y, Lim HS, Chung D, Choi JG, Yoon D. Risk evaluation of azithromycin-induced qt prolongation in real-world practice. *Biomed Res Int*. (2018) 2018:1574806. doi: 10.1155/2018/1574806
30. Chinese Society of Cardiology of Chinese Medical A. Expert consensus on clinical management of patients with emergent high-risk cardiovascular disease during the epidemic period of novel coronavirus pneumonia. *Chinese J Cardiol*. (2020) 48:E001. doi: 10.3760/cma.j.issn.0253-3758.2020.0001
31. Cooper LT Jr. Myocarditis. *N Engl J Med*. (2009) 360:1526–38. doi: 10.1056/NEJMra0800028
32. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. (2002) 105:1135–43. doi: 10.1161/hc0902.104353
33. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Ischemia/reperfusion. *Compr Physiol*. (2016) 7:113–70. doi: 10.1002/cphy.c160006
34. Ferro F, Elefante E, Baldini C, Bartoloni E, Puxeddu I, Talarico R, et al. Covid-19: the new challenge for rheumatologists. *Clin Exp Rheumatol*. (2020) 38:175–80.
35. Sarzi-Puttini P, Giorgi V, Sirotti S, Marotto D, Ardizzone S, Rizzardini G, et al. Covid-19, cytokines and immunosuppression: what can we learn from severe acute respiratory syndrome? *Clin Exp Rheumatol*. (2020) 38:337–42.
36. Ikonomidis I, Tzortzis S, Andreadou I, Paraskevaidis I, Katseli C, Katsimbri P, et al. Increased benefit of interleukin-1 inhibition on vascular function, myocardial deformation, and twisting in patients with coronary artery disease and coexisting rheumatoid arthritis. *Circ Cardiovasc Imaging*. (2014) 7:619–28. doi: 10.1161/CIRCIMAGING.113.001193
37. ClinicalTrials.gov. *A Study of Tocilizumab in Comparison to Etanercept in Participants With Rheumatoid Arthritis and Cardiovascular Disease Risk Factors*. Available online at: https://clinicaltrials.gov/ct2/show/results/nct01331837 (accessed July 13, 2017).
38. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med*. (2017) 377:1119–31. doi: 10.1056/NEJMoa1707914
39. Nidorf SM, Eikelboom JW, Budgeon CA, Thompson PL. Low-dose colchicine for secondary prevention of cardiovascular disease. *J Am Coll Cardiol*. (2013) 61:404–10. doi: 10.1016/j.jacc.2012.10.027
40. Tardif JC, Kouz S, Waters DD, Bertrand OF, Diaz R, Maggioni AP, et al. Efficacy and safety of low-dose colchicine after myocardial infarction. *N Engl J Med*. (2019) 381:2497–505. doi: 10.1056/NEJMoa1912388
41. Kleveland O, Kunszt G, Bratlie M, Ueland T, Broch K, Holte E, et al. Effect of a single dose of the interleukin-6 receptor antagonist tocilizumab on inflammation and troponin t release in patients with non-st-elevation myocardial infarction: a double-blind, randomized, placebo-controlled phase 2 trial. *Eur Heart J*. (2016) 37:2406–13. doi: 10.1093/eurheartj/ehw171
42. Everett BM, Cornel JH, Lainscak M, Anker SD, Abbate A, Thuren T, et al. Anti-inflammatory therapy with canakinumab for the prevention of hospitalization for heart failure. *Circulation*. (2019) 139:1289–99. doi: 10.1161/CIRCULATIONAHA.118.038010

43. Torre-Amione G, Anker SD, Bourge RC, Colucci WS, Greenberg BH, Hildebrandt P, et al. Results of a non-specific immunomodulation therapy in chronic heart failure (acclaim trial): a placebo-controlled randomised trial. *Lancet*. (2008) 371:228–36. doi: 10.1016/S0140-6736(08)60134-8
44. Strauer BE, Yousef M, Schannwell CM. The acute and long-term effects of intracoronary stem cell transplantation in 191 patients with chronic heart failure: the star-heart study. *Eur J Heart Fail*. (2010) 12:721–9. doi: 10.1093/eurjhf/hfq095
45. Patel AN, Henry TD, Quyyumi AA, Schaer GL, Anderson RD, Toma C, et al. Ixmyelocel-t for patients with ischaemic heart failure: A prospective randomised double-blind trial. *Lancet*. (2016) 387:2412–21. doi: 10.1016/S0140-6736(16)30137-4
46. Wojnicz R, Nowalany-Kozielska E, Wojciechowska C, Glanowska G, Wilczewski P, Niklewski T, et al. Randomized, placebo-controlled study for immunosuppressive treatment of inflammatory dilated cardiomyopathy: two-year follow-up results. *Circulation*. (2001) 104:39–45. doi: 10.1161/01.CIR.104.1.39
47. Frustaci A, Russo MA, Chimenti C. Randomized study on the efficacy of immunosuppressive therapy in patients with virus-negative inflammatory cardiomyopathy: the timic study. *Eur Heart J*. (2009) 30:1995–2002. doi: 10.1093/eurheartj/ehp249
48. Ridker PM, Luscher TF. Anti-inflammatory therapies for cardiovascular disease. *Eur Heart J*. (2014) 35:1782–91. doi: 10.1093/eurheartj/ehu203
49. Dick SA, Epelman S. Chronic heart failure and inflammation: What do we really know? *Circ Res*. (2016) 119:159–76. doi: 10.1161/CIRCRESAHA.116.308030
50. Westman PC, Lipinski MJ, Luger D, Waksman R, Bonow RO, Wu E, et al. Inflammation as a driver of adverse left ventricular remodeling after acute myocardial infarction. *J Am Coll Cardiol*. (2016) 67:2050–60. doi: 10.1016/j.jacc.2016.01.073
51. Frangogiannis NG. The inflammatory response in myocardial injury, repair, and remodelling. *Nat Rev Cardiol*. (2014) 11:255–65. doi: 10.1038/nrcardio.2014.28
52. Matsumori A. Cytokines in myocarditis and cardiomyopathies. *Curr Opin Cardiol*. (1996) 11:302–9. doi: 10.1097/00001573-199605000-00011
53. 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
54. Lazzerini PE, Acampa M, Capecchi PL, Fineschi I, Selvi E, Moscadelli V, et al. Antiarrhythmic potential of anticytokine therapy in rheumatoid arthritis: Tocilizumab reduces corrected qt interval by controlling systemic inflammation. *Arthritis Care Res (Hoboken)*. (2015) 67:332–9. doi: 10.1002/acr.22455
55. Kobayashi H, Kobayashi Y, Yokoe I, Kitamura N, Nishiwaki A, Takei M, et al. Heart rate-corrected qt interval duration in rheumatoid arthritis and its reduction with treatment with the interleukin 6 inhibitor tocilizumab. *J Rheumatol*. (2018) 45:1620–7. doi: 10.3899/jrheum.180065
56. National health commission of the people's republic of china. *The Diagnosis and Treatment Plan for Novel Coronavirus Pneumonia (6th version)*. Available online at: http://www.nhc.gov.cn/yzygj/s7653p/202002/8334a8326dd94d329df351d7da8aefc2.Shtml (accessed February, 19, 2020).
57. 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
58. Monzavi SM, Alirezaei A, Shariati-Sarabi Z, Tavakol Afshari J, Mahmoudi M, Dormanesh B, et al. Efficacy analysis of hydroxychloroquine therapy in systemic lupus erythematosus: a study on disease activity and immunological biomarkers. *Inflammopharmacology*. (2018) 26:1175–82. doi: 10.1007/s10787-018-0512-y
59. Hung YM, Wang YH, Lin L, Wang PYP, Chiou JY, Wei JC. Hydroxychloroquine may be associated with reduced risk of coronary artery diseases in patients with rheumatoid arthritis: a nationwide population-based cohort study. *Int J Clin Pract*. (2018) 72:e13095. doi: 10.1111/ijcp.13095
60. Hartman O, Kovanen PT, Lehtonen J, Eklund KK, Sinisalo J. Hydroxychloroquine for the prevention of recurrent cardiovascular events in myocardial infarction patients: Rationale and design of the oxi trial. *Eur Heart J Cardiovasc Pharmacother*. (2017) 3:92–7. doi: 10.1093/ehjcvp/pvw035
61. Yan X, Anzai A, Katsumata Y, Matsuhashi T, Ito K, Endo J, et al. Temporal dynamics of cardiac immune cell accumulation following acute myocardial infarction. *J Mol Cell Cardiol*. (2013) 62:24–35. doi: 10.1016/j.yjmcc.2013.04.023
62. Cunningham MW. Cardiac myosin and the th1/th2 paradigm in autoimmune myocarditis. *Am J Pathol*. (2001) 159:5–12. doi: 10.1016/S0002-9440(10)61665-3
63. Okura Y, Yamamoto T, Goto S, Inomata T, Hirono S, Hanawa H, et al. Characterization of cytokine and inos mrna expression *in situ* during the course of experimental autoimmune myocarditis in rats. *J Mol Cell Cardiol*. (1997) 29:491–502. doi: 10.1006/jmcc.1996.0293
64. Shiraishi M, Shintani Y, Shintani Y, Ishida H, Saba R, Yamaguchi A, et al. Alternatively activated macrophages determine repair of the infarcted adult murine heart. *J Clin Invest*. (2016) 126:2151–66. doi: 10.1172/JCI85782
65. Frangogiannis NG, Mendoza LH, Lindsey ML, Ballantyne CM, Michael LH, Smith CW, et al. Il-10 is induced in the reperfused myocardium and may modulate the reaction to injury. *J Immunol*. (2000) 165:2798–808. doi: 10.4049/jimmunol.165.5.2798
66. DeBerge M, Yeap XY, Dehn S, Zhang S, Grigoryeva L, Misener S, et al. Mertk cleavage on resident cardiac macrophages compromises repair after myocardial ischemia reperfusion injury. *Circ Res*. (2017) 121:930–40. doi: 10.1161/CIRCRESAHA.117.311327
67. Yang Z, Zingarelli B, Szabo C. Crucial role of endogenous interleukin-10 production in myocardial ischemia/reperfusion injury. *Circulation*. (2000) 101:1019–26. doi: 10.1161/01.CIR.101.9.1019
68. Watanabe K, Nakazawa M, Fuse K, Hanawa H, Kodama M, Aizawa Y, et al. Protection against autoimmune myocarditis by gene transfer of interleukin-10 by electroporation. *Circulation*. (2001) 104:1098–100. doi: 10.1161/hc3501.096190
69. Chen X, Zeng XH, Wang M, Chen L, Zhang N, Rao M, et al. Bcl2-like protein 12 is required for the aberrant t helper-2 polarization in the heart by enhancing interleukin-4 expression and compromising apoptotic machinery in cd4+ t cells. *Circulation*. (2018) 138:2559–68. doi: 10.1161/CIRCULATIONAHA.118.033890
70. Wong CK, Lam CW, Wu AK, Ip WK, Lee NL, Chan IH, et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. *Clin Exp Immunol*. (2004) 136:95–103. doi: 10.1111/j.1365-2249.2004.02415.x
71. Frangogiannis NG. Regulation of the inflammatory response in cardiac repair. *Circ Res*. (2012) 110:159–73. doi: 10.1161/CIRCRESAHA.111.243162
72. Kuhl U, Noutsias M, Seeberg B, Schultheiss HP. Immunohistological evidence for a chronic intramyocardial inflammatory process in dilated cardiomyopathy. *Heart*. (1996) 75:295–300. doi: 10.1136/hrt.75.3.295
73. Kawai C. From myocarditis to cardiomyopathy: Mechanisms of inflammation and cell death: Learning from the past for the future. *Circulation*. (1999) 99:1091–100. doi: 10.1161/01.CIR.99.8.1091
74. Batawi S, Tarazan N, Al-Raddadi R, Al Qasim E, Sindi A, Al Johni S, et al. Quality of life reported by survivors after hospitalization for middle east respiratory syndrome (mers). *Health Qual Life Outcomes*. (2019) 17:101. doi: 10.1186/s12955-019-1165-2
75. 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
76. Tanaka T, Ogata A, Narazaki M. Tocilizumab for the treatment of rheumatoid arthritis. *Expert Rev Clin Immunol*. (2010) 6:843–54. doi: 10.1586/eci.10.70
77. Bacchiega BC, Bacchiega AB, Usnayo MJ, Bedirian R, Singh G, Pinheiro GD. Interleukin 6 inhibition and coronary artery disease in a high-risk population: a prospective community-based clinical study. *J Am Heart Assoc*. (2017) 6:1–9. doi: 10.1161/JAHA.116.005038
78. McInnes IB, Thompson L, Giles JT, Bathon JM, Salmon JE, Beaulieu AD, et al. Effect of interleukin-6 receptor blockade on surrogates of vascular risk in rheumatoid arthritis: measure, a randomised, placebo-controlled study. *Ann Rheum Dis*. (2015) 74:694–702. doi: 10.1136/annrheumdis-2013-204345
79. Gabay C, McInnes IB, Kavanaugh A, Tuckwell K, Klearman M, Pulley J, et al. Comparison of lipid and lipid-associated cardiovascular risk marker changes after treatment with tocilizumab or adalimumab in

patients with rheumatoid arthritis. *Ann Rheum Dis*. (2016) 75:1806–12. doi: 10.1136/annrheumdis-2015-207872

80. Soubrier M, Pei J, Durand F, Gullestad L, John A. Concomitant use of statins in tocilizumab-treated patients with rheumatoid arthritis: a *post hoc* analysis. *Rheumatol Ther*. (2017) 4:133–49. doi: 10.1007/s40744-016-0049-8
81. Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: Results of the anti-tnf therapy against congestive heart failure (attach) trial. *Circulation*. (2003) 107:3133–40. doi: 10.1161/01.CIR.0000077913.60364.D2
82. Setoguchi S, Schneeweiss S, Avorn J, Katz JN, Weinblatt ME, Levin R, et al. Tumor necrosis factor-alpha antagonist use and heart failure in elderly patients with rheumatoid arthritis. *Am Heart J*. (2008) 156:336–41. doi: 10.1016/j.ahj.2008.02.025
83. Giudicessi JR, Noseworthy PA, Friedman PA, Ackerman MJ. Urgent guidance for navigating and circumventing the qtc prolonging and torsadogenic potential of possible pharmacotherapies for covid-19. *Mayo Clinic Proc*. (2020). doi: 10.1016/j.mayocp.2020.03.024. [Epub ahead of print].
84. Chen Feng HY, Zhijie Z, Jinling T, Jielai X, Siyan Z, Yang Z, et al. An urgent call for raising the scientific rigorousness of clinical trials on covid-19. *Chinese J Epidemiol*. (2020) 41:301–2. doi: 10.3760/cma.j.issn.0254-6450.2020.03.004

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COVID-19 Management and Arrhythmia: Risks and Challenges for Clinicians Treating Patients Affected by SARS-CoV-2

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The COVID-19 pandemic is an unprecedented challenge and will require novel therapeutic strategies. Affected patients are likely to be at risk of arrhythmia due to underlying comorbidities, polypharmacy and the disease process. Importantly, a number of the medications likely to receive significant use can themselves, particularly in combination, be pro-arrhythmic. Drug-induced prolongation of the QT interval is primarily caused by inhibition of the hERG potassium channel either directly and/or by impaired channel trafficking. Concurrent use of multiple hERG-blocking drugs may have a synergistic rather than additive effect which, in addition to any pre-existing polypharmacy, critical illness or electrolyte imbalance, may significantly increase the risk of arrhythmia and Torsades de Pointes. Knowledge of these risks will allow informed decisions regarding appropriate therapeutics and monitoring to keep our patients safe.

Keywords: COVID-19, QT, arrhythmia, hERG, drug safety, QTc

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INTRODUCTION

The novel coronavirus disease of 2019 (COVID-19) pandemic is uncharted territory for clinicians and healthcare systems alike. The potential for a high volume of severely unwell patients creates a significant challenge. In particular, the combination of severe infection, respiratory dysfunction, sepsis, shock, and/or haemodynamic instability (1–3) present significant potential for myocardial injury and potentially dangerous arrhythmia. Not only do these pathophysiological states promote the formation of arrhythmia, but unstable arrhythmia could present a significant threat to this cohort of patients who are at risk of haemodynamic instability.

Due to the potential strain on healthcare systems, it is likely that non-specialists will be involved in the management of COVID-19 patients including those experiencing arrhythmia. Similarly, clinicians may face situations where they are using multiple concurrent medications with which they may not be entirely familiar. Here, we attempt to summarize how the use of multiple different types of medication could contribute (synergistically, in some instances) to increase arrhythmic risk. In particular, we focus on the risk of drug-induced QT interval prolongation (commonly referred to as acquired long QT syndrome; aLQTS) which is a serious issue for many of the medications which are likely to be used.

While the use of drugs known to prolong the QT interval may well be necessary and unavoidable, an awareness and understanding of this risk should guide additional safety measures such as monitoring of the corrected QT interval (QTc) on 12-lead electrocardiogram (ECG) measurement. For cases of a prolonged QT interval there is a risk, particularly with a rate-corrected QT interval (QTc) of >500 ms (4), of dangerous arrhythmia including Torsades de Pointes (TdP); close monitoring and involvement of specialist cardiology input should be sought, as well as minimizing, where possible, the use of other known QT-prolonging medication.

INCREASED BASELINE ARRHYTHMIC RISK IN COVID-19 PATIENTS

From the data available, COVID-19 seems to cause more serious disease in older patients and those with comorbidities (1–3, 5–7). Zhou et al. (3) described hypertension, diabetes and coronary heart disease as the three most prevalent comorbidities in COVID-19 patients from two hospitals at the epicenter of the initial outbreak in Wuhan. Not only were these comorbidities associated with a significantly worse outcome, they are also, in combination with advanced age, significant risk factors for arrhythmia. Increasing age and co-morbidities also increase the likelihood of pre-existing polypharmacy, which may prove problematic in the context of additional potentially QT-prolonging medication. Unfortunately, published data of COVID-19 patient cohorts to date do not seem to include any ECG or specific arrhythmia data, although no doubt we will gain a better picture as our understanding of the disease and its management evolves.

INCREASED ARRHYTHMIC RISK OF COVID-19 INFECTION

SARS-CoV-2 is thought to gain entry to human host cells via the angiotensin converting enzyme 2 (ACE2) receptor, which is highly expressed in the heart and lungs and to which the viral spike protein has high affinity (8, 9). Interestingly, data during the previous SARS-CoV outbreak demonstrated ACE2-dependent cardiac infection and inflammation in both mouse and human hearts (10). The down-regulation of affected ACE2 receptors was suggested as a potential contributory factor to SARS-associated myocarditis and subsequent cardiomyopathy, with inflammation and fibrosis likely to provide a substrate for arrhythmia.

The risk of arrhythmia is likely to increase with the development of significant infection, and increase as the severity of the infection and/or systemic inflammatory response increases (11). Significant myocardial damage and fulminant myocarditis have been described (2, 3, 5), and cardiac arrest associated with ventricular arrhythmia (as well as non-shockable rhythms) is reported (7). Du et al. reported some form of arrhythmia present in 60% of a group of fatal cases, with cardiac arrest or malignant arrhythmia listed as the cause of death for over 10% of cases (12).

There is increasing awareness of the development in severely unwell patients of a hyperinflammatory state or cytokine storm (13) which can lead to multi-organ failure. Recent work strongly suggests this hypercytokinemia (in particular elevated levels of interleukin-6) further increases arrhythmic risk via multiple mechanisms, including, notably, hERG blockade (14) and QT prolongation (15, 16). Myocarditis itself is a heterogenous condition associated with a number of arrhythmic states including bradyarrhythmia and atrial or ventricular tachyarrhythmia (17). Data are urgently needed to describe the unique arrhythmic challenges of COVID-19 myocarditis.

Additionally, the multiple medications likely to receive significant use (antibiotics, anesthetic agents, anti-arrhythmic agents, and potentially specific agents to target COVID-19 such as anti-malarial or anti-viral medications) may indeed contribute to a pro-arrhythmic state in a patient group already at risk. Importantly, the additional risk of QT prolongation with some potential combinations of these medications may be synergistic rather than simply additive, due largely to their unique mechanisms of ion channel blockade. Finally, significant electrolyte disturbances, common in unwell patients, will further exacerbate arrhythmic risk (18).

CLINICAL MANAGEMENT OF COVID-19: SIGNIFICANT POTENTIAL FOR hERG BLOCKADE AND QT PROLONGATION

The hERG (or "Kv11.1") potassium channel (encoded by *human Ether-à-go-go Related Gene;* alternative nomenclature *KCNH2*) mediates the rapid component of cardiac delayed rectifier K^+ current (also known as I_{Kr}). Briefly, this channel is crucial to the repolarization phase of the cardiac action potential; we recommend an excellent review article (19). Inhibition of hERG is considered to be the most common and important mechanism of drug-induced QT prolongation (20, 21) and occurs through direct pharmacological channel block and/or impaired trafficking of hERG channels to the cell membrane. Consequently, hERG testing is a requirement during novel drug development: indeed, prolongation of the QT interval (and its association with dangerous TdP) has been the biggest cause of restriction or withdrawal of drugs already on the market (21–23).

Importantly, recent data including work from our group suggests that the effect on hERG (and thus on QT-prolongation) of multiple drugs may not be simply additive but synergistic (24, 25) (i.e., an effect in excess of the sum of their individual parts). This is particularly relevant when considering an older patient group who may already be taking hERG-blocking medication. In particular, the antimalarial medications chloroquine and hydroxychloroquine (26, 27) are receiving particular attention as antiviral agents: these drugs are multichannel blockers with particular effects on hERG and Kir2.1 and are likely to cause significant QT prolongation at the concentrations effective against SARS-CoV-2 *in vitro* (28–30), especially when combined with other antivirals (such as lopinavir/ritonavir) or antibiotics (macrolides and fluroquinolones being particularly notable) (26). Further information should be sought regarding novel antiviral agents (31) currently undergoing clinical trials; for example,

TABLE 1 | A list of medications which could be used in the management of COVID-19 which are also associated with risk of QT prolongation.

Type of medication	Drugs
Anesthetic agents	Propofol, sevoflurane
Antibiotic, antiviral or antifungal medication	Macrolides, fluoroquinolones, fluconazole, pentamidine, lopinavir/ritonavir*, favipiravir*
Anti-emetics	Domperidone, levomepromazine, ondansetron
Anti-arrhythmics	Amiodarone, flecainide, ibutilide, procainamide, quinidine, sotalol
Anti-psychotics (used for delirium)	Haloperidol, quetiapine, risperidone
Other potential therapies under consideration	Antimalarials such as chloroquine, hydroxychloroquine or mefloquine

*List intended to be illustrative as to risks and is not exhaustive. Further up to date information can be found online from trusted sources (www.crediblemeds.org). *possible rather than established risk.*

favipiravir has been associated with QT interval prolongation in a case report (32) and remdesivir awaits comprehensive testing.

Combination therapy with azithromycin and hydroxychloroquine is undergoing testing at present (33, 34). Notably, part of a Brazilian study comparing low vs. high dose chloroquine, in combination with ceftriaxone and azithromycin with or without oseltamivir, was terminated early due to safety concerns; with 25% of those in the high-dose arm showing QT prolongation (vs. 11% in the low-dose arm) and two patients in the high-dose arm experiencing ventricular tachycardia prior to death (35). Similarly, the head of a French cardiology unit reported they had prematurely terminated their hydroxychloroquine-azithromycin COVID-19 trial due to unacceptable QT prolongation (36). The randomized DisCoVeRy (NCT04315948), SOLIDARITY (EudraCT Number 2020-000982-18), and RECOVERY (EudraCT Number 2020-001113-21) studies will provide important evidence regarding the effectiveness and safety of various antiviral and antimalarial drugs in the treatment of COVID-19 patients (37).

Online resources can be consulted to clarify which drugs are associated with QT prolongation (see www.crediblemeds.org). **Table 1** lists medications associated with QT prolongation which may be commonly used in the management of COVID-19 [as taken from the updated WHO management guidance (38) with additions from authors' clinical experience from UK practice]. The list is not exhaustive but attempts to highlight potential areas of risk when using these medications, as well as their use in combination.

DISCUSSION

The recent publication by Ackerman et al. of urgent guidance and a practical flow-chart regarding safe use of QT-prolonging medication is very welcome, and should be consulted as an aid to manage risk in the setting of QT prolongation (39), as should the Heart Rhythm Society (HRS) Task Force update (40). Other resources provide valuable guidance for clinicians dealing with specific patient populations: those with congenital heart disease (41) or inherited arrhythmia syndromes (42). Of note, a case report has reported significant QT prolongation (620 ms) in a patient with COVID-19 treated with multiple hERG blocking medications (including levofloxacin, hydroxychloroquine and azithromycin), which was successfully managed with drug cessation and intravenous lignocaine (43). Separately, mexiletine has also been suggested to be effective in treating TdP associated with acquired long QT syndrome (44).

COVID-19 represents a step into the unknown: not only are we grappling to come to terms with effective management of this new disease, but so too with safe use of these treatments. Effective therapy will be welcome, but the use of multiple drugs in combination has to be exercised with caution as it may increase the risk of QT prolongation and Torsades de Pointes, largely via pharmacological hERG blockade. Knowledge of this risk enables clinicians to ensure adequate monitoring of the QT interval and management of arrhythmic risk, maximizing safety for our patients in this challenging time.

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All authors contributed to the concept, planning and writing of this mini-review, and approved the manuscript prior to submission.

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REFERENCES

1. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. (2020). doi: 10.1101/2020.02.06.20020974. [Epub ahead of print].
2. Yang X, Yu Y, Xu J, Shu H, Xia A, 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*. (2020). doi: 10.1016/S2213-2600(20)30079-5. [Epub ahead of print].
3. 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). doi: 10.1016/S0140-6736(20)30566-3. [Epub ahead of print].
4. Gibbs C, Thalamus J, Kristoffersen DT, Svendsen MV, Holla ØL, Heldal K, et al. QT prolongation predicts short-term mortality independent of comorbidity. *Europace*. (201) 21:1254–60. doi: 10.1093/europace/euz058
5. 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. *Intens Care Med*. (2020) 1–3. doi: 10.1007/s00134-020-05991-x. [Epub ahead of print].

6. Young BE, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. *JAMA*. (2020) 323:1488–94. doi: 10.1001/jama.2020.3204
7. 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
8. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intens Care Med*. (2020) 46:586–90. doi: 10.1007/s00134-020-05985-9
9. Crackower MA, Sarao R, Oliveira-dos-Santos AJ, Da Costa J, Zhang L. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*. (2002) 417:822–8. doi: 10.1038/nature00786
10. Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutanen SM, Penninger JM, et al. SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. *Eur J Clin Invest*. (2009) 39:618–25. doi: 10.1111/j.1365-2362.2009.02153.x
11. Shahreyar M, Fahhoum R, Akinseye O, Bhandari S, Dang G, Khouzam RN. Severe sepsis and cardiac arrhythmias. *Ann Transl Med*. (2018) 6:6. doi: 10.21037/atm.2017.12.26
12. Du Y, Tu L, Zhu P, Mu M, Wang R, Yang P, et al. Clinical features of 85 fatal cases of COVID-19 from Wuhan: a retrospective observational study. *Am J Respir Crit Care Med*. (2020). doi: 10.1164/rccm.202003-0543OC. [Epub ahead of print].
13. 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
14. Aromolaran AS, Srivastava U, Alí 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
15. Lazzerini PE, Boutjdir M, Capecchi PL. COVID-19, arrhythmic risk and inflammation: mind the gap! *Circulation*. (2020). doi: 10.1161/CIRCULATIONAHA.120.047293. [Epub ahead of print].
16. Lazzerini PE, Laghi-Pasini F, Boutjdir M, Capecchi PL. Cardioimmunology of arrhythmias: the role of autoimmune and inflammatory cardiac channelopathies. *Nat Rev Immunol*. (2019) 19:63–4. doi: 10.1038/s41577-018-0098-z
17. 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
18. Zeltser D, Justo D, Halkin A, Prokhorov V, Heller K, Viskin S. Torsade de Pointes due to noncardiac drugs. *Medicine*. (2003) 82:282–90. doi: 10.1097/01.md.0000085057.63483.9b
19. Vandenberg JI, Perry MD, Perrin MJ, Mann SA, Ke Y, Hill AP. hERG K^+ channels: structure, function, and clinical significance. *Physiol Rev*. (2012) 92:1393–478. doi: 10.1152/physrev.00036.2011
20. Recanatini M, Poluzzi E, Masetti M, Cavalli A, De Ponti F. QT prolongation through hERG K^+ channel blockade: current knowledge and strategies for the early prediction during drug development. *Med Res Rev*. (2005) 25:133–66. doi: 10.1002/med.20019
21. Hancox JC, McPate MJ, El Harchi A, Zhang Y. The hERG potassium channel and hERG screening for drug-induced torsades de pointes. *Pharmacol Ther*. (2008) 119:118–32. doi: 10.1016/j.pharmthera.2008.05.009
22. Lasser KE, Allen PD, Woolhandler SJ, Himmelstein DU, Wolfe SM, Bor DH. Timing of new black box warnings and withdrawals for prescription medications. *J Am Med Assoc*. (2002) 287:2215–20. doi: 10.1001/jama.287.17.2215
23. Roden DM. Drug-induced prolongation of the QT interval. *N Engl J Med*. (2004) 350:1013–22. doi: 10.1056/NEJMra032426
24. Wiśniowska B, Lisowski B, Kulig M, Polak S. Drug interaction at hERG channel: *In vitro* assessment of the electrophysiological consequences of drug combinations and comparison against theoretical models. *J Appl Toxicol*. (2018) 38:450–8. doi: 10.1002/jat.3552
25. El Harchi A, Butler AS, Zhang Y, Dempsey CE, Hancox JC. The macrolide drug erythromycin does not protect the hERG channel from inhibition by thioridazine and terfenadine. *Physiol Rep*. (2020) 8:e14385. doi: 10.14814/phy2.14385
26. Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. *J Crit Care*. (2020). doi: 10.1016/j.jcrc.2020.03.005. [Epub ahead of print].
27. Colson P, Rolain J-M, Lagier J-C, Brouqui P, Raoult D. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. *Int J Antimicrob Agents*. (2020) 105932. doi: 10.1016/j.ijantimicag.2020.105932. [Epub ahead of print].
28. Rodríguez-Menchaca AA, Navarro-Polanco RA, Ferrer-Villada T, Rupp J, Sachse FB, Tristani-Firouzi M, et al. The molecular basis of chloroquine block of the inward rectifier Kir2.1 channel. *Proc Natl Acad Sci USA*. (2008) 105:1364–8. doi: 10.1073/pnas.0708153105
29. Traebert M, Dumotier B, Meister L, Hoffmann P, Dominguez-Estevez M, Suter W. Inhibition of hERG K^+ currents by antimalarial drugs in stably transfected HEK293 cells. *Eur J Pharmacol*. (2004) 484:41–8. doi: 10.1016/j.ejphar.2003.11.003
30. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virol J*. (2005) 2:69. doi: 10.1186/1743-422X-2-69
31. Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). *Drug Discov Ther*. (2020) 14:58–60. doi: 10.5582/ddt.2020.01012
32. 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
33. Gautret P, Lagier J-C, 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) 105949. doi: 10.1016/j.ijantimicag.2020.105949. [Epub ahead of print].
34. Molina JM, Delaugerre C, Le Goff J, Mela-Lima B, Ponscarme D, Goldwirt L, et al. No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID-19 infection. *Médecine Mal Infect*. (2020). doi: 10.1016/j.medmal.2020.03.006. [Epub ahead of print].
35. Borba M, de Val FA, Sampaio VS, Alexandre MA, Melo GC, Brito M, et al. Chloroquine diphosphate in two different dosages as adjunctive therapy of hospitalized patients with severe respiratory syndrome in the context of coronavirus (SARS-CoV-2) infection: preliminary safety results of a randomized, double-blinded, phase IIb cl. *medRxiv*. (2020). doi: 10.1101/2020.04.07.20056424. [Epub ahead of print].
36. NEWSWEEK. *French Hospital Stops Hydroxychloroquine Treatment for COVID-19 Patient Over Major Cardiac Risk*. Available online at: https://www.newsweek.com/hydroxychloroquine-coronavirus-france-heart-cardiac-1496810 (accessed April 19, 2020).
37. Taccone FS, Gorham J, Vincent J-L. Hydroxychloroquine in the management of critically ill patients with COVID-19: the need for an evidence base. *Lancet*. (2020) doi: 10.1016/S2213-2600(20)30172-7. [Epub ahead of print].
38. World Health Organization. *Clinical Management of Severe Acute Respiratory Infection (SARI) When COVID-19 Disease Is Suspected: Interim Guidance* (2020).
39. 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). doi: 10.1016/j.mayocp.2020.03.024. [Epub ahead of print].
40. Heart Rhythm Society. *HRS COVID-19 Task Force Update*. Heart Rhythm Society. Available online at: https://www.hrsonline.org/COVID19-Challenges-Solutions/hrs-covid-19-task-force-message-qtc-guidance (accessed April 16, 2020).
41. Tan W, Aboulhosn J. The cardiovascular burden of coronavirus disease 2019 (COVID-19) with a focus on congenital heart disease. *Int J Cardiol*. (2020). doi: 10.1016/j.ijcard.2020.03.063. [Epub ahead of print].

42. Wu C-I, Postema PG, Arbelo E, Behr ER, Bezzina CR, Napolitano C, et al. SARS-CoV-2, COVID-19 and inherited arrhythmia syndromes. *Heart Rhythm*. (2020). doi: 10.1016/j.hrthm.2020.03.024. [Epub ahead of print].
43. Mitra RL, Greenstein SA, Epstein LM. An algorithm for managing QT prolongation in Coronavirus Disease 2019 (COVID-19) patients treated with either chloroquine or hydroxychloroquine in conjunction with azithromycin: possible benefits of intravenous lidocaine. *Heart Case Rep*. (2020). doi: 10.1016/j.hrcr.2020.03.016. [Epub ahead of print].
44. Badri M, Patel A, Patel C, Liu G, Goldstein M, Robinson VM, et al. Mexiletine prevents recurrent torsades de pointes in acquired long QT syndrome refractory to conventional measures. *JACC Clin Electrophysiol*. (2015) 1:315–22. doi: 10.1016/j.jacep.2015.05.008

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Key Strategies for Clinical Management and Improvement of Healthcare Services for Cardiovascular Disease and Diabetes Patients in the Coronavirus (COVID-19) Settings: Recommendations From the REPROGRAM Consortium

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Patients with cardiovascular disease and diabetes are at potentially higher risk of infection and fatality due to COVID-19. Given the social and economic costs associated with disability due to these conditions, it is imperative that specific considerations for clinical management of these patients be observed. Moreover, the reorganization of health services around the pandemic response further exacerbates the growing crisis around limited access, treatment compliance, acute medical needs, and mental health of patients in this specific subgroup. Existing recommendations and guidelines emanating from respective bodies have addressed some of the pressure points; however, there are variations and limitations *vis a vis* patient with multiple comorbidities such as obesity. This article will pull together a comprehensive assessment of the association of cardiovascular disease, diabetes, obesity and COVID-19, its impact on the health systems and how best health systems can respond to mitigate current challenges and future needs. We anticipate that in the context of this pandemic, the cardiovascular disease and diabetes

patients need a targeted strategy to ensure the harm to this group does not translate to huge costs to society and to the economy. Finally, we propose a triage and management protocol for patients with cardiovascular disease and diabetes in the COVID-19 settings to minimize harm to patients, health systems and healthcare workers alike.

Keywords: coronavirus disease 2019 (COVID-19), healthcare services, cardiovascular diseases (CVDs), diabetes, obesity, personal protective equipment (PPE), clinical algorithm

INTRODUCTION

On March 11th, 2020, coronavirus disease 2019 (COVID-19) was designated as a global pandemic by the World Health Organization (WHO). As of 28th May, 5,716,271 confirmed cases have been reported with ∼356,124 deaths globally from 188 countries (1). In a matter of months, this has escalated into an unprecedented public health as well as an economic crisis. Several studies have confirmed that patients with COVID-19 show distinctive and relatively significant comorbidities of diabetes, obesity and cardiovascular disease (CVD) (2–12). Furthermore, COVID-19 patients with diabetes, obesity and CVD conditions are at a higher risk of morbidity and mortality (6, 7, 13). Conversely, patients with diabetes, CVD and obesity are also at a higher risk of contracting COVID-19 infection (6, 7, 14). Broadly speaking, CVD, diabetes and obesity are associated with poor clinical outcomes (15, 16). Therefore, in the milieu of COVID-19; public health systems, carers, and healthcare providers must take appropriate measures to mitigate the infection risks in this population and consider health system reorganization and adoption of technologies to sustain ongoing management (17–19). The frontline healthcare workers triaging and managing COVID-19 patients should consider various risks and their compounding effects on the prognoses of patients with CVD, diabetes and/or obesity.

RISKS AND OUTCOMES OF COVID-19 IN THIS POPULATION

Independent of other medical problems such as CVD, patients with diabetes are at elevated risk for infection from COVID-19 by 2-to-3 fold (13). This may be attributed to the reduced functioning of the immune system caused by high blood glucose levels (13). Moreover, diabetes is often accompanied by CVD, obesity and old age, all of which are known to increase the risk of infection (14). Outcomes of infection by COVID-19 are also poor in this population. Of 72,314 patients from the Chinese Center for Disease Control and Prevention case series, case fatality rate (CFR) was elevated among those with diabetes and CVD; 7.3 and 10.5% respectively compared to an overall CFR of 2.3% (5). Outcomes are worse in people with poorly controlled diabetes, and in those with additional chronic medical conditions such as CVD and obesity (7, 13).

A recent meta-analysis of eight studies from China including 46,248 infected patients showed the most prevalent comorbidities were high blood pressure (17 ± 7%, 95% CI 14–22%) and diabetes (8 ± 6%, 95% CI 6–11%), followed by CVD (5 ± 4%, 95% CI 4–7%) (6). At this time, though the mechanism of these associations remains unclear, the potential explanations include CVD being more prevalent in those with advancing age, a functionally impaired immune system, elevated levels of angiotensin-converting enzyme 2 (ACE2), or a predisposition to COVID-19 for those with CVD (6). There is significant overlap in risk factors of CVD and venous thromboembolism (VTE); with CVD risk factors such as older age, smoking, and adiposity associated with high VTE risk (20). A recent Chinese study on 1026 COVID-19 patients reported that 40% ($n = 407$) at high risk of venous thromboembolism; and the high-risk patients who didn't receive prophylactic therapy (11%) developed venous thromboembolism (21).

As of 4th April 2020, the Intensive Care National Audit and Research Centre (ICNARC) in the United Kingdom (UK) received notification of 2,621 COVID-19 positive cases requiring critical care (22). Analysis of this data suggest a significantly greater number of COVID-19 positive cases, than "seasonal" non-COVID viral pneumonia patients, were obese, with body mass index (BMI) $\geq$30 kg/m^2 (38% compared to 31%, chi-square 28.2, $p < 0.00001$). The requirement for ventilatory support was equal between the obese and non-obese patients (76 and 68% of cases respectively, $p = 0.077$). Obesity was associated with higher mortality rates in critical care when compared to normal or underweight COVID-19 positive patients (58% compared to 45%, chi-square 8.3 $p = 0.004$). These data derive from the ICNARC case mix programme database. The case mix programme is the national clinical audit of patient outcomes from adult critical care coordinated by the ICNARC. For more information on the representativeness and quality of these data, we encourage readers to contact ICNARC (22).

GAPS, CHALLENGES, AND CONCERNS ABOUT THE MANAGEMENT OF CVD AND DIABETES

In the setting of COVID-19, specialist cardiologists and endocrinologists are confronted with a number of critical issues on management and treatment of CVD. There has been speculation regarding the risk associated with the use of ACE inhibitors (ACEi) and angiotensin receptor blockers (ARBs) in patients with COVID-19 (23, 24). This is particularly relevant to patients with diabetes and CVD, many of whom rely on such pharmacotherapy for the treatment of retinopathy, nephropathy and hypertension (14). Though the ACEi and ARB are commonly used in the management of CVDs (hypertension, coronary artery disease, congestive heart failure) and diabetes, there are

conflicting data from studies (13, 15, 16) demonstrating an increase or having minimal effect on ACE2 levels (25–29).

Poor glycaemic index is known to cause immune suppression through impaired neutrophil degranulation, deficient complement system and phagocytosis (30). The co-existence of CVD and diabetes is a known risk factor for several serious respiratory viral illnesses such as Influenza (31). With poor glycaemic control being correlated with worse prognosis in diabetic patients infected with COVID-19, glucose control is key to the prevention of infection and minimizing the severity of and morbidity caused by infection (32). However, the swift transition of primary health care provision from in-person to teleconsultations has led to many patients being unable to access services for regular check-ups, presumably due to lack of literacy and access to appropriate technology. Moreover, an increasing number of physicians have reported a sudden decrease in the incidence of myocardial infarction, stroke, and other acute conditions (33, 34). Given that the prevalence of these conditions would be invariably the same, if not elevated, in these circumstances, this indicates a problematic decline in the number of patients presenting to hospital with these critical conditions. Likewise, there are increasing concerns related to the postponement of elective cardiac and vascular surgeries. With pressure building on the available beds, it is imminent that only a select group of patients with clinical indication in which surgery cannot be postponed will receive the therapy (35). Furthermore, impact of postponement on those who will eventually receive prolonged and delayed surgery vis a vis their long-term morbidity is not known. In patients with diabetes, CVD or obesity, physical exercise is critical to improving patient outcomes (36). With the implementation of self-isolation however, the ability and motivation to engage in physical exercise are greatly diminished.

Healthcare workers responsible for the care of patients with diabetes and cardiovascular disorders infected with COVID-19 face threats to their own well-being, being at risk of exposure to a high viral load (37). Time is critical in acute myocardial or cerebral infarction. Given the reorganization of healthcare services, additional pressure on frontline services for COVID-19 cases, repurposing of other physicians to meet the demand, additional resources limitations are being realized across the spectrum in delivering time-critical reperfusion therapy (34). It is more likely that reperfusion services will also have time-constrained service hours, and due to palpable risks from COVID-19 positive patients to healthcare workers delivering reperfusion therapy, there will be significant negative impact and delays in reperfusion therapy. All patients with acute neuro/cardiovascular events, including acute myocardial infarction (AMI) and acute ischemic stroke (AIS) may be recommended to follow the overarching COVID-19 protocol to screen for any positive cases in order to minimize the risk to healthcare workers (34).

Refugees, undocumented immigrants and members of aboriginal communities also have limited provisions of access and medical relief in pandemic situations, due to structural factors and poor socioeconomic conditions that put them at compounded risk due to cardiovascular and diabetes comorbidities.

EXISTING RECOMMENDATIONS AND GUIDELINES FOR DIABETIC PATIENTS

Professional societies such as the American Association of Clinical Endocrinologists (38) and European society of endocrinology (39) are in agreement on the need for people with diabetes to prevent and prepare for the spread of COVID-19 by taking the regular precautions such as staying home as much as possible and washing hands regularly. The guidelines also advise people to continue taking their medication in order to maintain glucose control and to stock up on an additional 30-day supply of medication and supplies for monitoring blood glucose levels at home. However, there are no specific guidelines targeted at individuals with multiple comorbidities, such as obesity and CVD. As per the guidance given by the International Diabetes Federation in the context of COVID-19 pandemic, people with diabetes are among those high risk categories that can have serious illness (just like the flu) if they get the virus and it is best not to rush to the hospital, to avoid transmitting the virus to others and to allow priority arrangements to be made by medical personnel, if needed, instead of having to wait in line (40). The International Society for Pediatric and Adolescent Diabetes (ISPAD) has updated its guidelines recently on 19 March 2020 amidst the recent COVID-19 pandemic (41).

NHS clinical guidelines for the management of diabetic patients in COVID-19 recommend expedition of treatment and discharge of inpatients, and the use of virtual clinics and teleconsultations in primary and secondary care settings (42). However, guidelines fail to address the need for extra measures to be taken for care of patients with poor access and literacy with regards to technology. Moreover, elderly patients and those with chronic disability living in nursing homes or aged care facilities are at heightened risk of infection. These patients often have a high prevalence of comorbid cardiovascular and diabetes risk factors which makes them vulnerable during a pandemic such as COVID-19. Increasing reports of acts of microaggression, xenophobia and discrimination are surfacing since the inception of this pandemic. This is particularly relevant to specific populations such as south-Asians, who have high rates of diabetes (43).

CURRENT APPROACHES TO THE MANAGEMENT OF CARDIOVASCULAR PATIENTS

Current approaches to the management of this population aim to continue care of patients during COVID-19, while minimizing the risk of transmission to both healthcare workers and patients. The current protocol at academic medical centers in China for Acute Myocardial Infarction involves compulsory screening for fever and respiratory symptoms, and any patients with STEMI that have suspected or confirmed infection are treated with emergency intravenous thrombolysis, in the

absence of contraindications (44). For cardiologists in the operating theater, strict guidelines regarding hand hygiene and personal protective equipment (PPE) are followed, and the number of people in operating theater is minimized (44). The American College of Cardiology urges the implementation of telehealth in all cardiology clinics (45). Other societies including European Society of Cardiology, British Cardiovascular Society, Cardiac Society of Australian and New Zealand (CSANZ), High Blood Pressure Research Council of Australia (HBPRCA), Australian National Heart Foundation (NHF) and Australian and New Zealand Society of Cardiac and Thoracic Surgeons (ANZSCTS) also recommend use of telehealth services (17–19).

With regard to the use of ACEi and ARBs, several societies have highlighted that due to the limited nature of the evidence on this matter, it is advisable that ongoing management with such medication may continue in patients with diabetes and hypertension, unless otherwise clinically contra-indicated as per the case profile (46–48). Ongoing studies will bring clarity on the use of ACEi and ARBs in COVID-19 patients with diabetes and hypertension. A recent study reported a higher prevalence of CVD and more than 7% of patients suffer myocardial injury from the infection (22% of the critically ill) (49). Though ACE2 serves as the main gateway for infection, the role of ACEi or ARBs requires further investigation. Myocardial injury is present in more than a quarter of critical cases and presents in two patterns: acute myocardial injury and dysfunction on presentation; and myocardial injury that develops as the severity of illness intensifies (49–52). The continuation of clinically indicated ACEi and ARB medications is recommended based on the available evidence at this time though there are a number of promising treatments under investigation, but none with proven clinical efficacy to date. COVID-19 is proved to pose a challenge for heart transplantation, impacting donor selection, immunosuppression, and post-transplant management (52).

TABLE 1 | Summary of recommendations regarding COVID-19 in patients with diabetes and/or cardiovascular disease.

S. No	Stage of COVID-19 infection	Interventions/indications
1	Prevention of infection and containing pandemic	1. Wash your hands frequently with soap and water for 20 s or clean with alcohol-based hand rub 2. Maintain social distancing (2 meters or 6 feet) 3. Cough or sneeze into tissue or elbow 4. Avoid touching your face 5. Sanitize surfaces frequently
2	Symptomatic stage	1. If the patient is feeling unwell, he/she should stay at home 2. If the patient has fever, cough and/or difficulty breathing, seek medical attention and call in advance 3. Follow the directions of your local health authority
3	Controlling diabetes during illness	General sick day diabetes management principles (modified from ISPAD guidelines): 1. More frequent blood glucose and ketone (blood or urine) monitoring 2. Aim for a blood glucose level between 4 and 10 mmol/L (70–180 mg/dL) and blood ketones below 0.6 mmol/L when the child is ill 3. NEVER STOP INSULIN: If there is FEVER, insulin needs are usually higher 4. Monitor and maintain hydration with adequate salt and water balance 5. Treat underlying illness and symptoms (fever)
4.	URGENT specialist advice/referral to emergency	1. Fever or vomiting persists and/or weight loss continues, suggesting worsening dehydration and potential circulatory compromise 2. Fruity breath odor (acetone) persists or worsens / blood ketones remain elevated >1.5 mmol/L or urine ketones remain large despite extra insulin and hydration 3. The patient is becoming exhausted, confused, hyperventilating (Kussmaul breathing), or has severe abdominal pain 4. Identify COVID-19 patients who are at high-risk of venous thromboembolism (VTE), including those with prolonged immobility, overlapping cardiovascular disease (CVD) risk factors (adiposity, age and smoking) or with high estrogen levels (including those on exogenous hormone therapy). Consider initiating appropriate prophylaxis. If at higher risk of bleeding due to anticoagulation, adjust anticoagulation dose and duration as well as use of mechanical compression 5. Patients with body mass index (BMI) of 30 kg/m^2 or higher should be considered at high risk given the association of these patients with significantly higher mortality after COVID-19 infection. These patients need close monitoring over teleconsultation * 6. Patients who are at increased risk of QTc interval prolongation, life-threatening cardiac arrhythmic events and/or sudden cardiac death (e.g., COVID-19 positive patients with: (a) history of diabetes and/or CVD, and/or (b) those on post-exposure prophylaxis or treatment of COVID-19 using "off-label" drugs such as hydroxychloroquine, azithromycin and lopinavir/ritonavir)

Source:Prepared and adapted by the authors from the ISPAD guidelines.

ISPAD: International Society for Pediatric and Adolescent Diabetes.

**Based on the analysis of Intensive Care National Audit & Research Centre (ICNARC) United Kingdom data set (analyzed on April 4, 2020).*

*†Recommendations of the CVD and diabetes subcommittee of the COVID-19 Pandemic Health System **RE**silience **PROGRAM (REPROGRAM)** consortium.*

RISK OF LIFE-THREATENING CARDIAC ARRHYTHMIC EVENTS

Growing evidence suggests that COVID-19 is burdened by a higher risk of life-threatening cardiac arrhythmic events, especially in group of patients with diabetes and/or obesity, with important implications for survival (53). These life-threatening arrhythmias are also related to inflammation that can increase the duration of ventricular repolarization (QTc interval) (54). A particular attention to inflammation and arrhythmias is important in these patients that are frequently affected by QTc prolongation (55, 56). Therefore, key electrocardiogram (ECG) parameters such as QTc interval should be monitored in this subgroup of patients. Surveillance of QTc could potentially reduce the number of drug-induced ventricular arrhythmias and sudden cardiac deaths (57). This is particularly relevant as "off-label" agents such as hydroxychloroquine, azithromycin and lopinavir/ritonavir are being increasingly used in post-exposure prophylaxis or treatment of COVID-19 patients (57, 58). These drugs are proven to increase the risk of QTc interval prolongation, ventricular tachycardia (*torsades de pointes*) and sudden cardiac death (58).

RECOMMENDATIONS AND DISCUSSIONS

Patients with diabetes with multiple comorbidities, such as obesity and CVD, should take extra precaution for the prevention of possible infection risk due to COVID-19. They are recommended to be in virtual contact with their primary health carers, and to maintain glycaemic control with diligence. To ensure the maintenance of adequate glucose control in such exceptional circumstances, it is recommended that primary health care physicians take additional interest/responsibility to reach out to patients who have not presented for regular check-ups. The main recommendations for pediatric and adolescents with diabetes and CVD are summarized (**Table 1**). Due to the alarming decline in patients presenting with emergent conditions to hospitals and outpatient clinics, without any indications of a fall in prevalence of these conditions, we would request public health professions to take extra measures in reaching out to patients regarding the safety of coming to hospitals and the medical need to do so and benefit of getting timely acute reperfusion therapy in eligible patients. Given the aggravated risks, we propose a novel triage and management protocol that takes into account risks with CVD and diabetes (**Figure 1**).

Virtual delivery of group exercise classes could be organized for patients with diabetes, CVD or obesity, who are currently restricted by social isolation. Cross-department and peer-to-peer inter-specialty professional collaboration and communication are recommended to adapt existing pandemic preparedness and response strategies to manage patients with neuro cardiovascular emergencies. Special protection must be observed during interventions that produce aerosol (cardiopulmonary resuscitation). This may lower the risk of infection to healthcare workers and patients. Cardiovascular experts may brace themselves for deployments in different settings, for limited, extended or repurposed causes. The mobility of staff between COVID-19 treatment units and other patient facing consultation should be limited to avoid opportunities for nosocomial transmission. COVID-19 and patients with CVD, diabetes or obesity impact each other in compounding and negative dimensions. These patients are at increased risk of COVID-19 related hospitalization, morbidity and death; and those with COVID-19 also show propensity to increasing and emergent acute cardiovascular events. It is important to identify COVID-19 patients who are at high risk of VTE so that appropriate prophylaxis treatment could be initiated (22, 59). Anticoagulation should be considered for VTE prophylaxis. Given the high risk of bleeding in COVID-19 patients with high VTE risk, considerations should include adjustments in anticoagulant dose and duration as well as use of mechanical compressions (22).

Obesity is associated with severe COVID-19 (7–12, 22, 60). Moreover, obesity or higher BMI is known to be associated with a higher risk of CVD, diabetes and hypertension—which are independent predictors of poor outcomes in COVID-19 (50, 60). The analyses of ICNARC data suggest that BMI$\geq$30 kg/m^2 should be used as a prognostic indicator of mortality in critical care settings due to COVID-19 (22). Another recent study found a significant association of the prevalence of obesity (defined by BMI$\geq$30 kg/m^2) with severe COVID-19 (7). We recommend that clinicians should consider BMI$\geq$30 while estimating risks and stratifying patients for early and ongoing intervention. Center for Disease Control and Prevention (CDC) in the United States also list obesity, although with a relatively higher BMI cut-off of $\geq$40 kg/m^2, as an independent risk factor, of severe illness in COVID-19 (61). There are concerns this BMI cut-off ($\geq$40 kg/m^2) might mislead or compromise the safety of obese people at lower BMIs (60). The current consortium recommends BMI cut-off of 30 kg/m^2 in identifying patients with adverse COVID-19 prognosis. Surveillance of ECG parameters is recommended to potentially reduce the risk of life-threatening arrhythmic events and sudden cardiac death in COVID-19 positive patients especially those with history of diabetes and/or obesity and/or those on post-exposure prophylactic treatment (53–58).

In addition to clinical management, public health interventions must be adhered to such as masks (preferably N95), washing hands, social distancing. A New England Journal of Medicine study showed efficacy of face masks in preventing further transmission of Coronavirus from symptomatic individuals (62). It is evident from the guidance currently issued between World Health Organization (WHO), the CDC in United States, the Canadian Standards Association and Canadian Federal guidance (Canada), and the UK that differences exist in advice for healthcare workers to use respirators as opposed to surgical face masks (63–65). The UK initially advocated, "COVID-19 is classified as an airborne high consequence infectious disease in the UK", and instructed "ensure that staff who are assessing or caring for suspected COVID-19 cases are familiar with an FFP3 respirator conforming to EN149 [a protection level higher than N95], and that fit testing has been undertaken before using this equipment" (65). The current UK position aligns with WHO guidance although recommending

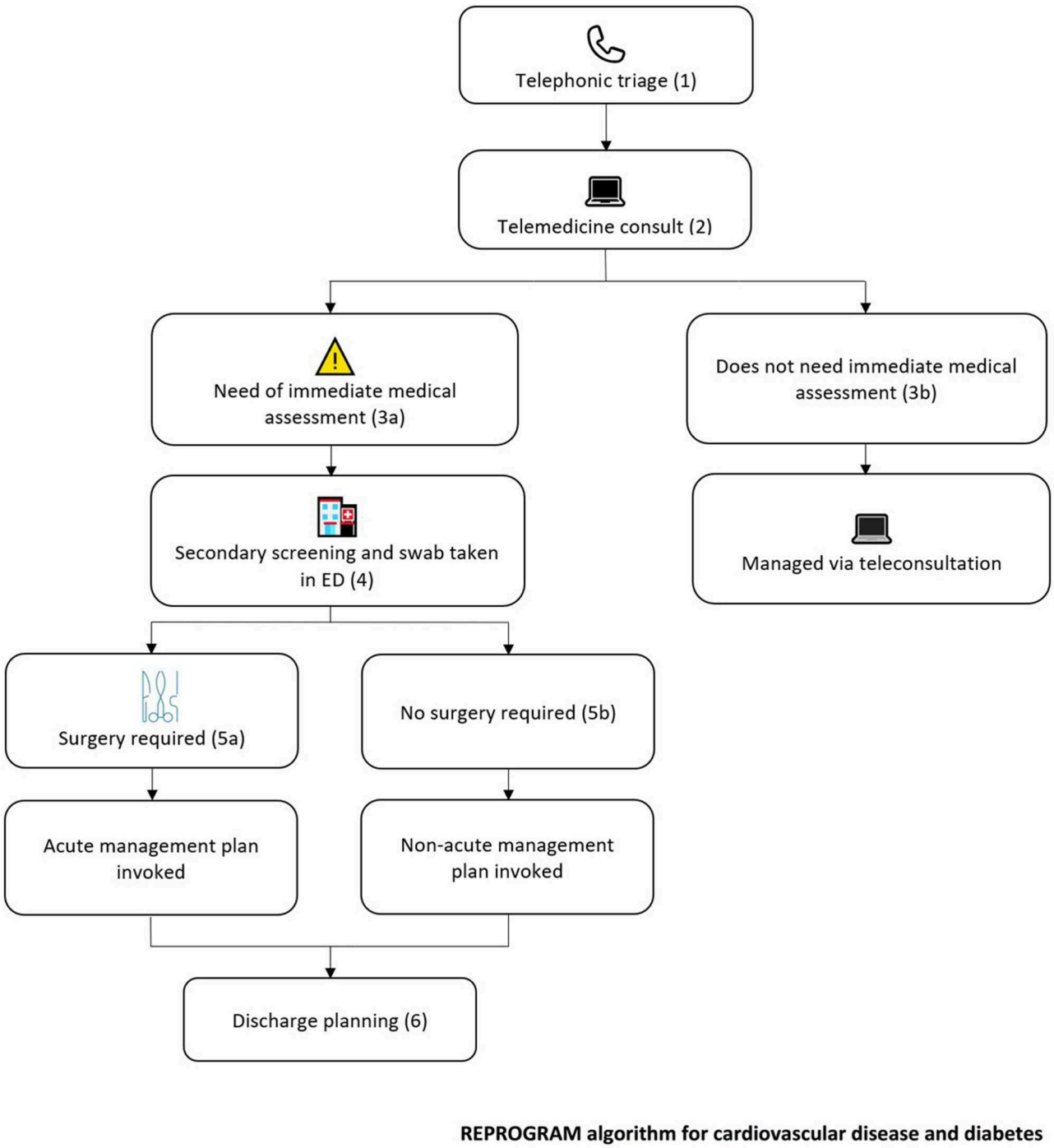

FIGURE 1 | Proposed cardiovascular disease and diabetes risk-adjusted, stage-wise, tele and in-hospital triage and management protocol. (1) All patients seeking outpatient or in-hospital appointment, except the emergency cases, must dial into the hospital for a triage over telephone for risk assessment of COVID-19 cases prior to consultation. The triage will be carried out by the relevant department officer, and will comprise a brief screening for signs, symptoms, and risk factors of COVID-19. Questions should address recent travel history, fever, cough, sore throat, shortness of breath, fatigue, aches and pains, headaches, runny or stuffy nose, diarrhea, sneezing, and loss of smell. Patients should be screened for their body mass index (BMI) and those with BMI $\geq$ 30 should be closely monitored and strongly advised to self-isolate and follow public health guidelines. Patients with BMI $\geq$ 30 are at significant risk of mortality after COVID-19 infection. (2) All patients, despite risk factors and symptoms, should be asked to attend a compulsory teleconsultation in order to minimize harm to both the patient and consultant. During the consultation, further assessment of COVID-19 symptoms can be made, and potential impact on underlying diabetes/cardiovascular disease should be assessed. (3a) Should there be a self-reported acute emergency by the patient, or a need for immediate medical attention as per the clinical judgement of the physician, the patient should be asked to

(Continued)

FIGURE 1 | present at the emergency department; (b) If immediate medical assessment is not required, management should be carried out via teleconsultation. (4) In the emergency department, the relevant steward must carry out secondary screening for COVID-19 symptoms. After screening, patients should undergo diagnostic testing for COVID-19. Drive-through testing facilities should be deployed for all patients, to minimize exposure to health systems, health workers and the community. Further imaging should also be carried out on patients, with extra precautions being taken to ensure proper cleaning of equipment when imaging COVID-19 positive patients. (5a) For patients who require surgery, the acute management plan should be invoked. If the patient is COVID-19 positive, measures must be taken to protect healthcare workers involved. Minimal number of staff should be involved at the direct interface and risk-minimization should be ensured for any peri-surgical procedures that might involve aerosol production. For COVID-19 negative patients, the routine management plan should be followed; (b) If no surgery is required, the non-acute management plan should be invoked. (6) A plan should be made to ensure proper quarantine of patients after discharge. This could include home isolation and telemonitoring. Patients should be advised to follow hand hygiene, wear masks and practice social distancing.

risk assessment by the individual healthcare worker within the guidance framework. The situation for Low- and Middle-Income Countries is made more difficult by a lack of resources and the uncertain availability of respiratory and PPE often intended as single use only.

Recently the CDC recommended wearing cloth face coverings in public settings where other social distancing measures are difficult to maintain (e.g., grocery stores and pharmacies), especially in areas of significant community-based transmission (66). Coronavirus like influenza and rhinovirus can possibly spread through short range aerosol transmission in exhaled breath. Therefore, this study reinforces the need for individual and public health strategies and the adoption of using face masks as a preventive intervention. The American Academy of Ophthalmology (AAO) recommends contact lens wearers to switch to wearing glasses for a while to limit the risk of COVID-19 infection (67).

Pandemics like COVID-19, SARS and Spanish flu invoke irrational and heightened fear which could be linked to incidents of xenophobia and discrimination (68). A public health crisis of this scale can quickly mutate into a social and political crisis. Therefore, it is warranted that the political and health systems leadership must continue transparent, open, and respectful communication with all communities, with special consideration for communities from marginalized and vulnerable backgrounds, as they tend to have a disproportionately poor cardiovascular and metabolic profile (68). Also, this subgroup of patients often have relatively poor access to health services and compromised provision of medical supplies, which is exacerbated in a public health crisis situation, more so for a sustained period as is the case with pandemics such as COVID-19 with an estimated mortality of 3.4% however the recent evidence suggests the rates are still evolving (69, 70).

Patients with comorbid CVD, diabetes and obesity are potentially vulnerable in a pandemic (68–70). It must be considered that a significant number of healthcare workers will have these same and other vulnerabilities due to pre-existing health conditions, therefore institutional policies should provide for redeployment away from COVID-19 patient direct contact or furlough. Some jurisdictions have developed national policy or workplace sector guidance in others there is likely a duty of care in law. Healthcare providers, health systems and political leadership must account for the heterogeneity, compounded infection and fatality risks, long-term complications and special considerations for ongoing management as well as the socio-economic factors that may interfere with the health and well-being of patients with CVD, diabetes and/or obesity. Technological innovation such as telemedicine along with public health strategies may mitigate some of these risks.

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'S NOTE

†The COVID19 pandemic is causing an unprecedented public health crisis impacting healthcare systems, healthcare workers and communities. The COVID-19 Pandemic Health System **RE**silience **PROGRAM (REPROGRAM)** consortium is a think-tank of leading international healthcare physicians, researchers and policymakers formed to champion the safety of healthcare workers, policy development and advocacy for global pandemic preparedness and action.

AUTHOR CONTRIBUTIONS

SBh devised the project, the main conceptual ideas and proof outline. SBh and AR wrote the first draft of the manuscript. SBh encouraged AR to investigate and supervised the findings of this work. All authors discussed the results and recommendations, and contributed to the final manuscript.

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REFERENCES

1. John Hopkins University. *COVID-19 Global Cases by the Center for Systems Science and Engineering (CSSE)*. Johns Hopkins University (2020). Available online at: https://coronavirus.jhu.edu/map.html (accessed April 6, 2020).
2. Yang X, Yu Y, Xu J, Shu H, Xia Ja, 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
3. Guan W-j, Ni Z-y, Hu Y, Liang W-h, Ou C-q, He J-x, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med.* (2020) 382:1708–20. doi: 10.1056/NEJMoa2002032
4. 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). doi: 10.1111/all.14238. [Epub ahead of print].
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 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA.* (2020) 323:1239–42. doi: 10.1001/jama.2020.2648
6. 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
7. Caussy C, Pattou F, Wallet F, Simon C, Chalopin S, Telliam C, et al. Prevalence of obesity among adult inpatients with COVID-19 in France. *Lancet Diab Endocrinol.* (2020). doi: 10.1016/S2213-8587(20)30160-1. [Epub ahead of print].
8. Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, et al. High prevalence of obesity in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) requiring invasive mechanical ventilation. *Obesity.* (2020). doi: 10.1002/oby.22831. [Epub ahead of print].
9. Bhatraju PK, Ghassemieh BJ, Nichols M, Kim R, Jerome KR, Nalla AK, et al. Covid-19 in critically ill patients in the seattle region - case series. *N Engl J Med.* (2020) 382:2012–22. doi: 10.1056/NEJMoa2004500
10. Mahase E. Covid-19: most patients require mechanical ventilation in first 24 hours of critical care. *BMJ.* (2020) 368:m1201. doi: 10.1136/bmj.m1201
11. Chen Q, Zheng Z, Zhang C, Zhang X, Wu H, Wang J, et al. Clinical characteristics of 145 patients with corona virus disease 2019 (COVID-19) in Taizhou, Zhejiang, China. *Infection.* (2020). doi: 10.1007/s15010-020-01432-5. [Epub ahead of print].
12. Lighter J, Phillips M, Hochman S, Sterling S, Johnson D, Francois F, et al. Obesity in patients younger than 60 years is a risk factor for COVID-19 hospital admission. *Clin Infect Dis.* (2020). doi: 10.1093/cid/ciaa415. [Epub ahead of print].
13. Barclay L, Nyarko E. *Are Diabetes, CVD Associated with Worse COVID-19 Prognosis?* Medscape (2020). Available online at: https://www.medscape.org/viewarticle/926097 (accessed April 5, 2020).
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. Hruby A, Hu FB. The epidemiology of obesity: a big picture. *Pharmacoeconomics.* (2015) 33:673–89. doi: 10.1007/s40273-014-0243-x
16. Leon BM, Maddox TM. Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. *World J Diabetes.* (2015) 6:1246–58. doi: 10.4239/wjd.v6.i13.1246
17. European Society of Cardiology (ESC). Available online at: https://www.escardio.org/Education/COVID-19-and-Cardiology (accessed May 28, 2020).
18. British Cardiovascular Society (BCS). Available online at: https://www.britishcardiovascularsociety.org/resources/covid-19-clinicians-hub (accessed May 28, 2020).
19. Zaman S, MacIsaac AI, Jennings GL, Schlaich M, Inglis SC, Arnold R, et al. Cardiovascular disease and COVID-19: Australian/New Zealand consensus statement. *Med J Aust [preprint].* (2020) 19. Available online at: https://www.mja.com.au/journal/2020/cardiovascular-disease-and-covid-19-australiannew-zealand-consensus-statement
20. Gregson J, Kaptoge S, Bolton T, Pennells L, Willeit P, Burgess S, et al. Cardiovascular risk factors associated with venous thromboembolism. *JAMA Cardiol.* (2019) 4:163–73. doi: 10.1001/jamacardio.2018.4537
21. Wang T, Chen R, Liu C, Liang W, Guan W, Tang R, et al. Attention should be paid to venous thromboembolism prophylaxis in the management of COVID-19. *Lancet Haematol.* (2020) 7:e362–3. doi: 10.1016/s2352-3026(20)30109-5
22. Intensive Care National Audit and Research Centre. *Report on 2249 patients critically ill with COVID-19.* Intensive Care National Audit and Research Centre (2020). Available online at: https://www.icnarc.org/About/Latest-News/2020/04/04/Report-On-2249-Patients-Critically-Ill-With-Covid-19 (accessed April 9, 2020).
23. Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. *Hypertension.* (2004) 43:970–6. doi: 10.1161/01.HYP.0000124667.34652.1a
24. Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, et al. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation.* (2005) 111:2605–10. doi: 10.1161/circulationaha.104.510461
25. Ocaranza MP, Palomera C, Román M, Bargetto J, Lavandero S, Jalil JE. Effect of hypertension on angiotensin-(1-7) levels in rats with different angiotensin-I converting enzyme polymorphism. *Life Sci.* (2006) 78:1535–42. doi: 10.1016/j.lfs.2005.07.026
26. Klimas J, Olvedy M, Ochodnicka-Mackovicova K, Kruzliak P, Cacanyiova S, Kristek F, et al. Perinatally administered losartan augments renal ACE2 expression but not cardiac or renal Mas receptor in spontaneously hypertensive rats. *J Cell Mol Med.* (2015) 19:1965–74. doi: 10.1111/jcmm.12573
27. Walters TE, Kalman JM, Patel SK, Mearns M, Velkoska E, Burrell LM. Angiotensin converting enzyme 2 activity and human atrial fibrillation: increased plasma angiotensin converting enzyme 2 activity is associated with atrial fibrillation and more advanced left atrial structural remodelling. *Europace.* (2017) 19:1280–7. doi: 10.1093/europace/euw246
28. Burchill LJ, Velkoska E, Dean RG, Griggs K, Patel SK, Burrell LM. Combination renin-angiotensin system blockade and angiotensin-converting enzyme 2 in experimental myocardial infarction: implications for future therapeutic directions. *Clin Sci.* (2012) 123:649–58. doi: 10.1042/cs20120162
29. Burrell LM, Risvanis J, Kubota E, Dean RG, MacDonald PS, Lu S, et al. Myocardial infarction increases ACE2 expression in rat and humans. *Eur Heart J.* (2005) 26:369–75. doi: 10.1093/eurheartj/ehi114
30. Witko-Sarsat V, Rieu P, Descamps-Latscha B, Lesavre P, Halbwachs-Mecarelli L. Neutrophils: molecules, functions and pathophysiological aspects. *Lab Invest.* (2000) 80:617–53. doi: 10.1038/labinvest.3780067
31. Gavin C, Meinke S, Heldring N, Heck KA, Achour A, Iacobaeus E, et al. The complement system is essential for the phagocytosis of mesenchymal stromal cells by monocytes. *Front Immunol.* (2019) 10:2249. doi: 10.3389/fimmu.2019.02249
32. Medscape. *Glucose Control Key With COVID-19 in Diabetes, Say Experts.* Medscape (2020). Available online at: https://www.medscape.com/viewarticle/927044 (accessed April 5, 2020).
33. Camporotondo R, Totaro R, Costantino I, Gnecchi M, Oltrona L. *Patients: Scared and Alone.* Pavia (2020). Available online at: https://www.escardio.org/Education/COVID-19-and-Cardiology/patients-scared-and-alone-pavia-italy (accessed April 5, 2020).
34. Bhaskar S, Sharma D, Walker AH, McDonald M, Huasen B, Haridas A, et al. Acute neurological care in the COVID-19 Era: the Pandemic Health System REsilience PROGRAM (REPROGRAM) Consortium Pathway. *Front Neurol.* (2020) 11:579. doi: 10.3389/fneur.2020.00579
35. American College of Surgeons. *COVID-19 Guidelines for Triage of Vascular Surgery Patients.* American College of Surgeons (2020). Available online at: https://www.facs.org/covid-19/clinical-guidance/elective-case/vascular-surgery (accessed April 5, 2020).
36. Colberg SR, Sigal RJ, Yardley JE, Riddell MC, Dunstan DW, Dempsey PC, et al. Physical activity/exercise and diabetes: a position statement of the American Diabetes Association. *Diabetes Care.* (2016) 39:2065–79. doi: 10.2337/dc16-1728

37. The Lancet. COVID-19: protecting health-care workers. *Lancet.* (2020) 395:922. doi: 10.1016/s0140-6736(20)30644-9
38. American Association of Clinical Endocrinologists. *AACE Position Statement: Coronavirus (COVID-19) and People with Diabetes*. American Association of Clinical Endocrinologists (2020). Available online at: https://www.aace.com/recent-news-and-updates/aace-position-statement-coronavirus-covid-19-and-people-diabetes-updated (accessed April 5, 2020).
39. European Society of Endocrinology. *A Statement from the European Society of Endocrinology COVID-19 and Endocrine Diseases*. European Society of Endocrinology (2020). Available online at: https://www.ese-hormones.org/about-us/our-communities/clinicians/covid-19-and-endocrine-disease-clinical-information-and-comment-from-ese/ (accessed April 5, 2020).
40. International Diabetes Federation (IDF). *COVID-19 Outbreak: Guidance for People with Diabetes*. International Diabetes Federation (2020). Available online at: https://www.idf.org/our-network/regions-members/europe/europe-news/196-information-on-corona-virus-disease-2019-covid-19-outbreak-and-guidance-for-people-with-diabetes.html (accessed April 7, 2020).
41. International Society for Pediatric and Adolescent Diabetes (ISPAD). *Coronavirus Infection (COVID-19) and Summary of Recommendations Regarding COVID-19 in Children with Diabetes*. International Society for Pediatric and Adolescent Diabetes (ISPAD) (2020). Available online at: https://www.ispad.org/page/CoronavirusinfectionCOVID-19 (accessed April 7, 2020).
42. National Health Service (NHS) UK. *Clinical Guide for the Management of People with Diabetes During the Coronavirus Pandemic*. National Health Service (NHS) UK (2020). Available online at: https://www.england.nhs.uk/coronavirus/wp-content/uploads/sites/52/2020/03/speciality-guide-diabetes-19-march-v2-updated.pdf (accessed April 5, 2020).
43. Devakumar D, Shannon G, Bhopal SS, Abubakar I. Racism and discrimination in COVID-19 responses. *Lancet.* (2020) 395:1194. doi: 10.1016/S0140-6736(20)30792-3
44. Jing Z-C, Zhu H-D, Yan X-W, Chai W-Z, 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
45. American College of Cardiology. *Telehealth: Rapid Implementation for Your Cardiology Clinic*. American College of Cardiology (2020). Available online at: https://www.acc.org/latest-in-cardiology/articles/2020/03/01/08/42/feature-telehealth-rapid-implementation-for-your-cardiology-clinic-coronavirus-disease-2019-covid-19 (accessed April 5, 2020).
46. European Society of Cardiology. *Position Statement of the ESC Council on Hypertension on ACE-Inhibitors and Angiotensin Receptor Blockers*. European Society of Cardiology (2020). Available online at: https://www.escardio.org/Councils/Council-on-Hypertension-(CHT)/News/position-statement-of-the-esc-council-on-hypertension-on-ace-inhibitors-and-ang (accessed April 5, 2020).
47. American Heart Association. *Patients Taking ACE-i and ARBs Who Contract COVID-19 Should Continue Treatment, Unless Otherwise Advised by Their Physician*. American Heart Association (2020). Available online at: https://newsroom.heart.org/news/patients-taking-ace-i-and-arbs-who-contract-covid-19-should-continue-treatment-unless-otherwise-advised-by-their-physician (accessed April 5, 2020).
48. European Society of Hypertension. *European Society of Hypertension Update on COVID-19*. European Society of Hypertension (2020). Available online at: https://www.eshonline.org/spotlights/esh-stabtement-on-covid-19/ (accessed April 5, 2020).
49. 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
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. 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
52. 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
53. 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). doi: 10.1056/NEJMc2010418. [Epub ahead of print].
54. Lazzerini PE, Boutjdir M, Capecchi PL. COVID-19, arrhythmic risk and inflammation: mind the gap! *Circulation.* (2020). doi: 10.1161/CIRCULATIONAHA.120.047293. [Epub ahead of print].
55. Omran J, Bostick BP, Chan AK, Alpert MA. Obesity and ventricular repolarization: a comprehensive review. *Prog Cardiovasc Dis.* (2018) 61:124–35. doi: 10.1016/j.pcad.2018.04.004
56. Kobayashi S, Nagao M, Asai A, Fukuda I, Oikawa S, Sugihara H. Severity and multiplicity of microvascular complications are associated with QT interval prolongation in patients with type 2 diabetes. *J Diabetes Investig.* (2018) 9:946–51. doi: 10.1111/jdi.12772
57. 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). doi: 10.1016/j.mayocp.2020.03.024. [Epub ahead of print].
58. Chorin E, Dai M, Shulman E, Wadhwani L, Bar-Cohen R, Barbhaiya C, et al. The QT interval in patients with COVID-19 treated with hydroxychloroquine and azithromycin. *Nat Med.* (2020). doi: 10.1038/s41591-020-0888-2. [Epub ahead of print].
59. Barbar S, Noventa F, Rossetto V, Ferrari A, Brandolin B, Perlati M, et al. A risk assessment model for the identification of hospitalized medical patients at risk for venous thromboembolism: the Padua Prediction Score. *J Thromb Haemost.* (2010) 8:2450–7. doi: 10.1111/j.1538-7836.2010.04044.x
60. Flint SW, Tahrani AA. COVID-19 and obesity lack of clarity, guidance, and implications for care. *Lancet Diab Endocrinol.* (2020) 8:474–5. doi: 10.1016/S2213-8587(20)30156-X
61. Government of UK. *Guidance: Staying Alert and Safe (Social Distancing).* Government of UK (2020). Available online at: https://www.gov.uk/government/publications/staying-alert-and-safe-social-distancing/staying-alert-and-safe-social-distancing#clinically-vulnerable-people (accessed May 28, 2020).
62. Leung NHL, Chu DKW, Shiu EYC, Chan K-H, McDevitt JJ, Hau BJP, et al. Respiratory virus shedding in exhaled breath and efficacy of face masks. *Nat Med.* (2020) 26:676–80. doi: 10.1038/s41591-020-0843-2
63. World Health Organization. *Infection Prevention and Control During Health Care when Novel Coronavirus (nCoV) Infection Is Suspected*. World Health Organization (2020). Available online at: https://www.who.int/publications-detail/infection-prevention-and-control-during-health-care-when-novel-coronavirus-(ncov)-infection-is-suspected-20200125 (accessed April 6, 2020).
64. Public Health Agency of Canada. *Infection Prevention and Control for Novel Coronavirus (2019-nCoV): Interim Guidance for Acute Healthcare Settings*. Public Health Agency of Canada (2020). Available online at: https://www.canada.ca/en/public-health/services/diseases/2019-novel-coronavirus-infection/health-professionals/interim-guidance-acute-healthcare-settings.html#a4.10 (accessed April 6, 2020).
65. Public Health England. *Guidance on Infection Prevention and Control for COVID-19*. Public Health England (2020). Available online at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/866112/COVID-19_Donning_guidance_web_v1_14_February_2020.pdf (accessed April 6, 2020).
66. The Center for Disease Control and Prevention (CDC). *Use of Cloth Face Coverings to Help Slow the Spread of COVID-19*. The Center for Disease Control and Prevention (CDC) (2020). Available online at: https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/diy-cloth-face-coverings.html (accessed on April 6, 2020).
67. American Academy of Ophthalmology (AAO). *Coronavirus Eye Safety*. American Academy of Ophthalmology (AAO) (2020). Available online at: https://www.aao.org/eye-health/tips-prevention/coronavirus-covid19-eye-infection-pinkeye (accessed April 7, 2020).

68. Ahorsu DK, Lin C-Y, Imani V, Saffari M, Griffiths MD, Pakpour AH. The fear of COVID-19 Scale: development and initial validation. *Int J Ment Health Addict*. (2020) 1–9. doi: 10.1007/s11469-020-00270-8
69. García-Basteiro AL, Chaccour C, Guinovart C, Llupià A, Brew J, Trilla A, et al. Monitoring the COVID-19 epidemic in the context of widespread local transmission. *Lancet Respir Med.* (2020) 8:440–2. doi: 10.1016/S2213-2600(20)30162-
70. World Health Organization. *Director-General's Opening Remarks at the Media Briefing on COVID-19.* World Health Organization (2020). Available online at: https://www.worldometers.info/coronavirus/coronavirus-death-rate/#ref-13 (accessed April 11, 2020).

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High Inflammatory Burden: A Potential Cause of Myocardial Injury in Critically Ill Patients With COVID-19

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Background: Myocardial injury is a severe complication of novel coronavirus disease (COVID-19), and inflammation has been suggested as a potential cause of myocardial injury. However, the correlation of myocardial injury with inflammation in COVID-19 patients has not been revealed so far.

Method: This retrospective single-center cohort study enrolled 64 critically ill patients with COVID-19. Patients were categorized into two groups by the presence of myocardial injury on admission. Demographic data, clinical characteristics, laboratory tests, treatments, and outcomes were analyzed in this study.

Result: Of these patients, the mean age was 64.8 ± 12.2 years old, and 34 (53.1%) were diagnosed with myocardial injury. Compared with non-myocardial injury patients, myocardial injury patients were older (67.8 ± 10.3 vs. 61.3 ± 13.3 years; $P = 0.033$), had more cardiovascular (CV) risk factors such as smoking (16 [47.06%] vs. 7 [23.33%]; $P = 0.048$) and were more likely to develop CV comorbidities (13 [38.2%] vs. 2 [6.7%]; $P = 0.003$). Scores on the Acute Physiology and Chronic Health Evaluation II (median [interquartile range (IQR)] 19.0 [13.25–25.0] vs. 13.0 [9.25-18.75]; $P = 0.005$) and Sequential Organ Failure Assessment systems (7.0 [5.0–10.0] vs. 4.5 [3.0–6.0]; $P < 0.001$) were significantly higher in the myocardial injury group. In addition, patients with myocardial injury had higher mortality than those without myocardial injury (29 [85.29%] vs. 18 [60.00%]; $P = 0.022$). Cox regression suggested that myocardial injury was an independent risk factor for high mortality during the time from admission to death (hazard ratio [HR], 2.06 [95% confidence interval (CI), 1.10–3.83]; $P = 0.023$). Plasma levels of high-sensitivity C-reactive protein (hs-CRP), interleukin (IL)-1β, interleukin-2 receptor (IL-2R), IL-6, IL-8, IL-10, and tumor necrosis factor-α (TNF-α) exceeded the normal limits, and levels of hs-CRP, IL-2R, IL-6, IL-8, and TNF-α were statistically higher in the myocardial injury group than in the non-myocardial injury group. Multiple-variate logistic regression showed that plasma levels of hs-CRP (odds ratio [OR] 6.23, [95% CI, 1.93–20.12], $P = 0.002$), IL-6 (OR 13.63, [95% CI, 3.33–55.71]; $P < 0.001$) and TNF-α (OR 19.95, [95% CI, 4.93–80.78]; $P < 0.001$) were positively correlated with the incidence of myocardial injury.

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Conclusion: Myocardial injury is a common complication that serves as an independent risk factor for a high mortality rate among in-ICU patients with COVID-19. A high inflammatory burden may play a potential role in the occurrence of myocardial injury.

Keywords: COVID-19, critical patients, myocardial injury, inflammation, In-ICU mortality

INTRODUCTION

Coronavirus disease 2019 (COVID-19), a novel coronavirus–infected pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has currently become a severe global health problem (1, 2). Myocardial injury was suggested to be prevalent in COVID-19 patients, which has contributed to fatal complications and high mortality rates (3–5). However, the mechanism underlying myocardial injury has not yet been confirmed. Recently, several studies revealed that COVID-19 patients were mostly in a high systemic inflammatory status with severe cytokine storms (e.g., high levels of interleukin [IL]-6, IL-8, and tumor necrosis factor-α [TNF-α]), which contributed to fatal complications (6–8). Given that inflammation has been revealed as a great contributor to all forms of myocardial injury (9), COVID-19-induced systemic inflammation was suggested to potentially cause myocardial injury in COVID-19 patients. In this study, we aimed to investigate the association of inflammation with myocardial injury in critically ill patients with COVID-19.

MATERIALS AND METHODS

Study Design and Participants

This single-center, retrospective, observational study was performed in a newly built intensive care unit (ICU) of Tongji Hospital (Sino-French New City Campus), Huazhong University of Science and Technology, Wuhan, China. This ICU was designated to treat critically ill patients with COVID-19. We retrospectively analyzed 64 COVID-19 patients admitted to the ICU in this study. The data cut-off for investigation of survival status was March 26, 2020. All the patients were confirmed as COVID-19 with a positive result on real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay of throat-swab specimens. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of PUMC Hospital. Written informed consent was waived due to the rapid emergence of this infectious disease. No potentially identifiable human images or data is presented in this study. Plasma levels of inflammatory cytokines test were finished within 24 h when patients were admitted. All the other laboratory tests were finished within 6 h after admission. All the data included in this study were part of routine patient care in ICU.

Data Collection and Study Design

The data collected in this study were extracted from electronic medical records reviewed by the clinical team from Peking Union Medical College Hospital (PUMCH). Patient data included demographics, survival time from ICU admission to death, baseline characteristics (i.e., prior medical illness, cardiovascular risk factors), in-ICU clinical information (i.e., vital signs, complications, and therapeutic measures), laboratory results and outcomes. We also documented patients' Acute Physiology and Chronic Health Evaluation II (APACHE II) and Sequential Organ Failure Assessment (SOFA) scores on admission to the ICU.

Outcome

Patients were categorized into two groups (myocardial injury vs. non-myocardial injury) based on their on-admission high-sensitivity cardiac troponin I (hs-cTnI) levels. The primary outcome was 28-day mortality after ICU admission. myocardial injury was defined as an elevated cardiac troponin value above the 99th percentile of the upper reference limit (34.2 ng/ml) according to the fourth Universal Definition of Myocardial Infarction (10). Prior cardiovascular (CV) disease was defined as coronary artery disease (CAD), myocardial infarction, heart failure or stroke, and in-ICU CV complications were defined as arrythmias (atrial tachycardia, atrial fibrillation, ventricular tachycardia, and/or ventricular fibrillation), cardiac arrest, cardiac shock or myocardial infarction.

Acute respiratory distress syndrome (ARDS) and acute kidney injury (AKI) were diagnosed according to the Berlin Definition and KDIGO clinical practice guidelines, respectively (11, 12).

Statistical Analysis

Categorical variables were presented as counts and percentages. Continuous variables were described as means ± standard deviations (SDs) for normally distributed data and medians (interquartile ranges [IQRs]) for non-normally distributed data. A two-sample *T*-test was used to assess whether there were significant differences in continuous variables when they were normally distributed; otherwise, the Mann-Whitney U-test was used. The χ^2 test was applied to test the differences in categorical variables, although Fisher's exact test was used for comparisons with small sample sizes. Kaplan-Meier (K-M) plots and Cox proportional hazards regression models were used for survival analysis, which was based on the time from ICU admission to death. The log-rank test was used to confirm the differences between K-M plots. Logistics regression was applied to test the contribution of inflammation to the incidence of myocardial injury. Concretely, we firstly involve single variate into logistics regression, and then put the variates with $P < 0.05$ into regression equation thereby giving the final result. Statistical significance was determined when two-sided α was <0.05. All statistical analyses were performed using SPSS 21.0 software (IBM, Armonk, NY).

RESULTS

General Characteristics of Critically Ill Patients With COVID-19

Night-nine adults admitted to the ICU from February 4 to March 3, 2020, were studied. After excluding 6 patients who were not admitted for COVID-19-related critical illness and 19 patients with incomplete data (1 patient had no troponin result and 18 patients had no inflammatory cytokines), we included 64 in-ICU patients in the final analysis (**Figure 1**).

Of these patients, 42 (65.6%) were men, the mean age was 64.8 ± 12.2 years (range, 26–92 years), and 47 patients reached the primary endpoint during the follow-up time. Prior CV diseases and CV risk factors were common in critical patients, as there were 13 patients (20.3%) with pre-existing CV diseases (CAD: 7 [10.9%]; heart failure: 2 [3.1%]; stroke: 8 [12.5%]) and 43 (67.2%) patients with 1 or more coexisting CV risk factors (hypertension: 35 [54.7%]; diabetes: 15 [23.4%]; smoking: 23 [35.9%]). ARDS was the most common in-ICU complication (62 [96.88%]), followed by AKI (21 [32.8%]) and CV complications (15 [23.4%]). Laboratory results showed that coagulation dysfunction and high inflammatory burden were common in these critical patients, as most coagulation indicators and inflammatory indicators were higher than the normal limits. In addition, plasma levels of N-terminal pro-B-type natriuretic peptide (NT-proBNP) and hs-cTnI were also significantly increased (**Table 2**). Fifty-two (81.3%) patients received invasive mechanical ventilation, and 19 (29.7%) received non-invasive mechanical ventilation. Immune therapies were commonly used in critical patients (glucocorticoids: 54 [84.4%]; tocilizumab: 7 [10.94%]). More detailed information is presented in **Tables 1**, **2**.

Differences Between Myocardial Injury Patients and Non-myocardial Injury Patients

In our study, 34 patients (53.1%) were diagnosed with myocardial injury. Compared with non-myocardial injury patients, the myocardial injury patients were significantly older (67.8 ± 10.3 vs. 61.3 ± 13.3 years; $P = 0.033$), more likely to have preexisting cardiovascular diseases (13 [38.2%] vs. 3 [10.0%]; $P = 0.009$), and had more CV risk factors (smoking: 16 [47.1%] vs. 7 [23.3%]; $P = 0.048$) and CV comorbidities (13 [38.2%] vs. 2 [6.7%]; $P = 0.003$) (**Table 1**). Concomitantly, patients with myocardial injury had higher APACHE II (19.0 [13.25–25.0] vs. 13.0 [9.25–18.75]; $P = 0.005$) and SOFA system scores than those of the non-myocardial injury group (7.0 [5.0–10.0] vs. 4.5 [3.0–6.0]; $P < 0.001$).

Regarding laboratory results, myocardial injury patients showed significant increases in the plasma levels of creatinine, blood urea nitrogen, D-dimer, high-sensitivity C-reactive protein (hs-CRP) (155.0 [78.3–210.9] vs. 45.0 [16.0–96.0]

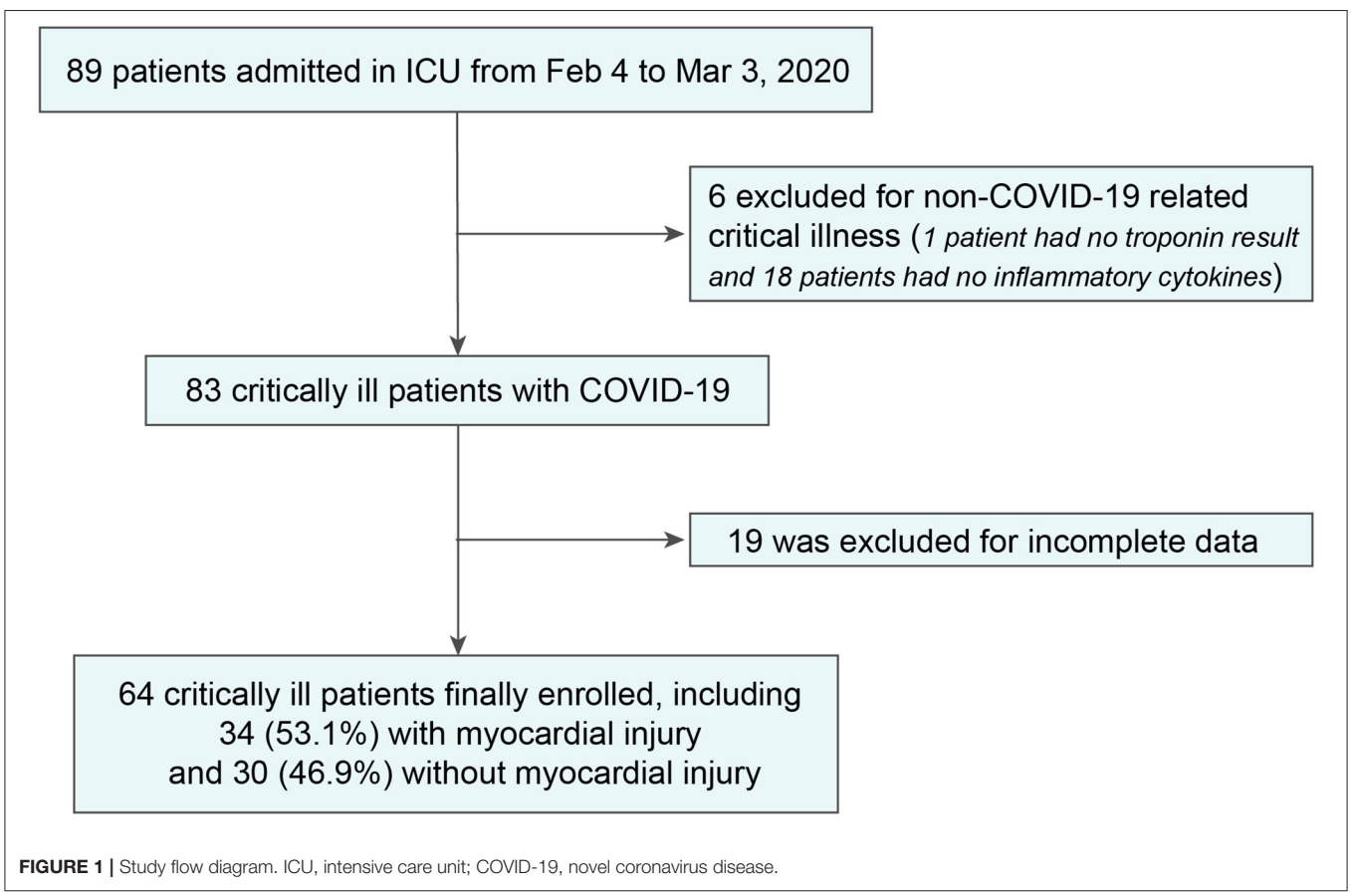


FIGURE 1 | Study flow diagram. ICU, intensive care unit; COVID-19, novel coronavirus disease.

TABLE 1 | Demographics and clinical characteristics of critically ill patients with COVID-19.

	Myocardial injury ($n = 34$)	Non-myocardial injury ($n = 30$)	Total ($n = 64$)	*P*-value
Age (yrs)	67.8 ± 10.3	61.3 ± 13.3	64.8 ± 12.2	0.033
Male	24 (70.6%)	18 (60.0%)	42 (65.6%)	0.37
Prior CV diseases				
CAD	6 (17.7%)	1 (3.3%)	7 (10.9%)	0.11
Heart failure	2 (5.9%)	0 (0.00%)	2 (3.1%)	0.49
Stroke	6 (17.7%)	2 (6.7%)	8 (12.5%)	0.27
CV risk factors				
Hypertension	22 (64.7%)	13 (43.3%)	35 (54.7%)	0.087
Diabetes	10 (29.4%)	5 (16.7%)	15 (23.4%)	0.23
Smoking	16 (47.1%)	7 (23.3%)	23 (35.9%)	0.048
Vital signs				
Fever	12 (35.3%)	14 (46.7%)	26 (40.6%)	0.36
HR (bpm)	112.9 ± 20.4	106.7 ± 18.5	110.0 ± 19.6	0.21
SBP (mmHg)	124.6 ± 26.3	127.8 ± 20.5	126.1 ± 23.6	0.60
DBP (mmHg)	74.8 ± 14.5	77.7 ± 14.2	76.2 ± 14.4	0.42
RR (times/min)	29.3 ± 8.8	27.5 ± 7.1	28.4 ± 8.0	0.38
Critical score				
APACHE II score*	19.0 (13.3–25.0)	13.0 (9.3–18.8)	15.0 (12.0–22.0)	0.005
SOFA score*	7.0 (5.0–10.0)	4.5 (3.0–6.0)	6.0 (4.0–8.0)	<0.001
Complications				
CV complications	13 (38.2%)	2 (6.7%)	15 (23.4%)	0.003
ARDS	34 (100.0%)	28 (93.3%)	62 (96.9%)	0.22
AKI	13 (38.2%)	8 (26.7%)	21 (32.8%)	0.33
Live dysfunction	6 (17.7%)	10 (33.3%)	16 (25.0%)	0.15
Symptom onset to ICU admission (days)	15.0 (11.0–23.0)	16.5 (9.3–23.5)	15.5 (10.0–23.3)	0.91
In-ICU therapy				
Non-invasive mechanical ventilation	12 (35.3%)	7 (23.3%)	19 (29.7%)	0.30
Invasive mechanical ventilation	26 (76.5%)	26 (86.7%)	52 (81.5%)	0.30
Immunoglobulin	26 (76.5%)	25 (83.3%)	51 (79.7%)	0.50
Glucocorticoids	26 (76.5%)	28 (93.3%)	54 (84.4%)	0.064
Vasoconstrictive agents	24 (70.6%)	18 (60.0%)	42 (65.6%)	0.37
Tocilizumab	2 (5.9%)	5 (16.7%)	7 (10.9%)	0.17
Death				
All-cause death	29 (85.3%)	18 (60.0%)	47 (73.4%)	0.022
Survival time*	7.0 (3.0–13.75)	19.0 (10.0–38.75)	11.5 (5.0-35.0)	0.002

**Continuous variables with non-normal distribution presented as "median (IQR)." CV, cardiovascular; CAD, coronary artery disease; ICU, intensive care unit. HR, heart rate; RR, respiratory rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; AKI, acute kidney injury; ARDS, acute respiratory distress syndrome. P-values present the differences between myocardial injury and non-myocardial injury patients.*

mg/L; $P < 0.001$), interleukin-2 receptor (IL-2R) (1152.0 [741.0–1679.0] vs. 731.0 [302.0–1224.5] pg/ml; $P = 0.02$), IL-6 (1144.7 ± 1466.7 vs. 204.4 ± 400.3 pg/ml; $P < 0.001$), IL-8 (48.5 [21.1–156.1] vs. 22.7 [14.4–42.9] pg/ml; $P = 0.015$) and TNF-α (19.8 [14.7–40.1] vs. 9.0 [7.1–11.0] pg/ml; $P < 0.001$) (**Table 2**). In addition, the levels of creatinine, blood urea nitrogen and D-dimer were also significantly increased in the myocardial injury group. However, no significant differences were found in the applications of in-ICU therapies between myocardial injury and non-myocardial injury patients (**Table 1**).

In the survival analysis, the mortality rate was much higher in the myocardial injury group than in the non-myocardial injury group (29 [85.29%] vs. 18 [60.00%]; $P = 0.022$). Furthermore, myocardial injury was demonstrated as an independent risk factor for reduced survival time from admission to death (hazard ratio [HR], 2.06 [95% confidence interval (CI), 1.10–3.83]; $P = 0.023$) by a multivariable adjusted Cox proportional hazard regression model adjusting for age, smoking history and pre-existing with CVD. The high mortality in the myocardial injury group was also shown in the K-M survival curves (log-rank test, $P = 0.003$) (**Figure 2**).

TABLE 2 | Laboratory tests between COVID-19 patients with and without myocardial injury.

	Normal range	Myocardial injury ($n = 34$)	Non-myocardial injury ($n = 30$)	Total ($n = 64$)	*P*-value
White blood count, $\times 10^9$/L	3.5–9.5	12.5 ± 5.1	11.1 ± 5.7	11.9 ± 5.4	0.30
Neutrophils* (%)	40.0–75.0	91.7 (88.7–95.1)	90.7 (83.2–93.4)	91.1 (85.9–94.0)	0.05
Lymphocytes, $\times 10^9$/L	1.1–3.2	0.5 ± 0.4	0.7 ± 0.4	0.6 ± 0.4	0.25
Hemoglobin, g/L	130.0–175.0	121.8 ± 21.7	123.0 ± 19.8	122.4 ± 20.7	0.82
Platelets, $\times 10^9$/L	125.0–350.0	155.0 ± 89.4	197.0 ± 105.6	174.7 ± 98.8	0.09
ALT*, U/L	≤41.0	26.0 (14.0–41.0)	27.5 (22.0–36.8)	29.0 (19.8–42.0)	0.89
Total bilirubin*, μmol/L	≤26.0	13.2 (9.6–21.2)	14.5 (8.0–18.7)	13.7 (8.7–19.0)	0.40
Albumin g/L	35.0–52.0	28.0 ± 4.3	30.5 ± 6.1	29.2 ± 5.3	0.065
Creatinine*, μmol/L	59.0–104.0	88.5 (71.5–124.0)	67.0 (48.5–86.0)	81.0 (58.0–107.8)	0.005
BUN*, mmol/L	3.6–9.5	10.2 (7.1–20.7)	7.1 (5.4–10.3)	7.8 (6.3–14.4)	0.013
Serum potassium, mmol/L	3.5–5.1	4.5 ± 0.8	4.5 ± 1.0	4.5 ± 0.9	0.84
PT*, s	11.5–14.5	17.3 (15.7–18.2)	15.4 (14.7–16.3)	16.15 (15.0–17.6)	0.005
APTT*, s	29.0–42.0	41.8 (38.4–45.3)	41.5 (37.4–45.1)	41.6 (37.5–45.2)	0.68
INR*	0.8–1.2	1.4 (1.2–1.5)	1.2 (1.1–1.3)	1.3 (1.2–1.4)	0.002
Fbg, g/L	2.0–4.0	4.5 ± 3.9	4.6 ± 2.1	4.5 ± 3.2	0.29
D-dimer*, mg/L	<0.5	21.0 (7.5–21.0)	3.7 (1.9–21.0)	14.7 (2.8–21.0)	0.005
hsCRP*, mg/L	<1.0	155.0 (78.3–210.9)	45.0 (16.0–96.0)	86.5 (34.7–194.3)	<0.001
IL1 β, pg/ml	<5.0	6.5 ± 4.4	5.2 ± 0.7	5.9 ± 3.3	0.53
IL2 R*, pg/ml	223.0–710.0	1152.0 (741.0–1679.0)	731.0 (302.0–1224.5)	1041.0 (554.3–1485.3)	0.02
IL-6, pg/ml	<7.0	982.2 ± 1517.9	204.4 ± 400.3	617.6 ± 1197.4	0.008
IL-8*, pg/ml	<62.0	48.5 (21.1–156.1)	22.7 (14.4–42.9)	29.4 (18.1–76.7)	0.015
IL-10*, pg/ml	<9.1	10.7 (6.3–24.0)	10.5 (5.1–15.5)	10.7 (5.5–19.6)	0.30
TNF-α*, pg/ml	<8. 1	19.8 (14.7–40.1)	9.0 (7.1–11.0)	13.8 (9.3–23.0)	<0.001
HscTnI*, ng/L	≤34.2	276.1 (139.1–909.7)	12.1 (4.7–18.9)	46.5 (12.1–374.1)	<0.001
NT-proBNP*, ng/L	<241.0	1947.5 (644.8–4393.5)	372.0 (73.8–836.5)	816.5 (254.5–2585.0)	<0.001

**Continuous variables with non-normal distribution presented as "median (IQR)." ALT, alanine aminotransferase; BUN, blood urea nitrogen; hsCRP, high-sensitivity C-reative protein; IL, interleukin; IL-2R, interleukin-2 receptor; TNF-α, tumor necrosis factor α; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio; Fbg, fibrinogen; hs-cTnI, high-sensitive cardiac troponin I; NT-proBNP, N-terminal pro-B-type natriuretic peptide. P-values present the differences between MI and non-MI patients.*

Association of High Inflammatory Burden With the Incidence of Myocardial Injury in Critically Ill Patients With COVID-19

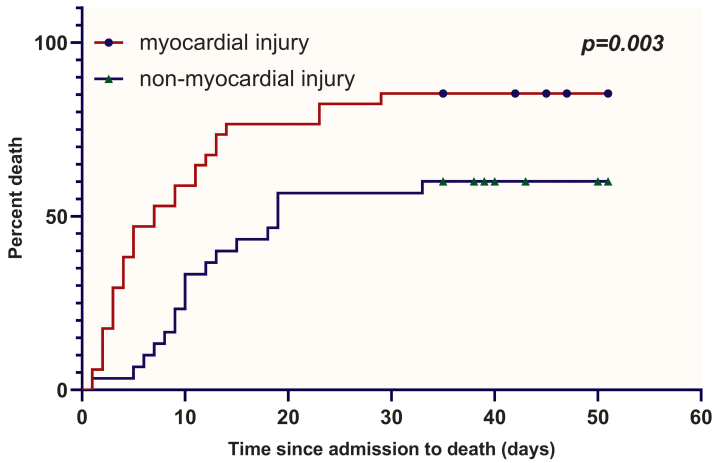
Most inflammatory biomarkers were significantly higher in COVID-19 patients with myocardial injury than in those without myocardial injury (**Table 2**). Consistently patients with higher inflammatory burden (plasma levels of inflammatory cytokines higher than the median levels) were also more likely to develop myocardial injury (**Figure 3**). To investigate the relation between high inflammatory burden with myocardial injury, we set the dependent variable to "myocardial injury" and set independent variables to the high/low inflammatory burden which was divided according to the cut-off of the median levels of inflammatory cytokines. In the univariate logistic regression analysis, we found that high plasma levels (higher than the median levels) of high-sensitivity C-reactive protein (hs-CRP) (odds ratio [OR] 10.80, [95% CI, 1.97–59.15]; $P = 0.006$), IL-6 (OR 9.13, [95% CI, 2.92–28.50]; $P < 0.001$), IL-8 (OR 7.27, [95% CI, 1.35–39.05]; $P = 0.021$) and TNF-α (OR 17.36, [95% CI, 3.04–99.20]; $P = 0.001$) were positively associated with the incidence of myocardial injury. We further entered these biomarkers into the multivariate logistic regression with adjusting variates of age, smoking history and pre-existing with CVD, and found that high plasma levels of hs-CRP (odds ratio [OR] 6.23, [95% CI, 1.93–20.12], $P = 0.002$), IL-6 (OR 13.63, [95% CI, 3.33–55.71]; $P < 0.001$), and TNF-α (OR 19.95, [95% CI, 4.93–80.78]; $P < 0.001$) were positively correlated with the incidence of myocardial injury (**Table 3**).

DISCUSSION

This study revealed that myocardial injury was associated with a high mortality rate in critically ill patients with COVID-19, and a high inflammatory burden was one of the potential causes of myocardial injury occurrence.

Of 64 in-ICU patients (42 males, 64.8 ± 12.2 years), 52 (81.5%) received invasive mechanical ventilation, and 47 (73.4%) died during the follow-up. A high incidence of COVID-19-induced myocardial injury was suggested by this study, since we found that 34 (53.1%) patients were diagnosed with myocardial injury, which is much higher than the incidence of myocardial injury in non-ICU patients (7.2% to 37.5%) (1, 4, 5). Myocardial injury usually contributes to various CV complications, such as cardiac dysfunction,

A



No. at risk

Patients with myocardial injury	34	14	8	5	4	1	0
Patients without myocardial injury	30	23	13	13	7	4	0

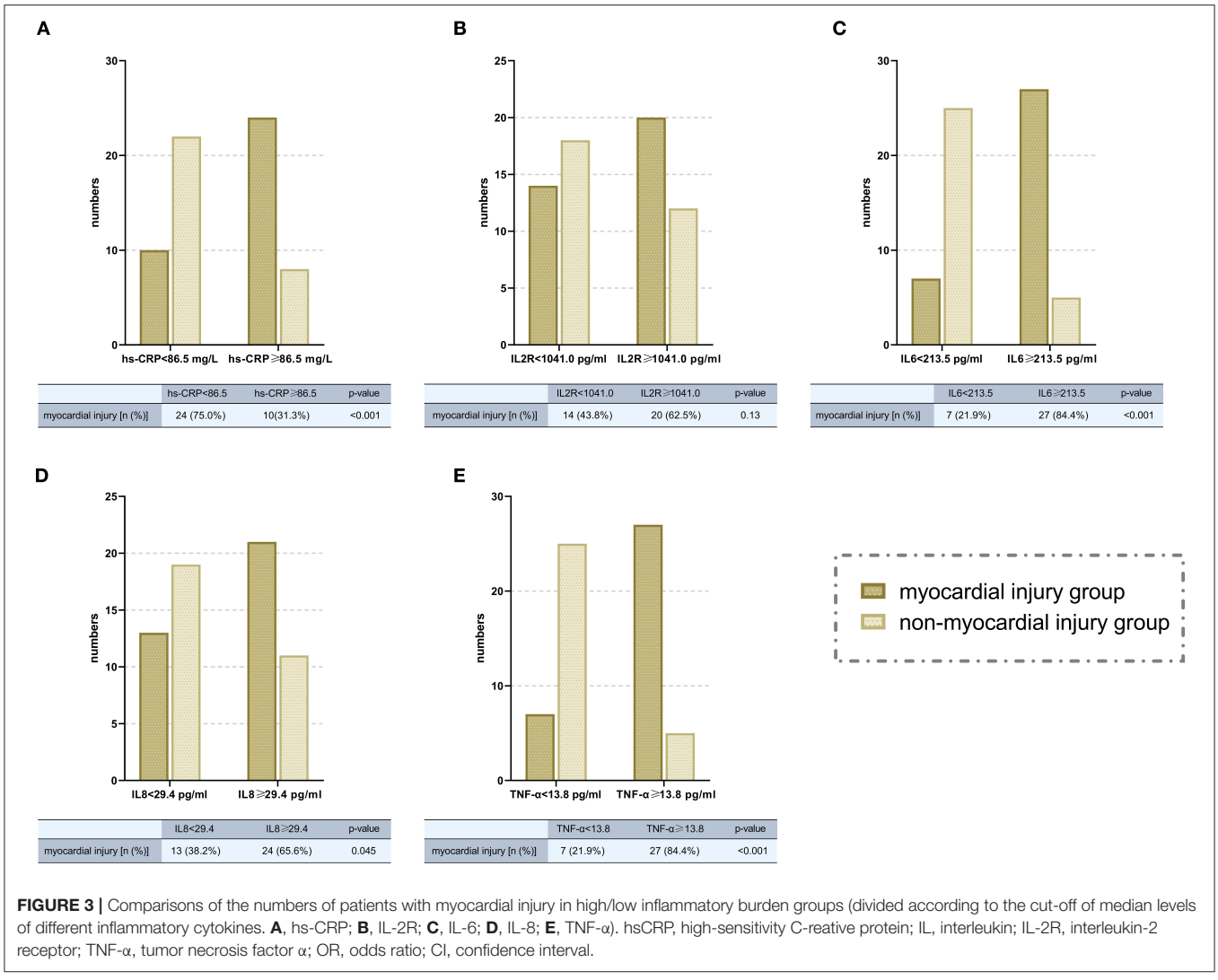
B

Group	Death number, (%)	Time since admission to death (days)	
		Duration median (IQR)	P-value
myocardial injury (n=34)	29 (85.29%)	7.0 (3.0-13.75)	0.002
non-myocardial injury (n=30)	18 (60.00%)	19.0 (10.0-38.75)	

FIGURE 2 | (A) K-M plot for patients with myocardial injury and without myocardial injury. **(B)** Comparisons of death number and time since admission to death between myocardial injury group and non-myocardial injury group.

arrhythmias and sudden death in patients with viral infectious diseases, which are associated with adverse events and a high mortality rate (13, 14). Several current studies have demonstrated that myocardial injury is associated with fatal outcomes and high mortality rates in hospitalized patients with COVID-19 (3, 5). In our study, in-ICU patients with myocardial injury were more likely to have preexisting cardiovascular diseases, develop cardiovascular complications, have higher APACHE-II/SOFA scores and have increased in-ICU mortality. In addition, Cox regression analysis suggested that myocardial injury was an independent risk factor for mortality, supporting that myocardial injury was associated with adverse events and high mortality rate in COVID-19 patients with critical illness.

Patients with COVID-19 were revealed to have a high systemic inflammatory status (1, 8). To date, the high systemic inflammation in hospitalized COVID-19 patients has been speculated as one of the potential causes of myocardial injury, as investigators found that hs-CRP levels positively correlated with plasma troponin levels in patients with COVID-19 (4). A similar finding was shown in our study. In addition to plasma hs-CRP, plasma levels of IL-1β, IL-2R, IL-6, IL-8, IL-10, and TNF-α were analyzed in this study, and IL-2R, IL-6, IL-8, and TNF-α were significantly increased in myocardial injury patients (**Table 2**). Moreover, patients with the high inflammatory burden were also shown to more likely develop myocardial injury (**Figure 3**). After univariate and multivariate logistic regression, the levels of hs-CRP, IL-6, and TNF-α were shown to be positively correlated with the incidence of myocardial injury, supporting the hypothesis that a high systemic inflammatory burden might contribute to myocardial injury in COVID-19 patients.



	hs-CRP<86.5	hs-CRP≥86.5	p-value
myocardial injury [n (%)]	24 (75.0%)	10(31.3%)	<0.001

	IL2R<1041.0	IL2R≥1041.0	p-value
myocardial injury [n (%)]	14 (43.8%)	20 (62.5%)	0.13

	IL6<213.5	IL6≥213.5	p-value
myocardial injury [n (%)]	7 (21.9%)	27 (84.4%)	<0.001

	IL8<29.4	IL8≥29.4	p-value
myocardial injury [n (%)]	13 (38.2%)	24 (65.6%)	0.045

	TNF-α<13.8	TNF-α≥13.8	p-value
myocardial injury [n (%)]	7 (21.9%)	27 (84.4%)	<0.001

FIGURE 3 | Comparisons of the numbers of patients with myocardial injury in high/low inflammatory burden groups (divided according to the cut-off of median levels of different inflammatory cytokines. **A**, hs-CRP; **B**, IL-2R; **C**, IL-6; **D**, IL-8; **E**, TNF-α). hsCRP, high-sensitivity C-reative protein; IL, interleukin; IL-2R, interleukin-2 receptor; TNF-α, tumor necrosis factor α; OR, odds ratio; CI, confidence interval.

TABLE 3 | Logistics regression for the association of inflammation with myocardial injury.

	Univariate			Multivariate		
	OR	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
hs-CRP ≥ 86.5 mg/L	10.80	1.97–59.15	0.006	6.23	1.93–20.12	0.002
IL-2R ≥ 1041.0 pg/ml	3.81	0.86–16.94	0.079	2.23	0.71–7.02	0.17
IL-6 ≥ 703.9 pg/ml	9.13	2.92–28.50	<0.001	13.63	3.33–55.71	<0.001
IL-8 ≥ 29.4 pg/ml	7.27	1.35–39.05	0.021	2.53	0.84–7.58	0.098
TNF-α ≥ 13.8 pg/ml	17.36	3.04–99.20	0.001	19.95	4.93–80.78	<0.001

OR, odds ratio; CI, confidence interval. hsCRP, high-sensitivity C-reative protein; IL, interleukin; IL-2R, interleukin-2 receptor; TNF-α, tumor necrosis factor α. Adjusted variates included age, smoking history, and pre-existing CVD.

Hypoxemia, septic shock, coagulation disorders and cardiac arrhythmias are potentially involved in the process of systemic high inflammatory burden-induced myocardial injury in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (15–17). These pathophysiological disorders were further illustrated by our study. In our patients, more than 95% of them developed ARDS with a significantly rapid heart rate, which caused an imbalance between cardiac metabolic demand and oxygen supply. Moreover, a prevalence of shock or insufficient peripheral perfusion was indicated by the common application of vasoconstrictive agents in our patients. Concomitantly, coagulation disequilibrium (higher D-dimer levels and longer PT) and the incidence of CV complications, including arrythmias, were also widely found

in these patients. Myocardial inflammation might be another cause of myocardial injury in coronavirus-infected patients (15). In a study performed with 21 autopsies of SARS-CoV infected patients, Oudit et al. reported increased inflammation in the myocardium of these patients associated with cardiac interstitial fibrosis and hypertrophy (18). For COVID-19, a brief case report suggested the potential occurrence of myocarditis in COVID-19 patients by describing a 53-year-old woman diagnosed with COVID-19 who developed acute myocarditis during hospitalization (19). However, COVID-19-induced viral myocarditis has not been supported by pathological data so far. In current autopsy reports of COVID-19 patients, researchers revealed that there was only a mild infiltration of inflammatory cells without substantial necrosis of cardiomyocytes (20, 21). It seems that systemic inflammation, but not localized myocardial inflammation plays a pivotal role in myocardial injury of COVID-19 patients. A similar conclusion was also given by another retrospective study that enrolled 112 COVID-19 patients, as they revealed that there were no typical signs of myocarditis on echocardiography, such as segmental wall motion abnormality, reduced LVEF or wall thickening, in COVID-19 patients with myocardial injury during hospitalization (5). The roles of myocardial inflammation in myocardial injury still need more investigation.

The mechanisms underlying the activation of inflammation in COVID-19 patients have been recently investigated by Zhang et al. (22). It has been suggested that COVID-19 induced the destruction of alveolar epithelial cells, which led to an increase in cell permeability and the release of virus. This result subsequently activated the innate immune system and induced the overproduction of cytokines (e.g., IL-6 and TNF-a), finally causing a systemic inflammatory response (22, 23). In this process, macrophage recruitment, which has been demonstrated to regulate SARS-CoV-2-induced inflammation (24, 25), was suggested to be one of the potential contributors, as interstitial mononuclear inflammatory infiltrates were observed in both lungs of patients with COVID-19. In addition, the plasma levels of macrophage-produced pro-inflammatory cytokines, such as IL-6 (26) and TNF-α (27), were shown to be significantly increased in patients with COVID-19, further supporting the contribution of macrophages to systemic inflammation in COVID-19 patients. In addition to macrophages, the activation of lymphocytes was also suggested as a factor in systemic inflammation in COVID-19 patients (6). Although decreased blood lymphocyte count of COVID-19 patients has been widely reported (4, 28), lymphocytes were shown to be activated as the increases in the expression of HLA-DR in $CD4^+$ and $CD8^+$ cells, the percentage of $CD4^+$ $CCR4^+$ $CCR6^+$ Th17 cells. The expression of cytotoxic particles (e.g., perforin and granulysin) in CD8 + T cells was demonstrated in an autopsy report of COVID-19 patients (21).

The efficiencies of anti-inflammatory treatments such as glucocorticoids, tocilizumab (TCZ) and anti-TNFα agents in COVID-19 patients were recently investigated by various registered cohort studies (6, 29). In this study, glucocorticoids and TCZ were applied in these patients. Glucocorticoids were widely applied during the outbreaks of several viral infectious diseases, such as SARS-CoV (30), Middle East respiratory syndrome (MERS)-CoV (31) and influenzas (32). However, the benefit derived from corticosteroids in the treatment of these diseases has not been revealed (33). For COVID-19, there are no clinical data indicating the benefits of corticosteroids, and the recommendation for their use is controversial (6, 33). Other investigators held a positive opinion for glucocorticoid usage, as systematic corticosteroid therapy in the first 3–5 days was shown to effectively inhibit severe inflammatory storms and alleviate critical symptoms in ICU patients with MERS (34). Currently, short-term systematic corticosteroid treatment (methylprednisolone, <1–2 mg/kg/d, 3–5 days) is recommended for the treatment of selective severe COVID-19 patients while being cautious of glucocorticoid-mediated immunosuppression, which delays the clearance of SARS-CoV-2 (35). In our study, systemic corticosteroid administration (methylprednisolone, 1–2 mg/kg/d × 5–7 days) was empirically used for patients with high inflammatory status. However, the efficiency is still not confirmed. Elucidating the benefit of glucocorticoids for COVID-19 patients is of immediate clinical importance. In contrast to glucocorticoids, TCZ, a recombinant human IL-6 monoclonal antibody, showed potential therapeutic value for COVID-19 patients. In a current clinical trial (clinical trial registration ID: ChiCTR2000029765), TCZ was administered once to 21 critical patients with COVID-19 at 400 mg intravenously. After a few days, the febrile patients' body temperature returned to normal, and all other symptoms improved significantly, in conjunction with better respiratory function, absorbed pulmonary lesions and lower plasma levels of hs-CRP (36). Moreover, several recent case reports also described the successful use of TCZ treatment in COVID-19 patients combined with other diseases (37, 38). In our study, TCZ was applied in several selective patients with high IL-6 levels. However, due to the insufficiency of related evidence, guidance and specialist consensus for the application of TCZ in COVID-19 patients is still lacking. Studies with larger populations are expected to further confirm the therapeutic value of TCZ against COVID-19 development. TNF-α inhibitors, such as infliximab (Remicade) and adalimumab (Humira), were not applied in our patients due to the lack of related information in COVID-19 patients. However, the therapeutic value of TNF-α for the severe immune-based pulmonary injury caused by SARS coronavirus has been implicated (39). Since high plasma levels of TNF-α have been widely observed in our patients, it is worth investigating the effects and safety of TNF-α inhibitors in the treatment of COVID-19.

This study still has several limitations. First, several pieces of cardiac information, such as echocardiography data and electrocardiography data, were lacking in this study, which limited the evaluation of myocardial injury. Second, plasma levels of certain inflammatory cytokines, such as granulocyte-colony stimulating factor, monocyte chemoattractant protein-1 and macrophage inflammatory protein 1-α (chemokine ligand 3), were not tested in our study. Finally, this study only involved 64 patients, and further studies with larger populations or multicenter study should be performed to confirm our results.

CONCLUSION

This study demonstrated that myocardial injury was a common complication of COVID-19, and myocardial injury was associated with the occurrence of adverse events and a high mortality rate. The positive correlation of high inflammatory burden with the incidence of myocardial injury was further revealed in critically ill patients with COVID-19 in this study.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the data which has been used is confidential. Requests to access the datasets should be directed to the corresponding author, camsww@163.com.

ETHICS STATEMENT

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of PUMC Hospital. Written informed consent was waived due to the rapid emergence of this infectious disease.

AUTHOR CONTRIBUTIONS

YS, PG, TR, WW, and SZ: concept and design. YS, PG, TR, HQ, FG, LC, and WW: acquisition, analysis, or interpretation of data. YS, WW, and SZ: drafting of the manuscript. YS, PG, TR, HQ, FG, LC, WW, and SZ: critical revision of the manuscript for important intellectual content. YS and WW: statistical analysis. WW and SZ: administrative, technical, material support, and supervision. All authors contributed to the article and approved the submitted version.

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*. (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
2. 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: 10.1101/2020.02.06.20020974
3. Bonow RO, Fonarow GC, O'Gara PT, Yancy CW. Association of Coronavirus Disease 2019 (COVID-19) with myocardial injury and mortality. *JAMA Cardiol*. (2020) doi: 10.1001/jamacardio.2020.1105. [Epub ahead of print].
4. 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) e201017. doi: 10.1001/jamacardio.2020.1017. [Epub ahead of print].
5. 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
6. Zhang W, Zhao Y, Zhang F, Wang Q, Li T, Liu Z, et al. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): the perspectives of clinical immunologists from China. *Clin Immunol*. (2020) 214:108393. doi: 10.1016/j.clim.2020.108393
7. 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. *Intens Care Med*. (2020) 46:846–8. doi: 10.1007/s00134-020-06028-z
8. Conti P, Ronconi G, Caraffa A, Gallenga CE, Ross R, Frydas I, et 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) 34:1. doi: 10.23812/CONTI-E
9. Trachtenberg BH, Hare JM. Inflammatory cardiomyopathic syndromes. *Circ Res*. (2017) 121:803–18. doi: 10.1161/CIRCRESAHA.117.310221
10. 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
11. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, et al. Acute respiratory distress syndrome: the Berlin definition. *JAMA*. (2012) 307:2526–33. doi: 10.1001/jama.2012.5669
12. Kellum JA, Lameire N. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). *Crit Care*. (2013) 17:204. doi: 10.1186/cc11454
13. Paddock CD, Liu L, Denison AM, Bartlett JH, Holman RC, Deleon-Carnes M, et al. Myocardial injury and bacterial pneumonia contribute to the pathogenesis of fatal influenza B virus infection. *J Infect Dis*. (2012) 205:895–905. doi: 10.1093/infdis/jir861
14. Yu CM, Wong RS, Wu EB, Kong SL, Wong J, Yip GW, et al. Cardiovascular complications of severe acute respiratory syndrome. *Postgrad Med*. (2006) 82:140–44. doi: 10.1136/pgmj.2005.037515
15. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of coronaviruses on the cardiovascular system: a review. *JAMA Cardiol*. (2020) doi: 10.1001/jamacardio.2020.1286. [Epub ahead of print].
16. Jou C, Shah R, Figueroa A, Patel JK. The role of inflammatory cytokines in cardiac arrest. *J Intens Care Med*. (2020) 35:219–24. doi: 10.1177/0885066618817518
17. 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
18. Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutanen SM, Penninger JM, et al. SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. *Eur J Clin Invest*. (2009) 39:618–25. doi: 10.1111/j.1365-2362.2009.02153.x
19. 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) doi: 10.1001/jamacardio.2020.1096. [Epub ahead of print].
20. Yao XH, Li TY, He ZC, Ping YF, Liu HW, Yu SC, et al. [A pathological report of three COVID-19 cases by minimally invasive autopsies]. *Zhonghua Bing Li Xue Za Zhi*. (2020) 49:411–17. doi: 10.3760/cma.j.cn112151-20200312-00193
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. Zhang C, Wu Z, Li JW, Zhao H, Wang GQ. 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
23. Fu Y, Cheng Y, Wu Y. Understanding SARS-CoV-2-mediated inflammatory responses: from mechanisms to potential therapeutic tools. *Virol Sin*. (2020) 1-6. doi: 10.1007/s12250-020-00207-4. [Epub ahead of print].
24. Hwang DM, Chamberlain DW, Poutanen SM, Low DE, Asa SL, Butany J. Pulmonary pathology of severe acute respiratory syndrome in Toronto. *Mod Pathol*. (2005) 18:1–10. doi: 10.1038/modpathol.3800247
25. Cameron MJ, Ran L, Xu L, Danesh A, Bermejo-Martin JF, Cameron CM, et al. Interferon-mediated immunopathological events are associated with atypical innate and adaptive immune responses in patients with severe acute respiratory syndrome. *J Virol*. (2007) 81:8692–706. doi: 10.1128/JVI.00527-07
26. Becker S, Quay J, Soukup J. Cytokine (tumor necrosis factor, IL-6, and IL-8) production by respiratory syncytial virus-infected human alveolar macrophages. *J Immunol*. (1991) 147:4307–12.
27. Gong JH, Sprenger H, Hinder F, Bender A, Schmidt A, Horch S, et al. Influenza A virus infection of macrophages. Enhanced tumor necrosis factor-alpha

(TNF-alpha) gene expression and lipopolysaccharide-triggered TNF-alpha release. *J Immunol.* (1991) 147:3507–13.
28. 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) e200950. doi: 10.1001/jamacardio.2020.0950. [Epub ahead of print].
29. Russell B, Moss C, George G, Santaolalla A, Cope A, Papa S, et al. Associations between immune-suppressive and stimulating drugs and novel COVID-19-a systematic review of current evidence. *Ecancermedicalscience.* (2020) 14:1022. doi: 10.3332/ecancer.2020.1022
30. Chen RC, Tang XP, Tan SY, Liang BL, Wan ZY, Fang JQ, et al. Treatment of severe acute respiratory syndrome with glucosteroids: the Guangzhou experience. *Chest.* (2006) 129:1441–52. doi: 10.1378/chest.129.6.1441
31. Arabi YM, Mandourah Y, Al-Hameed F, Sindi AA, Almekhlafi GA, Hussein MA, et al. Corticosteroid therapy for critically ill patients with middle east respiratory syndrome. *Am J Respir Crit Care Med.* (2018) 197:757–67. doi: 10.1164/rccm.201706-1172OC
32. Ni YN, Chen G, Sun J, Liang BM, Liang ZA. The effect of corticosteroids on mortality of patients with influenza pneumonia: a systematic review and meta-analysis. *Crit Care.* (2019) 23:99. doi: 10.1186/s13054-019-2395-8
33. Russell CD, Millar JE, Baillie JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. *Lancet.* (2020) 395:473–5. doi: 10.1016/S0140-6736(20)30317-2
34. Zhou W, Liu Y, Tian D, Wang C, Wang S, Cheng J, et al. Potential benefits of precise corticosteroids therapy for severe 2019-nCoV pneumonia. *Signal Transduct Target Ther.* (2020) 5:18. doi: 10.1038/s41392-020-0127-9
35. National Health Commission of the People's Republic of China. *The 5th Trial Version of Diagnosis and Treatment Scheme for Pneumonitis With 2019-nCoV Infection.* (2020). Available online at: http://www.nhc.gov.cn/yzygj/s7653p/202002/d4b895337e19445f8d728fcaf1e3e13a.shtml (accessed February 8, 2020).
36. Xu XL, Han MF, Li TT, Su W, Wang DS, Fu BQ, et al. *Effective Treatment of Severe COVID-19 Patients With Tocilizuma.* (2020). Available online at: http://chinaxiv.org/abs/202003.00026 (accessed March 11, 2020).
37. Mihai C, Dobrota R, Schroder M, Garaiman A, Jordan S, Becker MO, et al. COVID-19 in a patient with systemic sclerosis treated with tocilizumab for SSc-ILD. *Ann Rheum Dis.* (2020) 79:668–9. doi: 10.1136/annrheumdis-2020-217442
38. Zhang X, Song K, Tong F, Fei M, Guo H, Lu Z, et al. First case of COVID-19 in a patient with multiple myeloma successfully treated with tocilizumab. *Blood Adv.* (2020) 4:1307–10. doi: 10.1182/bloodadvances.2020001907
39. Tobinick E. TNF-alpha inhibition for potential therapeutic modulation of SARS coronavirus infection. *Curr Med Res Opin.* (2004) 20:39–40. doi: 10.1185/030079903125002757

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Renin-Angiotensin-Aldosterone System Blockers Are Not Associated With Coronavirus Disease 2019 (COVID-19) Hospitalization: Study of 1,439 UK Biobank Cases

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Background: Cardiometabolic morbidity and medications, specifically Angiotensin Converting Enzyme inhibitors (ACEi) and Angiotensin Receptor Blockers (ARBs), have been linked with adverse outcomes from coronavirus disease 2019 (COVID-19). This study aims to investigate, factors associated with COVID-19 positivity in hospital for 1,436 UK Biobank participants; compared with individuals who tested negative, and with the untested, presumed negative, rest of the cohort.

Methods: We studied 7,099 participants from the UK Biobank who had been tested for COVID-19 in hospital. We considered the following exposures: age, sex, ethnicity, body mass index (BMI), diabetes, hypertension, hypercholesterolaemia, ACEi/ARB use, prior myocardial infarction (MI), and smoking. We undertook comparisons between (1) COVID-19 positive and COVID-19 negative tested participants; and (2) COVID-19 tested positive and the remaining participants (tested negative plus untested, $n = 494{,}838$). Logistic regression models were used to investigate univariate and mutually adjusted associations.

Results: Among participants tested for COVID-19, Black, Asian, and Minority ethnic (BAME) ethnicity, male sex, and higher BMI were independently associated with a positive result. BAME ethnicity, male sex, greater BMI, diabetes, hypertension, and smoking were independently associated with COVID-19 positivity compared to the remaining cohort (test negatives plus untested). However, similar associations were observed when comparing those who tested negative for COVID-19 with the untested cohort; suggesting that these factors associate with general hospitalization rather than specifically with COVID-19.

Conclusions: Among participants tested for COVID-19 with presumed moderate to severe symptoms in a hospital setting, BAME ethnicity, male sex, and higher BMI are associated with a positive result. Other cardiometabolic morbidities confer increased risk of hospitalization, without specificity for COVID-19. ACE/ARB use did not associate with COVID-19 status.

Keywords: coronavirus disease 2019, UK Biobank, ethnicity, sex, obesity, cardiometabolic disease, Angiotensin Converting Enzyme inhibitors, Angiotensin Receptor Blockers

INTRODUCTION

Coronavirus disease 2019 (COVID-19), the clinical illness caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has reached pandemic levels. There has been growing recognition that patients with underlying cardiometabolic morbidities may be suffering higher rates of infection and a more severe disease course than the general population (1–3). Debate has ensued regarding whether these observations relate to the conditions themselves or the medications with which they are treated. In particular, some have suggested a mechanistic role for Angiotensin Converting Enzyme inhibitors (ACEi) or Angiotensin Receptor Blockers (ARBs) (4). However, recent reports have not produced convincing evidence for the specific association of ACEi/ARBs with poorer outcomes (4–6). Cardiometabolic diseases are common and ACEi/ARBs are used by many vulnerable patients. It is therefore important to better understand the augmented risk associated with cardiometabolic factors and ACEi/ARB use with COVID-19, to inform clinical practice and guidance to patients.

The UK Biobank (UKB) is a large cohort study comprising data from over 500,000 participants from across the UK, characterized in detail at baseline (2006–2010), and with linkages to Hospital Episode Statistic (HES) data. In response to the COVID-19 pandemic, the UKB facilitated rapid release of COVID-19 testing data for its participants through linkage with Public Health England (7), providing a unique opportunity to study the effects of many well-defined exposures on COVID-19 status.

The aim of this study is to investigate the association of demographic factors (age, sex, ethnicity), cardiometabolic profile [body mass index (BMI), diabetes, hypertension, hypercholesterolaemia, prior myocardial infarction (MI), smoking], and ACEi/ARB use with COVID-19 positivity in hospital using data from UKB.

METHODS

Setting and Study Population

UKB is a prospective cohort study including over 500,000 participants from across the UK. Individuals aged 40–69 years old identified via National Health Service (NHS) registers were recruited over a 4-year period between 2006 and 2010. Participants underwent detailed baseline assessment including characterization of socio-demographics, lifestyle, medical history, and a series of physical measures. The protocol is publicly available (8). Linkages with HES data permit longitudinal tracking of health outcomes for all participants with conditions recorded according to international classification of disease (ICD) codes. In addition, UKB has produced algorithmically defined outcome data for incidence of key illness, such as MI, through integration of data from multiple sources (9). The latest data release (24th June 2020) includes test results from 16th March to 14th June. In the UK, until the 18th of May 2020, testing was almost entirely limited to hospital settings, after this date, testing was extended to the community. Therefore, we consider a positive test performed up to the 18th of May as indicative of hospitalization, beyond this date we required explicitly labeling of the sample as "inpatient." Testing was based on a real-time polymerase chain reaction (RT-PCR) assay antigen test; for most participants the sample tested was from combined nose and throat swab; for patients in intensive care lower respiratory samples may have been used. Thus, we defined a cohort of participants who were tested for SARS-CoV-2 whilst admitted to hospital, and therefore are likely to have a relatively severe presentation.

Statistical Analysis

Statistical analysis was performed using R Version 3.6.2 (10), and RStudio Version 1.2.5019 (11). We considered the following exposures: age, sex, ethnicity, body mass index (BMI), diabetes, hypertension, high cholesterol, ACEi/ARB use, prevalent MI, and smoking. The cardiometabolic and demographic factors were selected based on existing reports of their potential association with COVID-19 outcomes (3, 12, 13). ACEi/ARBs were considered due to reports of potential mechanistic role of these medications in the clinical course of COVID-19 (4). We used age, sex, and ethnicity (White vs. BAME) as recorded at baseline. BMI was calculated from height and weight recorded at baseline. Smoking status was based on self-report. Hypertension, diabetes, and hypercholesterolaemia were defined through cross-checking across self-report and HES data. A list of ICD codes used is available in **Supplementary Table 1**. Information on prior MI was obtained from the UKB algorithmically defined health outcomes. ACEi/ARB use was determined from self-report (**Supplementary Table 2**). We considered the effect of ACEi and ARBs both separately and as an aggregate variable. We created three cohorts: test positives, test negatives, and the untested cohort (**Figure 1**). Individuals who were tested, but with unclear hospitalization status were excluded from the analysis. We firstly compared the COVID-19 test positive cohort with the combined

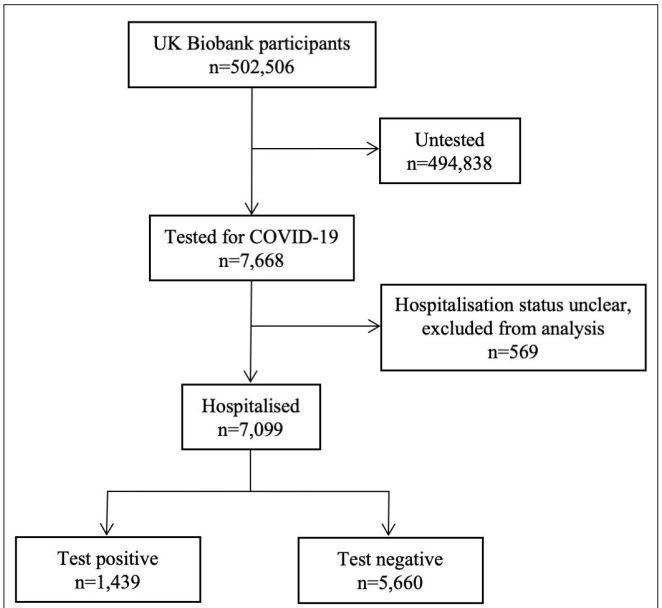

FIGURE 1 | Summary of COVID-19 testing and results for UK Biobank participants. Data includes COVID-19 test results from 16/03/2020 to 14/06/2020. During this time period, 7,688 participants, of the whole UK Biobank cohort ($n = 502,506$) have been tested for COVID-19. 7,099 were likely in a hospital setting, of whom 1,439 participants had a positive result and 5,660 tested negative. The remaining participants ($n = 494,838$) have not been tested.

cohort of test negatives and the untested UKB population. In order to investigate possible bias relating to hospitalization status, we also considered the importance of these exposure variables in two further comparisons: test positives vs. test negatives and test negatives vs. untested population. We used logistic regression models to elucidate univariate and then multivariate associations. There was no evidence of multicollinearity with variance inflation factor (VIF) <2.0 for all covariates. As the observed association with ethnicity was strong, we tested for potential interaction effects between ethnicity and all tested covariates in multivariate models. We present odds ratio (OR) for each exposure with the corresponding 95% confidence interval (CI) and *p*-value. Given the low background prevalence of COVID-19 positivity, the odds ratios can be interpreted as relative risks.

RESULTS

Baseline Characteristics

Of the 7,668 UKB participants tested for COVID-19, 7,099 were likely in a hospital setting and are included in this analysis (**Table 1**, **Figure 1**), of these 1,439 tested positive and 5,660 tested negative. There was no record of testing for the remainder of the UKB cohort ($n = 494,838$) (**Figure 1**).

In comparison to the untested cohort, the COVID-19 positive cohort were predominantly male (52.9% vs. 45.5%), had a greater proportion of BAME individuals (12.9% vs. 5.3%), and an all-round poorer cardiometabolic profile, with higher BMI, higher rates of smoking, prior MI, diabetes, hypertension, and high cholesterol; they also reported greater use of ACEi/ARB agents (21.8% vs. 14.3%). However, comparing the COVID-19 positive cohort with the tested negative cohort ($n = 5,660$), the differences were much less pronounced, as the test negative cohort also had a globally poorer cardiometabolic profile than the untested population.

Association of Exposures With COVID Status

COVID-19 Positive vs. Not COVID-19 Positive (Tested Negative Cohort Plus Untested Cohort)

We first tested whether there were univariate associations between exposures and COVID-19 positives ($n = 1,439$) vs. not COVID-19 positives (including tested negative and untested cohort, $n = 500,498$). Univariate associations were significant for all covariates considered, except age. In multivariate models, the independent predictors of COVID-19 positivity were younger age, male sex, BAME ethnicity, greater BMI, diabetes, hypertension, and smoking (**Table 2**, **Figure 2**: Comparison 1).

COVID-19 Positive vs. COVID-19 Tested Negative

We next considered associations between exposures and COVID-19 positives ($n = 1,439$) vs. tested negative cohort ($n = 5,660$). Within this sample, the univariate predictors of positivity were male sex, younger age, BAME ethnicity, greater BMI, and diabetes. These variables, with the exception of diabetes, remained statistically significant in the multivariate model with mutual adjustment for all other covariates (**Table 2**, **Figure 2**). The greatest magnitude of effect related to ethnicity; BAME individuals had almost twice the likelihood of a COVID-19 positive result compared to White ethnicities in the fully adjusted models [OR 1.95, 95% CI (1.60, 2.36)]. There was no evidence of interaction effect with ethnicity and any of the other covariates (**Supplementary Table 3**). Compared with women, men had 22% greater odds of a COVID-19 positive test [OR 1.22, 95% CI (1.08, 1.38)]. For every 5 kg/m^2 increase of BMI, there was 9% greater odds of COVID-19 positive status (**Table 2**, **Figure 2**: Comparison 2). There was a negative association with age, this may reflect older age of participants admitted to hospital for reasons other than COVID-19; alternatively, it may be an artifact of the data related to the narrow age range in the sample. Notably, there was no significant association between ACEi/ARB use and COVID-19 status, which was consistent when testing effect of ACEi and ARBs separately (**Supplementary Table 4**).

COVID-19 Tested Negatives vs. Untested Population

Finally, we investigated associations between the exposures with a negative test ($n = 5,660$) vs. untested UKB population ($n = 494,838$). There were significant univariate associations for all covariates considered. In the multivariate model, BAME ethnicity, older age, higher BMI, diabetes, hypertension, high cholesterol, previous MI, and smoking were significant predictors of a having a negative test, and therefore of presenting to hospital, perhaps with respiratory symptoms, compared to not being tested (**Table 2**, **Figure 2**: Comparison 3).

TABLE 1 | Baseline participant characteristics.

	COVID-19 tested (n = 7,099)	COVID-19 positive (n = 1,439)	COVID-19 negative (n = 5,660)	Untested population (n = 494,838)
Sex (Male)	3,525 (49.7%)	761 (52.9%)	2,764 (48.8%)	225,352 (45.5%)
Age*	69.11 (±8.65)	68.22 (±9.19)	69.34 (±8.49)	68.24 (±8.10)
White ethnicity	6,498 (91.5%)	1,242 (86.3%)	5,256 (92.9%)	465,681 (94.1%)
BAME ethnicity	562 (7.9%)	185 (12.9%)	377 (6.7%)	26,429 (5.3%)
BMI (kg/m^2)	27.66 [24.78, 31.13]	27.97 [25.18, 31.50]	27.58 [24.69, 31.02]	26.7 [±24.13, 29.89]
Smoking**	3,663 (51.6%)	732 (50.9%)	2,931 (51.8%)	221,478 (44.8%)
Prior MI	557 (7.8%)	103 (7.2%)	454 (8.0%)	20,227 (4.1%)
Diabetes	1,029 (14.5%)	241 (16.7%)	788 (13.9%)	38,046 (7.7%)
Hypertension	3,338 (47.0%)	676 (47.0%)	2,662 (47.0%)	171,415 (34.6%)
High cholesterol	2,388 (33.6%)	477 (33.1%)	1,911 (33.8%)	115,133 (23.3%)
ACEi†	1,117 (15.7%)	227 (15.8%)	890 (15.7%)	50,635 (10.2%)
ARB†	418 (5.9%)	87 (6.0%)	331 (5.8%)	20,416 (4.1%)

*Data are n (%), mean (standard deviation), or median [interquartile range]. COVID-19 data includes test results from 16/03/2020 to 14/06/2020 from hospital settings. *We report age of participants as of 01/04/2020. **Smoking includes current and previous smoking. †ACEi/ARB use is defined as a binary measure, defined as true if record of any of medications in* ***Supplementary Table 2****. ACEi, Angiotensin Converting Enzyme inhibitor; ARB, Angiotensin Receptor Blocker; BAME, Black, Asian, and Minority ethnic; BMI, body mass index; COVID-19, coronavirus 2019.*

TABLE 2 | Odds Ratios, 95% confidence intervals, and p-values for each exposure from univariate and multivariate logistic regression models in the three defined comparisons**.

	Comparison 1		Comparison 2		Comparison 3	
Predictors	**Univariate Models**	**Multivariate Model**	**Univariate Models**	**Multivariate Model**	**Univariate Models**	**Multivariate Model**
Male sex	1.34* [1.21, 1.49] 3.07×10^{-8}	1.19* [1.07, 1.32] 0.0017	1.18* [1.05, 1.32] 0.0061	1.22* [1.08, 1.38] 0.0012	1.14* [1.08, 1.20] 7.68×10^{-7}	1.00 [0.95, 1.06] 0.9759
Age (per 5 years)	1.00 [0.97, 1.03] 0.8620	0.96* [0.93, 1.00] 0.0316	0.93* [0.90, 0.96] 1.17×10^{-5}	0.94* [0.90, 0.97] 9.64×10^{-4}	1.09* [1.07, 1.11] 5.81×10^{-24}	1.03* [1.01, 1.05] 0.0013
BAME ethnicity	2.62* [2.23, 3.05] 4.58×10^{-34}	2.47* [2.10, 2.89] 5.58×10^{-28}	2.08* [1.72, 2.50] 1.59×10^{-14}	1.95* [1.60, 2.36] 2.07×10^{-11}	1.26* [1.14, 1.40] 1.29×10^{-5}	1.27* [1.14, 1.41] 1.70×10^{-5}
BMI (per 5kg/m2)	1.30* [1.24, 1.36] 2.19×10^{-29}	1.19* [1.13, 1.25] 7.63×10^{-11}	1.10* [1.04, 1.16] 3.62 × 10–4	1.09* [1.03, 1.16] 0.0031	1.19* [1.16, 1.22] 4.47×10^{-42}	1.09* [1.06, 1.12] 3.78×10^{-9}
Diabetes	2.39* [2.08, 2.74] 7.39×10^{-35}	1.52* [1.29, 1.79] 3.72×10^{-7}	1.24* [1.06, 1.45] 0.0066	1.17 [0.98, 1.41] 0.0882	1.94* [1.80, 2.09] 1.05×10^{-65}	1.34* [1.23, 1.46] 2.80×10^{-11}
Hypertension	1.66* [1.50, 1.84] 8.27×10^{-22}	1.25* [1.09, 1.43] 0.0010	1.00 [0.89, 1.12] 0.9704	0.98 [0.84, 1.14] 0.7727	1.68* [1.59, 1.77] 1.27×10^{-82}	1.28* [1.20, 1.37] 5.90×10^{-13}
High cholesterol	1.62* [1.45, 1.81] 5.20×10^{-18}	1.12 [0.97, 1.28] 0.1234	0.97 [0.86, 1.10] 0.6592	0.95 [0.81, 1.11] 0.5006	1.68* [1.59, 1.78] 3.31×10^{-75}	1.19* [1.11, 1.27] 1.52×10^{-6}
ACEi/ARB	1.65* [1.45, 1.87] 7.54×10^{-15}	1.04 [0.89, 1.22] 0.5885	1.01 [0.88, 1.17] 0.8563	0.99 [0.83, 1.19] 0.9468	1.64* [1.54, 1.75] 2.31×10^{-51}	1.04 [0.96, 1.13] 0.3193
Prior MI	1.79* [1.45, 2.17] 1.41×10^{-8}	1.18 [0.94, 1.46] 0.1377	0.88 [0.70, 1.10] 0.2770	0.85 [0.66, 1.08] 0.1893	2.05* [1.85, 2.25] 1.70×10^{-47}	1.39* [1.25, 1.54] 1.02×10^{-9}
Smoking	1.27* [1.15, 1.41] 4.58×10^{-6}	1.26* [1.13, 1.40] 3.02×10^{-5}	0.96 [0.86, 1.08] 0.5348	1.02 [0.90, 1.15] 0.7369	1.33* [1.26, 1.40] 5.91×10^{-26}	1.24* [1.17, 1.31] 9.40×10^{-15}

***Comparison 1: COVID-19 positive (n = 1,439) vs. not COVID-19 positive (tested negative plus untested cohort) (n = 494,838); Comparison 2: COVID-19 positive (n = 1,439) vs. COVID-19 test negative (n = 5,660); Comparison 3: COVID-19 test negative (n = 5,660) vs. untested population (n = 494,838). Results are odds ratio, 95% confidence interval, and p-value (from top to bottom) for each exposure. For continuous variables (age, BMI) coefficients refer to the effect on odds of the outcome per five unit increase in the exposures, i.e., 5-year increase in age and 5 kg/m^2 increase in BMI. The remaining exposures are set as binary measures with results showing effect of change from non-disease to disease states, male sex vs. female sex, BAME ethnicity vs. White ethnicity; smoking history (current/previous) vs. never smoked; ACEi/ARB use vs. no ACEi/ARB use on odds of the outcome. *Indicates p < 0.05. ACEi, Angiotensin Converting Enzyme inhibitor; ARB, Angiotensin Receptor Blocker; BMI, body mass index; coronavirus 2019: COVID-19; BAME, Black, Asian, and Minority ethnic; MI, myocardial infarction.*

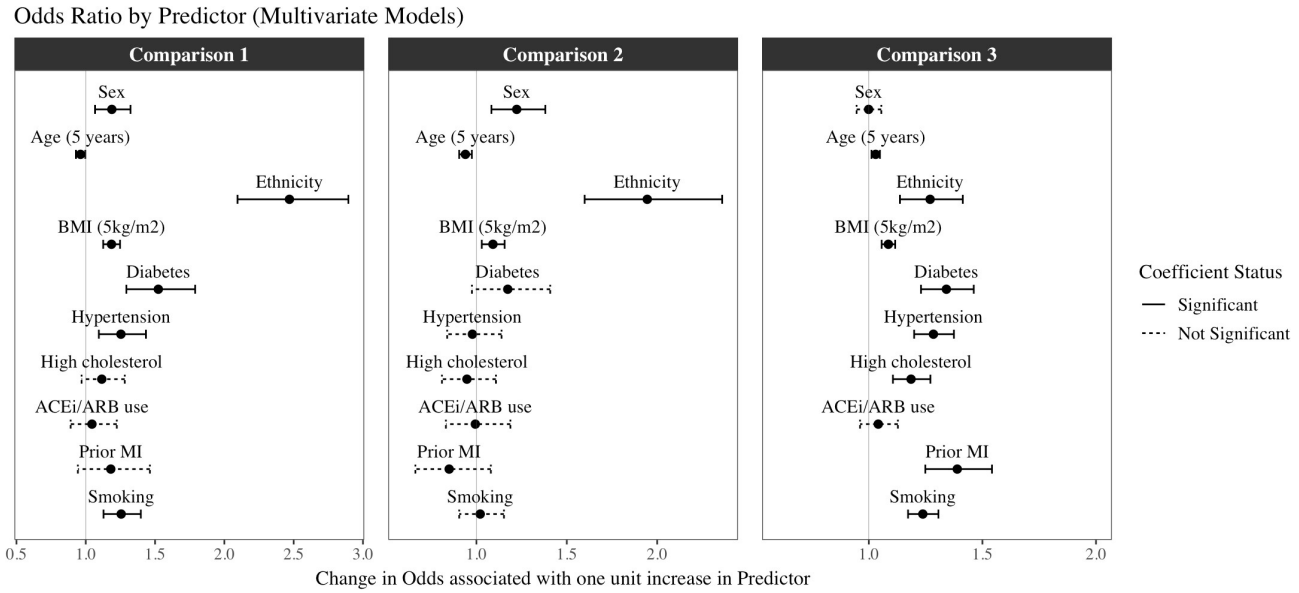

FIGURE 2 | Odds Ratios and 95% confidence intervals for each exposure from the multivariate logistic regression models in the three different comparisons*. *Comparison 1: COVID-19 positive (n = 1,439) vs. not COVID-19 positive (tested negative plus untested cohort) (n = 494,838); Comparison 2: COVID-19 positive (n = 1,439) vs. COVID-19 test negative (n = 5,660); Comparison 3: COVID-19 test negative (n = 5,660) vs. untested population (n = 494,838). Results are odds ratios with 95% confidence intervals. Dashed lines represent non-significant and solid lines statistically significant results, with threshold at $p < 0.05$.

DISCUSSION

Summary of Findings

In this analysis of 7,099 UKB participants tested for COVID-19 in a hospital setting, BAME ethnicity, younger age, male sex, greater BMI, diabetes, hypertension, and smoking were independently associated with COVID-19 positive test in comparison to the rest of the cohort (tested negatives plus untested). However, within the tested sample, a positive result was more likely for men, BAME individuals, younger ages, and with greater BMI. Indeed, when compared with the background population, the pattern of associations between exposures and COVID-19 positive was similar to that for COVID-19 test negative. These findings suggest that BAME ethnicity, male sex, and higher BMI have specific relevance to COVID-19, whilst the other exposure associations between COVID-19 positive and the remainder of the population reflect morbidities associated with general requirement for hospitalization, without specificity to COVID-19. Furthermore, as testing was in a hospital setting, these associations relate specifically to the more severe end of the COVID-19 manifestations requiring hospitalization. Notably, ACEi/ARB usage was not associated with COVID-19 status.

Comparison With Existing Literature

With the rapid global spread of COVID-19, understanding the determinants of infection risk and severity is a priority. Differences in ethnic background are known to contribute to differences in patterns of a number of diseases, including influenza (14), due to different genetic susceptibilities and environmental exposures (15). In the UK, national audit data demonstrates as many as one-third of COVID-19 patients admitted to intensive care are from BAME backgrounds; a rate which is disproportionate to their representation among the general UK population (16). In our study, BAME ethnicity had specific association with higher risk of COVID-19 positive status that appeared independent from often-quoted confounders of cardiovascular and metabolic morbidity that are known to be higher in prevalence in BAME cohorts (17). Having accounted for cardiometabolic morbidity, the possible explanations for this association remain numerous (18), gravitating around both genetic and social factors; behavioral, cultural, and socioeconomic differences, including health-seeking behavior and intergenerational cohabitation are all likely to play a role in the strong disparity observed in our study, providing key targets for both further research and public health policy. Initial studies, demonstrate complex interplay of biological and socio-economic factors and highlight need for urgent research in this area (19).

Since the first reports emerging from China at the beginning of the outbreak, it has been widely recognized that males suffer higher rates of infection and poorer outcomes compared to females; with reported distributions of approximately three-fifths men and two-fifths women (20, 21). The reasons for this are unclear. Animal studies demonstrate, that in mice infected with SARS-CoV, estrogen-deplete status either due to male gender or ovariectomy is associated with higher risk of acute respiratory distress syndrome (ARDS), indicating a possible protective role of estrogen signaling (22). Men are known to have higher burden of cardiovascular disease than women up to the perimenopausal years; and thus, lower cardiometabolic morbidity among women in the younger cohort has been postulated to contribute to better outcomes. However,

we demonstrate that in our study population, the association between male sex and higher infection rates was independent of cardiometabolic disease. Furthermore, male sex appears significant in our sample comprising an older cohort with almost all women being post-menopause, indicating that sex-differential disparities in COVID-19 disease severity relate to factors other than immediate-term estrogen exposure. Thus, our findings suggest that the higher risk of COVID-19 in men is not sufficiently explained by the estrogen pathway or greater burden of cardiometabolic disease.

Obesity is a global health issue, rising in prevalence and public health burden in both developed and developing countries. Patients who suffer from obesity are known to be at increased risk of a number of conditions, including cardiometabolic and respiratory disease, contributing to a poor physiological reserve. It is already known that patients with obesity have worse outcomes from influenza infection (23, 24). With the wealth of emerging research on COVID-19, concern has grown over the association between obesity and poor outcomes of infection (25); with studies consistently demonstrating higher rates of critical or intensive care requirement among individuals with higher BMI (26–28). Similar to ethnicity, the relationship between obesity and severe infection must be isolated from the confounding of obesity-related comorbidity. In our study, we demonstrate the distinct role of obesity from that of associated cardiometabolic diseases; with the major finding that obesity, and not its comorbidities, had independent and specific association with COVID-19 positivity. This is of important relevance, as mechanistic understanding of the reason behind this association may provide therapeutic insight. For example, obesity enhances risk of thrombosis, which has been a recent focus of interest given concern over a possible association between COVID-19 and prothrombotic intravascular coagulation (29). The results of our study provide useful information for risk stratification of patients, highlight important avenues for further research, and emphasize the public health-level importance of continued targeting of obesity.

Several reports hypothesize potential mechanistic links between ACEi/ARB usage and adverse outcomes from COVID-19 (4). SARS-CoV-2 has been shown to exhibit specific tropism for the angiotensin-converting enzyme 2 (ACE2) receptor; by which means it enters the cells and establishes itself in the host (30). The expression of ACE2 receptors in epithelial cells of the lung, intestine, kidney and endothelium may be increased in those treated with ACEi/ARBs, thereby facilitating entry and multisystem manifestations of COVID-19 (31, 32). The relationship between COVID19 infection risk and use of ACEi/ARBs has been a matter of debate since the early days of the outbreak, but recent studies have revealed a lack of independent association when morbidity variables, including atherosclerotic cardiovascular disease, heart failure and cardiometabolic diseases such as diabetes and hypertension were accounted for (4, 5). Furthermore, a recent study from Spain demonstrates no association between ACEi/ARB use and COVID-19 mortality or requirement for intensive care (33). Findings from our sample are consistent with these reports, demonstrating univariate association with ACEi/ARB use which becomes non-significant after adjustment for cardiometabolic and demographic factors.

Strengths and Limitations

UKB is a comprehensive data source, incorporating a large sample with linkages to prospectively tracked health outcomes recorded in a standardized manner using ICD codes, enabling reliable and up-to-date definition of morbidities. The rapid release of COVID-19 testing data provides a huge opportunity to examine association of a large number of exposures with COVID-19 status and outcomes. Due to the observational study design, we cannot comment on causal relationships from the results, however, the prospective nature of the study ensures confident temporal separation of exposure and outcome. Whilst analyses using the whole UK Biobank cohort of over 500,000 people may detect very small associations which are unlikely to be clinically significant, we studied a subset of much more modest sample size, with exposures and covariates chosen on the basis of prior literature and biological plausibility with the magnitude of relationships observed likely to be clinically meaningful. Further research in different cohorts would be helpful in better understanding the impact of the exposures studied. Whilst we can be reasonably confident about hospitalization status of the tested cohort in this study, there is uncertainty about the degree of symptoms. We acknowledge that there are local variations in testing approaches and that conclusions regarding disease severity drawn from hospitalization status alone have limitations. Studies in cohorts with more granular outcome data are needed. Furthermore, our results cannot be generalizable to asymptomatic or mildly symptomatic patients.

CONCLUSIONS

This work highlights specific associations of BAME ethnicity, male sex, and higher BMI with COVID-19 positive status, which were independent of other demographic or cardiometabolic factors. More detailed characterization of these associations in larger and more diverse cohorts is warranted, particularly with regards ethnicity. Investigation of potential biological pathways underlying these observed associations may provide insight into the mechanisms by which SARS-CoV-2 causes disease enabling more informed pursuit of potential therapeutic targets.

DATA AVAILABILITY STATEMENT

This study was performed using data from the UK Biobank under access application 2964. The UK Biobank is an open access research resource with data available on request to all bone fide researchers through the UK Biobank website: http://www.ukbiobank.ac.uk.

ETHICS STATEMENT

This study was covered by the ethics approval for UKB studies from the NHS National Research Ethics Service on 17th June

2011 (Ref 11/NW/0382) and extended on 10th May 2016 (Ref 16/NW/0274). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

This study was conceived by ZR-E, SP, and NH. The manuscript was written by ZR-E, MA, and MB. SP, NH, and CC advised on revisions of the manuscript. CM led on and conducted the statistical analysis. JC provided statistical advice. All co-authors read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2020.00138/full#supplementary-material

REFERENCES

1. 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
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). doi: 10.1001/jamacardio.2020.0950. [Epub ahead of print].
3. 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). doi: 10.1001/jamacardio.2020.1017. [Epub ahead of print].
4. 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
5. Mehra M, Desai S, Kuy S, Henry T, Patel A. Cardiovascular disease, drug therapy, and mortality in Covid-19 [Retracted]. *N Engl J Med.* (2020) 382:e102. doi: 10.1056/NEJMoa2007621
6. 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) 2019:1–6. doi: 10.1001/jamacardio.2020.1624
7. Armstrong J, Rudkin JK, Allen N, Crook DW, Wilson DJ, Wyllie DH, et al. Dynamic linkage of COVID-19 test results between Public Health England's Second Generation Surveillance System and UK Biobank. *Microb Genomics.* (2020). doi: 10.1099/mgen.0.000397. [Epub ahead of print].
8. UK Biobank. *Protocol for a Large-Scale Prospective Epidemiological Resource.* (2007). Available online at: https://www.ukbiobank.ac.uk/wp-content/uploads/2011/11/UK-Biobank-Protocol.pdf (accessed June 22, 2020).
9. Schnier C, Bush K, Nolan J, Sudlow C. *Definitions of Acute Myocardial Infarction and Main Myocardial Infarction Pathological Types UK Biobank Phase 1 Outcomes Adjudication Documentation on Behalf of UK Biobank Outcome Adjudication Group Definitions of Acute Myocardial Infarction.* (2017). Available online at: http://biobank.ndph.ox.ac.uk/showcase/showcase/docs/alg_outcome_mi.pdf (accessed June 22, 2020).
10. R Core Team (2019). *R: A Language and Environment for Statistical Computing.* Vienna: R Foundation for Statistical Computing. Available online at: https://www.R-project.org/
11. RStudio Team (2015). *RStudio: Integrated Development for R.* Boston, MA: RStudio, Inc. Available online at: http://www.rstudio.com/
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. *J Am Med Assoc.* (2020) 323:1061–9. doi: 10.1001/jama.2020.1585
13. 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
14. Zhao H, Harris RJ, Ellis J, Pebody RG. Ethnicity, deprivation and mortality due to 2009 pandemic influenza A(H1N1) in England during the 2009/2010 pandemic and the first post-pandemic season. *Epidemiol Infect.* (2015) 143:3375–83. doi: 10.1017/S0950268815000576
15. Lee C. 'Race' and 'ethnicity' in biomedical research: how do scientists construct and explain differences in health? *Soc Sci Med.* (2009) 68:1183–90. doi: 10.1016/j.socscimed.2008.12.036
16. *ICNARC Report on COVID-19 in Critical Care.* ICNARC COVID-19 Study Case Mix Program. Database (2020). p. 1–16. Available online at: https://www.icnarc.org/Our-Audit/Audits/Cmp/Reports (accessed June 15, 2020).
17. Tillin T, Hughes AD, Mayet J, Whincup P, Sattar N, Forouhi NG, et al. The relationship between metabolic risk factors and incident cardiovascular disease in Europeans, South Asians, and African Caribbeans: SABRE (Southall and Brent Revisited) - a prospective population-based study. *J Am Coll Cardiol.* (2013) 61:1777–86. doi: 10.1016/j.jacc.2012.12.046
18. Pareek M, Bangash MN, Pareek N, Pan D, Sze S, Minhas JS, et al. Ethnicity and COVID-19: an urgent public health research priority. *Lancet.* (2020) 395:1421–2. doi: 10.1016/S0140-6736(20)30922-3
19. Raisi-Estabragh Z, Mccracken C, Bethell MS, Cooper J, Cooper C, Caulfield MJ, et al. Greater risk of severe COVID-19 in Black, Asian and Minority Ethnic populations is not explained by cardiometabolic, socioeconomic or behavioural factors, or by 25(OH)-vitamin D status: study of 1326 cases from the UK Biobank. *J Public Health.* (2020) 25:1–10. doi: 10.1093/pubmed/fdaa095
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. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of Coronavirus Disease 2019 in China. *N Engl J Med.* (2020) 382:1708–20. doi: 10.1056/NEJMoa2002032
22. Channappanavar R, Fett C, Mack M, Ten Eyck PP, Meyerholz DK, Perlman S. Sex-based differences in susceptibility to severe acute respiratory syndrome coronavirus infection. *J Immunol.* (2017) 198:4046–53. doi: 10.4049/jimmunol.1601896

23. Green WD, Beck MA. Obesity impairs the adaptive immune response to influenza virus. *Ann Am Thorac Soc*. (2017) 14:406–9. doi: 10.1513/AnnalsATS.201706-447AW
24. Luzi L, Radaelli MG. Influenza and obesity: its odd relationship and the lessons for COVID-19 pandemic. *Acta Diabetol*. (2020) 57:759–64. doi: 10.1007/s00592-020-01522-8
25. Sattar N, McInnes IB, McMurray JJV. Obesity a risk factor for severe COVID-19 infection: multiple potential mechanisms. *Circulation*. (2020) 142:4–6. doi: 10.1161/CIRCULATIONAHA.120.047659
26. Petrilli CM, Jones SA, Yang J, Rajagopalan H, O'Donnell LF, Chernyak Y, et al. Factors associated with hospitalization and critical illness among 4,103 patients with COVID-19 disease in New York City. *medRxiv [Preprint]*. (2020). doi: 10.1101/2020.04.08.20057794
27. Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, et al. High prevalence of obesity in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) requiring invasive mechanical ventilation. *Obesity*. (2020) 28:1195–9. doi: 10.1002/oby.22831
28. Simonsick M, Ferrucci L, Resnick SM. Obesity in patients younger than 60 years is a risk factor for Covid-19 hospital admission. *Clin Infect Dis*. (2020). doi: 10.1093/cid/ciaa415. [Epub ahead of print].
29. 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
30. 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. doi: 10.1016/j.cell.2020.02.052
31. 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
32. 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. *Pharmacol Res*. (2017) 125:21–38. doi: 10.1016/j.phrs.2017.06.005
33. de Abajo FJ, Rodríguez-Martín S, Lerma V, Mejía-Abril G, Aguilar M, García-Luque A, 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

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REVIEW
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Covid-19 Kills More Men Than Women: An Overview of Possible Reasons

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The high mortality observed in Covid-19 patients may be related to unrecognized pulmonary embolism, pulmonary thrombosis, or other underlying cardiovascular diseases. Recent data have highlighted that the mortality rate of Covid-19 seems to be higher in male patients compared to females. In this paper, we have analyzed possible factors that may underline this sex difference in terms of activity of the immune system and its modulation by sex hormones, coagulation pattern, and preexisting cardiovascular diseases as well as effects deriving from smoking and drinking habits. Future studies are needed to evaluate the effects of sex differences on the prevalence of infections, including Covid-19, its outcome, and the responses to antiviral treatments.

Keywords: Covid-19, mortality, gender difference, immune system, sex hormones, smoking habit, coagulation pattern, cardiovascular diseases

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INTRODUCTION

All countries around the world are facing the COVID-19 emergency. As of June 22nd, more than 9,118,000 people have contracted the disease, and deaths have exceeded 471,000[1].

From a pathogenetic point of view, the progression of COVID-19 follows three main stages (1). The first stage, which approximately occurs in the initial 1–2 days, represents the phase in which the SARS-CoV-2 binds to epithelial cells and starts replicating. The human angiotensin-converting enzyme 2 (ACE2) receptor and TMPRSS2 are the main proteins involved in the cell entry of SARS-CoV-2 (2, 3). This phase is asymptomatic, and the innate immune response is limited. The second stage starts once the virus migrates down the respiratory tract. This phase is symptomatic with clear airway response and the innate immune response is triggered. An increase in the level of CXCL10 or other innate response cytokine is observed (4, 5). Indeed, this is the stage in which the so-called "cytokine storm" arises. Lastly, about 20% of patients with COVID-19 progress to a third stage, which is the most severe, and this stage is characterized by serious respiratory symptoms that include hypoxia, ground glass infiltrate, and progression to acute respiratory distress syndrome (ARDS). This stage can be further aggravated by organ failure and sepsis, potentially progressing to patient's death (6). At this stage, an aggressive immunomodulatory therapy is probably needed to prevent the onset of serious clinical consequences, such as the Disseminated intravascular coagulation (DIC) and the subsequent consumption coagulopathy (7). Indeed, as recently reported by a group of Italian researchers, the high mortality observed among Covid-19 patients may be somewhat due to unrecognized pulmonary embolism and pulmonary *in situ* thrombosis. Therefore, they suggested that a better understanding of Covid-19-related thromboembolic risk

[1] https://www.worldometers.info/coronavirus/ (accessed May 10, 2020).

would help to optimize diagnostic strategies but also the proper pharmacological management of patients with Covid-19 (8).

Data shared by Global Health 50/50, an internationally selected company that promotes gender equality in healthcare, revealed a higher proportion of deaths for Covid-19 in men than women in almost all countries. In Italy, according to data reported in the bulletin of integrated surveillance (update of April 23rd, 2020), deaths in men are approximately double compared to that of women (17.1 vs. 9.3%). Similar findings were reported in Greece, Holland, Denmark, Belgium, Spain, China, and the Philippines[2]. A study carried out by Liu et al. on 4,880 patients with respiratory symptoms or close contact with Covid-19 patients in a hospital in Wuhan showed that there was a significant higher rate in positivity to SARS-CoV-2 in males and the elderly population (>70 years), although only age was recognized as a risk factor (9). Similarly, a recent retrospective observational study showed that among critically ill patients with SARS-CoV-2, 67% were males and that the mortality rate was higher in males (10). In addition, a review of data related to 1,099 patients with Covid-19 showed that 58.1% were males. Furthermore, out of 173 severe cases, 57.8% concerned this population too (11). In addition, recent published data from a survival analysis (12) showed that men had a significantly higher mortality and exhibited worse symptoms than women. Lastly, Scully et al. recently reported that the case fatality rate for males is 1.7 times higher than for females ($P < 0.0001$) (13).

Considering that sex differences are frequently observed in many diseases, responses to drugs and the occurrence of adverse drugs reactions (14–17)—and that many reasons may underline these differences—in this paper, we aim to provide an overview of factors, including those influencing the immune system response, that possibly underline the sex and gender differences observed in Covid-19 patients. All those factors are summarized in **Table 1**.

TABLE 1 | Overview of sex- and gender-differences that could be responsible of increased mortality rate in men with Covid-19.

Activity of the immune system	■ Female patients seem to have an intense and prolonged innate, humoral, and cell-mediated immune response, leading to a faster and higher recognition of viral components ■ Preclinical studies showed that females might recover to a greater extent and are better protected from death during infections
Role of sex hormones	■ Testosterone shows suppressive effect on the immune function, while estrogen may have both suppressive and not suppressive effects depending on their levels ■ In men androgens deficiency is associated with increased levels of inflammatory cytokines and increased CD4+/CD8+ T-cell ratio ■ Estrogens are able to induce an upregulation in the expression of ACE2 ■ Exogenous estrogen increases the clotting risk in women and in biological males undergoing gender-affirming hormonal therapy ■ Sex hormones could also affect the response to antiviral treatments or vaccines
Prevalence of cardiovascular diseases	■ Women seem to have a higher risk and incidence of symptomatic supraventricular tachycardia and long QT syndrome compared with men ■ Men show higher risk of atrial fibrillation and sudden cardiac death and they are more affected by atherosclerotic cardiovascular disease compared with women
Coagulation pattern	■ Men have a 3.6-fold higher risk of recurrent VTE than women ■ Women show higher risk of VTE during fertile years
Smoking and drinking habits	■ Smoking habit is higher in men than women ■ Drinking habit is higher in men than women

SEX DIFFERENCES IN IMMUNE SYSTEM

Many studies, both preclinical and clinical, have analyzed the role of the sex in immune response patterns during viral infections. Few studies have proposed that the sex variability in the prevalence, pathogenesis, and response to viral infections can be related to the greater humoral and cell-mediated immune responses of females to viral antigens (18–20). This variability is probably the driver of a lower intensity and prevalence of viral infections in females than males. Indeed, female patients seem to be less susceptible to viral infections due to intense and prolonged innate, humoral, and cell-mediated immune responses. The higher activity of innate immune system in women, which is mediated by Toll-like receptors, retinoic acid-inducible gene I-like receptors, and nucleotide oligomerization domain-like receptors, may lead to a faster and higher recognition of viral components and consequently higher production of type 1 interferon (IFN) and inflammatory cytokines (IL-1, TNFs) (21).

For instance, in the United States, the 1918 influenza pandemic was associated with a higher mortality rate in men than women (22). Male gender could also be associated with higher mortality rate in herpes simplex virus-1 (HSV-1) respiratory infection. Indeed, Brown et al. evaluated the effects of sex on susceptibility to HSV-1 respiratory infection after repeated exhaustive exercise (treadmill running at 36 m/min) in CD-1 mice (86 males and 89 females). The results showed that the exercise stress was associated with increased morbidity and mortality in male mice, while only an increase in morbidity was observed in females. Authors suggested that females might recover to a greater extent and are ultimately better protected from death (23). Similar findings were found by another preclinical study carried out by Han et al. (24), while no sex differences were found for ocular HSV-1 infection (25).

On the other hand, the higher immune responses observed in females may lead to increased development of symptoms of infection in this population (26). For instance, with regard to influenza A viruses, even though men seemed to be more exposed to this infection, women showed higher mortality rates

[2]https://www.epicentro.iss.it/coronavirus/sars-cov-2-differenze-genere-importanza-dati-disaggregati

(27). During 2009 H1N1, a higher hospitalization rate was observed in young women (28). Furthermore, females had a 2-fold higher risk of death than males (29, 30). This could be the consequence of the higher immune response that leads to high levels of pro-inflammatory cytokines, including IL-1, IL-2, IL-6, G-CSF, IP-10, and TNFα, a condition that is defined as a "cytokine storm" and that seems to worsen symptoms of Covid-19 infections, such as ARDS, organ failure, and sepsis (31–33).

The Role of Sex Hormones

Apart from factors merely related to a higher/lower activity of innate, humoral, and cell-mediated immune responses and to the production of inflammatory cytokines, other factors, including sex hormones, may play a key role during response to viral infections (34). In women, the level of estrogen varies during the menstrual cycle and falls with menopause, while, in men, the level of testosterone remains stable up to 60 years of age. Sex hormones induce their effects through the binding with estrogen receptors (ERα and ERβ), the androgen receptor (AR), and progesterone receptors (PR-A and PR-B). Innate immune cells express those receptors to varying degrees (35). Some studies have demonstrated that testosterone exhibits a suppressive effect on the immune function, while estrogen may have both suppressive or not suppressive effects depending on their levels (36–38). Data from studies carried out in humans revealed that in men androgens deficiency is associated with increased levels of inflammatory cytokines and increased CD4+/CD8+ T-cell ratio compared to men with normal level of testosterone (39, 40). On the other hand, estrogens could affect several activities of the innate and adaptive immune responses, showing opposite effects on the immune system based on their level. Indeed, low doses of estrogens seem to induce monocyte differentiation into inflammatory DCs, leading to higher production of IL-4 and IFN-α and activate Th1-type and cell-mediated immune responses. On the contrary, high doses of estrogens show inhibitory activity on innate and pro-inflammatory immune responses and enhance Th2-type responses and humoral immune responses (36, 41). Given the multiple effects of female hormones on immune system functions, women may present different responses to viral infections during the course of their lives. For instance, during pregnancy, which represents a unique immunological state, women seem to undergo three different stages: an initial pro-inflammatory phase, a second one (corresponding to the second trimester of pregnancy), which is characterized by an anti-inflammatory state, and a third phase that is characterized by an increase of inflammatory processes, which are useful for uterine muscle contraction, for the delivery as well as for placenta rejection. The succession of these pro- and anti-inflammatory phases seems to be the results of T helper 1 (Th1)/T helper 2 (Th2) immune shifts that, in turn, could also reflect a change in sensitivity to infectious diseases among pregnant women (42). Indeed, the higher mortality rate of 2009 H1N1 in women was found for those in reproductive age (20–49 years), suggesting a role of gonadal hormones, especially during pregnancy (28). Lastly, sex hormones could also affect the response to antiviral treatments or vaccines. As reported by Klein (26), the efficacy of the HSV-2 vaccine and of the recombinant glycoprotein D (gD)-based HSV-2 vaccine against the development of symptoms associated with genital herpes was found to be higher in women than in men.

SEX DIFFERENCE IN CARDIOVASCULAR DISEASES

Recent literature data showed a higher prevalence of hypertension and coronary artery disease in patients with severe forms of Covid-19 (43, 44), suggesting that preexisting cardiovascular diseases may lead to a worse prognosis. According to Wu et al. (45), an arrhythmogenic effect of Covid-19, with occurrence of long and short QT syndrome, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia, could be expected (46). These life-threatening cardiac disorders can be the consequence of enhanced inflammation, which can increase the duration of ventricular repolarization, by affecting the QTc interval (47). On the other hand, heart injury can be induced by other mechanisms, including some deriving from the effects of ACE2 that is expressed in the lungs and in the cardiovascular system, while other deriving from the cytokine storm and hypoxaemia that results in myocardial cells damage[3]. Indeed, inflammatory cytokines, especially interleukin-6, increase the risk of QT interval prolongation and life-threatening arrhythmias (48). In addition, cytokines show a pro-atherogenic effect, including TNF-α, which activates NF-κB, p38 MAPK, and the transcription of proinflammatory genes for cytokines involved in the cytokine storm (49). Therefore, the role of cytokines in worsening the cardiovascular homeostasis of patients with Covid-19 cannot be excluded.

Some sex differences have been found in the incidence of cardiovascular diseases. Indeed, while women seem to have a higher risk and incidence of symptomatic supraventricular tachycardia and long QT syndrome, men show higher risk of atrial fibrillation and sudden cardiac death. Furthermore, epidemiological studies demonstrated that men are more affected by atherosclerotic cardiovascular disease compared with women. This difference can be imputable to the clinical risk profile, effects of sex hormones, and social attitude (50). Apart from sex differences in the production of inflammatory cytokines and in the incidence of cardiovascular diseases, recent evidence shows that a sex difference in virus-targeted mechanism could be hypothesized. As previously reported, the ACE2 receptor is essential for the cell entry of SARS-CoV-2, but it also represents an important enzyme of the renin-angiotensin system (RAS) that provides protective effects in many chronic conditions, like hypertension, cardiovascular diseases, and acute respiratory distress syndrome. All these clinical conditions represent risk factors for a worse prognosis in Covid-19 patients. Ruggieri and Gagliardi have recently reported that estrogens are able to

[3]https://www.nature.com/articles/s41569-020-0360-5#citeas

induce an upregulation in the expression of ACE2, which could explain better outcome and lower death rate in women compared to men[4]. Furthermore, as recently reported by Gagliardi et al., the gene that encodes for ACE2 is on the X chromosome, and XX cells over-express it (51). Furthermore, the results of a preclinical study that investigated the role of ACE2 in angiotensin (1–7)-induced hypertension and regulation of the RAS system in the kidney of wild type and Ace2 knockout mice revealed some sex differences in rising of mean arterial pressure, binding of glomerular AT1 receptor, and renal protein expression of the neutral endopeptidase neprilysin, suggesting that females may be protected from angiotensin (1–7)-induced hypertension (52).

Even though the ACE2 plays an essential role in the RAS system, it should be highlighted that a recent retrospective cohort study, carried out on 4,480 patients with Covid-19, showed that the prior use of ACE inhibitors or angiotensin receptor blockers (both acting on RAS) was not significantly associated with COVID-19 diagnosis neither with mortality or severe disease (53).

SEX DIFFERENCE IN COAGULATION PATTERN

The DIC is a life-threatening syndrome which leads to disseminated and uncontrolled activation of coagulation, thrombosis, and progressive consumption coagulopathy, leading to an increased bleeding risk. The DIC occurs frequently in almost 30–50% of patients with sepsis and 10% in patients with solid tumors, trauma, or obstetric calamities. Furthermore, the risk of DIC is higher in critically ill patients hospitalized in ICU, for whom the prevalence of DIC is about 8.5–34% (54). According to Tang et al., ∼71.4% of the non-survivor patients with Covid-19 matched the grade of overt-DIC (≥5 points) in later stages of SARS-Cov-2 pneumonia, and 76% of the non-survivors were males. On the contrary, only the 0.6% of survivors matched the DIC criteria during the hospital stay (55). Moreover, the DIC appears to be a driver of disease severity. As might be expected, it is a strong prognostic factor for poor outcome (55). Finally, *microthrombi* have been reported as autopsy findings in patients with Covid-19[5].

It is widely demonstrated that differential risks in men and women for cardiovascular disease exist, especially during premenopausal period due to female sex regulating hormones. Moreover, once reproductive risk factors are taken into account, men have a 3.6-fold higher risk of recurrent venous thromboembolism (VTE) than women (56). The pathophysiology behind this observation is unclear (57). Indeed, it is known that deficient coagulation problems, such as hemophilia, are under genetic control and sex-related, so one cannot exclude that hypercoagulability might be also affected by genetic factors (58). Furthermore, women show higher risk of VTE during fertile years, mainly as consequence of the effects mediated by pregnancy and oral contraceptive use. In this regard, literature data suggested that exogenous estrogen increases the clotting risk in women and in biological males undergoing gender-affirming hormonal therapy (59, 60).

GENDER DIFFERENCES IN SMOKING AND DRINKING HABITS

Compared to non-smokers, smokers generally show higher rates of respiratory diseases, including colds, influenza, bacterial pneumonia, and tuberculosis (61–64). Indeed, smoking habit leads to progressive lung damage, which exposes patients to higher risk of pulmonary bacterial and viral infections (65). This leads to higher risk of hospitalization due to influenza infection as well (66). Lastly, smoking represents the fourth leading cause of death in the world (67). In the context of Covid-19, smokers are more likely to contract the disease since the act of smoking implies that possibly contaminated fingers are in contact with lips, increasing the possibility of the SARS-CoV-2 virus being transmitted from hand to mouth (68, 69). Furthermore, smoking is also related to higher expression of ACE2, which is involved in the process of cell entry of the SARS-CoV-2 (70).

Generally, the percent of smoking habits is found to be higher in men than women, since the adolescent age, even though with differences among low- medium- and high-income countries (71, 72). In order to evaluate the association between smoking and Covid-19 outcomes, in terms of disease severity, need for mechanical ventilation or intensive care unit (ICU), hospitalization, and death, Vardavas et al. carried out a systematic review of studies on Covid-19 patients that included information on patients' smoking status. Authors highlighted that there were higher percentages of current and former smokers among patients who accessed to ICU, required mechanical ventilation, or who had died (73). Other studies are strongly needed to evaluate the prevalence of smokers among patients with severe Covid-19, but based on current knowledge it is possible to assume that smokers are likely to be at higher risk for severe SARS-CoV-2 infection. Therefore, smoking cessation awareness should be strongly encouraged in order to reduce the global impact of COVID-19 (74).

As for smoking habits, drinking is found to be higher in men than women. Indeed, women generally drink less and have a lower prevalence of drink problems than men (75). Alcohol-related liver disease represents one of the main causes of liver cirrhosis, associated with high mortality and morbidity. A recent study, which has analyzed the prevalence, severity and mortality of patients diagnosed with COVID-19 with underlying chronic liver diseases, showed that this disease is associated to higher severity and mortality also in Covid-19 patients[6].

[4]https://www.gendermedjournal.it/articoli.php?archivio=yes&vol_id=3351&id=33219

[5]https://www.preprints.org/manuscript/202002.0407/v2

[6]https://www.mdpi.com/2414-6366/5/2/80/htm

CONCLUSION

Covid-19 still represents a worldwide health emergency. In this paper, we have analyzed possible factors that may have contributed to a gender difference in Covid-19 clinical outcomes, especially in the rate of death. Among possible factors, those related to the activity of immune system and the role of sex hormones seem to be the most important. However, in our opinion, sex differences in cardiovascular diseases and coagulation patterns should be considered as well, especially considering the possible role of the cytokine storm in inducing vascular inflammation and atherosclerosis-related cardiovascular diseases but also gender differences in coagulation, which can be responsible of higher risk of thrombotic/thromboembolic phenomena in men compared to women. Further epidemiological studies will be needed to confirm this. Lastly, considering that women are often underrepresented in randomized clinical trials, future studies are needed to evaluate the effects of sex differences on the prevalence of infections, their outcome, and responses to antiviral treatments.

AUTHOR CONTRIBUTIONS

AC, FR, and GP drafted the work and revised it for important intellectual content, made substantial contributions to the acquisition, analysis, or interpretation of data for the work, approved the final version of the manuscript, developed the concept, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. *Eur Respir J*. (2020) 55:2000607. doi: 10.1183/13993003.00607-2020
2. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. *Methods Mol Biol*. (2015) 1282:1–23. doi: 10.1007/978-1-4939-2438-7_1
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: 271–280.e8. doi: 10.1016/j.cell.2020.02.052
4. Qian Z, Travanty EA, Oko L, Edeen K, Berglund A, Wang J, et al. Innate immune response of human alveolar type II cells infected with severe acute respiratory syndrome-coronavirus. *Am J Respir Cell Mol Biol*. (2013) 48:742–8. doi: 10.1165/rcmb.2012-0339OC
5. Wang J, Nikrad MP, Phang T, Gao B, Alford T, Ito Y, et al. Innate immune response to influenza A virus in differentiated human alveolar type II cells. *Am J Respir Cell Mol Biol*. (2011) 45:582–91. doi: 10.1165/rcmb.2010-0108OC
6. Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. *Mil Med Res*. (2020) 7:11. doi: 10.1186/s40779-020-00240-0
7. *COVID-19 and Haemostasis: A Position Paper From Italian Society on Thrombosis and Haemostasis (SISET)*. Available online at: http://www.sah.org.ar/pdf/covid-19/083-20_pre-publishing.pdf
8. 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
9. Liu R, Han H, Liu F, Lv Z, Wu K, Liu Y, et al. Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. *Clin Chim Acta*. (2020) 505:172–5. doi: 10.1016/j.cca.2020.03.009
10. 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) 8:475–481. doi: 10.1016/S2213-26002030079-5
11. Xie J, Tong Z, Guan X, Du B, Qiu H. Clinical characteristics of patients who died of coronavirus Disease 2019 in China. *JAMA New Open*. (2020) 3:e205619. doi: 10.1001/jamanetworkopen.2020.5619
12. Jin JM, Bai P, He W, Wu F, Liu XF, Han DM, et al. Gender differences in patients with COVID-19: focus on severity and mortality. *Front Public Health*. (2020) 8:152. doi: 10.3389/fpubh.2020.00152
13. Scully EP, Haverfield J, Ursin RL, Tannenbaum C, Klein SL. Considering how biological sex impacts immune responses and COVID-19 outcomes. *Nat Rev Immunol*. (2020) 20:442–7. doi: 10.1038/s41577-020-0348-8
14. Ferrajolo C, Sultana J, Ientile V, Scavone C, Scondotto G, Tari M, et al. Gender Differences in outpatient pediatric drug utilization: a cohort study from Southern Italy. *Front Pharmacol*. (2019) 10:11. doi: 10.3389/fphar.2019.00011
15. Sessa M, Mascolo A, Scavone C, Perone I, Di Giorgio A, Tari M, et al. Comparison of long-term clinical implications of beta-blockade in patients with obstructive airway diseases exposed to beta-blockers with different β1-adrenoreceptor selectivity: an Italian Population-Based Cohort Study. *Front Pharmacol*. (2018) 9:1212. doi: 10.3389/fphar.2018.01212
16. Scavone C, Di Mauro C, Ruggiero R, Bernardi FF, Trama U, Aiezza ML, et al. Severe cutaneous adverse drug reactions associated with allopurinol: an analysis of spontaneous reporting system in Southern Italy. *Drugs Real World Outcomes*. (2020) 7:41–51. doi: 10.1007/s40801-019-00174-7
17. Corrao S, Santalucia P, Argano C, Djade CD, Barone E, Tettamanti M, et al. Gender-differences in disease distribution and outcome in hospitalized elderly: data from the REPOSI study. *Eur J Intern Med*. (2014) 25:617–23. doi: 10.1016/j.ejim.2014.06.027
18. Klein SL, Jedlicka A, Pekosz A. The Xs and Y of immune responses to viral vaccines. *Lancet Infect Dis*. (2010) 10:338–49. doi: 10.1016/S1473-3099(10)70049-9
19. Khandaker G, Dierig A, Rashid H, King C, Heron L, Booy R. Systematic review of clinical and epidemiological features of the pandemic influenza A (H1N1) 2009. *Influenza Other Respir Viruses*. (2011) 5:148–56. doi: 10.1111/j.1750-2659.2011.00199.x
20. Puchhammer-Stockl E, Aberle SW, Heinzl H. Association of age and gender with alphaherpesvirus infections of the central nervous system in the immunocompetent host. *J Clin Virol*. (2012) 53:356–9. doi: 10.1016/j.jcv.2011.12.015
21. Ruggieri A, Gagliardi MC, Anticoli S. Sex-dependent outcome of hepatitis B and C viruses infections: synergy of sex hormones and immune responses? *Front Immunol*. (2018) 9:2302. doi: 10.3389/fimmu.2018.02302
22. Noymer A. The 1918 influenza pandemic affected sex differentials in mortality: Comment on Sawchuk. *Am J Phys Anthropol*. (2010). 143:499–500. doi: 10.1002/ajpa.21405
23. Brown AS, Davis JM, Murphy EA, Carmichael MD, Ghaffar A, Mayer EP. Gender differences in viral infection after repeated exercise stress. *Med Sci Sports Exerc*. (2004) 36:1290–5. doi: 10.1249/01.MSS.0000135798.72735.B3
24. Han X, Lundberg P, Tanamachi B, Openshaw H, Longmate J, Cantin E. Gender influences herpes simplex virus type 1 infection in normal and gamma interferon-mutant mice. *J Virol*. (2001) 75:3048–52. doi: 10.1128/JVI.75.6.3048-3052.2001
25. Riccio RE, Park SJ, Longnecker R, Kopp SJ. Characterization of sex differences in ocular herpes simplex virus 1 infection and herpes stromal keratitis pathogenesis of wild-type and herpesvirus entry mediator knockout mice. *mSphere*. (2019) 4:e00073-19. doi: 10.1128/mSphere.00073-19

26. Klein SL. Sex influences immune responses to viruses, and efficacy of prophylaxis and treatments for viral diseases. *Bioessays*. (2012) 34:1050–9. doi: 10.1002/bies.201200099
27. Klein SL, Pekosz A, Passaretti C, Anker M, Olukoya P. *Sex, Gender and Influenza*. Geneva: World Health Organization (2010). p. 1–58.
28. Klein SL, Passaretti C, Anker M, Olukoya P, Pekosz A. The impact of sex, gender and pregnancy on 2009 H1N1 disease. *Biol Sex Differ*. (2010) 1:5. doi: 10.1186/2042-6410-1-5
29. Randolph AG, Vaughn F, Sullivan R, Rubinson L, Thompson BT, Yoon G, et al. Critically ill children during the 2009–2010 influenza pandemic in the United States. *Pediatrics*. (2011) 128:e1450–8. doi: 10.1542/peds.2011-0774d
30. Zarychanski R, Stuart TL, Kumar A, Doucette S, Elliott L, Kettner J, et al. Correlates of severe disease in patients with 2009 pandemic influenza (H1N1) virus infection. *CMAJ*. (2010) 182:257–64. doi: 10.1503/cmaj.091884
31. Capuano A, Scavone C, Racagni G, Scaglione F, Italian Society of Pharmacology. NSAIDs in patients with viral infections, including Covid-19: victims or perpetrators? *Pharmacol Res*. (2020) 157:104849. doi: 10.1016/j.phrs.2020.104849
32. Scavone C, Brusco S, Bertini M, et al. Current pharmacological treatments for COVID-19: what's next? *Br J Pharmacol*. (2020) 1–12. doi: 10.1111/bph.15072
33. di Mauro G, Scavone C, Rafaniello C, Rossi F, Capuano A. SARS-Cov-2 infection: response of human immune system and possible implications for the rapid test and treatment. *Int Immunopharmacol*. (2020) 84:106519. doi: 10.1016/j.intimp.2020.106519
34. Roved J, Westerdahl H, Hasselquist D. Sex differences in immune responses: hormonal effects, antagonistic selection, and evolutionary consequences. *Horm Behav*. (2017) 88:95–105. doi: 10.1016/j.yhbeh.2016.11.017
35. Kadel S, Kovats S. Sex Hormones regulate innate immune cells and promote sex differences in respiratory virus infection. *Front Immunol*. (2018) 9:1653. doi: 10.3389/fimmu.2018.01653
36. Khan D, Ansar Ahmed S. The immune system is a natural target for estrogen action: opposing effects of estrogen in two prototypical autoimmune diseases. *Front Immunol*. (2016) 6:635. 10.3389/fimmu.2015.00635
37. Foo YZ, Nakagawa S, Rhodes G, Simmons LW. The effects of sex hormones on immune function: a meta-analysis. *Biol Rev Camb Philos Soc*. (2017) 92:551–71. doi: 10.1111/brv.12243
38. Trigunaite A, Dimo J, Jørgensen TN. Suppressive effects of androgens on the immune system. *Cell Immunol*. (2015) 294:87–94. doi: 10.1016/j.cellimm.2015.02.004
39. Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, Jones TH. The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. *J Clin Endocrinol Metab*. (2004) 89:3313–8. doi: 10.1210/jc.2003-031069
40. Bobjer J, Katrinaki M, Tsatsanis C, Lundberg Giwercman Y, Giwercman A. Negative association between testosterone concentration and inflammatory markers in young men: a nested cross-sectional study. *PLoS ONE*. (2013) 8:e61466. doi: 10.1371/journal.pone.0061466
41. Seillet C, Laffont S, Trémollières F, Rouquié N, Ribot C, Arnal JF, et al. The TLR-mediated response of plasmacytoid dendritic cells is positively regulated by estradiol *in vivo* through cell-intrinsic estrogen receptor α signaling. *Blood*. (2012) 119:454–64. doi: 10.1182/blood-2011-08-371831
42. Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. *Am J Reprod Immunol*. (2010) 63:425–33. doi: 10.1111/j.1600-0897.2010.00836.x
43. Guan WJ, Ni ZY, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. (2020) 382:1708–20. doi: 10.1056/NEJMoa2002032
44. 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
45. Wu CI, Postema PG, Arbelo E, Behr ER, Bezzina CR, Napolitano C, et al. SARS-CoV-2, COVID-19, and inherited arrhythmia syndromes. *Heart Rhythm*. (2020) S1547-5271(20)30285-X. doi: 10.1016/j.hrthm.2020.03.024
46. 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) NEJMc2010418. doi: 10.1056/NEJMc2010418. [Epub ahead of print].
47. Lazzerini PE, Boutjdir M, Capecchi PL. COVID-19, arrhythmic risk and inflammation: mind the gap!. *Circulation*. (2020). doi: 10.1161/CIRCULATIONAHA.120.047293
48. Aromolaran AS, Srivastava U, Alí 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
49. Welsh P, Grassia G, Botha S, Sattar N, Maffia P. Targeting inflammation to reduce cardiovascular disease risk: a realistic clinical prospect? *Br J Pharmacol*. (2017) 174:3898–913. doi: 10.1111/bph.13818
50. Tian J, Wang X, Tian J, Yu B. Gender differences in plaque characteristics of nonculprit lesions in patients with coronary artery disease. *BMC Cardiovasc Disord*. (2019) 19:45. doi: 10.1186/s12872-019-1023-5
51. Gagliardi MC, Tieri P, Ortona E, Ruggieri A. ACE2 expression and sex disparity in COVID-19. *Cell Death Discov*. (2020) 6:37. doi: 10.1038/s41420-020-0276-1
52. Ji H, de Souza AMA, Bajaj B, Zheng W, Wu X, Speth RC, et al. Sex-specific modulation of blood pressure and the renin-angiotensin system by ACE (Angiotensin-Converting Enzyme) 2. *Hypertension*. (2020). doi: 10.1161/HYPERTENSIONAHA.120.15276
53. Fosbøl EL, Butt JH, Østergaard 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) e2011301. doi: 10.1001/jama.2020.11301
54. Papageorgiou C, Jourdi G, Adjambri E, Walborn A, Patel P, Fareed J, et al. Disseminated intravascular coagulation: an update on pathogenesis, diagnosis, and therapeutic strategies. *Clin Appl Thromb Hemost*. (2018) 24(9 Suppl.):1076029618806424. doi: 10.1177/1076029618806424
55. 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
56. Tagalakis V. Sex may matter when it comes to the presenting location of deep vein thrombosis. *Thromb Res*. (2019) 173:164–5. doi: 10.1016/j.thromres.2018.12.001
57. Roach RE, 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*. (2015) 12:1593–600. doi: 10.1111/jth.12678
58. Bushnell CD. Stroke and the female brain. *Nat Clin Pract Neurol*. (2008) 4:22–33. doi: 10.1038/ncpneuro0686
59. Bischof E, Wolfe J, Klein SL. Clinical trials for COVID-19 should include sex as a variable. *J Clin Invest*. (2020) 130:3350–3352. doi: 10.1172/JCI139306
60. Getahun D, Nash R, Flanders WD, Baird TC, Becerra-Culqui TA, Cromwell L, et al. Cross-sex hormones and acute cardiovascular events in transgender persons: a cohort study. *Ann Intern Med*. (2018) 169:205–213. doi: 10.7326/M17-2785
61. Atto B, Eapen MS, Sharma P, Frey U, Ammit AJ, Markos J, et al. New therapeutic targets for the prevention of infectious acute exacerbations of COPD: role of epithelial adhesion molecules and inflammatory pathways. *Clin Sci*. (2019) 133:1663–703. doi: 10.1042/CS20181009
62. Eapen MS, Sharma P, Moodley YP, Hansbro PM, Sohal SS. Dysfunctional immunity and microbial adhesion molecules in smoking-induced pneumonia. *Am J Respir Crit Care Med*. (2019) 199:250–1. doi: 10.1164/rccm.201808-1553LE
63. Eapen MS, Sharma P, Sohal SS. Mitochondrial dysfunction in macrophages: a key to defective bacterial phagocytosis in COPD. *Eur Respir J*. (2019) 54:1901641. doi: 10.1183/13993003.01641-2019
64. Eapen MS, Sohal SS. Understanding novel mechanisms of microbial pathogenesis in chronic lung disease: implications for new therapeutic targets. *Clin Sci*. (2018) 132:375–9. doi: 10.1042/CS20171261
65. Lawrence H, Hunter A, Murray R, Lim WS, McKeever T. Cigarette smoking and the occurrence of influenza—Systematic review. *J Infect*. (2019) 79:401–6. doi: 10.1016/j.jinf.2019.08.014
66. Han L, Ran J, Mak YW, Suen LK, Lee PH, Peiris JSM, et al. Smoking and influenza-associated morbidity and mortality: a systematic review and meta-analysis. *Epidemiology*. (2019) 30:405–17. doi: 10.1097/EDE.0000000000000984

67. *World Health Organisation Chronic Obstructive Pulmonary Disease (COPD)*. (2020). Available online at: https://www.who.int/respiratory/copd/en/ (accessed March, 11 2020)
68. Istituto Superiore di Sanità. *Tobacco Smoking in the Age of COVID-19*. Available online at: https://www.epicentro.iss.it/en/coronavirus/sars-cov-2-addictions-smoking
69. Cai H. Sex difference and smoking predisposition in patients with COVID-19. *Lancet Respir Med*. (2020) 8:e20. doi: 10.1016/S2213-260020 30117-X
70. Li J, Zhang Y, Wang F, Liu B, Li H, Tang G, et al. Sex differences in clinical findings among patients with coronavirus disease 2019 (COVID-19) and severe condition. *medRxiv*. (2020) 02.27.20027524. doi: 10.1101/2020.02.27.200 27524
71. Zeman MV, Hiraki L, Sellers EM. Gender differences in tobacco smoking: higher relative exposure to smoke than nicotine in women. *J Womens Health Gend Based Med*. (2002) 11:147–53. doi: 10.1089/152460902753 645281
72. Han J, Chen X. A Meta-analysis of cigarette smoking prevalence among adolescents in China: 1981-2010. *Int J Environ Res Public Health*. (2015) 12:4617–30. doi: 10.3390/ijerph120504617
73. Vardavas CI, Nikitara K. COVID-19 and smoking: a systematic review of the evidence. *Tob Induc Dis*. (2020) 18:20. doi: 10.18332/tid/119324
74. Komiyama M, Hasegawa K. Smoking cessation as a public health measure to limit the coronavirus disease 2019 pandemic. *Eur Cardiol*. (2020) 15:e16. doi: 10.15420/ecr.2020.11
75. Ely M, Hardy R, Longford NT, Wadsworth ME. Gender differences in the relationship between alcohol consumption and drink problems are largely accounted for by body water. *Alcohol Alcohol*. (1999) 34:894–902. doi: 10.1093/alcalc/34.6.894

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SYSTEMATIC REVIEW
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Incidence of Venous Thromboembolism in Hospitalized Coronavirus Disease 2019 Patients: A Systematic Review and Meta-Analysis

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Background: Emerging evidence shows that coronavirus disease 2019 (COVID-19) is commonly complicated by coagulopathy, and venous thromboembolism (VTE) is considered to be a potential cause of unexplained death. Information on the incidence of VTE in COVID-19 patients, however, remains unclear.

Method: English-language databases (PubMed, Embase, Cochrane), Chinese-language databases (CNKI, VIP, WANFANG), and preprint platforms were searched to identify studies with data of VTE occurrence in hospitalized COVID-19 patients. Pooled incidence and relative risks (RRs) of VTE were estimated by a random-effects model. Variations were examined based on clinical manifestations of VTE (pulmonary embolism-PE and deep vein thrombosis-DVT), disease severity (severe patients and non-severe patients), and rate of pharmacologic thromboprophylaxis (≥60 and <60%). Sensitivity analyses were conducted to strengthen the robustness of results. Meta-regression was performed to explore the risk factors associated with VTE in COVID-19 patients.

Results: A total of 17 studies involving 1,913 hospitalized COVID-19 patients were included. The pooled incidence of VTE was 25% (95% CI, 19–31%; I^2, 95.7%), with a significant difference between the incidence of PE (19%; 95% CI, 13–25%; I^2, 93.2%) and DVT (7%; 95% CI, 4–10%; I^2, 88.3%; $P_{interaction} < 0.001$). Higher incidence was observed in severe COVID-19 patients (35%; 95 CI%, 25–44%; I^2, 92.4%) than that in non-severe patients (6%; 95 CI%, 3–10%; I^2, 62.2%; $P_{interaction} < 0.001$). The high rate of pharmacologic thromboprophylaxis in COVID-19 patients (≥60%) was associated with a lower incidence of VTE compared with the low pharmacologic thromboprophylaxis rate (<60%) (19 vs. 40%; $P_{interaction} = 0.052$). Severe patients had a 3.76-fold increased risk of VTE compared with non-severe patients (RR, 4.76; 95% CI, 2.66–8.50; I^2, 47.0%). Sensitivity analyses confirmed the robustness of the primacy results.

Conclusions: This meta-analysis revealed that the estimated VTE incidence was 25% in hospitalized COVID-19 patients. Higher incidence of VTE was observed in COVID-19 patients with a severe condition or with a low rate of pharmacologic thromboprophylaxis. Assessment of VTE risk is strongly recommended in COVID-19 patients, and effective measures of thromboprophylaxis should be taken in a timely manner for patients with high risk of VTE.

Keywords: COVID-19, venous thromboembolism, pulmonary embolism, incidence, thromboprophylaxis, anticoagulation

INTRODUCTION

Coronavirus disease 2019 (COVID-19) has spread globally, resulting in an unprecedented health crisis. As of 5 July 2020, there has been 11,125,245 cases of COVID-19 worldwide, of which 528,204 patients have died (1). Remarkably, emerging evidence shows that COVID-19 is commonly complicated by coagulopathy, and venous thromboembolism (VTE) is considered to be a potential cause of unexplained death, especially in severe COVID-19 patients (2, 3). A variety of potential risk factors of VTE exist among COVID-19 patients, including virus infection, respiratory failure, mechanical ventilation, and the use of a central venous catheter (4). The occurrence of VTE in COVID-19 patients, which includes pulmonary embolism (PE) and deep vein thrombosis (DVT), has been reported in several studies (5, 6). Thrombotic complications have been found in 31% of Intensive Care Unit (ICU) patients with COVID-19 in a Dutch teaching hospital (5), while 23% of PE incidence has been reported in a French hospital (7). At present, the incidence of VTE in this viral infection remains uncertain, however, understanding the precise incidence of VTE in COVID-19 patients is critically important for decision making on thromboprophylaxis. Accordingly, the present study summarizes all available evidence for a comprehensive and rigorous systematic review focused on VTE incidence in hospitalized COVID-19 patients, thus providing a panoramic view of this issue.

METHODS

This study was performed according to the standards of the Cochrane Handbook and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (8). All supporting data is available within the article and the **Supplementary File**.

Data Sources and Searches

The databases of PubMed, Embase, Cochrane Library databases, as well as the Chinese databases of the China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (VIP), and the WANFANG databases were electronically searched from inception to 8 May 2020, using search terms related to COVID-19. Full details of the search terms used are presented in **Table S1**. Preprint articles were retrieved from MedRxiv (https://www.medrxiv.org), BioRxiv (https://www.biorxiv.org), and SSRN (https://www.ssrn.com). The references of identified records were also screened manually to find any further relevant articles.

Study Selection and Outcomes

To be included, studies had to meet the following entry criteria: (1) included SARS-CoV-2 infected and hospitalized adult patients; (2) reported the data of VTE, PE, or DVT confirmed by computed tomography pulmonary angiography (CTPA) and/or ultrasonography. Two authors (CZ and LS) independently reviewed titles and abstracts of all studies, and assessed full texts of retrieved studies, with any discrepancies being resolved *via* consultation with a third author (ZG). The primary outcomes of this study were the incidence of VTE in hospitalized COVID-19 patients and corresponding relative risk in comparison between severe and non-severe patients. COVID-19 disease severity was defined according to the Clinical Management of COVID-19 (Interim guidance 27 May 2020) released by the World Health Organization (WHO). Criteria for severe cases included any of the following: (1) Respiratory rate >30 per min; (2) blood oxygen saturation (SPO2) < 93% at rest; (3) partial pressure of arterial oxygen to fraction of inspired oxygen ratio <300; or (4) more than 50% of lung infiltrates within 24–48 h. Patients needing mechanical respiratory support or presenting with septic shock or multiple organ dysfunction or failure constituted critical cases.

Data Extraction and Quality Assessment

All data from eligible studies were abstracted using *a priori* designed form, which included study characteristics (study name; country and period; population and number), clinical characteristics (mean age; gender ratio; previous VTE; the comorbidities of hypertension, diabetes, and cancer; and pharmacologic thromboprophylaxis rate), and data on VTE (occurrence number and total number of COVID-19 patients). The pharmacologic thromboprophylaxis rate was calculated as follows: the number of COVID-19 patients who received prophylactic anticoagulants (e.g., low molecular weight heparin [LMWH] or unfractionated heparin intravenously [UFH])/ total number of COVID-19 patients in the study. A rate of ≥60% was considered as a high proportion of pharmacologic thromboprophylaxis. The methodological quality of included studies was assessed according to the Newcastle-Ottawa Scale (NOS) (9). The NOS was modified according to our study design with a total of eight scores and the following six dimensions: (1)

representative of the cases; (2) ascertainment of the exposure; (3) ascertainment of the outcome; (4) ascertainment of the outcome for quality control; (5) control for factors of age and gender; and (6) control for factors related to VTE. A study could receive a maximum of one point for the first four dimensions and a maximum of two points for the last two dimensions. Total scores with ≥ 5 points represented a relatively good quality.

Data Synthesis and Statistical Analysis

All statistical analyses were performed using Stata version 13.0 (Statacorp, College Station, Texas, USA). The pooled incidence of VTE in hospitalized COVID-19 patients and associated 95% confidence intervals (95%CI) was calculated using a random-effects model, and relative risks (RRs) of VTE occurrence comparing severe with non-severe patients was also calculated. Heterogeneity among studies was assessed using the Cochran *Q*-test and I^2 index, with $I^2 > 50\%$ indicating considerable heterogeneity (10). Subgroup analysis was conducted by different manifestations of VTE (PE and DVT), severity of illness (severe patients and non-severe patients), and the pharmacologic thromboprophylaxis rate of patients (<60 and ≥60%). The interaction analysis (*P* for interaction) was applied to evaluate the risk difference of different subgroups. To strengthen the robustness of the results, leave-1-out sensitivity analyses were performed to explore whether a single study had an excessive influence on VTE incidence. Meta-regression was conducted to explore the potential risks associated with VTE. Funnel plots and Begg's test and Egger's test were carried out to qualitatively and quantitatively evaluate the presence of publication bias when more than 10 studies were available in a single analysis (11).

RESULTS

Study Selection and Study Characteristics

The process of study selection is outlined in **Figure 1**. A total of 4,449 records were identified through database searching, with 181 being from English-language databases, 31 from Chinese-language databases, and 4,237 from preprint platforms. Through reviewing the titles and abstracts, 28 duplicates were removed, and 4,272 records were excluded. The remaining 149 full-text articles were reviewed and 132 articles were excluded for the following reasons: irrelevant articles ($n = 27$), articles not reporting outcome of VTE ($n = 82$), case report or meta-analyses ($n = 16$), and repetition with another database ($n = 7$). Eventually, 17 retrospective studies (1, 5–8, 12–23) involving 1,913 hospitalized COVID-19 patients were included, 13 being from English-language databases, one from a Chinese-language database, and three from preprint platforms. Among them, six studies reported on patients in China, while 11 studies reported on patients in Europe, including the Netherlands, France, and Italy. The sample size of the involved studies varied from 16 to 420 patients. The detailed characteristics of included studies are presented in **Table 1**. Information of VTE and potential risk factors are summarized in **Table S2**. Of 17 studies, nine involved patients who had clinical suspicion of VTE and six included patients who were screened by CT or ultrasound. One study involved a population with both clinical suspicion and screening and one did not report the related information. Among 17 studies, 10 reported on patients who were prophylactically treated with anticoagulant therapy (**Table S3**), of which, five studies reported the dosage of LMWH (2,850 IU once to 6,000 IU twice). A high pharmacologic thromboprophylaxis rate was reported in seven studies, ranging from 66.7 to 100%.

Study Quality

All included studies satisfied the following risk bias items: representative of the cases; ascertainment of the exposure; ascertainment of the outcome; and ascertainment of the outcome for quality control. In total, 13 studies (82.3%) presented both age and gender ratio of the included patients, while seven studies (41.2%) reported more than three clinical characteristics (two points) and eight studies (47.1%) reported one or two clinical characteristics (one point). Accordingly, all 17 included studies were considered as being of relatively good quality (**Table S4**).

Incidence of VTE

Figure 2 provides the full view of VTE incidence in hospitalized COVID-19 patients. The overall pooled incidence of VTE was 25% (95% CI, 19–31%; I^2, 95.7%) (**Figure S1**). The incidence of PE and DVT was significantly different ($P_{interaction} < 0.001$), with the event rate being 19% (95% CI, 13–25%; I^2, 93.2%; **Figure S2**) and 7% (95% CI, 4–10%; I^2, 88.3%; **Figure S3**), respectively. Considering the disease severity of COVID-19, a higher incidence was observed in severe patients (35%; 95 CI%, 25–44%; I^2, 92.4%; **Figure S4**) than that in non-severe patients (6%; 95 CI%, 3–10%; I^2, 62.2%; **Figure S5**; $P_{interaction} < 0.001$). Because anticoagulation for thromboprophylaxis could decrease the occurrence of VTE, the high pharmacologic thromboprophylaxis rate of above 60% was associated with a lower incidence of VTE (19%; 95 CI%, 10–28%; I^2, 92.8%; **Figure S6**) when compared to the low pharmacologic thromboprophylaxis rate of below 60% (40%; 95 CI%, 20–60%; I^2, 89.7%; **Figure S6**; $P_{interaction} = 0.052$). Sensitivity analysis, by removing a single study at a time, confirmed the robustness of primacy results (**Table S5**).

Comparison of VTE Risk With Severe vs. Non-severe Patients

A total of 99 VTE events were found in 327 severe patients with the event rate of 30.3%. Comparatively, 34 VTE events were observed in 904 non-severe patients with a low event rate of 3.8%. Accordingly, severe patients were at a higher risk of VTE compared to non-severe patients (RR, 4.76; 95% CI, 2.66–8.50; I^2, 47.0%) (**Figure 3**). Leave-1-out sensitivity analysis was consistent with the primacy result (**Table S6**).

Risk Factors Associated With VTE

Meta-regression was conducted to assess the potential risk factors associated with VTE incidence. Seven variables (mean age, gender ratio, previous VTE, the comorbidities of hypertension, diabetes, cancer, and pharmacologic thromboprophylaxis rate)

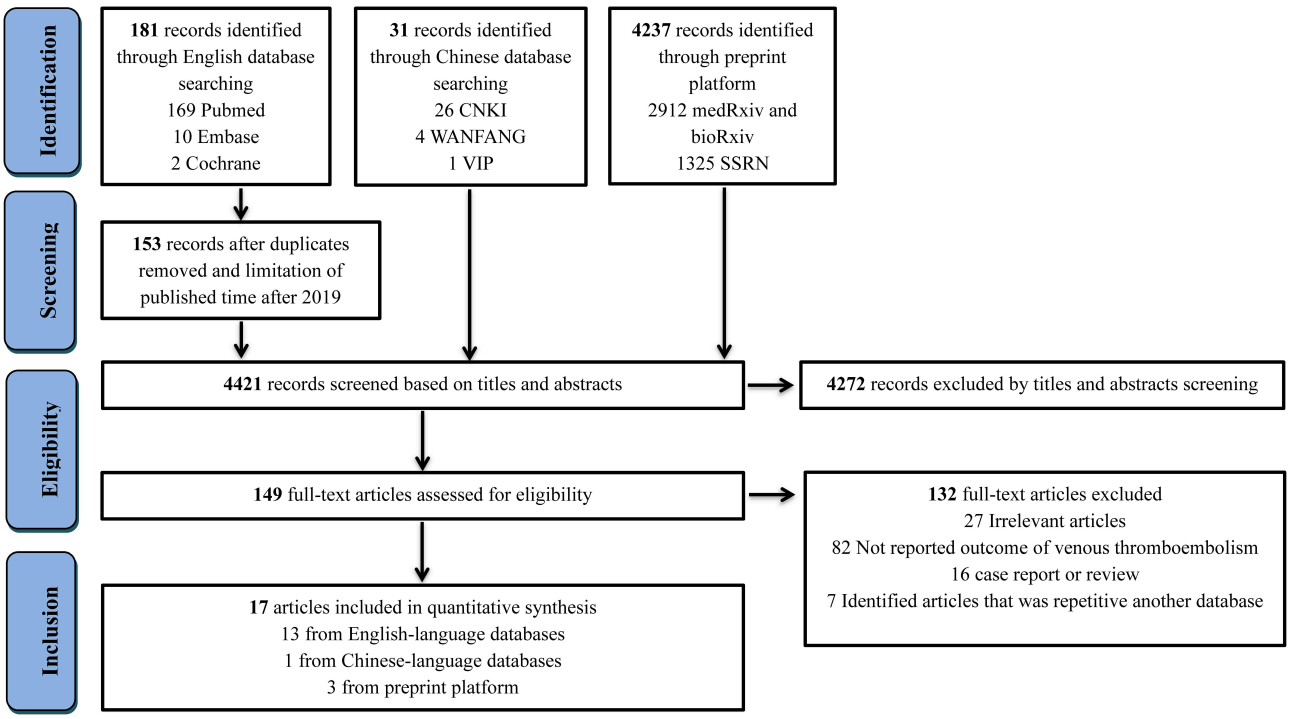

FIGURE 1 | Flow diagram for the selection of eligible studies. CNKI, China National Knowledge Infrastructure; VIP, China Science and Technology Journal Database.

were evaluated, and none of them were detected to be related to the incidence of VTE (**Table S7**).

Publication Bias

The funnel plots for VTE incidence as well as PE and DVT incidence were all asymmetrical on visual inspection. The corresponding *P*-values for the Egger's test were <0.001, <0.001, and <0.001, and the corresponding *P*-values for the Begg's test were 0.26, 0.009, and 0.003, respectively (**Figure S7**). Because of limited study numbers in comparison to severe and non-severe patients (seven studies), a funnel plot was not performed.

DISCUSSION

The true incidence of VTE in patients with COVID-19 remains uncertain. This is the first systematic review and meta-analysis to provide a comprehensive overview of VTE occurrence based on 17 retrospective studies involving 1,913 hospitalized COVID-19 patients. The overall VTE incidence was 25%, with the event rate of PE and DVT being 19 and 7%, respectively. Considering disease severity, a higher incidence was observed in severe patients (35%) than in non-severe patients (6%). Moreover, a high pharmacologic thromboprophylaxis rate of above 60% was associated with a lower incidence of VTE (19%) compared to a low pharmacologic thromboprophylaxis rate of below 60% (40%). Severe patients had a 3.76-fold increased risk of VTE compared to non-severe patients. The prevalence of VTE in COVID-19 patients seemed to be high, especially for severe patients. Therefore, it is important to improve the awareness of thromboprophylaxis for COVID-19 infection.

It was reported that a high proportion of severe and critically ill COVID-19 patients showed major coagulation disorders (24, 25). In our meta-analysis, a higher incidence of VTE was also found in severe patients (35%) than in non-severe patients (6%), with a risk ratio of 4.76. The results were similar to recent preliminary studies on COVID-19, in which the event rate of VTE for ICU patients was 2.18–4.42 folds than that of general ward patients (7, 20). In fact, the prevalence of VTE appeared to be higher in ICU patients than in patients in other disease conditions, with the mean rate of VTE diagnosis being 12.7% (26). The higher risk of VTE in ICU patients mainly resulted from both individual patient related risk factors (e.g., age, history of VTE, cancer) and ICU-specific risk factors (e.g., sedation, immobilization, central venous catheters) (27), therefore, pharmacological VTE prophylaxis is strongly recommended to critically ill patients by clinical guidelines (28). It is speculated that COVID-19 is probably an additional risk factor for VTE in hospitalized patients (29). As for severe or critically ill patients with COVID-19, the release of large amounts of inflammatory mediators and the application of hormones and immunoglobulin might exacerbate the blood hypercoagulability (23, 30). Rapid deterioration in oxygen saturation and increased dead space ventilation could also be factors of VTE events (31). Moreover, severe COVID-19 patients could present with a high fever, dehydration, as well as immobilization (32), which might also lead to VTE (33, 34). Therefore, underestimated prevalence

TABLE 1 | Characteristics of the included studies.

Study	Country/period	Population/number	Mean age (*y*)	Male (%)	Previous VTE (%)	Hypertension (%)	Diabetes (%)	Cancer (%)	Pharmacologic thromboprophylaxis rate (%)
Beun et al. (12)	Netherlands/NR	ICU/75	60.5	50	NR	NR	NR	NR	NR
Bi et al. (8)	China/2020.1.11–2020.3.10	Mild-moderate and severe-critical/420	NR	47.6	NR	11.7	5.7	0.2	NR
Chen et al. (1)	China/2020.1.1–2020.2.29	Mild-moderate and severe-critical/25	65	60	4	40	20	NR	80
Cui et al. (13)	China/2020.1.20–2020.3.22	ICU/81	59.9	46	NR	25	10	NR	0
Ding et al. (14)	China/2020.1.1–2020.2.3	NR/56	54.6	53.57	NR	NR	NR	NR	NR
Grillet et al. (7)	France/2020.3.15–2020.4.14	Non-ICU and ICU/100	66	70	NR	NR	20	20	NR
Helms et al. (15)	France/2020.3.3–2020.3.31	ICU/150	63	81.3	5.3	NR	NR	NR	66.7
Klok et al. (5)	Netherlands/NR-2020.4.5	ICU/184	64	76	NR	NR	NR	2.7	100
Li et al. (18)	China/2020.1.1–2020.2.13	Suspected PE/24	63	63.6	0	63.6	NR	NR	NR
Llitjos et al. (19)	France/2020.3.19–2020.4.11	ICU/26	68	77	4	85	NR	NR	31
Lodigiani et al. (20)	Italy/2020.2.13–2020.4.10	Non-ICU and ICU/362	66	68	0	47.2	22.7	6.4	Overall: 81.2%; ICU: 100%; Non-ICU: 78.3%
Leonard-Lorant et al. (17)	France/2020.3.1–2020.3.31	Non-ICU and ICU/106	63.3	66	NR	NR	NR	NR	39.6
Middeldorp et al. (21)	Netherlands/NR-2020.4.12	Non-ICU and ICU/198	61	66	5.6	NR	NR	3.5	100
Poissy et al. (6)	France/2020.2.27–2020.3.31	ICU/107	NR	NR	0.93	NR	NR	NR	NR
Ranucci et al. (22)	Italy/2020.3.8-NR	ICU/16	61	93.75	0	NR	NR	NR	100
Tavazzi et al. (16)	Italy/2020.2.21-NR	ICU/54	68	83	NR	NR	NR	NR	100
Xing et al. (23)	China/NR	Moderate and severe-critical/20	NR	60	NR	NR	NR	NR	NR

NR, not reported; ICU, intensive care unit; VTE, venous thromboembolism; PE, pulmonary embolism.

of VTE and inadequate thromboprophylaxis might exist among critically ill COVID-19 patients (35). It was reported that even on standard doses of thromboprophylaxis, the incidence of thrombotic complications was still as high as 31% for ICU patients with COVID-19 infection (5). Accordingly, routinely screening for VTE by CTPA or ultrasound, as well as the use of full-dose anticoagulation, are now recommended for critically ill COVID-19 patients by some experts.

To increase the awareness of thrombotic complications, the assessment of VTE risk should be strongly recommended, for the sake of taking timely and effective preventive measures for patients at high risk of VTE. It is recognized that prevention of VTE is required in all severe or critically ill patients in absence of anticoagulation contraindication (30, 36). For mild or moderate patients with COVID-19, determination of VTE risk might be exerted using the PADUA risk assessment model for medical patients and the CAPRINI prediction score for surgical patients, as there are currently no new VTE risk assessment models that are specialized for COVID-19 patients (30). Therefore, measures of thromboprophylaxis could be taken without delay in patients with high or moderate risk of VTE. Importantly, dynamic and repeated assessment for thrombotic risk should also be conducted in the course of treatment, including routine coagulation tests, concomitant medications, and invasive procedures, to adjust the antithrombotic regimen in a timely manner. Furthermore, the regular evaluation of bleeding risk should not be neglected in COVID-19 patients, and should be carefully balanced against the risk of thrombosis.

Anticoagulants are definitely the cornerstone for VTE prevention. Therefore, COVID-19 patients with high VTE risk

Variables	No. of studies	No. of Patients	No. of cases	Incidence (95%CI)	Incidence (95%CI)	I^2 (%)	*P interaction*
Overall	17	1913	299	0.25 (0.19-0.31)		95.7	
Manifestations							<0.001
Pulmonary embolism	14	1913	200	0.19 (0.13-0.25)		93.2	
Deep vein thrombosis	12	1913	99	0.07 (0.04-0.10)		88.3	
Disease severity							<0.001
Severe patients	15	929	253	0.35 (0.25-0.44)		92.4	
Non-severe patients	7	904	34	0.06 (0.03-0.10)		62.2	
Pharmacologic thromboprophylaxis rate							0.052
<60%	3	213	70	0.40 (0.20-0.60)		89.7	
≥60%	7	938	131	0.19 (0.10-0.28)		92.8	

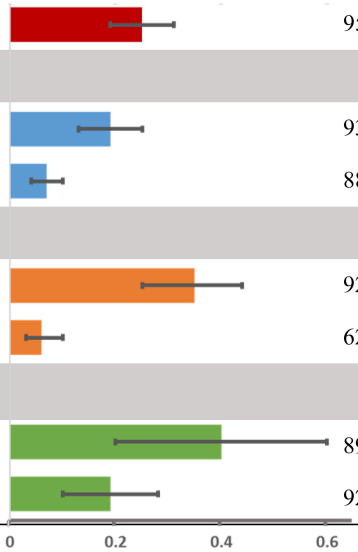

FIGURE 2 | Incidence of venous thromboembolism. No., number; 95% CI, 95% confidence interval.

Study	RR (95% CI)	RR (95% CI)	Severe, events (n)	N-severe, events (n)	Weight, %
Bi QF, et al		45.36 (2.58-797.88)	6/93	0/327	3.65
Chen JP, et al		3.19 (1.24-8.21)	6/8	4/17	17.75
Grillet F, et al		4.43 (1.91-10.26)	17/39	6/61	19.72
Lodigiani C, et al		2.18 (0.73-6.49)	4/48	12/314	15.37
Lorant IL, et al		3.62 (1.80-7.32)	24/48	8/58	22.5
Middeldrop S, et al		14.35 (5.31-38.77)	35/75	4/123	16.92
Xing CY, et al		4.41 (0.30-64.57)	7/16	0/4	4.1
Overall (I-squared=47.0%, *p*=0.079)		4.76 (2.66-8.5)	99/327	34/904	100

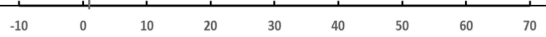

FIGURE 3 | Venous thromboembolism risk of severe patients vs. non-severe patients. n, number; RR, relative risk; 95% CI, 95% confidence interval.

should receive pharmacologic thromboprophylaxis, unless there are absolute contraindications (37, 38). As found in this study, the high pharmacologic thromboprophylaxis rate of above 60% was associated with a lower incidence of VTE (19 vs. 40%) compared with the low pharmacologic thromboprophylaxis rate of below 60%. A recent study involving 449 severe COVID-19 patients revealed that LMWH users appeared to be associated with better prognosis compared with non-users (39). Remarkably, prophylactic daily LMWH or twice daily subcutaneous UFH are now recommended for all hospitalized COVID-19 patients by the WHO as well as the International Society on Thrombosis and Haemostasis (ISTH) (23, 37, 40). Nevertheless, prophylactic dose of anticoagulation is supposed to be insufficient to contrast the hypercoagulable state presented by many COVID-19 patients in response to a cytokine storm syndrome (41). A substantial number of patients with standard doses of thromboprophylaxis could still suffer from thrombotic complications, which was also observed in some studies involved in our meta-analysis (5, 16, 20). These findings are strongly suggestive of a higher dose of anticoagulation for patients at high risk of VTE.

Given the relatively high VTE occurrence found in early reports, it might therefore be appropriate to conduct a universal thromboprophylaxis strategy for all hospitalized COVID-19 patients (42), however, more evidence is needed to support these considerations.

STRENGTHS AND LIMITATIONS

This is the first systematic review and meta-analysis that estimates the relatively precise incidence of VTE in hospitalized COVID-19 patients. A comprehensive search of English-language databases, Chinese-language databases, and preprint platforms was conducted, and a revised NOS tool was used to assess the study quality appropriately. Subgroup analyses were conducted by clinical manifestations, disease severity, as well as pharmacologic thromboprophylaxis rate, to explore the differences on VTE incidence. Nevertheless, several intrinsic limitations still remained in this study. First, in this meta-analysis, 17 retrospective studies with three being from preprint platforms were included. Because of the unexpected outbreak of COVID-19, timely information and initial experiences are urgently needed by medical workers to decide on the most optimal therapy for infected patients. Given that journal publications requires peer review and is a time-consuming process, preprints might provide a mechanism for rapidly communicating research, although they are recognized as being less reliable than peer reviewed journal publications. In order to perform a comprehensive meta-analysis, we analyzed as many studies as we could find in this field. Additionally, all studies included were retrospective, which could inevitably introduce heterogeneity to the results. Further studies published in journals as well as high quality studies are therefore needed to obtain more reliable results. Second, given the difficulty of performing CTPA or ultrasonography under strict isolation, it might be difficult to fully illuminate the exact prevalence and nature of VTE in COVID-19. Third, patient-level information about comorbidities and concomitant medication was unavailable to explore the potential risk factors of VTE. Furthermore, whether patients were on thromboprophylaxis or not, as well as different pharmacologic thromboprophylaxis rates, could also contribute to heterogeneity. In addition, the association between the occurrence of VTE and coagulation indicators, such as D-dimers and fibrin degradation products, was not assessed in this study.

CONCLUSION

This meta-analysis revealed that the estimated VTE incidence was 25% in hospitalized COVID-19 patients, with the incidence of PE and DVT being 19 and 7%, respectively. Higher incidence was observed in severe patients (35%) than in non-severe patients (6%). The high pharmacologic thromboprophylaxis rate was associated with a lower incidence of VTE compared with the low pharmacologic thromboprophylaxis rate. Assessment of VTE risk is therefore strongly recommended in COVID-19 patients, and effective measures of thromboprophylaxis should be taken for patients at high risk of VTE in a timely manner.

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

Z-CG and HX are the guarantors of the entire manuscript. CZ, LS, and K-JL contributed to the study conception and design, critical revision of the manuscript for important intellectual content, and final approval of the version to be published. M-MP, L-CK, ZZ, W-HG, and H-WL contributed to the data acquisition, analysis, and interpretation. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2020.00151/full#supplementary-material

REFERENCES

1. Chen J, Wang X, Zhang S, Liu B, Wu X, Wang Y, et al. Findings of acute pulmonary embolism in COVID-19 patients. *Lancet Infect Dis.* (2020). doi: 10.2139/ssrn.3548771.[Epub ahead of print].
2. 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
3. Wang T, Chen R, Liu C, Liang W, Guan W, Tang R, et al. Attention should be paid to venous thromboembolism prophylaxis in the management of COVID-19. *Lancet Haematol.* (2020) 7:e362–3. doi: 10.1016/S2352-3026(20)30109-5
4. Violi F, Pastori D, Cangemi R, Pignatelli P, Loffredo L. Hypercoagulation and antithrombotic treatment in coronavirus 2019: a new challenge. *Thromb Haemost*. (2020) 120:949–56. doi: 10.1055/s-0040-1710317
5. 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
6. Poissy J, Goutay J, Caplan M, Parmentier E, Duburcq T, Lassalle F, et al. Pulmonary embolism in COVID-19 patients: awareness of an increased prevalence. *Circulation*. (2020) 142:184–6. doi: 10.1161/CIRCULATIONAHA.120.047430

7. Grillet F, Behr J, Calame P, Aubry S. Acute pulmonary embolism associated with COVID-19 pneumonia detected by pulmonary CT Angiography. *Radiology*. (2020). doi: 10.1148/radiol.2020 201544. [Epub ahead of print].
8. Bi Q, Hong C, Meng J, Wu Z, Zhou P, Ye C, et al. Characterization of clinical progression of COVID-19 patients in Shenzhen, China. *MedRxiv*. (2020). doi: 10.1101/2020.04.22.20076190
9. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of non-randomized studies in meta-analyses. *Eur J Epidemiol.* (2010) 25:603–5. doi: 10.1007/s10654-010-9491-z
10. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557
11. 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 health care interventions: explanation and elaboration. *J Clin Epidemiol.* (2009) 62:e1–34. doi: 10.1016/j.jclinepi.2009.06.006
12. Beun R, Kusadasi N, Sikma M, Westerink J, Huisman A. Thromboembolic events and apparent heparin resistance in patients infected with SARS-CoV-2. *Int J Lab Hematol.* (2020) 42(Suppl.1):19–20. doi: 10.1111/ijlh.13230
13. 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
14. Ding Y, Huang Z, Zhao S, Li X, Wang X, Xie Y. Clinical and imaging characteristics of corona virus disease 2019 (COVID-19). *Radiol Practice.* (2020) 35:281–5. doi: 10.1016/j.jrid.2020.04.003
15. 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
16. Tavazzi G, Civardi L, Caneva L, Mongodi S, Mojoli F. Thrombotic events in SARS-CoV-2 patients: an urgent call for ultrasound screening. *Intensive Care Med.* (2020) 46:1121–3. doi: 10.1007/s00134-020-06040-3
17. Leonard-Lorant I. Acute pulmonary embolism in COVID-19 patients on CT angiography and relationship to D-dimer levels. *Radiology*. (2020). doi: 10.1148/radiol.2020201561. [Epub ahead of print].
18. Li T, Kicska G, Kinahan PE, Zhu C, Oztek MA, Wu W. Clinical and imaging findings in COVID-19 patients complicated by pulmonary embolism. *MedRxiv*. (2020). doi: 10.1101/2020.04.20.20064105
19. Llitjos JF, Leclerc M, Chochois C, Monsallier JM, Ramakers M, Auvray M, et al. High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. *J Thromb Haemost.* (2020) 18:1743–6. doi: 10.1111/jth.14869
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. Middeldorp S. Incidence of venous thromboembolism in hospitalized patients with COVID-19. *J Thromb Haemost*. (2020). doi: 10.1111/jth.14888. [Epub ahead of print].
22. 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
23. Xing C, Li Q, Du H, Kang W, Lian J, Yuan L. Lung ultrasound findings in patients with COVID-19 pneumonia. *Crit Care.* (2020) 24:174. doi: 10.1186/s13054-020-02876-9
24. 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
25. 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
26. Malato A, Dentali F, Siragusa S, Fabbiano F, Kagoma Y, Boddi M, et al. The impact of deep vein thrombosis in critically ill patients: a meta-analysis of major clinical outcomes. *Blood Transfus.* (2015) 13:559–68. doi: 10.2450/2015.0277-14
27. Minet C, Potton L, Bonadona A, Hamidfar-Roy R, Somohano CA, Lugosi M, et al. Venous thromboembolism in the ICU: main characteristics, diagnosis and thromboprophylaxis. *Crit Care.* (2015) 19:287. doi: 10.1186/s13054-015-1003-9
28. Schunemann HJ, Cushman M, Burnett AE, Kahn SR, Beyer-Westendorf J, Spencer FA, et al. American Society of Hematology 2018 guidelines for management of venous thromboembolism: prophylaxis for hospitalized and non-hospitalized medical patients. *Blood Adv.* (2018) 2:3198–225. doi: 10.1182/bloodadvances.2018022954
29. Zhang L, Feng X, Zhang D, Jiang C, Mei H, Wang J, et al. Deep vein thrombosis in hospitalized patients with coronavirus disease 2019 (COVID-19) in Wuhan, China: prevalence, risk factors, and outcome. *Circulation*. (2020) 142:114–28. doi: 10.1161/CIRCULATIONAHA.120.046702
30. Zhai Z, Li C, Chen Y, Gerotziafas G, Zhang Z, Wan J, et al. Prevention and treatment of venous thromboembolism associated with coronavirus disease 2019 infection: a consensus statement before guidelines. *Thromb Haemost.* (2020) 120:937–48. doi: 10.1055/s-0040-1710019
31. Aryal MR, Gosain R, Donato A, Pathak R, Bhatt VR, Katel A, et al. Venous thromboembolism in COVID-19: towards an ideal approach to thromboprophylaxis, screening, and treatment. *Curr Cardiol Rep.* (2020) 22:52. doi: 10.1007/s11886-020-01327-9
32. Subbarao K, Mahanty S. Respiratory virus infections: understanding COVID-19. *Immunity.* (2020) 52:905–9. doi: 10.1016/j.immuni.2020.05.004
33. Ettema HB, Kollen BJ, Verheyen CC, Buller HR. Prevention of venous thromboembolism in patients with immobilization of the lower extremities: a meta-analysis of randomized controlled trials. *J Thromb Haemost.* (2008) 6:1093–8. doi: 10.1111/j.1538-7836.2008.02984.x
34. Elias S, Hoffman R, Saharov G, Brenner B, Nadir Y. Dehydration as a possible cause of monthly variation in the incidence of venous thromboembolism. *Clin Appl Thromb Hemost.* (2016) 22:569–74. doi: 10.1177/1076029616649435
35. Hippensteel JA, Burnham EL, Jolley SE. Prevalence of venous thromboembolism in critically ill patients with COVID-19. *Br J Haematol.* (2020). doi: 10.1111/bjh.16908. [Epub ahead of print].
36. Song JC, Wang G, Zhang W, Zhang Y, Li WQ, Zhou Z. Chinese expert consensus on diagnosis and treatment of coagulation dysfunction in COVID-19. *Mil Med Res.* (2020) 7:19. doi: 10.1186/s40779-020-00247-7
37. 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
38. Moores LK, Tritschler T, Brosnahan S, Carrier M, Collen JF, Doerschug K, et al. Prevention, diagnosis and treatment of venous thromboembolism in patients with COVID-19: CHEST guideline and expert panel report. *Chest*. (2020). doi: 10.1016/j.chest.2020.05.559. [Epub ahead of print].
39. 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
40. 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
41. Porfidia A, Pola R. Venous thromboembolism and heparin use in COVID-19 patients: juggling between pragmatic choices, suggestions of medical societies. *J Thromb Thrombolysis*. (2020) 50:68–71. doi: 10.1007/s11239-020-02125-4
42. Spyropoulos AC, Levy JH, Ageno W, Connors JM, Hunt BJ, Iba T, et al. Scientific and standardization committee communication: clinical guidance on the diagnosis, prevention and treatment of venous thromboembolism in hospitalized patients with COVID-19. *J Thromb Haemost*. (2020). doi: 10.1111/jth.14929. [Epub ahead of print].

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Renin-Angiotensin System and Coronavirus Disease 2019: A Narrative Review

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Although clinical manifestations of the 2019 novel coronavirus disease pandemic (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-COV-2), are mainly respiratory symptoms, patients can also develop severe cardiovascular damage. Therefore, understanding the damage caused by SARS-COV-2 to the cardiovascular system and the underlying mechanisms is fundamental. The cardiovascular damage may be related to the imbalance of the renin-angiotensin-system (RAS) as this virus binds the Angiotensin-Converting-Enzyme 2 (ACE2), expressed on the lung alveolar epithelial cells, to enter into cells. Virus internalization may cause a downregulation of ACE2 on host cell surface that could lead to a local increased level of angiotensin II (AII) and a reduced level of angiotensin 1-7 (A1-7). An imbalance between these angiotensins may be responsible for the lung and heart damage. Pharmacological strategies that interfere with the viral attachment to ACE2 (umifenovir and hydroxychloroquine/chloroquine) or that modulate the RAS (analogous of A1-7 and ACE2, losartan) are in clinical development for COVID-19. The use of RAS inhibitors has also become a matter of public concern as these drugs may increase the mRNA expression and levels of ACE2 and impact the virulence and transmission of SARS-COV-2. Data on the effect of RAS inhibitors on ACE2 mRNA expression are scarce. Scientific societies expressed their opinion on continuing the therapy with RAS inhibitors in patients with COVID-19 and underlying cardiovascular diseases. In conclusion, RAS may play a role in SARS-COV-2-induced cardiac and pulmonary damage. Further studies are needed to better understand the role of RAS in COVID-19 and to guide decision on the use of RAS inhibitors.

Keywords: COVID-19, renin-angiotensin system, SARS-COV-2, heart damage, pulmonary damage, RAS inhibitors

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INTRODUCTION

The renin–angiotensin system (RAS) is a complex hormonal system composed by different mediators that can affect the cardiovascular, renal, immune, and nervous functions (1, 2). Many components of the RAS have been isolated from different tissues (3), including the lung (4). This system is composed by two pathways: the classic RAS and the non-classic RAS, which have

opposite activities, especially for renal, and cardiovascular functions (2, 5). A component of the non-classic RAS, the Angiotensin-Converting-Enzyme 2 (ACE2) present on the lung surface, has been discovered to be a functional receptor for coronaviruses, essential for triggering their infection (1). Severe acute respiratory syndrome coronavirus 1 (SARS-COV-1) and SARS-COV-2, which are responsible for the SARS and the more recent coronavirus disease 2019 (COVID-19), respectively, are both able to bind the ACE2 in the lung (6, 7). Patients affected with COVID-19 show respiratory and flu-like symptoms, which can be complicated by lymphopenia and interstitial pneumonia with high levels of pro-inflammatory cytokines that can lead to acute respiratory distress syndrome (ARDS) and organ failure (8). Although the clinical manifestations of COVID-19 are mainly represented by respiratory symptoms, some patients also developed severe cardiovascular damage (9). In addition, an increased risk of death was found in patients with cardiovascular diseases (9).

Understanding the mechanisms by which the RAS interacts with SARS-COV-2 is fundamental for the treatment of patients with cardiac diseases as showed in the context of metabolic diseases (10). Moreover, considering the interaction between these viruses and the ACE2, concerns were also raised about the use of RAS inhibitors in patients with COVID-19 as they may alter ACE2 mRNA expression and levels and, in this way, impact the virulence and transmission of SARS-COV-2 (11). Therefore, in this review, we aim to summarize the physiological role of the RAS, its implication in the SARS-COV-2 infection, the actual evidence and recommendation on the use of RAS inhibitors, and the ongoing researches of drugs with a potential for the treatment of COVID-19 and acting either by influencing the RAS or disrupting the viral attachment to ACE2.

CLINICAL CHARACTERISTICS OF COVID-19

First evidence regarding the clinical characteristics of patients with COVID-19 showed the presence of bilateral lung ground glass opacity on computed tomography (CT) imaging (12). CT abnormalities were observed in both asymptomatic or symptomatic patients with SARS-CoV-2 infection, making it a useful diagnostic tool. Asymptomatic individuals with CT abnormalities rarely developed severe pneumonia (13). Initial symptoms were fever, cough, dyspnea, myalgia or fatigue, sputum production, headache, hemoptysis, and diarrhea. In most severe cases, there was a progression to ARDS, to acute cardiac injury, to acute kidney injury (AKI), or to shock. Other symptoms that were identified pertained to the gastrointestinal system (nausea and diarrhea) (12). However, other studies showed a lower development of gastrointestinal symptoms (14, 15). Moreover, an increase in serum lactate dehydrogenase as marker of lung tissue damage was observed in COVID-19 patients (13), and it was associated with higher odds of severe disease (14). Additionally, older age and lymphopenia were identified as potential risk factors for severe COVID-19 (13).

CLASSIC AND NON-CLASSIC RAS

The classic RAS involves as main effector peptide the angiotensin II (AII), whose synthesis starts with the cleavage of angiotensinogen into angiotensin I (AI) by the renin and then its conversion into AII by the ACE (16) (**Figure 1**). Despite this represent the main pathway for the AII production, also other enzymes can be involved (5). The main effects of AII are explained by its interaction with three receptors (AT1, AT2, and nonAT1nonAT2). AT1 and AT2 are classified as G protein-coupled receptors (16), while nonAT1nonAT2 seems more prone to be an angiotensin clearance receptor or an angiotensinase (17). The stimulation of the AT1 receptor can induce vasoconstriction, increase the release of catecholamines and the synthesis of aldosterone (16). Moreover, AT1 receptors can stimulate fibrosis, inflammatory processes, reduction of collagenase activity, and expression of mitogen-activated protein kinase (MAPK) (2, 5). As pro-inflammatory action, these receptors seem to be involved in several pathways: down-regulation of the NADPH oxidase expression in smooth muscle cells; enhancement of the production of reactive oxygen species (ROS) and the activity of pro-inflammatory transcription nuclear factors like nuclear factor-kappaB (NF-kB) and E26 transformation-specific sequence (Ets) (18); release of different types of cytokines such as TNF-α, IL-6, and MCP-1 (19); shifting of the macrophage phenotype toward the pro-inflammatory M1 polarization state (20). The stimulation of AT2 receptors, instead, has a protective role in the RAS activation inducing anti-inflammatory, anti-oxidative, and anti-fibrotic effects (16).

The non-classic RAS involves, instead, other peptide mediators and enzymes. Specifically, the main mediator is the angiotensin 1-7 (A1-7), whose synthesis can involve two different pathways. One starts with the cleavage of AII into A1-7 by the carboxypeptidase ACE2, while another through the cleavage of AI into angiotensin 1–9 (A1–9) by ACE2 and its subsequent conversion into A1–7 by ACE (5) (**Figure 1**). Today, two forms of ACE2 are recognized, one soluble and another transmembrane, both contributing to the generation of A1-7. The A1–7 stimulates the G protein-coupled receptor MAS1, promoting the nitric oxide release (21), Akt phosphorylation (22), and anti-inflammatory effects (23). Moreover, the activation of MAS1 receptors, expressed on the macrophage surface, inhibits the inflammatory macrophage phenotype and the release of pro-inflammatory cytokines (5). Therefore, A1-7 is a component of a beneficial axis of the RAS that exerts opposite cardiovascular and renal effects compared to the ACE/AII/AT1 axis (24).

Interestingly, it has been found that human monocytes can express ACE and ACE2 and metabolize AI to multiple angiotensin peptides. In particular, classical monocytes ($CD14^{++}CD16^{-}$) produce both AII and A1–9/A1–7, whereas the non-classical subtype ($CD14^{+}CD16^{++}$) produces mainly A1–7 (25). This indicates that ACE and ACE2 participate to the inflammation also as components of a local RAS at sites infiltrated by monocytes/macrophages.

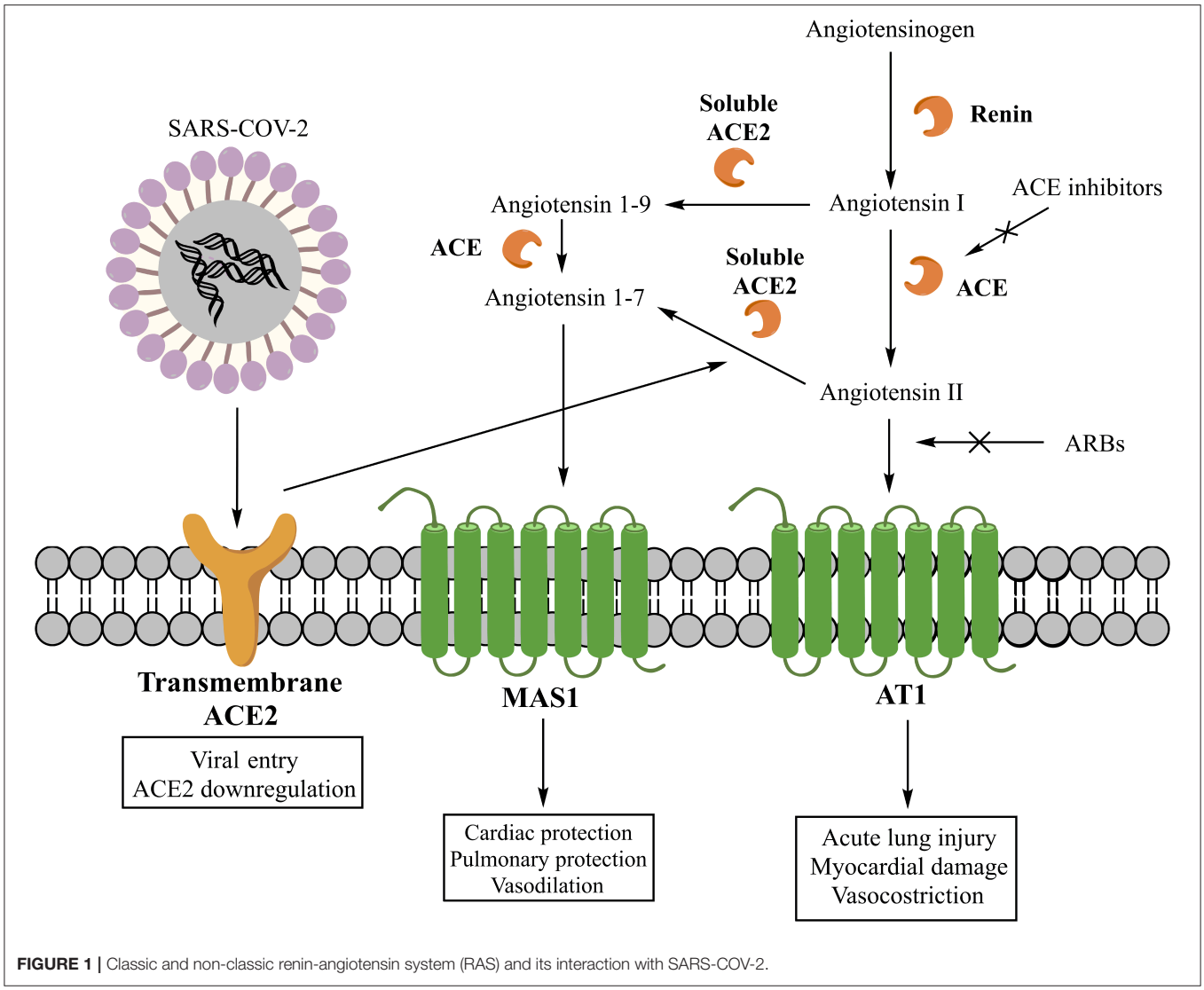

FIGURE 1 | Classic and non-classic renin-angiotensin system (RAS) and its interaction with SARS-COV-2.

SARS-COV-2 AND ACE2 IN THE LUNG

SARS-COV-2 is a betacoronavirus with a single-stranded positive-sense RNA genome encapsulated within a membrane envelope (26). The genome encodes for several structural proteins, including the glycosylated spike (S) protein that is a major inducer of host immune response. The S protein is also important because mediates host cell invasion by SARS-COV-2 via binding to the receptor protein ACE2 present on the surface of lung alveolar epithelial cells (host cells) (6, 27). The affinity of S protein binding region to the extracellular domain of ACE2 has been estimated of 15 nM (27, 28). The invasion process requires the activation of the S protein, which is facilitated by the human androgen-sensitive transmembrane serine protease type 2 (TMPRSS211) (6, 26). Specifically, TMPRSS211 cleaves the S protein and generates the S1 and S2 subunits. This is a critical step as both subunits are essential for viral entry in the host cells (28). S1 is the subunit recognized by ACE2 and the one that facilitates viral attachment, whereas S2 is the subunit that drives membrane fusion and viral internalization in the pulmonary epithelium (6). The greater virulence of SARS-COV-2 compared to SARS-COV-1 was supposed to be related to the higher affinity of S1 subunit for ACE2 (26, 28). In fact, a Cryo-EM structure analysis revealed that the affinity of the S protein of SARS-COV-2 to ACE2 is about 10–20 times greater than that observed with the S protein of SARS-COV-1 (27).

Another important consideration is that the ACE2 internalization mediated by SARS-COV-2 could potentially result in a reduced presence of ACE2 on cell surface, leading to the absence of a key factor for AII degradation and A1-7 synthesis. An imbalance between AII and A1-7 levels may further exacerbate the damage of lung provoked by SARS-COV-2. Therefore, a decrease in ACE2 may contribute to the reduction of pulmonary function and the increase of tissue fibrosis and inflammation due to COVID-19 (28). This hypothesis was already investigated with SARS-COV-1 infection,

which was associated with a reduced presence of ACE2 on cell membranes and an increased severity of lung injury (29). Because SARS-COV-1 and SARS-COV-2 share the same cellular invasion process, they may also share similar pathogenesis and pathological manifestations of lung injury (29).

SARS-COV-2 AND ACE2 IN THE HEART

Potentially, once the SARS-COV-2 enters the circulation, it can infect any tissue expressing the ACE2, including the heart or other cardiovascular tissues (28). Evidence showed that patients with COVID-19 had a high occurrence of cardiovascular symptoms, in addition to respiratory ones, and that these symptoms were also reported in patients without underlying cardiovascular diseases (30). The National Health Commission of China (NHC) reported that cardiovascular symptoms (such as heart palpitations and chest tightness) occurred at the beginning of the SARS-COV-2 infection in some of confirmed cases. Moreover, the 11.8% of patients who died for COVID-19 but without underlying cardiovascular diseases had substantial heart damage (30). These data suggest the necessity of involving cardiologists in the management of patients with COVID-19 (31). However, the real contribute of SARS-COV-2 in the development of myocardial injury is not clear (32). It is known that the infection itself may directly impact cardiovascular diseases and the development of cardiovascular complications (30, 33). Another factor that should be considered is also the expression in the tissue of TMPRSS211 or other proteases able to trigger the viral entry (6). Another hypothesis for the induction of heart damage considers the reduction of ACE2 caused by SARS-COV-2, which might exacerbate symptoms in patients with underlying cardiovascular diseases (28, 34). This could be due to the imbalance between the classic and non-classic RAS in favor of AII that may further compromise cardiac function apart from the viral infection (28). In fact, a preclinical study shows that ACE2 knockout animal models had a worse left ventricular remodeling in response to the AII-induced acute injury, suggesting a protective role of non-classic RAS in myocardial recovery (35). This finding may also explain the heart damage found in patients with COVID-19 but without cardiovascular diseases (30). To corroborate this hypothesis, a study demonstrated that the AII level in the plasma sample of SARS-COV-2 infected patients was markedly high and linearly associated with the viral load and lung injury (32). Moreover, another study found in the 35% of heart samples from patients with SARS the presence of viral RNA associated with a reduced ACE2 protein expression (36). Another proposed mechanism of myocardial injury includes the cytokine storm (32) as the systemic inflammatory response and immune system disorders during disease progression may be responsible for the myocardial damage (30). Also, in this case, other than the viral infection itself, a minor role in potentiating the inflammation might be played by the classic RAS cascade. Moreover, needs to be considered that also some drugs that are being investigated for COVID-19 are potential risk factors for the cardiovascular toxicity (31).

Finally, evidence showed that COVID-19 may produce a form of disseminated intravascular coagulation (DIC) as the presence of microthrombi have been reported from the autopsy of patients with COVID-19 (37). To date, the exact causes of DIC are many and unclear. Potential suggested mechanisms are as follows: inflammation (e.g., IL-6) stimulates the synthesis of fibrinogen (38); or the virus may directly bind to endothelial cells; or a mutual relationship between DIC and cytokine storm (wherein each exacerbates the other) exists.

CONCERNS, EVIDENCE AND RECOMMENDATION ON THE USE OF RAS INHIBITORS IN PATIENTS WITH COVID-19

Concerns were raised on the use of RAS inhibitors in patients with COVID-19 as the use of these drugs may determine an increase of ACE2 and then of SARS-COV-2 virulence (11, 30). Among drugs able to inhibit the RAS, there are renin inhibitors, ACE inhibitors, and the Angiotensin Receptor Blockers (ARBs). ACE inhibitors and ARBs are among drugs most commonly used worldwide for the treatment of cardiovascular diseases. Therefore, concerns on their use in patients with COVID-19 are even more important. Initial evidence showed that patients with COVID-19 and coexisting cardiovascular conditions had a more severe illness, a more frequent admission to the intensive care unit, were more prone to receive mechanical ventilation, or to die (11). The first hypothesis was that the medical management of these conditions, including the use of RAS inhibitors, may have contributed to the adverse health outcomes. So far, there is no rigorous report accounting for key factors as potential confounders in risk prediction; moreover, available evidence on the effect of RAS inhibitors on ACE2 mRNA expression and levels are conflicting and scarce, highlighting also the absence of data on lung-specific mRNA expression of ACE2 (11). Researches have also suggested that this effect of RAS inhibitors may not be uniform among molecules (11, 39). Moreover, even if there was a relationship between the RAS inhibition and the up-regulation of ACE2, there is no evidence demonstrating a causal relationship between the ACE2 activity and the SARS-COV-2 associated mortality (40). Furthermore, the presence of ACE2 on cell surface may not be the only factor participating in the infection process. In fact, additional co-factors might participate in the cell invasion process as SARS-COV-1 infection was not observed in some cells expressing ACE2 on the surface, whereas it was found in cells apparently without ACE2 (41). Moreover, the lethal outcome observed in patients with COVID-19 may also be driven by the severity of the lung damage. In this regard, a preclinical study suggested a beneficial role of RAS blockers in limiting the SARS-COV-1-induced lung injury (42), so that, a protective role is played by RAS inhibitors. This finding could rise a new hypothesis in which the activation of the classic RAS, rather than its inhibition, may predispose patients toward a more deleterious outcome.

Finally, another aspect that should be considered is the potential harm associated with the withdrawal of a RAS inhibitor in a patient with a stable cardiovascular condition. In fact,

RAS inhibitors are known to determine clinical benefits and to protect both myocardium and kidney. Therefore, their sudden withdrawal may expose patients to an unjustified risk related to decompensation and symptoms exacerbation, especially in high cardiovascular risk patients. In this regard, clinical trials have demonstrated a rapid relapse of the dilated cardiomyopathy or a decline of the clinical condition after the discontinuation of the pharmacological treatment with a RAS inhibitor (43).

Moreover, there are solid evidence on the effect of RAS inhibitors in reducing mortality in patients with cardiovascular diseases. These drugs are indeed the cornerstone therapy for a favorable prognosis in patients with heart failure, with the highest level of evidence in reducing mortality (44). Finally, Scientific Societies have expressed their opinion on the use of RAS inhibitors, highlighting the absence of evidence suggesting an eventual discontinuation of ACE-inhibitors, or ARBs in patients with COVID-19. Therefore, they recommend to continue the treatment with the usual anti-hypertensive agent in patients with COVID-19 (45–49). This recommendation has been supported by different observational studies published in the last few months. In this regards, a population-based case–control study carried out in the Lombardy region of Italy did not show any association between the use of ARBs or ACE-inhibitors with COVID-19 among all patients (adjusted odds ratio, 0.95 [95% confidence interval (CI), 0.86 to 1.05] for ARBs and 0.96 [95% CI, 0.87 to 1.07] for ACE inhibitors) or among patients with a severe or fatal course of the disease (adjusted odds ratio, 0.83 [95% CI, 0.63 to 1.10] for ARBs and 0.91 [95% CI, 0.69 to 1.21] for ACE inhibitors) (50).

Accordingly, another Italian nested case-control study showed no increased risk of being infected by SARS-COV-2 in patients treated with RAS inhibitors (51). Moreover, a case-population study showed that RAS inhibitors had an adjusted odds ratio for COVID-19 requiring admission to hospital of 0.94 (95% CI, 0.77 to 1.15) compared with users of other antihypertensive drugs (52). In relation to the mortality outcome, instead, a retrospective observational study showed similar mortality rates between the RAS inhibitor and non-RAS inhibitor cohorts (2.2 vs. 3.6%, adjusted hazard ratio [HR] 0.85; 95% CI, 0.28 to 2.58) (53). Similarly, a Korean nationwide population-based cohort study showed no difference for mortality between RAS inhibitors users and non-users (adjusted odds ratio, 0.88; 95% CI, 0.53 to 1.44) (54). Finally, a retrospective, multi-center study demonstrated a lower risk of COVID-19 mortality in inhospital patients with hypertension and hospitalized due to COVID-19 who received ACE inhibitor/ARB compared to those who did not receive an ACE inhibitor/ARB (adjusted HR, 0.37; 95% CI, 0.15 to 0.89) (55). Different other published studies supported the aforementioned findings (56–58). Moreover, it is ongoing an observational study that will enroll about 2,000 participants to assess if the chronic intake of RAS inhibitors modifies the prevalence and severity of clinical manifestations of COVID-19 (ClinicalTrials.gov identifier, NCT04331574).

Clinical trials are also ongoing to assess instead clinical benefits of continuing or not the treatment with ARBs or ACE inhibitors in patients with COVID-19 (NCT04330300, NCT04351581, NCT04353596, and NCT04329195). In particular, the NCT04330300 is a randomized, open label, parallel assignment clinical trial that will randomize patients with primary essential hypertension who are already taking ACE inhibitor/ARB to either switch to an alternative antihypertensive agent or continue with the ACE inhibitor/ARB treatment. The NCT04351581 is a randomized, single mask (outcome assessor), parallel assignment clinical trial that will randomize hospitalized patients with COVID-19 to continue or discontinue their treatment with the ACE inhibitor or ARB. The NCT04353596 is also a randomized, single mask (outcome assessor), parallel assignment clinical trial that will randomize symptomatic SARS-CoV2-infected patients to stop/replace the chronic treatment with the ACE inhibitor/ARB or to continue this chronic treatment. The NCT04329195 is instead a randomized, open label, parallel assignment clinical trial that will randomize patients with a history of cardiovascular disease treated with RAS blockers, and infected by SARS-CoV-2 to stop or continue the treatment with the RAS blocker. Moreover, the substudy of the Austrian Coronavirus Adaptive Clinical Trial (ACOVACT), which is a randomized, controlled, multicenter, open-label basket trial that aims to compare various antiviral treatments for COVID-19, will also compare the sub-arm with RAS blockade vs. no RAS blockade for patients with blood pressure >120/80 mmHg (NCT04351724). Characteristics of the ongoing clinical trials are showed in **Table 1**.

NEW PHARMACOLOGICAL APPROACHES FOR PREVENTING VIRAL ENTRY OF SARS-COV-2 WITH A FOCUS ON THE DISRUPTION OF S PROTEIN/ACE2 INTERACTION

To prevent viral infection, molecules like camostat mesylate, nafamostat mesylate, gabexate, umifenovir, and hydroxychloroquine/chloroquine are being considered (26). Nafamostat and camostat are inhibitors of the protease TMPRSS211 (26). Gabexate has instead multiple mechanisms of action. It has anticoagulant and anti-platelet activities on one hand, and it is a serine protease inhibitor with antiviral and anti-inflammatory properties on the other (59, 60).

While these drugs act on the protease inhibition, umifenovir and hydroxychloroquine/chloroquine directly influence the S protein/ACE2 interaction (**Table 2**) (26). Hydroxychloroquine and chloroquine, in addition to their use for malaria and autoimmune diseases, may be effective also for the treatment of COVID-19. These drugs are able to elevate endosomal pH and interfere with ACE2 glycosylation (26, 70). The efficacy of chloroquine was already demonstrated with SARS-COV-1 infection, in which the treatment was effective either if administrated prior or after the infection, suggesting that chloroquine may have both a prophylactic and therapeutic use (70). Moreover, preliminary *in vitro* results demonstrated that remdesivir and chloroquine are highly effective in the inhibition of SARS-COV-2 infection (71). Clinical findings also confirmed the efficacy of chloroquine in terms of reduction of exacerbation of pneumonia and duration of symptoms in a cohort of 100

TABLE 1 | Characteristics of ongoing clinical trials on drugs acting either by influencing the RAS or disrupting the viral attachment to ACE2 in patients with COVID-19.

Clinical trial number	Clinical phase; multicenter	Arms	Estimated enrollment	Primary outcome	Estimated study completion date
NCT04330300	4; No	• Experimental arm: switching to an alternative anti-hypertensive medication (specifically a calcium channel blocker or thiazide/thiazide-like diuretic at an equipotent blood pressure lowering dose). The choice of the alternative anti-hypertensive will be at the discretion of the patient's treating physician. • Comparator arm: continuing the treatment with ACE inhibitor/ARB	2,414	1. Number of COVID-19 positive participants who die, require intubation in intensive care unit, or require hospitalization for non-invasive ventilation at 12 months. Time from randomization to the first occurrence of any of the clinical events above.	March 1, 2021
NCT04351581	Not reported; No	• Experimental arm: continuing the treatment with ACE inhibitor/ARB. The clinicians will be encouraged to continue the medication throughout the hospital admission but it will be permissible for the clinician to stop treatment if necessary (e.g., due to hypotension). • Experimental arm: discontinuing the treatment with ACE inhibitor/ARB. If hypertensive treatment is necessary during hospital admission, the clinicians will first be encouraged to start non-ACE inhibitor/non-ARB treatment.	215	1. Days alive and out of hospital within 14 days after recruitment	December 2020
NCT04353596	4; Yes	• Experimental arm: chronic treatment with ACE inhibitor or ARB will be stopped or replaced. • Comparator arm: no intervention, which means to continue the treatment with ACE inhibitor or ARB.	208	1. Combination of maximum Sequential Organ Failure Assessment (SOFA) Score and death at 30 days. 2. Composite of admission to an intensive care unit, the use of mechanical ventilation, or all-cause death at 30 days.	May 15, 2022
NCT04329195	3; No	• Experimental arm: discontinuation of RAS blocker therapy • Comparator arm: continuation of RAS blocker therapy	554	1. Time to clinical improvement from day 0 to day 28 (improvement of two points on a seven-category ordinal scale, or live discharge from the hospital, whichever comes first)	August 9, 2020
NCT04351724 substudy	2/3; Yes	• Experimental arm: candesartan at 4 mg once daily and titrated to normotension • Comparator arm: non-RAS antihypertensive agents titrated to normotension. Those with normal blood pressure may be controlled without further treatment.	500	1. Sustained improvement (>48 h) of one point on the WHO Scale within 29 days (daily evaluation).	December 31, 2020
NCT04260594	4; Not reported	• Experimental arm: umifenovir tablets (2 tablets/time, 3 times/day for 14–20 days) + basic treatment • Comparator arm: basic treatment • The basic treatment is based on the condition of the patient.	380	1. Virus negative conversion rate in the first week	December 30, 2020
NCT04252885	4; No	• Experimental arm: standard treatment + lopinavir/ritonavir. Specifically, 50 participants are given ordinary treatment plus a regimen of lopinavir (200 mg) and ritonavir (50 mg) (oral, q12h, every time 2 tablets of each, taking for 7–14 days).	125	1. The rate of virus inhibition at Day 0, 2, 4, 7, 10, 14, and 21. Novel corona viral nucleic acid is measured in nose/throat swab at each time point.	July 31, 2020

(Continued)

TABLE 1 | Continued

Clinical trial number	Clinical phase; multicenter	Arms	Estimated enrollment	Primary outcome	Estimated study completion date
		• Comparator arm: standard treatment + umifenovir. Specifically, 50 participants are given ordinary treatment plus a regimen of umifenovir (100 mg) (oral, tid, 200 mg each time, taking for 7–14 days). • No intervention arm: standard treatment. Specifically, 25 cases are only given ordinary treatment.			
NCT04255017	4; No	• Experimental arm: addition of umifenovir (0.2 g once, 3 times a day for 2 weeks) • Experimental arm: addition of oseltamivir (75 mg once, twice a day for 2 weeks) • Experimental arm: addition of lopinavir/ritonavir (500 mg once, twice a day for 2 weeks) • No intervention arm: symptomatic supportive treatment	400	1. Rate of disease remission at 2 weeks. Defined for mild patients as fever, cough and other symptoms relieved with improved lung CT, and for severe patients as fever, cough and other symptoms relieved with improved lung CT, SPO2> 93% or PaO2/FiO2 > 300 mmHg (1 mmHg = 0.133 Kpa); 2. Time for lung recovery at 2 weeks. Defined as the comparison of the average time of lung imaging recovery after 2 weeks of treatment in each group.	July 1, 2020
NCT04350684	4; No	• Experimental arm: umifenovir + interferon-β 1a + lopinavir/ritonavir + single dose of hydroxychloroquine + standards of care • Comparator arm: interferon-β 1a + lopinavir/ritonavir + single dose of hydroxychloroquine + standards of care	40	1. Time to clinical improvement from the date of randomization until 14 days later. Improvement of two points on a seven-category ordinal scale (recommended by the World Health Organization: COVID-2019) R&D. Geneva: World Health Organization) or discharge from the hospital, whichever came first.	April 24, 2020
NCT04312009	2; Yes	• Experimental arm: losartan (50 mg daily, oral) • Control arm: placebo (microcrystalline methylcellulose, gelatin capsule, oral)	200	1. Difference in Estimated Positive End-expiratory Pressure (PEEP adjusted) P/F Ratio at 7 days. Outcome calculated from the partial pressure of oxygen or peripheral saturation of oxygen by pulse oximetry divided by the fraction of inspired oxygen (PaO2 or SaO2: FiO2 ratio). PaO2 is preferentially used if available. A correction is applied for endotracheal intubation and/or positive end-expiratory pressure. Patients discharged prior to day 7 will have a home pulse oximeter send home for measurement of the day 7 value, and will be adjusted for home O2 use, if applicable. Patients who died will be applied a penalty with a P/F ratio of 0.	April 1, 2021
NCT04311177	2; Yes	• Experimental arm: losartan (25 mg daily, oral) • Comparator arm: placebo (microcrystalline methylcellulose, gelatin capsule, oral)	580	1. Hospital Admission within 15 days. Outcome reported as the number of participants per arm admitted to inpatient hospital care due to COVID-19-related disease within 15 days of randomization.	April 1, 2021

(Continued)

TABLE 1 | Continued

Clinical trial number	Clinical phase; multicenter	Arms	Estimated enrollment	Primary outcome	Estimated study completion date
NCT04328012	2/3; Yes	• Experimental arm: lopinavir/ritonavir (400 mg/200 mg, oral, BID X 5–14 days depending on availability) • Experimental arm: hydroxychloroquine (400 mg BID on Day 0, and 200 mg BID Days 1–4, days 1–13 if available) • Experimental arm: losartan (25 mg, oral, daily X 5–14 days depending on availability) • Comparator arm: placebo (BID X 14 days)	4,000	1. National Institute of Allergy and Infectious Diseases COVID-19 Ordinal Severity Scale (NCOSS) at 60 days. Difference in NCOSS scores between the different treatment groups	April 1, 2021
NCT04335786	4; Yes	• Experimental arm: valsartan for 14 days at a dosage and frequency titrated to blood pressure with 80 mg or 160 mg tablets up to a maximum dose of 160 mg b.i.d. • Comparator arm: placebo for 14 days (matching 80 or 160 mg placebo tablets at a dosage and frequency titrated to systolic blood pressure)	651	1. First occurrence of intensive care unit admission, mechanical ventilation or death within 14 days. Death is defined as all-cause mortality	December 2021
NCT04360551	2; No	• Experimental arm: telmisartan (40 mg, oral, daily X 21 days) • Comparator arm: placebo (once daily X 21 days)	40	1. Maximum clinical severity of disease over the 21 day period of study. Based on a modified World Health Organization (WHO) COVID-19 7-point ordinal scale	June 30, 2021

subjects (72, 73). This finding led the China Authority to include these medicines in the recommendations for the prevention and treatment of COVID-19 pneumonia (73). Many other clinical studies are ongoing to evaluate the efficacy and safety of hydroxychloroquine for the pre-exposure prophylaxis, post-exposure prophylaxis, and treatment of COVID-19 (www.clinicaltrials.gov) (74). However, it should be noted that current evidence on the effects of chloroquine is conflicting. Authors of a recent systematic review underlined that, even though a rationale to justify clinical research on chloroquine in patients with COVID-19 exists, high-quality clinical trials are urgently needed (75). In addition, a further literature review (76) reported that there is limited *in vitro* evidence on the efficacy of this drug against SARS-COV-2 and that clinical data based on studies with small sample size and affected by methodological limitations (77, 78). Therefore, high quality randomized clinical trials are strongly needed. Umifenovir interferes instead with the attachment of viral envelope protein to host cells (26). Umifenovir is an antiviral agent actually authorized in Russia, but not in Europe, for the treatment of Influenza A and B. This drug is considered safe and it is patented for the SARS treatment (79). The opinion of the Italian Medicine Agency on this drug is that evidence on its efficacy are not sufficient to support its use in patients with COVID-19 (80). Currently, a randomized, open label, parallel assignment clinical study is evaluating the efficacy and safety of umifenovir for the treatment of pneumonia in patients infected with SARS-COV-2 (NCT04260594). In this study, patients will be randomized to receive umifenovir plus basic treatment or just the basic treatment (**Table 1**). Moreover, two clinical trials are ongoing to assess the efficacy and safety of umifenovir and lopinavir/ritonavir (NCT04252885) or umifenovir, oseltamivir, and lopinavir/ritonavir (NCT04255017). Specifically, the NCT04252885 is a randomized, open label, parallel assignment clinical trial that will randomize patients with SARS-COV-2 infection in three groups (2:2:1). One group will receive the standard treatment plus lopinavir/ritonavir; the second group will receive standard treatment plus umifenovir; finally, the third group will just receive the standard treatment. The NCT04255017 is instead a randomized, single mask (participants), parallel assignment clinical trial that will randomize COVID-19 patients in four arms. One arm will receive the treatment with umifenovir; the second arm will receive the treatment with oseltamivir; the third arm will receive the treatment with lopinavir/ritonavir; the last arm will just receive the symptomatic supportive treatment (**Table 1**). Another small, randomized, triple mask (Participant, Care Provider, Investigator), parallel assignment clinical trial will be conducted on patients who have a positive test confirming COVID-19 to evaluate the combined treatment with umifenovir, interferon-β 1a, lopinavir/ritonavir, single dose of hydroxychloroquine, and the standards of care compared to the same combined treatment without umifenovir (NCT04350684).

In addition, speculations were done on the possible use for COVID-19 of new compounds, never approved before, which

TABLE 2 | Mechanism of action, main adverse events and potential drug-drug interactions of inhibitors of viral invasion interfering with the S protein/ACE2 interaction, RAS inhibitors, and analogous ACE2 and A1-7 under clinical evaluation for the treatment of COVID-19.

Therapeutic class	Drugs	Main mechanism of action	Main adverse events	Drug-drug interactions	References
Inhibitors of S protein/ACE2 interaction	*Chloroquine/ Hydroxychloroquine*	Increase of endosomal pH and interference with ACE2 glycosylation	Cardiovascular disorders, including prolongation of QT	Digoxin, class IA and III antiarrhythmic, tricyclic antidepressants, antipsychotics	(61, 62)
	Umifenovir	Interference with the attachment of the viral protein to host cells	Gastrointestinal symptoms and increased transaminase	As UDP-glucuronosyltransferase 1A9 and 2B7 inhibitor, umifenovir can increase levels of its substrates (paracetamol, buprenorphine, etc.) Cytochrome 3A4 inducers can reduce umifenovir levels	(63, 64)
ARBs	*Losartan*	Blocks the AII-induced lung injury	Dizziness, anemia, renal failure, asthenia, hyperkaliemia	Fluconazole and Rifampicine can increase losartan levels, Potassium-sparing diuretics can increase the risk of hyperkaelemia	(65, 66)
Analogous of ACE2 and A1-7	*A1-7*	Restores the beneficial effect of the non-classic RAS	Headache, fatigue, injection site reaction	Not Available	(29, 67, 68)
	ACE2	Restores the beneficial effect of the non-classic RAS	Hypernatremia, rash, dysphagia, and pneumonia	Not Available	(69)

have shown the ability of interfering with S protein/ACE2 interaction (74). The compound SSAA09E2 showed the ability of blocking the early interaction of SARS-S protein with ACE2 in ACE2-expressing 293T cells (81). Moreover, the agent VE607 also showed a significant inhibition of SARS-pseudovirus entry in the same cellular model (82).

NEW PHARMACOLOGICAL PERSPECTIVE FOR COVID-19 ACTING ON THE RAS

Based on the beneficial role of the non-classic RAS, which seems lacking in patients with COVID-19, hypotheses have been made on the potential therapeutic approach of restoring the ACE2/A1-7 pathway. This hypothesis is based on preclinical evidence showing an improvement of oxygenation, reduction of inflammation, and reduction of tissue fibrosis after infusion of A1-7 in two models of ARDS (65, 83). Evidence also showed that the administration of the soluble human recombinant ACE2 was able to reverse the lung-injury process in preclinical models of other viral infections (84, 85). The rationale to administer soluble ACE2 is to stimulate the RAS protective pathway without increasing the ACE2 transmembrane form that could instead potentiate the viral entry into the cells. Clinical evidence on this aspect is scarce (86). A phase 2 trial conducted in patients with ARDS showed that ACE2 infusion safely reduced the AII level, but this trial was not powered enough to show efficacy in terms of pulmonary function (69). Restoring the ACE2 activity may also be beneficial for the myocardial protection in patients with COVID-19 (87). To date, clinical researches are ongoing to assess the clinical impact of a restoration of the non-classic RAS (ACE2 and A1-7) in patients with COVID-19. Is underway a controlled trial aimed to assess the efficacy, safety and clinical impact of A1-7 infusion in a cohort of COVID-19 patients requiring mechanical ventilation (NCT04332666). It was, instead, suspended a further clinical trial that aimed to assess preliminary biologic, physiologic, and clinical data with the use of ACE2 recombinant compared to the standard care in patients with COVID-19 (NCT04287686).

In addition, based on the organ protective effects of RAS inhibitors, many studies are being conducted to investigate their efficacy in COVID-19 patients. The beneficial effects of ACE inhibitors and ARB may be related to the prevalence of ACE2/A1-7 effects as demonstrated in experimental studies (88, 89). Moreover, experimental evidence strongly suggests that AII could promote acute lung injury induced by different coronaviruses, including SARS-COV-1 and SARS-COV-2 (42, 65). Therefore, the use of RAS inhibitors may block the deleterious effect associated with AII. Two trials are ongoing to investigate the role of losartan for the treatment of COVID-19 in patients who have not previously received a RAS inhibitor and are either hospitalized (NCT04312009) or not hospitalized (NCT04311177). In particular, both trails (NCT04312009 and NCT04311177) are randomized, quadruple mask (participant, care provider, investigator, outcomes assessor), parallel assignment clinical trials that will compare the treatment with losartan vs. placebo in COVID-19 patients, including those with ARDS. Moreover, a pragmatic adaptive, randomized, quadruple mask (participant, care provider, investigator, outcomes assessor), parallel assignment trial is comparing the

treatment with lopinavir/ritonavir, or hydroxychloroquine, or losartan vs. placebo in patients with COVID-19 (NCT04328012). Another randomized, quadruple mask (participant, care provider, investigator, outcomes assessor), parallel assignment clinical trial will evaluate the treatment with valsartan compared to placebo for the prevention of ARDS in hospitalized patients with COVID-19 (NCT04335786). Finally, a pilot, randomized, triple mask (participant, care provider, investigator), parallel assignment clinical trial is ongoing to assess the safety and efficacy of telmisartan compared to placebo for the mitigation of pulmonary and cardiac complications in COVID-19 patients (NCT04360551). Characteristics of the mentioned clinical trials are showed in **Table 1**. The mechanism of action, main adverse events and potential drug-drug interactions of RAS inhibitors and analogous of A1-7 and ACE2 under clinical evaluation for COVID-19 are summarized in **Table 1**.

Finally, other compounds that may be useful for the treatment of COVID-19, but not currently evaluated, are molecules that may adjust the imbalance between AT1 and AT2 receptors such as compound 21 (C-21), CGP-42112A, and L-163491 (26). C-21 and CGP-42112A are two agonists of AT2 receptors, whereas L-163491 has a dual action as a partial agonist of AT2 receptors and a partial antagonist of AT1 receptors (26).

CONCLUSION

The RAS may play a complex role in SARS-COV-2 infection. SARS-COV-2 internalization may cause a reduction of ACE2 on cell surface. A reduction in ACE2 can further contribute to the pulmonary function deterioration and the myocardial damage. However, there is a paucity of clinical evidence on the efficacy of restoring the ACE2 functionality for the treatment of viral-induced lung injury. A clinical trial is ongoing to evaluate the effect of A1-7 in COVID-19 patients. To date, there is no effective drug for the treatment of COVID-19 and few clinical data are available. Some clinical trials are ongoing to evaluate the efficacy of drugs that could interfere with the S protein/ACE2 interaction such as umifenovir and hydroxychloroquine/chloroquine.

Data instead on the increased mRNA expression and levels of ACE2 after treatment with RAS inhibitors are scarce and to date not associated with an increased mortality in patients with COVID-19. Currently, clinical trials are ongoing to investigate the use of a RAS inhibitor for the reduction of the lung damage in patients with COVID-19. Substantial evidence is needed to guide decision-making on the use of ACE inhibitors and ARBs in such patients, until then we need to base on the available data that place RAS inhibitors among the safe choices for cardiovascular diseases.

AUTHOR CONTRIBUTIONS

AM, CS, CR, CF, GR, LB, GP, FR, and AC: drafting the work, revising it for important intellectual content, final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately discussed. FR and AC developed the concept and designed the study. AM wrote the paper. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. 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
2. Mascolo A, Sessa M, Scavone C, De Angelis A, Vitale C, Berrino L, et al. New and old roles of the peripheral and brain renin–angiotensin–aldosterone system (RAAS): focus on cardiovascular and neurological diseases. *Int J Cardiol.* (2017) 227:734–42. doi: 10.1016/j.ijcard.2016.10.069
3. Skov J, Persson F, Frøkiær J, Christiansen JS. Tissue renin-angiotensin systems: a unifying hypothesis of metabolic disease. *Front Endocrinol.* (2014) 5:23. doi: 10.3389/fendo.2014.00023
4. Marshall R. The pulmonary renin-angiotensin system. *Curr Pharm Des.* (2005) 9:715–22. doi: 10.2174/1381612033455431
5. Mascolo A, Urbanek K, De Angelis A, Sessa M, Scavone C, Berrino L, et al. Angiotensin II and angiotensin 1–7: which is their role in atrial fibrillation? *Heart Fail Rev.* (2020) 25:367–80. doi: 10.1007/s10741-019-09837-7
6. 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
7. Li W, Moore MJ, Vasllieva 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
8. Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. *Mil Med Res.* (2020) 7:11. doi: 10.1186/s40779-020-00240-0
9. 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
10. Mori J, Oudit GY, Lopaschuk GD. SARS-CoV-2 perturbs the renin-angiotensin system and energy metabolism. *Am J Physiol Metab.* (2020) 319:E43–7. doi: 10.1152/ajpendo.00219.2020
11. Vaduganathan M, Vardeny O, Michel T, McMurray JJ V, 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
12. Jiang F, Deng L, Zhang L, Cai Y, Cheung CW, Xia Z. Review of the clinical characteristics of coronavirus disease 2019 (COVID-19). *J Gen Intern Med.* (2020) 35:1545–9. doi: 10.1007/s11606-020-05762-w
13. Tabata S, Imai K, Kawano S, Ikeda M, Kodama T, Miyoshi K, et al. Clinical characteristics of COVID-19 in 104 people with SARS-CoV-2 infection on the diamond princess cruise ship: a retrospective analysis. *Lancet Infect Dis.* (2020). doi: 10.1016/S1473-3099(20)30482-5. [Epub ahead of print].
14. Colaneri M, Sacchi P, Zuccaro V, Biscarini S, Sachs M, Roda S, et al. Clinical characteristics of coronavirus disease (COVID-19) early findings from a teaching hospital in Pavia, North Italy, 21 to 28 February 2020. *Eurosurveillance.* (2020) 25:2000460. doi: 10.2807/1560-7917.ES.2020.25.16.2000460

15. 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
16. Unger T. The role of the renin-angiotensin system in the development of cardiovascular disease. *Am J Cardiol.* (2002) 89:3A−9. doi: 10.1016/S0002-9149(01)02321-9
17. Karamyan VT, Arsenault J, Escher E, Speth RC. Preliminary biochemical characterization of the novel, non-AT1, non-AT2 angiotensin binding site from the rat brain. *Endocrine.* (2010) 37:442–8. doi: 10.1007/s12020-010-9328-2
18. 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
19. 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) 21:20–7. doi: 10.1038/sj.jhh.1002101
20. Yamamoto S, Yancey PG, Zuo Y, Ma LJ, Kaseda R, Fogo AB, et al. Macrophage polarization by angiotensin II-type 1 receptor aggravates renal injury-acceleration of atherosclerosis. *Arterioscler Thromb Vasc Biol.* (2011) 31:2856–64. doi: 10.1161/ATVBAHA.111.237198
21. Fraga-Silva RA, Pinheiro SVB, Gonçalves ACC, Alenina N, Bader M, Santos RAS, et al. The antithrombotic effect of angiotensin-(1-7) involves mas-mediated NO release from platelets. *Mol Med.* (2008) 14:28–35. doi: 10.2119/2007-00073.Fraga-Silva
22. Dias-Peixoto MF, Santos RAS, Gomes ERM, Alves MNM, Almeida PWM, Greco L, et al. Molecular mechanisms involved in the angiotensin-(1-7)/Mas signaling pathway in cardiomyocytes. *Hypertension.* (2008) 52:542–8. doi: 10.1161/HYPERTENSIONAHA.108.114280
23. da Silveira KD, Coelho FM, Vieira AT, Sachs D, Barroso LC, Costa VV, et al. Anti-inflammatory effects of the activation of the angiotensin-(1-7) receptor, MAS, in experimental models of arthritis. *J Immunol.* (2010) 185:5569–76. doi: 10.4049/jimmunol.1000314
24. Santos RAS, Ferreira AJ, Verano-Braga T, Bader M. Angiotensin-converting enzyme 2, angiotensin-(1-7) and mas: new players of the renin-angiotensin system. *J Endocrinol.* (2013) 216:R1–17. doi: 10.1530/JOE-12-0341
25. Rutkowska-Zapała M, Suski M, Szatanek R, Lenart M, Weglarczyk K, Olszanecki R, et al. Human monocyte subsets exhibit divergent angiotensin I-converting activity. *Clin Exp Immunol.* (2015) 181:126–32. doi: 10.1111/cei.12612
26. Liu C, Zhou Q, Li Y, Garner LV, Watkins SP, Carter LJ, et al. Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases. *ACS Cent Sci.* (2020) 6:315. doi: 10.1021/acscentsci.0c00272
27. 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
28. South AM, Diz D, Chappell MC. COVID-19, ACE2 and the cardiovascular consequences. *Am J Physiol Hear Circ Physiol.* (2020) 318:H1084–90. doi: 10.1152/ajpheart.00217.2020
29. Cheng H, Wang Y, Wang GQ. Organ-protective effect of angiotensin-converting enzyme 2 and its effect on the prognosis of COVID-19. *J Med Virol.* (2020) 96:726–30. doi: 10.1002/jmv.25785
30. 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
31. Driggin E, Madhavan MV, Bikdeli B, Chuich T, Laracy J, Bondi-Zoccai G, et al. Cardiovascular considerations for patients, health care workers, and health systems during the coronavirus disease 2019. (COVID-19) Pandemic. *J Am Coll Cardiol.* (2020) 75:2352–71. doi: 10.1016/j.jacc.2020.03.031
32. 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
33. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS-CoV - a target for vaccine and therapeutic development. *Nat Rev Microbiol.* (2009) 7:226–36. doi: 10.1038/nrmicro2090
34. Yousif MHM, Dhaunsi GS, Makki BM, Qabazard BA, Akhtar S, Benter IF. Characterization of angiotensin-(1-7) effects on the cardiovascular system in an experimental model of type-1 diabetes. *Pharmacol Res.* (2012) 66:269–75. doi: 10.1016/j.phrs.2012.05.001
35. Kassiri Z, Zhong J, Guo D, Basu R, Wang X, Liu PP, et al. Loss of angiotensin-converting enzyme 2 accelerates maladaptive left ventricular remodeling in response to myocardial infarction. *Circ Hear Fail.* (2009) 2:446–55. doi: 10.1161/CIRCHEARTFAILURE.108.840124
36. Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutanen SM, Penninger JM, et al. SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. *Eur J Clin Invest.* (2009) 39:618–25. doi: 10.1111/j.1365-2362.2009.02153.x
37. Luo W, Yu H, Gou J, Li X, Sun Y, Li J, et al. Clinical pathology of critical patient with novel coronavirus pneumonia (COVID-19) List of authors. *Preprints.* (2020). [Epub ahead of print].
38. Carty CL, Heagerty P, Heckbert SR, Jarvik GP, Lange LA, Cushman M, et al. Interaction between fibrinogen and IL-6 genetic variants and associations with cardiovascular disease risk in the cardiovascular health study. *Ann Hum Genet.* (2010) 74:1–10. doi: 10.1111/j.1469-1809.2009.00551.x
39. Mourad JJ, Levy BI. Interaction between RAAS inhibitors and ACE2 in the context of COVID-19. *Nat Rev Cardiol.* (2020) 17:313. doi: 10.1038/s41569-020-0368-x
40. Kuster GM, Pfister O, Burkard T, Zhou Q, Twerenbold R, Haaf P, et al. SARS-CoV2: should inhibitors of the renin-angiotensin system be withdrawn in patients with COVID-19? *Eur Hear J.* (2020) 41:1801–3. doi: 10.1093/eurheartj/ehaa235
41. Gu J, Korteweg C. Pathology and pathogenesis of severe acute respiratory syndrome. *Am J Pathol.* (2007) 170:1136–47. doi: 10.2353/ajpath.2007.061088
42. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. 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
43. Halliday BP, Wassall R, Lota AS, Khalique Z, Gregson J, Newsome S, et al. Withdrawal of pharmacological treatment for heart failure in patients with recovered dilated cardiomyopathy (TRED-HF): an open-label, pilot, randomised trial. *Lancet.* (2019) 393:61–73. doi: 10.1016/S0140-6736(18)32484-X
44. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 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. *Eur J Heart Fail.* (2016) 37:2129–200. doi: 10.1093/eurheartj/ehw128
45. Italian Society of Hypertension. *Farmaci antiipertensivi e rischio di COVID-19. Il comunicato della SIIA | SIIA*. Available online at: https://siia.it/notizie-siia/farmaci-antiipertensivi-e-rischio-di-covid-19-il-comunicato-della-siia/ (accessed April 4, 2020).
46. Italian Society of Cardiology. *GUIDA CLINICA COVID-19 PER CARDIOLOGI.* (2020). Available online at: https://www.sicardiologia.it/public/Documento-SIC-COVID-19.pdf (accessed April 20, 2020).
47. European Society of Cardiology. *Position Statement of the ESC Council on Hypertension on ACE-Inhibitors and Angiotensin Receptor Blockers.* (2020). Available online at: https://www.escardio.org/Councils/Council-on-Hypertension-(CHT)/News/position-statement-of-the-esc-council-on-hypertension-on-ace-inhibitors-and-ang (accessed April 20, 2020).
48. American Heart Association. *HFSA/ACC/AHA statement addresses concerns re: using RAAS antagonists in COVID-19.* (2020). Available online at: https://www.acc.org/latest-in-cardiology/articles/2020/03/17/08/59/hfsa-acc-aha-statement-addresses-concerns-re-using-raas-antagonists-in-covid-19 (accessed April 20, 2020).
49. Italian Society of Pharmacology. *SIF | Documento Informativo della Società Italiana di Farmacologia - Uso di Ace-Inibitori/Sartani ed infezione da COVID-19.* (2020). Available online at: https://www.sifweb.org/documenti/document_2020-03-13_documento-informativo-della-societa-italiana-di-farmacologia-uso-di-ace-inibitori-sartani-ed-infezione-da-covid-19 (accessed April 17, 2020).
50. 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

51. Gnavi R, Demaria M, Picariello Roberta, Dalmasso M, Ricceri F, Costa G. Therapy with agents acting on the renin-angiotensin system and risk of severe acute respiratory syndrome coronavirus 2 infection. *Clin Infect Dis*. (2020) 174:30–3. doi: 10.1093/cid/ciaa634
52. de Abajo FJ, Rodríguez-Martín S, Lerma V, Mejía-Abril G, Aguilar M, García-Luque A, 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
53. 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
54. 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) 22:ciaa624. doi: 10.1093/cid/ciaa624
55. Zhang P, Zhu L, Cai J, Lei F, Qin JJ, 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) 126:1671–81. doi: 10.1161/CIRCRESAHA.120.317242
56. 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
57. 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) 5:1–6. doi: 10.1001/jamacardio.2020.1624
58. 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
59. Yuksel M, Okajima K, Uchiba M, Okabe H. Gabexate mesilate, a synthetic protease inhibitor, inhibits lipopolysaccharide-induced tumor necrosis factor-α production by inhibiting activation of both nuclear factor-κB and activator protein-1 in human monocytes. *J Pharmacol Exp Ther*. (2003) 305:298–305. doi: 10.1124/jpet.102.041988
60. Tamura Y, Hirado M, Okamura K, Minato Y, Fujii S. Synthetic inhibitors of trypsin, plasmin, kallikrein, thrombin, C1r, and C1 esterase. *Biochim Biophys Acta*. (1977) 484:417–22. doi: 10.1016/0005-2744(77)90097-3
61. Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). *Drug Discov Ther*. (2020) 14:58–60. doi: 10.5582/ddt.2020.01012
62. Italian Medicine Agency. *Plaquenil, Summary of Product Characteristics*. Available online at: https://farmaci.agenziafarmaco.gov.it/aifa/servlet/PdfDownloadServlet?pdfFileName=footer_008055_013967_RCP.pdf&retry=0&sys=m0b1l3 (accessed April 8, 2020).
63. Wang M, Cai B, Li L, Lin J, Su N, Yu H, et al. [Efficacy and safety of arbidol in treatment of naturally acquired influenza] - pubmed. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao*. (2004) 26:289–93.
64. Liu X, Huang T, Chen JX, Zeng J, Fan XR, Xu-Zhu, et al. Arbidol exhibits strong inhibition towards UDP-glucuronosyltransferase (UGT) 1A9 and 2B7. *Pharmazie*. (2013) 68:945–50.
65. Wösten-Van Asperen RM, Lutter R, Specht PA, Moll GN, Van Woensel JB, Van Der Loos CM, et al. Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1-7) or an angiotensin II receptor antagonist. *J Pathol*. (2011) 225:618–27. doi: 10.1002/path.2987
66. Italian Medicine Agency. *Losaprex, Summary of Product Characteristics*. Available online at: https://farmaci.agenziafarmaco.gov.it/aifa/servlet/PdfDownloadServlet?pdfFileName=footer_004375_029393_RCP.pdf&retry=0&sys=m0b1l3 (accessed April 8, 2020).
67. Savage PD, Lovato J, Brosnihan KB, Miller AA, Petty WJ. Phase II trial of angiotensin-(1-7) for the treatment of patients with metastatic sarcoma. *Sarcoma*. (2016) 2016:1–7. doi: 10.1155/2016/4592768
68. Chappell MC. Emerging evidence for a functional angiotensin-converting enzyme 2-angiotensin-(1-7)-Mas receptor axis: more than regulation of blood pressure? *Hypertension*. (2007) 50:596–9. doi: 10.1161/HYPERTENSIONAHA.106.076216
69. Khan A, Benthin C, Zeno B, Albertson TE, Boyd J, Christie JD, et al. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit Care*. (2017) 21:234. doi: 10.1186/s13054-017-1823-x
70. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virol J*. (2005) 2:69. doi: 10.1186/1743-422X-2-69
71. 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
72. 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
73. Colson P, Rolain JM, Lagier JC, Brouqui P, Raoult D. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. *Int J Antimicrob Agents*. (2020) 55:105932. doi: 10.1016/j.ijantimicag.2020.105932
74. U.S. National Library of Medicine. *Search of: hydroxychloroquine | Covid-19 - List Results - ClinicalTrials.gov*. (2020). Available online at: https://clinicaltrials.gov/ct2/results?cond=Covid-19&term=hydroxychloroquine&cntry=&state=&city=&dist= (accessed April 6, 2020).
75. Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. *J Crit Care*. (2020) 57:279–83. doi: 10.1016/j.jcrc.2020.03.005
76. Gbinigie K, Frie K. Should chloroquine and hydroxychloroquine be used to treat COVID-19? a rapid review. *BJGP Open*. (2020) 4:bjgpopen20X101069. doi: 10.3399/bjgpopen20X101069
77. Chen J, Liu D, Liu L, Liu P, Xu Q, Xia L, et al. A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19). *J Zhejiang Univ*. (2020) 49:1–10.
78. Gautret P, Lagier J-C, 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
79. Blaising J, Polyak SJ, Pécheur EI. Arbidol as a broad-spectrum antiviral: an update. *Antiviral Res*. (2014) 107:84–94. doi: 10.1016/j.antiviral.2014.04.006
80. Italian Medicine Agency. *AIFA precisa: uso umifenovir su COVID-19 non autorizzato in Europa e USA, scarse evidenze scientifiche sull'efficacia*. (2020). Available online at: https://www.aifa.gov.it/web/guest/-/aifa-precisa-uso-umifenovir-su-covid-19-non-autorizzato-in-europa-e-usa-scarse-evidenze-scientifiche-sull-efficacia (accessed April 5, 2020).
81. Adedeji AO, Severson W, Jonsson C, Singh K, Weiss SR, Sarafianos SG. Novel inhibitors of severe acute respiratory syndrome coronavirus entry that act by three distinct mechanisms. *J Virol*. (2013) 87:8017–28. doi: 10.1128/JVI.00998-13
82. Kao RY, Tsui WHW, Lee TSW, Tanner JA, Watt RM, Huang JD, et al. Identification of novel small-molecule inhibitors of severe acute respiratory syndrome-associated coronavirus by chemical genetics. *Chem Biol*. (2004) 11:1293–9. doi: 10.1016/j.chembiol.2004.07.013
83. Zambelli V, Bellani G, Borsa R, Pozzi F, Grassi A, Scanziani M, et al. Angiotensin-(1-7) improves oxygenation, while reducing cellular infiltrate and fibrosis in experimental acute respiratory distress syndrome. *Intensive Care Med Exp*. (2015) 3:44. doi: 10.1186/s40635-015-0044-3
84. Zou Z, Yan Y, Shu Y, Gao R, Sun Y, Li X, et al. Angiotensin-converting enzyme 2 protects from lethal avian influenza A H5N1 infections. *Nat Commun*. (2014) 5:3594. doi: 10.1038/ncomms4594
85. Gu H, Xie Z, Li T, Zhang S, Lai C, Zhu P, et al. Angiotensin-converting enzyme 2 inhibits lung injury induced by respiratory syncytial virus. *Sci Rep*. (2016) 6:19840. doi: 10.1038/srep19840

86. Patel AB, Verma A. COVID-19 and angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: what is the evidence? *JAMA*. (2020) 323:1769–70. doi: 10.1001/jama.2020.4812
87. Basu R, Poglitsch M, Yogasundaram H, Thomas J, Rowe BH, Oudit GY. Roles of angiotensin peptides and recombinant human ACE2 in heart failure. *J Am Coll Cardiol*. (2017) 69:805–19. doi: 10.1016/j.jacc.2016.11.064
88. Chappell MC. Biochemical evaluation of the renin-angiotensin system: the good, bad, and absolute? *Am J Physiol - Hear Circ Physiol*. (2016) 310:H137–52. doi: 10.1152/ajpheart.00618.2015
89. Santos RAS, Oudit GY, Verano-Braga T, Canta G, Steckelings UM, Bader M. The renin-angiotensin system: going beyond the classical paradigms. *Am J Physiol Hear Circ Physiol*. (2019) 316:H958–70. doi: 10.1152/ajpheart.00723.2018

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OPINION
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Interdisciplinary Model for Scheduling Post-discharge Cardiopulmonary Care of Patients Following Severe and Critical SARS-CoV-2 (Coronavirus) Infection

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INTRODUCTION

As Covid-19 can severely implicate the respiratory and cardiovascular systems, potential pulmonary, and/or cardiovascular sequelae may be anticipated in patients following severe and critical SARS-CoV-2 infection meriting coordinated post-discharge management to identify residual effects and to mitigate potential worsening of pre-existing conditions. According to current literature, 14% of patients with SARS- CoV-2 infection require hospitalization, of these, 5–14% have severe and 2–5% have critical manifestations of infection (1–4). While Covid-19 is known to primarily cause substantial respiratory pathology in hospitalized patients, such as pneumonia (75%) and acute respiratory distress syndrome (ARDS) (15%), it can also result in systemic complications affecting multiple organ systems including the cardiovascular system such as venous and arterial thromboembolic events (10–25%; 31–59% of ICU patients), myocardial injury (20–30%, >25% of critically ill; >55% in those with pre-existing CVD), cardiomyopathy (7–33% of critically ill), arrhythmias (17%, 44% of ICU patients), and cerebrovascular disease (up to 8%). Additionally, acute kidney injury (9%), hepatocellular injury (19%), hyperglycaemia and ketosis, ocular symptoms, and dermatologic complications have been reported (2, 4–7).

Although long-term outcomes of patients surviving severe SARS-CoV-2 infection are unknown, these patients have the potential to suffer substantial sequelae comparable to those in patients surviving ARDS, sepsis, and other acute illnesses. Survival from sepsis, for example, is associated with increased risks for mortality up to 2 years, new cognitive impairment, new physical disability, recurrent infections, and continued health deterioration (4). Long-term sequelae observed in survivors of severe ARDS during H1N1 influenza include significant exertion dyspnea, decreased diffusion capacity across the blood-gas barrier, as well as reduced quality of life including reduced exercise capacity, anxiety, depression, and/ or development of post-traumatic stress disorder (8).

Follow-up CT imaging at 4 weeks in patients with Severe Acute Respiratory syndrome (SARS) showed that one third of patients with persistent respiratory symptoms had findings of fibrosis, including interlobular and intralobular reticulation, traction bronchiectasis and, more seldomly, honeycombing (9). In another CT study of convalescing SARS patients 51 days after symptom

start, follow-up CT showed air trapping (92%) ground-glass opacities GGO (90%) and reticulation (70%). While GGO and reticulation resolved by 5 months, air trapping caused by damage to ciliated respiratory epithelium persisted in 80% of patients (10). In Middle-East Respiratory Syndrome (MERS), 33% of patients showed evidence of lung fibrosis, affecting primarily the elderly, patients with prolonged ICU stays and those with greater lung involvement during the acute phase of infection (9).

With respect to cardiac sequelae following severe respiratory disease in recovered SARS patients, cardiac impairment was observed by echocardiography studies in short-term 30-days follow-up, especially in more critically ill patients (10). In the majority of patients with community-acquired pneumonia (CAP), cardiac injury was seen in 30-days follow-up, likely caused by myocardial oxygen supply and demand mismatch as well as an activated inflammation/coagulation system (11).

The European Society of Cardiology recognizes that SARS-CoV-2 infection has major implications on the cardiovascular system and that patients within the context of Covid-19 have increased risk of morbidity and mortality, especially those with established cardiovascular disease, common in patients with severe infection (12). Severe and critical SARS-CoV-2 infection is associated with acute myocardial injury, cardiac arrhythmias, likely caused by infection-induced myocarditis or ischemia, all with potential for new disease development. Following pneumonia, hypercoagulability, and systemic inflammatory activity can persist thus exposing patients to elevated long-term CV risk, justifying surveillance (12). An interdisciplinary model for scheduling follow-up care may serve as a practical tool for healthcare professionals to ensure that any infection-related sequelae following hospitalization for severe SARS-CoV-2 infection are identified and appropriately managed.

METHODOLOGY

European Center for Disease Prevention and Control reports (ECDC), Center for Disease Control (CDC USA) and National Institutes of Health (NIH USA) reports, WHO Interim Guidance Reports, and current 2020 PubMed articles evaluating SARS-CoV-2 virus manifestations, diagnosis, severity, and discharge criteria of patients with confirmed Covid-19 were reviewed. PubMed Articles describing short and long-term outcomes in SARS, MERS, pneumonia, acute respiratory syndrome ARDS, and sepsis were evaluated.

RATIONALE

The ECDC published a technical report in March 2020 comparing diverging international discharge and de-isolation criteria of patients hospitalized with Covid-19 found in national guidelines of Italy, China, Singapore, and the USA, and offered its own recommendations for discharge based on:

- Clinical criteria (e.g., no fever >3 days), improved respiratory symptoms, pulmonary imaging evidencing obvious absorption of inflammation, clinical assessment
- Laboratory evidence of SARS-CoV-2 clearance in respiratory samples, 2–4 negative RT- PCR tests for respiratory tract samples (nasopharynx and throat swabs with sampling interval $\geq$24 h) and if possible, serology with appearance of specific IgG (13).

With respect to post-discharge follow-up care, however, guidance is scant. The CDC China recommends that patients have follow-up visits 2 and 4 weeks after discharge, the National Centre for Infectious Diseases Singapore recommends clinic follow-up if indicated and daily wellness calls until day 14 after exposure, and the ECDC recommends 14 days of further isolation following discharge with regular health monitoring such as follow-up visits and phone calls, although specific guidance with respect to follow-up scope and content is not yet given (13).

The WHO report *Interim Guidance: Clinical Management of Covid 19,* released 27 May 2020 however anticipates potential sequelae in patients with severe and critical SARS-CoV-2 infection following treatment with mechanical ventilation, sedation, and/or prolonged bed rest based on evidence from general critical care populations. Post-intensive care syndrome (PICS) and severe respiratory illness may result in "a range of impairments including (but not limited to) physical deconditioning, reduced exercise tolerance, persisting fatigue, difficulties with activities of daily living, respiratory, swallow, cognitive, and mental health impairments" (14). According to WHO data, older people and patients of all ages with chronic diseases may be most susceptible to its impacts, including some patients recovering from severe COVID-19 who did not require admission to an ICU. The WHO recommends that patients must be referred for tailored inpatient, outpatient or community-based follow-up from post-acute to long term as indicated according to patient needs, with involvement of primary health care providers, relevant specialists, rehabilitation professionals, mental health, and psychosocial providers and social care services for coordinated care (14). A coordinated post-discharge care concept for patients surviving Covid-19 is therefore warranted to identify any cardiopulmonary sequelae and to mitigate possible worsening of preexisting disease following severe and critical SARS-Cov-2 infection. The literature has shown benefit of a well-structured transition phase to improve treatment outcomes and reduce readmission rates in management of other diseases such as heart failure, which may also develop in some patients following the infection (15–17).

While data examining residual effects after recovery from Covid-19 are still sparse, a number of sequelae especially affecting lung and heart function can be extrapolated from current literature. Initially defined by its pulmonary pathology and likely mediated via binding of SARS-CoV2 to ACE2 on

TABLE 1 | Interdisciplinary model for scheduling post-discharge cardiopulmonary care following severe and critical SARS-CoV-2 infection.

Discharge	❖ **Scheduling for patients following severe or critical SARS-CoV-2 infection** ❖ **Scheduling for patients following moderate SARS-CoV-2 infection with exacerbation/worsening of preexisting comorbidities**
Medical professionals at discharge	• Schedule lab work (see parameters below) • Schedule chest CT scan for 1–2 months post discharge (unenhanced low dose CT), at index hospital if possible; chest x-ray (CXR) if CT not available • Schedule follow-up with **General Practitioner** at 1–2 months post-discharge • Evaluate, prescribe, discuss dischargemedications, any O_2 use, care plan / appointments **with patient**, provide written instructions • If transfer to inpatient rehabilitation or long-term care facility, provide written instructions and care plan to managing physician • Involve **social worker**/**psychologist/nurse** where needed to address short, intermediate, long-term care/support (e.g., PTSD, psychological disorders, care provision) • Discuss potential participation and consent patient for any national/international registries or clinical trials and schedule appointments per protocol
1–2 months post-discharge	❖ **Diagnostic Testing, Care Coordination by GP/Internist,** ❖ **Referral and continued Follow-up when indicated**
Laboratory	• Complete Blood Count, C-Reactive Protein, LDH, AST/ALT, Urea, Glucose, Thrombin Time, Fibrinogen, Ferritin • Cardiac Biomarkers: CK, CK-MB, Troponin, NT-pBNP • For diabetes patients, also: Hemoglobin A1c • For Cancer patients, additional testing as instructed by managing oncologist
Radiologist	• Chest CT, or if not available, chest x-ray (CXR)
General practitioner/internist	• Clinical evaluation of symptoms (dyspnea, fatigue, psychological disorders) • Auscultation (determine signs of pulmonary fibrosis), • Oxygen saturation • ECG • Evaluation of laboratory, radiology and clinical findings, discussion with patient • Referral for further specialist examinations (e.g., **pulmonologist, cardiologist**) if indicated • Referral to **neurologist**, **nephrologist, endocrinologist** by suspicion of sequelae • Evaluate, prescribe, discuss discharge medications, any O_2 use, and care plan with patient, provide written instructions • Involve social worker/psychologist if further support needed
Pulmonologist (if indicated)	• CT evaluation and discussion with patient • Physical exam: signs and symptoms • Lung function test • 6 min walk test • Blood-gas test • Reevaluation of medications, O_2 use • Determine need for rehabilitation or intermediate/long-term care • Address primary/secondary prevention measures where applicable • Plan 6 and 12 month follow-up by any evidence of reduced functional capacity • Communicate findings and treatment plan to patient and general practitioner
Cardiologist (if indicated)	• Physical exam: signs and symptoms • ECG • Transthoracic echocardiography • Reevaluation/adjustments of medications • For patients with signs of heart failure: enrollment in heart failure program; for all HF patients: evaluate need for visiting heart failure nurse/rehabilitation program • For patients with arrhythmias, plan further evaluation (i.e., Holter monitoring, event recorder) • Address primary/secondary prevention measures where applicable • Schedule follow-up if appropriate • Communicate findings and treatment plan to patient and general practitioner
After 2 months	**Follow-up as needed and at the discretion of managing specialists**

CT, computed tomography; CXR, chest X-ray; LDH, lactate acid dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, Creatinkinase; CK-MB, Creatinkinase-MB; NT-pBNP, N-terminal pro b-type Natriuretic Peptide; O_2, oxygen; QoL, Quality of Life.

lung epithelia, Covid-19 may have significant effects on long term-outcome with respect to pulmonary function. According to Shi et al., lung abnormalities such as bilateral ground-glass opacities progressing to or coexisting with consolidations were observable in CT imaging within 1–3 weeks of SARS-Cov-2 infection (18). Pulmonary fibrosis may occur due to scarring of the lung tissue, as was observed in SARS and MERS, potentially causing significant reduction in lung function and exercise capacity (19, 20), thus warranting follow-up in surviving Covid-19 target populations. Thoracic imaging with chest radiography (CRX) and computed tomography (CT) are key tools for pulmonary disease diagnosis and management (21). CT, however, is more sensitive for detecting parenchymal lung disease, disease progression, and alternate diagnoses. Therefore, in patients with reduced lung capacity and radiological signs of fibrosis at 1–2 months, continued follow-up according to ATS/ACCP guidelines will be required.

Recent publications have also shown direct endothelial cell involvement of vascular beds of different organs by the SARS-CoV-2 virus (22). This should be considered as a reason for cardiovascular events, endotheliitis of lung, heart, kidney, and liver, as well as liver cell necrosis. Covid-19 is characterized by coagulation activation with a high rate of venous and arterial thromboembolic events, including venous thromboembolism, pulmonary embolism, disseminated intravascular coagulation, or cardiovascular events (23). Coagulation testing is therefore warranted and subsequent therapy may be indicated.

Covid-19 may induce new cardiac pathologies and/or exacerbate underlying cardiovascular disease (24). Thus, cardiologists will aim to evaluate residual cardiovascular effects and myocardial injury following SARS-Cov-2 infection. Systemic inflammatory response coupled with localized vascular inflammation may lead to plaque rupture and activation of coagulation cascades, endangering patients for acute coronary syndromes (25). Heart failure may develop following myocarditis, sepsis, or multi-organ failure during infection, or may be caused by treatment side effects. Inflammation and ACE2 downregulation with ensuing endothelial dysfunction can translate into diastolic dysfunction, while hypoxemia may lead to right ventricular dysfunction indicative of myocardial injury. Thus, transthoracic echocardiography may be considered to evaluate left and right ventricular global function, any regional dysfunction, end-diastolic cavity dimensions as well as pericardial thickening or effusion (26). Additionally, cardiac MRI may better reflect structural pathologies of inflammatory myocardial damage. Cardiac arrhythmias, possibly caused by metabolic disarray, hypoxia, neuro-hormonal, or inflammatory stress, have also been associated with the infection (27) and if present will need follow-up evaluation. Cardiologists should be aware of the risk for development of chronic thromboembolic pulmonary hypertension in patients who experienced pulmonary embolism during infection (28). Follow-up is also an opportunity to address primary and secondary prevention strategies for cardiovascular risk control in all patients.

As the short, intermediate and long-term effects of Covid-19 are unknown, patients should be encouraged to participate in national and international registries or clinical studies to facilitate study of this disease. The monitoring of immune effects is also of particular importance.

POST-DISCHARGE CARE MODEL

The proposed interdisciplinary model for scheduling post-discharge cardiopulmonary care of patients following SARS-Cov-2 infection (see **Table 1**) may serve as a practical guide for healthcare professionals to ensure that patients surviving severe and critical infection receive adequate cardiopulmonary follow-up care as we learn more about the residual and potentially chronic effects of the SARS-Cov-2 infection.

Target patient populations for post-discharge Covid-19 follow-up care include:

- Patients who experienced **severe illness*** defined as individuals who had respiratory frequency >30 breaths per minute, SpO_2 < 94% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) <300 mmHg, or lung infiltrates 50% (e.g., patients treated at an ICU requiring invasive ventilation or CPAP during SARS-CoV-2 infection)
- Patients who experienced **critical illness***, defined as individuals who had respiratory failure, septic shock, and/or multiple organ dysfunction (e.g., patients treated at an ICU requiring ECMO during SARS-CoV-2 infection)
- Patients with chronic conditions (e.g., COPD, cardiomyopathy, coronary artery disease, cancer, chronic kidney disease, hepatic disease, and uncontrolled diabetes) in the presence of disease exacerbation or progression during/following moderate*, severe and critical SARS-CoV-2 infection, where moderate infection is defined as individuals with evidence of lower respiratory disease by clinical assessment, imaging and a saturation of oxygen (SpO_2) > 94% on room air at sea level.

  * denotes NIH definitions of moderate, severe and critical illness (5).

As no guidelines on the timing of follow-up care for Covid-19 patients yet exist, this model schedules follow-up to occur at 1–2 months post-discharge based on several considerations. According to the previously cited radiological studies evaluating sequelae in patients following SARS and MERS infection, radiological follow-up was performed 1–2 months after start of infection (9, 10). In patients with confirmed pulmonary fibrosis, the American Thoracic Society recommends mid- to long-term follow up in 4–6-months intervals (29). With respect to cardiac involvement and the timing of follow-ups, the ACCF/AHA Guideline for the Management of Heart Failure was consulted with respect to recommendations for transition of care following hospitalization for acute cardiac decompensation. A follow-up visit within 7–14 days and/or a telephone follow-up within 3 days of discharge for acute cardiac decompensation is deemed a Class IIa recommendation (17). The 2016 *European Society of Cardiology Guidelines for the diagnosis and treatment of acute and chronic heart failure* detail the benefits of regular monitoring

of heart failure patients, especially during periods of instability or for optimization of medications, noting benefits especially in older patients. Although timing of follow-up is not detailed, the ESC recommends provision of written action plans and prescheduling follow-up appointments shortly after discharge of patients with acute heart failure to reduce readmission rates (16). Therefore, scheduling transition care for Covid-19 patients potentially suffering from residual cardiopulmonary effects of the infection shortly after discharge is merited. The planning of post-discharge evaluations in the dynamic context of a pandemic, however, must be adapted with respect to post-discharge isolation recommendations, logistics, resource utilization, and health care system overburden. Thus, evaluation within 1–4 weeks post discharge at the height of a pandemic may not feasible for many patients. The model below suggests scheduling follow-up at 1–2 months, if not sooner, according to need and availability.

CARE PATHWAY

Hospital discharge personnel coordinate follow-up laboratory and radiological examinations, schedule a subsequent appointment with the patient's general practitioner or internist, and provide patient with written instructions. The patient's primary care physician or internist will serve as follow-up care coordinator. The interdisciplinary model provides guidance for specialist referral and testing dependent upon the patient's signs and symptoms, as well as radiological and laboratory findings. Due to the association of a more severe course of Covid-19 in those patients with underlying comorbidities, especially those with concomitant cardiovascular and pulmonary diseases, timely follow-up is imperative to identify any worsening of conditions and to initiate or adapt guideline-recommended therapies.

COST ANALYSIS

We estimate that the costs per patient of the basic follow-up (Radiology, Lab, GP) to be € 1,026 according to the Austrian tariff system. In patients requiring specialist evaluation, an additional € 249 for pulmonary consultation and € 527 for cardiological consultation are estimated. However, cost-effectiveness cannot yet be determined until intermediate and long-term data become available for analysis.

CONCLUSION

Short, intermediate and long-term effects following severe and critical SARS-CoV-2 infection are unknown, and significant sequelae may be expected, especially in patient populations experiencing ARDS, sepsis, and/or multiple organ dysfunction, as well as patients with exacerbation or progression of preexisting pulmonary or cardiovascular disease. Coordinated post-discharge management of Covid-19 patients is essential to identify and manage potential pulmonary or cardiovascular sequelae and mitigate worsening of pre-existing conditions following infection. This interdisciplinary model for scheduling follow-up care may serve as a practical tool for healthcare professionals to ensure that patients receive adequate treatment and post-discharge care following hospitalization for severe and critical SARS-CoV-2 infection.

AUTHOR CONTRIBUTIONS

KK wrote the manuscript. ML, LM, AE, HS, JT, and HM revised the manuscript. UH provided supervision. BL and AD provided supervision and revised the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Stokes EK, Zambrano LD, Anderson KN, Marder EP, Raz KM, El Burai Felix S, et al. Coronavirus disease 2019 case surveillance—United States, January 22–May 30, 2020. *MMWR*. (2020) 69:759–65. doi: 10.15585/mmwr.mm6924e2
2. 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). doi: 10.1001/jama.2020.12839. [Epub ahead of print].
3. 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
4. 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
5. COVID-19 Treatment Guidelines Panel. *Coronavirus Disease 2019 (COVID-19) Treatment Guidelines*. National Institutes of Health. (2020). Available online at: https://www.covid19treatmentguidelines.nih.gov/ (accessed July 20, 2020).
6. Center for Disease Control. *Interim Clinical Guidance for Management of Patients with Confirmed Coronavirus Disease (COVID-19) National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases*. Available online at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html#clinical-course (accessed July 20, 2020).
7. Clerkin K, Fried J, Raikhelkar J, Sayer G, Griffin J, Masoumi A, et al. Covid-19 and cardiovascular disease. *Circulation*. (2020). 141:1648–55. doi: 10.1161/CIRCULATIONAHA.120.046941
8. Luyt C, Combes A, Becquemin MH, Beigelman-Aubry C, Hatem S, Brun A-L, et al. Long-term outcomes of pandemic 2009 influenza A(H1N1)-associated severe ARDS. *Chest*. (2012) 142:583–92. doi: 10.1378/chest.11-2196
9. Hosseiny M, Kooraki S, Gholamrezanezhad A, Reddy S, Myers L. Radiology perspective of coronavirus disease 2019 (COVID-19): lessons from severe acute respiratory syndrome and middle east respiratory syndrome. *Am J Roentgenol*. (2020) 214:1078–82. doi: 10.2214/AJR.20.22969
10. Li SS, Cheng C-w, Fu C-l, Chan Y-h, Lee M-p, Chan JW-m, et al. Left ventricular performance in patients with severe acute respiratory syndrome: a 30-days echocardiographic follow-up study. *Circulation*. (2003) 108:1798–803. doi: 10.1161/01.CIR.0000094737.21775.32
11. Frencken JF, van Baal L, Kappen TH, Donker DW, Horn J, van der Poll T, et al. Myocardial injury in critically ill patients with community-acquired pneumonia. A cohort study. *Ann Am Thorac Soc*. (2019) 16:606–12. doi: 10.1513/AnnalsATS.201804-286OC
12. The European Society for Cardiology. *ESC Guidance for the Diagnosis and Management of CV Disease during the COVID-19 Pandemic*. (2020). Available online at: https://www.escardio.org/Education/COVID-19-and-Cardiology/ESC-COVID-19-Guidance (accessed June 10, 2020).

13. European Centre for Disease and Control. *Technical Report: Novel Coronavirus (SARS-CoV-2) Discharge Criteria for Confirmed Covid-19 Cases-When Is it Safe to Discharge Cases Form the Hospital or End Home Isolation?* Available online at: https://www.ecdc.europa.eu/en/publications-data/covid-19-guidance-discharge-and-ending-isolation (accessed July 20, 2020).
14. World Health Organization. *Guidance Document: Clinical Management of Covid-19*. (2020). Available online at: https://www.who.int/publications/i/item/clinical-management-of-covid-19 (accessed July 20, 2020).
15. Mueller C, Bally K, Buser M, Flammer AJ, Gaspoz J-M, Mach F, et al. Roadmap for the treatment of heart failure patients after hospital discharge: an interdisciplinary consensus paper. *Swiss Med Wkly*. (2020) 150:w20159. doi: 10.4414/smw.2020.20159
16. Ponikowski P, Voors A, Anker S. 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 a special contribution of the Heart failure Association (HFA) of the ESC. *Eur Heart J*. (2016) 37:2129–200. doi: 10.1093/eurheartj/ehw128
17. Yancy C, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Colvin MM, et al. 2013 ACCF/AHA guideline for the management of heart of heart failure. A report from the American College of Cardiology Foundation/Amarican Heart Association Task Force on Practical Guidelines. *Circulation.* (2013) 136:e137–161. doi: 10.1161/CIR.0000000000000509
18. 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*. (2020) 20:425–34. doi: 10.1016/S1473-3099(20)30086-4
19. Venkataraman T, Frieman MB. The role of epidermal growth factor receptor (EGFR) signaling in SARS. *Antiviral Res*. (2017) 143:142–50. doi: 10.1016/j.antiviral.2017.03.022
20. Hui D, Ko F, Chan D, et al. The long-term impact of severe acute respiratory syndrome (SARS) on pulmonary function, exercise capacity and quality of life in a cohort of survivors. *CHEST J*. (2005) 128 doi: 10.1378/chest.128.4_MeetingAbstracts.148S-b
21. 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) 296:172–80. doi: 10.1148/radiol.2020201365
22. Varga Z, Flammer A., 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
23. 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
24. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of coronaviruses on the cardiovascular system: a review. *JAMA Cardiol*. (2020). doi: 10.1001/jamacardio.2020.1286
25. Hansson GK, Libby P, Tabas I. Inflammation and plaque vulnerability. *J Intern Med*. (2015) 278:483–93. doi: 10.1111/joim.12406
26. Moreo A, Pontone G, Gimelli A. *European Association of Cardiovascular Imaging, EACVI Webinar on COVID-19*. (2020). Available online at: https://www.escardio.org/Education/E-Learning/Webinars/EACVI-Webinar-on-COVID-19 (accessed April 30, 2020).
27. Driggin, E, Madhavan, M, Bikdeli, B, Chuich T, Laracy J, Biondi-Zoccai G, et al. Cardiovascular considerations for patients, health care workers and health systems during the coronavirus disease 2019 /Covid-19 pandemic. *JACC*. (2020) 75:31. doi: 10.1016/j.jacc.2020.03.031
28. Poissy J, Goutay J, Caplan M, Parmentier E, Duburcq T, Lassalle F, et al. Pulmonary embolism in COVID-19 patients: awareness of an increased prevalence. *Circulation*. (2020) 142:182–6. doi: 10.1161/CIRCULATIONAHA.120.047430
29. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management an official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respiratory Crit Care Med.* 183:788–824. doi: 10.1164/rccm.2009-040GL

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OPINION
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Innovation in Precision Cardio-Oncology During the Coronavirus Pandemic and Into a Post-pandemic World

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INTRODUCTION

Almost 2 million new cancer diagnoses will be made and more than 600,000 cancer deaths will occur in 2020, the equivalent of 5,000 new cases and 1,600 deaths daily (1). Juxtaposed with these staggering numbers is the prevalence of ∼17 million cancer survivors in the United States, with a projected estimate of 26 million in 2040 (2); advances in cancer treatments have significantly improved survival across cancers. With growing numbers of survivors comes a growing number of individuals at risk for or living with higher rates of cardiovascular disease than in the general population. In fact, cardiovascular disease is a leading cause of death in cancer survivors, second only to cancer recurrence or the development of new primary cancers (3). Consequently, Cardio-Oncology has emerged as a new field of medicine to specifically address cardiovascular care of cancer patients and survivors, with a particular focus on prevention.

Reminiscent of cardiovascular toxicities from cancer therapies, the recent coronavirus disease of 2019 (COVID-19) pandemic is a clear example of how cardiotoxicities can arise unexpectedly and how adaptable clinicians need to be to deal with a constant flow of new cardiotoxic agents and their complications. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has arisen as an emergent cardiotoxic agent, underlying COVID-19. By July 2020, more than 18 million confirmed cases and 600,000 deaths had been reported globally (4). In positive cases, direct and indirect cardiovascular (CV) injury has been noted as a prominent feature (5, 6), mediated by hypoxia, inflammation, demand ischemia, microvascular dysfunction, or thrombosis (7–10). Around the world, our patients have been physically and socially distancing themselves from others and avoiding physical entrance of health care facilities, in order to limit exposure in COVID-19. Correspondingly, health care institutions have restricted non-emergent in-person visits, to curb the rates of morbidity and mortality from COVID-19. Individuals with known CV disease or risk factors have been at greater risk of morbidity and mortality in COVID-19 (11–16), as is similar in Cardio-Oncology (17). Therefore, there is an urgent need for various avenues of innovation to predict cardiovascular risk and customize preventive, diagnostic, and management care plans in the setting of cancer therapies, especially during the pandemic and beyond. Here, we briefly describe forms of innovation implemented during the pandemic, as well as innovative tools being explored for utility beyond the pandemic (**Figure 1**).

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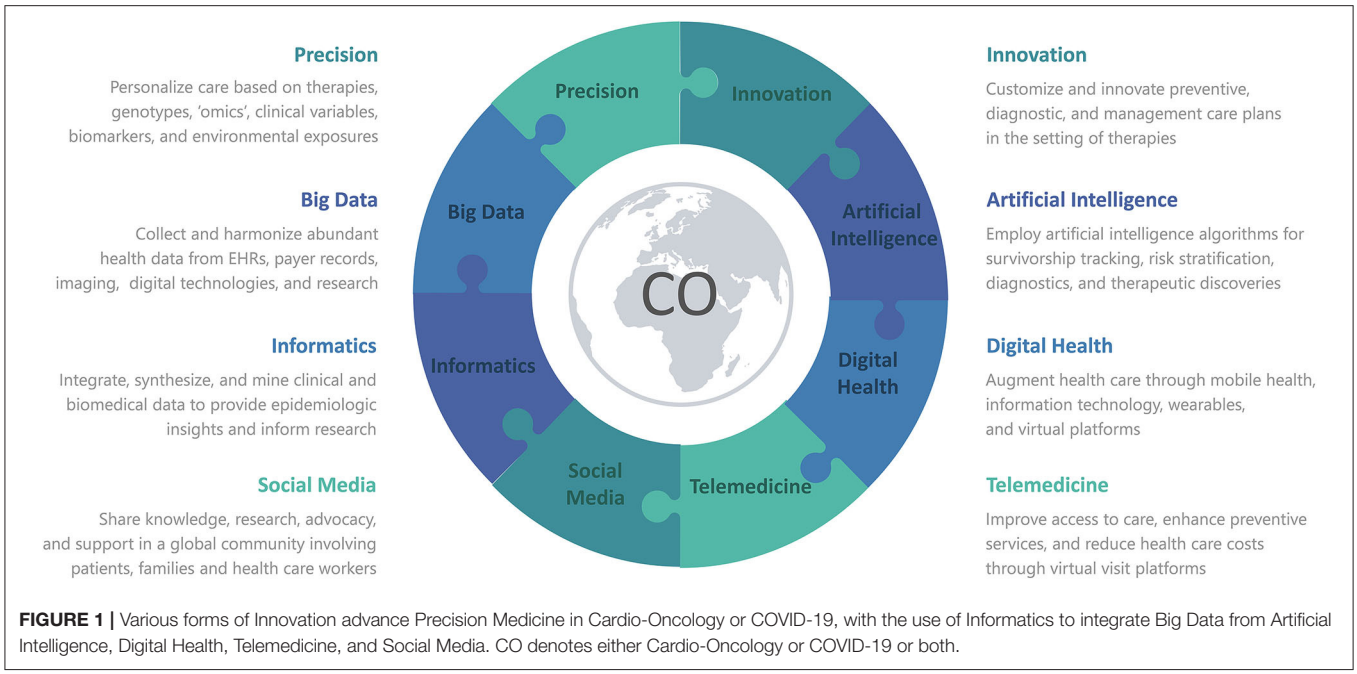

FIGURE 1 | Various forms of Innovation advance Precision Medicine in Cardio-Oncology or COVID-19, with the use of Informatics to integrate Big Data from Artificial Intelligence, Digital Health, Telemedicine, and Social Media. CO denotes either Cardio-Oncology or COVID-19 or both.

INNOVATION DURING THE PANDEMIC

Digital Health

Digital health technologies include mobile health (mHealth), wearable devices, health information technologies, wireless technologies, virtual platforms and applications, telehealth, telemedicine, artificial intelligence, machine learning, and personalized medicine, with a common goal of improving health care outcomes and efficiency (18). With more and more personalized health and lifestyle information available through digital technologies, care providers are better able to monitor patients' conditions in real time or by retrieving remote data recently stored by patients' local devices, identify treatment side effects, and personalize prevention and intervention strategies. Digital technologies can also empower and engage patients to proactively monitor their health while preventing unnecessary hospital visits, which is especially critical in times of a pandemic such as COVID-19 (19). With the implementation of shelter-in-place and subsequent rapid adaptation of virtual visits during this COVID-19 outbreak, the ability to remotely monitor patients' clinical conditions through digital technologies has become more important than ever.

Remote monitoring can enhance our care of cancer patients and survivors. For example, a wearable cardiac rhythm monitoring device such as an Apple watch can detect abnormal heart rates or rhythms (20). As atrial fibrillation is a common side effect of various cancer therapies, including multiple classes of novel tyrosine kinase inhibitors, the ability to detect this rhythm abnormality early and accurately through a wearable cardiac rhythm monitoring device would have an important impact in the ongoing care, as well as future treatment decisions, for cancer patients (21). Abnormal rhythm strips detected from these devices can now be shared and reviewed by the care team, which can potentially alter the treatment course and prevent undesirable toxicities. Another example is virtual cardiac rehabilitation and monitoring (22). Cancer therapies such as doxorubicin can cause myocardial injury and cardiac dysfunction, requiring close monitoring and preferably a tailored rehabilitation program as patients work to recover (23). Virtual rehab programs enable remote collection and evaluation of health data such as activity levels, blood pressures, heart rate/rhythms, and weight, which can be reviewed and acted upon when necessary by health care providers, allowing cancer patients and survivors to safely and efficiently recover from their cardiac complications. This has been of particular importance during COVID-19 pandemic, as many have avoided or limited outdoor physical activities. Guided virtual indoor rehabilitation would allow cancer patients and survivors to continue physical conditioning and rehabilitation and thereby remain physically active during the pandemic.

Digital technologies can provide the unique ability to quickly scale to larger populations with less time, money, and resources, and thereby facilitate near real-time data insights that allow for point-of-service execution (24). These technologies will be critical in caring for cancer patients and survivors, as their numbers continue to increase, with more cancer therapies and related cardiotoxicity profiles dynamically changing daily.

Telemedicine

Telemedicine or telehealth is the delivery of healthcare at a distance utilizing various technology platforms. Health care systems have recently devoted increased resources to implementation of telemedicine or telehealth services during the pandemic, building upon prior goals of improving access to specialty care, enhancing preventive services, reducing health care costs, and improving patient and provider safety

and satisfaction (25, 26). Numerous platforms have been actualized (27), including those embedded within electronic health records (e.g., In-Touch through EPIC) or third-party vendors such as *Doxy.me* or Zoom. Many of the software solutions are cloud-based, accessible (requiring only a desktop, tablet, or smartphone), and free, and have prioritized being HIPAA (Health Insurance Portability and Accountability Act)-compliant. However, security concerns have arisen with some vendors, leading to more careful attention to cybersecurity to enable telemedicine. Indeed, to facilitate wide-spread adoption of telemedicine, great emphasis on protection of patient information through cybersecurity technology will be key, in tandem with the persistence of government-supported regulations and initiatives.

Adoption of these platforms has been expedited during the pandemic to dramatically reduce in-person clinical visits and conform to social distancing (28). The US federal government has taken steps to support rapid and widespread utilization of telemedicine by allowing cross-state accreditation, developing new telemedicine billing codes, and temporarily reducing strict privacy restrictions while still protecting patients and providers (29). As a result, practices across the country converted to virtual clinics in a matter of weeks. This conversion has been especially important for our cardio-oncology patients, who are particularly vulnerable, given their high cardiovascular disease burden and immunocompromised states placing them at high risk for COVID-19 (30). Cardio-oncology, which relies heavily on the patient history and our understanding of cancer therapy regimens, is ideally suited to make the transition to telemedicine.

A recent report described the virtual adaptation of a Cardio-Oncology clinic (31). Suggestions for ensuring a successful patient-centered telemedicine visit include making eye contact with the patient, thanking the patient for inviting the provider into their home, and intentionally offering an excellent "webside" manner. It may become commonplace for initial cardio-oncology consultations to occur via a virtual platform, with follow-up visits (e.g., for reports on home blood pressures) occurring via telephone or secure messaging. Telemedicine could optimize cardio-oncologic care with (i) three-way video or teleconferences enabling the patient/oncologist/cardio-oncologist to collaboratively initiate treatment plans and monitoring algorithms similar to virtual multidisciplinary tumor boards, (ii) follow-up visits to monitor for hypertension and review cardiac function on surveillance imaging in patients on active cancer therapy, and (iii) access points to specialized cardio-oncologist expertise for oncologists in the community (32). While COVID-19 has exposed many limitations in our healthcare system, the expansion and integration of telemedicine in clinical practice will undoubtedly continue to play a larger role than ever before (33), and we are well-poised in cardio-oncology to help lead the way and benefit from this widespread adoption. The Association of American Medical Colleges has submitted a letter to the Centers for Medicare and Medicaid Services to appeal for the permanence of the widescale telemonitoring provisions made during the pandemic[1]. Bipartisan senators and other groups have also submitted similar letters in their respective spheres. With support from the senate and other governmental bodies, telemedicine will likely prevail after the pandemic.

Social Media

Social media provides an incredible opportunity for healthcare workers and patients and their families to share and exchange knowledge, research, and advocacy, and support in a global community. Spreading education and awareness on social media can propagate messages for prevention and disseminate discoveries and innovation (34–36). Online resources provide timely and timeless sources of information that can have tremendous impact for patients and health professionals if curated appropriately and accurately.

Social distancing during the COVID-19 pandemic has led to enhanced experiences of social networking online, as both patients and healthcare workers reached out to strengthen community and further buttress knowledge, for example, on Facebook (Facebook, Inc.; www.facebook.com) and Twitter (Twitter, Inc.; www.twitter.com) (37–42). Community and sharing of information were developed by patients among each other, healthcare workers among each other, and with cross-pollination between the two sets of communities as healthcare workers themselves became patients in the pandemic.

Social media integrated with the rise of telemedicine or telehealth, with creation of the hashtag #TelemedNow on Twitter (43), with associated twitter chats and threads. Individuals from various public and private healthcare sectors joined in the real-time discussions to share stories, successes, and challenges from implementing telemedicine or telehealth in response to COVID-19.

At no point did the impact of social media wane during the COVID-19 pandemic. In fact, social media became even more important for innovation, information, and prevention. Preventive Cardio-Oncology, Precision Cardio-Oncology, and other Cardio-Oncology tweets would spread across Twitter before the pandemic. These messages continued throughout the time of COVID-19, as preventive and innovative cardio-oncologic care of our patients remained of paramount value. Several pandemic-related Cardio-Oncology papers have been rapidly published, including one on the role of telehealth (31). Within a few hours, this paper was being disseminated on social media, to be assessed and validated or rebutted by healthcare workers and patients alike. Cardio-Oncology can learn much from the time of COVID-19. Rapid and persistent propagation of information can place relevant details in the palms of cancer patients and survivors and their healthcare providers in real-time. Such innovation should help protect the hearts and wellness of our patients and clinicians.

[1]https://www.aamc.org/system/files/2020-05/ocomm-hca-aamcletterto CMS5132020.pdf

INNOVATION BEYOND THE PANDEMIC

Artificial Intelligence

Much of digital health is driven by artificial intelligence. Remote monitoring, wearables, mobile health (mHealth), voice apps, voice analysis, and drones all depend on the simulation of human intelligence. All of these components can be useful in both the COVID-19 pandemic and the practice of Cardio-Oncology. Many of these technologies are also being explored for various scenarios in cardiology (44–51), and have great clinical utility for cardio-oncology and COVID-19. Remote monitoring from wearable biosensors and mHealth is being investigated to improve outcomes in heart rhythm and heart failure and other cardiovascular conditions (44, 46–50), and may have utility for COVID-19 (19, 52–58) and Cardio-Oncology (59–61). Voice apps and voice analysis have shown promise in cardiology for heart failure, ischemic heart disease, pulmonary hypertension, and other forms of cardiovascular disease (45, 62–64), as well as cardio-oncology (65), and have been considered for COVID-19. Drones built on artificial intelligence are being used to deliver healthcare equipment, medicines, personal protective equipment, and food, especially to remote areas with high rates of illness with COVID-19, and are also being dispatched to dense urban locations to urge pedestrians to maintain social distancing (66–68). Similar drones could be used to transport healthcare equipment, medicines, and supplies to cancer patients and survivors with limited mobilities and care support. Particularly in rural America, where advanced cancer and heart care services are limited (69), drones may facilitate delivery of point-of-care equipment and specialty medicines recommended by cardio-oncologists following remote assessment of cancer patients and survivors through virtual care.

Artificial intelligence algorithms could also be used to track cancer survivors and detect any early signs of cardiovascular risk features, saving lives of those who fought and overcame cancer years before. Other relevant AI applications currently being explored include (1) *in silico* screening to develop novel or repurposed therapeutics, (2) patient tracking by location or geography, (3) online voice apps on smartphones, tablets, and smart speakers to promote drug compliance as well as screen for new symptoms or disseminate educational information, and (4) big data predictive analytics to enhance prediction of disease incidence, severity, spread, and recovery (42, 70–80). There is a myriad of lessons to be learned from incredible technological progress being made during these epic times. The algorithms created or adapted for the era of COVID-19 should remain available for use and wide application in medicine, and especially in cardio-oncology, far beyond the pandemic.

Artificial intelligence has also been integrated with social media and interaction during the pandemic (42, 81). Twitter chatter has been monitored to assess individuals' self-reports of COVID-19 symptoms, testing experience, and recovery from illness (81). Gaps in care for symptomatic individuals have been revealed, due to limited testing capacity, and this has likely compromised accurate case counts of COVID-19 positivity at the city, state, and national, and global level. Interactive chatbots have utilized artificial intelligence to spread COVID-19 awareness and education and provide information and patient guidance (42). Analysis of social media chatter could help identify cancer patients and survivors with symptoms suggestive of cardiovascular toxicity and connect them with healthcare resources in cardio-oncology. Monitoring of social media channels could also help recruit patients into cohort studies and build national and international networks to optimize connectivity and care of cancer survivors.

Precision

Recent advances in multi-omics technologies may help us to collect in-depth large-scale data to better understand disease mechanisms, identify populations at risk, and discover preventive or therapeutic interventions (82). For example, the current state of the sequencing technologies renders whole genome sequencing to be performed in an accelerated and cost-effective (<$1,000) fashion (83). The consequent exponentially increasing genetic knowledge combined with deep cardiovascular phenotyping of cancer patients may allow us to identify genetic variants predicting either increased susceptibility or tolerance for specific drug-induced cardiotoxicity and thereby to risk stratify patients based on their genetic backgrounds (84). The same type of genomic data may also be applied and utilized to identify those at risk for COVID-19 complications. For example, a genome wide association study was recently completed on two case–control panels (835 patients and 1,255 control participants from Italy, and 775 patients and 950 control participants from Spain). The study identified COVID-19 susceptibility genetic loci (3p21.31 gene cluster) which could help risk stratify patients (85).

Additionally, novel biomarker discoveries may be possible through transcriptomics, metabolomics, or proteomics of patients' biological samples (e.g., serum), to complement current imaging-based screening strategies for early detection of cardiotoxicities (86) in cancer and in COVID-19. This is particularly relevant in the era of the COVID-19 pandemic, as we work to avoid clinical encounters or diagnostic studies such as echocardiography that would require in-person interactions (87). More refined biomarkers discovered through multi-omics investigations may allow physicians to closely and accurately monitor cardiotoxicities while minimizing in-person evaluations.

Finally, deeper understanding of ethnic disparities and socioeconomic factors may be achieved through population data-based epigenomics, environmentomics, or populomics, which in turn allows clinicians to assess patients holistically and tailor treatment strategies accordingly (88). Taken together, with accumulating comprehensive omics data, physicians may be able to deliver patients' individualized care based on their cancer therapies, genotypes, phenotypes, biomarker profiles, lifestyle, and surrounding environment, enabling precision cardio-oncology.

Big Data and Informatics

All aforementioned technologies have the potential to create an ever-increasing volume of data on our patients in the COVID-19 and post-pandemic world. Biomedical and clinical informatics

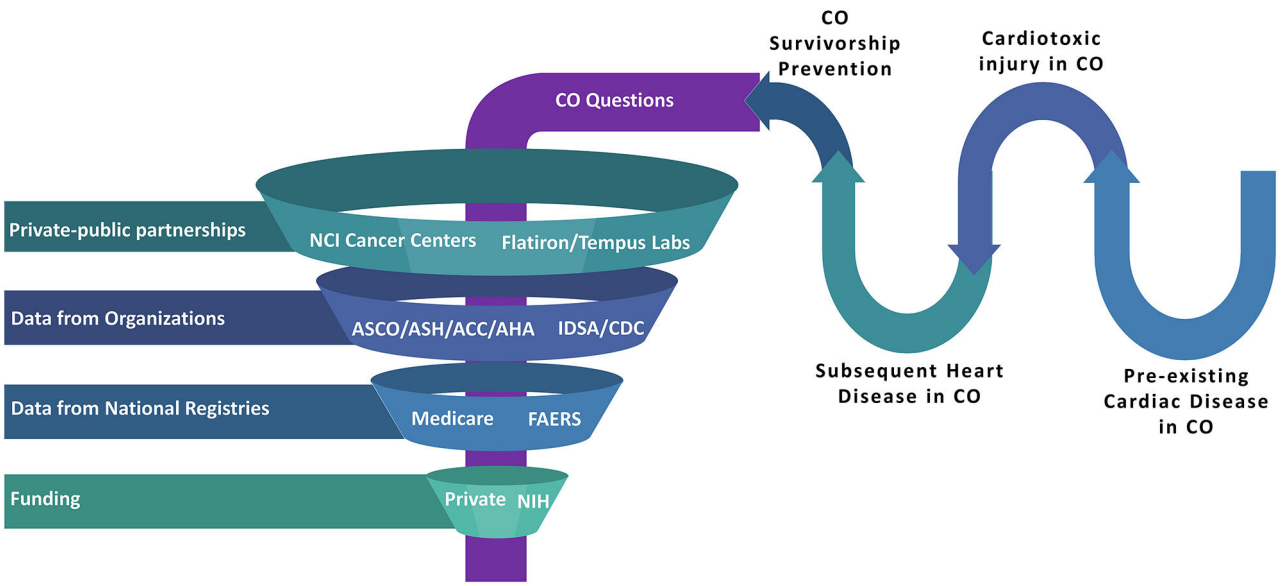

FIGURE 2 | Realms of Big Data such as national medical societies, data science companies (which specialize in patient electronic medical record data management), pharmaceutical industry partners, national databases, and multiple institutions can intersect with CO patient and survivor needs to optimize clinical care and research. This approach can be used to investigate cardiovascular toxic disease or therapy, heart disease predating or as a consequence of CO, survivorship and prevention initiatives, and other relevant themes. Regulatory processes will be needed to ensure preservation of privacy, fairness, inclusiveness, transparency, accountability, and appropriate oversight (79), to ensure the safety of our patients and their protected information. ACC, American College of Cardiology; AHA, American Heart Association; ASCO, American Society of Clinical Oncology; ASH, American Society of Hematology; CDC, Centers for Disease Control and Prevention; CO, either Cardio-Oncology or COVID-19 or both; FAERS, Food and Drug Administration Adverse Event Reporting System; IDSA, Infectious Diseased Society of America; NIH, National Institute of Health. CO denotes either Cardio-Oncology or COVID-19 or both.

can be useful for combining or mining the data and integrating data sources with the electronic health records. In addition, due to social distancing and reduced in-person work hours, traditional pathways of clinical research have been put on hold or disrupted completely. Big data generated from various government and non-government sources can supplement and help restart some of these endeavors amenable to informatics.

Claims-based information from Medicare registries, as well as Surveillance, Epidemiology, and End Results (SEER) databases, in addition to Truven and Healthcare Cost and Utilization Project (HCUP) datasets, can also reveal epidemiological insights regarding incidence, prevalence, trends, costs, and "codable" outcomes (89–91). The International Classification of Disease (ICD) version 10 and Healthcare Common Procedure Coding System (HCPCS) codes that have been created for COVID-19 will be helpful for capturing large-scale data on signs, symptoms, exposure, testing, diagnosis and treatment of this condition (92, 93). These codes may be used across the globe, including in countries which have nationalized healthcare system repositories like Sweden (94), Denmark (95), and the United Kingdom (96). These repositories can also overcome challenges faced when mining anti-cancer therapy information, since drug coverage in the US is heterogenous among insurance companies, resulting in more variability of administration of particular neoplastic drugs.

Several barriers to meaningful collection and use of big data are being quickly overcome during the pandemic, with rapid data-sharing. Challenges with physical recruitment of study participants for prospective studies have halted some pre-existing clinical trials or cohort studies. However, new trials and paradigms have emerged during the pandemic particularly in cancer patients, to facilitate digital clinical trials and cohort studies based on remote monitoring and virtual care (97, 98). Such paradigms enable novel methodology and also allow for continuation of biomedical inquiry in the midst of COVID-19. These tools will not be limited to the pandemic and will likely enrich our conduct of prospective studies in Cardio-Oncology.

Structured multi-pronged approaches should continue to be developed (**Figure 2**), similar to a vision for integrative and collaborative cardio-oncology practice and research laid out in the 2019 Global Cardio-Oncology summit meeting (99). Collective research and clinical practice targets in precision cardio-oncology could be divided among institutions and societies like the American College of Cardiology or American Heart Association, in partnership with large cancer centers. Industry partners should continue to sponsor clinical trials of anti-cancer therapies. Large oncological organizations such as the American Society of Clinical Oncology or the American Society of Hematology should also participate, and privately owned data science companies [e.g., Flatiron Health Inc. and Tempus Labs Inc. (100)] should create databases which are granular to the study of cardio-oncology epidemiology, multi-omics, and biomarkers to inform basic, translational and clinical research to further these aims. These companies work in the field of data management of patient electronic medical record

data into analyzable back ends with heavy focus in the field of oncology. However, in light of the recent major retractions of COVID-19 articles that used a large dataset from a private enterprise, a detailed public reporting of data source architecture, data dictionary, and signed attestation by all authors should be mandated while collaborating with private enterprises.

CONCLUSION

The COVID-19 pandemic has dramatically transformed health care and delivery, accelerating and actualizing a wide spectrum of technology solutions. Over the course of just a few weeks, outpatient practices across the country have been converted to virtual clinics to conform to social distancing. Digital technologies have also been rapidly incorporated into clinical care to further complement virtual care. Social media has played more important roles than ever in sharing and disseminating important health care information particularly relevant to cardiovascular complications of COVID-19. Healthcare and biomedical data, as well as precision health, have been assimilated through innovative ways to advance the care of our patients. These advances, along with the lessons learned through our experiences with COVID-19 will undoubtedly reshape our long-term care of patients and survivors in cardio-oncology.

Clinical implementation of these forms of innovation was heralded with the incorporation of "remote patient monitoring" or telemonitoring in the 2016 European Society of Cardiology guidelines for management of heart failure (101). The ACC and AHA have now followed suit and expanded indications for telehealth, remote monitoring, wearables, and other tools in digital medicine throughout the specialty of Cardiology during and after the pandemic (57). New guidelines and recommendations in subsequent years should also encourage the integration of remote monitoring, telemedicine, precision medicine, informatics, and other forms of digital health in electronic health records in Cardio-Oncology, among other medical and surgical specialties. Social media and artificial intelligence should also coalesce with these tools for synergistic monitoring, assessment, and health education. Such integration will help propel optimal care of patients and survivors further along the innovation spectrum in the Digital Post-Pandemic Era.

AUTHOR CONTRIBUTIONS

S-AB conceptualized the manuscript. S-AB, J-WR, AG, and VR drafted, edited, and approved the manuscript for publication. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin.* (2020) 70:7–30. doi: 10.3322/caac.21590
2. National Cancer Institute Office of Cancer Survivorship. *Statistics: National Cancer Institute.* (2019). Available online at: https://cancercontrol.cancer.gov/ocs/statistics/index.html (accessed June 30, 2020).
3. Mehta LS, Watson KE, Barac A, Beckie TM, Bittner V, Cruz-Flores S, et al. Cardiovascular disease and breast cancer: where these entities intersect: a scientific statement from the American heart association. *Circulation.* (2018) 137:e30–66. doi: 10.1161/CIR.0000000000000556
4. WHO. *WHO Coronavirus Disease (COVID-19) Dashboard 2020*. Available online at: https://covid19.who.int/ (accessed June 30, 2020).
5. Cheng P, Zhu H, Witteles RM, Wu JC, Quertermous T, Wu SM, et al. Cardiovascular risks in patients with COVID-19: potential mechanisms and areas of uncertainty. *Curr Cardiol Rep.* (2020) 22:34. doi: 10.1007/s11886-020-01293-2
6. Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutanen SM, Penninger JM, et al. SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. *Eur J Clin Invest.* (2009) 39:618–25. doi: 10.1111/j.1365-2362.2009.02153.x
7. Musher DM, Abers MS, Corrales-Medina VF. Acute infection and myocardial infarction. *N Engl J Med.* (2019) 380:171–6. doi: 10.1056/NEJMc1901647
8. 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) 5:1–6. doi: 10.1001/jamacardio.2020.1096
9. Libby P, Loscalzo J, Ridker PM, Farkouh ME, Hsue PY, Fuster V, et al. Inflammation, immunity, and infection in Atherothrombosis: JACC review topic of the week. *J Am Coll Cardiol.* (2018) 72:2071–81. doi: 10.1016/j.jacc.2018.08.1043
10. 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) 14:M20–2566. doi: 10.7326/M20-2566
11. Ganatra S, Hammond SP, Nohria A. The novel coronavirus disease (COVID-19) threat for patients with cardiovascular disease and cancer. *JACC CardioOncol.* (2020) 2:350–5. doi: 10.1016/j.jaccao.2020.03.001
12. 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
13. Ky B, Mann DL. COVID-19 clinical trials: a primer for the cardiovascular and cardio-oncology communities. *JACC CardioOncol.* (2020) 2:254–69. doi: 10.1016/j.jaccao.2020.04.002
14. Patel AB, Verma A. COVID-19 and angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: what is the evidence? *JAMA.* (2020) 323:1769–70. doi: 10.1001/jama.2020.4812
15. Asokan I, Rabadia SV, Yang EH. The COVID-19 pandemic and its impact on the cardio-oncology population. *Curr Oncol Rep.* (2020) 22:60. doi: 10.1007/s11912-020-00945-4
16. Lee LYW, Cazier JB, Starkey T, Turnbull CD, Kerr R, Middleton G, et al. COVID-19 mortality in patients with cancer on chemotherapy or other anticancer treatments: a prospective cohort study. *Lancet.* (2020) 395–1919–26. doi: 10.1016/S0140-6736(20)31173-9
17. Armenian SH, Lacchetti C, Barac A, Carver J, Constine LS, Denduluri N, et al. Prevention and monitoring of cardiac dysfunction in survivors of adult cancers: American society of clinical oncology clinical practice guideline. *J Clin Oncol.* (2017) 35:893–911. doi: 10.1200/JCO.2016.70.5400
18. Sharma A, Harrington RA, McClellan MB, Turakhia MP, Eapen ZJ, Steinhubl S, et al. Using digital health technology to better generate evidence and deliver evidence-based care. *J Am Coll Cardiol.* (2018) 71:2680–90. doi: 10.1016/j.jacc.2018.03.523
19. Keesara S, Jonas A, Schulman K. Covid-19 and health care's digital revolution. *N Engl J Med.* (2020) 382:e82. doi: 10.1056/NEJMp2005835
20. Perez MV, Mahaffey KW, Hedlin H, Rumsfeld JS, Garcia A, Ferris T, et al. Large-scale assessment of a smartwatch to identify atrial fibrillation. *N Engl J Med.* (2019) 381:1909–17. doi: 10.1056/NEJMoa1901183
21. Buza V, Rajagopalan B, Curtis AB. Cancer treatment-induced arrhythmias: focus on chemotherapy and targeted therapies. *Circ Arrhythm Electrophysiol.* (2017) 10:e005443. doi: 10.1161/CIRCEP.117.005443

22. Hwang R, Morris NR, Mandrusiak A, Bruning J, Peters R, Korczyk D, et al. Cost-utility analysis of home-based telerehabilitation compared with centre-based rehabilitation in patients with heart failure. *Heart Lung Circ.* (2019) 28:1795–803. doi: 10.1016/j.hlc.2018.11.010
23. Gilchrist SC, Barac A, Ades PA, Alfano CM, Franklin BA, Jones LW, et al. Cardio-oncology rehabilitation to manage cardiovascular outcomes in cancer patients and survivors: a scientific statement from the American heart association. *Circulation.* (2019) 139:e997–1012. doi: 10.1161/CIR.0000000000000679
24. Marceglia S, D'Antrassi P, Prenassi M, Rossi L, Barbieri S. Point of care research: integrating patient-generated data into electronic health records for clinical trials. *AMIA Annu Symp Proc.* (2017) 2017:1262–71.
25. Schwamm LH, Chumbler N, Brown E, Fonarow GC, Berube D, Nystrom K, et al. Recommendations for the implementation of telehealth in cardiovascular and stroke care: a policy statement from the American heart association. *Circulation.* (2017) 135:e24–44. doi: 10.1161/CIR.0000000000000475
26. Mann DM, Chen J, Chunara R, Testa PA, Nov O. COVID-19 transforms health care through telemedicine: evidence from the field. *J Am Med Inform Assoc.* (2020) 27:1132–5. doi: 10.1093/jamia/ocaa072
27. Hollander JE, Carr BG. Virtually perfect? Telemedicine for Covid-19. *N Engl J Med.* (2020) 382:1679–81. doi: 10.1056/NEJMp2003539
28. Gorodeski EZ, Goyal P, Cox ZL, Thibodeau JT, Reay RE, Rasmusson K, et al. Virtual visits for care of patients with heart failure in the Era of COVID-19: a statement from the heart failure society of America. *J Card Fail.* (2020) 26:448–56. doi: 10.1016/j.cardfail.2020.04.008
29. Centers for Medicare and Medicaid Services. *COVID-19 Emergency Declaration Blanket Waivers for Health Care Providers*. (2020). Available online at: https://www.cms.gov/files/document/summary-covid-19-emergency-declaration-waivers.pdf (accessed June 30, 2020).
30. Al-Shamsi HO, Alhazzani W, Alhuraiji A, Coomes EA, Chemaly RF, Almuhanna M, et al. A practical approach to the management of cancer patients during the novel coronavirus disease 2019 (COVID-19) pandemic: an international collaborative group. *Oncologist.* (2020) 25:e936–45. doi: 10.1634/theoncologist.2020-0213
31. Parikh A, Kumar AA, Jahangir E. Cardio-oncology care in the time of COVID-19 and the role of telehealth. *JACC Cardio Oncol.* (2020) 2:356–8. doi: 10.1016/j.jaccao.2020.04.003
32. Dharmarajan H, Anderson JL, Kim S, Sridharan S, Duvvuri U, Ferris RL, et al. Transition to a virtual multidisciplinary tumor board during the COVID-19 pandemic: University of Pittsburgh experience. *Head Neck.* (2020) 42:1310–6. doi: 10.1002/hed.26195
33. Wosik J, Fudim M, Cameron B, Gellad ZF, Cho A, Phinney D, et al. Telehealth transformation: COVID-19 and the rise of virtual care. *J Am Med Inform Assoc.* (2020) 27:957–62. doi: 10.1093/jamia/ocaa067
34. Basch CH, Hillyer GC, Meleo-Erwin ZC, Jaime C, Mohlman J, Basch CE. Preventive behaviors conveyed on youtube to mitigate transmission of COVID-19: cross-sectional study. *JMIR Public Health Surveill.* (2020) 6:e18807. doi: 10.2196/18807
35. Basch CE, Basch CH, Hillyer GC, Jaime C. The role of youtube and the entertainment industry in saving lives by educating and mobilizing the public to adopt behaviors for community mitigation of COVID-19: successive sampling design study. *JMIR Public Health Surveill.* (2020) 6:e19145. doi: 10.2196/19145
36. Sun K, Chen J, Viboud C. Early epidemiological analysis of the coronavirus disease 2019 outbreak based on crowdsourced data: a population-level observational study. *Lancet Digit Health.* (2020) 2:e201–8. doi: 10.1016/S2589-7500(20)30026-1
37. Limaye RJ, Sauer M, Ali J, Bernstein J, Wahl B, Barnhill A, et al. Building trust while influencing online COVID-19 content in the social media world. *Lancet Digit Health.* (2020) 2:e277–8. doi: 10.1016/S2589-7500(20)30084-4
38. Wahbeh A, Nasralah T, Al-Ramahi M, El-Gayar O. Mining physicians' opinions on social media to obtain insights into COVID-19: mixed methods analysis. *JMIR Public Health Surveill.* (2020) 6:e19276. doi: 10.2196/19276
39. Sesagiri Raamkumar A, Tan SG, Wee HL. Measuring the outreach efforts of public health authorities and the public response on facebook during the COVID-19 pandemic in early 2020: cross-country comparison. *J Med Internet Res.* (2020) 22:e19334. doi: 10.2196/preprints.19334
40. Lwin MO, Lu J, Sheldenkar A, Schulz PJ, Shin W, Gupta R, et al. Global sentiments surrounding the COVID-19 pandemic on twitter: analysis of twitter trends. *JMIR Public Health Surveill.* (2020) 6:e19447. doi: 10.2196/19447
41. Chen E, Lerman K, Ferrara E. Tracking social media discourse about the COVID-19 pandemic: development of a public coronavirus twitter data set. *JMIR Public Health Surveill.* (2020) 6:e19273. doi: 10.2196/19273
42. Ting DSW, Carin L, Dzau V, Wong TY. Digital technology and COVID-19. *Nat Med.* (2020) 26:459–61. doi: 10.1038/s41591-020-0824-5
43. Symplur. *#TelemedNow - Healthcare Social Media Analytics and Transcripts*. (2020). Available online at: https://www.symplur.com/healthcare-hashtags/telemednow/ (accessed June 30, 2020).
44. Sana F, Isselbacher EM, Singh JP, Heist EK, Pathik B, Armoundas AA. Wearable devices for ambulatory cardiac monitoring: JACC state-of-the-art review. *J Am Coll Cardiol.* (2020) 75:1582–92. doi: 10.1016/j.jacc.2020.01.046
45. Jadczyk T, Kiwic O, Khandwalla RM, Grabowski K, Rudawski S, Magaczewski P, et al. Feasibility of a voice-enabled automated platform for medical data collection: cardioCube. *Int J Med Inform.* (2019) 129:388–93. doi: 10.1016/j.ijmedinf.2019.07.001
46. Shufelt C, Dzubur E, Joung S, Fuller G, Mouapi KN, van Den Broek I, et al. A protocol integrating remote patient monitoring patient reported outcomes and cardiovascular biomarkers. *NPJ Digit Med.* (2019) 2:84. doi: 10.1038/s41746-019-0145-6
47. Treskes RW, van der Velde ET, Barendse R, Bruining N. Mobile health in cardiology: a review of currently available medical apps and equipment for remote monitoring. *Expert Rev Med Devices.* (2016) 13:823–30. doi: 10.1080/17434440.2016.1218277
48. Ong MK, Romano PS, Edgington S, Aronow HU, Auerbach AD, Black JT, et al. Effectiveness of remote patient monitoring after discharge of hospitalized patients with heart failure: the better effectiveness after transition – heart failure (BEAT-HF) randomized clinical trial. *JAMA Intern Med.* (2016) 176:310–8. doi: 10.1001/jamainternmed.2015.7712
49. Zakeri R, Morgan JM, Phillips P, Kitt S, Ng GA, McComb JM, et al. Impact of remote monitoring on clinical outcomes for patients with heart failure and atrial fibrillation: results from the REM-HF trial. *Eur J Heart Fail.* (2020) 22:543–53. doi: 10.1002/ejhf.1709
50. Sohn A, Speier W, Lan E, Aoki K, Fonarow G, Ong M, et al. Assessment of heart failure patients' interest in mobile health apps for self-care: survey study. *JMIR Cardio.* (2019) 3:e14332. doi: 10.2196/14332
51. Gensini GF, Alderighi C, Rasoini R, Mazzanti M, Casolo G. Value of telemonitoring and telemedicine in heart failure management. *Card Fail Rev.* (2017) 3:116–21. doi: 10.15420/cfr.2017:6:2
52. Greiwe J, Nyenhuis SM. Wearable technology and how this can be implemented into clinical practice. *Curr Allergy Asthma Rep.* (2020) 20:36. doi: 10.1007/s11882-020-00927-3
53. Zamberg I, Manzano S, Posfay-Barbe K, Windisch O, Agoritsas T, Schiffer E. a mobile health platform to disseminate validated institutional measurements during the COVID-19 outbreak: utilization-focused evaluation study. *JMIR Public Health Surveill.* (2020) 6:e18668. doi: 10.2196/18668
54. Timmers T, Janssen L, Stohr J, Murk JL, Berrevoets M. Using mHealth to support COVID-19 education, self-assessment, and symptom monitoring: an observational study in The Netherlands. *JMIR Mhealth Uhealth.* (2020) 8:e19822. doi: 10.2196/preprints.19822
55. Abeler J, Bäcker M, Buermeyer U, Zillessen H. COVID-19 contact tracing and data protection can go together. *JMIR Mhealth Uhealth.* (2020) 8:e19359. doi: 10.2196/19359
56. Annis T, Pleasants S, Hultman G, Lindemann E, Thompson JA, Billecke S, et al. Rapid implementation of a COVID-19 remote patient monitoring program. *J Am Med Inform Assoc.* (2020) 2020:ocaa097. doi: 10.1093/jamia/ocaa097
57. Varma N, Marrouche NF, Aguinaga L, Albert CM, Arbelo E, Choi JI, et al. HRS/EHRA/APHRS/LAHRS/ACC/AHA worldwide practice update for telehealth and arrhythmia monitoring during and after a pandemic. *J Am Coll Cardiol.* (2020). doi: 10.1002/joa3.12389. [Epub ahead of print].
58. Schinköthe T, Gabri MR, Mitterer M, Gouveia P, Heinemann V, Harbeck N, et al. A web- and app-based connected care solution for COVID-19 in- and

outpatient care: qualitative study and application development. *JMIR Public Health Surveill.* (2020) 6:e19033. doi: 10.2196/19033
59. Kim Y, Seo J, An SY, Sinn DH, Hwang JH. Efficacy and safety of an mhealth app and wearable device in physical performance for patients with hepatocellular carcinoma: development and usability study. *JMIR Mhealth Uhealth.* (2020) 8:e14435. doi: 10.2196/14435
60. Lynch BM, Nguyen NH, Moore MM, Reeves MM, Rosenberg DE, Boyle T, et al. A randomized controlled trial of a wearable technology-based intervention for increasing moderate to vigorous physical activity and reducing sedentary behavior in breast cancer survivors: the ACTIVATE trial. *Cancer.* (2019) 125:2846–55. doi: 10.1002/cncr.32143
61. Wright AA, Raman N, Staples P, Schonholz S, Cronin A, Carlson K, et al. The HOPE pilot study: harnessing patient-reported outcomes and biometric data to enhance cancer care. *JCO Clin Cancer Inform.* (2018) 2:1–12. doi: 10.1200/CCI.17.00149
62. Sara JDS, Maor E, Borlaug B, Lewis BR, Orbelo D, Lerman LO, et al. Non-invasive vocal biomarker is associated with pulmonary hypertension. *PLoS ONE.* (2020) 15:e0231441. doi: 10.1371/journal.pone.0231441
63. Maor E, Perry D, Mevorach D, Taiblum N, Luz Y, Mazin I, et al. Vocal biomarker is associated with hospitalization and mortality among heart failure patients. *J Am Heart Assoc.* (2020) 9:e013359. doi: 10.1161/JAHA.119.013359
64. Maor E, Sara JD, Orbelo DM, Lerman LO, Levanon Y, Lerman A. Voice signal characteristics are independently associated with coronary artery disease. *Mayo Clin Proc.* (2018) 93:840–7. doi: 10.1016/j.mayocp.2017.12.025
65. Hassoon A, Schrack J, Naiman D, Lansey D, Baig Y, Stearns V, et al. Increasing physical activity amongst overweight and obese cancer survivors using an alexa-based intelligent agent for patient coaching: protocol for the physical activity by technology help (PATH) trial. *JMIR Res Protoc.* (2018) 7:e27. doi: 10.2196/resprot.9096
66. Forum WE. *How Drones are Used for Life-Saving Healthcare 2020.* Available online at: https://www.weforum.org/agenda/2020/04/medicines-from-the-sky-how-a-drone-may-save-your-life/ (accessed June 30, 2020).
67. Transportation NCDo. *NCDOT Helping in Effort to Use Drones in COVID-19 Relief Efforts.* (2020). Available online at: https://www.ncdot.gov/news/press-releases/Pages/2020/2020-04-22-ncdot-drones-covid-19.aspx (accessed June 30, 2020).
68. Ye Q, Zhou J, Wu H. Using information technology to manage the COVID-19 pandemic: development of a technical framework based on practical experience in China. *JMIR Med Inform.* (2020) 8:e19515. doi: 10.2196/19515
69. Harrington RA, Califf RM, Balamurugan A, Brown N, Benjamin RM, Braund WE, et al. Call to action: rural health: a presidential advisory from the American heart association and american stroke association. *Circulation.* (2020) 141:e615–44. doi: 10.1161/CIR.0000000000000753
70. British Broadcasting Corporation. *Coronavirus: Covid-19 Detecting Apps Face Teething Problems 2020*. Available online at: https://www.bbc.com/news/technology-52215290 (accessed June 30, 2020).
71. National Public Radio. *Use of Smart Speakers in the US Increases During Quarantine.* Available online at: https://www.npr.org/about-npr/848319916/use-of-smart-speakers-in-the-u-s-increases-during-quarantine (accessed June 30, 2020).
72. Insilico Medicine. *In silico Medicine To Support The Drug Discovery Efforts Against Coronavirus 2019-nCoV*. (2020). Available online at: https://insilico.com/ncov-sprint (accessed June 30, 2020).
73. McCall B. COVID-19 and artificial intelligence: protecting health-care workers and curbing the spread. *Lancet Digit Health.* (2020) 2:e166–7. doi: 10.1016/S2589-7500(20)30054-6
74. Knight W. *How AI Is Tracking the Coronavirus Outbreak 2020.* Available online at: https://www.wired.com/story/how-ai-tracking-coronavirus-outbreak/ (accessed June 30, 2020).
75. Li L, Qin L, Xu Z, Yin Y, Wang X, Kong B, et al. Artificial intelligence distinguishes COVID-19 from community acquired pneumonia on chest CT. *Radiology.* (2020) 19:200905. doi: 10.1148/radiol.2020200905
76. Neri E, Miele V, Coppola F, Grassi R. Use of CT and artificial intelligence in suspected or COVID-19 positive patients: statement of the Italian Society of Medical and Interventional Radiology. *Radiol Med.* (2020) 2020:1–4. doi: 10.1007/s11547-020-01197-9
77. Rao ASRS, Vazquez JA. Identification of COVID-19 can be quicker through artificial intelligence framework using a mobile phone-based survey in the populations when cities/towns are under quarantine. *Infect Control Hosp Epidemiol.* (2020) 2020:1–5. doi: 10.1017/ice.2020.61
78. Alimadadi A, Aryal S, Manandhar I, Munroe PB, Joe B, Cheng X. Artificial intelligence and machine learning to fight COVID-19. *Physiol Genomics.* (2020) 52:200–2. doi: 10.1152/physiolgenomics.00029.2020
79. Ienca M, Vayena E. On the responsible use of digital data to tackle the COVID-19 pandemic. *Nat Med.* (2020) 26:463–4. doi: 10.1038/s41591-020-0832-5
80. Mei X, Lee HC, Diao KY, Huang M, Lin B, Liu C, et al. Artificial intelligence-enabled rapid diagnosis of patients with COVID-19. *Nat Med.* (2020). doi: 10.1038/s41591-020-0931-3. [Epub ahead of print].
81. Mackey T, Purushothaman V, Li J, Shah N, Nali M, Bardier C, et al. Machine learning to detect self-reporting of symptoms, testing access, and recovery associated with COVID-19 on Twitter: retrospective big data infoveillance study. *JMIR Public Health Surveill.* (2020) 6:e19509. doi: 10.2196/19509
82. Hasin Y, Seldin M, Lusis A. Multi-omics approaches to disease. *Genome Biol.* (2017) 18:83. doi: 10.1186/s13059-017-1215-1
83. Suwinski P, Ong C, Ling MHT, Poh YM, Khan AM, Ong HS. Advancing personalized medicine through the application of whole exome sequencing and big data analytics. *Front Genet.* (2019) 10:49. doi: 10.3389/fgene.2019.00049
84. Roden DM, McLeod HL, Relling MV, Williams MS, Mensah GA, Peterson JF, et al. Pharmacogenomics. *Lancet.* (2019) 394:521–32. doi: 10.1016/S0140-6736(19)31276-0
85. Ellinghaus D, Degenhardt F, Bujanda L, Buti M, Albillos A, Invernizzi P, et al. Genomewide association study of severe covid-19 with respiratory failure. *N Engl J Med.* (2020). doi: 10.1056/NEJMoa2020283. [Epub ahead of print].
86. Vohra A, Asnani A. Biomarker discovery in cardio-oncology. *Curr Cardiol Rep.* (2018) 20:52. doi: 10.1007/s11886-018-1002-y
87. Saleh M, Gabriels J, Chang D, Kim BS, Mansoor A, Mahmood E, et al. The 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
88. Saban KL, Mathews HL, deVon HA, Janusek LW. Epigenetics and social context: implications for disparity in cardiovascular disease. *Aging Dis.* (2014) 5:346–55. doi: 10.14336/AD.2014.0500346
89. National Cancer Institute. *Surveillance, Epidemiology, and End Results*. Available online at: https://seer.cancer.gov/ (accessed June 30, 2020).
90. International Business Machines. *Truven Health Analytics*. Available online at: https://www.ibm.com/watson-health/about/truven-health-analytics (accessed June 30, 2020).
91. Agency for Healthcare Research and Quality. *Healthcare Cost and Utilization Project.* Available online at: https://www.ahrq.gov/data/hcup/index.html (accessed June 30, 2020).
92. CDC. *ICD-10-CM Official Coding and Reporting Guidelines: CDC.* (2020). Available online at: https://www.cdc.gov/nchs/data/icd/COVID-19-guidelines-final.pdf (accessed March 31, 2020).
93. CMS. *Coverage and Payment Related to COVID-19 Medicare: CMS.* (2020). Available online at: https://www.cms.gov/files/document/03052020-medicare-covid-19-fact-sheet.pdf (accessed March 23, 2020).
94. Swedish registers › *Cancer.* (2020). Available online at: https://www.lupop.lu.se/lupop-for-researchers/registers/cancer (accessed March 3, 2020).
95. Schmidt M, Schmidt SAJ, Sandegaard JL, Ehrenstein V, Pedersen L, Sørensen HT. The Danish National patient registry: a review of content, data quality, and research potential. *Clin Epidemiol.* (2015) 7:449–90. doi: 10.2147/CLEP.S91125
96. Welch CA, Sweeting MJ, Lambert PC, Rutherford MJ, Jack RH, West D, et al. Impact on survival of modelling increased surgical resection rates in patients with non-small-cell lung cancer and cardiovascular comorbidities: a VICORI study. *Br J Cancer.* (2020). doi: 10.1038/s41416-020-0869-8. [Epub ahead of print].
97. Waterhouse DM, Harvey RD, Hurley P, Levit LA, Kim ES, Klepin HD, et al. Early impact of COVID-19 on the conduct of oncology clinical trials and long-term opportunities for transformation: findings from an American society of clinical oncology survey. *JCO Oncol Pract.* (2020) 16:417–21. doi: 10.1200/OP.20.00275

98. Goldsack JC, Izmailova ES, Menetski JP, Hoffmann SC, Groenen PMA, Wagner JA. Remote digital monitoring in clinical trials in the time of COVID-19. *Nat Rev Drug Discov.* (2020) 19:378–9. doi: 10.1038/d41573-020-00094-0
99. Lenihan DJ, Fradley MG, Dent S, Brezden-Masley C, Carver J, Filho RK, et al. Proceedings from the global cardio-oncology summit. *JACC CardioOncol.* (2019) 1:256. doi: 10.1016/j.jaccao.2019.11.007
100. *Real-World Data For The Precision Medicine Era*. Available online at: https://www.tempus.com/data-solutions/
101. 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/ehx158

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Cardiac Injury and Clinical Course of Patients With Coronavirus Disease 2019

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Background: Cardiac injury is recognized as one of the most common critical complications during exacerbation of coronavirus disease 2019 (COVID-19). This study aimed to investigate the effect of cardiac injury on the clinical course of COVID-19 and to examine its potential mechanism and treatments.

Methods and Results: A total of 222 hospitalized patients with COVID-19 from Wuhan were selected for the study during February 10 to March 28, 2020. Demographic, laboratory, and clinical data on admission and during hospitalization were compared between patients with COVID-19 with or without cardiac injury. On admission, cardiac injury ($n = 29$) was associated with advanced age, more underlying coronary artery disease, and a lower Pao_2. Troponin levels were correlated with inflammatory markers (C-reactive protein: $r = 0.348$, $P < 0.001$; interleukin 6: $r = 0.558$, $P < 0.001$) and d-dimer levels ($r = 0.598$, $P < 0.001$). During hospitalization, another six patients suffered from cardiac injury and cardiac injury ($n = 35$), resulting in higher rates of ventilation (invasive: 51.4 vs. 1.6%, $P < 0.001$; non-invasive: 31.4 vs. 1.1%, $P < 0.001$) and mortality (54.3 vs. 1.1%, $P < 0.001$). Cardiac injury on admission was a predictive factor for mortality (adjusted hazard ratio = 4.73, 95% confidence interval = 1.35–16.63, $P = 0.015$). Receiver operating characteristic curve analysis showed that, on admission, a troponin level of 36.35 pg/mL was predictive for mortality with a sensitivity of 73.7% and a specificity of 92.1%.

Conclusions: Cardiac injury complicates the disease course and increases the mortality rate of COVID-19. Troponin levels should be checked at admission and during hospitalization for triage, better monitoring, and managing those with COVID-19, especially in the most severe patients.

Condensed Abstract: Cardiac injury is not uncommon in COVID-19. In a cohort of 222 patients with COVID-19, cardiac injury was found in 29 patients on admission and in another 6 patients during hospitalization. The admission level of troponin was well-correlated with inflammatory factors and d-dimer levels and strongly predicted mortality. Cardiac injury is a manifestation secondary to hypoxia and systemic infection, but

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which nevertheless further complicates the disease course and increases the mortality rate. Troponin levels should be checked at admission and during hospitalization for triage, better monitoring, and managing those with COVID-19, especially in the most severe patients.

Keywords: COVID-19, cardiac injury, troponin, mortality, disease course

The escalating coronavirus disease 2019 (COVID-19) pandemic has evolved into a major public health crisis. There is a wide range of variation in reported mortality rates of COVID-19. The mortality rate of COVID-19 is 2.3% (1) according to a report from the Chinese Center for Disease Control and Prevention, but it is close to 10% in European countries, such as Italy and Spain (2). The mortality rate is <1% in population-based studies that have included mild and asymptomatic cases. In addition to severe respiratory distress, an overwhelming systemic inflammatory reaction that leads to multiple organ system failure appears to be the underlying cause for mortality of this disease (3). Cardiac involvement is one of the most common critical complications during exacerbation of COVID-19, especially in patients with underlying chronic cardiovascular disease (4, 5). Reports from China have shown that elevated troponin levels are associated with a worse outcome, and other biomarkers include lymphocytopenia and elevation of alanine aminotransferase, D-dimer, or interleukin 6 (IL-6) levels (6, 7). Patients with COVID-19 with underlying coronary artery disease and new cardiac injury showed the highest mortality rate (4). A recent case series of 18 patients with COVID-19 and ST-segment elevation cardiac injury from New York showed a mortality rate of up to 72% (8). Patients with non-coronary cardiac injury have a relatively higher death rate than those who are diagnosed with acute myocardial infarction. Early recognition of cardiac injury, close monitoring, and managing heart dysfunction may prevent excessive morbidity and mortality and improve prognosis of patients with COVID-19.

In a Chinese cohort of patients in Wuhan with COVID-19 who have not previously been studied, we investigated the clinical characteristics of these patients and the effect of COVID-19 on the clinical course and mortality rate from cardiac injury. We also discuss the potential mechanism and treatment strategy with the aim of decreasing the rate of fatality in patients with a severe form of COVID-19 infection.

METHODS

Study Population

All of the studied patients were hospitalized in the Sino-French New City Branch of Tongji Hospital in Wuhan, China, which was the epicenter in the initial COVID-19 outbreak. This branch was set up to accept the most critically ill patients since February 10, 2020. Doctors were urgently recruited from several provinces in China. The patients who were selected for the study were cared for by the team that originated from Jilin Province, China. Adult patients who were identified as having laboratory-confirmed COVID-19 infection were enrolled. We excluded patients whose cardiac enzymes were not checked and those with insufficient laboratory data.

All patients were admitted for severe pneumonia due to COVID-19 with unstable vital signs (saturation <93% at room air), or they had severe chronic underlying diseases. COVID-19 pneumonia was confirmed in all of the patients by reverse transcriptase–polymerase chain reaction (RT-PCR) assay or serological testing. All patients presented with positive computed tomographic findings (ground-glass opacities and consolidation with or without vascular enlargement, interlobular septal thickening, and air bronchogram sign) (9).

Laboratory Testing

Nasal pharyngeal swabs or upper or lower respiratory tract samples were collected in all of the patients to test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by nucleic acid RT-PCR. On admission, all patients had a comprehensive laboratory examination, including measurement of B-type natriuretic peptide (BNP) (normal limit = <241 pg/mL), high-sensitivity troponin I (hs-TnI) (normal limit = <34.2 pg/mL), blood cytology, biochemistry, blood gases, a coagulation panel, and inflammatory indicators. The entire laboratory test was repeated at least once a week or more frequently if the patient's condition was unstable.

Definitions

Cardiac injury was defined as an increase in troponin levels above the 99th percentile upper reference limit, regardless of new abnormalities in an electrocardiogram (ECG) and echocardiography (10). The diagnosis of myocardial infarction, especially type 2 myocardial infarction, was not made because of insufficient data in the period of initial urgency when medical teams for COVID-19 were being assembled. Acute respiratory distress syndrome (ARDS) was defined according to the Berlin definition. Acute kidney injury was identified according to the Kidney Disease: Improving Global Outcomes definition.

Treatment

Supportive treatment and antiviral medication (Chinese herbal medication and antiviral medication, including lopinavir/ritonavir, chloroquine phosphate, and arbidol) were provided to patients at the discretion of the individual physician. Low-dose (40 mg) methylprednisolone was administered for 3 to

Abbreviations: COVID-19, coronavirus disease 2019; C-reactive protein, CRP; hs-TnI, high-sensitivity troponin I; MI, myocardial infarction; ECMO, extracorporeal membrane oxygenation; ARDS, acute respiratory distress syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; BNP, B-type natriuretic peptide; IL-6, interleukin 6; ECG, electrocardiogram; CRRT, continuous renal replacement therapy.

5 days for unstable patients with persistent symptoms (febrile, increasing dyspnea, desaturation).

High-flow nasal catheter oxygenation or non-invasive mechanical ventilation was initiated when the patient's respiratory distress and/or hypoxemia was not improved after standard oxygen therapy. If the patient's condition did not respond to these non-invasive measures or even deteriorated within a short period of time (1–2 h), tracheal intubation and invasive mechanical ventilation were used.

Ventilation in the prone position was performed for more than 12 h per day. Indications of extracorporeal membrane oxygenation (ECMO) included the following: (1) When FIO_2 was >90% and the oxygenation index was < 80 mmHg for longer than 3 to 4 h; and (2) for patients with only respiratory failure when the airway platform pressure was ≥35 cmH_2O, the VV-ECMO mode was preferred, and if circulatory support was required, the VA-ECMO mode was used.

Endpoints

The primary endpoint was death or recovery. Follow-up was until March 28, 2020. Patients were considered as recovered and ready for discharge if the following criteria were met: temperature returned to normal for more than 3 days; respiratory symptoms were resolved; pulmonary imaging showed considerable resolution of inflammation; and there were two consecutive negative nuclei acid tests on respiratory tract samples, such as sputum and nasopharyngeal swabs (sampling interval of at least 24 h apart).

The data were retrospectively collected by two examiners from electronic medical record and were mutually checked for accuracy. All of the data were analyzed by investigators who were blinded to this study.

The protocol of this study was approved by the ethics committee of the First Teaching Hospital of Jilin University. Written informed consent was waived by the Ethics Commission for Emerging Infectious Diseases.

Statistical Analysis

Continuous variables are expressed as median [interquartile range (IQR)]. Categorical variables are presented as numbers and percentages. Continuous values were compared by the Mann–Whitney *U*-test. Comparison of categorical variables was performed by the χ^2 test or Fisher exact test as appropriate. The Spearman correlation coefficient was used to assess the linear correlation between hs-TnI levels and other laboratory results.

Receiver operating characteristic (ROC) curve analysis was used for predicting mortality and identifying the optimal cutoff value of plasma hs-TnI levels on the basis of Youden *J* statistic. Optimal cutoff values were defined as the points on the ROC curve where Youden index (sensitivity + specificity – 1) was the highest. Survival curves were plotted using the Kaplan–Meier method and compared between patients with cardiac injury and those without cardiac injury on admission using the log-rank test. The hazard ratio (HR) and 95% confidence interval (CI) were calculated using multivariate Cox regression models to identify independent predictors of all-cause mortality during hospitalization. To avoid overfitting in the model, five variables that have been reported to be associated with clinical outcomes by previous studies were chosen for multivariable analysis, including plasma hs-TnI levels, the percentage of lymphocytes, D-dimer levels, and IL-6 levels on admission, and age.

All reported *P*-values were two-sided, and $P < 0.05$ was considered statistically significant for all analyses. Statistics were calculated using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). Statistical charts were generated using Excel 2016 (Microsoft, Redmond, WA) or Prism 8 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Baseline Characteristics

A total of 222 patients with sufficient medical information were included in the final analysis. The median age of the patients was 63.0 years (IQR = 50.0–69.0 years), and 113 (50.9%) were men.

Cardiac Injury in Patients With COVID-19 Infection on Admission

Patients with elevated hs-TnI levels ($n = 29$) were older [median (IQR) = 70.0 (65.5–80.0] vs. 60.5 (48.0–67.0) years; $P < 0.001$], more likely to have hypertension (37.9 vs. 19.8%; $P = 0.029$) and coronary artery disease (24.1 vs. 3.7%; $P = 0.001$), and had a lower PaO_2 (92.58 ± 2.42 vs. 88.82 ± 9.98 mmHg; $P < 0.001$) compared with patients with normal hs-TnI levels ($n = 187$) (**Table 1**). The baseline characteristics and laboratory results are shown in **Table 1**. The white blood cell count [median (IQR) = 6.35 (5.38–10.21) vs. 5.7 (4.2–6.9) × 10^9/L; $P < 0.001$], neutrophil percentage [median (IQR) = 85.7% (74.3–92.3%) vs. 66.2% (56.3–75.0%); $P < 0.001$], and erythrocyte sedimentation rate [median (IQR) = 56.5 (36.0–87.0) vs. 30 (14–58) mm/h; $P < 0.001$] were significantly higher in patients with elevated hs-TnI levels than in those with normal hs-TnI levels. However, the lymphocyte percentage was significantly lower in patients with elevated hs-TnI levels than in those with normal hs-TnI levels [median (IQR) = 8.9% (5.2–13.5%) vs. 23.05% (15.8–31.5%), $P < 0.001$]. Patients with elevated hs-TnI levels had a lower estimated glomerular filtration rate [median (IQR) = 71.6 (44.1–96.35) vs. 94.5 (77.8–105.4) mL/min · 1.73 m^2, $P = 0.003$] and albumin levels [median (IQR) = 28.7 (23.8–31.3) vs. 36.3 (32.2–40.3) g/dL, $P < 0.001$] and higher aspartate aminotransferase levels [median (IQR) = 28.5 (23.25–46.25) vs. 23 (18–32) U/L, $P = 0.033$] and CK-MB levels [median (IQR) = 132 (55–294) vs. 61 (36.5–95.0) μg/L, $P = 0.049$] compared with patients with normal hs-TnI levels. High-sensitivity C-reactive protein levels [median (IQR) = 78.9 (10.2–11.4) vs. 5.3 (1.5–31.5) mg/dL, $P = 0.009$] as an inflammatory biomarker were significantly higher in patients with elevated hs-TnI levels than in those with normal hs-TnI levels. Moreover, patients with elevated hs-TnI levels had significantly higher levels of BNP [median (IQR) = 1,468 (382.5–5,651.5) vs. 65.0 (36.5–185.0) pg/mL, $P < 0.001$] and D-dimer [median (IQR) = 4.99 (2.3–21.0) vs. 0.6 (0.3–1.3) μg/mL, $P < 0.001$] than those with normal hs-TnI levels.

TABLE 1 | Baseline characteristics and laboratory results of patients with COVID-19.

Characteristic	All ($n = 222$)	No Cardiac injury ($n = 187$)	Cardiac injury ($n = 29$)	*P*-value comparing 29 to 187	New cardiac injury during hospitalization ($n = 6$)	*P*-value comparing 6 to 187
Age, median (IQR), years	63 (50, 69)	60.5 (48.0–67.0)	70 (65.5, 80.0)	<0.001	65.5 (65.0–70.5)	0.074
Gender (male), *n* (%)	113 (50.9)	96 (53.6)	12 (41.4)	0.221	5 (83.3)	0.223
Hypertension, *n* (%)	51 (23.0)	37 (19.8)	11 (37.9)	0.029	3 (50.0)	0.105
Diabetes, *n* (%)	30 (13.5)	23 (12.3)	7 (24.1)	0.086	0 (0.0)	1.000
Coronary artery disease, *n* (%)	14 (6.7)	7 (3.7)	7 (24.1)	0.001	0 (0.0)	1.000
Thyroid, *n* (%)	9 (4.1)	7 (3.7)	2 (6.9)	0.429	0 (0.0)	1.000
ACEI/ARB, *n* (%)	8 (3.6)	6 (3.2)	2 (6.9)	0.328	0 (0.0)	1.000
CCB, *n* (%)	13 (5.9)	9 (4.8)	3 (10.3)	0.226	1 (16.7)	0.276
Laboratory results on admission, median (IQR)						
White blood cell × 10^9/L	5.82 (4.34, 7.17)	5.7 (4.2–6.9)	6.35 (5.38, 10.21)	<0.001	5.1 (4.7–7.8)	0.955
Neutrophil, %	68.0 (57.7, 80.0)	66.2 (56.3–75.0)	85.7 (74.3, 92.3)	<0.001	82.0 (81.0–85.2)	0.003
Lymphocyte, %	21.3 (12.25, 29.35)	23.4 (15.8–31.5)	8.9 (5.2, 13.5)	<0.001	11.6 (10.0–12.5)	0.004
Erythrocyte sedimentation rate, mm/h	34 (16, 63)	30.0 (14.0–58.0)	56.5 (36.0, 87.0)	<0.001	50.0 (37.8–69.0)	0.137
Urea, mg/dL	4.9 (3.85, 6.6)	4.5 (3.8–6.2)	7.4 (4.5, 11.78)	0.023	7.8 (5.5–10.1)	0.044
Creatinine, μmol/L	71 (60, 88)	70.0 (60.0–87.0)	83 (61, 109)	0.076	66.5 (51.8–91.0)	0.751
eGFR, mL/(min·1.73 m2)	92.85 (74.92, 104)	94.5 (77.8–105.4)	71.6 (44.1, 96.35)	0.003	79.4 (67.2–91.5)	0.142
ALT, U/L	25 (16, 42)	26.0 (16.8–43.0)	25.5 (14.75, 37)	0.733	21.0 (18.0–24.0)	0.399
AST, U/L	24 (18.5, 32.5)	22.5 (18.0–32.0)	28.5 (23.25, 46.25)	0.033	24.0 (21.0–65.0)	0.298
TBIL, μmol/L	9.8 (7.1, 12.02)	9.5 (7.0–11.4)	10.60 (8.03, 13.88)	0.143	18.3 (13.3–22.6)	0.002
DBIL, μmol/L	3.9 (2.93, 5.2)	3.6 (2.9–4.7)	4.9 (3.43, 5.98)	0.031	10.4 (6.2–13.6)	0.0001
IBIL, μmol/L	5.3 (4.2, 7.1)	5.3 (4.2–6.8)	5.4 (4.28, 7.8)	0.813	8.1 (7.1–9.7)	0.042
LDH, U/L	237.5 (191.25, 314.5)	227.0 (189.0–290.5)	342 (205.5, 480)	0.012	382.0 (265.0–613.0)	0.034
Total cholesterol, mg/dL	4.08 (3.38, 4.82)	4.2 (3.6–4.9)	3.67 (2.83, 4.47)	0.044	3.6 (3.4–3.6)	0.115
Triglyceride, mg/dL	1.35 (1.02, 2.0)	1.3 (1.0–2.0)	1.50 (0.98, 2.33)	0.909	1.5 (1.4–1.6)	0.811
HDL, mg/dL	1.0 (0.81, 1.18)	1.0 (0.8–1.2)	0.92 (0.74, 1.05)	0.082	0.8 (0.7–0.9)	0.157
LDL, mg/Dl	2.63 (1.96, 3.03)	2.7 (2.0–3.1)	1.85 (1.41, 2.84)	0.024	1.9 (1.6–2.0)	0.029
Creatine kinase–MB fraction, μg/L	65.5 (35.75, 129.0)	61.0 (36.5–95.0)	132 (55, 294)	0.049	84.0 (31.5–154.5)	0.932
Potassium, mEq/L	4.29 (3.98, 4.61)	4.3 (4.0–4.6)	4.49 (3.77, 5.09)	0.297	4.4 (4.0–4.7)	0.760
Sodium, mEq/L	140.2 (138.3, 142.75)	139.9 (138.5–141.9)	141.15 (137.88, 144.9)	0.098	144.1 (141.3–144.1)	0.143
Ferritin, μg/L	565 (287.3, 1,109.5)	545.5 (269.2–936.0)	862 (453.8, 1,264.5)	0.123	2,305.0 (2,128.0–8,288.5)	0.006
HCO_3, mEq/L	24.55 (22.25, 26.08) ± 3.24	24.7 (23.0–26.1)	24.0 (20.15, 24.85)	0.051	26.2 (24.0–27.9)	0.393
C-reactive protein, mg/dL	10.2 (1.6, 49.8)	5.3 (1.5–31.5)	78.9 (10.2, 11.4)	0.009	124.8 (52.2–218.3)	0.005
BNP, pg/mL	115.5 (52.25, 668.0)	65.0 (36.5–185.0)	1,468 (382.5, 5,651.5)	<0.001	336.0 (236.0–734.5)	0.007
Albumin, g/dL	35.45 (31.07, 39.80)	36.3 (32.2–40.3)	28.7 (23.8, 31.3)	<0.001	33.4 (30.9–34.2)	0.134
d-Dimer, μg/mL	0.97 (0.41, 2.28)	0.6 (0.3–1.3)	4.99 (2.31, 21.0)	<0.001	12.2 (3.1–21.0)	0.002
IL-6, pg/mL	9.66 (2.94, 36.35)	7.4 (2.6–19.5)	43.39 (10.9, 108.01)	<0.001	72.8 (58.6–293.7)	<0.001
Pao_2, mean ± SD, mmHg	90.56 ± 6.85	92.58 ± 2.42	88.82 ± 9.98	<0.001	85.06 ± 13.88	<0.001

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; eGFR, estimated glomerular filtration rate; ALT, alanine transaminase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; LDH, lactic dehydrogenase; HDL, high density lipoprotein; LDL, low-density lipoprotein; BNP, B-type natriuretic peptide; IL-6, interleukin 6.

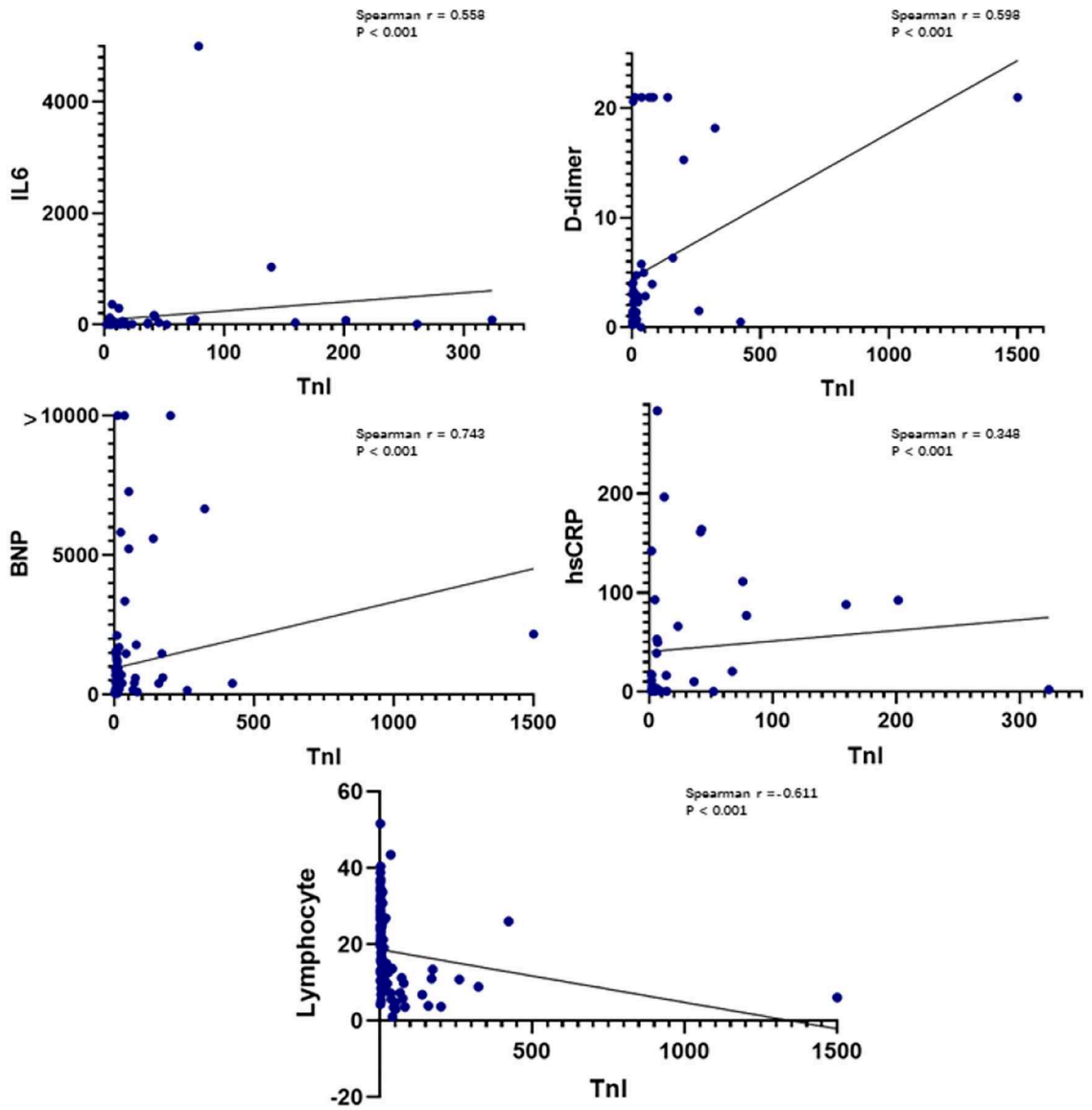

FIGURE 1 | Correlations between plasma hs-TnI levels on admission and IL-6 levels, D-dimer levels, lymphocytes, hs-CRP levels, and BNP levels. IL-6, interleukin 6; BNP, B-type natriuretic peptide; CRP, C-reactive protein; hs-TnI, high-sensitivity troponin I.

Correlations of the Baseline Troponin Level With Baseline Levels of Laboratory Biomarkers

Plasma hs-TnI levels were positively correlated with plasma IL-6 levels (Spearman $r = 0.558$, $P < 0.001$), plasma high-sensitivity C-reactive protein levels (Spearman $r = 0.348$, $P < 0.001$), plasma D-dimer levels (Spearman $r = 0.598$, $P < 0.001$), and plasma BNP levels (Spearman $r = 0.743$, $P < 0.001$) (**Figure 1**). Plasma hs-TnI levels were negatively correlated with the lymphocyte percentage (Spearman $r = -0.611$, $P < 0.001$).

Patients With Cardiac Injury During Hospitalization

Comparison of complications, treatment, and outcomes between patients with and those without cardiac injury during hospitalization is shown in **Table 2**. During hospitalization, six patients had newly developed elevation in troponin levels, and a total of 35 patients were diagnosed with cardiac injury. Patients with cardiac injury were significantly more likely to develop complications, including acute kidney injury (17.1 vs. 1.1%, $P < 0.001$) and ARDS (60.0 vs. 2.1%, $P < 0.001$) compared with those without cardiac injury. Results of the comparison of

TABLE 2 | Complications, treatment, and clinical outcome in patients with COVID-19 during hospitalization.

Characteristic	All ($n = 222$)	No cardiac injury ($n = 187$)	Cardiac injury ($n = 35$)	*P*-value
Complication				
AKI, *n* (%)	8 (3.6)	2 (1.1)	6 (17.1)	<0.001
ALF, *n* (%)	12 (5.4)	8 (4.3)	4 (11.4)	0.086
ARDS, *n* (%)	25 (11.3)	4 (2.1)	21 (60.0)	<0.001
Therapy				
Glucocorticoid, *n* (%)	62 (27.9)	44 (23.5)	18 (51.4)	0.001
Inotropics, *n* (%)	10 (4.5)	1 (0.5)	9 (25.7)	<0.001
LMWH, *n* (%)	15 (6.8)	4 (2.1)	11 (31.4)	<0.001
IVGc, *n* (%)	28 (12.6)	13 (7.0)	15 (42.9)	<0.001
Antivirus, *n* (%)	20 (9.0)	13 (7.0)	7 (20.0)	<0.001
Non-invasive mechanical ventilation, *n* (%)	13 (5.9)	2 (1.1)	11 (31.4)	<0.001
Invasive mechanical ventilation, *n* (%)	21 (9.5)	3 (1.6)	18 (51.4)	<0.001
CRRT, *n* (%)	10 (4.5)	1 (0.5)	9 (25.7)	<0.001
ECMO, *n* (%)	2 (0.9)	0 (0.0)	2 (5.7)	0.001
Clinical outcome				
Death, *n* (%)	21 (9.5)	2 (1.1)	19 (54.3)	<0.001

AST, aspartate aminotransferase; AKI, acute kidney injury; ALF, acute liver failure; ARDS, acute respiratory distress syndrome; LMWH, low molecular weight heparin; IVGc, intravenous glucocorticoids; CRRT, continuous renal replacement therapy; ECMO, extracorporeal membrane oxygenation.

baseline characteristics between patients with newly developed cardiac injury during hospitalization ($n = 6$) and patients without cardiac injury ($n = 187$) are presented in **Table 1**, which suggested patients with newly developed cardiac injury had higher baseline levels of hs-C-reactive protein, D-dimer, IL-6, ferritin and neutrophil percentage, and lower level of lymphocyte percentage.

Electrocardiograms were collected for 25 patients with cardiac injury. ST-T elevation was not found in any of the patients. Tachycardia was observed in all 25 patients. The next most common ECG abnormality was non-specific T-wave changes (32%). Atrial fibrillation was found in three patients. Limited bedside echocardiography was selectively performed in 24 patients. Among these patients, an ejection fraction <50% was observed in only one patient, and the most common finding was diastolic dysfunction (52%). Moderate tricuspid regurgitation was found in three patients.

With regard to treatment, the percentages of administering antiviral agents (20.05 vs. 7.0%, $P < 0.001$), glucocorticoids (51.4 vs. 23.5%, $P = 0.001$), inotropic agents (25.7 vs. 0.5%, $P < 0.001$), intravenous immunoglobulin (42.9 vs. 7.0%, $P < 0.001$), and low-molecular-weight heparin (31.4 vs. 2.1%, $P < 0.001$) were significantly higher in patients with elevated hs-TnI levels than in those with normal hs-TnI levels. Moreover, invasive and non-invasive mechanical ventilation (invasive: 51.4 vs. 1.6%, $P < 0.001$; non-invasive: 31.4 vs. 1.1%, $P < 0.001$) and continuous renal replacement therapy (25.7 vs. 0.5%, $P < 0.001$) were more frequently required in patients with cardiac injury than in those without cardiac injury. Notably, ECMO was used to support the most ill patients with cardiac injury among all of the included patients.

Among the 222 patients, 21 died during hospitalization, and 201 were discharged. A total of 19 (15/29 patients with cardiac injury on admission and 4/6 patients with new cardiac injury during hospitalization) of 35 (54.3%) patients died and 2 of 187 (1.1%) died in the cohorts with and without cardiac injury during hospitalization, respectively. Three patients suffered cardiac arrest before death. One patient died of ventricular fibrillation. All those four patients were in the group of cardiac injury.

A total of 169 tests of hs-TnI were checked for patients with cardiac injury during hospitalization. The median of hs-TNI was 149.3 (IQR = 42.9–489.7) pg/mL in patients died during hospitalization and 29.0 (IQR = 15.0–90.6) pg/mL in the survivors. Correlations between results of hs-TnI and time from admission in patients with cardiac injury are shown in **Figure 2**. Among patients with cardiac injury, 17 patients had continuously increased levels of hs-TnI during hospitalization compared with the levels of hs-TnI on admission, and the mortality of these patients was significantly higher than those patients without continuously increased hs-TnI [13/17 (76.5%) vs. 6/18 (33.3%), $P = 0.010$].

hs-TnI Levels on Admission and Predictors of Mortality in Patients With COVID-19 Infection

The mortality rate was higher in patients with elevated hs-TnI levels than in those with normal hs-TnI levels on admission (51.7 vs. 3.1%). Kaplan–Meier curves (**Figure 3A**) for mortality are shown in **Figure 3** (log-rank test, $P < 0.001$). The multivariate Cox proportional hazards model showed that the risk of mortality was significantly higher in patients with elevated hs-TnI levels than in those with normal hs-TnI levels (adjusted HR = 4.73, 95% CI = 1.35–16.63, $P = 0.015$). Additionally, increased D-dimer levels (μg/mL) on admission were associated with a higher risk

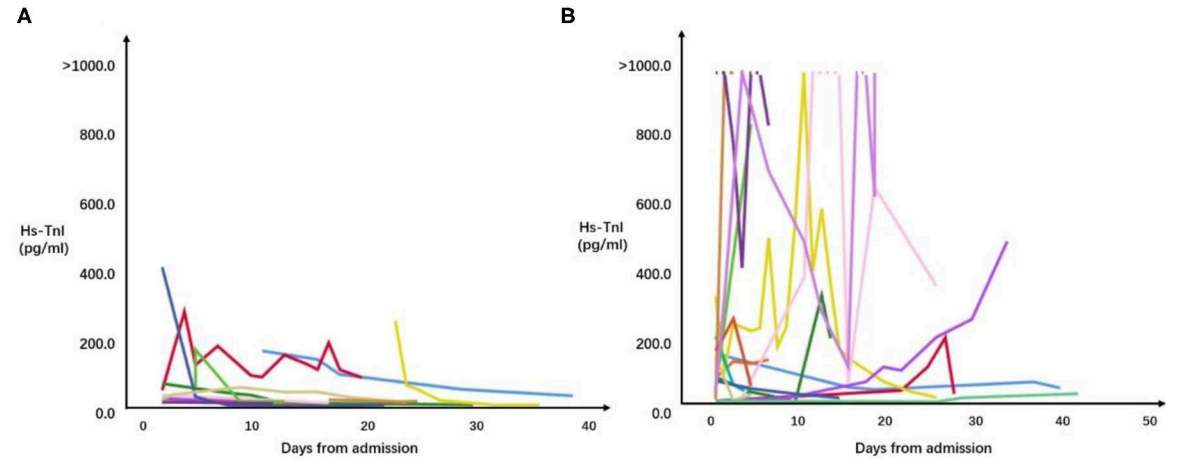

FIGURE 2 | Correlations between results of all hs-TnI checked and time from admission in patients hospitalized with COVID-19 and cardiac injury. **(A)** Correlations between results of all hs-TnI checked and time from admission in survivors. **(B)** Correlations between results of all hs-TnI checked and time from admission in patients who died during hospitalization.

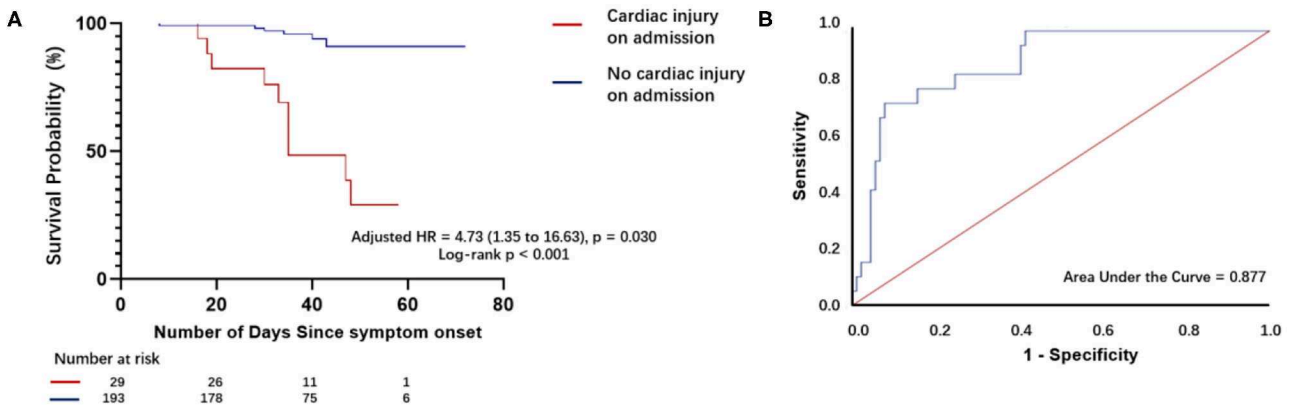

FIGURE 3 | Survival curves of patients with COVID-19 with and without cardiac injury on admission and receiver operating characteristic curve analysis for plasma hs-TnI levels as a predictor of death. **(A)** Kaplan–Meier curves with cumulative hazards of death for comparison of patients with and those without cardiac injury. The mortality rate was higher in patients with cardiac injury on admission compared with those without cardiac injury on admission. **(B)** Receiver operating characteristic (ROC) curve and the area under the ROC curve for plasma hs-TnI levels on admission to predict death.

TABLE 3 | Results of multivariable Cox regression analysis predicting death among 35 COVID-19 patients who had myocardial infarction at admission or during hospitalization vs. 187 COVID-19 patients who did not have myocardial infarction.

Variable	Hazard ratio	95% CI		*P*-value
		Lower	Upper	
Evaluated hs-TnI	4.73	1.35	16.63	0.015
d-Dimer (μg/mL)	1.10	1.02	1.17	0.011

of mortality (adjusted HR = 1.10, 95% CI: 1.02–1.17, $P = 0.011$) (**Table 3**).

Receiver operating characteristic analysis (**Figure 3B**) for plasma hs-TnI levels showed good discriminatory power for mortality of patients with laboratory-confirmed SARS-CoV-2 infection. The area under the ROC curve was 0.880. The cutoff for plasma hs-TnI levels with the highest prognostic value was identified as 36.35 pg/mL. The sensitivity and specificity of the cutoff value were 73.7 and 92.1%, respectively.

DISCUSSION

The clinical course of the 222 patients with COVID-19 infection showed that cardiac injury was relatively common. A total of 13% of the patients had cardiac injury on admission, and 15.8% of patients had cardiac injury during the complete course of hospitalization. On admission, cardiac injury was more common in patients with hypoxemia. Troponin levels were correlated with the levels of inflammatory factors. These

TABLE 4 | Previous studies which described reported incidence of cardiac injury and its association with mortality in COVID-19.

	Study 1 (9)	**Study 2 (6)**	**Study 3 (5)**	**Study 4 (4)**	**Study 5 (3)**	**Study 6 (24)**
First author	Shaobo Shi	Tao Guo	Dawei Wang	Fei Zhou	Tao Chen	Shaobo Shi
Publication date	3/25	3/27	2/7	3/9	3/26	5/6
Data source	Renmin Hospital of Wuhan University	Seventh Hospital of Wuhan City	Zhongnan Hospital of Wuhan University	Jinyintan Hospital and Wuhan Pulmonary Hospital	Tongji Hospital	Renmin Hospital of Wuhan University
Date of data collection	1/20–2/10	1/23–2/23	1/1–2/28	12/29–1/31	1/13–2/12	1/1–2/23
Sample size	416	187	138	191	274	671
Grouping	Cardiac injury ($n = 82$)/ no cardiac injury ($n = 334$)	Elevated TnT ($n = 52$)/normal TnT ($n = 135$)	ICU ($n = 36$) /non-ICU ($n = 102$)	Death ($n = 54$) /survivor ($n = 137$)	Deaths ($n = 113$) /recovered ($n = 161$)	Death ($n = 62$) /survivor ($n = 609$)
Study type	Cohort	Cohort	Descriptive	Case–control	Descriptive	Case–control
Overall age	64 (21–95)	58.50 (14.66)	56 (42–68)	56.0 (46.0–67.0)	62.0 (44.0–70.0)	63 (50–72)
Grouping age(year)	74 (34–95)/ 60 (21–90)	71.40 (9.43)/ 53.53 (13.22)	66 (57–78)/ 51 (37–62)	69.0 (63.0–76.0)/ 52.0 (45.0–58.0)	68.0 (62.0–77.0)/ 51.0 (37.0–66.0)	74 (66–81)/ 61 (49–70)
Overall hypertension (percentage)	30.5	32.6	31.2	30	34	29.7
Grouping hypertension (percentage)	59.8/23.4	63.5/20.7	58.3/21.6	48/23	48/24	59.7/26.6
Overall DM (percentage)	14.4	15.0	10.1	19	17	14.5
Grouping DM (percentage)	24.4/12.0	30.8/8.9	22.2/5.9	31/14	21/14	27.7/13.1
Overall CHD (percentage)	10.6	11.2	—	8	—	8.9
CHD (percentage)	29.3/6.0	32.7/3.0	—	24/1	—	33.9/6.4
Overall COPD (percentage)	2.9	2.1	2.9	3	—	3.4
COPD (percentage)	7.3/1.8	7.7/0	8.3/1.0	7/1	—	3.2/3.4
hs-TNI (pg/mL)	190 (80–1,120)/<6 (<6–9)	—	11.0 (5.6–26.4)/5.1 (2.1–9.8)	22.2 (5.6–83.1)/3.0 (1.1–5.5)	40.8 (14.7–157.8)/3.3 (1.9–7.0)	0.235 (0.042–1.996)/0.006 (0.006–0.011)
Cardiac injury (percentage)	19.7	27.8	—	—	—	75.8/9.7
Overall mortality (percentage)	13.7	23	4.3	28.3	14.1	9.2
Grouping mortality (percentage)	51.2/4.5	59.6/8.9	—	—	—	—

COVID-19, coronavirus disease 2019; DM, diabetes mellitus; CHD, chronic heart disease; COPD, chronic obstructive pulmonary disease; hs-Tni, high-sensitivity troponin I.

findings suggest that cardiac injury is most likely a secondary manifestation from respiratory distress and systemic infection. However, our findings could also be due to direct infection with the virus. During hospitalization, cardiac injury tended to be associated with multiorgan failure, more inotropic support and ventilator use, and consequent higher mortality. Along with elevated D-dimer levels, an increased troponin level up to 36 pg/mL on admission were predictive of death. Moreover, cardiac injury during hospitalization could, to some extent, become a confounding factor that contributes to mortality.

Comparison With Previous Studies on COVID-19 From Wuhan

Our findings are consistent with the literature. Before the submission of our study, there have been five relatively large cohort studies that reported the incidence of cardiac injury and its association with mortality (3–7). We compared these studies with our study (**Table 4**). The mortality rate in our study was lower than that in most of the other studies (9.5% in our study vs. 4.3–28.3%). One of the reasons for this difference between studies is that our patients presented with less comorbidities than the other studies (less hypertension and less cardiac injury). Moreover, the timing of enrollment in the study played a major role in explaining the improved outcome in our study. Our study recruited patients who were admitted after February 10, 2020, when medical resources and staff were more readily available in Wuhan. However, the previous studies analyzed patients who were afflicted with COVID-19 in the early stage of the Wuhan epidemic.

Possible Mechanism of Cardiac Injury

Cardiac injury is commonly found in patients with viral infection, and therefore, it is not unique for COVID-19 infection. Cardiac injury was found in 63.2% of patients who were infected with influenza A (H7N9) virus in China in the 2015–2017 outbreak, and these patients were associated with a high mortality rate (11). During the SARS and Middle East respiratory

syndrome outbreaks, evidence of cardiac involvement was also reported (12, 13).

Cardiac injury in COVID-19 infection could be due to many reasons. Several case reports have shown acute myocardial infarction (8), acute myocarditis (14), acute myopericarditis (15), reverse takotsubo syndrome (16), and pulmonary embolism (17) as the culprits for cardiac injury. However, cardiac injury in our cohort appeared to be due to demand ischemia in response to overwhelming inflammation and/or hypoxia. Elevation of troponin levels is proportional to the levels of inflammatory factors, among which IL-6 is the most significant predictive factor for prognosis.

Cardiac injury in the setting of COVID-19 infection appears to follow a different pattern from what is known in typical acute viral myocarditis, which usually occurs after 1 week of viral infection. COVID-19–infected patients show evidence of cardiac injury only a couple of days after diagnosis of pneumonia. Additionally, abnormal electrical conduction or deadly arrhythmia is much more common in patients with acute viral myocarditis than in COVID-19–infected patients in whom tachycardia is most frequently observed. Although our study and previous studies showed that malignant arrhythmia, especially cardiac arrest, may occur at the end of the disease owing to severe hypoxia or electrolyte disorder. Moreover, in contrast to fulminant myocarditis, circulatory support was rarely required in our cohort. A pathological report showed pronounced pulmonary edema with hyaline membrane formation in the lungs in those who died of ARDS due to COVID-19. However, there were no obvious histological changes in cardiac tissue, as reported in a previous case report (18). Endomyocardial biopsy from one patient with COVID-19 who presented with cardiogenic shock showed only low-grade myocardial inflammation, which was inconsistent with fulminant myocarditis (19). Coronavirus particles were also found from the biopsy, which suggested either transient viremia or infected macrophage migration from the lungs.

COVID-19 infection is distinguished from bacterial sepsis by showing more cardiac involvement than other organs, such as renal or liver impairment. There are several explanations for such a discrepancy. A previous study showed that SARS-CoV-2 shared angiotensin-converting enzyme 2 (ACE2) as the host cellular receptor for virus spike (S) protein, and the expression and distribution of ACE2 were key determinants for entry of the virus (20). Patients who suffer from heart failure at baseline have increased ACE2 expression at mRNA and protein levels. If patients are infected by SARS-CoV-2, they might have a higher risk of adverse cardiac events (e.g., a heart attack and becoming critically ill). Paradoxically, older individuals, especially those with preexisting cardiovascular comorbidities, are more susceptible and succumb to the more severe form of COVID-19 infection, even though ACE2 expression is notably reduced with aging. This seemingly implausible observation

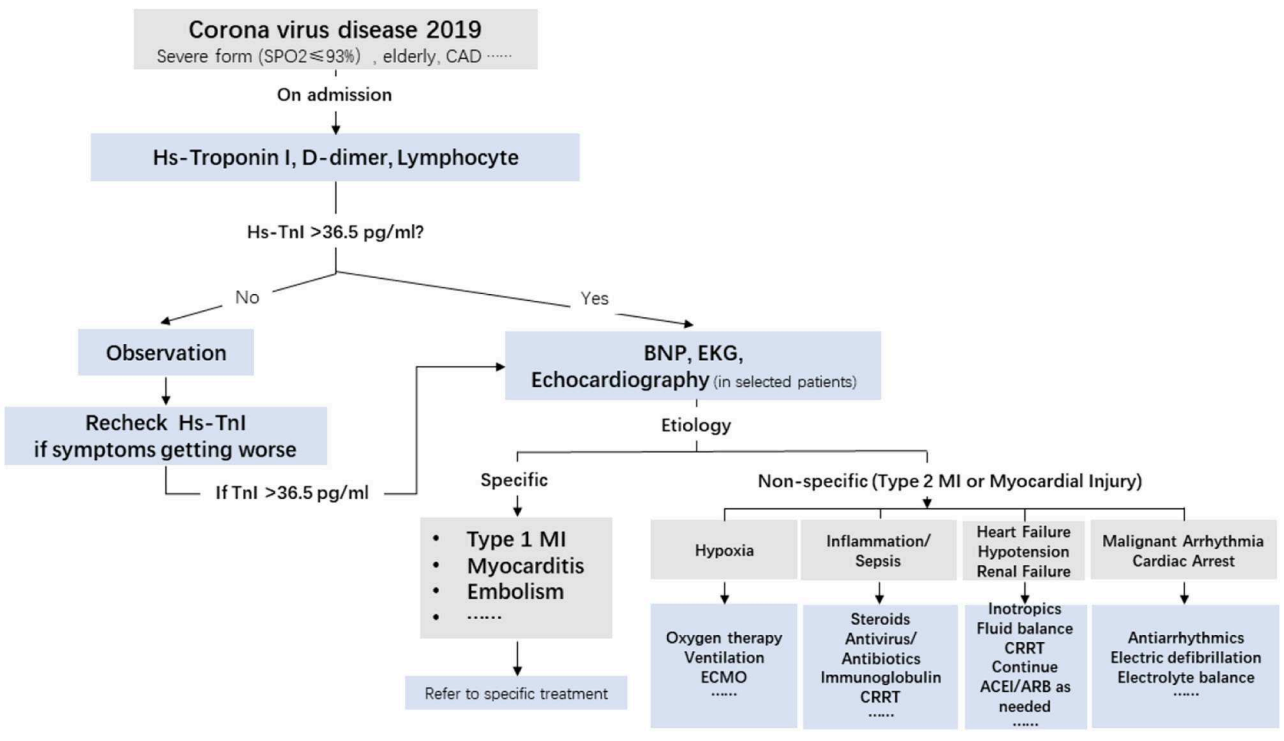

FIGURE 4 | Triage and treatment of patients with COVID-19 based on hs-TnI levels at admission and the etiology of cardiac injury. CAD, coronary artery disease; BNP, B-type natriuretic peptide; ECG, electrocardiogram; MI, myocardial infarction; CRRT, continuous renal replacement therapy; ECMO, extracorporeal membrane oxygenation; hs-TnI, high-sensitivity troponin I.

could be argued by emphasizing the other perspective of ACE function, of which upregulation could lead to a positive immune response (21).

Treatment Strategy of Cardiac Injury in Patients With COVID-19

Despite the fact that cardiac injury in COVID-19 infection is secondary to hypoxia and an inflammatory storm, cardiac involvement can portend a worse outcome and deteriorate the general well-being of infected patients. An example of this situation is that a rise in BNP levels indicates that normal heart function has become compromised. When severe pneumonia leads to septic shock found in COVID-19 infection, managing fluid balance becomes important. Similarly, cardiac injury and myocardial suppression might further predispose patients to volume and pressure overload, which could further deteriorate into severe pulmonary edema and cardiogenic shock. Therefore, early detection and intervention are effective in preventing adverse cardiac events (**Figure 4**).

Fortunately, myocardial suppression is reversible upon improvement of underlying respiratory distress. Although antiviral medication was anecdotally used in the patients in our study, some medications were discontinued in the middle of the course because of side effects. Steroids were used only when patients were febrile or their overall condition became worse. A low dosage of steroids for 1 week is suggested, while oral conversion and tapering are not necessary. Supportive treatment, such as immunoglobulin infusion, is frequently used to boost the immune system. Theoretically, IL-6 antibody could be used, but to date, there is no evidence that it is effective. To minimize inflammation, oxygenation is important. Discrete use of NIPV and early and prompt conversion to mechanical ventilation, while weighing the balance of applying ECMO to indicated patients, are paramount for decreasing the mortality rate. Our study showed that the longest ECMO support can last as long as 30 days, and patients still have a chance for extubation.

In our patients, angiotensin receptor blockers/ACE inhibitors were used in 3.6% of patients. Currently, there is no recommendation for stopping angiotensin receptor blockers/ACE inhibitors, especially for patients with heart failure. Continued administration of angiotensin receptor blockers/ACE inhibitors is recommended (22). Anticoagulation medication is frequently used in patients with cardiac injury, and antiplatelet medication is used only for patients with underlying coronary artery disease.

A comprehensive flowchart of triage and treatment of patients with COVID-19 patients based on troponin levels at admission and the etiology of cardiac injury is shown in the Central Illustration. A recent study showed that type 2 myocardial infarction and cardiac injury were associated with increased long-term mortality (23). Therefore, long-term follow-up of patients with COVID-19 and cardiac injury is required.

Study Limitations

A limitation of this study is its retrospective design, especially because we studied patients in an urgently constructed hospital for the Wuhan COVID-19 outbreak. Specific limitations are as follows. First, data collection, especially ECG and echocardiographic data, was not complete. Therefore, the rate of myocardial infarction (mainly type 2) could not be obtained. Second, based on available evidence of our cohort and other cohorts, cardiac injury is mainly due to an oxygen supply demand imbalance and an inflammatory response. A coronary angiogram is rarely required in this situation. Therefore, there was no evidence of coronary status. Third, most of the data regarding cardiac injury were from Wuhan, China. To date, data from Italy, Spain, and the United States are sparse: In view of a more advanced age and a higher mortality rate in those epidemic areas, better knowledge of the incidence of cardiac injury and its contribution to morbidity and mortality in COVID-19 may lead to an improved prognosis.

CONCLUSIONS

Cardiac injury is not uncommon and is relatively typical for COVID-19. Although cardiac injury is a manifestation secondary to systemic infection or hypoxia, it can complicate the disease course by compromising the patient's general condition and prolonging the course. We recommend checking troponin levels at admission and during hospitalization for better monitoring, managing, and predicating the prognosis of patients with COVID-19, especially in the most severe patients.

PERSPECTIVES

Competency in Medical Knowledge: Cardiac injury in COVID-19 is a manifestation secondary to hypoxia and systemic infection and further complicates the disease course and increases the mortality rate.

Competency in Patient Care: Troponin levels should be checked at admission and during hospitalization for triage, better monitoring, and managing patients with COVID-19, especially in the most severe patients.

Translational Outlook: Cardiac injury is common in severe cases of COVID-19, and long-term follow-up for patients who survive from the severe form of COVID-19 might be required.

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 protocol of this study was approved by the ethics committee of First teaching hospital of JiLin University. Written informed consent was waived by the Ethics Commission for emerging infectious diseases. The data was retrospectively collected by two examiners from electronic medical record, and mutually checked for the accuracy. All the data was analyzed by investigators who were blind to this study.

AUTHOR CONTRIBUTIONS

WL and YW designed the research. YZ and QT analyzed and interpreted the data. ZX performed the statistical analysis and wrote the manuscript. LW and GL critically revised the manuscript for key intellectual content. WL was responsible for the integrity of the work as a whole. All authors approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. 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
2. Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. *JAMA*. (2020) 323:1775–6. doi: 10.1001/jama.2020.4683
3. 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
4. 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) 5:1–8. doi: 10.1001/jamacardio.2020.1017
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. 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
7. 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
8. 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
9. Li Y, Xia L. Coronavirus Disease 2019. (COVID-19): role of Chest CT in diagnosis and management. *AJR Am J Roentgenol*. (2020). 214:1280–6. doi: 10.2214/AJR.20.22954
10. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Group CSD. Fourth universal definition of myocardial infarction 2018. *J Am Coll Cardiol*. (2018) 72:2231–64. doi: 10.1016/j.jacc.2018.08.1038
11. Gao C, Wang Y, Gu X, Shen X, Zhou D, Zhou S, et al. Association between cardiac injury and mortality in hospitalized patients infected with Avian Influenza A (H7N9) Virus. *Virus. Crit Care Med*. (2020) 48:451–458. doi: 10.1097/CCM.0000000000004207
12. 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
13. Alhogbani T. Acute myocarditis associated with novel Middle east respiratory syndrome coronavirus. *Ann Saudi Med*. (2016) 36:78–80. doi: 10.5144/0256-4947.2016.78
14. 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 insight. *Infection*. (2020) doi: 10.1007/s15010-020-01424-5. [Epub ahead of print].
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. 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) 41:1861–62. doi: 10.1093/eurheartj/ehaa286
17. Danzi GB, Loffi M, Galeazzi G, Gherbesi E. Acute pulmonary embolism and COVID-19 pneumonia: a random association? *Eur Heart J*. (2020) 41:1858. doi: 10.1093/eurheartj/ehaa254
18. 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
19. 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
20. 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
21. AlGhatrif M, Cingolani O, Lakatta EG. The Dilemma of Coronavirus Disease 2019, aging, and cardiovascular disease: insights from cardiovascular aging science *JAMA Cardiol*. (2020) 5:747–8. doi: 10.1001/jamacardio.2020.1329
22. Bavishi C, Maddox TM, Messerli FH. Coronavirus Disease 2019 (COVID-19) infection and renin angiotensin system blockers. *JAMA Cardiol*. (2020) 5:745–7. doi: 10.1001/jamacardio.2020.1282
23. Singh A, Gupta A, DeFilippis EM, Qamar A, Biery DW, Almarzooq Z, et al. Cardiovascular mortality after type 1 and Type 2 myocardial infarction in young adults. *J Am Coll Cardiol*. (2020) 75:1003–13. doi: 10.1016/j.jacc.2019.12.052

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COVID-19 and the UK Biobank—Opportunities and Challenges for Research and Collaboration With Other Large Population Studies

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Large population studies such as the UK Biobank provide great opportunities for understanding the pathophysiology, health impact and prognostic factors associated with COVID-19, a condition that has had significant impact on almost everyone around the world. We highlight the vast opportunities, challenges and limitations for research and collaboration from the UK Biobank and other large population studies in helping us better understand and manage both current and potential future pandemics.

Keywords: COVID-19, UK Biobank, population studies, precision medicine, epidemiology

BACKGROUND

The Coronavirus disease 2019 (COVID-19) pandemic has had a profound impact on health and the way people live globally. Our knowledge of the disease is increasing at a fast pace and thus far has largely been from observational studies and registries (1, 2), with an increasing number of clinical trials underway assessing treatment options, vaccination and other preventative strategies to limit the morbidity and mortality associated with it (www.covid-trials.org).

There have been reports that the disease has worse outcomes in those who are older, have cardiovascular disease, and may potentially be linked to certain medications, as well as socially disparate groups. The studies to date, whilst essential given the extraordinary circumstances, are prone to potential limitations inherent in clinical observational studies that generally lack systematic assessment and initially included mostly those who had been moderate or severely affected by COVID-19 and thus required hospitalization (3). The main presentations have been with cough and fever and confirmed cases were initially based on positive nasal and throat swabs for SARS-CoV-2 leading to respiratory failure. Oxygen support, non-invasive or invasive ventilation have been the main stay of treatment to date with reports of propensity to thromboembolic complications and potential cardiac manifestations (4).

LARGE POPULATION STUDIES

Large longitudinal population studies provide a powerful way of tracking the health of a large group of the population over time (5). The impact of factors such as environmental, genetic and lifestyle choices on health and outcomes can be assessed to enable researchers to better understand the

drivers for health and potential differences between groups of people. With the ultimate aim of improving health through public health policies and their delivery. A number of large population studies are under way around the world including the UK Biobank study, the China Kadoorie Biobank, USA Million Veteran Program, and the Prospective study of 500,000 adults in Chennai, India (6–9). Each study will have variations in the number of people enrolled, although these specific ones aim to involve between 500,000 or more adults. Each study varies in the populations enrolled (including age and ethnicity) and extent of factor measurement (imaging and genetic testing, for example). For the purpose of this manuscript we will discuss the UK Biobank study and other population studies to assess the opportunities and challenges in relation to the recent COVID-19 pandemic.

UK Biobank Cohort Study

The UK Biobank is a prospective cohort study with deep phenotype and genotype data collected for over 500,000 individuals aged between 40 and 69-years-old at recruitment between 2006 and 2010, from across England, Scotland and Wales (6). The rich dataset contains biological measurements, lifestyle questionnaires and health-related information, blood and urine biomarkers for all participants. Genome-wide genotype data collected on all participants are providing opportunities for genetic association discoveries and genetic basis of complex traits that could guide future therapeutic targets (10).

Additional information in a large subset are available or in the process of being collected, such as deep imaging (MRI of the heart, brain and abdomen, carotid ultrasound scanning and bone densitometry) in 100,000 with a target completion in 2023 (11, 12). Almost half of these participants have already been scanned. There is also funding confirmed to allow follow-up scanning in about 10,000 of these volunteers.

The number of UK Biobank participants scanned pre-COVID was under just below 50,000. The imaging centers stopped scanning participants on the 13th March due to COVID-19 and will resume scanning when deemed safe. Although only a 1/5 of the UK Biobank are planned to have imaging, it still provides detailed imaging information on 100,000 individuals which is substantial and unprecedented for any national biobank. Another advantage is the on-going rescanning effort which will enable the assessment of pre- and post-COVID changes.

Follow-up health information is provided by robust linkage to primary care electronic health records, death and cancer registries and hospital admission records. With increasing outcome information generated over time the epidemiological opportunities of the UK Biobank study will be vast.

The open source nature of the UK Biobank study is novel and therefore allow any researcher to benefit from the size and scope of the study through an application process. This is particularly commendable given longitudinal studies are notoriously expensive and logistically challenging to execute.

UK Biobanks and COVID-19

With the COVID-19 pandemic affecting so many people, the UK Biobank study provides great opportunity for epidemiological analysis and allow us to explore characteristics that are associated with poorer outcomes in COVID-19 patients along with those that may be protective. The association of lifestyle, comorbidities, medication and phenotypic information with outcomes will become an invaluable source as more data becomes available on those that are tested for presence of COVID-19, especially as the UK government plans to ramp up targets for testing in the general population and not just those admitted to hospital or health care workers (**Figure 1**).

Results of COVID-19 tests for UK Biobank participants are provided by Public Health England for participants residing in England. These are being updated on a weekly basis and include both positive and negative test results. On a monthly basis, information directly linked to primary care data, hospital inpatient data, and death data will be made available along with critical care data for those individuals that have been confirmed as having COVID-19. **Table 1** provides examples of large population studies and which studies are actively collecting COVID-19 related information. Even at the time of revising the manuscript for the journal it was clear that a large number of the Biobanks were taking active steps in increasing the COVID-19 related data to help us better understand the disease.

The UK Biobank for example has now also initiated a coronavirus antibody study where they will invite a representative sample of 20,000 of the total 500,000 participants who express an interest in participation. They will be asked to self-collect 0.5 mls of blood from finger prick for antibody testing. This will be repeated monthly for at least 6-months. Children and grandchildren of the participants, who are over the age of 18 years will also invited to provide blood samples for both antibody testing and genetic testing to additionally assess for genetic susceptibility in young adults.

OPPORTUNITIES

The growing COVID-19 related information for a cohort with a rich phenotype and genotype assessment along with regular outcome measure updates will allow researchers to define the relevance of wide-ranging genetic and non-genetic factors to severity and outcomes based on age, lifestyle, co-morbidities, prescribed medications, environmental, and regional factors. The outcome data now and in the future will provide a comprehensive analysis of the mortality rates and associated morbidity in the UK cohort. Particularly where the data are able to help identify risk factors that predispose to poorer outcomes and those that could be protective thus guiding lifestyle and prevention recommendations. This creates a colossal opportunity for detailed analysis of the cohort and the impact of the disease on longer term health and well-being of survivors that will guide future research and public health policies.

In those who have already undergone deep imaging phenotyping, follow-up scanning will provide novel insights in understanding the downstream, long-term effects of COVID-19 exposure on biological systems. Analysis of the subset of participants undergoing follow-up imaging could also provide

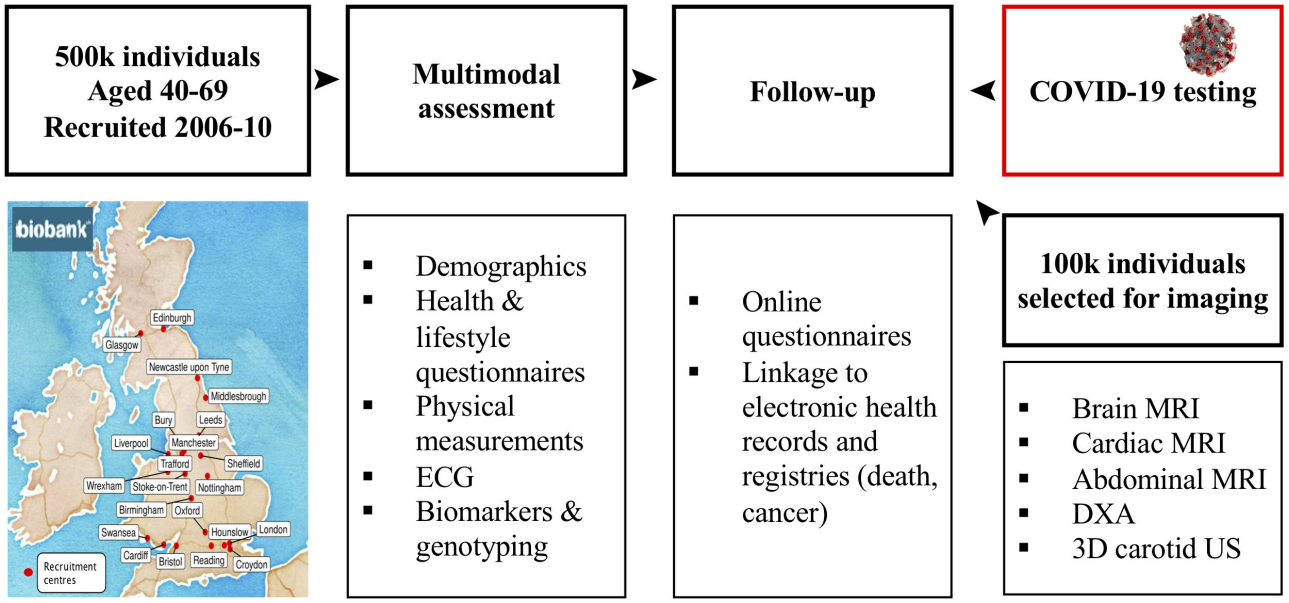

FIGURE 1 | Overview of the UK Biobank Study—A unique multimodal population study of 500,000 with recent addition of COVID-19 related data.

better understanding of pathophysiology using the pre- and post-COVID-19 imaging data.

The UK Biobank is already one of the largest contributors to an international consortium to investigate the genetic determinants of vulnerability to COVID-19, disease severity and outcomes (https://www.covid19hg.org). The second-round meta-analyses of the genome-wide association studies of COVID-19 status had been released. This initiative may not only enrich our knowledge of COVID-19 biology but provide the genetic evidence for drug targets and assist in the development of genetically informed risk assessment of COVID-19 susceptibility. The genetic data also allow the conduct of Mendelian randomization studies which permit evaluation of causality in observational settings (13).

CHALLENGES AND LIMITATIONS AND FUTURE PERSPECTIVES

There is already a large interest from researchers globally in the UK Biobank study which will lead to healthy competition for research and publication. As large groups of researchers may be working in silos on similar projects there may large efforts with those being quickest getting publications. Due to the need for timely submissions for publication there is a potential risk for less rigor or quality control checks during data cleaning and analysis (14).

The UK Biobank enrolled middle and older aged adults only and Caucasians making up the vast majority of participants, with limited number of other ethnicities (15). No participants were under the age of 40 at enrolment 16 years ago. Thus, only those who are about 56 years and older at the time of the COVID-19 pandemic are included. The recently proposed inclusion of children and grandchildren of participants for antibody testing will partly reduce this limitation. There is also evidence of healthy-volunteer bias in the UK Biobank cohort. Therefore, although the UK Biobank data are valid for the investigation of biological associations given its large sample size and the heterogeneity of measurements, it cannot be used to ascertain true disease prevalence in the population (16).

Impact of delayed uptake of population screening through swabbing in the UK in those with milder disease along with lack of systematic symptom data may limit the research potentials. There is also a chance that key findings may only be generated once we have passed the worst period of the pandemic.

Data sharing that allow combination of large cohorts from around the world including the UK Biobank study and other larger population initiatives will increase the richness of the data and allow better assessment of geographical variations, ethnic differences and similarities to better guide public health policies and ways of managing future pandemics.

CONCLUSIONS

COVID-19 has had a global impact and will change our health care approaches in the future. The UK Biobank population study can offer great opportunities given the detailed systematic nature of the assessments along with the growing linkage to the current COVID-19 testing and outcome data. The true potentials of the UK Biobank and other large population-based research studies will become evident as the data accumulate over time and may be enhanced further by linking large population-based studies which can allow limitations such as ethnic and geographical differences and guide optimisation of public health policies.

TABLE 1 | Overview of Large Biobanks around the world and where COVID-19 related data is already becoming available.

Country	Year enrolled	Number enrolled	Additional ongoing Enrolment	Variables collected							SARS-CoV-2 information being made available	Funding	Additional information
				Biological measures	Surveys	Blood	Urine	Stool	Scalp hair	Genetics			
UK Biobank	2007–2010	500k	Subset of 100k with imaging data (2014–2023) Subset 50k exercise stress test with ECG Subset 100k with Activity monitor (2013–2015)	Anthropometrics Blood pressure Lung vital capacity Bone density Intra ocular pressure	Self-completed lifestyle and general health	+	+	−	−	500k with microarray 50k WES (planned 130k 2020) WGS planned	Yes COVID-19 test results (hospitalized and public screening) with linking to electronic health records. Tested positive −1,150, tested negative 6,118 (June 2020) Serial antibody testing in 20k participants and invitation to their children and grandchildren underway	UK DoH MRC Wellcome Trust £62M to date	Phased releases for imaging, WES, WGS data. Primary care data, Hospital-linked admissions Cancer registry data linked Death status linked
China Kadoorie Biobank	2004–2008	510k 30–79 y/o	Subgroup 25k tested every few years	Baseline clinical variables	Medical and lifestyle	+	−	−	−	∼100k with candidate array (384 SNPs) Up to ∼100k with GWAS array (700k SNPs)	No	Chinese government	Joint venture between University of oxford and Chinese Academy of Medical Sciences 8 years follow-up data available
China Taizhou Biobank	2004	100k	Planned	Anthropometrics Tissue Disease-oriented	Interviewer-conducted surveys	+	−	−	−	Unknown	No	Chinese government	Fudan University Institute of Health Sciences Includes CSF, frozen tissue, FFPE
National Cancer Tissue Biobank Chennai, India*	500,000			Cancer biobank							–		Public-private partnership
USA Million Veteran Program	2011 825k	Target 1M	–	EHR	Self-completed Lifestyle Health	+	−	−	−	GWAS array WES WGS	Yes Questionnaire on participants physical and mental health and experiences underway (June 2020) Summary data on COVID-19 deaths, active and convalescent cases	DoH	United States Department of Veterans Affairs

(Continued)

TABLE 1 | Continued

Country	Year enrolled	Number enrolled	Additional ongoing Enrolment	Variables collected							SARS-CoV-2 information being made available	Funding	Additional information
				Biological measures	Surveys	Blood	Urine	Stool	Scalp hair	Genetics			
USA All of Us	2015	∼350k to date	Goal to target 1M by 2022	Anthropometrics EHR	Self-completed questionnaires	+	+	−	−		Yes Antibody testing planned in 10,000 COVID-19 Questionnaire on participants physical and mental health and experiences underway Collection of relevant electronic health record data from >200k participants	NIH Google Verily life Sciences	Was built on the NHGRI "The American project," launched as "Precision Medicine Initiative" 2016 and renamed to "All of Us" in 2016
Lifelines Cohort Study Netherlands	2006–2013	167k 25–50 y/o and 3 generations invited (includes offspring, partners and parents	Add on studies reviewed on request e.g., Omics profiling agreed in subset of 10k	Anthropometrics Blood pressure ECG Lung vital capacity Cognitive	Lifestyle Health Personality Work Living environment	+	+	+	+	GWAS array completed Planned microbiome	Yes COVID-19 Questionnaires completed by > 70,000 participants on physical and mental health and experiences (June 2020)		30-year longitudinal study
deCODE Iceland	1996-present	230k to date	Planned enrolment entire Icelandic population of 364k	Medical records Genealogical records	Unknown	+	−	−	−	GWAS array (337k) WES WGS (15k)	Yes, 9,199 individuals invited (symptomatic and their contacts), 1,221 (13.3%) positive for SARS-CoV-2 10,797 population volunteers (0.8% positive) + Randomly selected screening of 2,283 (0.6% positive) Virus sequenced from 643 individuals (April 2020)	Private initiative Amgen	
Finland Fingen	2018–2024	Planned 500k	No	EHR		+	−	−	—	GWAS arrary (500k)	Yes 264 cases tested positive for COVID-19 (June 11 release) 203,376 populations controls	Finnish Universities and Private partners	Private includes pharmaceutical companies 230k samples collected to date

*DoH, Department of Health; ECG, electrocardiograph, FFPE, formalin-fixed, paraffin-embedded; GWAS, genome wide association studies; k, Thousand; NHGRI, National Human Genome Research Institute; SNP, single nucleotide polymorphisms; WES, whole exome sequencing; WGS, whole genome sequencing; y/o, Years-old; *limited information available.*

DATA AVAILABILITY STATEMENT

Publicly available data from the UK Biobank study was analyzed in this study. The datasets are available to researchers through an open application via https://www.ukbiobank.ac.uk/register-apply/.

AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to conception and design, involved in drafting the manuscript or revising it critically for important intellectual content, and given final approval of the version to be published. All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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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. Jordan RE, Adab P, Cheng KK. Covid-19: Risk factors for severe disease and death. *BMJ*. (2020) 368:m1198 doi: 10.1136/bmj.m1198
3. Wynants L, Van Calster B, Collins GS, Riley RD, Heinze G, Schuit E, et al. Prediction models for diagnosis and prognosis of covid-19 infection: systematic review and critical appraisal. *BMJ*. (2020) 369:m1328. doi: 10.1136/bmj.m1328
4. Available online at: https://bestpractice.bmj.com/topics/en-gb/3000168/pdf/3000168/Coronavirusdisease2019~%28COVID-19%29.pdf (accessed June 5, 2020).
5. Szklo M. Population-based cohort studies. *Epidemiol Rev*. (1998) 20:81–90. doi: 10.1093/oxfordjournals.epirev.a017974
6. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. (2015) 12:1001779. doi: 10.1371/journal.pmed.1001779
7. Chen Z, Chen J, Collins R, Guo Y, Peto R, Wu F, et al. China Kadoorie Biobank of 0.5 million people: survey methods, baseline characteristics and long-term follow-up. *Int J Epidemiol*. (2011) 40:1652–66. doi: 10.1093/ije/dyr120
8. Gaziano JM, Concato J, Brophy M, Fiore L, Pyarajan S, Breeling J, et al. Million veteran program: a mega-biobank to study genetic influences on health and disease. *J Clin Epidemiol*. (2016) 70:214–23. doi: 10.1016/j.jclinepi.2015.09.016
9. Gajalakshmi V, Peto R, Kanimozhi VC, Whitlock G, Veeramani D. Cohort profile: the Chennai prospective study of mortality among 500 000 adults in Tamil Nadu, South India. *Int J Epidemiol*. (2007) 36:1190–5. doi: 10.1093/ije/dym091
10. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, et al. The UK Biobank resource with deep phenotyping and genomic data. *Nature*. (2018) 562:203–9. doi: 10.1038/s41586-018-0579-z
11. Petersen SE, Matthews PM, Francis JM, Robson MD, Zemrak F, Boubertakh R, et al. UK Biobank's cardiovascular magnetic resonance protocol. *J Cardiovasc Magn Reson*. (2016) 18:8. doi: 10.1186/s12968-016-0227-4
12. Alfaro-Almagro F, Jenkinson M, Bangerter NK, Andersson JLR, Griffanti L, Douaud G, et al. Image processing and Quality Control for the first 10,000 brain imaging datasets from UK Biobank. *Neuroimage*. (2018) 166:400–24. doi: 10.1016/j.neuroimage.2017.10.034
13. Koellinger PD, De Vlaming R. Mendelian randomization: the challenge of unobserved environmental confounds. *Int J Epidemiol*. (2019) 48:665–671. doi: 10.1093/ije/dyz138
14. Ioannidis JPA. Why most published research findings are false. *PLoS Med*. (2005) 2:e124. doi: 10.1371/journal.pmed.0020124
15. Khanji MY, Jensen MT, Kenawy AA, Raisi-Estabragh Z, Paiva JM, Aung N, et al. Association between recreational cannabis use and cardiac structure and function. *JACC Cardiovasc Imaging*. (2020) 13:886–8. doi: 10.1016/j.jcmg.2019.10.012
16. Thompson SG, Willeit P. *UK Biobank Comes of Age*. (2015). doi: 10.1016/S0140-6736(15)60578-5

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QTc Prolongation Risk Evaluation in Female COVID-19 Patients Undergoing Chloroquine and Hydroxychloroquine With/Without Azithromycin Treatment

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Women have higher risk for developing TdP in response to ventricular repolarization prolonging drugs. Hundreds of trials are administering chloroquine and hydroxychloroquine with/without azithromycin to COVID-19 patients. While an overall prolonged QTc has been reported in COVID-19 patients undergoing these treatments, the question on even higher QTc elevation risk in thousands of female COVID-19 patients undergoing these treatments remains unanswered. We therefore explore data reported and shared with us to evaluate safety and efficacy of antimalaria pharmacotherapies in female COVID-19 patients. Although we observed longer mean QTc intervals in female patients in 2 of the 3 cohorts reviewed, the sex disproportionality in COVID-19 hospitalizations precludes a clear sex mediated QTc interval elevation risk association in the female COVID-19 patients undergoing acute treatment regimens. Adoption of study designs that include observation of sex mediated differential triggering of cardiac electrical activity by these drugs is warranted.

Keywords: COVID, QTc changes, hydroxychloroquine, chloroquin, azithromycin (AZM), QTc, hydroxychloroquine (HCQ), women

INTRODUCTION

Female gender is a known risk factor for QTc prolongation and one of the highest pro-arrhythmic risk factors (1–3). Women are at a significantly greater risk than men for developing the potentially fatal ventricular arrhythmia torsades de pointes (TdP) in response to certain drugs that prolong ventricular repolarization (1, 4). TdP occurs three times more commonly in women than in men, and female gender is also an independent risk factor for the incidence of syncope and sudden death in the inherited long QT syndrome (LQTS) (5, 6).

In the current coronavirus disease 2019 (COVID-19) pandemic crisis, without validated treatment options available for management, hundreds of trials are administering chloroquine and hydroxychloroquine with/without azithromycin to COVID-19 patients in monitored settings,

TABLE 1 | Corrected QT (QTc) Interval prolongation review from different studies.

Drug/s	N	Sex F, (%)	Baseline QTc (ms)	QTc max (ms)	ΔQTc (ms)	QTc> 500 (%)	TdP	QTc based Rx Red./Term. (n)	Efficacy	Study (ref.)
CQ	95	(34)	432 (360–505)	466 (383–549)	34 (25–43)	23	No	22	Unknown	22
Total	40	(20)	414 (392–428)	454 (420–480)	35 (10–66)	17.5	No	17	Unknown	27
HCQ	22									
HCQ + AZ	18									
HCQ + AZ	251	(25)	439 ± 29	473 ± 36	34 ± 35	13	1 VT (TdP?)	8	Unknown	9
Total	90	(49)	455 (430–474)	476 (445–500)	21 (1–39)	20	1TdP	10	Unknown	24
HCQ	90		474 (454–487)	479.5 (443.5–501.5)	5.5 (14–31)					
HCQ + AZ	53		442 (427–461)	458 (449–492)	23 (10–40)					
CQ + AZ + Os	81	(25)	424.7 (27.4)	Unknown	Unknown	15	2 VT	Unknown	Maybe	26
Low Dose	40		421.9 (24.0)	Unknown	Unknown	11			Lethality	
High Dose	41		427.8 (31.0)	Unknown	Unknown	18.9				

while associated benefits and risks remained debated. Chloroquine, hydroxychloroquine, and azithromycin are individually implicated in prolonging corrected heart rate (QTc), a predictor of TdP (7–10). Concurrent use of QTc altering drugs can result in synergistic increase in risk of ventricular arrhythmias and sudden death (11, 12), a setting recreated by the current COVID-19 treatment regimens.

Factors associated with increased QT prolongation cardiotoxicity risk in females include hormonal mediated differentiation in cardiac electrical activities, greater genetic predisposition for Long QT Syndrome (LQTS) and a higher propensity for drug acquired LQTS driven by drug-drug Interactions (13). The baseline QTc is longer in women than in men (14–16). Endogenous estrogen is associated with QTc lengthening, while testosterone and progesterone shorten the action potential (16, 17). Small QTc prolongation also has been reported with some fourth generation oral contraceptives (18). Women have a higher predisposition to genetic mutations that potentiate TdP and have a higher risk of TdP with Long QT syndrome (LQTS) type 1 and type 2, caused by mutations in potassium channel gene KCNQ1 (KvLQT1) and mutations in potassium channel gene KCNH2 (also known as hERG), respectively (19, 20).

Furthermore, female gender is increasingly recognized as an independent risk factor for acquired LQTS which mainly occurs on exposure to an environmental stressor, most common being an adverse drug reaction leading to drug induced LQTS (DI-LQTS). The mechanisms underlying QT prolongation by medications in acquired LQTS almost always involve blockage of the inward potassium rectifier (IKr) channel, also known as the human ether-a-go-go-related gene (hERG) channel (21). IKr channel controls the movement of potassium out of the myocytes and conducts a rapid IKr current, a critical current in the phase 3 repolarization of the cardiac action potential (22). LQTS and the QT interval prolongation relative to the administration of IKr blockers is greater in women and accompanied by a propensity of drug-induced polymorphic ventricular arrhythmia (5, 23). The estrogen-mediated reduced repolarization reserve in women is believed to be responsible for their higher susceptibility to DI-LQTS (5).

Although the higher risk in females is well-recognized, mechanisms underlying these sex-based risk differences are notably poorly understood. Using a combined experimental and computational approach using "male" and "female" computational model representations of human ventricular cardiac myocytes, a recent study provides first evidence linking structure to function mechanisms underlying higher risk for acquired long-QT-dependent arrhythmias in females (24). Structural modeling presented two distinct, plausible mechanisms of estrogen action enhancing torsadogenic effects: estradiol interaction with hERG mutations in the pore loop containing G604 or with common TdP-related blockers in the intra-cavity binding site. The model predicted increased risk for arrhythmia in females when acute sympathetic nervous system discharge was applied in the settings of both inherited and acquired long-QT syndrome.

Another study models prediction of potential cardiac adverse events caused by combination COVID-19 treatments by combining simulations of pharmacokinetics (PK) with quantitative systems pharmacology (QSP) modeling of ventricular myocytes (25). Their simulation results predicted that drug combinations can lead to greater cellular action potential prolongation compared to drugs given in isolation. The simulations of different patient groups also predicted that females with pre-existing heart disease are especially susceptible to drug-induced arrhythmias, compared males with disease or healthy individuals of either sex.

Despite the high cardiotoxicity risk associated with combination treatments of Chloroquine, hydroxychloroquine and azithromycin in female COVID-19 patients, clinical data supportive of this risk outcome is still not available. We provide

Abbreviations: CQ, Chloroquine; HCQ, Hydroxychloroquine; AZ, Azithromycin; Os, Oseltamivir; ms, milliseconds; ΔQTc, Change in corrected QT interval; VT, Polymorphic ventricular tachycardia; TdP, torsades de pointes; Rx, Drug; Red., Reduction in dose; Term., Termination.

first look at clinical QTc data elongation patterns in male vs. female COVID-19 patients from multiple clinical trials across different geographical regions.

RESULTS AND DISCUSSION

With surveillance of the COVID-19 management interventions, studies are now reporting safety concerns, including the risk of QTc prolongation in patients receiving these treatments (**Table 1**). Re-iterating the predicate hypotheses, all these studies report clinically relevant QTc interval increase in COVID-19 patients receiving these pharmacotherapies and recommend continuous QTc interval monitoring and strict cutoffs for therapy cessation (9, 26–32). While an overall prolonged QTc is observed in COVID-19 patients undergoing these treatments, the question on even higher QTc elevation risk in thousands of female COVID-19 patients undergoing these treatments remains unanswered. We therefore look deeper into the data reported (28, 30) and additional data shared with us (9, 26, 27, 29, 31) to assess if sex mediated disparities in the QTc alterations exist in COVID-19 treatments.

In recent report from on New York and Italy COVID-19 patient cohort, Chorin et al. (9, 29) reported significantly prolonged QTc intervals in COVID-19 patients treated with hydroxychloroquine and azithromycin. Additional data shared with us on a cohort of 251 patients shows that the average baseline QTc in male patients was 441 ± 30 ms while maximum QTc during treatment (QTc max) was 476 ± 36 ms (**Table 2A**). For the female patients, mean baseline QTc of 438 ± 26 and QTc max of 468 ± 38 were observed. This translates into an average increase of 35 ms in the male patients and 30 ms in female patients after treatment with hydroxychloroquine and azithromycin. Sample QTc elevation illustrations in two female patients are shown (**Table 2D**).

Another retrospective, observational cohort conducted on 95 COVID-19 patients in Netherlands also reported clinically relevant prolonged QTc intervals in patients undergoing chloroquine therapy (26). Additional data shared with us reveals that female patients made up 34% of the cohort and their average baseline QTc of 438 ms (346–514) was elevated to 476 ms (415–600) post-chloroquine administration (manual interpretation). In male patients, a baseline mean QTc of 429 ms (369–609) increased to 461 ms (385–557) post-treatment. An increase of 32 ms in male patients ($n = 63$) and 38 ms in female patients ($n = 32$) was recorded during chloroquine treatment (**Table 2B**). Eight of 32 females (25%) had a QTC > 500 ms post-chloroquine vs. 14 of 64 males (22%).

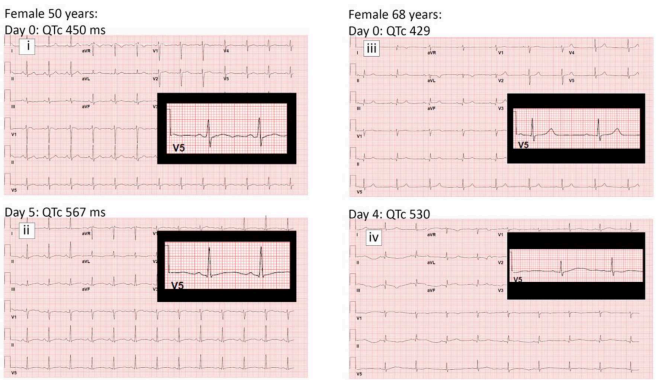
Recent publication by Bessière et al. (31) on data from 49 COVID-19 ICU patients in France reveals QTc interval increase in 93% patients after the administration of the antiviral therapy with hydroxychloroquine alone or in combination with azithromycin. The overall median baseline QTc was 414 ms and max QTc after antiviral therapy was 454 ms. This cohort include 20% female patients and a 52 ms mean increase was observed in their baseline QTc average of 411 ± 26 ms to a maximal average value of 463 ± 29 during treatment. The baseline QTc average of 413 ± 30 in males was elevated to 452 ± 45 after treatment (**Table 2C**). None of the female patients showed QTc >500 ms.

Data from New York state department of health retrospective cohort of 1,438 patients hospitalized COVID-19 patients treatment with hydroxychloroquine, azithromycin, or both, indicated that cardiac arrest was more likely in patients receiving hydroxychloroquine and azithromycin, compared to hydroxychloroquine alone and azithromycin alone (27). Additional data (personal communication) on male and female patient distribution revealed QTc prolongation was observed in 13.0% male and 12.0% female patients in the HCQ and azithromycin cohort. In the HCQ alone cohort prolongation was observed in 16.8% males and 16.7% female patients. For the azithromycin alone cohort, they observed 7.8% percent prolongation in males and 9.2% prolongation in female patients and in the no treatment

TABLE 2 | (A–C) QTc prolongation comparison in male and female Coronavirus Disease 2019 patients.

	Total	Males	Females
(A) Chorin et al. (9)			
Number	251	188	63
Baseline QTc (ms)	439 ± 29	441 ± 30	438 ± 26
QTc max (ms)	473 ± 36	476 ± 36	468 ± 38
ΔQTc (ms)	34 ± 35	NA	NA
(B) van den Broek et al. (26)			
Number	95	32	63
Baseline QTc (ms)	432 (360–505)	429 (369–609)	438 (346–514)
QTc max (ms) (95% CI)	466 (383–549)	461 (385–557)	476 (415–600)
ΔQTc (ms) (95% CI)	34 (25–43)	32 (21–44)	38 (22–54)
(C) Bessière et al. (31)			
Number	40	32	8
Baseline QTc (ms)	414 (392–428)	413 ± 30	411 ± 26
QTc max (ms)	454 (420–480)	452 ± 45	463 ± 29
ΔQTc (ms)	35 (10–66)	38 ± 36	52 ± 31

(D)



(D) QTc interval prolongation sample illustrations in 2 female patients. Inset images show magnification of lead V5. (i). Baseline ECG for a 50 years old female patient before the initiation of HY/AZ. QTc interval = 450 ms. (ii). QTc interval prolonged to 567 ms on day 5. (iii) Baseline ECG for another 68 years old female patient before the initiation of HY/AZ. QTc interval = 429 ms. (iv). QTc interval prolonged to 530 ms on day 4. NA, Not Available.

cohort, 11.7% prolongation in males and 5.1% prolongation in female patients.

CONCLUSIONS

Although we observed longer mean QTc intervals in female patients in 2 of the 3 cohorts reviewed, no conclusive sex mediated QTc interval elongation is apparent amongst the COVID-19 patients undergoing acute chloroquine and hydroxychloroquine with/without azithromycin treatment regimens (9, 26, 27, 29, 31). Since a greater proportion of COVID-19 patients admitted to the hospitals are males, the sex disproportionality in hospitalizations precludes a distinctive risk association in the female COVID-19 patients (27). None of the studies included had an outcome measure of investigating sex mediated differential QTc response in COVID-19 patients and given the diverse study designs, our retrospective, observational analysis lacks statistical validation. Additionally, the results could also be skewed by co-consumption of other medications and underlying co-morbidities. While validation from optimally designed trials is still required, adoption of study designs that include observation of sex mediated differential triggering of cardiac electrical activity by these drugs is warranted.

AUTHOR CONTRIBUTIONS

SG: research and writing. LJ: data from NYC COVID-19 trial. MB: data form Netherlands COVID-19 trial. MC: data from France COVID 19 trial. GB: women health contribution. JK: cardiology contribution. KM: project conception, coordination, and writing. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Makkar RR, Fromm BS, Steinman RT, Meissner MD, Lehmann MH. Female gender as a risk factor for torsades de pointes associated with cardiovascular drugs. *JAMA*. (1993) 270:2590–7. doi: 10.1001/jama.1993.03510210076031
2. Bednar MM, Harrigan EP, Ruskin JN. Torsades de pointes associated with nonantiarrhythmic drugs and observations on gender and QTc. *Am J Cardiol.* (2002) 89:1316–9. doi: 10.1016/S0002-9149(02)02337-8
3. Lehmann MH, Hardy S, Archibald D, Quart B, MacNeil DJ. Sex difference in risk of torsade de pointes with d,l-Sotalol. *Circulation.* (1996) 94:2535–41. doi: 10.1161/01.CIR.94.10.2535
4. Pham TV, Rosen MR. Sex, hormones, and repolarization. *Cardiovasc Res.* (2002) 53:740–51. doi: 10.1016/S0008-6363(01)00429-1
5. Drici MD, Clément N. Is gender a risk factor for adverse drug reactions? *Drug Saf.* (2001) 24:575–85. doi: 10.2165/00002018-200124080-00002
6. Darpo B, Karnad DR, Badilini F, Florian J, Garnett CE, Kothari S, et al. Are women more susceptible than men to drug-induced QT prolongation? Concentration-QTc modelling in a phase 1 study with oral rac-sotalol. *Br J Clin Pharmacol.* (2014) 77:522–31. doi: 10.1111/bcp.12201
7. Projean D, Baune B, Farinotti R, Flinois JP, Beaune P, Taburet AM, et al. *In vitro* metabolism of chloroquine: identification of CYP2C8, CYP3A4, and CYP2D6 as the main isoforms catalyzing N-desethylchloroquine formation. *Drug Metab Dispos.* (2003) 31:748–54. doi: 10.1124/dmd.31.6.748
8. Hancox JC, Hasnain M, Vieweg WVR, Crouse ELB, Baranchuk A. Azithromycin, cardiovascular risks, QTc interval prolongation, torsade de pointes, and regulatory issues: a narrative review based on the study of case reports. *Ther Adv Infect Dis.* (2013) 1:155–65. doi: 10.1177/2049936113501816
9. Chorin E, Wadhwani L, Magnani S, Dai M, Shulman E, Nadeau-Routhier C, et al. QT interval prolongation and torsade de pointes in patients with COVID-19 treated with Hydroxychloroquine/Azithromycin. *Heart Rhythm.* (2020). doi: 10.1016/j.hrthm.2020.05.014. [Epub ahead of print].
10. Negoescu, Thornback A, Wong E, Ostor AJ. *Long QT and Hydroxychloroquine; A Poorly Recognised Problem in Rheumatology Patients.* American College of Rheumatology Abstracts (2013).
11. Ray WA, Murray KT, Hall K, Arbogast PG, Stein CM. Azithromycin and the Risk of Cardiovascular Death. *N Engl J Med.* (2012) 366:1881–90. doi: 10.1056/NEJMoa1003833
12. Owens RC Jr. Risk assessment for antimicrobial agent-induced QTc interval prolongation and torsades de pointes. *Pharmacotherapy.* (2001) 21:301–19. doi: 10.1592/phco.21.3.301.34206
13. Misra K. Drug induced QT interval prolongation risk factors for female patients undergoing current treatments for COVID-19. *CV Network.* (2020) 19:12–36. Available online at: https://0901.nccdn.net/4_2/000/000/076/de9/CV-Network-Vol-19-No-1-March-2020.pdf
14. Rautaharju PM, Zhou SH, Wong S, Calhoun HP, Berenson GS, Prineas R, et al. A. Sex differences in the evolution of the electrocardiographic QT interval with age. *Can J Cardiol.* (1992) 8:690–5.
15. Merri M, Benhorin J, Alberti M, Locati E, Moss AJ. Electrocardiographic quantitation of ventricular repolarization. *Circulation.* (1989) 80:1301–8. doi: 10.1161/01.CIR.80.5.1301
16. Nakagawa M, Ooie T, Takahashi N, Taniguchi Y, Anan F, Yonemochi H, et al. Influence of menstrual cycle on QT interval dynamics. *Pacing Clin Electrophysiol.* (2006) 29:607–13. doi: 10.1111/j.1540-8159.2006.00407.x
17. Saito T, Ciobotaru A, Bopassa JC, Toro L, Stefani E, Eghbali M. Estrogen contributes to gender differences in mouse ventricular repolarization. *Circ Res.* (2009) 105:343–52. doi: 10.1161/CIRCRESAHA.108.190041
18. Sedlak T, Shufelt C, Iribarren C, Lyon LL, Noel Bairey Merz C. Oral contraceptive use and the ECG: evidence of an adverse QT effect on corrected QT interval. *Ann Noninvasive Electrocardiol.* (2013) 18:389–98. doi: 10.1111/anec.12050
19. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, et al. Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. *Circulation.* (2000) 102:1178–85. doi: 10.1161/01.CIR.102.10.1178
20. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, et al. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. *Circulation.* (2001) 103:89–95. doi: 10.1161/01.CIR.103.1.89
21. Kannankeril P, Roden DM, Darbar D. Drug-induced long QT syndrome. *Pharmacol Rev.* (2010) 62:760–81. doi: 10.1124/pr.110.003723
22. Nachimuthu S, Assar MD, Schussler JM. Drug-induced QT interval prolongation: mechanisms and clinical management. *Ther Adv Drug Saf.* (2012) 3:241–53. doi: 10.1177/2042098612454283
23. Hreiche R, Morissette P, Turgeon J. Drug-induced long QT syndrome in women: review of current evidence and remaining gaps. *Gender Med.* (2008) 5:124–35. doi: 10.1016/j.genm.2008.05.005
24. Yang Z, Prinsen JK, Bersell KR, Shen W, Yermalitskaya L, Sidorova T, et al. Azithromycin causes a novel proarrhythmic syndrome. circulation. *Arrhythm Electrophysiol.* (2017) 10. doi: 10.1161/CIRCEP.115.003560
25. Varshneya M, Irurzun-Arana I, Campana C, Dariolli R, Gutierrez A, Pullinger TK, et al. Investigational treatments for COVID-19 may increase

ventricular arrhythmia risk through drug interactions. *medRxiv*. [Preprint] (2020). doi: 10.1101/2020.05.21.20109397
26. van den Broek MP, Möhlmann JE, Abeln BG, Liebregts M, van Dijk VF, van de Garde EM. Chloroquine-induced QTc prolongation in COVID-19 patients. *Neth Heart J.* (2020) 28:406–9. doi: 10.1007/s12471-020-01429-7
27. Rosenberg ES, Dufort EM, Udo T, Wilberschied LA, Kumar J, Tesoriero J, et al. Association of treatment with hydroxychloroquine or azithromycin with in-hospital mortality in patients with COVID-19 in New York State. *JAMA*. (2020) 323:2493–502. doi: 10.1001/jama.2020.8630
28. Mercuro NJ, Yen CF, Shim DJ, Maher TR, McCoy CM, Zimetbaum PJ, et al. Risk of QT interval prolongation associated with use of hydroxychloroquine with or without concomitant azithromycin among hospitalized patients testing positive for coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020). doi: 10.1001/jamacardio.2020.1834. [Epub ahead of print].
29. Chorin E, Dai M, Shulman E, Wadhwani L, Bar-Cohen R, Barbhaiya C, et al. The QT interval in patients with COVID-19 treated with hydroxychloroquine and azithromycin. *Nat Med.* (2020) 26:808–9. doi: 10.1038/s41591-020-0888-2
30. 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
31. Bessière F, Roccia H, Delinière A, Charrière R, Chevalier P, Argaud L, et al. Assessment of QT intervals in a case series of patients with coronavirus disease 2019 (COVID-19) infection treated with hydroxychloroquine alone or in combination with azithromycin in an intensive care unit. *JAMA Cardiol.* (2020) 1:e201787. doi: 10.1001/jamacardio.2020.1787
32. Goyal P, Choi JJ, Pinheiro LC, Schenck EJ, Chen R, Jabri A, et al. Clinical characteristics of covid-19 in New York City. *N Engl J Med.* (2020) 382:2372–4. doi: 10.1056/NEJMc2010419

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The Dual Role of Echocardiography in the Diagnosis of Acute Cardiac Complications and Treatment Monitoring for Coronavirus Disease 2019 (COVID-19)

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The Coronavirus Disease 2019 (COVID-19) pandemic, being caused by an easily and rapidly spreading novel betacoronavirus, has created a state of emergency for people, the scientific community, healthcare systems and states, while the global financial consequences are still unfolding. Cardiovascular complications have been reported for COVID-19-infected patients and are associated with a worse prognosis. ECG and biomarkers may raise suspicion of cardiac involvement. However, transthoracic echocardiography is a fast and reliable bedside method to establish the diagnosis of cardiac complications, including acute coronary syndromes, pericarditis, myocarditis, and pulmonary embolism. Early detection of cardiac dysfunction by speckle tracking echocardiography during off-line analysis may be used to identify a high-risk population for development of heart failure in the acute setting. Precautionary measures are mandatory for operators and equipment to avoid viral dispersion. No specific treatment is yet available for severe acute respiratory syndrome coronavirus 2 (SARS-CoV 2), and a variety of antiviral, immune-modifying, and antioxidant agents are therefore under intense investigation. Echocardiography, including assessment of myocardial deformation, may provide a useful tool to monitor the effects of the various treatment regimens on cardiac function both acutely and in the midterm.

Keywords: coronavirus, transthoracic echo, Coronavirus disease 2019, SARS-CoV 2, global longitudinal strain, antiviral treatment, anti-inflammatory treatment

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INTRODUCTION

A novel enveloped, single-stranded, positive-sense RNA betacoronavirus belonging to the family of coronaviruses has been identified as the causative agent of the novel viral pneumonia that started in the city of Wuhan, Hubei Province, China, on December 12, 2019, (1) and has turned into a global health emergency. The Coronavirus Disease 2019 pandemic counts, as of June 4, 2020, over 6.5 million confirmed cases in 188 countries and regions in the world and 384.815 fatalities (2), rapidly doubling the number of deaths within a month. The consequences of the pandemic in terms of its effects on the world population and global economy are still unfolding. The disease varies considerably from an asymptomatic or mild form without pneumonia to mild

forms of pneumonia to severe pneumonia with lung consolidation that can lead to respiratory failure, sepsis, and multiorgan failure (3). Compared to the previous two coronaviruses that cause severe disease in humans, SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome), SARS exhibits environmental stability (4) and MERS requires close and prolong contact for contamination (5), while the current coronavirus shows easy and high transmissibility, partly related to the high viral load early in the course of the disease (6).

Cardiovascular complications are relatively common, occurring in up to 25% of COVID-19 patients (7, 8) (**Table 1**). Myocardial injury is associated with a 37% in-hospital mortality even in patients without prior cardiovascular disease (9, 15). Cases of acute myocarditis have been reported presenting either as fulminant myocarditis or with symptoms mimicking an acute coronary syndrome (ACS) (16, 17). Pathology evidence of myocardial infiltration by a limited number of monocytes, lymphocytes, and/or neutrophils (18), and rarely associated epicarditis (19) may be suggestive of either activation of the systemic immune response or myocardial inflammatory infiltration due to viral-induced myocyte lysis. Patients presenting with ST elevation myocardial infarction (STEMI) either as an initial manifestation of the disease or during the course of hospitalization for COVID-19 disease (20) were treated with primary percutaneous intervention (PCI). Interestingly, 39% of patients had no evidence of obstructive coronary artery disease on coronary angiography, a finding that questions thrombolysis as a therapeutic alternative to timely coronary angiography and possibly primary PCI. Myocardial injury (11, 14) may also be attributed to myocardial supply/demand mismatch precipitated by hypoxemia, hypotension, tachycardia, and an uncontrolled inflammatory response, leading to cytokine release syndrome (10).

Pulmonary embolism (12, 13, 21, 22) occurs frequently occurring in up to a quarter of all COVID-19 patients despite prophylactic antithrombotic treatment. The activation of the coagulation cascade by inflammatory cytokines, direct endothelial injury of lung microcirculation, antiplatelet activation, and suppression of the fibrinolytic system are all involved synergistically in the mechanism of venous thrombosis (23). Sudden hemodynamic compromise, the need for increased oxygen supplementation in discordance with radiological disease severity or elevations in D-dimers, especially >1 g/l, should prompt further diagnostic work-up with Computed Tomography Pulmonary Angiography (CTPA) to confirm pulmonary embolism.

ECHOCARDIOGRAPHY FOR THE DIAGNOSIS OF ACUTE CARDIOVASCULAR COMPLICATIONS DURING THE COURSE OF COVID-19

Echocardiography is a first-line imaging method to diagnose overt and subtle myocardial dysfunction (14). Localized wall motion abnormalities may be suggestive of a culprit coronary artery lesion leading to an ACS, whereas a diffuse pattern of abnormal segmental longitudinal myocardial strain by echocardiography may support the diagnosis of myocarditis over this of an acute coronary syndrome. Signs indicative of acute pulmonary embolism (PE) should be sought. Right ventricular dilatation from a PLAX view or a basal RV/LV ratio > 1 in a four chamber view can be easily measured as well as pulmonary artery diameter from a short axis view. RV dysfunction can be assessed both qualitatively and with the integration of simple and fast measurements of TDI and TAPSE. Right ventricular systolic hypokinesis is associated with

TABLE 1 | Cardiovascular complications in COVID 19 hospitalized patients in selected studies.

Study	City, Country	Total study population	Acute myocarditis	DVT /Pulmonary embolism	Acute cardiac injury*	ACS	Ischemic stroke
Huang et al. (6)	Wuhan, China	41 patients	–	–	5/41 (12%)		
Yang et al. (7)	Wuhan, China	52 critically ill ICU patients	–	–	12/52 (23%)		
Shi et al. (8)	Wuhan, China	416 hospitalized patients	–	–	82/416 (19.7%)		
Guao et al. (9)	Wuhan, China	187 hospitalized patients	-	-	52/187 (27.8%)		
Middeldorp et al. (10)	Amsterdam, the Netherlands	198 hospitalized patients	–	39/198 (20%) 13% DVT 6.6%PE			
Bombard et al. (11)	Paris, France	135 patients	–	32/135 (24% PE)			
Lodigiani et al. (12)	Milan, Italy	388 hospitalized patients	–	26/388 (6.7%) 16/388 (4.4% VTE) 10/388 (2.8% PE)		4/388 (1.1%)	9/388 (2.5%)
Inciardi et al. (13)	Brescia Lombardy Italy	99 hospitalized patients	–	12/99 (12%)		3/99 Arterial thromboembolism	
Chen et al. (14)	Wuhan, China	274 hospitalized patients	–		89/203 (44%)		

**hs Trop≥99th percentile, ECG changes, Echocardiography abnormalities.*

worse 30–day prognosis (24). The presence of a Mc-ConnelI sign increases the sensitivity for the diagnosis though specificity on its own is only 33% (25). Echocardiographic evidence of RV pressure overload expressed by systolic and diastolic septal flattening can be noticed (26). Estimation of pulmonary artery systolic pressure from tricuspid systolic gradient and a usually increased inferior vena cava diameter combined with a short acceleration time and midsystolic notch in the PW Doppler

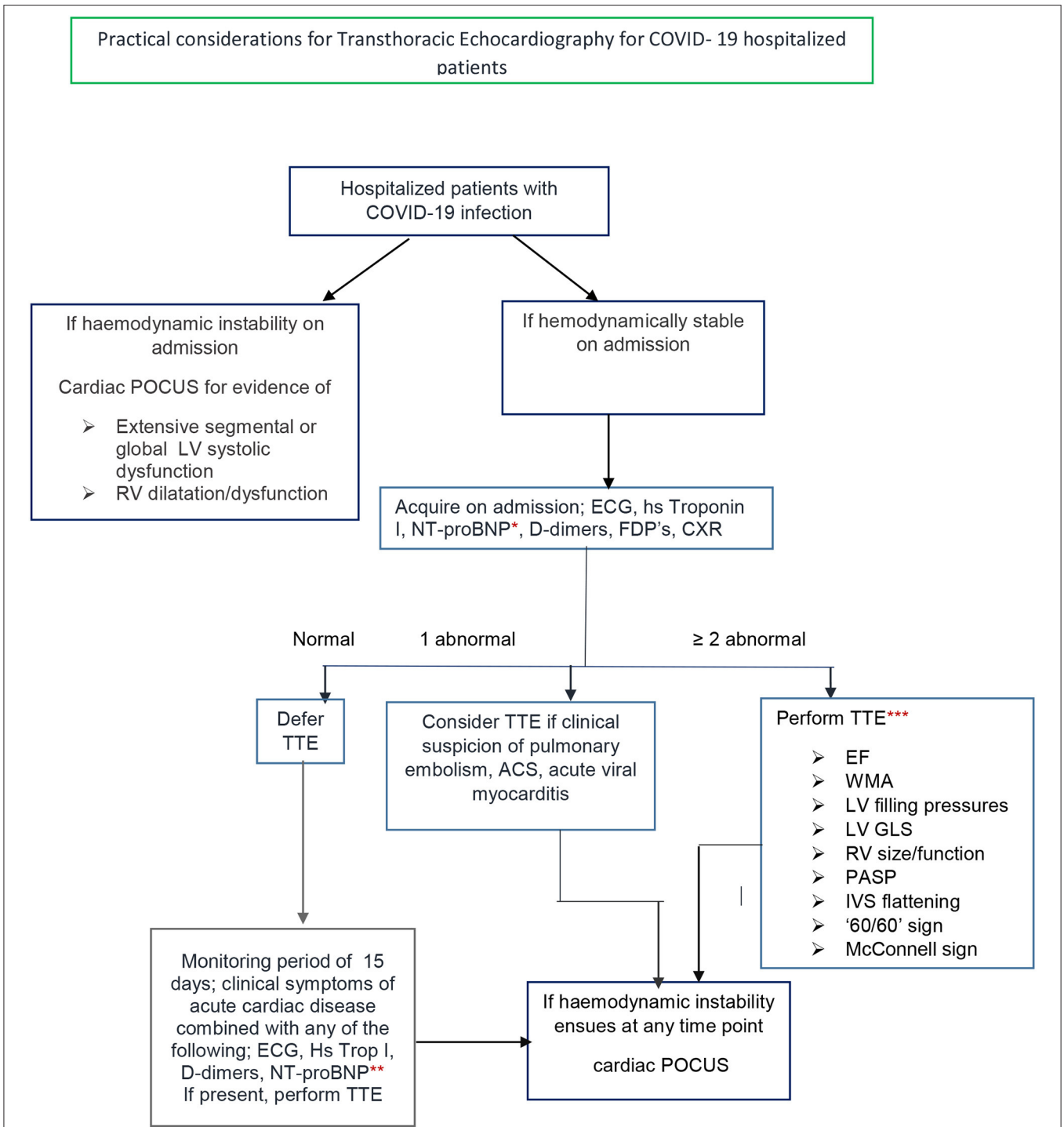

FIGURE 1 | Transthoracic echocardiography for hospitalized COVID 19 disease patients. *NT-pro BNP level on admission ≥300 pg/ml (9). **NT-pro BNP level during hospitalization ≥940 pg/ml (16, 17). ***One experienced sonographer/cardiologist. Use PPE. Disinfect equipment in and out of the ward. EF, ejection fraction; WMA, wall motion abnormality; LV, left ventricle; RV, right ventricle, IVS, interventricular septum; GLS, global longitudinal strain by offline analysis; Cardiac POCUS, cardiac point of care cardiac ultrasound.

of the RVOT('60/60; sign) (26) further indicates increased PA pressure and proximal thromboemboli (27). In clinical practice, hemodynamic instability should lead to a cardiac Point of care Ultrasound (POCUS) (28, 29) to determine the presence of left ventricular dysfunction and/or right ventricular dilatation or a large pericardial effusion (**Figure 1**). Handheld echo devices are the most suitable for this indication as they are portable, more easily disinfected compared to traditional ultrasound machines, while images can be stored and transferred to a PC. On the other hand, in an hemodynamically stable patient with COVID-19 infection, a high clinical suspicion of cardiac involvement, supported by one abnormal diagnostic parameter acquired on admission-that is ECG, CXR, hs Troponin I, NT-proBNP, D-dimers, FDP's—should lead to consideration of a transthoracic echocardiogram (TTE). Solely one abnormal laboratory test cannot be the only criterion for a TTE in a stable COVID-19 infection patient, since their positive predictive value for a specific disease may be low, especially in patients with concomitant chronic diseases and thus lead to a TTE patients' without cardiac involvement. For example, D-dimers may be elevated in various diseases where activation of coagulation and fibrinolysis is present such as cancer patients with COVID 19 (30) or chronic kidney disease (31). For NT-pro BNP values, a level greater that 300 pg/ml (32) should be considered as abnormal. Although, when taking age into consideration, a higher NTproBNP level >1,800 pg/ml should be used to suspect acute heart failure in patients older than >75 years, (33, 34), anything lower than those NT pro-BNP values, at a cut-off level of 940 pg/ml, has been related to adverse outcomes in critically ill patients admitted to ICU (35). Elevated hs cardiac Troponin I is even more specific to myocardial injury than CTnT (36) and may be attributed to multiple and overlapping mechanisms. Cardiac Troponin I can be measured on admission and during hospitalization of COVID- 19 patients in conjunction to NT-pro BNP and thus guide the need for TTE.

On the contrary, when two of the initial diagnostic parameters are abnormal, a complete TTE should be performed to diagnose possible cardiac involvement and ventricular dysfunction. Even the combination of two elevated laboratory biomarkers alone, should lead to a complete TTE, as hsTrop T and NT-proBNP levels were linearly correlated and considerably increased in non-survivor COVID-19 patients (15). As cardiac complications may occur within 15 days of admission monitoring of NT-proBNP, troponins and D-dimers in combination with the patients' clinical status are recommended throughout this period (37).

Notable considerations exist when estimating left and right ventricular systolic function, one being the presence of tachycardia, related to numerous factors such as fever, hypoxemia, cytokine production, and systemic inflammation. Ventricular systolic function is negatively affected in the presence of tachycardia due to the force–frequency relationship. Diastolic function estimations parameters are also affected by tachycardia, since fusion of transmitral E and A waves makes estimation of their ratio and the DT time inaccurate (38). In that case, a TR maximum velocity jet for estimation of PA systolic pressure may be an indicator of LV filling pressures. An impaired global longitudinal strain of the LV or RV may also indicate the initiation of myocardial damage particularly in patients with elevated troponins.

Echocardiography exams should be performed by experienced practitioners to ensure quick acquisition of high quality images (24) and thus minimize possible viral exposure.

TEE carries a high risk of spreading aerosolized viral material within an exam environment. It should be avoided during the pandemic (39). It should be only carried out when there is an absolute indication (e.g., bacterial endocarditis), and the results are expected to modify patient's management. In that case, the exam should be carefully designed by the patient's medical team (40).

CONSIDERATIONS FOR HEALTH CARE PROFESSIONALS

Safety is of utmost importance for the personnel involved in echocardiography of suspected or confirmed COVID-19 patients. Frequent and meticulous handwashing is mandatory. Personal Protective Equipment (PPE) should be used depending on the risk level. Face masks, headcovers, eye shields, gloves, gowns, and shoe covers should be used when examining high risk patients. Detailed description for the PPE is provided by WHO, ASE and EACVI (24, 34, 41). Institutions provide their own detailed protocols in line with international societies' guidance and local experience.

CONSIDERATIONS FOR EQUIPMENT DISINFECTION

Equipment used for Echocardiography studies should be thoroughly disinfected at the end of the exam, in the examination room and again at the hallway (8). Dedicated machines for scanning suspected or confirmed patients may be preferable at this time. Manufacturer's guidance for proper disinfection of the different types of machines should be followed as well as instructions given by certain disinfectant producers.

ECHOCARDIOGRAPHY FOR MONITORING TREATMENT EFFECT ON MYOCARDIAL FUNCTION

A number of different pharmacological agents (42)—antivirals, investigational antivirals, and immune-system-mediating agents—are currently under investigation for COVID-19 treatment in 1,833 clinical trials enrolled at Clinical Trials.gov as of May 30, 2020, under the search terms COVID-19 and SARS-CoV-2.

Chloroquine and hydroxychloroquine are used as antimalarial chemotherapeutic agents. They are also used in the treatment of different autoimmune diseases due to their multitargeted mechanism of action. They inhibit release of inflammatory cytokines by mononuclear cells (43) and interfere with Toll-like receptor signaling pathways and cyclic GMP-AMP (cGamP) synthase (cGaS) activity. In COVID-19 infection, it has been

shown *in vitro* that chloroquine can inhibit viral binding to ACE2 receptor (44). They are both contraindicated in G6PD deficiency. QT prolongation and possible TdP may occur, especially in patients with hypokalemia, hypomagnesemia, hypocalcemia, or on concomitant use of QT prolonging drugs. Serious cardiac side effects occur especially in high cumulative doses after long term treatment, though low cumulative doses (45) may also result in heart failure. Conduction disorders were the main side effect reported in a systematic review (38), affecting 85% of patients. Other non-specific adverse cardiac events include ventricular hypertrophy (22%), hypokinesia (9.4%), heart failure (26.8%), pulmonary arterial hypertension (3.9%), and valvular dysfunction (7.1%), which can be readily ruled in by echocardiography (37). Both agents increase the bioavailability of metoprolol via inhibition of CYP2D6-catalyzed pathways (46). Frequent ECG is recommended, while TTE may reveal early myocardial dysfunction leading to possible treatment discontinuation. A number of ongoing clinical trials (47, 48) examine the therapeutic benefit hydroxychloroquine in COVID-19-infected patients as well as its role in chemoprophylaxis for exposed healthcare workers (49).

Recombinant human angiotensin converting enzyme 2 (ACE−2) has experimental (50) and clinical data (51) on the attenuation of acute lung injury by lessening angiotensin II levels and possibly IL-6.

Convalescent plasma treatment may be promising in terms of viral load and even mortality (52).

Corticosteroids have conflicting evidence for their effect on SARS CO-V 2 infection as they may delay viral clearance from blood and respiratory tract based on data from previous coronaviruses outbreaks (53). On the contrary, a small retrospective clinical trial of early, low-dose, short-term administration of methylprednisolone was associated with improved outcomes in patients with COVID 19 pneumonia (54), revealing the need for further clinical studies.

Remdesevir, a nucleotide analog inhibiting viral RNA polymerases, has an Emergency Use Authorization (EUA) from FDA for suspected or confirmed COVID 19 adult and children patients with severe disease since May 2020 (55). It has been shown to inhibit SARS and MERS in an *in vitro* model of human epithelial airway cells (56) and is also under clinical investigation (57–60). Preliminary results in limited number of patients point to further clinical studies (61).

The combination lopinavir/ritonavir is used in treating HIV-1 infection—they are both aspartase protease inhibitors, and ritonavir increases its plasma half-life. This drug combination has been reported to reduce viral load in clinical case reports (62), although the first clinical trial did not show statistically significant benefit (63). HAART (Highly Active Antiretroviral Treatment), especially protease inhibitors, have been associated with endothelial dysfunction and subclinical atherosclerosis (64–66). HAART may promote metabolic factors such as hyperlipidemia and induce atherosclerotic lesion formation through a CD-36 dependent accumulation of cholesterol in macrophages (30, 67, 68). These mechanisms, combined with the possible myocardial injury associated with the infection itself (9), may contribute to vascular and myocardial dysfunction. Therefore, for patients that have recovered from SARS-CoV-2 infection under protease inhibitor treatment, vascular, and ventricular function should be assessed by TTE at the end of the treatment and possibly at a 3- to 6-month intervals. The combination can also promote QT and PR interval prolongation as well as second and third degree AV block (11). Moreover, lopinavir/ritonavir are CYP3A4 inhibitors. They therefore cannot be used concomitantly with chloroquine (69), while antiplatelet and anticoagulant drugs may need dose adjustment or monitoring (59). Combination therapy of lopinavir /ritonavir, ribavirin and interferon b-1b was superior to lopinavir/ritonavir in a phase 2 clinical trial in terms of symptom alleviation, viral shedding, and hospital stay (70).

Monoclonal antibodies, such as tocilizumab (71), sarilumab (72), and bevasizumab (73), are under investigation to control the cytokine surge associated with the severe form of COVID-19 infection manifested as acute respiratory distress syndrome and multiorgan failure. IL-6 inhibition with biological agents such as tocilizumab and sarilumab may show a beneficial effect in controlling the excessive cytokine production (74) and evolution to alveoli consolidation. As has been recently shown in mechanically ventilated patients with COVID-19 infection (75), excessive IL-6 production is associated with lymphopenia and immunoparesis as assessed by low expression of the humanleukocyte antigen (HLA)-DR on CD14-monocytes, and this effect is reversed by tocilizumab. Additionally, IL-1b production is major factor contributing to the macrophage activation syndrome (Haemophagocytic lymphohistiocytosis syndrome) which characterizes significant number of the critically ill COVID-19 infected patients. Another possible mechanism for monoclonal antibodies beneficial effect could be mediated by preserving endothelial glycocalyx integrity. Damage of endothelial glycocalyx increases vascular permeability to circulating blood cell inflammatory markers and proteins (76) and may thus mediate lung injury and initiate SARS in COVID-19 as has been previously shown in septic patients. Anti-inflammatory treatment may exert beneficial effect on endothelial glycocalyx and thus may offer protection from evolution to alveoli exudation (77). Moreover, anti-inflammatory treatment with tocilizumab but also anakinra—an IL 1 receptor antagonist—exhibit beneficial effects on vascular function and myocardial function (78), as has been shown in patients with rheumatoid arthritis.

Additional protective mechanisms for IL-6 and IL-1 inhibitors may be related to regulation of ROS production, which hampers cellular functions, such as with the proteasome, leading to impaired endogenous protein degradation and mitochondrial dysfunction, augmenting the damage promoted by the direct interaction of SARS-COV proteins with the proteasome (79). ROS may activate the STAT/IL-6 axis (80) and promote IL-8 expression in pulmonary epithelial cells stimulated with lipid-associated membrane proteins from Mycoplasma pneumonia (81), triggering cytokine release and immune cell infiltration in the lung cells. Agents with inherent antioxidant properties such as N-acetylcysteine (NAC) and vitamin C may also be shown to be effective. The beneficial anti-inflammatory effect of monoclonal antibody treatment on myocardial function

in COVID-19 infected patients may by easily monitored by an improvement in global longitudinal strain (GLS) toward normal values (81), as has been previously shown in patients with rheumatoid arthritis and uncontrolled inflammation (78, 79). The lowest expected normal values for GLS are −16.7% in men and −17.8% in women, according to a recently proposed consensus document, and these are similar to the values reported after remission of the acute inflammatory exacerbations by biological agents in patients with rheumatoid arthritis (78, 79).

CONCLUSION

The COVID-19 pandemic, still unfolding around the world, has created a significant worldwide human, scientific, financial, and psychological burden, requiring innovative and cooperative strategies to combat the pandemic and its unprecedented consequences. TTE is required to guide clinical management of patients with abnormal ECG and/or biomarkers, as it may diagnose early cardiac involvement in the acute setting. Antiviral, anti-inflammatory, and antioxidant treatment agents as well as hyperimmune plasma are being investigated in a multitude of clinical trials. Echocardiography provides a valid method to monitor myocardial effect of potential treatments for COVID-19 during hospitalization and in the mid-term follow up.

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

II had the original idea of the manuscript. JP reviewed the biomarkers and treatment section. A-RV wrote the initial version of the manuscript including figure. All authors offered comments on the manuscript's sections.

REFERENCES

1. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. (2020) 395:565–74. doi: 10.1016/S0140-6736(20)30251-8
2. Johns Hopkins Coronavirus Resource Center. Available online at: https://coronavirus.jhu.edu
3. He F, Deng Y, Li W. Coronavirus disease 2019 (COVID-19): what we know? *J Med Virol*. (2020) 92:719–25. doi: 10.1002/jmv.25766
4. Rabenau HF, Cinatl J, Morgenstern B, Bauer G, Preiser W, Doerr HW. Stability and inactivation of SARS coronavirus. *Med Microbiol Immunol*. (2005) 194:1–6. doi: 10.1007/s00430-004-0219-0
5. Mackay IM, Arden KE. MERS coronavirus: diagnostics, epidemiology and transmission. *Virol J*. (2015) 12:222. doi: 10.1186/s12985-015-0439-5
6. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis*. (2020) 20:565–74. doi: 10.1016/S1473-3099(20)30196-1
7. 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
8. Yang X, Yu Y, Xu J, Shu H, Jia'an X, 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
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. Guzik T, Mohiddin S, 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
11. 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
12. Bompard F, Monnier H, Saab I, Tordjman M, Abdoul H, Fournier L, et al. Pulmonary embolism in patients with Covid-19 Pneumonia. *Eur Respir J*. (2020) 56:2001365. doi: 10.1183/13993003.01365-2020
13. Middeldorp S, Coppens M, van Haaps TF, Foppen M, Vlaar AP, Müller M, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. *J Thromb Haemost*. (2020) 18:1995–2002. doi: 10.20944/preprints202004.0345.v1
14. 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
15. 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) 5:1–8. doi: 10.1001/jamacardio.2020.1017
16. 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
17. 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) 5:1–6. doi: 10.1001/jamacardio.2020.1096
18. National Health Commission of the People's Republic of China. *Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment*. 7th ed. Available online at: http://kjfy.meetingchina.org/msite/news/show/cn/3337.html 2020
19. Schaller T, Hirschbühl K, Burkhardt K, Braun G, Trepel M, Märkl B, et al. Post mortem examinations of patients with COVID-19. *JAMA*. (2020) 323:2518–20. doi: 10.1001/jama.2020.8907
20. Stefanini GG, Montorfano M, Trabattoni D, Andreini D, Ferrante G, Ancona M, et al. ST-elevation myocardial infarction in patients with COVID-19: clinical and angiographic outcomes. *Circulation*. (2020) 141:2113–6. doi: 10.1161/CIRCULATIONAHA.120.047525
21. 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
22. Inciardi R, 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
23. Iba T, Levy J, Levi M, Connors JM, Thachil J. Coagulopathy of coronavirus disease 2019. *Crit Care Med*. (2020). doi: 10.1097/CCM.0000000000004458
24. Kucher N, Rossi E, De Rosa M, Goldhaber SZ. Prognostic role of echocardiography among patients with acute pulmonary embolism and a systolic arterial pressure of 90 mm Hg or higher. *Arch Intern Med*. (2005) 165:1777–81. doi: 10.1001/archinte.165.15.1777

25. Casazza F, Bongarzoni A, Capozi A, Agostoni O. Regional right ventricular dysfunction in acute pulmonary embolism and right ventricular infarction. *Eur J Echocardiogr*. (2005) 6:11–4. doi: 10.1016/j.euje.2004.06.002
26. Konstantinides S, Meyer G, Becattini C, Bueno H, Geersing G-J, Harjola V-P, 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
27. Torbicki A, Kurzyna M, Ciurzynski M, Pruszczyk P, Pacho R, Kuch-Wocial A, et al. Proximal pulmonary emboli modify right ventricular ejection pattern. *Eur Respir J*. (1999) 13:616–21. doi: 10.1183/09031936.99.13361699
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. *J Am Soc Echocardiogr*. (2020) 33:648–53. doi: 10.1016/j.echo.2020.04.001
29. Johri AM, Galen B, Kirkpatrick JN, Lanspa M, Mulvagh S, Thamman R. ASE statement on point-of-care ultrasound (POCUS) during the 2019 novel coronavirus pandemic. *J Am Soc Echocardiogr*. (2020) 33:670–3. doi: 10.1016/j.echo.2020.04.017
30. Mehta V, Goel S, Kabarriti R, Cole D, Goldfinger M, Acuna-Villaorduna A, et al. Case fatality rate of cancer patients with COVID-19 in a New York Hospital system. *Cancer Discov*. (2020) 10:935–41. doi: 10.1158/2159-8290.CD-20-0516
31. Sharain K, Hoppensteadt D, Bansal V, Singh A, Fareed J. Progressive increase of inflammatory biomarkers in chronic kidney disease and end-stage renal disease. *Clin Appl Thromb Hemost*. (2013) 19:303–8. doi: 10.1177/1076029612454935
32. Ponikowski P, Voors AA, Anker S, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J*. (2016) 37:2129–200. doi: 10.1093/eurheartj/ehw128
33. Fu S, Ping P, Zhu Q, Ye P, Luo L. Brain natriuretic peptide and its biochemical, analytical, and clinical issues in heart failure: a narrative review. *Front Physiol*. (2018) 9:692. doi: 10.3389/fphys.2018.00692
34. Ikonomidis I, Nikolaou M, Dimopoulou I, Paraskevaidis I, Lekakis J, Mavrou I, et al. Association of left ventricular diastolic dysfunction with elevated NT-pro-BNP in general intensive care unit patients with preserved ejection fraction: a complementary role of tissue Doppler imaging parameters and NT-pro-BNP levels for adverse outcome. *Shock*. (2010) 33:141–8. doi: 10.1097/SHK.0b013e3181ad31f8
35. Kotanidou A, Karsaliakos P, Tzanella M, Mavrou I, Kopterides P, Papadomichelakis E, et al. Prognostic importance of increased plasma amino-terminal pro-brain natriuretic peptide levels in a large noncardiac, general intensive care unit population. *Shock*. (2009) 31:342–7. doi: 10.1097/SHK.0b013e31818635b6
36. Thygesen K, Alpert J, Jaffe A, Chaitman BR, Bax JJ, Morrow DA, et al. Universal definition of myocardial infarction 2018. *Eur Heart J*. (2019) 40:237–69. doi: 10.1093/eurheartj/ehy462
37. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, 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
38. Nagueh S, Smiseth OA, Appleton C, Byrd BF III, 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 Echocardiogr*. (2016) 29:277–314. doi: 10.1016/j.echo.2016.01.011
39. Skulstad H, Cosyns B, Popescu BA, Galderisi M, Salvo GD, Donal E, et al. COVID-19 pandemic and cardiac imaging:EACVI recommendations on precautions, indications, prioritization, and protection for patients and healthcare personnel. *Eur Heart J Cardiovasc Imaging*. (2020) 21:592–8. doi: 10.1093/ehjci/jeaa072
40. British Society of Echocardiography; bsecho.org/covid19.
41. World Health Organization. *CORONAVIRUS DISEASE (COVID-19) Outbreak: Rights, Roles and Responsibilites of Health Workers Including Key Considerations for Occupational Safety and Health*.
42. Klerkin J, Fried JA, Raikhelkar JK, Sayer G, Griffin JM, Masoumi A et al. Coronavirus disease 2019 (COVID-19) and cardiovascular disease. *Circulation*. (2020) 141:1648–55. doi: 10.1161/CIRCULATIONAHA.120.046941
43. Schrezenmeier E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. *Nat Rev Rheumatol*. (2020) 16:155–66. doi: 10.1038/s41584-020-0372-x
44. 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
45. Chatre L, 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
46. Somer M, Kallio J, Pesonen U, Pyykkö K, Huupponen R, Scheinin M, et al. Influence of hydroxychloroquine on the bioavailability of oral metoprolol. *Br J Clin Pharmacol*. (2000) 49:549–54. doi: 10.1046/j.1365-2125.2000.00197.x
47. 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
48. ClinicalTrials.gov. *Hydroxychloroquine for the Treatment of Patients With Mild to Moderate COVID-19 to Prevent Progression to Severe Infection or Death*. Identifier: NCT04323631.
49. Clinical trials.gov. *Chemoprophylaxis of SARS-CoV-2 Infection (COVID-19) in Exposed Healthcare Workers (COVIDAXIS)*. Identifier: NCT04328285. Available online at: https://clinicaltrials.gov/ct2/show/NCT043\penalty-\@M28285
50. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*. (2005) 436:112–6. doi: 10.1038/nature03712
51. Khan A, Benthin C, Zeno B, Albertson TE, Boyd J, Christie JD, et al. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit Care*. (2017) 21:234. doi: 10.1186/s13054-017-1823-x
52. Bloch EM, Shoham S, Casadevall A, Sachais BS, Shaz B, Winters JL, et al. Deployment of convalescent plasma for the prevention and treatment of COVID-19. *J Clin Invest*. (2020) 130:2757–65. doi: 10.1172/JCI138745
53. Russel CD, Millar JE, Baillie JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. *Lancet*. (2020) 395:473–5. doi: 10.1016/S0140-6736(20)30317-2
54. Wang Y, Jiang W, He Q, Wang B, Zhou P, Dong N, et al. A retrospective cohort study of methylprednisolone therapy in severe patients with COVID-19 Pneumonia. *Signal Transduct Target Ther*. (2020) 5:57. doi: 10.1038/s41392-020-0158-2
55. https://www.fda.gov/media/137564/
56. Ko WC, Rolain JM, Lee NY, Chen PL, Huang CT, Lee PI, et al. Arguments in favour of remdesivir for treating SARS-CoV-2 infections. *Int J Antimicrob Agents*. (2020) 55:105933. doi: 10.1016/j.ijantimicag.2020.105933
57. ClinicalTrials.gov. *Severe 2019-nCoV Remdesivir RCT*. Identifier: NCT04257656 (2020). Available online at: https://clinicaltrials.gov/ct2/show/NCT04257656
58. ClinicalTrials.gov. *Mild/Moderate 2019-nCoV Remdesivir RCT*. NCT04252664 (2020). Available online at: https://clinicaltrials.gov/ct2/show/NCT04252664
59. ClinicalTrials.gov. *Study to Evaluate the Safety and Antiviral Activity of Remdesivir (GS-5734^{TM}) in Participants With Severe Coronavirus Disease (COVID-19)*. Identifier: NCT04292899 (2020). Available online at: https://clinicaltrials.gov/ct2/show/NCT04292899
60. ClinicalTrials.gov. *Study to Evaluate the Safety and Antiviral Activity of Remdesivir (GS-5734^{TM}) in Participants With Moderate Coronavirus Disease (COVID-19) Compared to Standard of Care Treatment*. Identifier: NCT04292730 (2020). Available online at: https://clinicaltrials.gov/ct2/show/NCT04292730
61. Grein J, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, et al. Compassionate use of Remdesevir for patients with Severe Covid−19. *N Engl J Med*. (2020) 382:2327–36. doi: 10.1056/NEJMc2015312
62. Lim J, Jeon S, Shin H-Y, Kim MJ, Seong YM, Lee WJ, et al. Case of the index patient who caused tertiary transmission of coronavirus disease 2019

in Korea: the application of lopinavir/ritonavir for the treatment of COVID-19 Pneumonia monitored by quantitative RT-PCI. *J Korean Med Sci*. (2020) 35:e79. doi: 10.3346/jkms.2020.35.e79

63. 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
64. Palios J, Ikonomidis I, Lekakis J, Tsiodras S, Poulakou G, Antoniadou A, et al. Microcirculatory vascular dysfunction in HIV-1 infected patients receiving highly active antiretroviral therapy. *Microcirculation*. (2010) 17:303–10. doi: 10.1111/j.1549-8719.2010.00023.x
65. Lekakis J, Tsiodras S, Ikonomidis I, Palios J, Poulakou G, Rallidis L, et al. HIV-positive patients treated with protease inhibitors have vascular changes resembling those observed in atherosclerotic cardiovascular disease. *Clin Sci*. (2008) 115:189–96. doi: 10.1042/CS20070353
66. Lekakis J, Ikonomidis I, Palios J, Tsiodras S, Karatzis E, Poulakou G, et al. Association of highly active antiretroviral therapy with increased arterial stiffness in patients infected with human immunodeficiency virus. *Am J Hypertens*. (2009) 22:828–34. doi: 10.1038/ajh.2009.90
67. Tsiodras S, Mantzoros C, Hammer S, Samore S. Effects of protease inhibitors on hyperglycemia, hyperlipidemia, and lipodystrophy: a 5-year cohort study. *Arch Intern Med*. (2000) 160:2050–6. doi: 10.1001/archinte.160.13.2050
68. Dressman J, Kincer J, Matveev SV Guo L, Greenberg RN, Guerin T, et al. HIV protease inhibitors promote atherosclerotic lesion formation independent of dyslipidemia by increasing CD36-dependent cholesteryl ester accumulation in macrophages. *J Clin Invest*. (2003) 111:389–39. doi: 10.1172/JCI200316261
69. Naksuk N, Lazar S, Peeraphatdit T, Bee T. 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
70. Hung IF, Lung KC, Tso EY, Liu R, Chung TW, Chu MY, et al. Triple combination of interferon beta-1b, lopinavir–ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. *Lancet*. (2020) 395:1695–704. doi: 10.1016/S0140-6736(20)31042-4
71. Clinical Trials.gov. *Tocilizumab in COVID−19 Pneumonia (TOCIVID-19) NCT04317092*. Available online at: https://clinicaltrials.gov/ct2/show/NCT04317092
72. ClinicalTrials.gov. *Evaluation of the Efficacy and Safety of Sarilumab in Hospitalized Patients With COVID−19 NCT04315298*. (2020). Available online at: https://clinicaltrials.gov/ct2/show/NCT04315298
73. ClinicalTrials.gov. *Bevacizumab in Severe or Critically Severe Patients With COVID−19 Pneumonia-RCT (BEST-RCT) NCT04305106*. (2020). Available online at: https://clinicaltrials.gov/ct2/show/NCT04305106
74. Chen L, Liu HG, Liu W, Liu J, Liu K, Shang J, et al. Analysis of clinical features of 29 patients with 2019 novel coronavirus pneumonia. *Zhonghua Jie He He Hu Xi Za Zhi*. (2020) 43:203–8. doi: 10.3760/cma.j.issn.1001-0939.2020.03.013
75. 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
76. Becker BF, Jacob M, Leipert S, Salmon AH, Chappell D. Degradation of the endothelial glycocalyx in clinical settings: searching for the sheddases. *Br J Clin Pharmacol*. (2015) 80:389–402. doi: 10.1111/bcp.12629
77. Ikonomidis I, Pavlidis G, Katsibri P, Andreadou I, Triantafyllidi E, Tsoumani M, et al. Effects of interleukin 6 inhibitor tocilizumab on endothelial glycocalyx, vascular and myocardial function compared to prednisolone. *Eur Heart J*. (2019) 40:ehz745.0837. doi: 10.1093/eurheartj/ehz745.0837
78. Ikonomidis I, Pavlidis G, Katsimbri P, Andreadou I, Triantafyllidi H, Tsoumani M, et al. Differential effects of inhibition of interleukin 1 and 6 on myocardial, coronary and vascular function. *Clin Res Cardiol*. (2019) 108:1093–101. doi: 10.1007/s00392-019-01443-9
79. Wang, Q, Li C, Zhang Q, Wang T, Li J, Guan W, et al. Interactions of SARS Coronavirus Nucleocapsid Protein with the host cell proteasome subunit p42. *Virol J*. (2010) 7:99. doi: 10.1186/1743-422X-7-99
80. Choi SY, Lim JW, Shimizu T, Kuwano K, Kim JM, Kim H, et al. Reactive oxygen species mediate Jak2/Stat3 activation and IL-8 expression in pulmonary epithelial cells stimulated with lipid-associated membrane proteins from Mycoplasma pneumoniae. *Inflamm Res*. (2012) 61:493–501. doi: 10.1007/s00011-012-0437-7
81. Sugimoto T, Dulgheru R, Bernard AIlardi F, Contu L, Addetia K, et al. Echocardiographic reference ranges for normal left ventricular 2D strain: results from the EACVI NORRE Study. *Eur Heart J Cardiovasc Imaging*. (2017) 18:833–40. doi: 10.1093/ehjci/jex140

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frontiers in Cardiovascular Medicine

OPINION
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In-hospital Routes of Acute Heart Failure Admissions During COVID-19

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Keywords: acute heart failure, ARDS, COVID-19, inhospital routes, emergency department

Coronavirus disease 2019 (COVID-19) is a new viral infection causing acute respiratory distress syndrome (ARDS) that has spread around the world counting 32,429,965 cases and 985,823 deaths as of September 26, 2020[1]. Because of the high percentage of COVID-19–related hospital admission, in Italy a reduction in cardiovascular disease hospitalization was observed, which could contribute to an increased rate of cardiovascular death out-of-hospital (1, 2). Acute heart failure (AHF) syndromes are characterized by a rapid symptom onset requiring fast hospitalization and treatment. Similarly, COVID-19–related ARDS needs hospital admission both for diagnosis and for treatment. However, dyspnea represents a common symptom of these pathological conditions, and for this reason, there is an unmet need of established in-hospital route to better manage the two diseases and to reduce in-hospital infection spread.

First, after presentation to the emergency department (ED), it is mandatory to distinguish two different routes: one is for known positive coronavirus patients and the other for unknown coronavirus patients. In case of positive coronavirus patients, they should recover in the "red zone" of the ED, where AHF patients should undergo clinical, laboratory, and instrumental assessment by clinicians with the support of a cardiologist advisor provided with showerproof single-use coat, gloves, facial protection, and FFP3 mask (3, 4). AHF diagnosis should be done following the latest guidelines criteria (5). Venous blood sample, arterial blood gas analysis, electrocardiogram, and chest x-ray are mandatory to identify the main diagnosis of each patient (viral infection ARDS or cardiovascular disease or cardiovascular involvement during COVID-19). Chest computed tomography scan should be performed according to clinical suspect of interstitial pneumonia. Monitoring electrocardiogram should be useful because of the high risk of arrhythmias in COVID-19 patients. In these patients, echocardiography should be performed in case of new-onset AHF (without medical history of HF), suspected pericardial tamponade, suspected acute pulmonary embolism, suspected AHF associated to acute coronary syndrome (ACS), and suspected acute valvular heart diseases. However, there are some limitations to using echocardiography in COVID-19 patients: (1) high risk of clinician contamination during echocardiography; (2) higher rate of echocardiography failure due to severe respiratory distress. The first concern should be avoided using the latest fast-echo protocol for COVID-19 patients with handheld tablet ultrasound, which provides appropriate information about cardiac conditions, limiting contact and contamination (6). The second concern should be overcome by the use of a contrast agent that enhances the identification/exclusion of ventricular thrombosis, abnormalities in wall motions, and computation of left ventricular ejection fraction (7). AHF treatment should be shared between the cardiologist advisor and ED clinicians, taking into account renal function deterioration, diuresis, electrolytes unbalance, and ARDS complication. ARDS complication should be managed in the "red zone" of the intensive care unit (ICU) together with ICU clinicians. In case

[1] https://covid19.who.int/

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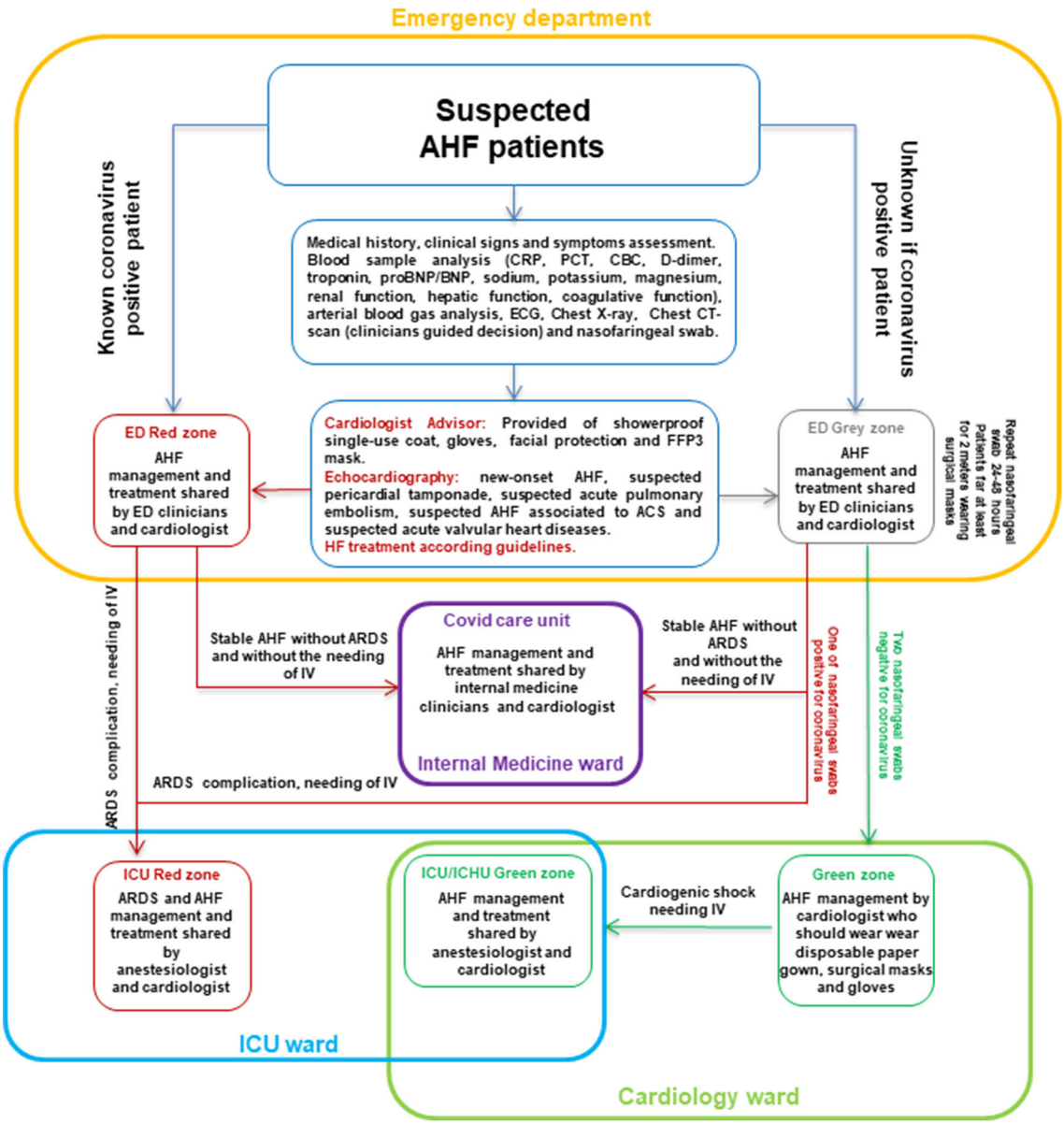

FIGURE 1 | Flow chart for AHF patients hospital admission during COVID-19. ACS, acute coronary syndrome; AHF, acute heart failure; ARDS, acute respiratory distress syndrome; BNP, B-type natriuretic peptide; CBC, count blood cell; CRP, C-reactive protein; CT, computed tomography; ECG, electrocardiogram; ED, emergency department; ICU, intensive care unit; ICHU, intensive care heart unit; IV, invasive ventilation; PCT, procalcitonin.

of coronavirus-positive AHF patients, who do not show ARDS and do not require invasive ventilation (IV), these patients should be allocated into internal medicine ward, which should be organized as COVID-19 care unit. In this unit, patients should be managed by internal medicine clinicians together with a cardiologist advisor. In case of unknown for COVID-19 AHF patients, it is necessary to limit infection among patients and health care workers. It should be optimal to recognize a "gray zone" within the ED where patients should be screened for coronavirus. In this "gray zone," patients should be far at least for 2 m, and healthcare workers should wear showerproof single-use coat, gloves, facial protection, and FFP3 mask (3, 4). Recovered patients should undergo nasopharyngeal swabbing at hospital admission and after 24–48 h. The cardiologist advisor should support clinicians for AHF diagnosis and treatment during coronavirus infection assessment, wearing showerproof single-use coat, gloves, facial protection, and FFP3 mask. In case of positive coronavirus nasopharyngeal swab, AHF patients should be managed in the "red zone" together with ED clinicians; these patients should be transferred to the ICU or COVID-19 care unit according to ARDS complications and the need for IV. In case of two negative coronavirus nasopharyngeal swab results,

AHF patients could be transferred in the cardiology ward ("green zone") and should be managed and treated by cardiologists. In the "green zone," cardiologist and other healthcare workers should wear disposable paper gown, surgical masks, and gloves. All patients may wear surgical masks during the hospitalization period. Cardiac biomarkers (D-dimer, troponin, and natriuretic peptide) monitoring should be performed in all patients to recognize treatment efficacy, worsening heart failure, and acute cardiovascular complication related or not to COVID-19, such as acute pulmonary embolism or ACS (**Figure 1**).

AUTHOR CONTRIBUTIONS

GR give a substantial contribution in conception, design, writing, and revising the manuscript. MF and AP revising manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. 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
2. Baldi E, Sechi GM, Mare C, Canevari F, Brancaglione A, Primi R,. Out-of-Hospital Cardiac Arrest during the Covid-19 Outbreak in Italy. *N Engl J Med.* (2020) 383:496–8. doi: 10.1056/NEJMc2010418
3. Centers for Disease Control and Prevention. *Discontinuation of Transmission-Based Precautions and Disposition of Patients With COVID-19 in Healthcare Settings (Interim Guidance)*. (2020). Available online at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/disposition-hospitalized-patients.html
4. Ong SWX, Tan YK, Chia PY, Lee TH, Ng OT, Wong MSY. Surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) from a symptomatic patient. *JAMA*. (2020) 323:1610–2. doi: 10.1001/jama.2020.3227
5. 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
6. McMahon SR, De Francis G, Schwartz S, Duvall WL, Arora B, Silverman DI. Tablet-based limited echocardiography to reduce sonographer scan and decontamination time during the COVID-19 pandemic. *J Am Soc Echocardiogr*. (2020) 33:895–9. doi: 10.1016/j.echo.2020.05.005
7. Argulian E, Sud K, Bohra C, Vogel B, Garg V, Talebi S, et al. Safety of ultrasonic enhancing agents in patients with COVID-19. *J Am Soc Echocardiogr*. (2020) 33:906–8. doi: 10.1016/j.echo.2020.04.022

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Comorbidities, Cardiovascular Therapies, and COVID-19 Mortality: A Nationwide, Italian Observational Study (ItaliCO)

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Background: Italy has one of the world's oldest populations, and suffered one the highest death tolls from Coronavirus disease 2019 (COVID-19) worldwide. Older people with cardiovascular diseases (CVDs), and in particular hypertension, are at higher risk of hospitalization and death for COVID-19. Whether hypertension medications may increase the risk for death in older COVID 19 inpatients at the highest risk for the disease is currently unknown.

Methods: Data from 5,625 COVID-19 inpatients were manually extracted from medical charts from 61 hospitals across Italy. From the initial 5,625 patients, 3,179 were included in the study as they were either discharged or deceased at the time of the data analysis. Primary outcome was inpatient death or recovery. Mixed effects logistic regression models were adjusted for sex, age, and number of comorbidities, with a random effect for site.

Results: A large proportion of participating inpatients were ≥65 years old (58%), male (68%), non-smokers (93%) with comorbidities (66%). Each additional comorbidity increased the risk of death by 35% [$_{adj}$OR = 1.35 (1.2, 1.5) $p < 0.001$]. Use of ACE inhibitors, ARBs, beta-blockers or Ca-antagonists was not associated with significantly increased risk of death. There was a marginal negative association between ARB use and death, and a marginal positive association between diuretic use and death.

Conclusions: This Italian nationwide observational study of COVID-19 inpatients, the majority of which ≥65 years old, indicates that there is a linear direct relationship between the number of comorbidities and the risk of death. Among CVDs, hypertension and pre-existing cardiomyopathy were significantly associated with risk of death. The use of hypertension medications reported to be safe in younger cohorts, do not contribute significantly to increased COVID-19 related deaths in an older population that suffered one of the highest death tolls worldwide.

Keywords: COVID-19, comorbidities, ACE inhibitors, mortality, cohort study

INTRODUCTION

Italy, after Japan, tops the list of the world's oldest countries, with over 22% of its population aged 65 or older (1). Italy has been one of the hardest hit countries during the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. As of September 1 2020 over 35,500 persons had died due to Coronavirus disease 2019 (COVID-19), especially in the northern regions of the country, with 84.5% of deaths occurring in patients age 70 or older (Istituto Superiore di Sanitá, ISS, https://www.epicentro.iss.it/coronavirus/), and a crude case fatality rate in the region Lombardy of 18.3% (2). Previous study showed that comorbid conditions play a relevant role in increasing the risk of death in patients with COVID-19 (3–9). In particular, hypertension and underlying cardiovascular diseases (CVDs) have been strongly associated with death in COVID-19 inpatients (7, 10, 11), and case fatality rates tend to be high in older people and hypertensive individuals (12). Indeed, the prevalence of CVDs in COVID-19 patients across studies ranges from 8 to 42% (13). Hypertension, heart failure (HF) and ischemic heart disease are often treated with renin-angiotensin-aldosterone system (RAAS) blockers such as angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs). The use of ACE inhibitors/ARBs in patients with COVID-19 or at risk of infection with the virus is currently a subject of intense debate (14, 15), due to the evidence that SARS-CoV-2 uses the ACE2 receptor for entry into target cells (16). ACE2 and its related axis are an endogenous counter-regulatory system, with effects opposite to those of the ACE axis (17, 18). Nonetheless, ACE inhibitor/ARBs have also been associated with a reduction of mortality and re-hospitalization in patients with cardiovascular diseases due to their anti-thrombotic and anti-inflammatory effects, and protective effects against endothelial dysfunction (13). Whether the use of ACE inhibitors and ARB affects the mortality of COVID-19 patients has been debated. The only and largest survey published so far assessing the association between comorbidities, use of ACE inhibitors/ARBs, and COVID-19 death included 4,480 patients from Denmark (12). The authors found no evidence that either ACE inhibitors or ARB increased the risk for death among persons hospitalized for COVID-19. However, the COVID-19-related death burden in Denmark has been tremendously lower than Italy (628 vs. 35,595, respectively, as of September 1 2020) which poses questions on the heterogeneity of the Italian and Danish populations and the ways COVID-19 hit and was handled by the two countries. A second study from China also excluded subjects aged ≥75 years (19). To date, there is no study available of the relation between chronic use of ACE inhibitors and ARB, considered separately, and mortality in hospitalized COVID-19 patients that includes sufficient patients in the older age group, and that accounts for concomitant cardiovascular therapies or comorbid conditions. Moreover, due to higher fatality of COVID-19 infection in patients affected by CVDs, there is an unmet need to understand the link between cardiovascular therapies, CVD and COVID-19 severity and mortality.

In this study, we describe baseline characteristics and factors associated with death among 3,179 patients hospitalized for COVID-19, who were either discharged or died, and who were residents of 19 out of Italy's 21 regions, including the main islands. In assessing potential risks for death, we specifically assessed the comorbidities and the role of pre-hospitalization ACE inhibitors and ARBs and other commonly used CVD medications (such as beta-blockers, calcium channel blockers and diuretics). By age distribution, our sample is representative of older people, that are most severely affected by COVID-19 since the beginning of the pandemic (5, 6).

METHODS

Patient Inclusion

Data for 5,625 patients hospitalized for COVID-19 and with a positive nasopharyngeal swab for SARS-CoV-2 virus were manually extracted from medical charts from 61 hospitals across Italy. Patients were included in this analysis if they had been either discharged or had died at the time of ascertainment (n = 3,179, **Figure 1**, 56 sites). The status for each patient was reported at the time of data collection by the local investigators and represents an assessment of the patient's condition between March 25 and April 22, 2020. All the patients' information was obtained by manual review of the medical charts by the attending physician or nurse during their shifts. Each participating center was provided, upon enrollment, with a database to fill with patients' demographic, social, and clinical information and detailed instructions about the data collection. Smoking history was manually extracted from the chart for each patient. Information about smoking was not available for 316 patients. The collection and analysis of data in the registry have been deemed exempt from ethics review.

FIGURE 1 | Italian Cartographic representation of the study subjects: Cartographic representation of the patients in this study cohort, with the area of each red circle proportional to the combined number of patients from each compact metropolitan area.

Comorbidities

Investigators manually extracted information about preexisting comorbidities known or suspected to be associated with COVID-19 mortality from the chart of each patient that was still hospitalized in their hospital or discharged within 30 days from the collection of the data. Information was available for atrial fibrillation, blood cancer, organ cancer, coronary artery disease, cardiomyopathy, chronic heart failure, chronic obstructive pulmonary disease (COPD), chronic renal failure, diabetes, hypertension, obesity, and stroke. We used a count

of the reported number of comorbidities for each patient to assess their combined effect on mortality. Patients missing comorbidity information were excluded from these analyses ($n = 17$, **Figure 2**).

Cardiovascular Medications

For this study, we specifically targeted extraction of detailed information from the patient's chart regarding use of ACE inhibitors and ARB at the time of admission. We also extracted information about other medications usually prescribed for hypertension (beta-blockers, diuretics, and Ca-antagonists).

Statistics

A generalized linear mixed model, mixed-effects logistic regression, was used to assess the relations of sex, age, comorbidity count and hypertension medication use to death

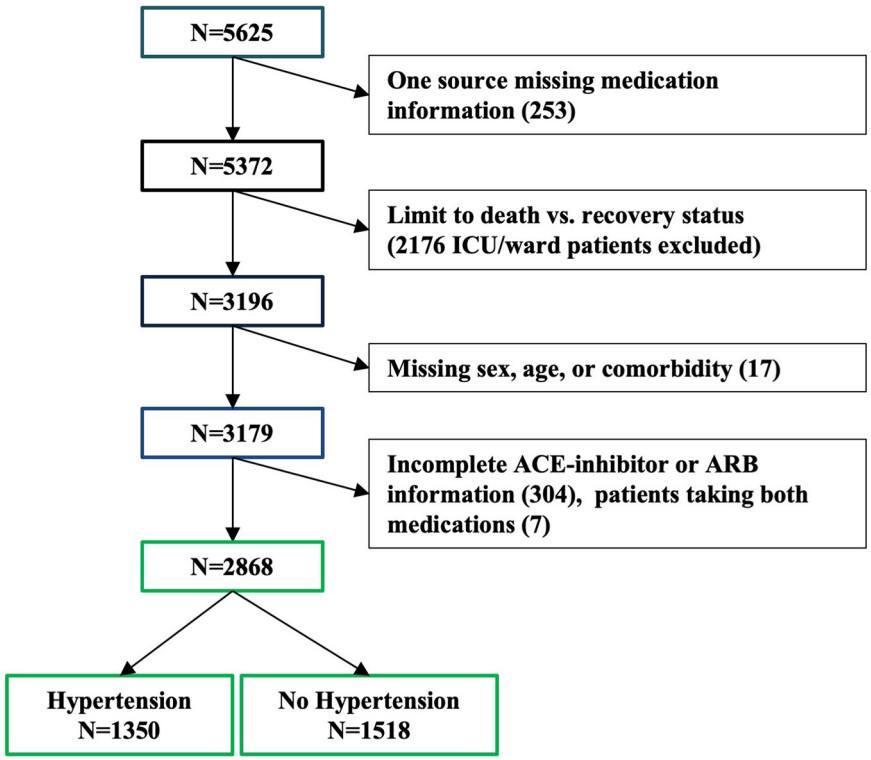

FIGURE 2 | Flow chart of patient sample sizes.

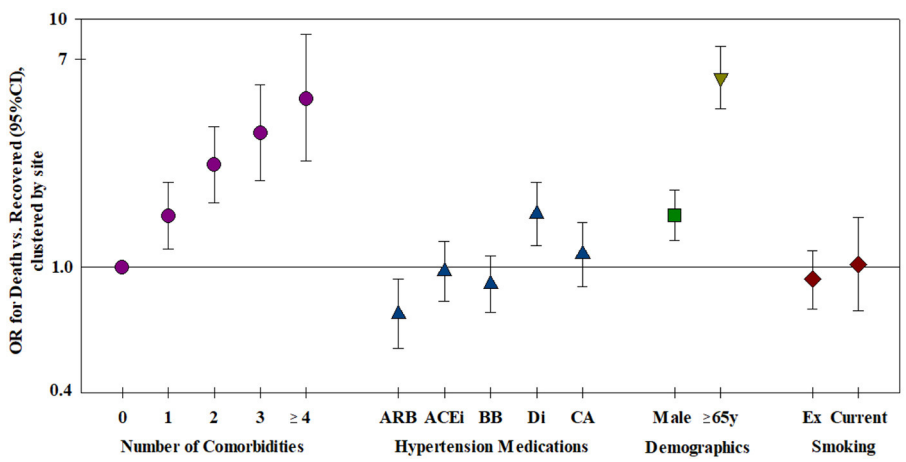

FIGURE 3 | Risk factors for mortality—all risk factors were included in the model, clustered by site ($n = 2,868$). ARB, Angiotensin receptor blocker; ACEi, Angiotensin converting enzyme inhibitor; BB, Beta-blocker; Di, Diuretic; CA, Ca-antagonist.

relative to recovery (STATA 16, StataCorp, College Station, TX, USA). The primary outcome was inpatient mortality. Since data were clustered by hospital site, site was included in the models as a random effect to account for potential within site correlation of patient characteristics. The number of patients contributed by each hospital site varied, ranging from 2 to 242 patients (**Supplementary Figure 1**). A dummy category for those patients missing smoking information was included in the model for **Figure 3**.

RESULTS

There were 3,179 patients with complete data for sex, age, status, and comorbidities (**Table 1**); 2,282 (71.8%) had been discharged from the hospital and 897 (28.2%) had died. The median age was 69.0 years, with an interquartile range of 57 to 78 years (**Supplementary Figure 2**).

Risk Factors for Death

The relation of age and death was non-linear; very few patients under the age of 50 died (**Table 1**). Males were more likely to die than females and patients aged ≥65 years were over six times more likely to die compared to younger patients (**Table 2**). Current smoking was unrelated to death in either univariate or multivariable analyses (**Figure 3**). Hypertension (47.2%) was the most frequent comorbidity, followed by diabetes (16.3%), coronary artery disease (11.3%), atrial fibrillation (8.1%), and obesity (6.9%) (**Table 1**). In a model including all comorbidities and adjusted for sex, age and site, the comorbidities cardiomyopathy, COPD, chronic renal failure, hypertension, obesity, organ cancer, and stroke were all independent risk factors for death (**Table 3**). Interestingly, while only 5.9% of COVID-19 inpatients had COPD, the latter was highly and significantly associated with increased risk of death (**Table 3**). In addition to evaluating the relation of the individual comorbidities to death, the count of comorbidities reported for each patient was strongly associated with risk of death (**Table 2**), with the odds for death increasing by 35% for each additional comorbidity (evaluated as an ordinal count; adjOR =1.35 [1.2, 1.5] $p < 0.001$), after adjusting for sex, age, and site.

Analysis of Variability Across Geographic Regions and Hospitals

There were differences in the number of comorbidities across the hospitals and regional areas that provided data (**Supplementary Table 1**). Patients from the Central Italy were reported to have the fewest number of comorbidities and those from the Northeastern region to have the most. Therefore, in order to assess whether the geographic/hospital variation in diagnostic labeling of comorbidities could have influenced the meaning of the associations made between comorbidities and mortality, in addition to the random effect adjustment for hospital site, we added regional area to the multivariable model shown in **Table 2**. There was no appreciable change in the relation of any risk factor to death, including comorbidity count, after this additional adjustment (data not shown).

TABLE 1 | Characteristics of all patients, recovered patients and deceased patients.

Characteristic	Group	All patients (n = 3,179)		Recovered (n = 2,282)		Deceased (n = 897)	
		N	%	*N*	%	*N*	%
Sex	Female	1,008	31.7	764	33.5	244	27.2
	Male	2,171	68.3	1,518	66.5	653	72.8
Age	<20	10	0.3	10	0.4	0	0
	20<30	35	1.1	34	1.5	1	0.1
	30<40	102	3.2	101	4.4	1	0.1
	40<50	260	8.2	250	11.0	10	1.1
	50<60	547	17.2	497	21.8	50	5.6
	60<70	707	22.2	572	25.1	135	15.1
	70<80	828	26.1	525	23.0	303	33.8
	80+	690	21.7	293	12.8	397	44.3
Age	<65	1,320	41.5	1,205	52.8	115	12.8
	≥65	1,859	58.5	1,077	47.2	782	87.2
Each comorbidity[a]	Atrial fibrillation	256	8.1	142	6.2	114	12.7
	Blood cancer	28	0.9	19	0.8	9	1.0
	Coronary artery disease	359	11.3	186	8.2	173	19.3
	Cardiomyopathy	105	3.3	54	2.4	51	5.7
	Chronic heart failure	119	3.7	59	2.6	60	6.7
	COPD	188	5.9	98	4.3	90	10.0
	Chronic renal failure	157	4.9	72	3.2	85	9.5
	Diabetes	518	16.3	319	14.0	199	22.2
	Hypertension	1,500	47.2	960	42.1	540	60.2
	Obesity	218	6.9	163	7.1	55	6.1
	Organ cancer	135	4.3	82	3.6	53	5.9
	Stroke	107	3.4	52	2.3	55	6.1
Comorbidities[b] Count	0	1,096	34.5	939	41.2	157	17.5
	1	1,043	32.8	756	33.1	287	32.0
	2	644	20.3	388	17.0	256	28.5
	3	274	8.6	144	6.3	130	14.5
	≥4	122	3.8	55	2.4	67	7.5
Smoking[c]	Never	1,963	68.6	1,437	68.9	526	67.7
	Ex	692	24.2	496	23.8	196	25.2
	Current	208	7.3	153	7.3	55	7.1
Race[d]	Caucasian	2,983	97.5	2,133	96.6	850	99.8
	Not caucasian	77	2.5	75	3.4	2	0.2
Regional areas	Lombardia	1,397	43.9	901	39.5	496	55.3
	Northeastern	634	19.9	481	21.1	153	17.1
	Northwestern	380	12.0	272	11.9	108	12.0
	Central	400	12.6	340	14.9	60	6.7
	Southern	368	11.6	288	12.6	80	8.9

[a] *Percentage with each comorbidity was calculated as the number with the comorbidity divided by the total for that column.*

[b] *Number of comorbidities were summed for each patient and included: atrial fibrillation, blood cancer, organ cancer, coronary artery disease, cardiomyopathy, chronic heart failure, COPD, chronic renal failure, diabetes, hypertension, obesity and stroke.*

[c] *316 patients were missing smoking information.*

[d] *119 patients were missing race information.*

TABLE 2 | Proportion of patient groups who died vs. recovered at the time of data collection.

Characteristic	Group	All patients (n = 3,179)									
						Univariate			Multivariable		
		Outcome				Death			Death		
		Total *N*	Recovered *N*	Deceased *N*	Deceased %	OR[a]	95%CI	*P*	$_{adj}$OR[c]	95%CI	*P*
Sex	Female	1,008	764	244	24.2	ref			ref		
	Male	2,171	1,518	653	30.1	1.42	1.2, 1.7	0.001	1.56	1.3, 1.9	<0.001
Age	<65	1,320	1,205	115	8.7	ref			ref		
	≥65	1,859	1,077	782	42.1	8.25	6.4, 10.6	<0.001	6.35	4.9, 8.2	<0.001
Comorbidities[b]	0	1,096	939	157	14.3	ref			ref		
	1	1,043	756	287	27.5	2.59	2.0, 3.3	<0.001	1.71	1.3, 2.2	<0.001
	2	644	388	256	39.8	4.54	3.5, 6.0	<0.001	2.52	1.9, 3.4	<0.001
	3	274	144	130	47.5	6.69	4.7, 9.4	<0.001	3.29	2.3, 4.7	<0.001
	≥4	122	55	67	54.9	9.26	5.7, 14.9	<0.001	4.49	2.7, 7.4	<0.001

Univariate and multivariable estimates for the relation of sex, age, and number of comorbidities to patient mortality, with clustering for site.

[a] *Odds ratio for death estimated with clustering for site (56 sites).*

[b] *Number of comorbidities were summed for each patient and included: atrial fibrillation, blood cancer, organ cancer, coronary artery disease, cardiomyopathy, chronic heart failure, COPD, chronic renal failure, diabetes, hypertension, obesity and stroke.*

[c] *Multivariable model included sex, age divided into those <65 and those ≥65 years old, and the number of comorbidities as a categorical covariate with clustering for site as a random effect.*

TABLE 3 | Multivariable model for the risk of death associated with each comorbidity after adjustment for sex and age, clustering for site as a random effect.

Comorbidity	All patients (n = 3,179)		
	Risk of death		
	$_{adj}$OR	95%CI	*P*
Atrial fibrillation	0.97	0.69, 1.36	0.874
Blood Cancer	0.93	0.29, 2.97	0.901
Coronary artery disease	1.11	0.83, 1.49	0.471
Cardiomyopathy	**1.85**	**1.11, 3.11**	**0.019**
Chronic heart failure	0.74	0.44, 1.23	0.240
COPD	**1.93**	**1.31, 2.85**	**0.001**
Chronic renal failure	**1.71**	**1.09, 2.67**	**0.019**
Diabetes	1.21	0.93, 1.58	0.149
Hypertension	**1.24**	**1.00, 1.53**	**0.049**
Obesity	**2.03**	**1.30, 3.17**	**0.002**
Organ cancer	**1.67**	**1.04, 2.68**	**0.032**
Stroke	**2.00**	**1.22, 3.27**	**0.006**
Male	**1.85**	**1.47, 2.33**	**<0.001**
Age, years	**1.10**	**1.09, 1.11**	**<0.001**

P < 0.05 are indicated in bold.

Risk of Death by Hypertension Medication Use

Of the 3,179 patients, 2,868 had complete information for hypertension medication use. Patients with no comorbidities were less likely to use ARBs and ACE inhibitors but there was no trend for increased use of ACE inhibitors or ARBs among patients with one or more comorbidities (**Supplementary Table 2**). The use of diuretics, beta-blockers and Ca-antagonists increased significantly with the number of comorbidities reported for each patient. Comorbidities were strongly associated with age but not with sex (**Supplementary Table 2**).

Most of the 951 patients taking either ACE inhibitors or ARB at admission had hypertension, 87.9 and 90.3%, respectively, and beta-blocker use was reported for 29.9%, diuretic use for 24.3% and Ca-antagonist use for 22.8%. After adjustment for age, sex, number of comorbidities, smoking and site, we found no increased risk of death associated with the use of ACE inhibitors, ARBs, beta-blockers or Ca-antagonists (**Figure 3** and **Table 4**). There was a marginal negative association between ARB use and a marginal positive association between diuretic use and death (**Figure 3**, $p = 0.025$ and $p = 0.020$, respectively).

DISCUSSION

This is the first and largest Italian countrywide study to date of COVID-19 inpatients, the majority of which was aged over 65, who either died or were discharged from hospital in 19 out of the 21 Italian regions, including the major islands (**Figure 1**). We found, in line with previous publications (3–9), that pre-existing comorbidities are major risk factors for death in COVID-19 patients. We report that the number of comorbidities is linearly and strongly associated with the risk of COVID 19-related death. However, after adjusting for comorbidities, age, and sex, we report that the lack of an association between risk of inpatient death due to COVID-19 and use of CVD medications reported in younger patients (3–9), extends to the older population at highest risk for COVID-19. Diuretics were associated with a marginal increased risk of death, and ARBs

TABLE 4 | List of risk factors included in the model, clustered for site as a random effect.

Characteristic	Group	Patients with complete hypertension medication information (n = 2,868)						
						Death		
		Total *N*	Recovered *N*	Deceased *N*	Deceased %	$_{adj}$OR	95%CI	*P*
Comorbidities[b]	0	982	845	137	14.0	ref		
	1	927	690	237	25.6	1.64	1.19, 2.24	0.020
	2	595	365	230	38.7	2.64	1.85, 3.78	<0.001
	3	250	129	121	48.4	3.56	2.29, 5.55	<0.001
	≥4	114	50	64	56.1	4.88	2.73, 8.72	<0.001
ARB	No	2,446	1,767	679	27.8	ref		
	Yes	422	312	110	26.1	0.65	0.47, 0.89	0.025
ACE inhibitor	No	2,339	1,742	597	25.5	ref		
	Yes	529	337	192	36.3	0.97	0.73, 1.29	0.929
Beta-blocker	No	2,247	1,702	545	24.3	ref		
	Yes	621	377	244	39.3	0.85	0.65, 1.12	0.244
Diuretic	No	2,456	1,870	586	23.9	ref		
	Yes	412	209	203	49.3	1.66	1.23, 2.25	0.020
Ca-antagonist	No	2,488	1,842	646	26.0	ref		
	Yes	380	237	143	37.6	1.13	0.84, 1.53	0.773
Sex	Female	904	684	220	24.3	ref		
	Male	1,964	1,395	569	29.0	1.65	1.29, 2.09	<0.001
Age	<65years	1,207	1,100	107	8.9	ref		
	≥65 years	1,661	979	682	41.1	5.92	4.47, 7.83	<0.001
Smoking[c]	No	1,771	1,293	478	27.0	ref		
	Ex	642	470	172	26.8	0.89	0.68, 1.17	0.412
	Current	187	135	52	27.8	1.04	0.66, 1.62	0.879
	Unknown	268	181	87	32.5	1.65	1.08, 2.52	0.020

The risk factors for mortality are also listed in ***Figure 3****.*

[a]*Multivariable model included sex, age, number of comorbidities as a categorical covariate, smoking and each hypertension medication, with clustering for site as a random effect (55 sites); a dummy category for patients with missing smoking history was included in the model.*

[b]*Number of comorbidities were summed for each patient and included: atrial fibrillation, blood cancer, organ cancer, coronary artery disease, cardiomyopathy, chronic heart failure, COPD, chronic renal failure, diabetes, hypertension, obesity and stroke.*

[c]*Patients who were missing information about smoking were included as a separate smoking category in the model.*

with a marginal decreased risk of death in this sample. Each comorbidity increased mortality risk independently from age and gender. Among CVD, cardiomyopathies and hypertension were related to a poor outcome. These findings are in line with Inciardi et al. (20) who showed that, in the Northern Italian population, COVID-19 patients with pre-existing CVD had an increased rate of death compared to patients without CVD. The underlying systemic inflammation in patients with CVD (21) might contribute to the increase immune responses and inflammatory cascade known to lead to a worse prognosis in COVID-19 patients (22).

Prior therapy with ACE inhibitors/ARBs was not related to worse prognosis in this cohort. The use of ACE inhibitors and ARB in patients with COVID-19 has been called into question by some (14), due to the evidence that SARS-CoV-2 uses the ACE2 receptor for entry into target cells (16). On the other hand, it has been recently shown that treatment with ACE inhibitors and ARBs does not increase ACE2 plasma levels in patients with heart failure (23). A recent study by Fosbol et al. (12) showed no association between risk of mortality in the Danish population and ACE inhibitor/ARB use. However, the COVID-19-related death burden in Denmark has been ∼57-fold lower than Italy (https://coronavirus.jhu.edu/data/mortality), which calls for further studies investigating the characteristics of the Italian COVID-19 population. Additionally, Fosbol et al. did not compare the use of ACE inhibitors to the use of ARB, and the data were computed from national electronic health record review. Here we report that a similar conclusion is applicable to the Italian population. As the north of Italy, and especially the region Lombardia, suffered from one of the highest COVID-19 mortality rates worldwide (2), our data (carefully collected by manual review of medical charts from Italian 56 hospital distributed throughout the peninsula) are particularly important in order to understand the characteristics of the Italian COVID-19 patients with regional specificity. Also, in our study, a higher number of inpatients taking either ACEi or ARB than the Danish cohort was enrolled, thus allowing a stronger statistical power to rule out the individual effects of ACE inhibitors and ARB treatments. In another study, Reynolds et al. reported that previous treatment with ACE inhibitors or ARBs was not

associated with a higher risk of testing positive for COVID-19 (10). Similarly, Mancia et al. recently showed that the use of ARBs and ACE inhibitors was more frequent among patients from one Italian region (Lombardy) who were infected with SARS-CoV-2 than among a large population of controls who were matched for age, sex, and place of residence (9). However, in the same study, neither ACE inhibitors nor ARBs showed an independent association with COVID-19 in patients with mild-to-moderate disease or in those with severe disease. Neither of these two latter studies had mortality as primary outcome, but rather the likelihood of the subjects of testing positive for COVID-19, or experiencing severe manifestations of COVID-19.

In a study of 5,700 patients hospitalized with COVID-19 in the New York City area, the mortality rates for patients with hypertension not taking an ACE inhibitors or ARBs at admission, taking an ACE inhibitor, or taking an ARB were comparable (8). Moreover, among 1,128 hospitalized COVID-19 patients with hypertension from Hubei, China, the inpatient use of ACE inhibitors/ARB was reported to be associated with lower risk of all-cause mortality compared with ACE inhibitors/ARB non-users (19). However, in the first study (8) the results were unadjusted for known confounders, including age, sex, race, ethnicity, and comorbidities. In the second study (19), the sample-size included only 188 patients who received ACE inhibitors/ARB, and thus it did not have the power to test the effects of ACE inhibitors and ARBs separately.

This is the first study assessing the safety of CVD medications, and in particular ACE inhibitors and ARB studied separately, in a nation-wide representative sample of COVID-19 inpatients, mostly aged >65. We provide strong evidence suggesting that, regardless of a person's risks for COVID19, the five drugs most frequently used for the treatment of CVDs in outpatient settings are not associated with increased inpatient mortality due to COVID-19.

Among CVD therapies, diuretics were associated with a marginally significant increased risk of mortality in our final models. However, the proportion of patients who were using diuretics at the time of admission increased markedly with the number of comorbidities in each patient. Although we adjusted for number of comorbidities, it is likely that residual confounding may be present. Similarly, use of ARBs was associated with a small decrease in death rates after adjustment for confounders, but the effect was marginally significant and may have been influenced by unaccounted confounding.

Our study has several strengths: (1) it is the first Italian country-wide study describing the characteristics of a highly heterogeneous cohort of COVID-19 inpatients both in terms of severity of the clinical manifestations, and also in terms of geographical distribution. We believe that the analyses performed in this study are clinically informative given that the north of Italy suffered from one of the highest mortalities for COVID-19 worldwide; and (2) the availability of a sample representative of the older people (>65 years of age) at highest risk for morbidity and mortality due to COVID-19. Limitations include: (1) the observational nature of the study, and the fact that data on comorbidities were collected by manual review of the medical charts with no objective assessment; this methodological limitation did not allow the disentanglement of the independent associations of antihypertensive medications with mortality (i.e., adequate control for disease related-, hypertension-, and other medication use- related variables); (2) lack of other outcome measures apart from death or hospital discharge, and of information about the cause of death of the patients (such as severe respiratory distress, acute kidney injury, myocardial infarction, pulmonary, and systemic thromboembolism); (3) the absence of information about specific treatments received during hospitalization and in-hospital ACE inhibitors/ARBs continuation or discontinuation; (4) the lack of a severity score for COVID-19 patients during hospitalization; and (5) the potential bias introduced by excluding patients that were still hospitalized at the time of data collection. However, we felt that including the latter could have introduced an even bigger bias, because these individuals could have had associations between the variables analyzed and the outcome that could be different from patients that were included because they had an outcome.

In summary, the results of our nationwide Italian study of a population of COVID19 inpatients, that suffered from one of the highest mortality rates worldwide, confirms that the number of comorbidities appears to be independently associated with increased COVID-19-related death. Among cardiovascular comorbidities both hypertension and pre-existing cardiomyopathy were associated with COVID-19 risk of death. Nonetheless, we provide reassuring evidence that use of commonly-used CVD medications is not associated with increased risk of death due to COVID-19, regardless of the person's risk for the disease due to age, sex, or comorbid conditions. Our findings show that there is no need to interrupt treatments for CVD in COVID-19 patients; in particular, the treatment with ACE inhibitors/ARBs should be continued in order to reduce potential cardiovascular derangement in COVID-19 patients. Further studies are needed in order to shed light onto the relationship between CVD therapies, underlying CVD, and prognosis in COVID-19 patients.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Arizona IRB waiver #2003521629. The ethics committee approvals/waivers were also obtained from each of the participating hospitals. The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

FP, GR, EB, MB, MCa, BC, MCo, AC, FD'A, ED'E, GF, SGa, SGu, SH, MK, LM, AP, RP, PP, VP, MP, CT, and RT collected the data. FP, DAS, SGu, JCW, and FDiM analyzed the data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2020.585866/full#supplementary-material

REFERENCES

1. International Institute for Applied Systems Analysis (IIASA). Aging Demographic Data Sheet: Laxenburg, AI: International Institute for Applied Systems Analysis (IIASA) (2018).
2. Odone A, Delmonte D, Scognamiglio T, Signorelli C. COVID-19 deaths in Lombardy, Italy: data in context. *Lancet Public Health.* (2020) 5:e310. doi: 10.1016/S2468-2667(20)30099-2
3. 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
4. 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: 10.1183/13993003.01227-2020
5. Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. *JAMA.* (2020) 323:1775–6. doi: 10.1001/jama.2020.4683
6. 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
7. 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
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. 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
10. 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
11. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol.* (2020) 17:259–260. doi: 10.1038/s41569-020-0360-5
12. 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
13. 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
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. 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
16. 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.e278. doi: 10.1016/j.cell.2020.02.052
17. Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, et al. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature.* (2012) 487:477–81. doi: 10.1038/nature11228
18. 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
19. Zhang P, Zhu L, Cai J, Lei F, Qin JJ, 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) 126:1671–81. doi: 10.1161/CIRCRESAHA.120.317242

20. 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
21. Paquissi FC. The role of inflammation in cardiovascular diseases: the predictive value of neutrophil-lymphocyte ratio as a marker in peripheral arterial disease. *Ther Clin Risk Manag.* (2016) 12:851–60. doi: 10.2147/TCRM.S107635
22. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet.* (2020) 395:1033–14. doi: 10.1016/S0140-6736(20)30628-0
23. Sama IE, Ravera A, Santema BT, van Goor H, Ter Maaten JM, Cleland JGF, et al. Circulating plasma concentrations of angiotensin-converting enzyme 2 in men and women with heart failure and effects of renin-angiotensin-aldosterone inhibitors. *Eur Heart J.* (2020) 41:1810–7. doi: 10.1093/eurheartj/ehaa373

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REVIEW
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Vascular Manifestations of COVID-19 – Thromboembolism and Microvascular Dysfunction

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The coronavirus pandemic has reportedly infected over 31.5 million individuals and caused over 970,000 deaths worldwide (as of 22nd Sept 2020). This novel coronavirus, officially named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), although primarily causes significant respiratory distress, can have significant deleterious effects on the cardiovascular system. Severe cases of the virus frequently result in respiratory distress requiring mechanical ventilation, often seen, but not confined to, individuals with pre-existing hypertension and cardiovascular disease, potentially due to the fact that the virus can enter the circulation via the lung alveoli. Here the virus can directly infect vascular tissues, via TMPRSS2 spike glycoprotein priming, thereby facilitating ACE-2-mediated viral entry. Clinical manifestations, such as vasculitis, have been detected in a number of vascular beds (e.g., lungs, heart, and kidneys), with thromboembolism being observed in patients suffering from severe coronavirus disease (COVID-19), suggesting the virus perturbs the vasculature, leading to vascular dysfunction. Activation of endothelial cells via the immune-mediated inflammatory response and viral infection of either endothelial cells or cells involved in endothelial homeostasis, are some of the multifaceted mechanisms potentially involved in the pathogenesis of vascular dysfunction within COVID-19 patients. In this review, we examine the evidence of vascular manifestations of SARS-CoV-2, the potential mechanism(s) of entry into vascular tissue and the contribution of endothelial cell dysfunction and cellular crosstalk in this vascular tropism of SARS-CoV-2. Moreover, we discuss the current evidence on hypercoagulability and how it relates to increased microvascular thromboembolic complications in COVID-19.

Keywords: COVID-19, endothelium, pericyte, coronavirus, thromboembolism

INTRODUCTION

In January 2020, the Center for Disease Control recognized a new coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is believed to have originated from the Wuhan city in Hubei province, China. As of the 22nd September 2020, over 31.5 million people worldwide have been infected, with currently over 970,000 deaths recorded (1). According

to the World Health Organization (WHO) the total case fatality rate (CFR) is 3.1%, but this varies significantly depending on geographical location. For example, the USA have a CFR of 2.9% (6,740,464 cases), whereas the United Kingdom and Italy have significantly higher CFRs of 10.6% (394,261 cases) and 12.0% (298,156 cases), respectively (1). The SARS-CoV-2 infection gives rise to COVID-19 disease, which typically results in fever, respiratory distress (shortness of breath and cough) (2–4), and subsequent respiratory failure. Symptoms often arise between 2 and 14 days after infection (5), and the risk of mortality due to COVID-19 appears greater in older individuals (6), and in individuals with comorbidities, such as hypertension (7), coronary artery disease (CAD), and diabetes mellitus.

Despite patients reporting with symptoms relating to fever and respiratory distress, there is growing evidence for the involvement of the cardiovascular system. Patients often exhibit elevated cardiac biomarkers such as cardiac troponin I/T (hs-cTnI/hs-cTnT) (3, 4, 6, 8–11) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels (8, 12), which suggest myocardial damage and ventricular/atrial dysfunction. However, the impact of COVID-19 on the vasculature is largely unknown, but there are case reports of viral infection of the endothelium (13), as well as elevated markers of coagulation, such as D-dimer in COVID-19 patients (14), which itself may indicate a significant risk of pulmonary thromboembolism (PTE) in patients.

The focus of this review is to detail the effects of SARS-CoV-2 and COVID-19 disease on the vasculature, whilst discussing the potential direct and indirect mechanisms which lead to endothelial damage and dysfunction. Moreover, we also discuss the pathogenesis of COVID-19 associated thromboembolism and its consequences upon the cardiovascular system and COVID-19 disease progression.

EPIDEMIOLOGY OF COVID-19 AND CARDIOVASCULAR RISK

Patient cohort studies show that there is a large prevalence of patients with COVID-19 who have comorbidities, such as hypertension (17–57% of all patients) and cardiovascular disease (CVD) (11–21% of all patients) (3, 15–17). Patients with hypertension or CAD are not only at greater risk of infection, and admission to hospital, but having one or more of these comorbidities also appears to increase the risk of progression of the disease (15). In a Chinese cohort, it was observed that in COVID-19 patients, 30% of them had hypertension (14). In the non-survivors, the incidence of hypertension was greater than that of survivors (48 vs. 23% of patients), and this was even more pronounced for incident coronary heart disease (24 vs. 1% of patients) (14). Hypertension and pre-existing CVD were also more common comorbidities in patients requiring admission to the intensive care unit (ICU) (18).

The initial evidence of the cardiovascular impact of COVID-19 was provided in cross-sectional cohort studies which observed significantly elevated hs-cTnI and hs-cTnT levels, suggestive of myocardial injury in these patients (14, 18, 19). High levels of these cardiac biomarkers are related to worse prognosis of the disease (19, 20), with a number of studies demonstrating a higher risk of admission to ICU (10), requirement for mechanical ventilation (12), and incidence of arrhythmias and death from COVID-19 (3, 4, 10, 12, 19) in those with elevated circulating hs-cTnI or hs-cTnT levels. Moreover, the mortality risk associated with elevated hs-TnI/T was greater than that observed for advanced age, pre-existing diabetes, respiratory disorders, and CAD (10, 12). The elevations in hs-TnI/T are also associated with elevated levels of NT-ProBNP and C-reactive protein (CRP), suggesting the myocardial injury observed in COVID-19 patients may be linked with ventricular dysfunction and inflammation (12). There are several potential reasons for the elevated cardiac injury observed in COVID-19 patients with worsening outcomes. These include direct viral infection of the myocardium, the use of anti-viral medications (18), the side-effects of the COVID-19 associated cytokine storm (21), or likely a combination of the three. Viral entry is likely, as SARS-CoV-2 is known to enter human cells via binding of the transmembrane protein, the angiotensin-converting enzyme 2 (ACE2) receptor, which is highly expressed in both the lungs and the heart (22). In fact, due to this mechanism of entry, there has been debate on the use and potential benefit of the use of ACE inhibitors in patients with cardiac injury and/or hypertension (23), with the American Heart Association, The Heart Failure Society of America, and the American College of Cardiology publishing a joint consensus statement for the treatment of COVID-19 patients with ACE inhibitors (24).

Cardiovascular events, such as incidences of acute coronary syndrome (ACS) or acute myocardial infarction (AMI) in COVID-19 patients have been demonstrated (25), indicating that the impact of COVID-19 on the cardiovascular system leads to cardiovascular-related mortality. The root causes of COVID-19 ACS/AMI remain unknown, but could be due to the elevated myocardial demand as a result of the infection, akin to type 2 MI, cytokine-induced atherosclerotic plaque instability and rupture, or non-plaque thrombosis (25–27). Although, as documented, there is a clear impact of the virus on the myocardium, either directly or indirectly; however, the potential role of the vasculature in COVID-19 associated cardiovascular complications has been relatively overlooked, and may be prognostically important in these patients. In fact, in a recent study by Chen et al. (28) using a single cell atlas of the human myocardium showed that ACE2 is expressed on pericytes in the heart (28), suggesting that viral infection of pericytes, which surround the endothelial lining of blood vessels, could lead to microvascular inflammation in the heart tissue, resulting in non-obstructive MI. Therefore, the following sections will investigate the impact of COVID-19 on vascular tissues, specifically endothelial cells and pericytes, and the

subsequent involvement of these tissues on thrombotic risk in COVID-19.

COVID-19 AND ENDOTHELIAL CELL DYSFUNCTION

Initial SARS-CoV-2 infection occurs within the lung epithelia, whereby serine proteases, most notably transmembrane protease serine 2 (TMPRSS2), cathepsin B, and cathepsin L1, prime the SARS-CoV-2 spike glycoprotein, which is followed by ACE2-mediated viral entry (29). Infection of lung alveoli allows SARS-CoV-2 to enter the systemic circulation, subsequently predisposing multiple organs to potential infection. Co-expression of both key serine proteases and ACE2 is required for successful infection of cells by SARS-CoV-2 (29). Multiple organs contain cells which co-express ACE2 and these serine proteases, including the lungs, heart, kidneys, liver, and the vasculature (30–32).

Microvascular dysfunction and the role of the vascular endothelium is increasingly implicated in the acute respiratory distress syndrome (ARDS) and systemic impact of SARS-CoV-2 infection. Endothelial cells protect the cardiovascular system and are crucial in regulating vascular homeostasis, preventing coagulation, controlling blood flow, and regulating oxidative stress and inflammatory reactions (33, 34). There is growing evidence of a vascular involvement in the pathogenesis of severe COVID-19, with imaging studies revealing perfusion abnormalities within the brains of patients with COVID-19 presenting with neurological issues (35), in addition to perfusion abnormalities within the lungs of COVID-19 pneumonia patients (36). Moreover, cross-sectional studies have reported a high incidence of coagulopathies, characterized by elevated D-dimer and fibrinogen concentrations, which lead to thrombotic events and are associated with poor outcomes (37, 38), thus demonstrating the potential involvement of endothelial cells in the pathophysiological consequences of COVID-19.

Endothelial Cell Involvement in COVID-19

Involvement of endothelial cells in the pathophysiology of COVID-19 goes beyond coagulation derangements, with SARS-CoV-2 being shown to directly infect engineered human blood vessel organoids and human kidney organoids *in vitro* (39). This has been confirmed, *in vivo*, by histological studies demonstrating viral infiltration into endothelial cells, with Varga et al. (13) reporting endothelial cell involvement across multiple organs (e.g., lungs, heart, intestines, kidneys, and liver) in three patients; two of whom died (multisystem organ failure; myocardial infarction, and subsequent cardiac arrest, respectively) and one survived. Viral infection of endothelial cells was observed in a transplanted kidney of one patient with evidence of endothelial cell inflammation (endothelialitis) within cardiac, small bowel, lung, and liver tissue of two patients. Furthermore, one other patient demonstrated endothelialitis of the submucosal vessels within the small intestine, which was accompanied by a reduced left ventricular ejection fraction. These findings demonstrate direct viral infection of endothelial cells and endothelialitis within multiple tissue beds in patients with COVID-19.

Although limited by a small sample size, the findings of Varga et al. (13) are supported by Ackermann et al. (40), who reported severe endothelial injury, viral infection, and disrupted cell membranes in seven lungs obtained post-mortem from individuals who died from COVID-19. When compared to seven lungs from individuals who died from influenza, microthrombi were nine times as prevalent in the lungs from the COVID-19 individuals. Furthermore, widespread microthrombi was accompanied by microangiopathy and occlusion of alveolar capillaries (40), which is in line with other studies (41), and can predispose organs to microinfarcts (42). An unexpected finding was the observation of intussusceptive angiogenesis, in which the degree was associated with the duration of hospitalization (40). Intussusceptive angiogenesis is the formation of new vessels, via non-sprouting angiogenesis, and is constructed of an endothelial-lined "pillar" spanning the vessel lumen, which significantly alters the microcirculation (43). Cytoplasmic vacuolisation and cell detachment in pulmonary arteries (44), in addition to pulmonary capillary injury featuring neutrophil infiltration and fibrin deposition (41, 45) has also been reported, further demonstrating local endothelial cell perturbations within lung tissue. Moreover, renal post-mortem histopathological analysis by Su et al. (46) found endothelial cell swelling with foamy degeneration in 19% of patients, with 12% demonstrating a few areas of segmental fibrin thrombus in glomerular capillary loops that is associated with severe endothelial injury.

Considering endothelial dysfunction leads to impaired systemic microvascular function, it seems likely that involvement of the vascular system's first line of defense (endothelial cells) precipitates and propagates the systemic damage observed in severe cases of COVID-19, through altered vascular integrity, vascular inflammation, and via disruption of coagulation and inflammatory pathways (13, 33). The mechanisms for this have not yet been fully elucidated and are varied due to the heterogenic nature in which the virus affects individuals. Cardiometabolic comorbidities associated with poorer prognosis in COVID-19 patients have a strong association with pre-existing endothelial dysfunction (i.e., hypertension and CAD) (47, 48). It is therefore evident that understanding the role of endothelial cells in SARS-CoV-2 infection is crucial to identifying potential therapeutic strategies to combat the virus and improve patient outcomes. The role of endothelial cells and potential mechanisms of endothelial cell dysfunction in COVID-19 are depicted in **Figure 1**.

Potential Mechanisms of Endothelial Dysfunction in COVID-19

Angiotensin-Converting Enzyme 2 (ACE2)

ACE2 is an endogenous negative regulator of the renin-angiotensin system (RAS) and has been identified as the key receptor facilitating viral entry of SARS-COV-2 (49, 50), along with key serine proteases to prime the spike glycoprotein of the virus, most notably TMPRSS2 (29), which is expressed by endothelial cells (30). ACE2 is widely expressed in cells throughout the body, from the respiratory tree to the vascular

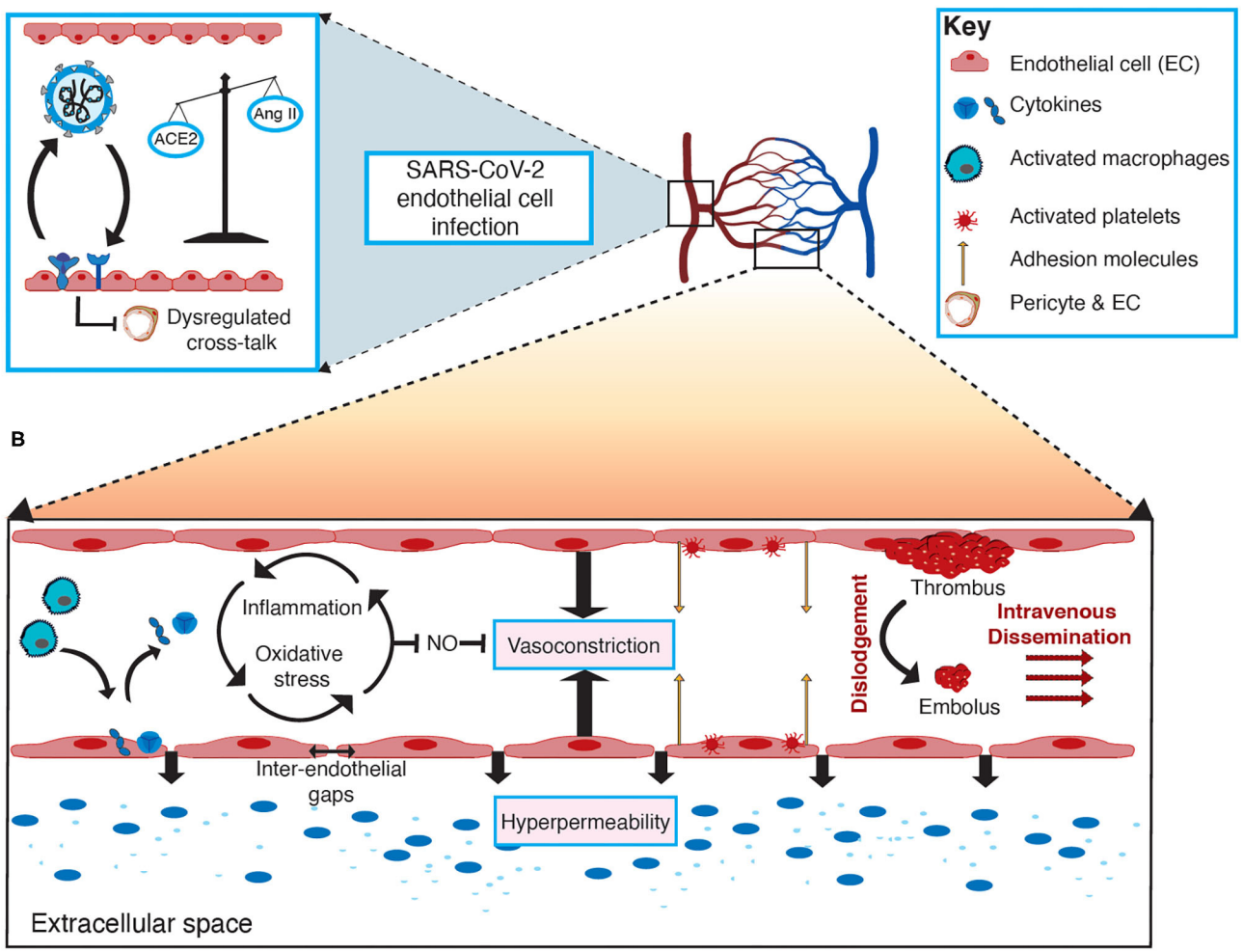

FIGURE 1 | The role of endothelial cells and mechanisms of endothelial cell dysfunction in COVID-19. **(A)** SARS-CoV-2 infects endothelial cells through angiotensin-converting enzyme 2 (ACE2) mediated viral entry, facilitated by TMPRSS2 priming the SARS-CoV-2 spike glycoprotein. Infection of endothelial cells may result in a downregulation of ACE2, promoting an imbalance between ACE2 and angiotensin II (AngII) levels, in favor of AngII. Moreover, infection of either endothelial cells or pericytes will perturb the crosstalk between these two cells, thus contributing to endothelial cell dysfunction. **(B)** In severe cases of COVID-19, activated macrophages release various cytokines (e.g., soluble interleukin 2-receptor [IL-2R], interleukin-6 [IL-6] and tumor necrosis factors [TNFs]), which are attributed to the exaggerated immune-mediated cytokine storm and can result in vascular inflammation (endothelialitis) as a result of increased adhesion molecule expression on endothelial cells and inter-endothelial gaps, thus promoting vascular hyperpermeability. Activated endothelial cells can contribute to the cytokine storm by releasing various cytokines in response to damage and dysfunction, contributing to a vicious cycle of inflammation and oxidative stress that inhibits the release of vasoactive factors (e.g., nitric oxide [NO]), thus favoring vasoconstriction and further contributing to vascular permeability. Abnormal activation of platelets and endothelial cells is the key process leading to thrombosis, which represents the role of endothelial cell dysfunction in the pathogenesis of thromboembolism in COVID-19 patients. Subsequently, the dislodgement of thrombotic clots creates a mobile embolus that disseminates intravenously, thereby leading to thromboembolic complications in COVID-19.

system, heart, kidneys, liver, gut, central nervous system, and retina, and is recognized as eliciting protective effects, particularly against CVD (49). The expression of ACE2 in many organs allows relatively easy transport of the virus throughout the body (51). Consequently, interference of the physiological processes associated with ACE2 by viral entry of SARS-CoV-2 is likely to explain the multi-organ dysfunction pertaining to endothelial cells that is seen in severe cases of COVID-19.

A downregulation in the expression of ACE2, as a result of viral entry into cells, disrupts the regulation balance between angiotensin II (Ang II) and ACE2, indirectly affecting the vasculature. This imbalance facilitates an elevation in the expression of Ang II, subsequently promoting an atherogenic state across the cardiovascular system, especially inflammation and oxidative stress, whilst also elevating blood pressure by stimulating an increase in sympathetic nervous system

activity (52). This is supported by studies reporting marked elevations in plasma AngII concentrations in patients with COVID-19 (53) and also being linked to disease severity in patients infected with novel influenza A (54). This pathophysiological increase in Ang II and without the modulator and protective effects of Ang 1-7, results in downstream elevation of plasminogen activator inhibitor-1 (PAI-1) from endothelial cells, further accelerating vascular inflammation and the facilitation of the coagulation cascade (42), thus resulting in endothelial damage (55). Elevated PAI-1 is a hallmark of endothelial dysfunction, promoting increases in circulating endothelial microvesicles, resulting from endothelial shedding via activated cells, which pose a risk of thromboembolic events (56, 57).

Some have argued that following cell entry of SARS-CoV-2, down-regulation of ACE2 receptors may result in an indirect activation of the kallikrein-bradykinin pathway, thereby promoting an increase in vascular permeability and thus leading to oedema and microcirculatory dysfunction (33, 58, 59). It has been suggested that kinin inhibition may be a potential therapeutic approach to reducing vascular leakage into the lung, and therefore, oedema (60). Kinin inhibition may, therefore, promote endothelial repair through reducing vascular permeability, although whether this is an effective therapeutic approach is yet to be confirmed within the literature. In contrast to this, consistent reports of hypokalaemia in patients with severe COVID-19 (61, 62) suggest an increase in aldosterone, via elevations in Ang II, resulting in an increase in ACE, which acts to metabolize bradykinin (63). Therefore, the role of bradykinin in the pathogenesis of microvascular dysfunction in COVID-19 is questionable and more likely a result of the effects of Ang II, stemming from a downregulation of ACE2 after viral entry into cells. Moreover, given that hypokalaemia is associated with ventricular arrhythmias that are commonly observed in COVID-19 (18), it is plausible that this is a contributing mechanism to both endothelial dysfunction and arrhythmogenesis.

The Cytokine Storm

The mechanisms involved in the pathogenesis of microvascular dysfunction in COVID-19 patients, although not yet fully understood, are likely not solely attributed to direct viral infection of endothelial cells. Endocytosis or membrane fusion of SARS-CoV-2 to cells either leads to cell damage or apoptosis which activates the immune response and the release of various cytokines promoting an exaggerated inflammatory environment (42). Moreover, endothelial cells regulate local and systemic inflammatory reactions and immune responses (33) and activation of these cells via the exaggerated immune-mediated inflammatory response of SARS-CoV-2 may present an indirect mechanism of endothelial damage and dysfunction among the COVID-19 patient population. Endothelial cells produce various cytokines and chemokines and have been identified as central regulators of an exaggerated systemic inflammatory response, or "cytokine storm" (64), a common feature of severe SARS-CoV-2 infection (65).

More severe cases of COVID-19 are associated with progressive lung damage which has, in part, been attributed to this cytokine storm (65–67), leading to a loss of vascular barrier integrity and likely promoting pulmonary oedema, thereby causing endothelialitis, and activation of coagulation pathways. Cross-sectional studies have consistently demonstrated marked elevations in pro-inflammatory markers, such as soluble interleukin-2 receptor (IL-2R), interleukin-6 (IL-6), CRP, and tumor necrosis factors (TNF) (6, 12, 68). This marked elevation in pro-inflammatory markers has been linked with mortality and promotes inter-endothelial gaps and thus vascular hyperpermeability (69, 70), along with exacerbating oxidative stress. IL-6 in particular is associated with increased vascular permeability, a hallmark of the inflammatory response (71, 72), and IL-6 levels are directly correlated with the severity and mortality of COVID-19 (14, 73, 74). Moreover, IL-6, along with other cytokines released from activated macrophages, such as IL-1β, activate endothelial cells via elevations in adhesion molecules (42) leading to a myriad of vascular disturbances including leukocyte tethering to the vascular bed, platelet aggregation and coagulation derangements.

Oxidative Stress

An overproduction of reactive oxygen species (ROS) in infected cells is a key factor in viral replication of respiratory viruses and subsequent tissue damage (75). Following viral infection, endothelial activation and regulation of adhesion molecules leads to neutrophil activation, which results in the production of a plethora of histotoxic mediators including ROS (59). This has implications for the onset and progression of the cytokine storm since, as described above, endothelial cells are key orchestrators of cytokine overload. The ensuing oxidative stress, defined as a systemic imbalance between ROS (or free radicals) and antioxidants, causes an increased expression of prothrombotic and cell-surface adhesion molecules (76). Oxidative stress may therefore be linked to the pathogenesis and severity of COVID-19 infections (77) and peri-endothelial ROS production in COVID-19 may, therefore, contribute to the multi-organ failure associated with severe disease, which seems likely given that it has previously been demonstrated in the pathogenesis of other viral infections, such as SARS-CoV and influenza (78, 79), and ARDS (80). The elevation in ROS accumulation promotes oxidative stress and nuclear factor kappa B (NF-κB) signaling, with the potential for dysregulated antioxidant mechanisms, such as Nrf2 and antioxidant response element signaling, promoting the release of various endothelial genes, such as endothelin and adhesion molecules, thus favoring vasoconstriction and increased vascular permeability (81, 82).

The elevation in free radical production, potentially as a combined result of increased Ang II expression, pro-inflammatory responses, and a reduced capacity for free radical scavenging by impaired antioxidant signaling, impairs endothelial function. Elevated superoxide concentrations, promoted by the release of mitochondrial-derived ROS is a hallmark of oxidative stress, which facilitates the quenching of nitric oxide (NO) and the formation of the secondary free radical, peroxynitrite, in turn reducing NO bioavailability (83). Moreover, this process uncouples endothelial

nitric oxide synthase, which further elevates superoxide production, contributing to the pro-oxidant environment of the vasculature. Such elevations in oxidative stress would promote antioxidant signaling, however, numerous respiratory viral infections, such as respiratory syncytial virus, human metapneumovirus, and influenza, have perturbed antioxidant defense mechanisms by inhibiting antioxidant enzyme induction (84). Interestingly, it has been proposed that Nrf2 activators could be a potential therapeutic strategy for inhibiting viral entry of SARS-CoV-2 (85), and may also pose a benefit to endothelial repair and functioning by the scavenging of free radicals, reducing oxidative stress, and inhibiting pro-inflammatory signaling.

Coagulation Cascade

Perturbations to the endothelium may result in vascular leakage and promote inflammation, but also predispose the vasculature to a pro-coagulant state. Indeed, a common manifestation in patients with COVID-19 is the presence of coagulation abnormalities and instances of thromboembolism, which has been associated with disease severity and a higher incidence of mortality (38), whilst also increasing the risk of MI and stroke. The endothelium plays an important role in the prevention of thromboembolic events by regulating the coagulation cascade, achieved, in part, via inhibition of various tissue factors by a Kunitz-type protease inhibitor, known as the tissue factor pathway inhibitor (TFPI) that resides on the endothelial cell surface (34). The transmembrane protein tissue factor is required for *in vivo* coagulation by the binding and activation of various tissue factors (i.e., activation of factor Xa) promoting prothrombin conversion to thrombin, and thus the conversion of fibrinogen to fibrin (34, 86), inhibiting TFPI and promoting clot formation. TFPI is predominantly bound to the microvasculature (87), however, it has been demonstrated to play a role in the regulation of arterial thrombosis in mice (86).

Marked coagulation derangements have been reported in a single-center cross-sectional study by Goshua et al. (88) who assessed markers of endothelial cell and platelet activation, namely circulating von Willebrand factor (vWF), soluble P-selectin and soluble thrombomodulin, in critically and non-critically ill COVID-19 patients. They observed that endotheliopathy is present in COVID-19 and is associated with increased mortality, with a suggestion that soluble thrombomodulin concentrations may predict mortality and clinical outcomes in COVID-19 patients. It was suggested that the coagulopathy observed in their data was distinctly separate from disseminated intravascular coagulation (DIC) and should be considered an endotheliopathy (88). The notion of a "COVID-19 coagulopathy" is supported by a number of other studies. DIC has been reported to be characteristic of COVID-19, however, its presentation is different to that regularly observed in sepsis-induced DIC. In sepsis-induced DIC, marked thrombocytopenia is observed with a mild elevation in D-dimer concentrations (89), which is in contrast to DIC observed in COVID-19 patients (90). This is supported by only 14.7% (22 of 150) of patients scoring positive on the "sepsis-induced coagulopathy score" (90). DIC has been linked with multi-organ system failure within the COVID-19 population (38, 91, 92), demonstrating a pro-coagulant state of the vasculature. Furthermore, mild thrombocytopenia can be found in 70 to 95% of patients with severe COVID-19, however, it has not been found to be an important predictor of outcome (21, 93). Therefore, the presence of coagulopathy within patients with COVID-19 should be considered as an endotheliopathy, rather than traditional DIC.

Cellular Cross-Talk: Endothelial Cells and Pericytes

Pericytes share a basement membrane with endothelial cells, which is formed, maintained, and remodeled successfully through cellular cross-talk between these two cells, demonstrating that pericytes and endothelial cells have an extensive linkage and are key for maintaining basement membrane, and thus vascular barrier integrity. This has been confirmed by cell-to-cell interaction analysis, demonstrating that endothelial cells are the main cross-talking cell with pericytes within cardiac tissue, with a predominant role of angiopoietin ligands (ANGPT1/2) and Tie receptor 2 (TIE2) maintaining endothelial cell stability and function in capillary vessels (28). A balance between ANGPTs and TIE2 is key for the maintenance of endothelial stability and vascular integrity (28, 94); therefore, it is possible that a breakdown of the cross-talk between pericytes and endothelial cells disrupts this balance and results in a compromised vasculature that is prone to a pro-inflammatory, pro-coagulant state. Whilst these findings were observed in normal heart tissue, this is supported by a pericyte-specific infection by SARS-CoV-2 in experimental (95) and human histological studies (96).

Whilst there is evidence of a direct viral infection of endothelial cells, some have argued that endothelial cell dysfunction is a result of pericyte infection. Cardot-Leccia et al. (96) reported wall thickening of the venules and alveolar capillaries in lung tissue of a deceased COVID-19 patient, accompanied by a marked decrease in pericytes, compared to normal lung parenchyma. Combined with the findings of He et al. (95) and the highly infectious potential of pericytes demonstrated by single cell RNA sequencing studies (28), these data seem to support a potential "pericyte hypothesis" as a mechanism for microvascular dysfunction in the pathogenesis of COVID-19. Moreover, infection and loss of pericytes would result in a dysregulation of the cross-talk between pericytes and endothelial cells, promoting capillary endothelial dysfunction, which would explain the wall thickening of venules and capillaries observed in the data from Cardot-Leccia et al. (96). Taken together, pericytes seem to have the potential as a highly infectious cell population for SARS-CoV-2 and may contribute to endothelial dysfunction by promoting an imbalance between ANGPT1/2 and TIE2, perturbing vascular barrier integrity and increasing vascular permeability. However, the notion that it is solely pericytes that are infected and induce endothelial dysfunction is unlikely considering the compelling histological data presented within the literature (13, 40).

COVID-19 AND THE COAGULATION CASCADE - RISK OF THROMBOEMBOLIC EVENTS

There is evidence to suggest increased risk of thrombotic complications and stroke (both are hereafter referred to as thromboembolism for simplicity) in COVID-19 (97). At the mechanistic level, both venous and arterial thrombosis have been attributed to activation of inflammation and hypoxia, platelet activation, endothelial dysfunction, and circulatory stasis. However, the impact of thromboembolic complications on the prognosis of COVID-19, clinical course of thromboembolic disorders in these patients, and the impact of prophylactic and therapeutic anticoagulation therapies in COVID-19 are not well-known.

Epidemiological Burden of Thromboembolism in COVID-19

The prevalence of neurologic manifestations, including cerebrovascular diseases, was reported at 36.4% in an earlier retrospective case series from Wuhan, China (98). In patients presenting with confirmed or suspected COVID-19, thromboembolism is prevalent at 20.4% (99). In the same study, six of the patients with laboratory findings demonstrated elevated D-dimer levels (>7,000 mg/L) and 40% of the patients had pulmonary thromboembolism. Another series showed that 67% of thromboembolic complications are ischaemic in origin, while 33% are haemorrhagic (100). In the pediatric population, thromboembolic complications are not common. For instance, elevation of D-dimer was not found in children with SARS-CoV-2 compared to other inflammatory multisystem syndromes (101), and no thromboembolic event was found in children and adolescents in a large, multicentre European cohort (102).

In addition to a prior history of stroke, patients with COVID-19 develop incident thromboembolism. The incidence rates of acute thromboembolic complications are reported between 5 and 32.5% in retrospective cohorts (103, 104). Underlying cardiovascular risk factors, including diabetes, hypertension, and a history of CVD, are implicated as univariate correlates (103). D-dimer levels at hospital admission are also significantly correlated with incident thromboembolism, with a negative predictive value of more than 90% (104). In a prospective cohort of 150 French COVID-19 patients vs. a historic cohort of 233 non-COVID-19 controls, COVID-19 ARDS independently predicted thromboembolic complications and pulmonary thromboembolism even after propensity score matching (90).

The comorbid nature of thromboembolic lesions in patients with COVID-19 underscores some underlying predisposition to SARS-CoV-2 infection. Indeed, thromboembolic complications have been associated with depressed immune function and increased post-stroke infections. Infection rates ranging from 18.7 to 43.7% have been reported in patients with intracerebral hemorrhage (105, 106), with respiratory infections predicting almost six-fold higher risk of future thromboembolism (106). A 1-unit increment in National Institutes of Health Stroke Scale (NIHSS) was associated with 23% increased risk of COVID-19 positivity. Interestingly, in a retrospective multicentre study of stroke patients (107), 28% were later diagnosed with COVID-19. However, the true burden of thromboembolism COVID-19 remains unknown and will, hopefully, be answered by larger prospective studies.

Impact of Thromboembolic Complications on COVID-19 Prognosis

The presence of underlying or incident thromboembolic complications is associated with poor prognosis of COVID-19. A history of thromboembolism is reported in 2.3 to 22% of severe cases compared to 0 to 6% in non-severe cases (108). Patients with prior neurologic thromboembolic complications are shown to have a 2.5-fold increased risk of COVID-19 severity (108) and D-dimer is often elevated above reference range in hospitalized cases (17). These patients are usually older, have a higher number of comorbidities, have a higher prevalence of ARDS, and are more likely to be non-invasively ventilated (109). Data also shows that patients with more severe COVID-19 have higher incidence rates of thromboembolic complications. For instance, 31% of patients admitted to the ICU developed thromboembolic complications during follow-up in one Dutch study (110). Yearly increment in age and prior coagulopathy, defined as prothrombin time >3 s or activated partial thromboplastin time (aPPT) >5 s, are shown as independent predictors of incident thromboembolic complications in severe COVID-19 (110). Diagnosis of pulmonary thromboembolism in ICU patients with COVID-19 is more common (at 21%) compared to 7% admitted due to influenza or 6% for all ICU patients (111).

Additionally, the association between a history of thromboembolic complications and mortality has been analyzed in COVID-19 patients. The burden of underlying coagulopathy was reported in 50% of non-survivors in the Wuhan cases (14), with a D-dimer >1,000 ng/mL (reference range ≤250 ng/mL) shown to be an independent predictor of 18-fold greater risk of in-hospital mortality (14). A multicentre cohort from the US showed that the coagulation component of the SOFA score is associated with 64% greater odds of 28-day in-hospital death in a multivariable adjusted model (112). These observations are further supported by the results of a meta-analysis (113), which show a 2.4-fold elevated risk of mortality in COVID-19 patients with cerebrovascular disease, defined as stroke and brain infarction. Overall, these data highlight the risk, and subsequent poor prognosis of thromboembolism in COVID-19.

Coagulation Cascades and the Mechanisms of Thrombosis in COVID-19

While significant associations have been noted for thromboembolism and SARS-CoV-2 infection and worsening of COVID-19, a causal relationship is not well-defined. However, there are data to suggest some mechanistic underpinnings (**Figure 2**). Laboratory investigations have demonstrated significant elevations of markers of coagulation cascades, such as D-dimer, aPPT, fibrinogen, and factor VIII. D-dimer ≥2,600 ng/mL and failure of clot lysis at 30 min on

thromboelastography predicted future thromboembolic events in ICU patients with c-statistic of 0.78 and 0.74, respectively (114). This highlights the fact that shutdown of fibrinolysis occurs in COVID-19. In addition to coagulation markers, endothelial dysfunction may underlie the increased risk of thromboembolism in COVID-19 as both vWF activity and vWF antigen are increased in COVID-19 ARDS compared to non-COVID-19 ARDS (90).

Thromboembolic complications might also be precipitated by underlying cardiovascular injury. For example, patients with co-existing ST-elevation MI and COVID-19 have significantly increased rates of thromboembolic complications, affecting multiple vessels and stents, thrombus grade post-percutaneous coronary intervention (115). Additionally, cardiac arrhythmias play an important role in the development of thromboembolic events, due in part to the shared underlying myocardial substrate (116). Cardiomyopathy, consisting of mechanical dysfunction, structural remodeling, and electrophysiological changes, is a common cause of both intracardiac thrombus and cardiac arrhythmogenic substrate formation (116). The presence of right-heart echodensity on transoesophageal and transthoracic echocardiography has been reported in COVID-19 patients (117–119). Interestingly, intracardiac thrombus coexisted with persistent tachycardia, global hypokinesis, left ventricular dysfunction, and right ventricular dilatation and reduced systolic function (117–119). Taken together, this indicates that thromboembolism in COVID-19 might be mediated via cardiac-specific pathologies.

At the mechanistic level, thromboembolic complications may arise due to activation of inflammation and hypoxia, platelet activation, endothelial dysfunction, and circulatory stasis in COVID-19. Inflammatory overdrive and hypoxia may induce abnormalities of coagulation, the third component of the Virchow triad. On necropsy, areas of diffuse and extensive inflammatory infiltrations have detectable thromboemboli and microemboli (120). Direct infection of immune cells with SARS-CoV led to activation of monocyte-macrophage differentiation, coagulation pathway upregulation, and increased cytokine production (121). SARS-CoV-2 might drive thromboembolic mechanisms by its utilization of the ACE-2 receptor, which is needed to clear Ang II from the circulation. Increased Ang II could, in turn, drive the release of vWF from endothelial cells and platelet activation via involvement of Na^+/H^+ exchanger (122). Finally, the presence of auto-antibodies, such as lupus anticoagulant, might drive activated coagulation pathways and thromboembolic risk (123).

Direct activation of platelets by SARS-CoV-2 is a likely pathway for the development of thromboembolism. Hottz et al. (124) reported platelet activation and formation of platelet-monocyte aggregates in patients with severe but not in mild COVID-19. Similar findings were observed when platelets from COVID-19 negative patients were treated with plasma from COVID-19 positive patients (124). Platelets from COVID-19 patients induces *ex vivo* expression of tissue factor (TF) in monocytes (124), indicating a likely reprogramming event during SARS-CoV-2 infection. Indeed, this hypothesis is supported by pre-publication evidence reporting the presence of SARS-CoV-2 RNA in platelets of COVID-19 patients, which were shown to be hyperactivated and aggregated at a lower threshold of *in vitro* thrombin stimulation (125). Platelets from COVID-19 degranulate, which correlates with reduced platelet factor 4 and serotonin levels, and release extracellular vesicles to participate in coagulation (125). Consequently, platelet reprogramming could facilitate the transmission of SARS-CoV-2 and promote thrombo-inflammation. Indeed, thrombo-inflammation mediated by distinct patterns of platelet and neutrophil activations, neutrophil-platelet aggregate formation, and neutrophil extracellular traps has been reported in COVID-19 pneumonia (126).

Prophylaxis and Management of Thromboembolism in COVID-19

Given the high burden of comorbidities and mortality in patients with thromboembolic complications, proper and adequate anticoagulation is highly warranted. Current management of patients with severe COVID-19 includes subcutaneous low molecular weight heparin (LMWH), suspicion of venous thromboembolism in those with high D-dimer levels and rapid respiratory deterioration, and consideration of therapeutic anticoagulation in those in whom diagnostic testing is not possible and there is no apparent bleeding risk (127, 128). A retrospective series showed no mortality benefit with LMWH compared to non-users (129). However, in those with a high sepsis-induced coagulopathy score and markedly elevated D-dimer level, 28-day mortality was lower among users (129). There is also consideration of experimental interventions, such as plasma exchange or administration of anti-inflammatory drugs, in clinical trial settings.

Nevertheless, there are several unknowns with the management of thromboembolism and associated complications in COVID-19. For instance, will prophylactic as compared to therapeutic anticoagulation result in a better outcome in these patients? A prospective cohort recently demonstrated significant reduction in pro-coagulants 7 days after thromboprophylaxis (130). However, the study was very limited by sample size. In another study, patients on prophylactic anticoagulation had higher venous thromboembolism than the therapeutic anticoagulant arm, although the latter group had a higher overall incidence of thromboembolic events, including pulmonary embolism (131). It is envisaged that these issues will be answered in ongoing clinical trials, such as the COVID-19 HD, a randomized controlled trial comparing high-dose vs. low-dose LMWH (132).

SUMMARY

In addition to the known impact on the respiratory system, emerging evidence strongly implicates COVID-19 as a vascular disease. Patients with pre-existing cardiovascular conditions which are commonly characterized by endothelial dysfunction are particularly at risk of downstream complications and COVID-19-associated mortality. Endothelial cell dysfunction, inflammation, and damage are implicated as a consequence

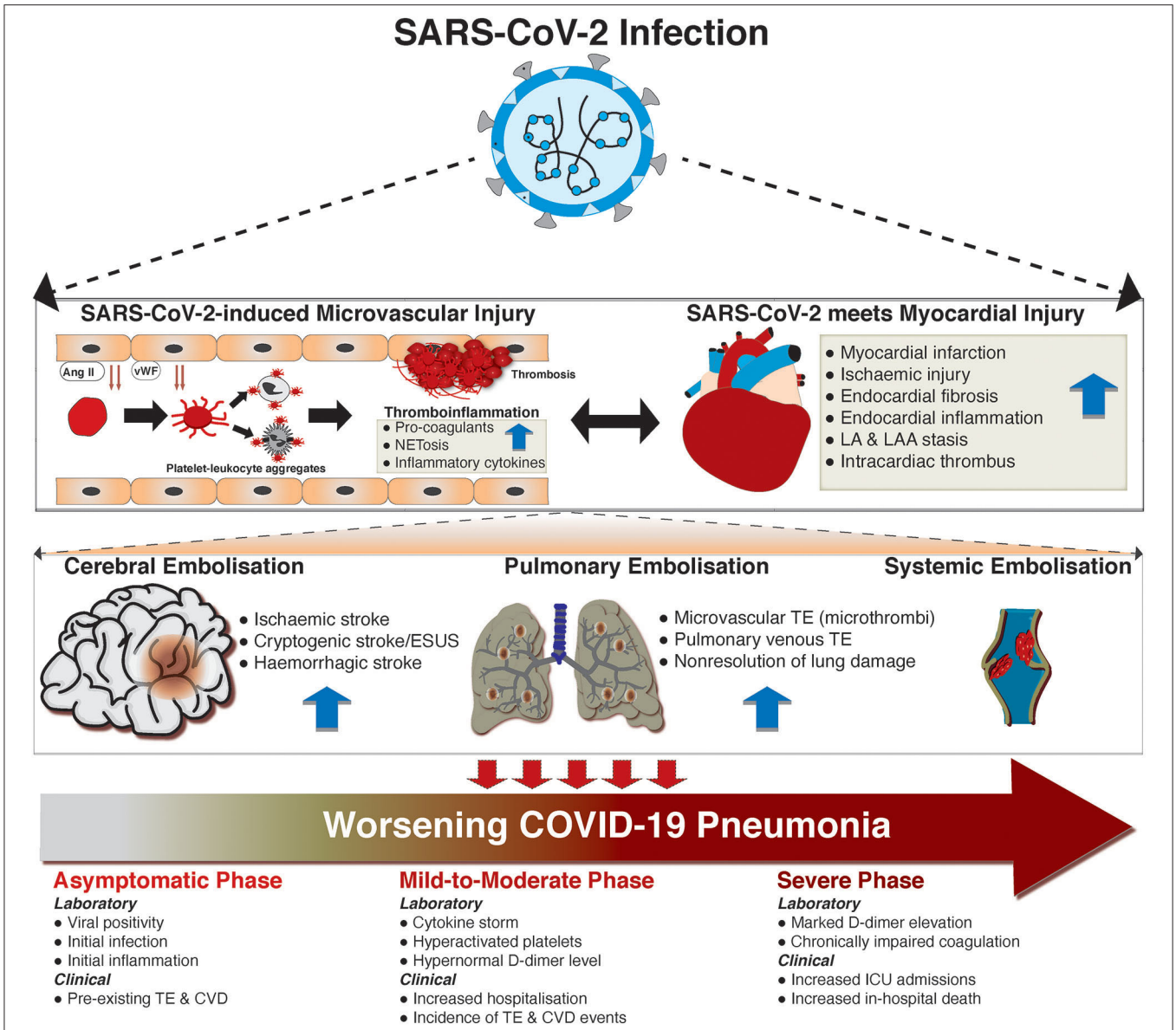

FIGURE 2 | The development and consequences of thromboembolism in COVID-19. The thromboembolic implications of SARS-CoV-2 are best conceptualized in three key stages. First, lung infection of SARS-CoV-2 can spill over, with a consequent cardiovascular tropism of the virus. Within the vascular beds, the increased level of Ang II, which occurs due to SARS-CoV-2 mediated depletion of ACE2, could drive the dysfunction of endothelial cells. This, and other independent pathways (i.e., direct infection of endothelial cells), could lead to the release of von Willebrand factors (vWF), which can activate circulating platelets via adhesive glycoprotein receptors (i.e., gpIb). Activated platelets form aggregates with monocytes and neutrophils, leading to enhanced production of pro-coagulants, inflammatory cytokines, and neutrophil-extracellular traps (NETosis). Within the heart, SARS-CoV-2 infection can directly and indirectly (via cytokine storm) lead to myocardial ischaemia, myocardial infarction, endocardial dysfunction (via inflammation and subsequent fibrosis), and blood stasis in the left atrial atrium (LA) and left atrial appendage (LAA). These can, in turn, lead to intracardiac thrombus. Moreover, thromboinflammation within the vascular beds can drive myocardial injury and vice versa. In the second stage, the dislodgement of thrombus creates mobile embolus, which can be carried to the brain (causing stroke), pulmonary vasculature (causing pulmonary thromboembolism [TE]), or systemically (causing venous thrombosis). Importantly, the presence of thromboembolic complications can lead to progressive COVID-19 disease (in the third conceptual stage). The presence of underlying cardiovascular disease (CVD; i.e., TE) could predispose individuals to SARS-CoV-2 infection via inflammatory derangement. Coexistence of SARS-CoV-2 infection and TE can lead to dysregulated inflammation and coagulation disorders, manifesting with high symptom burden and hospitalization, and increased *de novo* incidence of TE and other CVDs. Consequently, TE and CVDs predispose COVID-19 patients to worse outcomes, including prolonged intensive care unit (ICU) stay and in-hospital mortality.

of the disease, which likely results in elevated ACS/AMI and thromboembolic risk in COVID-19 patients. Direct viral infection of the endothelium, as well as the surrounding pericytes, via the ACE2 receptor, are likely to be causative factors, as well as the deleterious effects of the supraphysiological increase of pro-inflammatory factors, the so called "cytokine storm."

Clinicians and research scientists should consider monitoring the vascular effects of the disease to help identify and manage patients, which may highlight individuals at risk of cardiovascular complications. Despite therapeutic anticoagulation, COVID-19 patients remain at a high risk of both systemic and pulmonary venous thromboembolism. This highlights the need for, perhaps, a more aggressive anticoagulant therapy, and monitoring. Studies should explore the benefits of using D-dimer levels to guide treatment of thromboembolic complications. Further work is needed to determine how best to manage vascular inflammation in COVID-19 patients, which has the potential to significantly improve clinical outcomes in this pandemic.

AUTHOR CONTRIBUTIONS

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

REFERENCES

1. WHO. *WHO Coronavirus Disease (COVID-19) Dashboard*. (2020). Available online at: https://covid19.who.int/
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. 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
4. 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
5. Centers for Disease Control and Prevention. *Coronavirus Disease 2019*. (COVID-19)—symptoms (2020). Available onlie at: https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html
6. 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
7. 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: 10.1183/13993003.01227-2020
8. 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
9. 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
10. 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
11. 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].
12. 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) 5:1–8. doi: 10.1001/jamacardio.2020.1017
13. 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
14. 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
15. 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
16. Li B, Yang J, Zhao F, Zhi L, Wang X, Liu L, et al. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. *Clin Res Cardiol.* (2020) 109:531–8. doi: 10.1007/s00392-020-01626-9
17. 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
18. 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
19. 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
20. 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. doi: 10.1016/j.pcad.2020.04.008
21. 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
22. Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front Med*. (2020) 14:185–92. doi: 10.1007/s11684-020-0754-0
23. 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
24. Patients Taking ACE-i and ARBs who Contract COVID-19 Should Continue Treatment, Unless Otherwise Advised by their Physician (2020). Available online at: https://hfsa.org/patients-taking-ace-i-and-arbs-who-contract-covid-19-should-continue-treatment-unless-otherwise
25. 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
26. 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
27. Libby P, Loscalzo J, Ridker PM, Farkouh ME, Hsue PY, Fuster V, et al. Inflammation, immunity, and infection in atherothrombosis: JACC review topic of the week. *J Am Coll Cardiol.* (2018) 72:2071–81. doi: 10.1016/j.jacc.2018.08.1043
28. 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
29. 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
30. Aimes RT, Zijlstra A, Hooper JD, Ogbourne SM, Sit ML, Fuchs S, et al. Endothelial cell serine proteases expressed during vascular

morphogenesis and angiogenesis. *Thromb Haemost.* (2003) 89:561–72. doi: 10.1055/s-0037-1613388
31. Pan XW, Xu D, Zhang H, Zhou W, Wang LH, Cui XG. Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: a study based on single-cell transcriptome analysis. *Intensive Care Med.* (2020) 46:1114–6. doi: 10.1007/s00134-020-06026-1
32. Sungnak W, Huang N, Becavin C, Berg M, Queen R, Litvinukova M, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat Med.* (2020) 26:681–7. doi: 10.1038/s41591-020-0868-6
33. Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. *Nat Rev Immunol.* (2007) 7:803–15. doi: 10.1038/nri2171
34. van Hinsbergh VW. Endothelium–role in regulation of coagulation and inflammation. *Semin Immunopathol.* (2012) 34:93–106. doi: 10.1007/s00281-011-0285-5
35. Helms J, Kremer S, Merdji H, Clere-Jehl R, Schenck M, Kummerlen C, et al. Neurologic Features in Severe SARS-CoV-2 Infection. *N Engl J Med.* (2020) 382:2268–70. doi: 10.1056/NEJMc2008597
36. Lang M, Som A, Mendoza DP, Flores EJ, Reid N, Carey D, et al. Hypoxaemia related to COVID-19: vascular and perfusion abnormalities on dual-energy CT. *Lancet Infect Dis.* (2020). doi: 10.1016/S1473-3099(20)30367-4. [Epub ahead of print].
37. 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
38. 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
39. Monteil V, Kwon H, Prado P, Hagelkruys A, Wimmer RA, Stahl M, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell.* (2020) 181:905–13 e7. doi: 10.1016/j.cell.2020.04.004
40. 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
41. Magro C, Mulvey JJ, Berlin D, Nuovo G, Salvatore S, Harp J, et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. *Transl Res.* (2020) 220:1–13. doi: 10.1016/j.trsl.2020.04.007
42. 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
43. Mentzer SJ, Konerding MA. Intussusceptive angiogenesis: expansion and remodeling of microvascular networks. *Angiogenesis.* (2014) 17:499–509. doi: 10.1007/s10456-014-9428-3
44. Copin MC, Parmentier E, Duburcq T, Poissy J, Mathieu D, Lille C-I, et al. Time to consider histologic pattern of lung injury to treat critically ill patients with COVID-19 infection. *Intensive Care Med.* (2020) 46:1124–6. doi: 10.1007/s00134-020-06057-8
45. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, et al. Targeting potential drivers of COVID-19: neutrophil extracellular traps. *J Exp Med.* (2020) 217:e20200652. doi: 10.1084/jem.20200652
46. Su H, Yang M, Wan C, Yi LX, Tang F, Zhu HY, et al. Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney Int.* (2020) 98:219–27. doi: 10.1016/j.kint.2020.04.003
47. Harvey A, Montezano AC, Touyz RM. Vascular biology of ageing-Implications in hypertension. *J Mol Cell Cardiol.* (2015) 83:112–21. doi: 10.1016/j.yjmcc.2015.04.011
48. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation.* (2001) 104:2673–8. doi: 10.1161/hc4601.099485
49. 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
50. 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
51. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, 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
52. Cheng H, Wang Y, Wang GQ. Organ-protective effect of angiotensin-converting enzyme 2 and its effect on the prognosis of COVID-19. *J Med Virol.* (2020) 92:726–30. doi: 10.1002/jmv.25785
53. 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
54. Huang F, Guo J, Zou Z, Liu J, Cao B, Zhang S, et al. Angiotensin II plasma levels are linked to disease severity and predict fatal outcomes in H7N9-infected patients. *Nat Commun.* (2014) 5:3595. doi: 10.1038/ncomms4595
55. Verdecchia P, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *Eur J Intern Med.* (2020) 76:14–20. doi: 10.1016/j.ejim.2020.04.037
56. Brodsky SV, Malinowski K, Golightly M, Jesty J, Goligorsky MS. Plasminogen activator inhibitor-1 promotes formation of endothelial microparticles with procoagulant potential. *Circulation.* (2002) 106:2372–8. doi: 10.1161/01.CIR.0000033972.90653.AF
57. George JN, Pickett EB, Saucerman S, McEver RP, Kunicki TJ, Kieffer N, et al. Platelet surface glycoproteins. Studies on resting and activated platelets and platelet membrane microparticles in normal subjects, and observations in patients during adult respiratory distress syndrome and cardiac surgery. *J Clin Invest.* (1986) 78:340–8. doi: 10.1172/JCI112582
58. Bossi F, Peerschke EI, Ghebrehiwet B, Tedesco F. Cross-talk between the complement and the kinin system in vascular permeability. *Immunol Lett.* (2011) 140:7–13. doi: 10.1016/j.imlet.2011.06.006
59. 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
60. van de Veerdonk FL, Netea MG, van Deuren M, van der Meer JW, de Mast Q, Bruggemann RJ, et al. Kallikrein-kinin blockade in patients with COVID-19 to prevent acute respiratory distress syndrome. *eLife.* (2020) 9:e57555. doi: 10.7554/eLife.57555
61. Bansal M. Cardiovascular disease and COVID-19. *Diabetes Metab Syndr.* (2020) 14:247–50. doi: 10.1016/j.dsx.2020.03.013
62. Chen D, Li X, Song Q, Hu C, Su F, Dai J, et al. Assessment of Hypokalemia and Clinical Characteristics in Patients With Coronavirus Disease 2019 in Wenzhou, China. *JAMA Network Open.* (2020) 3:e2011122. doi: 10.1001/jamanetworkopen.2020.11122
63. Henry BM, Vikse J, Benoit S, Favaloro EJ, Lippi G. Hyperinflammation and derangement of renin-angiotensin-aldosterone system in COVID-19: a novel hypothesis for clinically suspected hypercoagulopathy and microvascular immunothrombosis. *Clin Chim Acta.* (2020) 507:167–73. doi: 10.1016/j.cca.2020.04.027
64. Teijaro JR, Walsh KB, Cahalan S, Fremgen DM, Roberts E, Scott F, et al. Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection. *Cell.* (2011) 146:980–91. doi: 10.1016/j.cell.2011.08.015
65. 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
66. Pedersen SF, Ho YC. SARS-CoV-2: a storm is raging. *J Clin Investigation.* (2020) 130:2202–3. doi: 10.1172/JCI137647
67. 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
68. Li X, Wang L, Yan S, Yang F, Xiang L, Zhu J, et al. Clinical characteristics of 25 death cases with COVID-19: a retrospective review of medical records

in a single medical center, Wuhan, China. *Int J Infect Dis.* (2020) 94:128–32. doi: 10.1016/j.ijid.2020.03.053
69. Jose RJ, Manuel A. COVID-19 cytokine storm: the interplay between inflammation and coagulation. *Lancet Respir Med.* (2020) 8:e46–7. doi: 10.1016/S2213-2600(20)30216-2
70. Meduri GU, Kohler G, Headley S, Tolley E, Stentz F, Postlethwaite A. Inflammatory cytokines in the BAL of patients with ARDS. Persistent elevation over time predicts poor outcome. *Chest.* (1995) 108:1303–14. doi: 10.1378/chest.108.5.1303
71. Alsaffar H, Martino N, Garrett JP, Adam AP. Interleukin-6 promotes a sustained loss of endothelial barrier function via Janus kinase-mediated STAT3 phosphorylation and *de novo* protein synthesis. *Am J Physiol Cell Physiol.* (2018) 314:C589–602. doi: 10.1152/ajpcell.00235.2017
72. Desai TR, Leeper NJ, Hynes KL, Gewertz BL. Interleukin-6 causes endothelial barrier dysfunction via the protein kinase C pathway. *J Surg Res.* (2002) 104:118–23. doi: 10.1006/jsre.2002.6415
73. 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
74. 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
75. Khomich OA, Kochetkov SN, Bartosch B, Ivanov AV. Redox biology of respiratory viral infections. *Viruses.* (2018) 10:392. doi: 10.3390/v10080392
76. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: harms and benefits for human health. *Oxid Med Cell Longev.* (2017) 2017:8416763. doi: 10.1155/2017/8416763
77. Delgado-Roche L, Mesta F. Oxidative stress as key player in severe acute respiratory syndrome coronavirus (SARS-CoV) infection. *Arch Med Res.* (2020) 51:384–7. doi: 10.1016/j.arcmed.2020.04.019
78. Allen IC, Scull MA, Moore CB, Holl EK, McElvania-TeKippe E, Taxman DJ, et al. The NLRP3 inflammasome mediates *in vivo* innate immunity to influenza a virus through recognition of viral RNA. *Immunity.* (2009) 30:556–65. doi: 10.1016/j.immuni.2009.02.005
79. Chen IY, Moriyama M, Chang MF, Ichinohe T. Severe acute respiratory syndrome coronavirus viroporin 3a activates the NLRP3 inflammasome. *Front Microbiol.* (2019) 10:50. doi: 10.3389/fmicb.2019.00050
80. Kellner M, Noonepalle S, Lu Q, Srivastava A, Zemskov E, Black SM. ROS signaling in the pathogenesis of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). In: Wang YX, editor. *Pulmonary Vasculature Redox Signaling in Health and Disease*. Cham: Springer International Publishing. (2017). p. 105–37. doi: 10.1007/978-3-319-63245-2_8
81. 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
82. Masaki T, Sawamura T. Endothelin and endothelial dysfunction. *Proc Jpn Acad Ser B Phys Biol Sci.* (2006) 82:17–24. doi: 10.2183/pjab.82.17
83. Schiffrin EL. Oxidative stress, nitric oxide synthase, and superoxide dismutase: a matter of imbalance underlies endothelial dysfunction in the human coronary circulation. *Hypertension.* (2008) 51:31–2. doi: 10.1161/HYPERTENSIONAHA.107.103226
84. Komaravelli N, Casola A. Respiratory Viral Infections and Subversion of Cellular Antioxidant Defenses. *J Pharmacogenomics Pharmacoproteomics.* (2014) 5:1000141.
85. Hassan SM, Jawad MJ, Ahjel SW, Singh RB, Singh J, Awad SM, et al. The Nrf2 activator (DMF) and Covid-19: is there a possible role? *Med Arch.* (2020) 74:134–8. doi: 10.5455/medarh.2020.74.134-138
86. White TA, Johnson T, Zarzhevsky N, Tom C, Delacroix S, Holroyd EW, et al. Endothelial-derived tissue factor pathway inhibitor regulates arterial thrombosis but is not required for development or hemostasis. *Blood.* (2010) 116:1787–94. doi: 10.1182/blood-2009-10-250910
87. Osterud B, Bajaj MS, Bajaj SP. Sites of tissue factor pathway inhibitor (TFPI) and tissue factor expression under physiologic and pathologic conditions. On behalf of the Subcommittee on Tissue factor Pathway Inhibitor (TFPI) of the Scientific Standardization Committee of the ISTH. *Thromb Haemost.* (1995) 73:873–5. doi: 10.1055/s-0038-1653884
88. Goshua G, Pine AB, Meizlish ML, Chang CH, Zhang H, Bahel P, et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. *Lancet Haematol.* (2020) 7:e575–82. doi: 10.1016/S2352-3026(20)30216-7
89. Levi M, Scully M. How I treat disseminated intravascular coagulation. *Blood.* (2018) 131:845–54. doi: 10.1182/blood-2017-10-804096
90. 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
91. Porfidia A, Pola R. Venous thromboembolism in COVID-19 patients. *J Thromb Haemost.* (2020) 18:1516–7. doi: 10.1111/jth.14842
92. Zhu J, Ji P, Pang J, Zhong Z, Li H, He C, et al. Clinical characteristics of 3062 COVID-19 patients: a meta-analysis. *J Med Virol.* (2020) 92:1902–14. doi: 10.1002/jmv.25884
93. 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
94. Bilimoria J, Singh H. The Angiopoietin ligands and Tie receptors: potential diagnostic biomarkers of vascular disease. *J Recept Signal Transduct Res.* (2019) 39:187–93. doi: 10.1080/10799893.2019.1652650
95. He L, Mäe MA, Muhl L, Sun Y, Pietilä R, Nahar K, et al. Pericyte-specific vascular expression of SARS-CoV-2 receptor ACE2 - implications for microvascular inflammation and hypercoagulopathy in COVID-19. *bioRxiv*. 2020:2020.05.11.088500. doi: 10.1101/2020.05.11.088500
96. Cardot-Leccia N, Hubiche T, Dellamonica J, Burel-Vandenbos F, Passeron T. Pericyte alteration sheds light on micro-vasculopathy in COVID-19 infection. *Intensive Care Med.* (2020) 46:1777–8. doi: 10.1007/s00134-020-06147-7
97. 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
98. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, et al. Neurologic manifestations of hospitalized patients with Coronavirus Disease 2019 in Wuhan, China. *JAMA Neurol.* (2020) 77:1–9. doi: 10.1001/jamaneurol.2020.1127
99. 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) 8:awaa240. doi: 10.1093/brain/awaa240
100. Morassi M, Bagatto D, Cobelli M, D'Agostini S, Gigli GL, Bnà C, et al. Stroke in patients with SARS-CoV-2 infection: case series. *J Neurol.* (2020) 267:2185–92. doi: 10.1007/s00415-020-09885-2
101. Whittaker E, Bamford A, Kenny J, Kaforou M, Jones CE, Shah P, et al. Clinical characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. *JAMA.* (2020) 324:259–69. doi: 10.1001/jama.2020.10369
102. Götzinger F, Santiago-García B, Noguera-Julián A, Lanaspa M, Lancella L, Calò Carducci FI, et al. COVID-19 in children and adolescents in Europe: a multinational, multicentre cohort study. *Lancet Child Adolescent Health.* (2020) 4:653–61. doi: 10.1016/S2352-4642(20)30177-2
103. Li Y, Li M, Wang M, Zhou Y, Chang J, Xian Y, et al. Acute cerebrovascular disease following COVID-19: a single center, retrospective, observational study. *Stroke Vascular Neurol.* (2020) 5:279–84. doi: 10.1136/svn-2020-000431
104. Artifoni M, Danic G, Gautier G, Gicquel P, Boutoille D, Raffi F, et al. Systematic assessment of venous thromboembolism in COVID-19 patients receiving thromboprophylaxis: incidence and role of D-dimer as predictive factors. *J Thrombosis Thrombolysis.* (2020) 50:211–6. doi: 10.1007/s11239-020-02146-z
105. Murthy SB, Moradiya Y, Shah J, Merkler AE, Mangat HS, Iadacola C, et al. Nosocomial infections and outcomes after intracerebral hemorrhage: a population-based study. *Neurocrit Care.* (2016) 25:178–84. doi: 10.1007/s12028-016-0282-6
106. Melmed KR, Boehme A, Ironside N, Murthy S, Park S, Agarwal S, et al. Respiratory and blood stream infections are associated with subsequent

venous thromboembolism after primary intracerebral hemorrhage. *Neurocritical Care*. (2020). doi: 10.1007/s12028-020-00974-8
107. Kihira S, Schefflein J, Chung M, Mahmoudi K, Rigney B, Delman BN, et al. Incidental COVID-19 related lung apical findings on stroke CTA during the COVID-19 pandemic. *J NeuroInterventional Surg.* (2020) 12:669–72. doi: 10.1136/neurintsurg-2020-016188
108. Aggarwal G, Lippi G, Michael Henry B. 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
109. Qin C, Zhou L, Hu Z, Yang S, Zhang S, Chen M, et al. Clinical characteristics and outcomes of COVID-19 patients with a history of stroke in Wuhan, China. *Stroke.* (2020) 51:2219–23. doi: 10.1161/STROKEAHA.120.030365
110. Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers D, Kant KM, et al. Confirmation of the high cumulative incidence of thrombotic complications in critically ill ICU patients with COVID-19: an updated analysis. *Thrombosis Res.* (2020) 191:148–50. doi: 10.1016/j.thromres.2020.04.041
111. 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
112. 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). doi: 10.1001/jamainternmed.2020.3596
113. 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 Cerebrovascular Dis.* (2020) 29:104949. doi: 10.1016/j.jstrokecerebrovasdis.2020.104949
114. Wright FL, Vogler TO, Moore EE, Moore HB, Wohlauer MV, Urban S, et al. Fibrinolysis shutdown correlation with thromboembolic events in severe COVID-19 infection. *J Am Coll Surgeons.* (2020) 231:193–203. doi: 10.1016/j.jamcollsurg.2020.05.007
115. 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
116. Goldberger JJ, Arora R, Green D, Greenland P, Lee DC, Lloyd-Jones DM, et al. Evaluating the atrial myopathy underlying atrial fibrillation: identifying the arrhythmogenic and thrombogenic substrate. *Circulation.* (2015) 132:278–91. doi: 10.1161/CIRCULATIONAHA.115.016795
117. Janus SE, Hajjari J, Cunningham MJ, Hoit BD. COVID19: a case report of thrombus in transit. *Eur Heart J Case Reports*. (2020) 4. doi: 10.1093/ehjcr/ytaa189
118. Sethi SS, Zilinyi R, Green P, Eisenberger A, Brodie D, Agerstrand C, et al. Right ventricular clot in transit in COVID-19: implications for the pulmonary embolism response team. *JACC: Case Reports.* (2020) 2:1391–6. doi: 10.1016/j.jaccas.2020.05.034
119. Horowitz JM, Yuriditsky E, Henderson IJ, Stachel MW, Kwok B, Saric M. Clot in transit on transesophageal echocardiography in a prone patient with COVID-19 acute respiratory distress syndrome. *CASE.* (2020) 4:200–3. doi: 10.1016/j.case.2020.05.007
120. 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: a prospective cohort study. *Annals Internal Med.* (2020) 173:268–77. doi: 10.7326/M20-2003
121. Ng LF, Hibberd ML, Ooi EE, Tang KF, Neo SY, Tan J, et al. A human *in vitro* model system for investigating genome-wide host responses to SARS coronavirus infection. *BMC Infect Dis.* (2004) 4:34. doi: 10.1186/1471-2334-4-34
122. Huck V, Niemeyer A, Goerge T, Schnaeker EM, Ossig R, Rogge P, et al. Delay of acute intracellular pH recovery after acidosis decreases endothelial cell activation. *J Cell Physiol.* (2007) 211:399–409. doi: 10.1002/jcp.20947
123. Bowles L, Platton S, Yartey N, Dave M, Lee K, Hart DP, et al. Lupus Anticoagulant and Abnormal Coagulation Tests in Patients with Covid-19. *N Engl J Med.* (2020) 383:288–90. doi: 10.1056/NEJMc2013656
124. Hottz ED, Azevedo-Quintanilha IG, Palhinha L, Teixeira L, Barreto EA, Pão CRR, et al. Platelet activation and platelet-monocyte aggregates formation trigger tissue factor expression in severe COVID-19 patients. *Blood*. (2020) 136. doi: 10.1182/blood.2020007252
125. Zaid Y, Puhm F, Allaeys I, Naya A, Oudghiri M, Khalki L, et al. Platelets can associate with SARS-Cov-2 RNA and are hyperactivated in COVID-19. *Circulation Res*. (2020). doi: 10.1101/2020.06.23.20137596. [Epub ahead of print].
126. Nicolai L, Leunig A, Brambs S, Kaiser R, Weinberger T, Weigand M, 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
127. Konstantinides SV, Meyer G, Becattini C, Bueno H, Geersing GJ, Harjola VP, et al. ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS): The Task Force for the diagnosis and management of acute pulmonary embolism of the European Society of Cardiology (ESC). *Eur Heart J*. (2019) 41:543–603. doi: 10.1183/13993003.01647-2019
128. Zhai Z, Li C, Chen Y, Gerotziafas G, Zhang Z, Wan J, et al. Prevention and treatment of venous thromboembolism associated with coronavirus disease 2019 infection: a consensus statement before guidelines. *Thrombosis Haemostasis.* (2020) 120:937–48. doi: 10.1055/s-0040-1710019
129. 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 Thrombosis Haemostasis.* (2020) 18:1094–9. doi: 10.1111/jth.14817
130. 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 Thrombosis Haemostasis.* (2020) 18:1747–51. doi: 10.1111/jth.14854
131. Llitjos JF, Leclerc M, Chochois C, Monsallier J-M, Ramakers M, Auvray M, et al. High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. *J Thrombosis Haemostasis.* (2020) 18:1743–6. doi: 10.1111/jth.14869
132. Marietta M, Vandelli P, Mighali P, Vicini R, Coluccio V, D'Amico R. Randomised controlled trial comparing efficacy and safety of high versus low Low-Molecular Weight Heparin dosages in hospitalized patients with severe COVID-19 pneumonia and coagulopathy not requiring invasive mechanical ventilation (COVID-19 HD): a structured summary of a study protocol. *Trials.* (2020) 21:574. doi: 10.1186/s13063-020-04475-z

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MINI REVIEW
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Blood Glucose Control Strategy for Type 2 Diabetes Patients With COVID-19

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Since December 2019, coronavirus disease 2019 (COVID-19) caused by a novel coronavirus has spread all over the world affecting tens of millions of people. Another pandemic affecting the modern world, type 2 diabetes mellitus is among the major risk factors for mortality from COVID-19. Current evidence, while limited, suggests that proper blood glucose control may help prevent exacerbation of COVID-19 even in patients with type 2 diabetes mellitus. Under current circumstances where the magic bullet for the disease remains unavailable, it appears that the role of blood glucose control cannot be stressed too much. In this review the profile of each anti-diabetic agent is discussed in relation to COVID-19.

Keywords: COVID-19, diabetes mellitus, antidiabetic agents, healthy diet, exercise

CORONAVIRUS DISEASE 2019 PANDEMIC

Coronavirus disease 2019 (COVID-19), caused by coronavirus SARS coronavirus 2 (SARS-CoV-2), was originally identified in Wuhan, China in December 2019 (1). Since then, the disease has spread all over the world at a tremendous rate and the number of confirmed cases already exceeded 13 million, killing more than 570 thousand people (2). Though measures are being taken in affected countries, such as lockdown of major cities, to control the pandemic, the numbers are still growing day by day (3, 4).

SARS-CoV-2 belongs to the Betacoronavirus genus as SARS coronavirus (SARS-CoV) and MERS coronavirus (MERS-CoV) do (5). As with SARS-CoV, SARS-CoV-2 spike glycoprotein interacts with and binds to human angiotensin-converting enzyme 2 (ACE2) when the virus enters into target cells (6). While ACE2, a type I transmembrane glycoprotein, serves as a functional receptor for SARS-CoV-2, ACE2 is also shown to play a protective role against acute respiratory distress syndrome (ARDS) and SARS pathogenesis by catalyzing angiotensin I and angiotensin II to angiotensin (1-9) and angiotensin (1-7), respectively (7). However, its overall impact on COVID-19 remains to be further elucidated.

A clue could be found with angiotensin-converting enzyme inhibitors (ACE inhibitors) and angiotensin II receptor blockers (ARBs). Since several data suggested that these agents can increase ACE2 expression through their influence on the level of angiotensin II, there was concern over their potential negative influence on COVID-19 morbidity, severity and mortality rates (8). However,

multiple large-scaled studies show that their use affected none of these rates (9–11). Actually, at present, almost all medical associations, including the International Society of Hypertension, American College of Cardiology, American Heart Association and Heart Failure Society of America, have published statements recommending the continued use of ACE inhibitors and ARBs (12, 13).

COVID-19 is thought unlikely to become severe in a majority of cases. In fact, a recent meta-analysis of 47,344 patients with COVID-19 in China shows that the risks of severity and mortality were 18.0 and 3.2%, respectively, among these patients, while these rates increased if patients had comorbidities (14).

COVID-19 AND DIABETES

Diabetes mellitus is another global pandemic affecting 463 million people worldwide (15). In the face of the COVID-19 pandemic, two facts need to be taken to heart. First, nutrition and exercise therapy represent the cornerstone of diabetes management (16). However, the resultant need for home confinement to control the pandemic, as well as for a new lifestyle to reduce the risk of infection, is shown to reduce physical activity and increase sweet food consumption (17, 18), while, now more than ever, the importance of healthy diet and exercise needs to be stressed. Second, which concerns the main theme of this review, diabetes is among the comorbidities associated with increased risk of COVID-19 (19). According to a recent meta-analysis, COVID-19 patients who had diabetes at baseline had increased severity and mortality (HR, 2.11 [CI, 1.40–3.18], 1.69 [CI, 1.22–2.33], respectively), although the prevalence of diabetes in the affected population did not seem to differ from that in the non-affected population in Asia (20). Fortunately, however, it is indicated in a retrospective multicenter study conducted in China that proper blood glucose control may reduce not only the severity of COVID-19 but mortality from the disease in patients with pre-existing diabetes showing improvements in systemic inflammation as measured by serum inflammation markers (21). Actually, in the study, during the 28-day observation period, patients with favorable glycemic control (3.9–10 mmol/L) had a significantly lower mortality rate compared to those with poorly glycemic control (the lowest BG level, >3.9 mmol/L or the highest BG level, >10 mmol/L) (HR 0.14 [CI, 0.03–0.60])(21). In the current situation where COVID-19 and diabetes are so prevalent that they combine to affect a high proportion of patients and where there is no critical medicine or vaccine for COVID-19, the fact that blood glucose control has a role to play in reducing the severity of the disease as well as mortality from the disease, cannot be stressed too much (21).

STRATEGY FOR BLOOD GLUCOSE CONTROL

Then, how should we achieve blood glucose control? First, we would suggest that PCR-confirmed asymptomatic type 2 diabetic patients with COVID-19 or those with mild self-limiting COVID-19 should continue with their current prescription because, to date, there is no evidence to suggest that certain glucose-lowering agents interact with or worsen the disease in patients with asymptomatic or mild COVID-19 (22). This strategy is supported by the fact that hyperglycemia itself is likely to lead to greater severity of COVID-19 and higher mortality from the disease (23, 24). In patients with moderate to severe symptoms who need hospital admission, however, given the pathophysiological and clinical characteristics of COVID-19, some drugs may not be deemed favorable, due to their side effects that could potentially adversely affect the course of the illness. In this review focused on biguanides, thiazolidinediones, sulfonylureas, dipeptidyl peptidase-4 (DPP4) inhibitors, glucagon-like peptide-1 receptor (GLP-1R) agonists, sodium-glucose cotransporter-2 (SGLT2) inhibitors and insulin, their profiles will be discussed in relation to COVID-19.

BIGUANIDES

Biguanides, represented by metformin, is one of the most frequently prescribed oral glucose-lowering agents. Metformin mainly functions by activating AMP-activated protein kinase (AMPK) through inhibition of the respiratory chain of mitochondria thereby subsequently reducing gluconeogenesis in the liver (25). In most situations, metformin is a well-tolerated drug with a relatively low rate of adverse effects. However, as it inhibits mitochondrial respiration and increases lactate production, it may induce lactic acidosis in some patients receiving it, with nearly half of all patients developing lactic acidosis dying from it. Of note, while the risk of lactic acidosis is increased in patients with renal or hepatic impairment, dehydration, shock, hypoxic states, sepsis and advanced age (26), these conditions are often found to be present in patients with severe COVID-19 (27). In addition, up to half of all hospitalized COVID-19 patients are shown to suffer deep venous thrombosis (DVT) thus often requiring the use of contrast-enhanced CT for DVT assessment (28). When transient renal impairment occurs following injection of an iodinated contrast agent in patients receiving a biguanide, however, the renal excretion of the drug is decreased, and their lactic acid levels increased, thus placing these patients at risk of lactic acidosis (29). On the contrary, a recent retrospective study performed in China showed that metformin-treated patients hospitalized for COVID-19 had a lower mortality rate compared to non-metformin-treated patients (30, 31). Thus, overall, while diabetic patients with asymptomatic or mild COVID-19 may continue current metformin therapy and further interventional studies should be conducted to prove or disprove this recommendation, it appears that, as a rule, metformin should be withdrawn in hospitalized patients.

THIAZOLIDINEDIONES

Thiazolidinediones are shown to achieve their blood glucose-lowering effect by activating peroxisome proliferator-activated receptor γ (PPARγ) thereby increasing insulin sensitivity (32). In addition to their glucose-lowering effects, thiazolidinediones are also shown to exert immunomodulatory effects (33). Given

that immune hyperactivity is considered to be involved in the pathophysiology of COVID-19, it appears reasonable to assume that they may have some positive impact on the disease progression (33–36). However, there are also some concerns. First, while its clinical impact is not known, pioglitazone has the potential to enhance ACE2 expression in the liver, adipose tissue and skeletal muscle (37, 38), suggesting that the use of thiazolidinediones may affect not only the prevalence of COVID-19 but the mortality from the disease. Second, thiazolidinediones also act on the collecting tubule to increase water and sodium reabsorption by enhancing the expression of the epithelial Na^+ channel, thus causing edema and fluid retention (39, 40). This adverse effect may be enhanced in patients with COVID-19, given that the disease is sometimes shown to damage the kidneys and myocardium (41, 42). Therefore, it appears that, for the time being at least, it is advisable to avoid using thiazolidinediones in hospitalized patients.

SULFONYLUREAS AND GLINIDES

Sulfonylureas, the oldest oral antidiabetic drugs, are shown to promote insulin release from pancreatic β cells by binding to and closing the ATP-sensitive potassium channel resulting in depolarization of the plasma membrane and increased calcium influx thus leading to insulin exocytosis (43). Again, glinides represent viable options in managing postprandial hyperglycemia due to their rapid and short-lasting insulinotropic effects (44, 45). However, sulfonylureas are known to cause hypoglycemia at a non-negligible rate with the risk shown to increase in acute settings (46), thus making their use inappropriate in patients with severe COVID-19.

DIPEPTIDYL PEPTIDASE-4 INHIBITORS

DPP4 inhibitors exert their anti-diabetic effects by inhibiting DPP4, which degrades incretin hormones, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), thus elevating blood levels of these hormones. Since these hormones stimulate insulin release glucose-dependently, DPP4 inhibitors lower blood glucose levels with no significant risk of hypoglycemia (43). Although DPP4 is considered to be a functional receptor of MERS-CoV (47), a sibling of SARS-CoV-2, there is no evidence to date to show that it interacts with SARS-CoV-2. Actually, a case control study conducted in Italy showed no association between the exposure to DPP4 inhibitor and the risk of hospitalization due to COVID-19 (48). Of note, DPP4 inhibitors exert anti-inflammatory effects without increasing the risk of infectious disease and thus may prove protective against COVID-19-induced lung injury (49–51). To confirm these hypotheses, a phase 4 clinical trial of linagliptin vs. insulin is currently underway to compare their effectiveness not only in achieving glucose control but in preventing the progression of COVID-19 in type 2 diabetic patients with mild to moderate COVID-19 (52). While, given its glucose-dependent effects and the risk of hypoglycemia thought to be relatively low with this DPP-4 inhibitor, the use of DPP-4 inhibitors as a class may be deemed relatively safe in these patients mild to moderate COVID-19, consideration should be given to switching to insulin in patients with severe COVID-19.

GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISTS

While the mechanism of action of GLP-1R agonists is not fully understood, it seems likely that it involves cAMP signaling pathways and intracellular glucose metabolism in restoring β-cell glucose sensitivity (53). Due to this mechanism, GLP-1R agonists are assumed to lower blood glucose with relatively low risk of hypoglycemia (54). In addition, they help reduce food intake and body weight and thus may often be beneficial for diabetic patients who tend to be overweight (53–55). Also, their cardio- and renoprotective profile may prove beneficial for patients with COVID-19 (56, 57). In addition, of the GLP-1 agonists, liraglutide has the potential to increase lung ACE2 expression, while the net effect of ACE2 on COVID-19 still remains unknown (58). Nevertheless, GLP-1R agonists may as well be withdrawn from diabetic patients requiring hospitalization with COVID-19, given that their frequent adverse events, gastrointestinal symptoms (e.g., nausea, diarrhea or vomiting) are likely to worsen dehydration and, as a consequence, cause renal failure, which often occur in patients with COVID-19 (27, 53).

SODIUM-GLUCOSE COTRANSPORTER-2 INHIBITORS

Unlike other oral glucose-lowering agents described above, this class of drugs exert their effects, independently of insulin, by blocking sodium-glucose cotransporter 2 (SGLT2) in the renal proximal tubules from reabsorbing filtered glucose, i.e., by increasing glucose excretion thereby decreasing levels of blood glucose (59). Moreover, SGLT2 inhibitors are known to have cardio- and renoprotective effects (60–62) and have the potential to improve systemic metabolism, thus possibly preventing respiratory failure and organ dysfunction associated with COVID-19. To test this speculation, a phase 3 international, multicenter, double-blind, randomized, placebo-controlled trial of dapagliflozin is now underway in hospitalized, mild-to-moderate COVID-19 patients with preexisting comorbidities (63). However, an international expert panel warns that increased glucose excretion may also lead to fluid loss, possibly resulting in worsening of dehydration and onset of diabetic ketoacidosis in diabetic patients with COVID-19 (22, 64). Based on these findings, therefore, consideration should be given to discontinuing SGLT2 inhibitors in diabetic patients with COVID-19 at high risk of respiratory failure and thrombosis.

INSULIN ANALOGS

The only hormone available to lower blood levels of glucose, insulin is released from pancreatic β cells sensitized by glucose influx through glucose transporter type 2 (GLUT2) and stimulates the uptake of carbohydrates, peptides and lipids by

TABLE 1 | Ongoing trials evaluating the antidiabetic drugs in COVID-19 patients.

Clinical trial number	Clinical phase/Multicenter	Arms	Target number of patients	Primary outcome	Estimated date of study completion
NCT04341935	4/No	• Experimental arm: linagliptin + standard of care insulin regimen as per hospital protocol during hospitalization for up to 14 days • Active comparator arm: standard of care insulin regimen as per hospital protocol during hospitalization for up to 14 days	20	1. Changes in glucose levels	March 30, 2021
NCT04371978	3/No	• Experimental arm: linagliptin + standard of care insulin regimen as per hospital protocol during their entire hospitalization • Nonintervention arm: standard of care insulin regimen as per hospital protocol during their entire hospitalization	100	1. Time to clinical change	September 30, 2021
NCT04365517	3/No	• Active comparator arm: sitagliptin + nutritional therapy with or without insulin treatment • Nonintervention arm: nutritional therapy with or without insulin treatment	170	1. Time for clinical improvement 2. Clinical parameters of acute lung disease 3. Biochemical parameter of acute lung disease	December 30, 2020
NCT04473274	4/No	• Experimental arm: pioglitazone 15–30 mg daily oral or enteral during hospitalization for up to 30 days + standard of care • Standard of care	20	1. Adverse events outcomes without attribution 2. Adverse events attributable	June 1, 2021
NCT04510194	2 (prevention), 3 (treatment)/ No	Prevention • Experimental arm: metformin (500 mg; twice daily) • Comparator: placebo Treatment • Experimental: metformin (500 mg; twice daily) • Placebo	1,522	1. Rate of death due to COVID-19 2. Rate of hospitalization due to COVID-19 3. Rate of emergency department utilization 4. Rate of urgent care utilization	September 2021
NCT04350593	3/Yes	• Active comparator: dapagliflozin 10 mg daily • Placebo comparator arm: dapagliflozin matching placebo 10 mg daily	900	1. Time to first occurrence of either death from any cause or new/worsened organ dysfunction through 30 days of follow up	December 2020

other cells. Simultaneously, it inhibits hepatic gluconeogenesis and glycolysis, thus causing a rapid drop of blood glucose (65, 66). In normal settings, the goal of insulin therapy is to reproduce physiologic insulin secretion using long-acting analogs as basal insulin release and rapid-acting analogs as prandial insulin release (67). Unlike other oral antidiabetic agents, insulin has been used in critically ill patients whose prognosis is shown to improve to a greater extent with conventional insulin therapy than with intensive insulin therapy (68, 69). While insulin therapy prior to admission was shown to be associated with higher mortality in patients with COVID-19 (70, 71), there is a possibility that blood glucose control with insulin therapy during hospitalization leads to reductions in the risk of sever disease in these patients (72). Of course, when using insulin, close monitoring of blood glucose levels is essential, because it is sometimes associated with hypoglycemic events thus possibly raising mortality rates in critically ill patients (73, 74). However, given the current circumstances where there is no accumulated evidence to support the use of other agents, insulin appears to be the best choice for diabetic inpatients with COVID-19.

ONGOING STUDIES

While the possible harms and benefits of antidiabetic drugs have been summarized in the context of COVID-19, some of these recommendations remain rather hypothetical, because of the paucity of current evidence. Again, several clinical trials are now underway to investigate the actual effect of these drugs in controlling blood glucose diabetic patients with COVID-19, with some of these drugs expected to prevent exacerbation of COVID-19 even in non-diabetic patients. The characteristics of these trials are summarized in **Table 1**. These include NCT04341935 and NCT04371978, both randomized open label studies in diabetic patients with COVID-19, with the former intended to prove the efficacy of the investigational drugs in controlling blood glucose, and the latter aimed to reveal the efficacy of the study drugs in improving the severity of the disease (52, 75); NCT04365517, also a randomized controlled open label study designed to investigate the potential respiratory role of the DPP4 inhibitor sitagliptin in diabetic patients suffering from pneumonia due to COVID-19 (76). Through these studies, DPP4 inhibitors may be

shown to be effective in controlling blood glucose in diabetic patients with COVID-19. Again, a non-randomized matching cohort study, NCT04473274 is intended to investigate the safety of the thiazolidinedione pioglitazone in patients with relative hyperglycemia requiring hospital admission due to COVID-19 (77) and may be able to address the speculative concern posed above. NCT04510194 is a large-scale, randomized, quadruple blinded study evaluating metformin not only for its therapeutic but for its preventive role against COVID-19 (78). Given the large sample size and the reliable study design, as well as the fact that, to date, virtually no drug has been proved to prevent the infection itself, the results of this study are worth paying attention to. Furthermore, another large-scale study, NCT04350593 should be of particular interest, given the unavailability of any established treatment for the disease (63). Again, overall, the outcomes of these trials may help further optimize blood glucose control strategy for diabetic patients with COVID-19.

CONCLUSION

The present review was an attempt to summarize the profiles of currently available antidiabetic agents and their role in maintaining blood glucose control in hospitalized diabetic patients with COVID-19. While it remains important to continue current regimens for glucose control in patients with mild, self-limiting COVID-19, it appears that insulin may be a good choice for patients with severe COVID-19, while DPP4 inhibitors may also prove to be a good choice, along with insulin, for patients with mild to moderate disease, pending the results of clinical trials currently underway. At any rate, given that the COVID-19 pandemic is unlikely to end any time soon and that diabetes is closely associated with disease progression, continued efforts need to be made to accumulate evidence that guides the use of antidiabetic agents in diabetic patients with COVID-19 and to establish the best possible approach to achieving blood glucose control in these patients.

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REFERENCES

1. 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
2. *Coronavirus Disease (COVID-19) Outbreak Situation*. World Health Organization (2020). Available online at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/ (accessed July 14, 2020).
3. Lopez L, Rodo X. The end of social confinement and COVID-19 re-emergence risk. *Nat Hum Behav.* (2020) 4:746–55. doi: 10.1038/s41562-020-0908-8
4. Scala A, Flori A, Spelta A, Brugnoli E, Cinelli M, Quattrociocchi W, et al. Time, space and social interactions: exit mechanisms for the Covid-19 epidemics. *Sci Rep.* (2020) 10:13764. doi: 10.1038/s41598-020-70631-9
5. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet.* (2020) 395:565–74. doi: 10.1016/S0140-6736(20)30251-8
6. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature.* (2020) 581:215–20. doi: 10.1038/s41586-020-2180-5
7. Imai Y, Kuba K, Ohto-Nakanishi T, Penninger JM. Angiotensin-converting enzyme 2 (ACE2) in disease pathogenesis. *Circ J.* (2010) 74:405–10. doi: 10.1253/circj.CJ-10-0045
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. 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
10. 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
11. 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
12. *A Statement From the International Society of Hypertension on COVID-19*. International Society of Hypertension (2020). Available online at: https://ish-world.com/news/a/A-statement-from-the-International-Society-of-Hypertension-on-COVID-19/ (accessed August 6, 2020).
13. *HFSA/ACC/AHA Statement Addresses Concerns Re: Using RAAS Antagonists in COVID-19*. American College of Cardiology (2020). Available online at: https://www.acc.org/latest-in-cardiology/articles/2020/03/17/08/59/hfsa-acc-aha-statement-addresses-concerns-re-using-raas-antagonists-in-covid-19 (accessed August 6, 2020).
14. Hu Y, Sun J, Dai Z, Deng H, Li X, Huang Q, et al. Prevalence and severity of corona virus disease 2019 (COVID-19): a systematic review and meta-analysis. *J Clin Virol.* (2020) 127:104371. doi: 10.1016/j.jcv.2020.104371
15. *IDF Diabetes Atlas Ninth Edition.* (2019). Available online at: https://www.diabetesatlas.org/en/ (accessed July 14, 2020).
16. American Diabetes A. 4. Lifestyle management: standards of medical care in diabetes-2018. *Diabetes Care.* (2018) 41(Suppl. 1):S38–50. doi: 10.2337/dc18-S004
17. Martinez-Ferran M, de la Guia-Galipienso F, Sanchis-Gomar F, Pareja-Galeano H. Metabolic impacts of confinement during the COVID-19 pandemic due to modified diet and physical activity habits. *Nutrients.* (2020) 12:1549. doi: 10.3390/nu12061549
18. Ruiz-Roso MB, de Carvalho Padilha P, Mantilla-Escalante DC, Ulloa N, Brun P, Acevedo-Correa D, et al. Covid-19 confinement and changes of adolescent's dietary trends in Italy, Spain, Chile, Colombia and Brazil. *Nutrients.* (2020) 12:1807. doi: 10.3390/nu12061807
19. Yan Y, Yang Y, Wang F, Ren H, Zhang S, Shi X, et al. Clinical characteristics and outcomes of patients with severe covid-19 with diabetes. *BMJ Open Diabetes Res Care.* (2020) 8:e001343. doi: 10.1136/bmjdrc-2020-001343
20. Singh AK, Gillies CL, Singh R, Singh A, Chudasama Y, Coles B, et al. Prevalence of co-morbidities and their association with mortality in patients with COVID-19: a systematic review and meta-analysis. *Diabetes Obes Metab.* (2020). doi: 10.1111/dom.14124. [Epub ahead of print].
21. 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 Metab.* (2020) 31:1068–77.e3. doi: 10.1016/j.cmet.2020.04.021

22. Bornstein SR, Rubino F, Khunti K, Mingrone G, Hopkins D, Birkenfeld AL, et al. Practical recommendations for the management of diabetes in patients with COVID-19. *Lancet Diabetes Endocrinol.* (2020) 8:546–50. doi: 10.1016/S2213-8587(20)30152-2
23. Bode B, Garrett V, Messler J, McFarland R, Crowe J, Booth R, et al. Glycemic characteristics and clinical outcomes of COVID-19 patients hospitalized in the United States. *J Diabetes Sci Technol.* (2020) 14:813–21. doi: 10.1177/1932296820924469
24. Iacobellis G, Penaherrera CA, Bermudez LE, Bernal Mizrachi E. Admission hyperglycemia and radiological findings of SARS-CoV2 in patients with and without diabetes. *Diabetes Res Clin Pract.* (2020) 164:108185. doi: 10.1016/j.diabres.2020.108185
25. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. *Diabetologia.* (2017) 60:1577–85. doi: 10.1007/s00125-017-4342-z
26. DeFronzo R, Fleming GA, Chen K, Bicsak TA. Metformin-associated lactic acidosis: current perspectives on causes and risk. *Metabolism.* (2016) 65:20–9. doi: 10.1016/j.metabol.2015.10.014
27. 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
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/CIRCULATIONAHA.120.046702
29. Jain V, Sharma D, Prabhakar H, Dash HH. Metformin-associated lactic acidosis following contrast media-induced nephrotoxicity. *Eur J Anaesthesiol.* (2008) 25:166–7. doi: 10.1017/S026502150700097X
30. Luo P, Qiu L, Liu Y, Liu XL, Zheng JL, Xue HY, et al. Metformin treatment was associated with decreased mortality in COVID-19 patients with diabetes in a retrospective analysis. *Am J Trop Med Hyg.* (2020) 103:69–72. doi: 10.4269/ajtmh.20-0375
31. Scheen AJ. Metformin and COVID-19: from cellular mechanisms to reduced mortality. *Diabetes Metab.* (2020). doi: 10.1016/j.diabet.2020.07.006. [Epub ahead of print].
32. Soccio RE, Chen ER, Lazar MA. Thiazolidinediones and the promise of insulin sensitization in type 2 diabetes. *Cell Metab.* (2014) 20:573–91. doi: 10.1016/j.cmet.2014.08.005
33. Ciavarella C, Motta I, Valente S, Pasquinelli G. Pharmacological (or synthetic) and nutritional agonists of PPAR-gamma as candidates for cytokine storm modulation in COVID-19 disease. *Molecules.* (2020) 25:2076. doi: 10.3390/molecules25092076
34. Tay MZ, Poh CM, Renia 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
35. Jagat JM, Kalyan KG, Subir R. Use of pioglitazone in people with type 2 diabetes mellitus with coronavirus disease 2019 (COVID-19): boon or bane? *Diabetes Metab Syndr.* (2020) 14:829–31. doi: 10.1016/j.dsx.2020.06.015
36. Carboni E, Carta AR, Carboni E. Can pioglitazone be potentially useful therapeutically in treating patients with COVID-19? *Med Hypotheses.* (2020) 140:109776. doi: 10.1016/j.mehy.2020.109776
37. Zhang W, Li C, Liu B, Wu R, Zou N, Xu YZ, et al. Pioglitazone upregulates hepatic angiotensin converting enzyme 2 expression in rats with steatohepatitis. *Ann Hepatol.* (2013) 12:892–900. doi: 10.1016/S1665-2681(19)31294-3
38. Zhang W, Xu YZ, Liu B, Wu R, Yang YY, Xiao XQ, et al. Pioglitazone upregulates angiotensin converting enzyme 2 expression in insulin-sensitive tissues in rats with high-fat diet-induced non-alcoholic steatohepatitis. *ScientificWorldJournal.* (2014) 2014:603409. doi: 10.1155/2014/603409
39. Guan Y, Hao C, Cha DR, Rao R, Lu W, Kohan DE, et al. Thiazolidinediones expand body fluid volume through PPARgamma stimulation of ENaC-mediated renal salt absorption. *Nat Med.* (2005) 11:861–6. doi: 10.1038/nm1278
40. Satirapoj B, Watanakijthavonkul K, Supasyndh O. Safety and efficacy of low dose pioglitazone compared with standard dose pioglitazone in type 2 diabetes with chronic kidney disease: a randomized controlled trial. *PLoS ONE.* (2018) 13:e0206722. doi: 10.1371/journal.pone.0206722
41. Ronco C, Reis T, Husain-Syed F. Management of acute kidney injury in patients with COVID-19. *Lancet Respir Med.* (2020) 8:738–42. doi: 10.1016/S2213-2600(20)30229-0
42. 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
43. Tahrani AA, Barnett AH, Bailey CJ. Pharmacology and therapeutic implications of current drugs for type 2 diabetes mellitus. *Nat Rev Endocrinol.* (2016) 12:566–92. doi: 10.1038/nrendo.2016.86
44. Owens DR, McDougall A. Repaglinide: prandial glucose regulation in clinical practice. *Diabetes Obes Metab.* (2000) 2(Suppl. 1):S43–8. doi: 10.1046/j.1463-1326.2000.0022s.x
45. International Hypoglycaemia Study G. Minimizing hypoglycemia in diabetes. *Diabetes Care.* (2015) 38:1583–91. doi: 10.2337/dc15-0279
46. Pasquel FJ, Fayfman M, Umpierrez GE. Debate on insulin vs. non-insulin use in the hospital setting-is it time to revise the guidelines for the management of inpatient diabetes? *Curr Diab Rep.* (2019) 19:65. doi: 10.1007/s11892-019-1184-8
47. Raj VS, Mou H, Smits SL, Dekkers DH, Muller MA, Dijkman R, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature.* (2013) 495:251–4. doi: 10.1038/nature12005
48. Fadini GP, Morieri ML, Longato E, Bonora BM, Pinelli S, Selmin E, et al. Exposure to dipeptidyl-peptidase-4 inhibitors and COVID-19 among people with type 2 diabetes: a case-control study. *Diabetes Obes Metab.* (2020). doi: 10.1111/dom.14097. [Epub ahead of print].
49. Varin EM, Mulvihill EE, Beaudry JL, Pujadas G, Fuchs S, Tanti JF, et al. Circulating levels of soluble dipeptidyl peptidase-4 are dissociated from inflammation and induced by enzymatic DPP4 inhibition. *Cell Metab.* (2019) 29:320–34.e5. doi: 10.1016/j.cmet.2018.10.001
50. 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
51. Goossen K, Graber S. Longer term safety of dipeptidyl peptidase-4 inhibitors in patients with type 2 diabetes mellitus: systematic review and meta-analysis. *Diabetes Obes Metab.* (2012) 14:1061–72. doi: 10.1111/j.1463-1326.2012.01610.x
52. *Effects of DPP4 Inhibition on COVID-19.* (2020). Available online at: clinicaltrials.gov/ct2/show/NCT04341935 (accessed July 14, 2020).
53. Drucker DJ. Mechanisms of action and therapeutic application of glucagon-like peptide-1. *Cell Metab.* (2018) 27:740–56. doi: 10.1016/j.cmet.2018.03.001
54. Aroda VR. A review of GLP-1 receptor agonists: evolution and advancement, through the lens of randomised controlled trials. *Diabetes Obes Metab.* (2018) 20(Suppl. 1):22–33. doi: 10.1111/dom.13162
55. Htike ZZ, Zaccardi F, Papamargaritis D, Webb DR, Khunti K, Davies MJ. Efficacy and safety of glucagon-like peptide-1 receptor agonists in type 2 diabetes: a systematic review and mixed-treatment comparison analysis. *Diabetes Obes Metab.* (2017) 19:524–36. doi: 10.1111/dom.12849
56. Bethel MA, Patel RA, Merrill P, Lokhnygina Y, Buse JB, Mentz RJ, et al. Cardiovascular outcomes with glucagon-like peptide-1 receptor agonists in patients with type 2 diabetes: a meta-analysis. *Lancet Diabetes Endocrinol.* (2018) 6:105–13. doi: 10.1016/S2213-8587(17)30412-6
57. Kristensen SL, Rorth R, Jhund PS, Docherty KF, Sattar N, Preiss D, et al. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet Diabetes Endocrinol.* (2019) 7:776–85. doi: 10.1016/S2213-8587(19)30249-9
58. Romani-Perez M, Outeirino-Iglesias V, Moya CM, Santisteban P, Gonzalez-Matias LC, Vigo E, et al. Activation of the GLP-1 receptor by liraglutide increases ACE2 expression, reversing right ventricle hypertrophy, and improving the production of SP-A and SP-B in the lungs of type 1 diabetes rats. *Endocrinology.* (2015) 156:3559–69. doi: 10.1210/en.2014-1685
59. Heerspink HJ, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium Glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects, potential mechanisms, and clinical applications. *Circulation.* (2016) 134:752–72. doi: 10.1161/CIRCULATIONAHA.116.021887

60. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. *N Engl J Med.* (2019) 380:347–57. doi: 10.1056/NEJMoa1812389
61. Neuen BL, Young T, Heerspink HJL, Neal B, Perkovic V, Billot L, et al. SGLT2 inhibitors for the prevention of kidney failure in patients with type 2 diabetes: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol.* (2019) 7:845–54. doi: 10.1016/S2213-8587(19)30256-6
62. Thomas MC, Cherney DZI. The actions of SGLT2 inhibitors on metabolism, renal function and blood pressure. *Diabetologia.* (2018) 61:2098–107. doi: 10.1007/s00125-018-4669-0
63. *Dapagliflozin in Respiratory Failure in Patients With COVID-19 (DARE-19).* (2020). Available online at: clinicaltrials.gov/ct2/show/NCT04350593 (accessed July 14, 2020).
64. Fralick M, Schneeweiss S, Patorno E. Risk of diabetic ketoacidosis after initiation of an SGLT2 inhibitor. *N Engl J Med.* (2017) 376:2300–2. doi: 10.1056/NEJMc1701990
65. Mathieu C, Gillard P, Benhalima K. Insulin analogues in type 1 diabetes mellitus: getting better all the time. *Nat Rev Endocrinol.* (2017) 13:385–99. doi: 10.1038/nrendo.2017.39
66. Tokarz VL, MacDonald PE, Klip A. The cell biology of systemic insulin function. *J Cell Biol.* (2018) 217:2273–89. doi: 10.1083/jcb.201802095
67. Cahn A, Miccoli R, Dardano A, Del Prato S. New forms of insulin and insulin therapies for the treatment of type 2 diabetes. *Lancet Diabetes Endocrinol.* (2015) 3:638–52. doi: 10.1016/S2213-8587(15)00097-2
68. Investigators N-SS, Finfer S, Chittock DR, Su SY, Blair D, Foster D, et al. Intensive versus conventional glucose control in critically ill patients. *N Engl J Med.* (2009) 360:1283–97. doi: 10.1056/NEJMoa0810625
69. Griesdale DE, de Souza RJ, van Dam RM, Heyland DK, Cook DJ, Malhotra A, et al. Intensive insulin therapy and mortality among critically ill patients: a meta-analysis including NICE-SUGAR study data. *CMAJ.* (2009) 180:821–7. doi: 10.1503/cmaj.090206
70. Cariou B, Hadjadj S, Wargny M, Pichelin M, Al-Salameh A, Allix I, et al. Phenotypic characteristics and prognosis of inpatients with COVID-19 and diabetes: the CORONADO study. *Diabetologia.* (2020) 63:1500–15. doi: 10.1007/s00125-020-05180-x
71. 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
72. Sardu C, D'Onofrio N, Balestrieri ML, Barbieri M, Rizzo MR, Messina V, et al. Outcomes in patients with hyperglycemia affected by COVID-19: can we do more on glycemic control? *Diabetes Care.* (2020) 43:1408–15. doi: 10.2337/dc20-0723
73. Umpierrez G, Korytkowski M. Diabetic emergencies–ketoacidosis, hyperglycaemic hyperosmolar state and hypoglycaemia. *Nat Rev Endocrinol.* (2016) 12:222–32. doi: 10.1038/nrendo.2016.15
74. Investigators N-SS, Finfer S, Liu B, Chittock DR, Norton R, Myburgh JA, et al. Hypoglycemia and risk of death in critically ill patients. *N Engl J Med.* (2012) 367:1108–18. doi: 10.1056/NEJMoa1204942
75. *Efficacy and Safety of Dipeptidyl Peptidase-4 Inhibitors in Diabetic Patients With Established COVID-19.* (2020). Available online at: clinicaltrials.gov/ct2/show/NCT04371978 (accessed September 4, 2020).
76. *The Effect of Sitagliptin Treatment in COVID-19 Positive Diabetic Patients (SIDIACO).* (2020). Available online at: clinicaltrials.gov/ct2/show/NCT04365517 (accessed September 4, 2020).
77. *GlitazOne Treatment for Coronavirus HypoxiA, a Safety and Tolerability Open Label With Matching Cohort Pilot Study (GOTCHA).* (2020). Available online at: clinicaltrials.gov/ct2/show/NCT04473274 (accessed September 4, 2020).
78. *MET-Covid Trial–METformin for Prevention and Outpatient Treatment of COVID-19.* (2020). Available online at: clinicaltrials.gov/ct2/show/NCT04510194 (accessed September 4, 2020).

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Myocardial Injury at Early Stage and Its Association With the Risk of Death in COVID-19 Patients: A Hospital-Based Retrospective Cohort Study

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Background: There are growing evidence demonstrating that coronavirus disease 2019 (COVID-19) is companied by acute myocardial injury. However, the associations of SARS-CoV-2-induced myocardial injury with the risk of death and prognosis after discharge in COVID-19 patients are unclear.

Methods: This prospective cohort study analyzed 355 COVID-19 patients from two hospitals in different regions. Clinical and demographic information were collected and prognosis was followed up.

Results: Of 355 hospitalized patients with COVID-19, 213 were mild, 90 severe, and 52 critically ill patients. On admission, 59 (16.7%) patients were with myocardial injury. Myocardial injury was more popular in critically ill patients. Univariate and multivariate logistic regression revealed that male, older age and comorbidity with hypertension were three crucial independent risk factors predicting myocardial injury of COVID-19 patients. Among 59 COVID-19 patients with myocardial injury, 25 (42.4%) died on average 10.9 days after hospitalization. Mortality was increased among COVID-19 patients with myocardial injury (42.4 vs. 3.38%, $RR = 12.542$, $P < 0.001$). Follow-up study observed that 4.67% COVID-19 patients with myocardial injury were not fully recovered in 14 days after discharge.

Conclusion: Myocardial injury at early stage elevates mortality of COVID-19 patients. Male elderly patients with hypertension are more vulnerable to myocardial injury. SARS-CoV-2-induced myocardial injury has not completely recovered in 14 days after discharge.

Keywords: severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), coronavirus disease 2019 (COVID-19), myocardial injury, prognosis, death

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is a newly recognized infectious disease caused by the newly discovered Severe Acute Respiratory Syndrome- Coronavirus-2 (SARS-CoV-2). COVID-19 firstly broke out in Wuhan city, Hubei province in China since December 2019 (1). Now, it has been pandemic all over the world. Up to April 19th, China has cumulatively diagnosed 84,225 cases and over 4,642 deaths, while the number of cases in other countries is growing rapidly with a total of 2,277,363 confirmed cases and 157,572 death cases (2). American and Europe have gradually become the epicenter of the pandemic and the entire world is suffering great public health crisis from SARS-CoV-2, which is more severe than 2003 SARS disaster (3).

Some studies have found that SARS-CoV-2 transmitted through not only droplets or direct contact but also feces (4, 5). Evidence pointing to the person-to-person transmission has occurred among close contacts in hospital and family (6). As reported by other researchers and our team found that patients with COVID-19 present primarily with fever, diarrhea, fatigue, dry cough, lymphopenia and radiographic evidence of pneumonia (7–9). The Chinese Center for Disease Control and Prevention recently revealed that the risk of death of mild COVID-19 patients was relatively low in a large Chinese population-based study, the fatality rate was significantly elevated among critically ill COVID-19 cases (10). Previous studies found that SARS-CoV-2 mainly evoked severe acute respiratory syndrome. More and more researches revealed that most cases were prone to suffer multiple organ injuries, such as immune system disorder, acute kidney injure and even liver dysfunction (11–13). The clinical characteristics of myocardial injury caused by SARS-CoV-2 are gradually being recognized. However, the associations between SARS-CoV-2-induced myocardial injury with the risk of death and the prognosis after discharge in patients with COVID-19 remain unknown.

The aim of this study was to investigate SARS-CoV-2-induced myocardial injury, its associations with the risk of death and prognosis in the short term after discharge. Our results suggested that male elderly COVID-19 patients with hypertension were more vulnerable to myocardial injury. We found that myocardial injury at early stage increased the risk of death among COVID-19 patients. This study firstly provides evidence that SARS-CoV-2 induced myocardial injury has not completely recovered in 14 days after discharge.

METHODS

Study Design and Participants

In the present study, 200 patients with COVID-19 were recruited in Union Hospital of Huazhong University of Science and Technology in Wuhan city from January 1 to January 30, 2020. Another 155 patients with COVID-19 were recruited from the Second People's Hospital of Fuyang City in Anhui province. Union Hospital of Huazhong University of Science and Technology and Second People's Hospital of Fuyang City were assigned responsibility for the treatment of patients with COVID-19 by the Anhui government and Wuhan government. All patients were laboratory confirmed positive of SARS-CoV-2 injection by RT-PCR on pharyngeal swab specimens. Diagnosis and treatment of COVID-19 were based on the New Coronavirus Pneumonia Prevention and Control Program (6th edition) published by the National Health Commission of China. Laboratory examination, epidemiological investigation, imaging examination, electrocardiogram assessment and etiological examination were performed in all COVID-19 patients. When patients were diagnosed with SARS-CoV-2 injection, patients were isolated and began to cure based on the guide. Next, conventional observation of patients' conditions, antivirotic treatment, antibacterial treatment when patients accompanied with bacterial injection were carried among COVID-19 patients, such as interferon, lopinavir, ritonavir, and chloroquine phosphate were used. Moreover, some severe and critically ill patients may receive respiratory support with mechanical ventilation, rescue treatment using ECMO, circulation supportive or Chinese medicine treatment. Except for the above-mentioned drugs, glucocorticoid was also used in the severe and critically ill patients. The patients could be discharged who met the following criteria: body temperature was normal more than 3 days, respiratory symptoms were ameliorated, diffuse infiltration was absorbed using CT imaging and two consecutive nucleic acid detections of SARS-CoV-2 virus in nasopharyngeal swab and sputum were negative with 24 h interval. There was no death case with COVID-19 in the Second People's Hospital of Fuyang City. At last, 150 cured cases were performed follow-up examination in 14 days after discharge in the Second People's Hospital of Fuyang City, biochemical indexes and blood routine were detected. This study was approved by the institutional ethics board of Union Hospital of Huazhong University of Science and Technology, and Second People's Hospital of Fuyang City. All COVID-19 patients were eligible in this study. Oral consent was obtained from patients or patients' next of kin.

Data Collection

The medical record of each COVID-19 patient was collected. Patient's data including demographics, comorbidities (chronic obstructive pulmonary disease, hepatic disease, cardiovascular disease, hypertension, diabetes and other disease), patient's signs and symptoms, and laboratory test results were collected. The date of onset and outcomes were recorded.

Laboratory Testing

Patient's pharyngeal swab specimens were measured. Real-time RT-PCR was used to detect viral nucleic acid using COVID-19 nucleic acid detection kits following experimental instructions (Shanghai bio-germ Medical Technology Co Ltd). Troponin (Tn), aspartate aminotransferase (AST), creatine kinase (CK), creatine kinase isoenzyme (CKMB), lactate dehydrogenase (LDH), oxygenation index (PaO_2/FiO_2), C-reactive protein (CRP) were examined on admission. Myocardial injury was defined as troponin beyond normal range (14). A complete blood routine and blood chemistries were conducted in all COVID-19 patients.

Statistical Analysis

All statistical analyses were performed using SPSS19.0 software. Categorical variables were expressed with frequencies and percentages. Continuous variables were shown using median and mean values. All categorical variables were compared for the study outcome by the Fisher exact test or χ^2 test, and continuous variables were compared using the *t*-test or the Mann-Whitney *U* test, as appropriate. Logistic regression analysis between myocardial injure with different parameters and the prognosis were performed. Statistical significance was determined at $P < 0.05$.

RESULTS

Demographic and Clinical Characteristics

All 355 COVID-19 patients' clinical information was collected and evaluated. As shown in **Table 1**, mild case, defined as oxygenation index higher than 300, was 213 (60.0%). For severe case, whose oxygenation index was from 200 to 300, was 90 (25.4%). For critically ill case, whose oxygenation index was lower than 200, accounted for 14.6% (**Table 1**). Moreover, the demographic characteristics were then analyzed. As shown in **Table 2**, 162 (45.6%) were female and 193 (54.4%) were male. There were 96 patients younger than 39 years old, 144 patients aged between 40 and 59, and 115 patients older than 60 years old. Of 355 patients with COVID-19, 230 (64.8%) patients had hypertension, 208 (58.6%) patients had diabetes, and 20 (5.63%) had chronic heart disease.

Association of Myocardial Injury With the Severity of COVID-19 Patients

COVID-19 firstly broke out in Wuhan City of China. Because the number of patients was large and medical resources were limited, only electrocardiogram assessment of partially patients were performed (data not shown). We found that the patients with left and right ventricular dysfunction were more in severe and critically ill patients than those in mild patients with COVID-19. Diastolic dysfunction was observed in only a small number of patients with COVID-19. Only a few patients had mild pericardial effusion without other clinical or electrocardiographic signs of pericarditis. The association between myocardial injury and the severity of COVID-19 was evaluated in patients. Myocardial injury indexes, including Tn, CK, CKMB, LDH, and AST, were analyzed. As shown in **Table 1**, the level of CK were higher in critically ill patients than those in mild and severe patients. The number of CKMB-positive patients were more in critically ill patients than those of mild patients. The levels of LDH and AST were the lowest in the mild patients with COVID-19. Moreover, the levels of LDH and AST were higher in the critically ill patients than those of in severe patients. The levels of Tn were gradually increased in parallel with the severity of COVID-19. The results indicated that 22 (10.3%) COVID-19 patients were with myocardial injury at early stage in mild patients. Nineteen (21.1%) patients with myocardial injury were in severe patients and 18 (34.6%) patients with myocardial injury were in critically ill patients. Furthermore, the associations between oxygenation index and myocardial injury markers were analyzed. As shown in **Supplementary Table 1**, no association between oxygenation index with CK and CKMB was observed. There was a negative association between AST ($r = -0.249$, $P = 0.001$), LDH ($r = -0.431$, $P < 0.001$) and Tn ($r = -0.221$, $P = 0.038$) with oxygenation index among COVID-19 patients. Additionally, the associations between inflammatory cytokine and myocardial injury markers were analyzed. The results indicated that CRP was positively correlated with AST ($r = 0.241$, $P = 0.004$), LDH ($r = 0.457$, $P < 0.001$) and CK ($r = -0.198$, $P = 0.018$) (**Supplementary Table 1**).

Male Elderly COVID-19 Patients With Hypertension Are More Vulnerable to Myocardial Injury

The effects of demographic characteristics on myocardial injury markers were analyzed. As shown in **Table 2**, the level of CK were higher in males than in females. There was no difference of CKMB, LDH, AST, and Tn between females and males. Further analysis showed that CK, CKMB, LDH, AST, and Tn were lower in patients younger than 39 years old than those of older patients. Moreover, we found that CK, CKMB, LDH, AST, and Tn were higher in patients older than 60 than those between 40 and 59 years old (**Table 2**). The effects of comorbidities on myocardial functional indexes were then analyzed. As shown in **Table 2**, CKMB-positive patients were more in COVID-19 patients with hypertension than those without hypertension. Besides, the level of CK was elevated in COVID-19 patients with diabetes compared with those without diabetes. LDH and AST were increased in COVID-19 patients with chronic heart disease compared with those without heart disease. There was no difference of Tn in patients with hypertension and diabetes or not. The levels of Tn were higher in patients with heart disease than those in patients without heart disease. In addition, the risk factors of myocardial injury were analyzed using univariate and multivariate logistic regression among COVID-19 patients. In the univariate logistic regression analysis, the *OR* of male gender was 2.012 (95% CI: 1.125, 3.599), the *OR* of age was 1.434 (95% CI: 1.041, 1.976) and the *OR* of hypertension was 3.393 (95% CI: 1.441, 7.989) for myocardial injury (**Table 3**). In the multivariate logistic regression analysis, the *OR* of male gender was 2.349 (95% CI: 1.135, 4.312), the *OR* of age was 1.332 (95% CI: 1.014, 2.123) and the *OR* of hypertension was 2.958 (95% CI: 1.331, 5.636) for myocardial injury (**Table 3**).

Myocardial Injury at Early Stage Elevates the Risk of Death of COVID-19 Patients

The effects of myocardial injury at the early stage on the risk of death were analyzed using multivariate logistic regression after adjusted age, sex, and comorbidities. An shown in **Table 4**, among 59 COVID-19 patients with myocardial injury, 42.4% were died. In multivariate logistic regression analysis, the fatality rate was higher among COVID-19 patients with myocardial injury than those without myocardial injury (42.4 vs. 3.38%; *RR* = 12.542, *95% CI*: 6.367, 24.708; $P < 0.001$).

TABLE 1 | The associations between the severity and myocardial injury indexes.

Parameters	Mild	Severe	Critically ill
Cases, *N*	213	90	52
CK (U/L)	70.0 (47.0, 101.0)	72.0 (47.3, 160.0)	149 (70.0, 319.0)$^{**\#\#}$
CKMB, *N* (%)	69 (33.7)	51 (59.3)	38 (74.5)*
LDH (U/L)	222.0 (186.0, 272.0)	299.0 (226.0, 370.0)**	442.0 (277.5, 630.3)$^{**\#\#}$
AST (U/L)	26.0 (20.0, 35.0)	29.0 (22.8, 54.0)*	49.0 (35.0, 80.0)$^{**\#\#}$
Tn (ng/mL)	0.01 (0, 0.02)	0.04 (0.02, 0.06)*	0.07 (0.05, 0.12)$^{**\#\#}$
Myocardial injury cases (%)	22 (10.3)	19 (21.1)*	18 (34.6)$^{**\#}$

*Compared with "Mild", *P < 0.05, **P < 0.01; Compared with "Severe", $^{\#}$P < 0.05, $^{\#\#}$P < 0.01.*
Myocardial injury was defined as troponin beyond normal range.

TABLE 2 | The effects of demographic characteristics and complications on myocardial injury indexes.

	Cases	CK (U/L)	CKMB, *N* (%)	LDH (U/L)	AST (U/L)	Tn (ng/mL)
Gender						
Female	162	59.5 (40.0, 97.0)	79 (49.1)	236.0 (188.5, 341.0)	26.0 (20.0, 39.0)	0.04 (0.02, 0.06)
Male	193	92.0 (59.5, 153.5)**	76 (41.5)	254.0 (201.5, 337.5)	30.0 (23.0, 47.0)*	0.05 (0.02, 0.07)
Age						
<39	96	75.5 (41.5, 101.5)	19 (19.8)	209.0 (172.0, 261.0)	23.0 (19.0, 29.0)	0.03 (0.01, 0.05)
40–59	144	69.0 (47.0, 114.0)**	54 (37.8)**	246.0 (200.3, 327.5)**	28.0 (22.0, 45.0)*	0.05 (0.02, 0.08)**
>60	115	99.0 (58.0, 194.0)$^{**\#\#}$	82 (71.3)$^{**\#\#}$	296.0 (223.0, 450.5)$^{**\#\#}$	36.0 (23.0, 57.5)$^{**\#\#}$	0.08 (0.06, 0.10)$^{**\#\#}$
Hypertension						
Yes	230	72.0 (47.0, 166.0)	89 (73.0)	270.0 (189.0, 403.0)	31.5 (21.0, 58.8)	0.05 (0.02, 0.07)
No	125	77.0 (50.0, 122.0)	69 (30.7)**	239.0 (195.8, 312.8)	27.0 (21.0, 39.0)*	0.04 (0.01, 0.06)
Diabetes						
Yes	208	89.0 (50.0, 170.0)	95 (66.4)	270.0 (177.3, 389.5)	32.0 (23.0, 58.0)	0.04 (0.02, 0.07)
No	147	72.0 (49.0, 113.8)*	63 (30.9)	237.0 (197.0, 306.0)	26.0 (21.0, 37.5)**	0.05 (0.02, 0.08)
Heart disease						
Yes	20	78.0 (47.0, 184.0)	12 (63.2)	346.0 (223.0, 480.0)	35.0 (21.0, 71.0)	0.06 (0.03, 0.09)
No	335	77.0 (49.0, 125.5)	146 (44.5)	245.0 (193.8, 323.8)*	28.0 (21.0, 43.0)	0.04 (0.01, 0.07)*

*Cases in gender, compared with "Female", *P < 0.05, **P < 0.01.*
*Cases in age, compared with "<39", *P < 0.05, **P < 0.01; compared with "40–59", $^{\#\#}$P < 0.01.*
*Cases in hypertension, diabetes, and heart disease, compared with "Yes", *P < 0.05, **P < 0.01.*

TABLE 3 | Logistic regression analysis the risk factors of myocardial injury among COVID-19 patients.

	Univariate logistic regression analysis				Multivariate logistic regression analysis			
	β	Wald	*P-value*	OR (95% CI)	*β*	Wald	*P-value*	OR (95% CI)
Male	0.699	5.556	0.018	2.012 (1.125, 3.599)	0.717	5.662	0.017	2.349 (1.135, 4.312)
Age	−1.304	5.003	0.028	1.434 (1.041, 1.976)	0.031	12.136	0.001	1.332 (1.014, 2.123)
Hypertension	1.222	7.814	0.005	3.393 (1.441, 7.989)	1.160	6.773	0.009	2.958 (1.331, 5.636)
Diabetes	0.660	3.118	0.077	1.936 (0.930, 4.029)	0.498	1.658	0.198	1.646 (0.771, 3.513)
Heart disease	−0.016	0.000	0.984	0.984 (0.202, 4.788)	−0.791	1.083	0.298	0.454 (0.102, 2.010)

TABLE 4 | The association between myocardial injury and death risk among COVID-19 patients.

Myocardial injury	Cases	Death (%)	RR (95% CI)	*P-value*
Yes	59	25 (42.4)	12.542 (6.367, 24.708)	<0.001
No	296	10 (3.38)	1	—

Adjusted for age, sex, and comorbidities.

Myocardial Markers Remain Abnormal in 14 Days After Discharge

The recovery of myocardial injury was investigated in every patient with COVID-19. The levels of myocardial markers were compared between on admission and in 14 days after discharge in the Second People's Hospital of Fuyang City. As shown in **Table 5**, there was no significant difference in the levels of CK, CKMB, AST, and Tn among COVID-19 patients between on admission and after discharge, whereas LDH was decreased in 14 days after discharge than on admission. On admission, 5 (3.3%) cases with CK, 9 (6.1%) cases with CKMB, 56 (38.1%) cases with LDH, 19 (12.3%) cases with AST, and 20 (13.0%) cases with Tn were above the normal range. In all, there was 20 (13.0%) COVID-19 patients with myocardial injury. The prognosis of COVID-19 patients' myocardial injury markers was followed up in 14 days after discharge in the Second People's Hospital of Fuyang City. We found that 2 (1.33%) patients with CKMB, 25 (16.7%) patients with LDH, 25 (15.3%) patients with AST, and 7 (4.67%) patients with Tn remained above the normal range. Further analysis showed that 4.67% patients with COVID-19 continuously accompanied with myocardial injury in 14 days after discharge (**Table 5**).

DISCUSSION

This study mainly investigated SARS-CoV-2-induced myocardial injury, its associations with mortality and the prognosis in 14 days after discharge. The major results of this study include: (1) Myocardial injury is more popular in the critically ill patients with COVID-19; (2) Male elderly COVID-19 patients with hypertension are more vulnerable to myocardial injury; (3) Myocardial injury at early stage elevates the risk of death of COVID-19 patients; (4) Myocardial injury of 4.67% COVID-19 patients has not completely recovered in 14 days after discharge.

More and more studies have demonstrated that COVID-19 patients were accompanied with multiple organ injuries, mainly including acute liver injury, acute kidney injury, respiratory failure and even lymphopenia (12, 13, 15). In the present study, myocardial injury was evaluated through measuring biochemical indexes, such as CK, CKMB, LDH, AST, and Tn. We found that these five myocardial injury markers were higher in critically ill patients than those in mild and severe patients with COVID-19. Moreover, the cases of myocardial injury were more in critically ill patients than those in mild and severe patients with COVID-19. Myocardial injury was more popular in critically ill patients. These results provide evidence that myocardial injury at early stage is positively associated with the severity of COVID-19 patients.

The previous studies have revealed that older age patients have more severe symptoms and signs (12, 16). In the present study, the effects of demographic characteristics on myocardial injury were analyzed. Although no difference of CKMB, LDH, and Tn were observed between females and males, CK and AST was higher in males than those in females. In addition, the number of CKMB-positive cases was more in older patients than younger patients. Several reports indicated that comorbidities elevated the risk of death and the severity of COVID-19 patients (17, 18). The present study found that 64.8% patients were with hypertension, 58.6% patients were with diabetes and 5.63% patients were with chronic heart disease. In order to investigate the influence of comorbidities on myocardial injury, the markers of myocardial injury were analyzed among COVID-19 patients. This study found that the number of CKMB-positive cases was more in patients with hypertension than those without hypertension. In addition, the level of serum CK was slightly increased in patients with diabetes. The level of serum LDH was significantly higher in patients with chronic heart disease as compared with those without heart disease. The level of Tn was obviously increased in patients with heart disease than those without heart disease. These results indicate that male, older age and comorbidities may aggravate myocardial injury of COVID-19 patients. In order to further analyze the associations between myocardial injury with demographic characteristics and comorbidities, the univariate and multivariate logistic regression were performed. Our results indicated that male, older age, comorbidity with hypertension were three independent risk factors of myocardial injury. Generally speaking, male elderly COVID-19 patients with hypertension are more vulnerable to myocardial injury.

The effect of myocardial injury on the prognosis of COVID-19 patients is not yet clear. The association between myocardial injury and the risk of death was analyzed among COVID-19 patients. The present study found that the mortality was higher

TABLE 5 | Myocardial injury indexes on admission and after discharge among COVID-19 patients.

Myocardial injury indexes	On admission (*N* = 154)			Discharge (*N* = 150)		
	Median	Below the range, *N* (%)	Above the range, *N* (%)	Median	Below the range, *N* (%)	Above the range, *N* (%)
CK (U/L)	65.0 (44.0, 96.0)	45 (29.8)	5 (3.3)	63.0 (47.0, 82.0)	43 (28.6)	0$^{\#}$
CKMB (U/L)	8.0 (5.0, 13.0)	0	9 (6.1)	7.0 (4.0, 11.0)	0	2 (1.33)$^{\#}$
LDH (U/L)	230.0 (194.0, 278.0)	1 (0.7)	56 (38.1)	203.0 (176.0, 242.0)**	0	25 (16.7)$^{\#\#}$
AST (U/L)	24.0 (20.0, 31.0)	23 (15.0)	19 (12.3)	22.0 (19.0, 30.0)	11 (7.5)	23 (15.3)
Tn (ng/mL)	0.04 (0.02, 0.06)	0	20 (13.0)	0.03 (0.01, 0.04)	0	7 (4.67)$^{\#}$

*Compared with "Median values" among COVID-19 patients on admission, **$P < 0.01$.*
Compared with "Above the range" among COVID-19 patients on admission, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

in COVID-19 patients with myocardial injury than those without myocardial injury. Myocardial injury on admission obviously elevates the risk of death among COVID-19 patients. This is an urgent issue which is worthy of studying whether myocardial injury recovers during a short-period after discharge. In this work, 150 COVID-19 patients were tracked and markers of myocardial injury were detected. The rate of myocardial injury between on admission and in 14 days after discharge were compared among COVID-19 patients from the Second People's Hospital of Fuyang City. Although no remarkably difference of the levels of serum CK, CKMB, AST, and Tn were observed between on admission and in 14 days after discharge, the level of serum LDH was obviously decreased in 14 days after discharge. In spite of the abnormal number of CKMB, LDH and Tn were decreased, 1.33% patients with CKMB, 16.7% patients with LDH, and 4.67% patients with Tn were above the normal range. Our results indicate that myocardial injury of 4.67% patients with COVID-19 were not fully recovered in 14 days after discharge. Therefore, whether SARS-CoV-2 causes continuous myocardial injury is needed to perform further follow-up research in the future clinical work.

The mechanism of which SARS-CoV-2 induces myocardial injury is scarcely clear. The previous study found that CRP was evidently increased in the critically ill patients (19, 20). CRP is an acute-phase protein in response to inflammatory cytokines after infections. High level of CRP partially reflects the severity of inflammation and evokes cytokine storm, which largely enhances vascular permeability and impairs organ function. Our results found that CRP was positively correlated with the levels of AST, LDH and CK, indicating that SARS-CoV-2-induced cytokine storm may be one of the mechanisms of myocardial injury. Both our research and other team found that SARS-CoV-2 injection reduced oxygenation index and caused respiratory function failure (9). Continuous blood hypoxia induces acidosis and excess generation of reactive oxygen species, ultimately damaging myocardial cell. In the present study, we found that oxygenation index was negatively associated with AST, LDH and Tn among COVID-19 patients, suggesting that respiratory function failure may contribute, at least partially, to SARS-CoV-2-evoked myocardial injury. Increasing data demonstrate that angiotension converting enzyme (ACE)2, as a receptor for SARS-CoV-2, exerts a significant role in the pathogenesis of COVID-19 patients (21–23). Recently, a report found that ACE2 was also expressed in cardiocytes (24). Hence, these evidences don't exclude that SARS-CoV-2 evokes myocardial injury partially through directly damaging myocardial cells.

In brief, this research mainly analyzed the associations between SARS-CoV-2-induced myocardial injury with mortality and the prognosis in 14 days after discharge based on a retrospective cohort study. Nevertheless, there are some flaws in this study. Firstly, the patients were only from two different regions and the sample size was mild. A larger sample size from multicenter in China is needed in the future study. Secondly, COVID-19 firstly broke out in Wuhan City of China. In that circumstances, the number of patients was large and medical resources were limited. Due to the higher transmissibility and high mortality of COVID-19, only partially patients' electrocardiogram assessment was performed. All COVID-19 patients' coronary angiography was not conducted. Therefore, we can't ascertain whether the elevation of troponin is due to myocarditis or to acute coronary syndromes. Moreover, we also cannot exclude that patients died for acute coronary syndromes or because of heart failure after a massive myocarditis. Thirdly, SARS-CoV-2 injection not only evokes inflammation storm, but also damages myocardial cells directly. Elevation of inflammatory cytokines also damage myocardial cells. So, the present study can't ascertain the exact mechanism of SARS-CoV-2 inducing myocardial injury.

CONCLUSION

In summary, the present study mainly analyzed SARS-CoV-2-evoked myocardial injury among COVID-19 patients in two hospitals from different region. These results showed that SARS-CoV-2-induced myocardial injury was more general in critically ill patients. Furthermore, male elderly COVID-19 patients with hypertension were more vulnerable to myocardial injury. Our results firstly suggest that myocardial injury at early stage elevates the risk of death of COVID-19 patients. What's more, SARS-CoV-2-evoked myocardial injury has not completely recovered in 14 days after discharge. Therefore, it is essential to further investigate whether SARS-CoV-2 results in a long-term myocardial injury.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

All procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975. This study was approved by the institutional ethics board of Union Hospital of Huazhong University of Science and Technology, and Second People's Hospital of Fuyang City. All COVID-19 patients were eligible in this study. Oral consent was obtained from patients or patients' next of kin.

AUTHOR CONTRIBUTIONS

D-XX and HZ designed the research. LF, X-YL, JF, H-XX, YX, M-DL, F-FL, and YL conducted the research. LF, X-YL, and JF analyzed the data. D-XX and LF wrote the paper and had primary responsibility for final content. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2020.590688/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. World Health Organization. *Coronavirus Disease (COVID-19) Pandemic*. (2020). Available online at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (accessed April 19, 2020).
3. World Health Organization. *WHO Director-General's Opening Remarks at the Media Briefing on COVID-19*. (2020). Available online at: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-mission-briefing-on-covid-19 (accessed March 13, 2020).
4. 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
5. 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
6. 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
7. 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
8. Fu L, Fei J, Xu S, Xiang HX, Xiang Y, Tan ZX, et al. Influence factors of the risk of death among COVID-19 patients in Wuhan, China: a hospital-based case-cohort study. *medRxiv [Preprint].* (2020) 035329. doi: 10.1101/2020.03.13.20035329
9. Fei J, Fu L, Li Y, Xiang HX, Xiang Y, Li MD, et al. Reduction of lymphocyte at early stage elevates severity and the risk of death of COVID-19 patients: a hospital-based case-cohort study. *medRxiv [Preprint].* (2020) 050955. doi: 10.5114/aoms.2020.99006
10. 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
11. Li Y, He F, Zhou N, Wei J, Ding Z, Wang L, et al. Organ function support in patients with coronavirus disease 2019: Tongji experience. *Front Med.* (2020) 14:232–48. doi: 10.1007/s11684-020-0774-9
12. Fu L, Fei J, Xu S, Xiang HX, Xiang Y, Tan ZX, et al. Acute liver injury and its association with the risk of death of patients with COVID-19: a hospital-based prospective case-cohort study. *medRxiv [Preprint].* (2020) 050997. doi: 10.1101/2020.04.02.20050997
13. Xu S, Fu L, Fei J, Xiang HX, Xiang Y, Tan ZX, et al. Acute kidney injury at early stage as a negative prognostic indicator of patients with COVID-19: a hospital-based retrospective analysis. *medRxiv [Preprint].* (2020) 042408. doi: 10.1101/2020.03.24.20042408
14. 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
15. Spiezia L, Boscolo A, Poletto F, Cerruti L, Tiberio I, Campello E, et al. COVID-19-related severe hypercoagulability in patients admitted to intensive care unit for acute respiratory failure. *Thromb Haemost.* (2020) 120:996–1000. doi: 10.1055/s-0040-1710018
16. 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
17. Du Y, Tu L, Zhu P, Mu M, Wang R, Yang P, et al. Clinical features of 85 fatal cases of COVID-19 from Wuhan: a retrospective observational study. *Am J Respir Crit Care Med.* (2020) 201:1372–9. doi: 10.1164/rccm.202003-0543OC
18. 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
19. Xu B, Fan CY, Wang AL, Zou YL, Yu YH, He C, et al. Suppressed T cell-mediated immunity in patients with COVID-19: a clinical retrospective study in Wuhan, China. *J Infect.* (2020) 81:e51–60. doi: 10.1016/j.jinf.2020.04.012
20. Chen X, Zhao B, Qu Y, Chen Y, Xiong J, Feng Y, et al. Detectable serum SARS-CoV-2 viral load (RNAaemia) is closely correlated with drastically elevated interleukin 6 (IL-6) level in critically ill COVID-19 patients. *Clin Infect Dis.* (2020) ciaa449. doi: 10.1101/2020.02.29.20029520
21. 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. doi: 10.1016/j.cell.2020.02.052
22. Fan CB, Li K, Ding YH, Lu LW, Wang JQ. ACE2 expression in kidney and testis may cause kidney and testis damage after 2019-nCoV infection. *medRxiv [Preprint].* (2020). doi: 10.1101/2020.02.12.20022418
23. Rajapaksha IG, Gunarathne LS, Asadi K, Cunningham SC, Sharland A, Alexander IE, et al. Liver-targeted angiotensin converting enzyme 2 therapy inhibits chronic biliary fibrosis in multiple drug-resistant gene 2-knockout Mice. *Hepatol Commun.* (2019) 3:1656–73. doi: 10.1002/hep4.1434
24. Minato T, Nirasawa S, Sato T, Yamaguchi T, Hoshizaki M, Inagaki T, et al. B38-CAP is a bacteria-derived ACE2-like enzyme that suppresses hypertension and cardiac dysfunction. *Nat Commun.* (2020) 11:1058. doi: 10.1038/s41467-020-14867-z
25. Fu L, Li XY, Fei J, Xiang Y, Xiang HX, Li MD, et al. Myocardial injury at early stage and its association with death risk of patients with COVID-19: a hospital-based prospective case-cohort study. *Res Square [Preprint].* (2020). doi: 10.21203/rs.3.rs-34902/v1

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OPINION
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Sacubitril/Valsartan: Potential Impact of ARNi "Beyond the Wall" of ACE2 on Treatment and Prognosis of Heart Failure Patients With Coronavirus Disease-19

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Keywords: COVID-19, natriuretic peptide, ARNi, cardiovascular diseases, HFrEF—heart failure with reduced ejection fraction

INTRODUCTION

From the beginning of the SARS-CoV-2 pandemia, the type 2 angiotensin-converting enzyme (ACE2), probably the most "unloved and neglected" member of the renin-angiotensin-aldosterone (RAAS) family, has attracted increasing attention since it has been shown as the cell receptor through which the virus enters into the cells (1).

The physiological action of ACE2, a membrane protein expressed in the heart, lungs, kidneys, liver, and intestine, consists in degrading angiotensin II (Ang II) to angiotensin (1-7), a heptapeptide with a potent vasodilator function through the Mas receptor able to counterbalance the Ang II effects on vasoconstriction, sodium retention, and fibrosis (1). Previous studies have shown that Ang II type 1 receptor (AT1R) blockers (ARBs), ACE inhibitors (ACEI), and mineralocorticoid receptor antagonists (MRA) may up-regulate the expression of ACE2 both in acute and chronic settings of cardiovascular diseases (CVDs), such as hypertension, heart failure (HF) and myocardial infarction (1). These data have generated concern during the early phases of the pandemia, since it has been speculated that the increase in ACE2 level may have contributed to disease virulence and to adverse outcomes particularly in subjects affected by chronic coexisting conditions, namely hypertension, coronary artery disease, HF, and diabetes, who commonly received treatment with RAAS inhibitors and who were characterized by a worse clinical course (2).

On the other hand, it has been observed that the binding between coronavirus and ACE2 leads to ACE2 downregulation, resulting in an unopposed production of Ang II by ACE, contributing to lung damage as a consequence of AT1R mediated inflammation, fibrosis, thrombosis, vasoconstriction, and increased vascular permeability. According to these findings, RAAS inhibitors and, in particular, ARBs may even protect against COVID-19 acute lung injury (1). As a matter of fact, epidemiological studies conducted in large populations of COVID-19 patients demonstrated that ARBs or ACE inhibitors had no association with a severe or fatal course of the disease (3–5).

EVIDENCE SUPPORTING THE POTENTIAL BENEFICIAL ROLE OF ARNi IN HF PATIENTS WITH COVID-19

Natriuretic peptides (NPs), which include atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP), along with their N-terminal counterparts, may play an important protective role in COVID-19 disease. NPs are released as a consequence of increased volume overload and myocytes stress and, through their vasorelaxant, diuretic, and

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effects, are able to counterbalance RAAS and sympathetic nervous system actions, ultimately regulating blood pressure, electrolytes, and water homeostasis (6). At the vascular level, NPs reduce cellular growth and proliferation, preserving endothelial function and integrity as well as vascular tone, and they oppose blood clotting, inflammation, angiogenesis, and atherosclerosis progression (6). Apart from their well-described systemic hemodynamic and autocrine/paracrine functions within the cardiovascular system, NPs also play an important protective role in the lungs. In fact, ANP reduces lung endothelial permeability caused by inflammation and oxidative stress, avoiding the development of acute respiratory distress syndrome and improving arterial oxygenation during mechanical ventilation (7). According to this evidence, it has been proposed that COVID-19 patients with deficiencies in the NP system, mainly obese subjects and black people, may have an increased risk of developing severe lung complications.

Of interest, a bidirectional interaction between NPs, particularly ANP, and ACE2 has been demonstrated in experimental models. ANP, through cyclic guanosine monophosphate (cGMP) production, inhibited the Ang II-mediated activation of the extracellular signal regulated kinase (ERK1/ERK2) pathway and upregulated the mitogen-activated protein kinase phosphatase (MKP1), finally preventing the decrease in ACE2 mRNA synthesis (8). On the other hand, Ang-(1-7), the product of ACE2 activity, stimulated ANP secretion through the Mas receptor/phosphatidylinositol 3-kinase/protein kinase B (Mas/PI3K/Akt) pathway, thus reducing cardiac hypertrophy and fibrosis and potentially avoiding COVID-19 pulmonary damage (8).

Furthermore, consistently with the well-known prognostic role of NPs, it has been demonstrated that NT-proBNP level represents an independent risk factor of in-hospital death in patients with severe COVID-19, its levels being significantly higher among those patients who experienced severe clinical conditions, and increasing further during hospitalization in subjects who died, without significant changes among survivors (9).

Apart from the known pathogenetic, diagnostic, and prognostic implications in the cardiovascular system (10), NPs have relevant therapeutic properties. In this context, a field of great interest may be represented by the potential impact on the clinical course of the COVID-19 disease

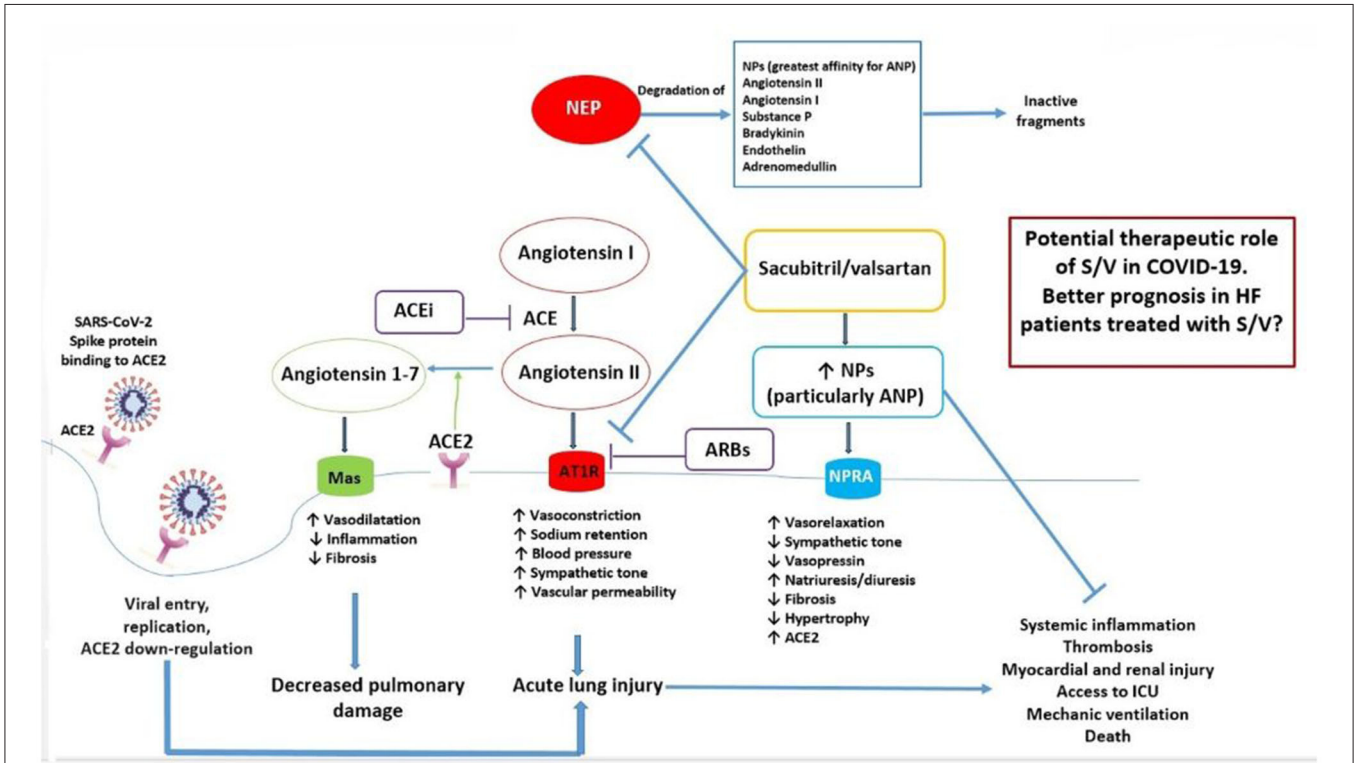

FIGURE 1 | Mechanisms underlying the potential beneficial effects of sacubitril/valsartan in HF patients with COVID-19. Ang 1-7, produced by ACE2 from Ang II, and NPs, particularly ANP, may protect from acute lung injury, systemic inflammation, and adverse outcomes during SARS-CoV-2 infection. On the other hand, local activation of the RAAS system may mediate injury responses to viral insults. S/V inhibits both the AT1R and neprilysin, which degrades NPs. As a consequence, S/V may exert an important protective function from adverse clinical course, mediated by an increase of ANP and by AT1R blockade, in HF patients with COVID-19. ACE, angiotensin converting enzyme; ACE2, type 2 angiotensin converting enzyme; ACE-i, ACE inhibitor; ANP, atrial natriuretic peptide; ARB, angiotensin type I receptor blocker; AT1R, angiotensin type I receptor; HF, heart failure; ICU, intensive care unit; NEP, neprilysin; NPs, natriuretic peptides; NPR-A, natriuretic peptide receptor A; S/V, sacubitril/valsartan.

and on its outcome of a treatment with sacubitril/valsartan (S/V), a member of the new pharmacological class of AT1R/neprilysin inhibitors (ARNi). S/V is now recognized as a cornerstone of the therapeutic management of HF with reduced ejection fraction (HFrEF) due to the impressive benefits on cardiovascular death and HF hospitalization (11).

The beneficial effects of S/V in HFrEF were confirmed in recent real-life clinical studies showing a significant reduction of cardiac death and HF rehospitalization, an improvement of echocardiographic parameters, such as left ventricular EF, systolic volume, and systolic pulmonary arterial pressure, of renal function and of quality of life (12–14). Moreover, S/V treatment can be safely started during hospitalization in daily clinical practice with no evidence of increased risk of hypotension, worsening of renal function and hyperkalaemia (15).

With regard to the trend of different NPs levels after the initiation of S/V, NT-proBNP level decreases as a consequence of the improvement of cardiac function and haemodynamic status, representing a useful biomarker of treatment response; BNP level slightly increases due to its relatively low affinity to neprilysin, whereas ANP level consistently and substantially increases both in human studies and in experimental models, mediating most of the benefits of neprilysin inhibition (16, 17).

According to these evidences, an approach based on early administration of S/V has been proposed in the therapeutic management of all COVID-19 hospitalized patients to avoid an adverse clinical course (18).

PERSPECTIVES

Based on the ability of S/V to increase ANP level while antagonizing the Ang II/AT1R effects, we propose a major protective role of this class of drugs in HFrEF patients, the only current indication for the use of ARNi, when affected by COVID-19 disease (**Figure 1**). In order to test the expected beneficial role of S/V in COVID-19, a retrospective analysis of existing registries of hospitalized COVID-19 patients could help to find out whether, among subjects affected by HFrEF, those who were already treated with S/V presented a lower disease incidence, better prognosis, and clinical course (particularly in terms of intensive care unit access, mechanical ventilation, and death), compared to patients who received other medications, including ACEI/ARBs. Furthermore, a call to action is requested to test the potential benefits of S/V in HFrEF patients affected by COVID-19 through new prospective randomized clinical trials.

AUTHOR CONTRIBUTIONS

SR, GG, and MV contributed to the conception and design, acquisition of data, or analysis and interpretation of data, drafted the article, and approved the final version to be published. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Battistoni A, Volpe M. Might renin-angiotensin system blockers play a role in the COVID-19 pandemic? *Eur Heart J Cardiovasc Pharmacother*. (2020) 6:248–51. doi: 10.1093/ehjcvp/pvaa030
2. Volpe M, Battistoni A, the board of the Italian Society of Cardiovascular Prevention, Bellotti P, Bellone S, Bertolotti M, et al. Recommendations for cardiovascular prevention during the Sars-Cov-2 pandemic: an executive document by the board of the Italian society of cardiovascular prevention. *High Blood Press Cardiovasc Prev*. (2020) 30:1–5. doi: 10.1007/s40292-020-00401-1
3. 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
4. Iaccarino G, Grassi G, Borghi C, Ferri C, Salvetti M, Volpe M, et al. 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
5. Volpe M, Battistoni A. Systematic review of the role of renin-angiotensin system inhibitors in late studies on Covid-19: a new challenge overcome? *Int J Cardiol*. (2020) 321:150–4. doi: 10.1016/j.ijcard.2020.07.041
6. Volpe M, Rubattu S, Burnett J Jr. Natriuretic peptides in cardiovascular diseases: current use and perspectives. *Eur Heart J*. (2014) 35:419–25. doi: 10.1093/eurheartj/eht466
7. Mitaka C, Hirata Y, Nagura T, Tsunoda Y, Amaha K. Beneficial effect of atrial natriuretic peptide on pulmonary gas exchange in patients with acute lung injury. *Chest*. (1998) 114:223–28. doi: 10.1378/chest.114.1.223
8. Gallagher PE, Ferrario CM, Tallant EA. Regulation of ACE2 in cardiac myocytes and fibroblasts. *Am J Physiol Heart Circ Physiol*. (2008) 295:2373–9. doi: 10.1152/ajpheart.00426.2008
9. Gao L, Jiang D, Wen XS, Cheng XC, Sun M, He B, et al. Prognostic value of NT-proBNP in patients with severe COVID-19. *Respir Res*. (2020) 21:83. doi: 10.1186/s12931-020-01352-w
10. Rubattu S, Volpe M. Natriuretic peptides in the cardiovascular system: multifaceted roles in physiology, pathology and therapeutics. *Int J Mol Sci*. (2019) 20:E3991. doi: 10.3390/ijms20163991
11. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, et al. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N Engl J Med*. (2014) 371:993–1004. doi: 10.1056/NEJMoa1409077
12. Polito MV, Silverio A, Rispoli A, Vitulano G, Auria F, De Angelis E, et al. Clinical and echocardiographic benefit of sacubitril/valsartan in a real-world population with HF with reduced ejection fraction. *Sci Rep*. (2020) 10:6665. doi: 10.1038/s41598-020-63801-2
13. Mentz RJ, Xu H, O'Brien EC, Thomas L, Alexy T, Gupta B, et al. PROVIDE-HF primary results: patient-reported outcomes investigation following initiation of drug therapy with entresto (sacubitril/valsartan) in heart failure. *Am Heart J*. (2020) 230:35–43. doi: 10.1016/j.ahj.2020.09.012
14. Spannella F, Marini M, Giulietti F, Rosettani G, Francioni M, Perna GP, et al. Renal effects of sacubitril/valsartan in heart failure with reduced ejection fraction: a real life 1-year follow-up study. *Intern Emerg Med*. (2019) 14:1287–97. doi: 10.1007/s11739-019-02111-6
15. López-Azor JC, Vicent L, Valero-Masa MJ, Esteban-Fernández A, Gómez-Bueno M, Pérez Á, et al. Safety of sacubitril/valsartan initiated during hospitalization: data from a non-selected cohort. *ESC Heart Fail*. (2019) 6:1161–6. doi: 10.1002/ehf2.12527

16. Rubattu S, Cotugno M, Forte M, Stanzione R, Bianchi F, Madonna M, et al. Effects of dual angiotensin type 1 receptor/neprilysin inhibition vs. angiotensin type 1 receptor inhibition on target organ injury in the stroke-prone spontaneously hypertensive rat. *J Hypertens.* (2018) 36:1902–14. doi: 10.1097/HJH.0000000000001762
17. Ibrahim NE, McCarthy CP, Shrestha S, Gaggin HK, Mukai R, Szymonifka J, et al. Effect of neprilysin inhibition on various natriuretic peptide assays. *J Am Coll Cardiol.* (2019) 73:1273–84. doi: 10.1016/j.jacc.2018.12.063
18. Acanfora D, Ciccone MM, Scicchitano P, Acanfora C, Casucci G. Neprilysin inhibitor-angiotensin II receptor blocker combination (sacubitril/valsartan): rationale for adoption in SARS-CoV-2 patients. *Eur Heart J Cardiovasc Pharmacother*. (2020) 6:135–6. doi: 10.1093/ehjcvp/pvaa028
19. Rubattu S, Gallo, Volpe M. Sacubitril/valsartan: potential impact of ARNi "beyond the Wall" of ACE2 on treatment and prognosis of heart failure patients with COVID-19. *Authorea. [Preprint].* (2020). doi: 10.22541/au.160157528.86277450

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ORIGINAL RESEARCH
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Assessment of Clinical Pharmacists' Assistance for Patients With Established Cardiovascular Diseases During the COVID-19 Pandemic: Insights From Southern India

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Objectives: We aimed to assess the clinical pharmacist-initiated telephone-based patient education and self-management support for patients with cardiovascular disease during the nationwide lockdown during COVID-19 pandemic.

Methods: A prospective single-center telephone-based cross-sectional study was conducted among patients at the Cardiology Department and its speciality clinic at a 1,800-bed tertiary care hospital in Southern India. A validated 8-item clinical pharmacist aided on-call questionnaire with two Domains was administered during and after lockdown (15 March and 8 June 2020). Clinical pharmacist-provided educational assistance on self-management practices were in accordance with the guidelines of Indian Council of Medical Research (ICMR) and World Health Organization. Comparisons was performed using sign test and association of responses were analyzed using the Goodman and Kruskal's gamma test. All the tests were two-tailed, $p < 0.05$ was considered to be statistically significant.

Results: Of the 1,080 patients, 907 consented with a response rate of (83.9%) and 574 (96.36%) patients were analyzed post-intervention. Majority of the patients were male (54.7%) and had Acute Coronary Syndrome [NSTEMI (42.10%), STEMI (33.92%) and Unstable Angina (9.86)]. The majority of subjects had at least two co-morbid conditions [(Type II Diabetes (48.33%), Hypertension (50.11%)] and were rural population (82.5%) as self-employed (43.1%) with a middle-class economy (31.6%). In the Domain-1 of checklist the awareness toward complications caused by COVID-19 in cardiovascular diseases ($Z = -19.698$, $p = 0.000$) and the importance of universal safety precautions enhanced after clinical pharmacist assistance [($Z = -8.603$, $p = 0.000$) and ($Z = -21.795$, $p = 0.000$)]. In Domain-II of checklist there was a significant improvement in patients awareness toward fatal complications caused by COVID-19 ($Z = -20.543$, $p = 0.000$), maintenance of self-hygiene ($Z = -19.287$, $p = 0.000$), practice of universal safety precautions ($Z = -16.912$, $p = 0.000$) and self-isolation ($Z = -19.545$, $p = 0.000$). The results of our study population varied from baseline evaluation (41.7%, $n = 907$) to post-intervention (95%, $n = 574$) based on Literacy, employment status and economic status.

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Conclusions: The proactive role of clinical pharmacists in providing instructional services in collaboration with cardiologist during the pandemic circumstances increased patients understanding and mitigated infection exposure among patients, health care professionals and also assuring the continuity of care in patients with established cardiovascular diseases.

Keywords: COVID-19, cardiovascular diseases, clinical pharmacist, SARS-CoV- 2, corona virus 19

INTRODUCTION

In the last two decades, clustering and incidence of severe acute respiratory infections are one of the major threats to public health. Coronavirus disease (COVID-19) was first recorded in Wuhan, China, by the end of December 2019. Since then, COVID-19 has rapidly spread around the world. The COVID-19 was declared as a global pandemic on 11th March 2020 by the World Health Organization. COVID-19 has a major impact on public health and has a direct or indirect impact on social and economic activities. The exponential increase in the number of patients with COVID-19 in the past 6 months has overwhelmed health-care systems across the world. This is due to an inadequate understanding of the dynamic interplay of shifting epidemiology, publicity, pandemic prevention strategies, risk identification, and public health behavior (1). Cardiovascular disease is common comorbidity observed in patients infected with SARS or MERS (10 and 30% prevalence, respectively) (2). Currently, there is no promising evidence from randomized clinical trials (RCTs) that any potential therapy improves outcomes in patients with either suspected or confirmed COVID-19. Neither clinical trial data is supporting any prophylactic therapy.

The pre-existing cardiovascular disease seems to be linked with worse outcomes and increased risk of death in patients with COVID-19. Patients requiring intensive treatment had a significantly higher prevalence of chronic health conditions such as diabetes, cardiovascular and cerebrovascular disease (3). Moreover, COVID-19 itself can cause induce myocardial injury, arrhythmia, acute coronary syndrome and venous thromboembolism (4). Providing clinical care for patients with chronic cardiovascular disease and other comorbidities during pandemic times is challenging. Telehealth is an ideal platform to deliver clinical care during disasters and pandemics. Telemedicine negated the risk of COVID-19 exposure or transmission (5). In India, providing healthcare is a challenge, telemedicine ensures the safety of patients and health workers, especially when there is a risk of infection (6). India's digital health policy advocates the use of digital tools and focuses significantly on the use of telemedicine services, particularly at the grassroots level in the health and wellness Centers, where a mid-level provider/health worker can connect patients to doctors through technology platforms to provide timely and best possible care (7).

Citizens can make informed choices, defend themselves and comply with prescribed practices by focusing on what can be done during COVID-19 and when adequate resources are accessible, easily understood and communicated via reliable and accessible networks (8). Therefore, through collaboration between clinical pharmacist and cardiologist, we aimed to provide educational assistance regarding self-management practices in patients with existing cardiovascular diseases to mitigate exposure to COVID-19 infection.

METHODS

Study Design and Participants

A prospective single-center telephone-based cross-sectional study was conducted among patients at the Cardiology Department and its speciality clinic at a 1,800-bed tertiary care hospital in Southern India serving 37 specialities. A validated 8-item clinical pharmacist aided on-call questionnaire with two Domains (**Table 1**) was administered during and after lockdown (15 March and 8 June 2020). Majority of the participants with acute coronary syndrome were the subset population of an ongoing clinical study and are currently being followed up. Clinical pharmacist-provided educational assistance on self-management practices was in accordance with the guidelines of Indian Council of Medical Research (ICMR) and World Health Organization.

Reliability and Validity of the Questionnaire

Initially, the questionnaire was validated by selected faculty and research team using facial and content validation methods to ensure readability. To assess overall reliability, the internal consistency of individual items in each questionnaire domain was examined by the researchers. The questionnaire consists of two domains and eight questions pertaining to awareness and knowledge of subjects toward COVID-19. Each question consists of two responses which was scored as Yes is 1 and No is 2. The score for the questionnaire range between 8 and 16, for the purpose of identifying the status of awareness and knowledge, participants are divided into high knowledge (8–12) and low knowledge (13–16) categories that has been derived by cumulative score. Finally, the survey questionnaire was administered to patients by a clinical pharmacist to facilitate better understanding. Higher score (>12) for the questionnaire indicates that patients have lack of awareness and knowledge which indicates the need for educational assistance. This telemedicine questionnaire of clinical pharmacists to assess awareness and knowledge regarding COVID-19 for patients with established cardiovascular diseases was self-developed with scoring, there are no references identified to cite this conjecture.

TABLE 1 | 8-item telemedicine questionnaire checklist of clinical pharmacists to assess awareness and knowledge regarding COVID-19 for patients with established cardiovascular diseases.

S no	Questions	Response	Score
Domain-I: Assessment of awareness			
1	Are you aware of the spread and impact of Novel corona virus 2019?	YES	1
		NO	2
2	Are you aware of the complications caused by Novel corona virus among patients with cardiovascular diseases?	YES	1
		NO	2
3	Are you aware of your present and past medical history	YES	1
		NO	2
4	Are you aware of the importance of universal safety precautions to prevent getting infected from Novel corona virus?	YES	1
		NO	2
Domain-II: Assessment of knowledge			
5	Do u know that Novel corona virus cause (SARS-nCoV-19) life threatening fatal complications among patients with cardiovascular complications and other co-morbid conditions?	YES	1
		NO	2
6	Do you know that self-isolation and maintenance of hygiene can aid in preventing infection from Novel corona virus cause (SARS-CoV-2)?	YES	1
		NO	2
7	Do you know how to follow universal safety precautions to prevent getting infected from Novel corona virus?	YES	1
		NO	2
8	Do you know that self-quarantine is a procedure followed by people who are at risk during epidemic?	YES	1
		NO	2

The following questions in the domain-I and II are related to assess awareness and knowledge toward COVID-19 or SARS nCov-II infection in patients with established cardiovascular diseases by a clinical pharmacist through telephone.

This questionnaire is copyrighted and can be used as a tool for patients with established cardiovascular diseases without any changes and other clinical groups (can be modified accordingly) to assess awareness and knowledge about COVID-19 or SARS nCov-II infection.

The score for this questionnaire range between 8 and 16, participants are divided into high knowledge (8–12 score) and low knowledge (9–12) categories that has been derived by cumulative scores. Higher score (>12) for this questionnaire indicates that patients have lack of Awareness and Knowledge which indicates the need for educational assistance.

Sampling Method

This study followed a non-probability sampling method among the target population (subjects with established cardiovascular diseases at a tertiary care hospital).

Outcome

The primary outcome of the study is to identify the impact of the clinical pharmacist-initiated educational guidance on COVID-19 pandemic among patients with established cardiovascular disease. The secondary outcome is to ensure continuity of care and compliance with the prescribed drugs.

Statistical Analysis

Data were entered in MS Office Excel 2019 and analyzed using the IBM SPSS Statistics Version 25. Continuous variables were presented as mean ± standard deviation (SD). Categorical variables were presented as absolute numbers and percentages. Comparisons between baseline and post assistance scores among the individuals were performed using sign test, Association of Responses with socio-demographic variables were analyzed using the Goodman and Kruskal's gamma test. All tests were two-tailed, $p < 0.05$ was considered to be statistically significant.

RESULTS

Of the 1,080 patients contacted by telephone, the response rate at the baseline was 907 (83.9%) and 574 (63.28%) post-intervention. The majority (54.7%) of the study population were male and had at least two co-morbid conditions (44.56%) in the age group (61–80 years) (**Table 2**). The patients in the study had Acute Coronary Syndrome [NSTEMI (42.10%), STEMI (33.92%) and UA (9.86%)] followed by associated comorbidities as described in **Table 3**. The questionnaire developed was administered during and after nationwide lockdown. In the Domain-1 the patients were aware of the spread of COVID-19 ($p = 0.000$) and their current condition ($p = 0.000$). However, majority of them were not aware of the complications caused by COVID-19 among patients with cardiovascular diseases ($Z = -19.698$, $p = 0.000$) and the importance of universal safety precautions, their awareness enhanced after clinical pharmacist assistance [(Yes = 85.01 vs. 98.08%, No = 14.99 vs. 1.92%, Z = −8.603, $p = 0.000$) and (Yes = 11.84 vs. 94.94%, No = 88.15 vs. 5.05%, $Z = -21.795$, $p = 0.000$)]. In Domain-II regarding knowledge aspect majority of the patient's knowledge improved regarding fatal complications caused by COVID 19 (Yes = 22.12 vs. 95.98%, No = 77.87 vs. 4.01%, $Z = -20.543$, $p = 0.000$), the process of self-isolation, maintenance of self-hygiene (Yes

TABLE 2 | Descriptive Summary of Demographics ($N = 574$).

S. no.	Parameter		Summary[#] ($N = 574$)
1.	Age (in years)	21–40	30 (5.32%)
		41–60	254 (44.34%)
		61-80	257 (44.56%)
		81–100	33(5.76%)
2.	Gender	Male	314 (54.7%)
		Female	260 (45.23%)
3.	Literacy	Below high school	206 (36%)
		High school & above	287 (50%)
		Graduate & above	81 (14%)
4.	Economic status	Lower class	62 (10.7%)
		Upper-low class	254 (44.1%)
		Middle class	181 (31.6%)
		Upper class	77 (13.6%)
5.	Employment status	Salaried	111 (19.4%)
		Self-employed	248 (43.1%)
		Homemaker	215 (37.5%)
6.	Marital status	Married	530 (92.2%)
		Divorced/Widowed	51 (8.8%)
7.	Location	Urban	100 (17.5%)
		Rural	473 (82.5%)
8.	Smoking habit	Smokers	123 (21.5%)
		Non-smokers	450 (78.5%)
9.	Alcoholism	Occasional	153 (26.7%)
		Chronic	78 (13.5%)
		Non-alcoholics	401 (69.8%)
10	Time spent on call per patient	22.54 ± 11.23 min[a]	

[#]Data represented as number (proportion), [a]data represented as Mean ± SD, SD: Standard Deviation.

TABLE 3 | Clinical parameters.

S. no.	Parameter		Summary[#] ($N = 574$)
1.	Acute coronary syndrome (ACS)	UA	57 (9.86%)
		NSTEMI	242 (42.10%)
		STEMI	195 (33.92%)
2.	Venous thromboembolism (VTE)	DVT	48 (8.4%)
		PE	33 (5.8%)
3.	T2DM		277 (48.33%)
4.	HTN		288 (50.11%)
5.	Kidney disease		72 (12.56%)
6.	T2DM + HTN		292 (50.86%)
7.	COPD		211 (36.73%)
8.	Depression		22 (3.8%)
9.	Atrial fibrillation		17 (2.9%)

T2DM, Type 2 Diabetes Mellitus; HTN, Hypertension; COPD, Chronic obstructive pulmonary disease; UA, Unstable angina; NSTEMI, non-ST segment elevation myocardial infarction; STEMI, ST-Elevation Myocardial Infarction.
[#]Data represented as number (proportion).

= 33.97 vs. 99.12%, No = 66.02 vs. 0.88%, $Z = -19.287$, $p = 0.000$), the importance of universal safety precaution (Yes = 44.94 vs. 94.94%, No = 55.06 vs. 5.06%, $Z = -16.912$, $p = 0.000$) and regarding self-quarantine (Yes = 25.08 vs. 91.98%, No = 74.91 vs. 8.02%, $Z = -19.545$, $p = 0.000$) depicted in **Table 4**. The individual responses of the patients for every question at baseline was evaluated to correlate the association of sociodemographic variables with awareness and knowledge which demonstrated that the responses of the patients varied based on Literacy, employment status and economic status as represented in **Table 5**.

DISCUSSION

Pandemics and epidemics are a widespread problem then and now as COVID-19. During such periods, people in the community face several challenges. Lack of awareness and consciousness often leads to an uneasy attitude which could adversely affect the patients with established cardiovascular complications. Different stakeholders in their respective countries are working together to "flatten the curve" by joint prevention initiatives led by the WHO. With a practically sufficient global lockdown, Pharmacists appear to be the first contact point for meeting the health requirements of the public (8).

We studied the role of clinical pharmacists' assistance for patients with established cardiovascular diseases during the COVID-19. The principal findings in our study at initial assessment were (1) Most of the patients were aware of their medical condition (CVD and comorbidities), (2) Most of the patients were aware of SARS-CoV-2 (COVID-19) infection, (3) majority of the patients were unaware of fatal complications caused by COVID-19 and association of COVID severity with CVD and comorbidities, (4) most of them were unaware of the importance of universal safety precautions, (5) majority of them don't know that self-quarantine is a procedure followed by people who are at risk during the epidemic.

Pharmacists continue to play their role in promoting continuity of pharmaceutical care, as well as supporting governments for disseminating information on precautions related to COVID-19 spread (13). Pharmacists are an integral part of health care performing exceptional roles in past pandemics and health crises, with some, such as Ebola and Zika, posing global health security risks (9). In this study after assessing the awareness and knowledge we provided educational assistance which helped our patients to gain (1) knowledge regarding fatal complications caused by COVID 19, (2) the process of self-isolation, (3) maintenance of self-hygiene, (4) the importance of universal safety precaution and (5) regarding self-quarantine practice. The Chinese Centre for Disease Control and Prevention recently published the largest COVID-19 case series in mainland China; the overall fatality rate was 2.3% (1,023 deaths among 44,672 confirmed cases), but the mortality rate in

TABLE 4 | Comparison of on call checklist responses before and after the clinical pharmacist assistance/intervention.

S No	Questions	Baseline responses	Post assistance/intervention responses			*Z*-value	*P*-value
			Yes	No	Total		
Domain-I:							
Q1	Are you aware of the spread and impact of Novel corona virus 2019?	Yes	487	0	487	−8.603	0.000*
		No	76	11	87		
		Total	563	11	574		
Q2	Are you aware of the complications caused by Novel corona virus among patients with cardiovascular diseases?	Yes	109	0	109	−19.698	0.000*
		No	390	75	465		
		Total	499	75	574		
Q3	Are you aware of your present and past medical history?	Yes	563	0	563	–	1.000
		No	0	11	11		
		Total	563	11	574		
Q4	Are you aware of the importance of universal safety precautions to prevent getting infected from Novel corona virus?	Yes	68	0	68	−21.795	0.000*
		No	477	29	506		
		Total	545	29	574		
Domain-II:							
Q5	Are you aware of the importance of universal safety precautions to prevent getting infected from Novel corona virus?	Yes	127	0	127	−20.543	0.000*
		No	424	23	447		
		Total	551	23	574		
Q6	Do u know that Novel corona virus cause (SARS-nCoV-19) life threatening fatal complications among patients with cardiovascular complications and other co-morbid conditions?	Yes	195	0	195	−19.287	0.000*
		No	374	5	379		
		Total	569	5	574		
Q7	Do you know how to follow universal safety precautions to prevent getting infected from Novel corona virus?	Yes	257	0	257	−16.912	0.000*
		No	288	29	317		
		Total	545	29	574		
Q8	Do you know that self-quarantine is a procedure followed by people who are at risk during epidemic?	Yes	144	0	144	−19.545	0.000*
		No	384	46	430		
		Total	528	46	574		

**Statistically significant p-value (2-tailed, < 0.05) has been obtained by performing Sign test.*

patients with underlying CVD reached 10.5% (10). However, these results emphasize the potential risk of fatality in our patients with established cardiovascular disease and provide evidence regarding the need for intensive treatment on the infection (11).

The other measure which has been mentioned a lot in recent weeks is hand hygiene. The World Health Organization (WHO) regards handwashing with soap and water and friction with hydroalcoholic gel as the most effective measures for the prevention of infections and antimicrobial resistance (12). Research in major public universities following the H1N1 influenza pandemic reported inadequate compliance with preventive measures, such as residence at home when the virus is ill to prevent transmission in 2009 linked to the results of this study (14). Researchers can work with public agencies/health departments to set up information and awareness centers through participatory groups that may have significant population effects. Our study helped to implement preventive measures, such as isolation, quarantine and community confinement, early identification of cases, social assistance and the provision of patient-specific instructions.

As a consequence of COVID-19, the need for social distancing forced us to use all the resources in our toolbox, and telehealth is one of them that accelerated its adoption globally (15). Telehealth strategies should be encouraged with a view to increasing access and providing care to the patients with chronic diseases to promote continuity of their care which made us adapt the new normal practices. We need to make a conscious effort to avoid any possible worsening of the digital divide, and the government needs to take that responsibility in this case (16).

What was wonderful about it was during this pandemic when our patients are generally considered to be at greater risk of having more severe COVID-19 disease and when they have been asked to stay at home, through virtual

TABLE 5 | Association of baseline responses with socio-demographic variables among study participants.

Question	Response	Literacy					Employment status					Economic status					
		1	2	3	Gamma	*p*-value	1	2	3	Gamma	*p*-value	1	2	3	4	Gamma	*p*-value
Q1	Yes	138	270	79	−0.776	0.000*	83	230	177	−0.043	0.693	55	223	153	56	0.252	0.008*
	No	68	17	2			28	18	38			7	31	28	21		
Q2	Yes	13	70	26	−0.532	0.000*	20	49	40	0.000	0.999	35	208	157	66	−0.329	0.000*
	No	193	217	55			91	199	175			27	46	24	11		
Q3	Yes	198	285	80	−0.556	0.055*	99	233	197	−0.042	0.761	46	246	175	70	−0.310	0.047*
	No	8	2	1			12	15	18			16	8	6	7		
Q4	Yes	4	32	32	−0.767	0.000*	26	20	22	0.266	0.021*	38	227	171	70	−0.465	0.000*
	No	202	255	49			85	228	193			24	27	10	7		
Q5	Yes	21	70	36	−0.520	0.000*	32	41	54	−0.008	0.925	44	38	26	19	0.336	0.000*
	No	185	217	45			79	207	161			18	216	155	58		
Q6	Yes	51	102	42	−0.323	0.000*	24	74	97	−0.337	0.000*	29	101	106	41	−0.196	0.003*
	No	155	185	39			87	174	118			33	153	75	36		
Q7	Yes	67	175	15	−0.076	0.275	47	113	97	−0.025	0.720	26	77	102	52	−0.379	0.000*
	No	137	113	67			64	135	118			36	177	79	25		
Q8	Yes	48	82	14	0.014	0.864	22	63	59	−0.114	0.157	19	53	54	7	0.094	0.222
	No	158	205	67			89	185	156			43	201	127	70		

**Statistically significant p-value has been derived from application of Goodman and Kruskal's gamma test.*

Literacy: 1: Below high school, 2: High school & above, 3: Graduate & above. Employment:1: Salaried, 2: Self-employed, 3: Homemaker. Economic status: 1: Lower class, 2: Upper-Low class, 3: Middle class, 4: Upper class.

visits, we are still able to maintain their continuity of care, evaluate their COVID-19 awareness and knowledge, and provide instructional assistance and mitigate their exposure to infection. Overall, the results reflect what might develop into a new standard of future health care, particularly during contagious outbreaks (17). In this difficult time, hopelessness is the mother of acceptance, virtual practices have become a new normal. But hopefully, as we emerge from this pandemic, the telemedicine infrastructure will remain and benefit those in need.

Strengths and Limitations

During the times of stretched clinical resources due to COVID-19, our research results helped to add new ways to reduce COVID-19 spread in patients with established cardiovascular diseases. Although our study is a single-center study involving a clinical pharmacist, despite its limitations, the results in our study suggest that the extended role of the clinical pharmacist may also be beneficial to other clinical groups. In addition, more awareness amongst the study patients could also be attributed to govt initiated awareness programmes on COVID-19 (18). The results of this study may not be generalizable beyond India due to differences in clinical pharmacist practice worldwide.

CONCLUSIONS

The clinical pharmacist may, however, play a pro-active role in promoting patient-specific treatment decisions by serving as a resource for physicians and other health care professionals to mitigate adverse events caused by SARS nCoV-2 infection in patients with established cardiovascular disease. The enhanced role of clinical pharmacists in providing instructional services should mitigate infection transmission during the COVID-19 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 institutional ethics committee has approved this study and participants have been informed of the purpose of the study before participating and voluntary consent is obtained virtually. All procedures performed in this study involving human participants were consistent with the Declaration of Helsinki 1964 and its subsequent amendments or comparable ethical standards.

AUTHOR CONTRIBUTIONS

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

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REFERENCES

1. Taggart J, Williams A, Dennis S, Newall A, Shortus T, Zwar N, et al. A systematic review of interventions in primary care to improve health literacy for chronic disease behavioral risk factors. *BMC Family Pract*. (2012) 13:49. doi: 10.1186/1471-2296-13-49
2. 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
3. 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
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) 20:1–6. doi: 10.1038/s41569-020-0413-9
5. Monaghesh E, Hajizadeh A. The role of telehealth during COVID-19 outbreak: a systematic review based on current evidence. *BMC Pub Health*. (2020) 20:1193. doi: 10.1186/s12889-020-09301-4
6. Shadmi E, Chen Y, Dourado I, Faran-Perach I, Furler J, Hangoma P, et al. Health equity and COVID-19: global perspectives. *Int J Equity Health*. (2020) 19:1–6. doi: 10.1186/s12939-020-01218-z
7. *Telemedicine Practice Guidelines*. Available online at: https://www.mohfw.gov.in/pdf/Telemedicine.pdf (accessed April 16, 2020).
8. Okan O, Bollweg TM, Berens EM, Hurrelmann K, Bauer U, Schaeffer D. Coronavirus-related health literacy: a cross-sectional study in adults during the COVID-19 infodemic in Germany. *Int J Environ Res Pub Health*. (2020) 17:5503. doi: 10.3390/ijerph17155503
9. Li H, Zheng S, Liu F, Liu W, Zhao R. Fighting against COVID-19: innovative strategies for clinical pharmacists. *Res Soc Administ Pharm*. (2020). doi: 10.1016/j.sapharm.2020.04.003
10. 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
11. 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
12. Safety WP, World Health Organization. *WHO Guidelines on Hand Hygiene in Health Care*. Geneva: World Health Organization (2009).
13. Bukhari N, Rasheed H, Nayyer B, Babar ZU. Pharmacists at the frontline beating the COVID-19 pandemic. *J Pharmaceut Policy Practice*. (2020) 13:8. doi: 10.1186/s40545-020-00210-w
14. Morens DM, Taubenberger JK, Harvey HA, Memoli MJ. The 1918 influenza pandemic: lessons for 2009 and the future. *Crit Care Med*. (2010) 38(Suppl. 4):e10. doi: 10.1097/CCM.0b013e3181ceb25b
15. Fisk M, Livingstone A, Pit SW. Telehealth in the context of COVID-19: Changing Perspectives in Australia, the United Kingdom, and the

United States. *J Med Internet Res*. (2020) 22:e19264. doi: 10.2196/19264
16. Ramsetty A, Adams C. Impact of the digital divide in the age of COVID-19. *J Am Med Inform Associat*. (2020) 27:1147–8. doi: 10.1093/jamia/ocaa078
17. Tahan HM. Essential case management practices amidst the novel coronavirus disease 2019 (COVID-19) crisis: part 2: end-of-life care, workers' compensation case management, legal and ethical obligations, remote practice, and resilience. *Prof Case Manage*. (2020). doi: 10.1097/NCM.0000000000000455
18. World Health Organization. *Coronavirus Disease 2019 (COVID-19) Situation Report* (2020). p. 72.

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Pandemic Perspective: Commonalities Between COVID-19 and Cardio-Oncology

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Overlapping commonalities between coronavirus disease of 2019 (COVID-19) and cardio-oncology regarding cardiovascular toxicities (CVT), pathophysiology, and pharmacology are special topics emerging during the pandemic. In this perspective, we consider an array of CVT common to both COVID-19 and cardio-oncology, including cardiomyopathy, ischemia, conduction abnormalities, myopericarditis, and right ventricular (RV) failure. We also emphasize the higher risk of severe COVID-19 illness in patients with cardiovascular disease (CVD) or its risk factors or cancer. We explore commonalities in the underlying pathophysiology observed in COVID-19 and cardio-oncology, including inflammation, cytokine release, the renin-angiotensin-aldosterone-system, coagulopathy, microthrombosis, and endothelial dysfunction. In addition, we examine common pharmacologic management strategies that have been elucidated for CVT from COVID-19 and various cancer therapies. The use of corticosteroids, as well as antibodies and inhibitors of various molecules mediating inflammation and cytokine release syndrome, are discussed. The impact of angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) is also addressed, since these drugs are used in cardio-oncology and have received considerable attention during the COVID-19 pandemic, since the culprit virus enters human cells *via* the angiotensin converting enzyme 2 (ACE2)

receptor. There are therefore several areas of overlap, similarity, and interaction in the toxicity, pathophysiology, and pharmacology profiles in COVID-19 and cardio-oncology syndromes. Learning more about either will likely provide some level of insight into both. We discuss each of these topics in this viewpoint, as well as what we foresee as evolving future directions to consider in cardio-oncology during the pandemic and beyond. Finally, we highlight commonalities in health disparities in COVID-19 and cardio-oncology and encourage continued development and implementation of innovative solutions to improve equity in health and healing.

Keywords: cardio-oncology, COVID-19, pandemic, telemedicine, inflammation, cytokine release syndrome, right ventricle, health disparities

INTRODUCTION

In early 2020, the World Health Organization (WHO) designated the new, highly contagious, and unnervingly fatal disease COVID-19 caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) a global pandemic. By June 1, 2020, the WHO reported more than 6 million confirmed cases and 370,000 deaths across nearly 220 countries and territories, with the US having the highest number of confirmed cases (1.7 million) and deaths (100,000) (1).

Although initially thought to be primarily a lung disease, COVID-19 also involves marked toxicity to the cardiovascular system. As data has emerged, it has become clear to our cardio-oncology group (2–7) that much of the cardiovascular toxicity reported in COVID-19 is also observed in cardio-oncology, with overlap in underlying pathophysiology. Additionally, pharmacologic options frequently used or currently being studied in cardio-oncology are also proving beneficial in COVID-19. This begs the question of whether evaluating commonalities in the toxicities, pathophysiology, and pharmacology of COVID-19 and cardio-oncology would be informative for advancing understanding and avenues for research in cardio-oncology, as well as COVID-19. Cardio-oncology is an emerging field in medicine focused on the prevention, surveillance, detection, and management of injury to the cardiovascular system from cancer therapies or from cancer itself. The cardiovascular injuries are inflicted by an exogenous source, primarily pharmacologic or radiologic cancer therapy. In COVID-19, the cardiovascular injuries are also incited by an exogenous source, primarily SARS-CoV-2. Due to the exogenous nature of the original source of injury, in addition to pathophysiology mediating the injury, some authors refer to these cardiovascular injuries in COVID-19 as "toxicities" (8–10), which is also the term conventionally used in cardio-oncology (11–13). While cancer therapies and SARS-CoV-2 are two very different entities, the havoc they both wreak on the cardiovascular system is thought-provoking.

In this perspective, we share the overarching viewpoint that these commonalities exist and are intriguing, and consequently, the dynamic research efforts surrounding COVID-19 may be able to inform new understanding and avenues for investigation in cardio-oncology. A clear understanding of the mechanisms of various forms of CVT in cardio-oncology remains elusive. Development of novel concepts, paradigms, and drug utilization trends based on observations identified in CVT related to COVID-19 may help advance research and clinical practice in cardio-oncology. To this end, we first present cardiovascular toxicities common to COVID-19 and cardio-oncology, then we expound on underlying pathophysiology. This is followed by description of pharmacologic options being pursued in both COVID-19 and cardio-oncology. Finally, we discuss ramifications of these commonalities in the context of Cardio-Oncologic care and research in the pandemic and beyond (**Table 1**).

COMMON TOXICITIES IN COVID-19 AND CARDIO-ONCOLOGY

CVT in COVID-19 and Cardio-Oncology

In COVID-19, SAR-CoV-2 causes direct and indirect cardiovascular injury, which typically manifests as cardiomyopathy, myopericarditis, ischemia, or arrythmia (14–25). SARS-CoV-2 has been discovered in cardiac tissue (15, 25), similar to SARS-CoV-1 infection in which 35% of patients had viral RNA expressed in cardiac tissue (26). Patients with pre-existing CVD and cancer or CVD risk factors (e.g., diabetes mellitus, chronic kidney disease, obesity, and advanced age) are among those at highest risk of poor outcomes, i.e., increased morbidity and mortality from COVID-19 (10, 22, 27–30). According to a retrospective analysis of 72,314 cases in China, patients with pre-existing CVD morbidities had a five-fold increase in mortality, and a COVID-19-related death rate of 10.5% (22). Indirectly, patients with CVD morbidities are inherently more susceptible to the adverse effects of viral infection and the body's adaptive response. The systemic effects of COVID-19 causing fever, hypoxia, hypotension, and tachycardia may not be well-tolerated in patients with underlying cardiomyopathy or obstructive coronary artery disease, and this may manifest as further myocardial injury, and increased incidence of decompensated heart failure and type II myocardial infarction (20). Evidence of myocardial injury (e.g., elevated troponin), is common in patients hospitalized with COVID-19 (10). When present, elevated cardiac biomarkers such as brain natriuretic peptide and serum troponin have been associated with increased mortality in patients with COVID-19 (18).

TABLE 1 | Mechanisms, concepts, and paradigms: commonalities in toxicity, pathophysiology, and pharmacology of cardio-oncology and COVID-19.

Common topic of study	Cardio-oncology	COVID-19
Mechanisms of left ventricular cardiomyopathy	Elucidate mechanisms and optimal management of left ventricular systolic dysfunction in Cardio-Oncology	Elucidate mechanisms and optimal management of left ventricular systolic dysfunction in COVID-19
Immune system activation	Analyze pathophysiology and optimal management of immune response, cytokine release syndrome, and autoimmune adverse effects from ICIs or CAR-T cell therapy	Analyze pathophysiology and optimal management of immune response, cytokine release syndrome, and related adverse effects in COVID-19
Long-term sequelae of inflammation	Investigate long-term implications of inflammation induced by neoplastic agents	Investigate long-term implications of myocardial inflammation in COVID-19
Endothelial dysfunction	Interrogate role of endothelial dysfunction in ischemic and cardiomyopathic cardiovascular injuries from cancer drugs	Interrogate role of endothelial dysfunction in ischemic and cardiomyopathic cardiovascular injuries from COVID-19
Coagulopathy and anticoagulation	Study the burden, mechanisms, and optimal management of coagulopathy (arterial or venous) with need for anticoagulation or antiplatelet therapy in Cardio-Oncology	Study the burden, mechanisms, and optimal management of coagulopathy and microthrombosis with beneficial response to anticoagulation in COVID-19
Role of RV and RVAD	Explore significance of RV systolic dysfunction after anthracycline therapy	Explore significance of RV systolic dysfunction in severe COVID-19 infection
Prognostic value of RV strain	Evaluate utility of RV strain to predict outcomes following anthracycline therapy	Evaluate utility of RV strain to predict COVID-19 severity/mortality
Utility of steroid therapy and biologics	Determine the effectiveness and timing of steroid treatment and monoclonal antibodies for inflammation- or immune-related adverse events from ICIs or CAR-T cells	Determine the effectiveness and timing of steroid treatment and monoclonal antibodies for inflammation-related adverse CV events in COVID-19
Neurohormonal therapy	Establish cardioprotective contributions of neurohormonal therapies	Establish whether neurohormonal therapies are protective in COVID-19
Potential drug Interactions	Appraise the extent and impact of potential drug interactions between Cardiology drugs and Oncology drugs	Appraise the extent and impact of potential drug interactions between Cardiology drugs and COVID-19 drugs
Impact of health disparities	Assess underlying factors and solutions to address health disparities in cardiovascular toxicities observed in Cardio-Oncology	Assess underlying factors and solutions to address health disparities observed in cardiovascular injuries in COVID-19
Precision of risk prediction	Develop precise methods of predicting cardiovascular toxicities and prognosis	Develop precise methods of predicting risk and overall prognosis in COVID-19

CAR-T Cells, Chimeric Antigen Receptor T-Cells; COVID-19, Coronavirus Diseases of 2019; CV, cardiovascular; ICI, Immune Checkpoint Inhibitor; RV, Right Ventricle; RVAD, Right Ventricular Assist Device.

Similarly, a wide spectrum of cancer therapies has been associated with CVT, such as cardiomyopathy, myopericarditis, ischemia, and arrhythmias (11, 31). Radiation therapy can lead to all of these toxicities in the absence of chemotherapy. Various chemotherapy and targeted cancer therapy regimens can also result in CVT. Anthracyclines most commonly associate with cardiomyopathy, and can also bring about conduction abnormalities, myocarditis, or pericardial disease. Tyrosine kinase inhibitors commonly associate with hypertension, and less commonly with cardiomyopathy or ischemia. Immune checkpoint inhibitors are most notorious for myocarditis, and can also prompt pericarditis, cardiomyopathy, conduction abnormalities, and ischemia. Many other CVT are noted in cardio-oncology, with a variety of drug classes. In addition, tachycardia and elevated biomarkers may also portend poor prognosis in cardio-oncology (32, 33). There is therefore much overlap of CVT and prognostic factors in cardio-oncology with CVT and prognostic biomarkers in COVID-19. Furthermore, some types of cancers and cancer treatments weaken patients' immune systems and increase risk of any infection. Cancer patients' immunosuppression often also associates with blunted or delayed symptoms, which could in turn delay urgent therapy and increase mortality in COVID-19.

Interesting to consider is any potential synergistic CVT in patients on cancer therapies in COVID-19. A prospective cohort of 800 cancer patients with COVID-19 analyzed in late April 2020 linked COVID-19 mortality with older age, male gender and comorbidities such as hypertension and cardiovascular disease (14). There was no association between receipt of cytotoxic chemotherapy, targeted therapies, radiation therapy, or other cancer therapies and COVID-19 mortality in this cohort. Similarly, a retrospective cohort of 928 cancer patients from the USA, Canada and Spain associated advanced age, smoking, progressive malignancy and increased comorbidities COVID-19 mortality, but failed to show associations with cancer type and type of anticancer therapy with COVID-19 mortality (34).Thus,

the role of chemotherapy and other cancer systemic therapies in COVID-19 mortality remains uncertain.

Emerging Role of Right Ventricular Failure

Recent studies emerging in parallel in the pandemic and in cardio-oncology indicate that the RV may play an important role in the prognosis of patients with COVID-19 or CVT from cancer therapy; RV failure generally associates with worse outcomes in a variety of populations, and patients with COVID-19 or CVT from cancer therapies may be no different (35–38). While the mechanisms of insult to the RV in COVID-19 are different from those in cardio-oncology, similar changes are noted in the ventricle and these may have prognostic value. Importantly, RV longitudinal strain (RVLS) has emerged as a key player in the prediction of RV failure in both COVID-19 and cardio-oncology (36, 38).

In COVID-19, RVLS inversely associates with myocardial injury, mechanical ventilation, acute respiratory distress syndrome (ARDS), and mortality, as well as signs of systemic inflammation such as heart rate, D-dimer, and C-reactive protein, as well as thromboembolism (38). In addition to RVLS, RV dilation and systolic dysfunction also predict mortality in COVID19 (39). Abnormalities in RV strain, size, and systolic function in COVID-19 may result from ARDS, pulmonary hypertension with increased pulmonary vascular resistance due to acute lung injury or thromboembolism, in addition to CO_2 retention, positive pressure ventilation, or other causes of acute myocardial injury (19, 40–46). In one COVID-19 study, of 10 patients with RV dilation, 50% had PE noted on CTA; and of 21 total deaths in that COVID-19 cohort, 62% had RV dilation (39). Some patients with apparent ARDS do not respond as expected to low pressure ventilation strategies per ARDSNet ventilation protocols (47). Prone positioning in COVID-19 improves oxygenation and reduces the risk and need for mechanical ventilation or extracorporeal membrane oxygenation (ECMO) in patients on mechanical ventilation, but the maneuver appears to also reduce the risk of RV failure in ARDS including COVID-19 (48–50). Anecdotally, we have observed that the typical progression from hypoxemic respiratory failure to multi-system organ failure with escalating pressor requirements can be blunted with insertion of a percutaneous right ventricular assist device (RVAD) connected to an oxygenator. In all cases, the pressor requirement has been eliminated upon initiation of RVAD flows. These observations are consistent with our experiences in using these devices to treat other forms of RV failure which are frequently misdiagnosed as distributive shock.

In cardio-oncology, changes are also noted in RV strain, structure, function, and size in patients with breast cancer and lymphoma who receive anthracycline chemotherapy (36, 51). Although the left ventricle is more commonly studied, the RV also shows impairment in contractility, with temporal changes of decreased RVLS and increased right ventricular end systolic volume (RVESV) preceding reduction in right ventricular ejection fraction (RVEF) (36). Additionally, patients with end-stage heart failure as a result of cardiomyopathy from anthracycline therapy benefit from RV assist device support (52). The underlying pathophysiology of RV dysfunction in anthracycline CVT is likely similar to LV dysfunction. LV dysfunction results from release of cytokines and inflammatory markers, related to generation of reactive oxygen species, disruption of mitochondrial biogenesis, and activation of apoptosis, and double-stranded DNA breaks (53–55). This is a recent and novel area of inquiry in cardio-oncology. Additional studies are needed to determine whether RV size, function, and longitudinal strain can predict CVT and mortality in cardio-oncology (36), as has been found in COVID-19.

Health Disparities in CVT

A multi-ethnic study of more than 3,500 individuals with COVID-19 was published in the New England Journal of Medicine (NEJM) (56). While <40% of patients in the study were hospitalized, African Americans composed almost 80% of inpatients admitted with COVID-19 and associated CVT. A higher rate of comorbidities associated with the risk for hospitalization, and African Americans had higher rates of comorbidities. This is similar to general trends in health disparities, in which African Americans have higher rates of CVD, obesity, hypertension, and diabetes than Caucasians (57), and are therefore at higher risk for CVT related to COVID-19. These disparities were found to associate with inequities in socioeconomic demographics in the NEJM report, as in prior studies (56, 57). Notably, ACE (I/D) polymorphisms have been implicated in COVID-19 and related CVT, and vary across racial groups (58). However, this alone does not explain the disparities observed in COVID-19. The D/D polymorphism that associates with the development and severity of sarcoidosis (59), which is more prevalent, complex, and mortal in African-Americans (60), is the same polymorphism that is suggested to associate with protection in COVID-19 (61–63). Nevertheless, African Americans have had the highest proportions of severe and fatal illness from COVID-19 and consequent CVT. In the same way, CVT in cardio-oncology has been reported at higher rates in African Americans, with similar underlying reasons (64–68).

Implications of Common Toxicities

It is worth continuing to study shared toxicities in COVID-19 and cardio-oncology. For example, increased attention to emerging special topics such as RV strain, function, and predictive value in COVID-19 may help elucidate sequelae of commonalities to optimize care and survival of our patients in COVID-19 and also in cardio-oncology (**Figure 1**). Perhaps studying the pathophysiology and host characteristics in patients with abnormal RV size, function, and longitudinal strain in COVID-19 could also help us better understand the pathophysiology of abnormal RV size, function, and longitudinal strain in some patients after anthracycline therapy. Further, it will be important to address the disproportionate percentages of African-Americans with severe and fatal CVT related to both COVID-19 (69–73) and cancer therapies in cardio-oncology (64–68).

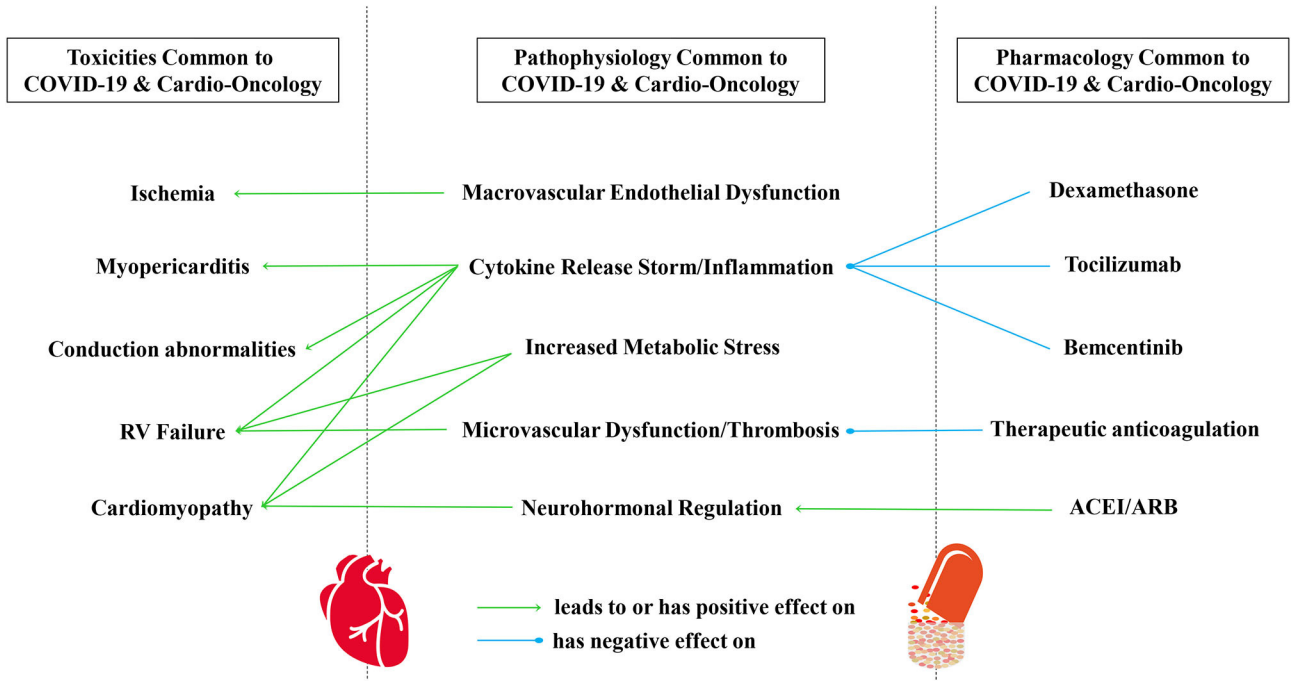

FIGURE 1 | A conceptual framework of commonalities in CV toxicities, pathophysiology, and pharmacology common to COVID-19 and cardio-oncology. CV toxicities common to COVID-19 and cardio-oncology include myocardial injury, cardiomyopathy, myopericarditis, ischemia, conduction abnormalities, and RV failure, in part mediated by immune system activation, cytokine release syndrome, and arterial and venous coagulopathy. All of these are also examples of oncologic CV toxicities that can result from pharmacologic or radiation therapies. Indeed, the pathophysiology and modulation of SARS-CoV-2 infection remains under investigation, with components of viral infection/invasion, macrovascular endothelial dysfunction, cytokine release syndrome/inflammation, microvascular dysfunction/thrombosis, neurohormonal regulation, coagulopathy, and increased metabolic stress. Several pharmacologic considerations have risen to the surface during the pandemic, involving steroids, cancer immunotherapy, biologic antibodies and inhibitors, drug repurposing, the role of cyp450 and drug transporters in drug-drug interactions, anticoagulation, and neurohormonal regulation. ARB, Angiotensin Receptor Blocker; ACEI, Angiotensin Converting Enzyme Inhibitor; CV, Cardiovascular; COVID-19, Coronavirus Disease of 2019; RV, Right Ventricle; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus.

COMMON PATHOPHYSIOLOGY IN COVID-19 AND CARDIO-ONCOLOGY

Pathophysiology of COVID-19 and Cardio-Oncology

Potential mechanisms of cardiovascular injury in COVID-19 include hemodynamic derangement or hypoxemia, increased metabolic stress, demand ischemia, microvascular dysfunction or thrombosis due to hypercoagulability, or systemic inflammation and cytokine storm, which may also destabilize existing coronary artery plaques (16, 74–76). Although not yet demonstrated with SARS-CoV-2, an autopsy study of people who died from SARS-CoV-1 infection demonstrated that 35% of patients had viral RNA expressed in cardiac tissue (26). Further, a recent study illustrated *in vitro* direct infection of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) by SARS-CoV-2 (77). Microscopy and RNA-sequencing provided evidence that SARS-CoV-2 enters hiPSC-CMs *via* the cell surface receptor ACE2. The study also demonstrated that in response to SARS-CoV-2 infection, the hiPSC-CMs upregulated the innate immune response and antiviral clearance gene pathways, in addition to downregulating ACE2 expression.

ACE2 receptors are the SARS-CoV-2 entry point into human cells (10, 78). Patients with pre-existing CVD or CV risk factors, which associate with heightened systemic inflammation, have higher levels of ACE2 receptor expression than the general population (10, 79, 80). In normal physiology, ACE2 is counter-regulatory and anti-inflammatory (79, 80). Interestingly, a particular angiotensin converting enzyme (ACE) genetic polymorphism (D/D), although not a ACE2 polymorphism, associates with decreased ACE2 levels and has been suggested to be protective in patients with COVID-19 (61–63). The physiologic effects of ACE and ACE2 are typically in some degree of homeostatic equilibrium, with ACE mediating inflammation, oxidative stress, and vasoconstriction, and ACE2 also being vasodilatory (81). SARS-CoV-2 may remove ACE2 from this homeostatic pathway due to both the virus and the receptor being internalized from the cell surface in COVID-19 (81).

The inflammatory response elicited by SARS-CoV-2 is implicated in direct suppression of cardiac contractility (75). Evidence of new contractile dysfunction was reported in ∼30% of patients with critical illness related to COVID-19, and cardiac or circulatory shock is a common pathway to fatal outcomes (82, 83). This is reminiscent of CVT in cardio-oncology, in which increased metabolic stress, cytokine

release, inflammation, macrovascular endothelial dysfunction, microvascular dysfunction, thrombosis, and neurohormonal dysregulation can all result in impairment of cardiac contractility underlying cardiomyopathy.

Immune System Activation

Two recent studies evaluating immunologic characteristics of peripheral blood samples from COVID-19 patients have emerged from China (84, 85). In these studies, severe cases of COVID-19 were associated with depletion of CD8+ T-cells, suggesting that upregulation of immune checkpoint molecules that downregulate T-cells may play an important role in impairing the immune response to the virus. These early studies should be interpreted with caution given the small sample sizes, and continued investigation will shed light on the mechanisms of immune dysregulation induced by COVID-19.

Immune checkpoint inhibitors (ICIs) are drugs that target immune checkpoint molecules such as programmed death 1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). These drugs have dramatically improved overall survival for patients with a wide range of malignancies (86). Inflammatory cytokines, such as interferon-γ and type I interferons, induce PD-L1 expression on immune and tumor cells (87). Interaction of the PD-L1 and PD-1 proteins leads to T-cell exhaustion, and blockade of this interaction with PD-1/PD-L1 inhibitors restores effector function to CD8+ T-cells, allowing for destruction of malignant cells. Chief among concerns with ICIs during the pandemic is whether ICIs can increase COVID-19-related complications, particularly CVT. A retrospective study found patients receiving ICIs to be at higher risk of hospitalization and severe outcomes from COVID-19 (88). Strong conclusions are difficult to draw from this small, retrospective, single-center study in which only 31 patients received ICIs. A prospective observational study from the UK Coronavirus Center Monitoring Project found no association between COVID-19 mortality and ICI treatment in the 44 patients who received ICIs (89). Ongoing large-scale prospective data may shed further light on this interaction.

Many cancer patients receiving ICIs possess comorbidities that enhance risk for poor outcomes related to COVID-19. ICIs and COVID-19 can cause overlapping organ toxicities, particularly pulmonary and cardiac, which inform risk-benefit decisions on ICI use during the pandemic. ICIs can induce immune-mediated cardiotoxicity, including myocarditis, pericarditis, heart failure, arrhythmias, and MI. These events are uncommon, occurring in <3% of patients who receive ICIs, but carry high risk of mortality (90).

The pathophysiology of the immunologic mechanisms of cardiotoxicity with ICIs and COVID-19 likely differ, but macrophages may play roles in both pathways, which could contribute to anecdotal response to glucocorticoid responsiveness for ICI and COVID-19 toxicities (**Table 2**). The renin-angiotensin system has been implicated in the pathophysiology of both COVID-19 and tumorigenesis, with data suggesting the RAAS pathway promotes an immunosuppressive tumor microenvironment (91–93). However, much like COVID-19, the impact of ACE inhibitors on survival outcomes with ICIs is currently unclear (94). Given the prevalence of ICI use, it is essential to exert a coordinated effort to track COVID-19 incidence in patients receiving ICIs, as well as rates of pulmonary and cardiac sequelae and mortality to truly understand the long-term impact of the virus on this large population.

TABLE 2 | Clinical characteristics of similar CV toxicity in ICI therapy, CAR T-cell therapy, and COVID-19.

	ICI and CV toxicity	CAR-T cell and CV toxicity	COVID-19 and CV toxicity
Incidence	<1%	NA/unknown	NA/unknown
Pathophysiology	T-cell mediated	Cytokine storm, high IL-6	Hypoxia, Cytokine storm
Risk of VTE	High	High	High
Management	Steroid	Tocilizumab, and/or steroid	Supportive management, +/− dexamethasone
ACEI/ARB continue	Yes	Yes	Yes
Long-term CV effect	Unknown	Unknown	Unknown

ACEI, ACE Inhibitor; ARB, Angiotensin Receptor Blocker; CAR-T Cells, Chimeric Antigen Receptor T-Cells; CRS, Cytokine Release Syndrome; CV, Cardiovascular; COVID-19, Coronavirus Diseases of 2019; ICI, Immune Checkpoint Inhibitor; IL-6, Interleukin 6; NA, Not Applicable; VTE, Venous Thromboembolism.

Cytokine Release Syndrome

In COVID-19, the inflammatory cytokine IL-6 has also been shown to play a role in critically ill patients, in whom "cytokine release storm" or "cytokine release syndrome" (CRS) pathophysiology leads to cardiopulmonary complications and multisystem failure (95). Clinical manifestations of CRS include fever, chills, fatigue, myalgias, arthralgias, nausea, vomiting, and diarrhea (96). In the patient with CRS, cardiovascular manifestations include tachycardia, hypotension, elevated troponin, heart failure, and in severe cases, cardiogenic shock (96, 97). IL-6 could possibly mediate cardiac dysfunction and hemodynamic instability (98). In general, IL-6 elevation has associated with cardiovascular complications such as atherosclerosis, MI, and heart failure.

IL-6 and other cytokines are key components of the human body host defense system against infection, yet high levels of these cytokines in a hyperinflammatory response can lead to CRS (99, 100). Cytokine release syndrome can be a fatal complication due to exaggerated inflammatory response in COVID-19, partially mediated by immune cells fighting the viral infection by increasing inflammatory cytokines *via* activation of intracellular NF-κB (101), but also in large part mediated by the ACE2 and AT1 receptors, which are generally highly expressed on epithelial cells in the lung and endothelium (20, 102–104). A main function of ACE2 is to convert angiotensin II (Ang II) into angiotensin-(1-7), a counter-regulatory peptide that dampens the inflammatory effects of Ang

II *via* AT1 (101, 105). After the SARS-CoV-2 S-protein attaches to ACE2 on respiratory epithelium, ACE2 is down-regulated (77). The resulting SARS-CoV-2-mediated imbalance of serum Ang II/angiotensin-(1-7) drives net activation of AT1 signaling [which is dependent on serum Ang II/angiotensin-(1-7)] in pulmonary epithelial cells. It is well-established that COVID-19 infection causes hyperactivation of the angiotensin 1 receptor (AT1), which leads to disproportionate activation of nucleotide-binding domain-like receptor protein 3 (NLRP3) inflammasome in lung epithelial cells and endothelium (106, 107), as well as activation of STAT3 and the NF-κB pathway, producing potent pro-inflammatory cytokines IL-6, IL-1β, and IL-18 (101, 108–110). The detrimental pathophysiological consequence of the hyperinflammatory response includes enhanced activation of reactive oxygen species (ROS) release, fibrosis, vasoconstriction, and programmed cell death, that contribute to the CRS pathophysiology. Interestingly, ACE2 and AT1 are known to be expressed at extremely low levels on hematopoietic stem cells (HSC) and endothelial progenitor cells (EPC) (111). A recent study demonstrated for the first time that ACE2 is expressed on very small embryonic-like stem cells (VSELs) (112). Pre-clinical data demonstrated that interaction of ACE2 receptor with the COVID-19 spike protein activated the NLRP3 inflammasome in VSELs and HSC leading to programmed cell death (112); the contribution of this to CRS is yet unclear.

Unlike traditional chemotherapy, CAR-T cell therapy is a novel form of immunotherapy in cardio-oncology to treat individuals with refractory hematologic malignancies, and is commonly associated with toxicity related to CRS. CAR-T cell therapy utilizes genetically engineered T-cells to attack cancer cells (113). The activation of CAR-T cells when engaged with antigen in a malignant cell leads to its CAR-T cell proliferation, which further activates monocytes and macrophages, leading to release of proinflammatory cytokines and chemokines such as IL-6, IL-8, IL-10, interferon-gamma (INF-y), monocyte chemoattractant protein-1b, and granulocyte-macrophage colony-stimulating factor (114, 115). These proinflammatory cytokines are potential mediators for CRS in patients with cancer, with a similar cascade in patients with COVID-19.

Coagulopathy

Arterial and venous coagulopathy has emerged as an important factor in COVID-19 pathophysiology and cardio-oncology, especially in critically ill patients (116–120), in part related to underlying endothelial cell dysfunction and inflammation in patients with COVID-19 or cancer (117, 121, 122).

Severe COVID-19 infection requiring critical care admission has been associated with increased incidence of venous thromboembolism (VTE) (117, 123), due to hyperinflammation and a hypercoagulable state (124, 125). The incidence has been reported to be 3 to 4-fold greater than in the general population (117, 123). In critically ill patients in the general population, the cumulative incidence of VTE is around 9.6% (126, 127), while in COVID-19 patients it is reported to be between 31 and 42% (117, 123). Thrombotic events in COVID-19 mostly categorize VTE, but in some patients, a significant number of arterial thrombosis are also being reported. In one study, 3.7% of the 31% reported cases had ischemic strokes, while in another study population two ischemic strokes and one limb ischemia were reported (117, 123). Endotheliitis with underlying hyperinflammation, along with hypoxia leading to increased blood viscosity, are suspected to cause increased coagulopathy in severe COVID-19 infection (128, 129). Excess cytokine release also results in macroscopic or microscopic endothelial injury, leading to a prothrombotic state (130). Elevation of D-dimer above normal values on admission or over time during the disease process has been associated with poor outcomes in patients with severe COVID-19 (125). Close monitoring of D-dimer, aPTT/PT, fibrinogen, and platelet count in hospitalized COVID-19 patients is recommended as derangement of these coagulation parameters can be an early sign of disseminated intravascular coagulation (DIC) (125).

A similar phenomenon is observed in cancer patients (131). Similar factors are associated with thrombosis, with circulating microparticles, procoagulants, and endothelial dysfunction contributing to disruption of normal blood flow and hyperviscosity (120, 132, 133). Cancer also poses a 4 times increased risk of VTE as compared to general population while chemotherapy increases the risk to 6.5 times (134). Patients who receive CAR-T cell therapy are also at increased risk for venous thromboembolism, potentially mediated by CRS and high levels of IL-6 (135, 136), in the setting of underlying hypercoagulability due to the presence of the cancer itself. Other pharmacologic cancer therapies can also associate with thrombosis. Cisplatin and tyrosine kinases often lead to coronary or peripheral arterial thrombosis related to endothelial injury, thromboxane synthesis, and platelet activation and aggregation, placing patients at 1.5- to 1.7-fold or as high as 6-fold increased risk of acute coronary syndromes [see review in expert consensus statement (137)].

Endothelial Dysfunction

Furthermore, many chemotherapeutics associate with endothelial dysfunction and consequent ischemia in the absence of thrombosis. In these cases, ischemia is due to vasospasm. This phenomenon can be caused by 5-fluorouracil (5-FU), capecitabine (5-FU pro-drug), paclitaxel, docetaxel, cisplatin (especially when combined with bleomycin or vincristine), cyclophosphamide, and tyrosine kinase inhibitors (e.g., sorafenib and sunitinib) [see review in expert consensus statement (137)]. Undiagnosed underlying coronary artery disease is thought to be a likely pre-disposing condition. Likewise, endothelial dysfunction and consequent ischemia in the absence of thrombosis are also suspected in some patients with COVID-19 who present with ACS and non-obstructed coronary arteries; severe hypoxia, CRS, plaque rupture, vasospasm, and microthromboembolism are also on the differential in these patients (25, 138, 139).

Implications of Common Pathophysiology

Shared pathophysiology in COVID-19 and cardio-oncology also have important implications. For example, ICIs and CAR-T cells used as cancer therapy can lead to excessive activation of the immune system and inflammation and subsequently autoimmune and inflammatory adverse CV effects. Despite the

favorable responses from CAR-T cell and ICI therapy in cardio-oncology, we still have limited evidence and understanding of CVT from these immunotherapies or their long-term impact. We can potentially fill our knowledge gap on CVT due to CRS related to CAR-T cell therapy, or supranormal activation of the immune system related to ICIs, in cardio-oncology by pursuing a better understanding of the inflammatory pathophysiology from the ongoing COVID-19 pandemic. The reverse is also true, and long-term sequelae of CRS on the cardiovascular system should be investigated and addressed in patients who have had COVID-19 or in cancer patients who have received CAR-T cell therapy. Similarly, coagulopathy in COVID-19 is an emerging topic with the majority of evidence stemming from observational studies and autopsies (128). The hypercoagulability of cancer, which is often treated by cardio-oncologists, can be informative for COVID-19, given a role for anticoagulation to address thromboembolism in these hypercoagulable states. Additionally, the role of endothelial dysfunction can be further elucidated in both COVID-19 and cardio-oncology with anticipated shared vascular pathophysiology, albeit with different mechanisms of endothelial injury.

COMMON PHARMACOLOGY IN COVID-19 AND CARDIO-ONCOLOGY

Corticosteroids

Given the robust inflammatory response induced by COVID-19, corticosteroids are under investigation and have been demonstrating promising efficacy for treating the disease. Dexamethasone has recently garnered significant international attention for the treatment of COVID-19 with the pre-print publication (not yet peer reviewed) of the phase 3 RECOVERY trial. Patients were randomized to dexamethasone at 6 mg daily for up to 10 days vs. standard care. Dexamethasone significantly reduced deaths in patients who required supplemental oxygen or mechanical ventilation (140). Notably, the pre-print manuscript does not quantify the number of patients with cancer included in the analysis and may be difficult to generalize to an oncology population with COVID-19.

Corticosteroids have been mainstays of treatment for immune-related adverse events (irAEs) induced by ICIs and CAR-T cells in cancer patients, owing to their ability to rapidly dampen inflammation and quickly reverse irAEs (141, 142). In the widely utilized, evidence-based irAE management guidelines published by the American Society of Clinical Oncology (ASCO), high-dose corticosteroids are recommended as first-line management of most grade 2 or higher irAEs (143). For cardiovascular irAEs, including myocarditis, pericarditis, heart failure, and vasculitis, high-dose corticosteroids are recommended for any grade of toxicity (141, 142, 144). Thus, steroids may be helpful to quell activated immune responses leading to CVT due to various endogenous sources, whether cancer therapy or COVID-19.

Biologic Antibodies and Inhibitors

Tocilizumab is an IL-6 receptor antagonist and is indicated as the first-line agent for the management of CRS in cancer patients (145–148). The use of tocilizumab in COVID-19 is an extrapolation based on the evidence of promising outcomes from using the drug to treat CRS from CAR-T cell therapy in cancer patients. Off-label use of tocilizumab is an option used in the management of severe cases of COVID-19 on compassionate grounds, supported by a case series from China (149) and a pilot open, single-arm multicenter study from Italy (150), particularly if tocilizumab is administered within 6 days of admission (HR 2.2, 95% CI 1.3–6.7, $p < 0.05$) (150). Additionally, a large retrospective cohort study demonstrated that tocilizumab decreased risk of death or minimized risk for invasive mechanical ventilation in patients with severe COVID-19 (adjusted HR 0.61, 95% CI 0.40–0.92; $p = 0.020$) (151). A smaller, retrospective cohort study demonstrated a significantly shorter need for vasopressor support in severely ill COVID-19 patients who received tocilizumab (152).

Cardiac dysfunction due to CRS is largely reversible, and in severe cases mitigated by tocilizumab (153). In some severe cases not responding to tocilizumab, the corticosteroid is added. In rare cases, when the patient does not respond to tocilizumab or steroid, other agents such as anakinra (IL-1R inhibitor) and etanercept (anti-TNFα) are potential options to hinder inflammatory pathways (114, 154). Siltuximab is a chimeric monoclonal antibody that also binds IL-6; however, no studies have been published on its use in the management of CRS in cancer patients to date (96).

Next generation novel immunotherapeutics could also affect COVID-19-related incidence and outcomes. For instance, AXL is a receptor tyrosine kinase which mediates tumor invasion, metastasis, and epithelial-mesenchymal transition. AXL also negatively modulates cancer immune responses through signaling pathways involving dendritic cells, natural killer cells and macrophages (155). Given its role in cancer metastasis and immune function, numerous AXL inhibitors are being used in clinical trials to treat advanced malignancies. AXL mediates viral entry into cells and modulates inflammatory responses induced by viral infections (156, 157). AXL is also overexpressed on myocardial cells in patients with heart failure and in patients who experience LV remodeling after STEMI (158). It is conceivable that through immune and cardiovascular impacts, investigational drugs that target AXL may impact outcomes of cancer patients with COVID-19 infection, and clinical trial sponsors and investigators should be encouraged to track and study COVID-19-infected trial patients to better understand these complex interactions. To this end, bemcentinib, an oral AXL inhibitor under investigation as a cancer immunotherapeutic, has recently been repurposed to combat COVID-19 as part of the Accelerating COVID-19 Research & Development (ACCORD) platform in the United Kingdom.

Interestingly, human antibodies have been isolated from the convalescent serum of COVID-19 survivors and when coupled have been shown to be protective. Perhaps the use of these emerging dual antibodies may be as efficacious for COVID-19 patients as the dual antibodies trastuzumab and pertuzumab have been for breast cancer patients. Developing therapeutics from antibodies such as these may help provide safer effective

options for COVID-19 patients and facilitate avoiding potential or proven drug-drug interactions.

Role of CYP450 and Drug Transporters

The antiviral drug remdesivir is an investigational drug being used to treat COVID-19, and concomitant use with drugs that are strong CYP3A4 inducers is not recommended (159). The CYP450 enzyme system (which includes CYP3A4) forms the backbone for metabolism of multiple drugs and plays a vital role in metabolism of numerous cardio-oncologic drugs including beta blockers, calcium channel blockers, statins, cyclophosphamide, docetaxel, cisplatin, and tyrosine kinase inhibitors (160). In particular, the antiandrogen drugs apalutamide and enzalutamide used to treat prostate cancer are strong CYP3A4 inducers (161–163). Both agents can associate with CVT, such as atrial fibrillation, hypertension, and ischemic heart disease, especially in individuals with pre-existing cardiovascular diseases (161–164). Concurrent use of remdesivir with these drugs should be avoided at the interface of COVID-19 and cardio-oncology. Of note, in cardio-oncology, the calcium channel blockers diltiazem and verapamil are moderate inhibitors of CYP3A4, which also metabolizes cancer pharmacologic drugs such as doxorubicin, imatinib, and ibrutinib (160). *In vitro*, remdesivir is a substrate for CYP2C8, CYP2D6, and CYP3A4, and an inhibitor of CYP3A4, as well as a substrate for p-glycoprotein and organic anion transporting polypeptides 1B1 (OATP1B1) and an inhibitor of OATP1B1 (165). P-glycoprotein and OATP1B1 are membrane transporters known to help mediate drug-drug interactions. However, remdesivir generally has a low potential for clinically significant drug-drug interactions mediated by the CYP450 system or drug transporters (165–167), since remdesivir functions as a prodrug that is rapidly metabolized to the active bioavailable form (165, 168).

Anticoagulation

Empiric therapeutic anticoagulation associates with better prognosis in severe COVID-19 cases, with improved in-hospital mortality in retrospective analyses (129, 169). While practice has varied across centers during the pandemic, anticoagulation should be considered based on COVID-19 patient factors and risk stratification (119).

Empiric prophylactic anticoagulation also associates with better outcomes in cancer patients who are hospitalized and have reduced mobility, or are ambulatory and have (170, 171):

- advanced or metastatic pancreatic cancer,
- intermediate-high VTE risk based on cancer type or Khorana score,
- or treatment with immunomodulatory drugs and steroids or other systemic antineoplastic therapies.

Neurohormonal Drugs: ACEIs and ARBs

It has been suggested that ACE inhibitors may counteract resulting unopposed ACE-mediated effects in COVID-19 (81). Thus, the influence of these vasoactive and cardiovascular remodeling drugs on the risk and severity of COVID-19 has been under investigation, with some studies suggesting benefit, juxtaposed with initial speculations about harm (28, 172–181). It is unknown whether polymorphisms in ACE, or polymorphisms in ACE2 that may contribute to COVID-19 prognosis [see pre-prints (182, 183)], also determine the prognosis of patients in cardio-oncology treated with RAAS regulators, such as ACE inhibitors and ARBs.

It is important to note that ACEIs and ARBs have established benefits in protecting the myocardium. They are among first-line therapy for various CVT (e.g., cardiomyopathy, hypertension, and myocardial infarction) in cancer patients and survivors, along with beta blockers, to mitigate symptoms and prolong survival (184). Withdrawal of these agents instigates clinical decompensation in high-risk patients, such as rapid relapse of dilated cardiomyopathy in cancer patients with CVT due to neoplastic agents (28, 172, 185). Consequently, patients receiving ACEIs or ARBs should continue ACEI/ARB therapy during the COVID-19 pandemic (172, 186, 187). Taken together, these findings suggest overlapping utility of these drugs in both cardio-oncology and COVID-19.

Implications of Common Pharmacology

It is also important to study shared pharmacologic management opportunities in COVID-19 and cardio-oncology. Corticosteroids and immunomodulatory drugs such as tocilizumab and bemcentinib and other analogous therapies are being used or studied in cardio-oncology and have also been repurposed during the pandemic to temper the inflammation milieu initiated by SARS-CoV-2. Further, thousands of cancer patients are currently enrolled in clinical trials combining ICIs with investigational novel therapeutics across the world, accounting for another special risk population in the COVID-19 pandemic. Ongoing clinical trials on anti-interleukins in COVID-19 patients (188, 189) (NCT04330638, NCT04317092) will also help us to elucidate benefits and outcomes. Accordingly, an algorithm has been proposed to incorporate anti-inflammatory agents such as tocilizumab, canakinumab (IL-1β monoclonal antibody), anakinra, etanercept, and infliximab (TNFα monoclonal antibody) to curb CRS in acute COVID-19 infection (190). The antiviral remdesivir carries a low risk of modulating the membrane drug transporter p-glycoprotein and the cytochrome protein 450 family of enzymes and potentially interacting with CV and cardio-oncology drugs. Nevertheless, caution is recommended with the combined use of any drugs that modulate p-glycoprotein or CYP450, due to their potential for drug-drug interactions and resultant effects on the CV system in cardio-oncology and COVID-19. Therapeutic anticoagulants are another class of medications found to be useful in both COVID-19 and cardio-oncology, due to their beneficial effects on thromboembolism. Clinical studies are being pursued to determine the impact of direct oral anticoagulants or aspirin and statin to limit arterial or venous thrombotic risk in cancer (120). Regulators of the RAAS (primarily ACEI/ARB) have also taken centerstage, as many patients are on these medications to treat hypertension or other common comorbidities that increase the risk of a more severe course in those with COVID-19. There has been debate about whether these RAAS modulator drugs

augment the risk of COVID-19 infection, given the virus' use of the ACE receptor to enter host cells. ACE receptor gene polymorphisms have also been implicated in the prediction of disease severity, with questionable regulation by the ACEI/ARB drug class. Pursuit of observational studies and clinical trials continues to help elucidate the impact of ACEIs and ARBs in the pandemic (27). Further study should also define the interplay between SARS-CoV-2 and RAAS and explore any differential effects of ACEI vs. ARB therapy and tumor specific responses (91, 93, 94, 185). Future collaboration among basic and clinical scientists should focus on the biological rationale for the treatment of COVID-19 patients as well as limited understanding with respect to the interaction of RAAS inhibitors, ACE2 levels and SARS CoV-2 infectivity in humans (27).

DISCUSSION

As we recover from the COVID-19 pandemic, we should not let opportunities for learning surreptitiously slip from our grasp. The myriad of overlap of CV toxicities, pathophysiology, and innovative management in COVID-19 and cardio-oncology provide multiple paths for exploration that could lead to greater understanding of both COVID-19 and CVT noted in cardio-oncology (**Figure 1**). It would behoove us in cardio-oncology to continue to closely study these toxicities, pathophysiology, and pharmacologic options in COVID-19 to help update our understanding in cardio-oncology. Cardio-oncology continues to expand as a relatively new medical subspecialty. Knowledge gaps in CVT toxicity, pathophysiology, and pharmacology in cardio-oncology may benefit from the application of novel concepts, paradigms, and drug use from overlapping forms of CVT in COVID-19 (**Table 1**).

In addition to short-term morbidity and mortality, patients who recover from COVID-19 infection may be at increased risk of future incident CVD and CVD-related complications (191, 192). Severe acute respiratory syndrome coronavirus (SARS-CoV-1) and Middle East respiratory syndrome coronavirus (MERS-CoV) infection have been implicated in causing diabetes, hypertension and altered lipid metabolism (193–195). The increase in CVD risk profile combined with the possibility of viral-mediated impairment in cardiac and/or pulmonary function may combine to further increase the risk and complexity of future CVD events. Aggressive risk factor modification and prophylactic therapy may prove important in mitigating long-term CVT. Optimal prevention and management of CVT will require a multidisciplinary approach with close collaborations among various medical specialties and researchers.

As our current practices change and new questions arise, future studies in cardio-oncology should focus on studying the emerging cardiovascular epidemiology of COVID-19, as well as the impact of changed practices on the health of patients with cancer and CVD. To reduce the rate of transmission while providing safe and timely care for patients with cancer and CVD, temporary recommendations favored telehealth visits in telecardio-oncology and deferral of non-urgent procedures, similar to the rest of the population (10, 196). Cardiac imaging surveillance was limited to patients who were more likely to have abnormal testing or at higher risk for cancer-related CVT, particularly if test results would guide initiation of cardioprotective medications or impact cancer therapy delivery (197).

Of utmost importance is ensuring equity in our distribution of hope, health, and healing in the midst of and beyond the pandemic, as we extend lessons learned from COVID-19 to cardio-oncology. Ethnic health disparities during the pandemic have amplified a pre-existing broken healthcare structure, with disproportionate percentages of African-Americans severely and fatally affected by COVID-19 (69–73). The pandemic has been set on a backdrop of inequity, in which African-American cancer patients are known to be more susceptible to CVT following cancer therapies (64–68). The higher risk for African Americans in both COVID-19 and cardio-oncology is of multifactorial etiology, including higher rates of CVD and CVD risk factors, which are often also underdiagnosed and undertreated (57, 69, 198–206). Underlying causes of the plethora of inequalities in healthcare are largely structural and socioeconomic and reflect our imperfections as a society, with socioeconomic status being a risk factor for CVD, CVT, and COVID-19 (198, 207, 208). We must recognize the imbalance of comorbidities and sociodemographics in ethnic populations, in order to make equitable progress in the post-pandemic era. The lasting impact of COVID-19 in cardio-oncology need not be the challenges we faced while caring for our patients during the pandemic. The long-term sequelae should be steps we have taken to optimize quality and quantity of life for all.

Thus, there are several areas of overlap, similarity, and interaction in the toxicity, pathophysiology, and pharmacology profiles in COVID-19 and cardio-oncology syndromes. Learning more about either will likely provide some level of insight into both, with further illumination of CVT mechanisms and new paradigms of drug utilization to help guide research and clinical practice in both COVID-19 and cardio-oncology. Such an approach can be informative peri-pandemic, and should perhaps be pursued long after the pandemic, to assess for evidence of long-term independent or synergistic CV effects in survivors of COVID-19 and cancer, with equity at the forefront of our efforts.

DATA AVAILABILITY STATEMENT

The original contributions generated in the study are included in the article, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

S-AB conceived, designed, and helped draft the manuscript. SZ, PM, JT, BT, DI, EW, GA, JR, JS, and DJ helped draft the manuscript. BK and MW helped design and draft the manuscript. All authors revised and approved the manuscript.

REFERENCES

1. WHO. *WHO Coronavirus Disease (COVID-19) Dashboard*. (2020). Available online at: https://covid19.who.int/ (accessed October 01, 2020).
2. Brown SA. Preventive cardio-oncology: the time has come. *Front Cardiovasc Med*. (2019) 6:187. doi: 10.3389/fcvm.2019.00187
3. Brown SA, Rhee JW, Guha A, Rao VU. Innovation in precision cardio-oncology during the coronavirus pandemic and into a post-pandemic world. *Front Cardiovasc Med*. (2020) 7:145. doi: 10.3389/fcvm.2020.00145
4. Brown SA, Sandhu N, Herrmann J. Systems biology approaches to adverse drug effects: the example of cardio-oncology. *Nat Rev Clin Oncol*. (2015) 12:718–31. doi: 10.1038/nrclinonc.2015.168
5. Brown SA, Nhola L, Herrmann J. Cardiovascular toxicities of small molecule tyrosine kinase inhibitors: an opportunity for systems-based approaches. *Clin Pharmacol Ther*. (2017) 101:65–80. doi: 10.1002/cpt.552
6. Brown SA, Ray JC, Herrmann J. Precision cardio-oncology: a systems-based perspective on cardiotoxicity of tyrosine kinase inhibitors and immune checkpoint inhibitors. *J Cardiovasc Transl Res*. (2020) 13:402–16. doi: 10.1007/s12265-020-09992-5
7. Brown SA, Okwuosa TM, Barac A, Volgman AS. The role of angiotensin-converting enzyme inhibitors and β-blockers in primary prevention of cardiac dysfunction in breast cancer patients. *J Am Heart Assoc*. (2020) 9:e015327. doi: 10.1161/JAHA.119.015327
8. 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
9. 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
10. Ganatra S, Hammond SP, Nohria A. The novel coronavirus disease (COVID-19) threat for patients with cardiovascular disease and cancer. *JACC CardioOncology*. (2020) 2:350–5. doi: 10.1016/j.jaccao.2020.03.001
11. Chang HM, Moudgil R, Scarabelli T, Okwuosa TM, Yeh ETH. Cardiovascular complications of cancer therapy: best practices in diagnosis, prevention, and management: part 1. *J Am Coll Cardiol*. (2017) 70:2536–51. doi: 10.1016/j.jacc.2017.09.1096
12. Barac A, Murtagh G, Carver JR, Chen MH, Freeman AM, Herrmann J, et al. Cardiovascular health of patients with cancer and cancer survivors: a roadmap to the next level. *J Am Coll Cardiol*. (2015) 65:2739–46. doi: 10.1016/j.jacc.2015.04.059
13. Bonaca MP, Olenchock BA, Salem JE, Wiviott SD, Ederhy S, Cohen A, et al. Myocarditis in the setting of cancer therapeutics: proposed case definitions for emerging clinical syndromes in cardio-oncology. *Circulation*. (2019) 140:80–91. doi: 10.1161/CIRCULATIONAHA.118.034497
14. Cheng P, Zhu H, Witteles RM, Wu JC, Quertermous T, Wu SM, et al. Cardiovascular risks in patients with COVID-19: potential mechanisms and areas of uncertainty. *Curr Cardiol Rep*. (2020) 22:34. doi: 10.1007/s11886-020-01293-2
15. 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
16. 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
17. Kochav SM, Coromilas E, Nalbandian A, Ranard LS, Gupta A, Chung MK, et al. Cardiac arrhythmias in COVID-19 infection. *Circ Arrhythm Electrophysiol*. (2020) 13:e008719. doi: 10.1161/CIRCEP.120.008719
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. 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
20. 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
21. Zhu H, Rhee JW, Cheng P, Waliany S, Chang A, Witteles RM, et al. Cardiovascular complications in patients with COVID-19: consequences of viral toxicities and host immune response. *Curr Cardiol Rep*. (2020) 22:32. doi: 10.1007/s11886-020-01292-3
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 72314 cases from the Chinese center for disease control and prevention. *JAMA*. (2020) 323:1239–42. doi: 10.1001/jama.2020.2648
23. 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
24. 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
25. 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
26. Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutanen SM, Penninger JM, et al. SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. *Eur J Clin Invest*. (2009) 39:618–25. doi: 10.1111/j.1365-2362.2009.02153.x
27. Ky B, Mann DL. COVID-19 clinical trials: a primer for the cardiovascular and cardio-oncology communities. *JACC CardioOncol*. (2020) 5:501–17. doi: 10.1016/j.jacbts.2020.04.003
28. Patel AB, Verma A. COVID-19 and angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: what is the evidence? *JAMA*. (2020) 323:1769–70. doi: 10.1001/jama.2020.4812
29. Asokan I, Rabadia SV, Yang EH. The COVID-19 pandemic and its impact on the cardio-oncology population. *Curr Oncol Rep*. (2020) 22:60. doi: 10.1007/s11912-020-00945-4
30. Lee LYW, Cazier JB, Starkey T, Turnbull CD, Kerr R, Middleton G, et al. COVID-19 mortality in patients with cancer on chemotherapy or other anticancer treatments: a prospective cohort study. *Lancet*. (2020) 395:1919–26.
31. Chang HM, Okwuosa TM, Scarabelli T, Moudgil R, Yeh ETH. Cardiovascular complications of cancer therapy: best practices in diagnosis, prevention, and management: part 2. *J Am Coll Cardiol*. (2017) 70:2552–65. doi: 10.1016/j.jacc.2017.09.1095
32. Singh D, Thakur A, Tang WH. Utilizing cardiac biomarkers to detect and prevent chemotherapy-induced cardiomyopathy. *Curr Heart Fail Rep*. (2015) 12:255–62. doi: 10.1007/s11897-015-0258-4
33. Meinardi MT, Van Der Graaf WT, Gietema JA, Van Den Berg MP, Sleijfer DT, De Vries EG, et al. Evaluation of long-term cardiotoxicity after epirubicin containing adjuvant chemotherapy and locoregional radiotherapy for breast cancer using various detection techniques. *Heart*. (2002) 88:81–2. doi: 10.1136/heart.88.1.81
34. Kuderer NM, Choueiri TK, Shah DP, Shyr Y, Rubinstein SM, Rivera DR, et al. Clinical impact of COVID-19 on patients with cancer (CCC19): a cohort study. *Lancet*. (2020) 395:1907–18. doi: 10.1016/S0140-6736(20)31187-9
35. Sanz J, Sánchez-Quintana D, Bossone E, Bogaard HJ, Naeije R. Anatomy, function, and dysfunction of the right ventricle: JACC state-of-the-art review. *J Am Coll Cardiol*. (2019) 73:1463–82. doi: 10.1016/j.jacc.2018.12.076
36. Zhao R, Shu F, Zhang C, Song F, Xu Y, Guo Y, et al. Early detection and prediction of anthracycline-induced right ventricular cardiotoxicity by 3-dimensional echocardiography. *JACC CardioOncol*. (2020) 2:13–22. doi: 10.1016/j.jaccao.2020.01.007
37. Nagata Y, Wu VC, Kado Y, Otani K, Lin FC, Otsuji Y, et al. Prognostic value of right ventricular ejection fraction assessed by transthoracic 3D echocardiography. *Circ Cardiovasc Imaging*. (2017) 10:e005384. doi: 10.1161/CIRCIMAGING.116.005384
38. 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
39. 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

40. Mekontso Dessap A, Boissier F, Charron C, Bégot E, Repessé X, Legras A, et al. Acute cor pulmonale during protective ventilation for acute respiratory distress syndrome: prevalence, predictors, and clinical impact. *Intensive Care Med*. (2016) 42:862–70. doi: 10.1007/s00134-015-4141-2
41. Bull TM, Clark B, McFann K, Moss M. Pulmonary vascular dysfunction is associated with poor outcomes in patients with acute lung injury. *Am J Respir Crit Care Med*. (2010) 182:1123–8. doi: 10.1164/rccm.201002-0250OC
42. Mekontso Dessap A, Charron C, Devaquet J, Aboab J, Jardin F, Brochard L, et al. Impact of acute hypercapnia and augmented positive end-expiratory pressure on right ventricle function in severe acute respiratory distress syndrome. *Intensive Care Med*. (2009) 35:1850–8. doi: 10.1007/s00134-009-1569-2
43. 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
44. Xie Y, Wang X, Yang P, Zhang S. COVID-19 complicated by acute pulmonary embolism. *Radiol Cardiothoracic Imaging*. (2020) 2:e200067. doi: 10.1148/ryct.2020200067
45. Rotzinger D, Beigelman-Aubry C, von Garnier C, Qanadli S. Pulmonary embolism in patients with COVID-19: time to change the paradigm of computed tomography. *Thromb Res*. (2020) 190:58–9. doi: 10.1016/j.thromres.2020.04.011
46. Poissy J, Goutay J, Caplan M, Parmentier E, Duburcq T, Lassalle F, et al. Pulmonary embolism in COVID-19 patients: awareness of an increased prevalence. *Circulation*. (2020) 142:184–6. doi: 10.1161/CIRCULATIONAHA.120.047430
47. Network ARDS. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med*. (2000) 342:1301–8. doi: 10.1056/NEJM200005043421801
48. Vieillard-Baron A, Charron C, Caille V, Belliard G, Page B, Jardin F. Prone positioning unloads the right ventricle in severe ARDS. *Chest*. (2007) 132:1440–6. doi: 10.1378/chest.07-1013
49. Guérin C, Reignier J, Richard J-C, Beuret P, Gacouin A, Boulain T, et al. Prone positioning in severe acute respiratory distress syndrome. *N Engl J Med*. (2013) 368:2159–68. doi: 10.1056/NEJMoa1214103
50. Ghelichkhani P, Esmaeili M. Prone position in management of COVID-19 patients; a commentary. *Arch Acad Emerg Med*. (2020) 8:e48.
51. Boczar KE, Aseyev O, Sulpher J, Johnson C, Burwash IG, Turek M, et al. Right heart function deteriorates in breast cancer patients undergoing anthracycline-based chemotherapy. *Echo Res Pract*. (2016) 3:79–84. doi: 10.1530/ERP-16-0020
52. Oliveira GH, Dupont M, Naftel D, Myers SL, Yuan Y, Tang WH, et al. Increased need for right ventricular support in patients with chemotherapy-induced cardiomyopathy undergoing mechanical circulatory support: outcomes from the INTERMACS Registry (interagency registry for mechanically assisted circulatory support). *J Am Coll Cardiol*. (2014) 63:240–8. doi: 10.1016/j.jacc.2013.09.040
53. Vejpongsa P, Yeh ET. Topoisomerase 2β: a promising molecular target for primary prevention of anthracycline-induced cardiotoxicity. *Clin Pharmacol Ther*. (2013) 95:45–52. doi: 10.1038/clpt.2013.201
54. Nebigil CG, Désaubry L. Updates in anthracycline-mediated cardiotoxicity. *Front Pharmacol*. (2018) 9:1262. doi: 10.3389/fphar.2018.01262
55. Moazeni S, Cadeiras M, Yang EH, Deng MC, Nguyen KL. Anthracycline induced cardiotoxicity: biomarkers and "Omics" technology in the era of patient specific care. *Clin Transl Med*. (2017) 6:17. doi: 10.1186/s40169-017-0148-3
56. Price-Haywood EG, Burton J, Fort D, Seoane L. Hospitalization and mortality among black patients and white patients with covid-19. *N Engl J Med*. (2020) 382:2534–43. doi: 10.1056/NEJMsa2011686
57. Carnethon MR, Pu J, Howard G, Albert MA, Anderson CAM, Bertoni AG, et al. Cardiovascular health in African Americans: a scientific statement from the American Heart Association. *Circulation*. (2017) 136:e393–423. doi: 10.1161/CIR.0000000000000534
58. Ned RM, Yesupriya A, Imperatore G, Smelser DT, Moonesinghe R, Chang MH, et al. The ACE I/D polymorphism in US adults: limited evidence of association with hypertension-related traits and sex-specific effects by race/ethnicity. *Am J Hypertens*. (2012) 25:209–15. doi: 10.1038/ajh.2011.182
59. Fløe A, Hoffmann HJ, Nissen PH, Møller HJ, Hilberg O. Genotyping increases the yield of angiotensin-converting enzyme in sarcoidosis–a systematic review. *Dan Med J*. (2014) 61:A4815.
60. Mirsaeidi M, Machado RF, Schraufnagel D, Sweiss NJ, Baughman RP. Racial difference in sarcoidosis mortality in the United States. *Chest*. (2015) 147:438–49. doi: 10.1378/chest.14-1120
61. Trojanowicz B, Ulrich C, Fiedler R, Martus P, Storr M, Boehler T, et al. Modulation of leucocytic angiotensin-converting enzymes expression in patients maintained on high-permeable haemodialysis. *Nephrol Dial Transplant*. (2018) 33:34–43. doi: 10.1093/ndt/gfx206
62. Delanghe JR, Speeckaert MM, De Buyzere ML. The host's angiotensin-converting enzyme polymorphism may explain epidemiological findings in COVID-19 infections. *Clin Chim Acta*. (2020) 505:192–3. doi: 10.1016/j.cca.2020.03.031
63. Cao Y, Li L, Feng Z, Wan S, Huang P, Sun X, et al. Comparative genetic analysis of the novel coronavirus (2019-nCoV/SARS-CoV-2) receptor ACE2 in different populations. *Cell Discov*. (2020) 6:11. doi: 10.1038/s41421-020-0147-1
64. Hasan S, Dinh K, Lombardo F, Kark J. Doxorubicin cardiotoxicity in African Americans. *J Natl Med Assoc*. (2004) 96:196–9.
65. Lotrionte M, Biondi-Zoccai G, Abbate A, Lanzetta G, D'Ascenzo F, Malavasi V, et al. Review and meta-analysis of incidence and clinical predictors of anthracycline cardiotoxicity. *Am J Cardiol*. (2013) 112:1980–4. doi: 10.1016/j.amjcard.2013.08.026
66. Finkelman BS, Putt M, Wang T, Wang L, Narayan H, Domchek S, et al. Arginine-nitric oxide metabolites and cardiac dysfunction in patients with breast cancer. *J Am Coll Cardiol*. (2017) 70:152–62. doi: 10.1016/j.jacc.2017.05.019
67. Litvak A, Batukbhai B, Russell SD, Tsai HL, Rosner GL, Jeter SC, et al. Racial disparities in the rate of cardiotoxicity of HER2-targeted therapies among women with early breast cancer. *Cancer*. (2018) 124:1904–11. doi: 10.1002/cncr.31260
68. Baron KB, Brown JR, Heiss BL, Marshall J, Tait N, Tkaczuk KH, et al. Trastuzumab-induced cardiomyopathy: incidence and associated risk factors in an inner-city population. *J Card Fail*. (2014) 20:555–9. doi: 10.1016/j.cardfail.2014.05.012
69. Yancy CW. COVID-19 and African Americans. *JAMA*. (2020) 323:1891–2. doi: 10.1001/jama.2020.6548
70. Haynes N, Cooper LA, Albert MA, Cardiologists AoB. At the heart of the matter: unmasking and addressing COVID-19's toll on diverse populations. *Circulation*. (2020) 142:105–7. doi: 10.1161/CIRCULATIONAHA.120.048126
71. Dorn AV, Cooney RE, Sabin ML. COVID-19 exacerbating inequalities in the US. *Lancet*. (2020) 395:1243–4. doi: 10.1016/S0140-6736(20)30893-X
72. Douglas M, Katikireddi SV, Taulbut M, McKee M, McCartney G. Mitigating the wider health effects of covid-19 pandemic response. *BMJ*. (2020) 369:m1557. doi: 10.1136/bmj.m1557
73. Chung RY, Dong D, Li MM. Socioeconomic gradient in health and the covid-19 outbreak. *BMJ*. (2020) 369:m1329. doi: 10.1136/bmj.m1329
74. Musher DM, Abers MS, Corrales-Medina VF. Acute infection and myocardial infarction. Reply. *N Engl J Med*. (2019) 380:e21. doi: 10.1056/NEJMc1901647
75. Libby P, Loscalzo J, Ridker PM, Farkouh ME, Hsue PY, Fuster V, et al. Inflammation, immunity, and infection in atherothrombosis: JACC review topic of the week. *J Am Coll Cardiol*. (2018) 72:2071–81. doi: 10.1016/j.jacc.2018.08.1043
76. 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
77. Sharma A, Garcia G, Arumugaswami V, Svendsen CN. Human iPSC-derived cardiomyocytes are susceptible to SARS-CoV-2 infection. *bioRxiv*. (2020). doi: 10.1101/2020.04.21.051912

78. 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
79. Perlot T, Penninger JM. ACE2—from the renin-angiotensin system to gut microbiota and malnutrition. *Microbes Infect*. (2013) 15:866–73. doi: 10.1016/j.micinf.2013.08.003
80. Anguiano L, Riera M, Pascual J, Valdivielso JM, Barrios C, Betriu A, et al. Circulating angiotensin-converting enzyme 2 activity in patients with chronic kidney disease without previous history of cardiovascular disease. *Nephrol Dial Transpl*. (2015) 30:1176–85. doi: 10.1093/ndt/gfv025
81. South AM, Diz DI, Chappell MC. COVID-19, ACE2, and the cardiovascular consequences. *Am J Physiol Heart Circ Physiol*. (2020) 318:H1084–H90. doi: 10.1152/ajpheart.00217.2020
82. 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
83. 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) 46:1294–7. doi: 10.1007/s00134-020-06028-z
84. Zheng M, Gao Y, Wang G, Song G, Liu S, Sun D, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell Mol Immunol*. (2020) 17:533–5. doi: 10.1038/s41423-020-0402-2
85. Zheng HY, Zhang M, Yang CX, Zhang N, Wang XC, Yang XP, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. *Cell Mol Immunol*. (2020) 17:541–3. doi: 10.1038/s41423-020-0401-3
86. Haslam A, Prasad V. Estimation of the percentage of US patients with cancer who are eligible for and respond to checkpoint inhibitor immunotherapy drugs. *JAMA Netw Open*. (2019) 2:e192535. doi: 10.1001/jamanetworkopen.2019.2535
87. Garcia-Diaz A, Shin DS, Moreno BH, Saco J, Escuin-Ordinas H, Rodriguez GA, et al. Interferon receptor signaling pathways regulating PD-L1 and PD-L2 expression. *Cell Rep*. (2019) 29:3766. doi: 10.1016/j.celrep.2019.11.113
88. Robilotti EV, Babady NE, Mead PA, Rolling T, Perez-Johnston R, Bernardes M, et al. Determinants of COVID-19 disease severity in patients with cancer. *Nat Med*. (2020) 26:1218–23. doi: 10.1038/s41591-020-0979-0
89. Lee LYW, Cazier JB, Starkey T, Turnbull CD, Kerr R, Middleton G, et al. COVID-19 mortality in patients with cancer on chemotherapy or other anticancer treatments: a prospective cohort study. *Lancet*. (2020) 395:1919–26. doi: 10.1016/S0140-6736(20)31173-9
90. Oren O, Yang EH, Molina JR, Bailey KR, Blumenthal RS, Kopecky SL. Cardiovascular health and outcomes in cancer patients receiving immune checkpoint inhibitors. *Am J Cardiol*. (2020) 125:1920–6. doi: 10.1016/j.amjcard.2020.02.016
91. Xie G, Cheng T, Lin J, Zhang L, Zheng J, Liu Y, et al. Local angiotensin II contributes to tumor resistance to checkpoint immunotherapy. *J Immunother Cancer*. (2018) 6:88. doi: 10.1186/s40425-018-0401-3
92. Pinter M, Jain RK. Targeting the renin-angiotensin system to improve cancer treatment: implications for immunotherapy. *Sci Transl Med*. (2017) 9:eaan5616. doi: 10.1126/scitranslmed.aan5616
93. Hicks BM, Filion KB, Yin H, Sakr L, Udell JA, Azoulay L. Angiotensin converting enzyme inhibitors and risk of lung cancer: population based cohort study. *BMJ*. (2018) 363:k4209. doi: 10.1136/bmj.k4209
94. Medjebar S, Richard C, Fumet J-D, Malo J, Elkrief A, Blais N, et al. Angiotensin-converting enzyme inhibitor prescription is associated with decreased progression-free survival (PFS) and overall survival (OS) in patients with lung cancers treated with PD-1/PD-L1 immune checkpoint blockers. *Am Soc Clin Oncol*. (2019) 37:e20512. doi: 10.1200/JCO.2019.37.15_suppl.e20512
95. Cascella M, Rajnik M, Cuomo A, Dulebohn SC, Di Napoli R. *Features, Evaluation and Treatment Coronavirus (COVID-19)*. Treasure Island, FL: StatPearls Publishing Copyright©, LLC (2020).
96. Yáñez L, Sánchez-Escamilla M, Perales MA. CAR T cell toxicity: current management and future directions. *Hemasphere*. (2019) 3:e186. doi: 10.1097/HS9.0000000000000186
97. Neelapu SS, Tummala S, Kebriaei P, Wierda W, Gutierrez C, Locke FL, et al. Chimeric antigen receptor T-cell therapy—assessment and management of toxicities. *Nat Rev Clin Oncol*. (2018) 15:47–62. doi: 10.1038/nrclinonc.2017.148
98. 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
99. Huang L, Zhao X, Qi Y, Li H, Ye G, Liu Y, et al. Sepsis-associated severe interleukin-6 storm in critical coronavirus disease 2019. *Cell Mol Immunol*. (2020) 17:1092–4. doi: 10.1038/s41423-020-00522-6
100. Liu Z, Li J, Chen D, Gao R, Zeng W, Chen S, et al. Dynamic interleukin-6 level changes as a prognostic indicator in patients with COVID-19. *Front Pharmacol*. (2020) 11:1093. doi: 10.3389/fphar.2020.01093
101. 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
102. Liu Z, Xiao X, Wei X, Li J, Yang J, Tan H, et al. Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential intermediate hosts of SARS-CoV-2. *J Med Virol*. (2020) 92:595–601. doi: 10.1002/jmv.25726
103. Ratajczak MZ, Kucia M. SARS-CoV-2 infection and overactivation of Nlrp3 inflammasome as a trigger of cytokine "storm" and risk factor for damage of hematopoietic stem cells. *Leukemia*. (2020) 34:1726–9. doi: 10.1038/s41375-020-0887-9
104. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. 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
105. Leisman DE, Deutschman CS, Legrand M. Facing COVID-19 in the ICU: vascular dysfunction, thrombosis, and dysregulated inflammation. *Intensive Care Med*. (2020) 46:1105–8. doi: 10.1007/s00134-020-06059-6
106. Ren XS, Tong Y, Ling L, Chen D, Sun HJ, Zhou H, et al. NLRP3 gene deletion attenuates angiotensin II-induced phenotypic transformation of vascular smooth muscle cells and vascular remodeling. *Cell Physiol Biochem*. (2017) 44:2269–80. doi: 10.1159/000486061
107. Chen IY, Moriyama M, Chang MF, Ichinohe T. Severe acute respiratory syndrome coronavirus viroporin 3a activates the NLRP3 inflammasome. *Front Microbiol*. (2019) 10:50. doi: 10.3389/fmicb.2019.00050
108. Schieffer B, Bünte C, Witte J, Hoeper K, Böger RH, Schwedhelm E, et al. Comparative effects of AT1-antagonism and angiotensin-converting enzyme inhibition on markers of inflammation and platelet aggregation in patients with coronary artery disease. *J Am Coll Cardiol*. (2004) 44:362–8. doi: 10.1016/j.jacc.2004.03.065
109. Luther JM, Gainer JV, Murphey LJ, Yu C, Vaughan DE, Morrow JD, et al. Angiotensin II induces interleukin-6 in humans through a mineralocorticoid receptor-dependent mechanism. *Hypertension*. (2006) 48:1050–7. doi: 10.1161/01.HYP.0000248135.97380.76
110. Swanson KV, Deng M, Ting JP. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat Rev Immunol*. (2019) 19:477–89. doi: 10.1038/s41577-019-0165-0
111. Park TS, Zambidis ET. A role for the renin-angiotensin system in hematopoiesis. *Haematologica*. (2009) 94:745–7. doi: 10.3324/haematol.2009.006965
112. 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. *Stem Cell Rev Rep*. (2020) 20:1–12. doi: 10.1007/s12015-020-10010-z
113. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med*. (2017) 377:2531–44. doi: 10.1056/NEJMoa1707447
114. Giavridis T, van der Stegen SJC, Eyquem J, Hamieh M, Piersigilli A, Sadelain M. CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. *Nat Med*. (2018) 24:731–8. doi: 10.1038/s41591-018-0041-7
115. Shimabukuro-Vornhagen A, Gödel P, Subklewe M, Stemmler HJ, Schlößer HA, Schlaak M, et al. Cytokine release syndrome. *J Immunother Cancer*. (2018) 6:56. doi: 10.1186/s40425-018-0343-9
116. 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

117. 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
118. Cook D, Meade M, Guyatt G, Walter S, Heels-Ansdell D, Warkentin TE, et al. Dalteparin versus unfractionated heparin in critically ill patients. *N Engl J Med*. (2011) 364:1305–14. doi: 10.1056/NEJMoa1014475
119. 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. *J Am Coll Cardiol*. (2020) 75:2950–73. doi: 10.1016/j.jacc.2020.04.031
120. Navi BB, Reiner AS, Kamel H, Iadecola C, Okin PM, Elkind MSV, et al. Risk of arterial thromboembolism in patients with cancer. *J Am Coll Cardiol*. (2017) 70:926–38. doi: 10.1016/j.jacc.2017.06.047
121. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet*. 395:1417–8. doi: 10.1016/S0140-6736(20)30937-5
122. Bick RL. Cancer-associated thrombosis. *N Engl J Med*. (2003) 349:109–11. doi: 10.1056/NEJMp030086
123. 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
124. Henry BM, Vikse J, Benoit S, Favaloro EJ, Lippi G. Hyperinflammation and derangement of renin-angiotensin-aldosterone system in COVID-19: a novel hypothesis for clinically suspected hypercoagulopathy and microvascular immunothrombosis. *Clin Chim Acta*. (2020) 507:167–73. doi: 10.1016/j.cca.2020.04.027
125. Connors JM, Levy JH. COVID-19 and its implications for thrombosis and anticoagulation. *Blood*. (2020) 135:2033–40. doi: 10.1182/blood.2020006000
126. Cook D, Crowther M, Meade M, Rabbat C, Griffith L, Schiff D, et al. Deep venous thrombosis in medical-surgical critically ill patients: prevalence, incidence, and risk factors. *Crit Care Med*. (2005) 33:1565–71. doi: 10.1097/01.CCM.0000171207.95319.B2
127. Zhang C, Zhang Z, Mi J, Wang X, Zou Y, Chen X, et al. The cumulative venous thromboembolism incidence and risk factors in intensive care patients receiving the guideline-recommended thromboprophylaxis. *Medicine (Baltimore)*. (2019) 98:e15833. doi: 10.1097/MD.0000000000015833
128. Becker RC. COVID-19 update: covid-19-associated coagulopathy. *J Thromb Thrombolysis*. (2020) 50:54–67. doi: 10.1007/s11239-020-02134-3
129. 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
130. Iba T, Levy JH. Inflammation and thrombosis: roles of neutrophils, platelets and endothelial cells and their interactions in thrombus formation during sepsis. *J Thromb Haemost*. (2018) 16:231–41. doi: 10.1111/jth.13911
131. Thachil J, Falanga A, Levi M, Liebman H, Di Nisio M, Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis. Management of cancer-associated disseminated intravascular coagulation: guidance from the SSC of the ISTH. *J Thromb Haemost*. (2015) 13:671–5. doi: 10.1111/jth.12838
132. Khorana AA, Kuderer NM, Culakova E, Lyman GH, Francis CW. Development and validation of a predictive model for chemotherapy-associated thrombosis. *Blood*. (2008) 111:4902–7. doi: 10.1182/blood-2007-10-116327
133. Key NS, Khorana AA, Kuderer NM, Bohlke K, Lee AYY, Arcelus JI, et al. Venous thromboembolism prophylaxis and treatment in patients with cancer: ASCO clinical practice guideline update. *J Clin Oncol*. (2020) 38:496–520. doi: 10.1200/JCO.19.01461
134. Lee AY, Levine MN. Venous thromboembolism and cancer: risks and outcomes. *Circulation*. (2003) 107(Suppl. 1):I17-21. doi: 10.1161/01.CIR.0000078466.72504.AC
135. Liu H, Yang Y, Jiang J, Wang X, Zhang C, Jiang Y, et al. Coexistence of a huge venous thromboembolism and bleeding tendency in cytokine release syndrome during CAR-T therapy. *Onco Targets Ther*. (2019) 12:8955–60. doi: 10.2147/OTT.S223697
136. Hashmi H, Mirza A-S, Darwin A, Logothetis C, Garcia F, Kommalapati A, et al. Incidence and management of venous thrombo-embolism (VTE) associated with CD19-directed chimeric antigen receptor (CAR) T-cell therapy: a single institution experience. *Biol Blood Marrow Transplant*. (2020) 26:S265. doi: 10.1016/j.bbmt.2019.12.430
137. Iliescu CA, Grines CL, Herrmann J, Yang EH, Cilingiroglu M, Charitakis K, et al. SCAI Expert consensus statement: evaluation, management, and special considerations of cardio-oncology patients in the cardiac catheterization laboratory (endorsed by the cardiological society of india, and sociedad Latino Americana de Cardiologia intervencionista). *Catheter Cardiovasc Interv*. (2016) 87:E202–23. doi: 10.1002/ccd.26379
138. 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
139. 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
140. Horby P, Lim WS, Emberson J, Mafham M, Bell J, Linsell L, et al. Effect of dexamethasone in hospitalized patients with COVID-19: preliminary report. *medRxiv*. (2020) NEJMoa2021436. doi: 10.1101/2020.06.22.20137273
141. Ghosh AK, Chen DH, Guha A, Mackenzie S, Walker JM, Roddie C. CAR T cell therapy–related cardiovascular outcomes and management. Systemic disease or direct cardiotoxicity? *JACC CardioOncol*. (2020) 2:97–109. doi: 10.1016/j.jaccao.2020.02.011
142. Mahmood SS, Fradley MG, Cohen JV, Nohria A, Reynolds KL, Heinzerling LM, et al. Myocarditis in patients treated with immune checkpoint inhibitors. *J Am Coll Cardiol*. (2018) 71:1755–64. doi: 10.1016/j.jacc.2018.02.037
143. Brahmer JR, Lacchetti C, Thompson JA. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American society of clinical oncology clinical practice guideline summary. *J Oncol Pract*. (2018) 14:247–9. doi: 10.1200/JOP.18.00005
144. Hu JR, Florido R, Lipson EJ, Naidoo J, Ardehali R, Tocchetti CG, et al. Cardiovascular toxicities associated with immune checkpoint inhibitors. *Cardiovasc Res*. (2019) 115:854–68. doi: 10.1093/cvr/cvz026
145. Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med*. (2014) 6:224ra25. doi: 10.1126/scitranslmed.3008226
146. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med*. (2014) 371:1507–17. doi: 10.1056/NEJMoa1407222
147. Le RQ, Li L, Yuan W, Shord SS, Nie L, Habtemariam BA, et al. FDA approval summary: tocilizumab for treatment of chimeric antigen receptor T cell-induced severe or life-threatening cytokine release syndrome. *Oncologist*. (2018) 23:943–7. doi: 10.1634/theoncologist.2018-0028
148. Abboud R, Keller J, Slade M, DiPersio JF, Westervelt P, Rettig MP, et al. Severe cytokine-release syndrome after t cell-replete peripheral blood haploidentical donor transplantation is associated with poor survival and anti-IL-6 therapy is safe and well tolerated. *Biol Blood Marrow Transplant*. (2016) 22:1851–60. doi: 10.1016/j.bbmt.2016.06.010
149. Luo P, Liu Y, Qiu L, Liu X, Liu D, Li J. Tocilizumab treatment in COVID-19: a single center experience. *J Med Virol*. (2020) 92:814–8. doi: 10.1002/jmv.25801
150. Sciascia S, Aprà F, Baffa A, Baldovino S, Boaro D, Boero R, et al. Pilot prospective open, single-arm multicentre study on off-label use of tocilizumab in patients with severe COVID-19. *Clin Exp Rheumatol*. (2020) 38:529–32.
151. Guaraldi G, Meschiari M, Cozzi-Lepri A, Milic J, Tonelli R, Menozzi M, et al. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. *Lancet Rheumatol*. (2020) 2:e474–84. doi: 10.1016/S2665-9913(20)30173-9
152. Kewan T, Covut F, Al-Jaghbeer MJ, Rose L, Gopalakrishna KV, Akbik B. Tocilizumab for treatment of patients with severe COVID-19: a retrospective cohort study. *EClinicalMedicine*. (2020) 24:100418. doi: 10.1016/j.eclinm.2020.100418
153. Alvi RM, Frigault MJ, Fradley MG, Jain MD, Mahmood SS, Awadalla M, et al. Cardiovascular events among adults treated with chimeric

antigen receptor T-cells (CAR-T). *J Am Coll Cardiol*. (2019) 74:3099–108. doi: 10.1016/j.jacc.2019.10.038
154. Maude SL, Barrett D, Teachey DT, Grupp SA. Managing cytokine release syndrome associated with novel T cell-engaging therapies. *Cancer J*. (2014) 20:119–22. doi: 10.1097/PPO.0000000000000035
155. Zhu C, Wei Y, Wei X. AXL receptor tyrosine kinase as a promising anti-cancer approach: functions, molecular mechanisms and clinical applications. *Mol Cancer*. (2019) 18:153. doi: 10.1186/s12943-019-1090-3
156. Morizono K, Xie Y, Olafsen T, Lee B, Dasgupta A, Wu AM, et al. The soluble serum protein Gas6 bridges virion envelope phosphatidylserine to the TAM receptor tyrosine kinase Axl to mediate viral entry. *Cell Host Microbe*. (2011) 9:286–98. doi: 10.1016/j.chom.2011.03.012
157. Fujimori T, Grabiec AM, Kaur M, Bell TJ, Fujino N, Cook PC, et al. The Axl receptor tyrosine kinase is a discriminator of macrophage function in the inflamed lung. *Mucosal Immunol*. (2015) 8:1021–30. doi: 10.1038/mi.2014.129
158. Caldentey G, García De Frutos P, Cristóbal H, Garabito M, Berruezo A, Bosch X, et al. Serum levels of growth arrest-specific 6 protein and soluble AXL in patients with ST-segment elevation myocardial infarction. *Eur Heart J Acute Cardiovasc Care*. (2019) 8:708–16. doi: 10.1177/2048872617740833
159. National Institutes of Health. *Coronavirus Disease 2019 (COVID-19) Treatment Guidelines*. (2020). Available online at: https://www.covid19treatmentguidelines.nih.gov/ (accessed October 01, 2020).
160. Fatunde OA, Brown SA. The role of CYP450 drug metabolism in precision cardio-oncology. *Int J Mol Sci*. (2020) 21:604. doi: 10.3390/ijms21020604
161. Iacovelli R, Verri E, Cossu Rocca M, Aurilio G, Cullurà D, De Cobelli O, et al. The incidence and relative risk of cardiovascular toxicity in patients treated with new hormonal agents for castration-resistant prostate cancer. *Eur J Cancer*. (2015) 51:1970–7. doi: 10.1016/j.ejca.2015.06.106
162. Lu-Yao G, Nikita N, Keith SW, Nightingale G, Gandhi K, Hegarty SE, et al. Mortality and hospitalization risk following oral androgen signaling inhibitors among men with advanced prostate cancer by pre-existing cardiovascular comorbidities. *Eur Urol*. (2020) 77:158–66. doi: 10.1016/j.eururo.2019.07.031
163. Saltalamacchia G, Frascaroli M, Bernardo A, Quaquarini E. Renal and cardiovascular toxicities by new systemic treatments for prostate cancer. *Cancers (Basel)*. (2020) 12:1750. doi: 10.3390/cancers12071750
164. Angiolillo DJ, Fernández-Ortiz A, Bernardo E, Barrera Ramírez C, Sabaté M, Fernandez C, et al. Platelet aggregation according to body mass index in patients undergoing coronary stenting: should clopidogrel loading-dose be weight adjusted? *J Invasive Cardiol*. (2004) 16:169–74.
165. Yang K. What do we know about remdesivir drug interactions? *Clin Transl Sci*. (2020) 13:842–4. doi: 10.1111/cts.12815
166. 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
167. 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
168. Siegel D, Hui HC, Doerffler E, Clarke MO, Chun K, Zhang L, et al. Discovery and synthesis of a phosphoramidate prodrug of a pyrrolo[2,1-f][triazin-4-amino] adenine C-nucleoside (GS-5734) for the treatment of ebola and emerging viruses. *J Med Chem*. (2017) 60:1648–61. doi: 10.1021/acs.jmedchem.6b01594
169. Paranjpe I, Fuster V, Lala A, Russak A, Glicksberg BS, Levin MA, et al. Association of treatment dose anticoagulation with in-hospital survival among hospitalized patients with COVID-19. *J Am Coll Cardiol*. (2020) 76:122–4. doi: 10.1016/j.jacc.2020.05.001
170. Farge D, Frere C, Connors JM, Ay C, Khorana AA, Munoz A, et al. 2019 international clinical practice guidelines for the treatment and prophylaxis of venous thromboembolism in patients with cancer. *Lancet Oncol*. (2019) 20:e566–81. doi: 10.1016/S1470-2045(19)30750-8
171. Key NS, Bohlke K, Falanga A. Venous thromboembolism prophylaxis and treatment in patients with cancer: ASCO clinical practice guideline update summary. *J Oncol Pract*. (2019) 15:661–4. doi: 10.1200/JOP.19.00368
172. 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
173. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*. (2005) 436:112–6. doi: 10.1038/nature03712
174. Khan A, Benthin C, Zeno B, Albertson TE, Boyd J, Christie JD, et al. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit Care*. (2017) 21:234. doi: 10.1186/s13054-017-1823-x
175. Zhang P, Zhu L, Cai J, Lei F, Qin JJ, 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) 126:1671–81. doi: 10.1161/CIRCRESAHA.120.317242
176. 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
177. 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
178. 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
179. de Abajo FJ, Rodríguez-Martín S, Lerma V, Mejía-Abril G, Aguilar M, García-Luque A, 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
180. Alashi A, Lang R, Seballos R, Feinleib S, Sukol R, Cho L, et al. Reclassification of coronary heart disease risk in a primary prevention setting: traditional risk factor assessment. *Cardiovasc Diagn Ther*. (2019) 9:214–20. doi: 10.21037/cdt.2019.04.05
181. 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. *medRxiv*. (2020). doi: 10.1101/2020.05.17.20104943
182. Stawiski EW, Diwanji D, Suryamohan K, Gupta R, Fellouse FA, Sathirapongsasuti F, et al. Human ACE2 receptor polymorphisms predict SARS-CoV-2 susceptibility. *bioRxiv*. (2020). doi: 10.1101/2020.04.07.024752
183. Calcagnile M, Forgez P, Iannelli A, Bucci C, Alifano M, Alifano P. ACE2 polymorphisms and individual susceptibility to SARS-CoV-2 infection: insights from an in silico study. *bioRxiv*. (2020). doi: 10.1101/2020.04.23.057042
184. Gulati G, Heck SL, Ree AH, Hoffmann P, Schulz-Menger J, Fagerland MW, et al. Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2 × 2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol. *Eur Heart J*. (2016) 37:1671–80. doi: 10.1093/eurheartj/ehw022
185. Sommerstein R, Kochen MM, Messerli FH, Grani C. Coronavirus disease 2019 (COVID-19): do angiotensin-converting enzyme inhibitors/angiotensin receptor blockers have a biphasic effect? *J Am Heart Assoc*. (2020) 9:e016509. doi: 10.1161/JAHA.120.016509
186. Cardiology ACo. HFSA/ACC/AHA statement addresses concerns re: using RAAS antagonists in COVID-19. *J Card Fail*. (2020) 26:370. doi: 10.1016/j.cardfail.2020.04.013
187. Simone Gd. *Position Statement of the ESC Council on Hypertension on ACE-Inhibitors and Angiotensin Receptor Blockers*. European Society of Cardiology (2020).
188. National Cancer Institute N. *COVID-19 and Its Implications for Thrombosis and Anticoagulation: ClinicalTrials.gov Identifier: NCT04317092*. (2020). Available online at: https://clinicaltrials.gov/ct2/show/NCT04317092 (accessed October 01, 2020).
189. Bart N. Lambrecht UH, Ghent. *ClinicalTrials.gov*. (2020). Available online at: https://www.clinicaltrials.gov/ct2/show/NCT04330638 (accessed April 24, 2020).

190. 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
191. 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
192. 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
193. 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
194. Zhang Z, He G, Filipowicz NA, Randall G, Belov GA, Kopek BG, et al. Host lipids in positive-strand RNA virus genome replication. *Front Microbiol*. (2019) 10:286. doi: 10.3389/fmicb.2019.00286
195. Heaton NS, Randall G. Dengue virus-induced autophagy regulates lipid metabolism. *Cell Host Microbe*. (2010) 8:422–32. doi: 10.1016/j.chom.2010.10.006
196. Parikh A, Kumar AA, Jahangir E. Cardio-oncology care in the time of COVID-19 and the role of telehealth. *JACC CardioOncol*. (2020) 2:356–8. doi: 10.1016/j.jaccao.2020.04.003
197. Calvillo-Arguelles O, Abdel-Qadir H, Ky B, Liu JE, Lopez-Mattei JC, Amir E, et al. Modified routine cardiac imaging surveillance of adult cancer patients and survivors during the COVID-19 pandemic. *JACC CardioOncol*. (2020) 2:345–9. doi: 10.1016/j.jaccao.2020.04.001
198. Karlamangla AS, Merkin SS, Crimmins EM, Seeman TE. Socio-economic and ethnic disparities in cardiovascular risk in the United States, 2001–2006. *Ann Epidemiol*. (2010) 20:617–28. doi: 10.1016/j.annepidem.2010.05.003
199. Rosamond WD, Chambless LE, Heiss G, Mosley TH, Coresh J, Whitsel E, et al. Twenty-two year trends in incidence of myocardial infarction, CHD mortality, and case-fatality in four US communities, 1987 to 2008. *Circulation*. (2012) 125:1848–57. doi: 10.1161/CIRCULATIONAHA.111.047480
200. Lackland DT. Racial differences in hypertension: implications for high blood pressure management. *Am J Med Sci*. (2014) 348:135–8. doi: 10.1097/MAJ.0000000000000308
201. Braithwaite D, Tammemagi CM, Moore DH, Ozanne EM, Hiatt RA, Belkora J, et al. Hypertension is an independent predictor of survival disparity between African-American and white breast cancer patients. *Int J Cancer*. (2009) 124:1213–9. doi: 10.1002/ijc.24054
202. Breathett K, Liu WG, Allen LA, Daugherty SL, Blair IV, Jones J, et al. African Americans are less likely to receive care by a cardiologist during an intensive care unit admission for heart failure. *JACC Heart Fail*. (2018) 6:413–20. doi: 10.1016/j.jchf.2018.02.015
203. Taylor AL, Ziesche S, Yancy C, Carson P, D'Agostino R, Ferdinand K, et al. Combination of isosorbide dinitrate and hydralazine in blacks with heart failure. *N Engl J Med*. (2004) 351:2049–57. doi: 10.1056/NEJMoa042934
204. Giblin EM, Adams KF, Jr, Hill L, Fonarow GC, Williams FB, Sharma PP, et al. Comparison of hydralazine/nitrate and angiotensin receptor neprilysin inhibitor use among black versus nonblack americans with heart failure and reduced ejection fraction (from CHAMP-HF). *Am J Cardiol*. (2019) 124:1900–6. doi: 10.1016/j.amjcard.2019.09.020
205. Frierson GM, Howard EN, DeFina LE, Powell-Wiley TM, Willis BL. Effect of race and socioeconomic status on cardiovascular risk factor burden: the cooper center longitudinal study. *Ethn Dis*. (2013) 23:35–42.
206. Liu Q, Leisenring WM, Ness KK, Robison LL, Armstrong GT, Yasui Y, et al. Racial/ethnic differences in adverse outcomes among childhood cancer survivors: the childhood cancer survivor study. *J Clin Oncol*. (2016) 34:1634–43. doi: 10.1200/JCO.2015.66.3567
207. Caplin DA, Smith KR, Ness KK, Hanson HA, Smith SM, Nathan PC, et al. Effect of population socioeconomic and health system factors on medical care of childhood cancer survivors: a report from the childhood cancer survivor study. *J Adolesc Young Adult Oncol*. (2017) 6:74–82. doi: 10.1089/jayao.2016.0016
208. Blumenthal D, Fowler EJ, Abrams M, Collins SR. Covid-19—implications for the health care system. *N Engl J Med*. (2020) 383:1483–8. doi: 10.1056/NEJMsb2021088

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Lipid Profile Features and Their Associations With Disease Severity and Mortality in Patients With COVID-19

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Background: Emerging studies have described and analyzed epidemiological, clinical, laboratory, and radiological features of COVID-19 patients. Yet, scarce information is available regarding the association of lipid profile features and disease severity and mortality.

Methods: We conducted a prospective observational cohort study to investigate lipid profile features in patients with COVID-19. From 9 February to 4 April 2020, a total of 99 patients (31 critically ill and 20 severely ill) with confirmed COVID-19 were included in the study. Dynamic alterations in lipid profiles were recorded and tracked. Outcomes were followed up until 4 April 2020.

Results: We found that high-density lipoprotein-cholesterol (HDL-C) and apolipoprotein A-1 (apoA-1) levels were significantly lower in the severe disease group, with mortality cases showing the lowest levels ($p < 0.0001$). Furthermore, HDL-C and apoA-1 levels were independently associated with disease severity (apoA-1: odds ratio (OR): 0.651, 95% confidence interval (CI): 0.456–0.929, $p = 0.018$; HDL-C: OR: 0.643, 95% CI: 0.456–0.906, $p = 0.012$). For predicting disease severity, the areas under the receiver operating characteristic curves (AUCs) of HDL-C and apoA-1 levels at admission were 0.78 (95% CI, 0.70–0.85) and 0.85 (95% CI, 0.76–0.91), respectively. For in-hospital deaths, HDL-C and apoA-1 levels demonstrated similar discrimination ability, with AUCs of 0.75 (95% CI, 0.61–0.88) and 0.74 (95% CI, 0.61–0.88), respectively. Moreover, patients with lower serum concentrations of apoA-1 (<0.95 g/L) or HDL-C (<0.84 mmol/l) had higher mortality rates during hospitalization (log-rank $p < 0.001$). Notably, levels of apoA-1 and HDL-C were inversely proportional to disease severity. The survivors of severe cases showed significant recovery of apoA-1 levels at the end of hospitalization (vs. midterm apoA-1 levels, $p = 0.02$), whereas the mortality cases demonstrated continuously lower apoA-1 levels throughout hospitalization. Correlation analysis revealed that apoA-1 and HDL-C levels were negatively correlated with both admission levels and highest concentrations of C-reactive protein and interleukin-6.

Conclusions: Severely ill COVID-19 patients featured low HDL-C and apoA-1 levels, which were strongly correlated with inflammatory states. Thus, low apoA-1 and HDL-C levels may be promising predictors for severe disease and in-hospital mortality in patients suffering from COVID-19.

Keywords: HDL-C, apoA-1, inflammation, lipid, COVID-19

INTRODUCTION

As Coronavirus Disease 2019 (COVID-19) continues to spread worldwide, millions of people across hundreds of countries have been impacted. Epidemiological data show that although most cases are mild, severely ill patients rapidly progress to acute respiratory disease, multi-organ failure, and septic shock, with a remarkably increased mortality rate. Therefore, early identification of risk factors for COVID-19 severity and progression is of great importance.

Mounting evidence suggests that an impaired immune function and hyper-inflammatory response are characteristics of COVID-19 severity and mortality (1–3). Systemic inflammation and sepsis are prevalent metabolic disorders accompanying severe COVID-19 (4). Furthermore, proteome analysis suggests that patients with severe COVID-19 display dysregulated lipid metabolism (5). Dyslipidemia is associated with damage to the immune, respiratory, and cardiovascular systems, along with high levels of proinflammatory cytokines. Furthermore, dyslipidemia is casually associated with increased risk of thrombotic complications, endothelial dysfunction, and higher platelet activity (6). Thus, lipid dysregulation may contribute to morbidity and mortality from COVID-19 infection. However, the characteristics and dynamic changes in lipid profiles in COVID-19 patients, as well as their predictive value in disease severity and mortality, remain largely unknown.

Here, we performed an observational cohort study to investigate the lipid profile features of patients with COVID-19 and illuminate the associations between lipid features and disease severity/mortality.

MATERIALS AND METHODS

Study Population

This observational cohort study prospectively included 99 COVID-19-confirmed inpatients treated from 9 February to 4 April 2020 in Leishenshan Hospital, an urgently constructed hospital designated for COVID-19 patients located in Wuhan, China. All patients were diagnosed with COVID-19 according to interim guidance provided by the World Health Organization (WHO) (7). COVID-19 severity was classified according to the Guidelines on the Diagnosis and Treatment of COVID-19 released by the National Health Commission of China (version 7). Criteria for severe cases included any of the following: (1) respiratory rate $\geq$ 30 per min; (2) blood oxygen saturation (SPO_2) $\leq$ 93% at rest; (3) partial pressure of arterial oxygen to fraction of inspired oxygen ratio <300; (4) more than 50% of lung infiltrates within 24–48 h; or (5) patients needing mechanical respiratory support or presenting with septic shock or multi-organ dysfunction or failure. All patients had a definite outcome (discharged, continued treatment, deceased) before data analysis.

Data Collection

Time from symptom onset to hospitalization and length of hospital stay were recorded. All epidemiological, clinical, laboratory, and outcome data were collected with standardized data collection forms from the electronic medical records system at Leishenshan Hospital. Personal history, including comorbidities, was confirmed with patients or family members. For information not available from the electronic medical records, researchers also communicated directly with patients or their families to obtain additional epidemiological and symptom data. Lipid profiles, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), apolipoprotein A-1 (apoA-1), and apolipoprotein B (apoB), were first determined within 24 h of admission. A subset of patients had multiple lipid and cytokine metrics (i.e., collected more than once); therefore, these data were included for longitudinal analysis. Dynamic alterations in the above indicators were recorded. The Sequential Organ Failure Assessment (SOFA) score (https://www.mdcalc.com/sequential-organ-failure-assessmment-sofa-score) were calculated for each participant on admission. Two researchers independently reviewed the forms to double-check the data collected.

Outcome Definition

Outcomes were followed up until 4 April 2020. The primary outcome in the study was defined as in-hospital death.

Statistics Analysis

No preliminary sample size calculation was evaluated, considering the observational nature of our study about this emerging infectious disease. Continuous variables were expressed as medians with interquartile ranges (IQR) and compared using unpaired Student's *t*-test or Mann-Whitney U test. Categorical data were expressed as absolute values and percentages and were compared using chi-square or Fisher's exact tests. Univariate and multivariable analyses were conducted to examine the associations between lipids and disease severity. To assess the discrimination ability of each lipid marker for outcome, receiver operating characteristic (ROC) curves were calculated, and the optimal cutoff values were determined by maximizing the Youden index. Spearman tests were used to analyze the correlations between lipids and inflammatory factors. Survival differences among groups with different lipid concentrations were compared by Kaplan-Meier analysis using

the log-rank test. Significance levels were set based on two-sided $\alpha < 0.05$. Data analyses were performed in statistical packages R (The R Foundation; http://www.r-project.org; version 3.6.1) and SPSS 22.0. Diagrams were plotted by GraphPad Prism 8.0 (GraphPad Software, USA).

RESULTS

Baseline Characteristics

A total of 99 laboratory-confirmed COVID-19 patients were prospectively enrolled in this study. As shown in **Table 1**, the median time from symptom onset to admission was comparable between mild and severe cases [20.00 (IQR: 14.00–26.00) days vs. 19.00 (IQR, 10.25–30.00) days, $p = 0.841$] as well as between severe-surviving and severe-non-surviving groups [20.00 (IQR: 10.50–30.00) days vs. 17.00 (IQR, 10.00–30.00) days, $p = 0.663$]. Compared with mild cases, severely ill patients were older (severe: median 70.5 years: IQR, 61.3–81.8 vs. mild: 52 years: IQR, 42.0–62.0) and more likely to have comorbidities (severe: 84% vs. mild: 59.2%) and higher SOFA scores (severe: median, 5, IQR, 2–7 vs. mild: median, 0, IQR, 0–1). No sex differences were found between the mild and severe groups. Fourteen patients received mechanical ventilation in the severe group, whereas no mechanical ventilation was used in the mild cases. A total of 15 severe group patients died in hospital. Mechanical ventilation was more frequently applied among non-survivors. Severe-non-surviving cases presented significantly higher SOFA scores (median, 8.00, IQR, 7.50–10.00) than severe-surviving cases (median, 3.00, IQR, 1.25–5.00). Statin and antiviral treatment were similar among the groups. However, corticosteroid and antibiotic use differed significantly between severe and mild patients. Of note, more deceased patients received corticosteroid therapy compared with severe-surviving patients. The time from symptom onset to admission was comparable between the mild and severe groups [20 IQR (14–26) days vs. 19 IQR (10.25–30) days, $p = 0.841$] as well as between the severe-surviving and severe-non-surviving groups [20 IQR (10.5–30) days vs. 17 IQR (10–30) days, $p = 0.663$]. Mild patients experienced a longer hospitalization stay compared to severe patients [20 IQR (15–25) days vs. 15 IQR (9–20.5) days, $p = 0.012$]. Length of hospitalization was similar between the severe-surviving and severe-non-surviving groups [15 IQR (9–22.5) days vs. 15 IQR (10–18.5) days, $p = 0.706$].

Laboratory Parameters and Lipid Variation on Admission

For major laboratory characteristics, mild and severe COVID-19 cases demonstrated significant deviation in terms of blood cell proportions, coagulation functions, cardiac and renal functions, inflammatory indicators, and lipid profiles. Hierarchical clustering was performed to visualize the differences in laboratory parameters between mild and severe COVID-19 patients. The resulting heatmap illustrated different enrichment in blood indicators between mild and severe cases (**Figure 1**, **Supplementary Figure 1**). Notably, inflammatory cytokines, which are organ injury-associated indicators, were found at higher concentrations in the severe cases, whereas certain blood indicators, including lymphocytes, erythrocytes, hemoglobin, and albumin, were higher in the mild group.

In terms of lipid profiles, we detected lower concentrations of HDL-C, apoA-1, LDL-C, and TC in the severe group compared with the mild group **(Figures 2A–D)**. The TG level was significantly increased in the severe-non-surviving cases compared with the severe-surviving cases (**Figure 2E**), while HDL-C, apoA-1, LDL-C, TC and apoB concentrations were comparable between these two groups **(Figures 2A–D,F)**.

Lipid Profiles and Risk of Severe Condition

Based on the distinct lipid profile features between the severe and mild cases, we performed univariate and multivariate logistic regression analyses to explore the associations between lipid concentrations and disease severity. According to univariate analysis, TC, HDL-C, and apoA-1 levels were associated with severe disease as both continuous and categorical variables (divided by tertiles), whereas LDL-C and TG did not reach statistical significance. Remarkably, based on multivariate analysis, we found that apoA-1 (OR: 0.651 95% CI: 0.456–0.929, $p = 0.018$) and HDL-C (OR: 0.643 95% CI: 0.456–0.906, $p = 0.012$) were still independently associated with severity after adjusting for well-recognized risk factors: i.e., age and albumin, D-dimer, C-reactive protein (CRP), and interleukin-6 (IL-6) levels (**Table 2**). Moreover, patients with the highest tertile of HDL-C and apoA-1 displayed the lowest risk for severe COVID-19. Even after considering comorbidities and SOFA scores for further adjustment, apoA-1 and HDL-C levels remained independently associated with severe status of the disease (**Supplementary Table 1**). The ROC curves confirmed the significant predictive value of HDL-C and apoA-1 for the presence of severe cases. As shown in **Table 3**, apoA-1 $\leq$ 1.16 g/L predicted severity with a specificity of 0.86, sensitivity of 0.66, and area under ROC curve (AUC) of 0.85 (95% CI: 0.76–0.91; $p < 0.001$). An optimal serum HDL-C cut-off of 1.00 mmol/L provided diagnostic specificity and sensitivity of 75.5 and 68.2%, respectively, for severe cases. TC also displayed prognostic capability, but LDL-C, apoB, and TG showed weak discrimination of the severe condition.

Association of Lipid Biomarkers With COVID-19 Mortality

We further detected the predictive performance of lipid profiles for in-hospital death. Notably, ROC analysis revealed that HDL-C and apoA-1 remained valuable for predicting in-hospital death. At a threshold of 0.95 g/L, the AUC of the ROC curve of apoA-1 for death was 0.74 (95% CI 0.61–0.88, $p = 0.002$). With a cut-off of 0.84 mmol/L, the AUC of HDL-C for death was 0.75 (95% CI: 0.61–0.88, $p = 0.002$) (**Table 4**). Moreover, the Kaplan-Meier survival curves and log-rank tests demonstrated that patients with lower apoA-1 or HDL-C levels had a higher rate of in-hospital mortality (divided according to the best threshold) **(Figure 3)**.

Dynamic Alterations in Lipid Profiles and Associations With Inflammatory Indicators

Figure 4 shows the changes in inflammatory factors and lipid profiles in the mild, severe-surviving, and severe-non-surviving

TABLE 1 | Clinical characteristics and laboratory assessments in COVID-19 patients.

	Mild ($n = 49$)	Severe($n = 50$)	p-value	Severe ($n = 50$)		p-value
				Severe-surviving ($n = 35$)	Severe-non-surviving ($n = 15$)	
Age, years	52.00 (42.00–62.00)	70.50 (61.25–80.75)	<0.001	69.00 (61.50–80.50)	73.00 (63.50–78.50)	0.695
Male, *n*%	26 (53.06%)	34 (68.00%)	0.128	26 (74.29%)	8 (53.33%)	0.191
SOFA score	0 (0–1)	5.0 (2.0–7.0)	<0.001	3.00 (1.25–5.00)	8.00 (7.50–10.00)	<0.001
Mechanical ventilation, n%	0 (0.00%)	15 (30.00%)	<0.001	5 (14.29%)	10 (66.67%)	<0.001
Symptom to admission duration, days	20.00 (14.00–26.00)	19.00 (10.25–30.00)	0.841	20.00 (10.50–30.00)	17.00 (10.00–30.00)	0.663
Length of hospitalization, days	20.00 (15.00–25.00)	15.00 (9.00–20.50)	0.012	15.00 (9.00–22.50)	15.00 (10.00–18.50)	0.706
Symptom						
- Fever, *n*%	38 (77.55%)	28 (56.0%)	0.023	20 (57.14%)	8 (53.33%)	0.804
- Diarrhea, *n*%	9 (18.37%)	6 (12.0%)	0.377	6 (17.14%)	0 (0.00%)	0.160
- Fatigue, *n*%	13 (26.53%)	19 (38.0%)	0.222	16 (45.71%)	3 (20.00%)	0.117
- Cough, *n*%	29 (59.18%)	26 (52.0%)	0.472	19 (54.29%)	7 (46.67%)	0.760
- Chest pain, *n*%	19 (38.78%)	23 (46.0%)	0.467	18 (51.43%)	5 (33.33%)	0.355
- Dyspnea, *n*%	13 (26.53%)	24 (48.0%)	0.027	21 (60.00%)	3 (20.00%)	0.014
Comorbidities, *n*%	29 (59.18%)	42 (84.0%)	0.006	27 (77.14%)	15 (100.00%)	0.086
- Diabetes, *n*%	7 (14.29%)	24 (48.00%)	<0.001	16 (45.71%)	7 (46.67%)	1.000
- Hypertension, *n*%	18 (36.73%)	28 (56.00%)	0.085	19 (54.29%)	9 (60.00%)	0.765
- Pulmonary disease, *n*%	5 (10.20%)	6 (12.00%)	0.563	4 (11.43%)	2 (13.33%)	0.849
- Heart failure, *n*%	3 (6.12%)	14 (26.00%)	0.007	11 (31.43%)	3 (20.00%)	0.507
- CKD, *n*%	0 (0.00%)	16 (32.00%)	<0.001	11 (31.43%)	5 (33.33%)	1.000
- CAD, *n*%	1 (2.04%)	13 (26.00%)	<0.001	10 (28.57%)	3 (20.00%)	0.728
- Tumor, *n*%	3 (6.12%)	4 (8.00%)	0.716	2 (5.71%)	2 (13.33%)	0.574
- Autoimmune disease, *n*%	0 (0.00%)	2 (4.00%)	0.157	1 (2.86%)	1 (6.67%)	0.514
- Dyslipidemia, *n*%	4 (8.16%)	8 (16.00%)	0.147	4 (11.43%)	4 (26.67%)	0.178
Laboratory findings						
- Leukocytes × 10^9/L	5.68 (4.67–7.02)	7.42 (5.27–10.41)	<0.001	7.33 (5.68–9.68)	9.69 (5.00–14.82)	0.403
- Neutrophil × 10^9/L	3.04 (2.61–3.94)	6.01 (3.96–8.91)	<0.001	5.64 (3.96–7.28)	8.00 (4.26–11.82)	0.182
- Lymphocyte × 10^9/L	1.66 (1.04–2.26)	0.83 (0.67–1.24)	<0.001	0.90 (0.71–1.33)	0.70 (0.28–0.89)	0.020
- Platelets × 10^9/L	199.00 (171.00–256.00)	199.00 (133.75–274.50)	0.378	216.00 (174.00–281.00)	117.00 (80.50–152.50)	0.003
- Erythrocytes × 10^{12}/L	4.13 (3.87–4.51)	3.35 (2.83–3.80)	<0.001	3.31 (2.88–3.77)	3.39 (2.55–3.74)	0.594
- Hemoglobin, g/L	128.00 (119.00–137.00)	103.00 (84.00–117.50)	<0.001	105.0–3.74 (84.50–120.00)	101.00 (84.00–112.00)	0.775
- CRP, mg/L	0.81 (0.52–2.61)	33.91 (9.14–82.47)	<0.001	22.66 (6.26–63.94)	69.53 (30.16–114.89)	0.014
- Procalcitonin, ng/mL	0.03 (0.02–0.04)	0.32 (0.09–1.04)	<0.001	0.16 (0.09–0.52)	0.87 (0.44–1.53)	0.017
- ESR, mm/H	12.00 (7.00–23.00)	43.00 (21.25–60.75)	<0.001	42.00 (21.50–59.50)	44.00 (17.00–67.50)	0.916
- SAA, mg/L	5.00 (5.00–5.30)	54.78 (13.61–214.33)	<0.001	35.44 (9.61–244.24)	102 (32.4–270.46)	0.016
- PT, s	11.40 (10.90–11.70)	12.10 (11.43–13.55)	<0.001	12.10 (11.35–13.60)	12.10 (11.55–14.40)	0.491
- INR	0.98 (0.93–1.01)	1.05 (0.98–1.18)	<0.001	1.05 (0.97–1.19)	1.05 (0.99–1.27)	0.484
- Fibrinogen, g/L	2.66 (2.40–2.95)	4.04 (3.21–5.60)	<0.001	3.99 (3.24–5.72)	4.75 (3.25–5.60)	0.832
- D-Dimer, mg/L	0.29 (0.15–0.59)	2.94 (1.64–4.09)	<0.001	2.31 (1.45–3.74)	4.03 (2.57–6.42)	0.088
- BNP, pg/mL	7.00 (6.00–13.87)	117.66 (28.15–342.00)	<0.001	119.79 (26.27–593.00)	115.22 (33.84–189.34)	0.695
- Hs-cTnI, ng/ml	0.01 (0.01–0.01)	0.03 (0.01–0.06)	<0.001	0.02 (0.01–0.06)	0.03 (0.03–0.06)	0.078
- ALT, μ/L	28.00 (19.00–42.00)	21.00 (12.50–29.50)	0.059	21.00 (13.00–27.00)	24.00 (16.50–36.50)	0.532
- AST,μ/L	20.00 (17.00–26.00)	24.00 (18.00–32.75)	0.053	22.00 (18.00–31.00)	28.00 (18.50–44.00)	0.385
- Albumin, g/L	38.10 (36.10–41.30)	30.50 (28.40–35.68)	<0.001	30.50 (28.80–34.55)	29.40 (25.10–34.90)	0.346
- TBIL, μmol/L	9.24 (7.40–12.70)	9.40 (6.55–14.10)	0.607	8.40 (6.35–11.65)	14.10 (7.25–18.10)	0.159
- Glucose, mmol/L	4.69 (4.38–5.03)	5.97 (4.88–8.20)	<0.001	5.73 (4.89–7.48)	6.69 (4.62–12.05)	0.498

(Continued)

TABLE 1 | Continued

	Mild ($n = 49$)	Severe($n = 50$)	p-value	Severe ($n = 50$)		p-value
				Severe-surviving ($n = 35$)	Severe-non-surviving ($n = 15$)	
- BUN, mmol/L	4.70 (4.00–5.30)	8.70 (5.32–15.60)	<0.001	7.20 (4.60–11.05)	14.40 (8.80–37.40)	0.026
- Creatinine, μmol/L	60.20 (50.70–70.40)	82.20 (56.73–154.83)	<0.001	75.00 (56.25–108.45)	98.20 (68.20–235.20)	0.295
- Total cholesterol, mmol/L	4.52 (3.63–4.9)	3.51 (2.90–4.48)	<0.001	3.59 (2.98–4.48)	3.18 (2.58–4.25)	0.553
- Triglycerides, mmol/L	1.21 (0.81–1.80)	0.96 (0.70–1.62)	0.114	0.90 (0.70–1.38)	1.00 (0.82–2.71)	0.010
- LDL-C, mmol/L	2.57 (2.04–2.96)	2.16 (1.58–2.68)	0.016	2.19 (1.64–2.83)	1.76 (1.49–2.64)	0.494
- HDL-C, mmol/L	1.18 (1.00–1.42)	0.94 (0.74–1.12)	<0.001	0.97 (0.76–1.08)	0.77 (0.61–0.99)	0.112
- apoA-1, g/L	1.42 (1.22–1.64)	1.01 (0.79–1.23)	<0.001	1.03 (0.80–1.25)	0.84 (0.64–1.19)	0.277
- apoB, g/L	0.93 (0.79–1.08)	0.80 (0.69–1.14)	0.205	0.86 (0.73–1.14)	0.70 (0.66–1.07)	0.277
- IL.6, pg/mL	1.29 (0.75–3.37)	38.45 (12.59–80.07)	<0.001	23.84 (10.55–41.88)	124.90 (58.45–241.45)	<0.001
- IL.1β, pg/mL	3.00 (2.00–3.29)	3.75 (3.00–5.00)	0.009	3.00 (3.00–4.07)	5.00 (3.67–6.32)	0.023
- IL.8, pg/mL	6.00 (3.80–8.60)	16.70 (13.00–27.80)	<0.001	16.00 (11.50–22.00)	28.40 (19.50–49.00)	0.005
- IL.10, pg/mL	3.00 (2.00–3.56)	4.01 (3.00–8.97)	<0.001	4.00 (3.00–7.55)	8.20 (3.43–15.00)	0.146
- IL2R, U/mL	0.31 (0.22–0.43)	0.81 (0.57–1.65)	<0.001	0.72 (0.58–1.42)	1.56 (0.60–2.94)	0.147
- TNF α, pg/mL	6.50 (5.50–7.16)	10.61 (7.75–14.73)	<0.001	10.70 (7.45–14.38)	11.50 (8.50–19.45)	0.427
Treatment, *n*%						
Antibiotic therapy	17 (34.70%)	50 (100%)	<0.001	35 (100%)	15 (100%)	–
Antiviral therapy	47 (95.92%)	48 (96.00%)	0.984	34 (97.14%)	14 (93.33%)	0.529
Use of corticosteroids	0 (0%)	19 (38.00%)	<0.001	10 (28.57%)	9 (60.00%)	0.036
Statin	8 (16.32%)	15 (30.00%)	0.107	11 (31.42%)	4 (26.67%)	0.736

Categorical data are expressed as absolute values and percentages and were compared using chi-square or Fisher exact tests. Continuous variables were expressed as medians with interquartile ranges (IQR) and compared by unpaired Student's t-test or Mann-Whitney U test. AST, aspartate aminotransferase; ALT,alanine aminotransferase; BUN, blood urea nitrogen; BNP, brain natriuretic peptide; Hs-cTnI, hypersensitive troponin I;CRP, C-reactive protein; CKD, chronic kidney disease; CAD, coronary artery disease; ESR, erythrocyte sedimentation rate; SAA, serum amyloid A; IL, interleukin; IL2R, interleukin2 receptor; LDL-C, low-densitylipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; apoA-1, apolipoproteinA-1; apoB, apolipoproteinB; INR,international standard ratio; PT, prothrombin time; TNF α, tumor necrosis factorα; TBIL, total bilirubin; SOFA, Sequential Organ Failure Assessment.

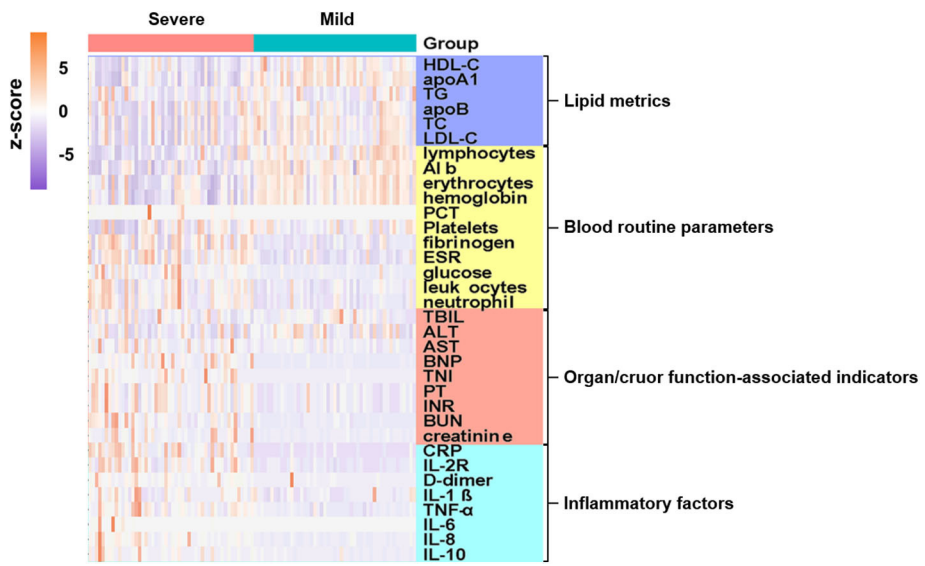

FIGURE 1 | Admission characteristics of laboratory parameters between mild and severe COVID-19 patients. Hierarchical clustering was applied based on laboratory parameters. Heatmap indicates enriched concentration of laboratory indicators in mild and severe cases. Levels of laboratory metrics were scaled by calculating z-scores (subtracting mean, then dividing by standard deviation of each row). Laboratory metrics were categorized into four major groups, i.e., lipid metrics, routine blood parameters, organ/cruor function-associated indicators, and inflammatory factors, with color bars on right side of plot indicating each analyte category. Y-axis represents laboratory values after z-scoring by row; x-axis represents individual cases. Annotations show severe cases in pink and mild cases in cyan.

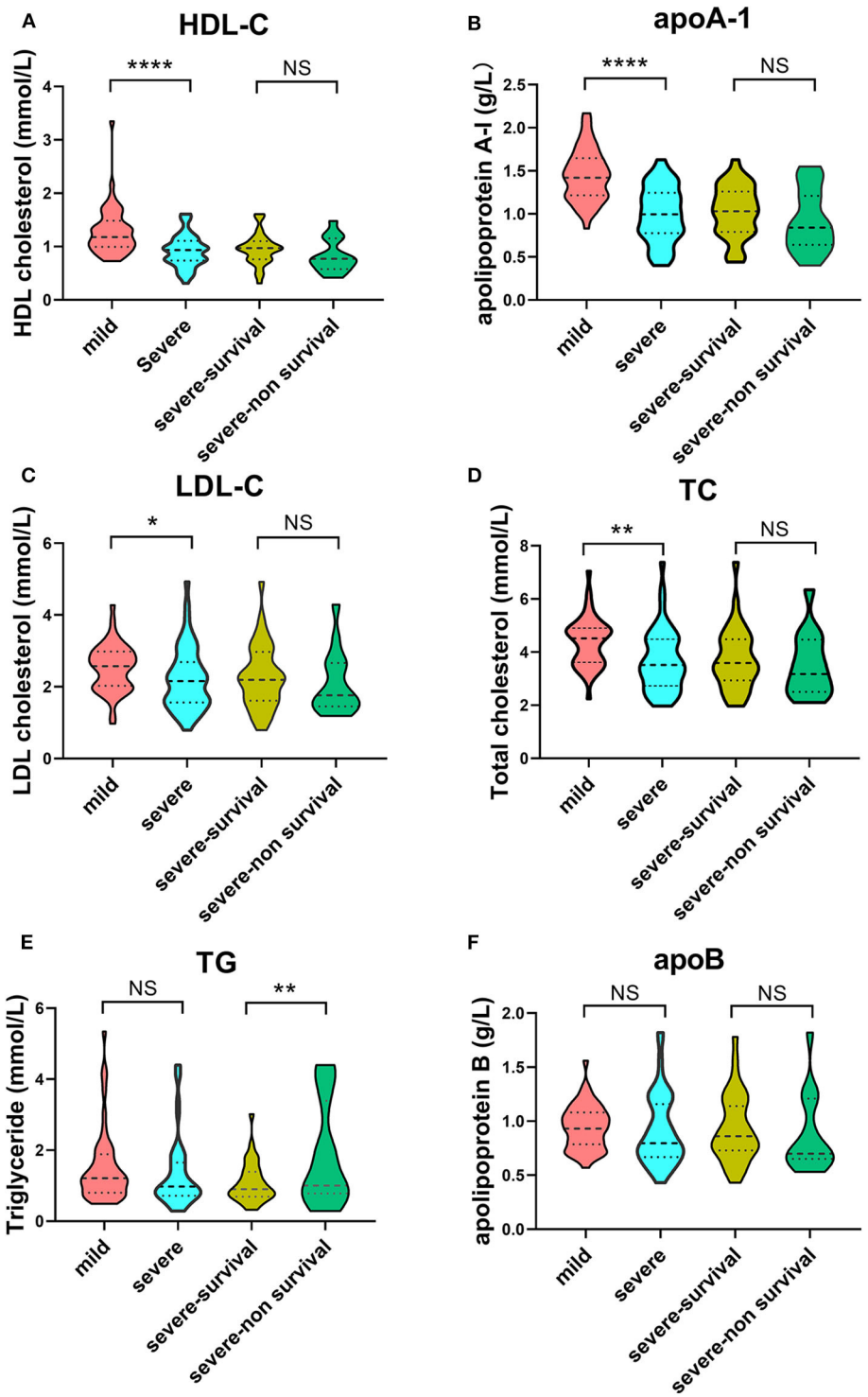

FIGURE 2 | Violin plots of lipid features of mild vs. severe and severe survivors vs. severe non-survivors. Plots demonstrate lipid concentration within each group. Horizontal dotted lines represent first and third quartiles; horizontal dashed lines within plot indicate median of lipid levels. Dunnett's test was applied to assess significance of differences with mild cases serving as the control. (****$p < 0.0001$, **$p < 0.01$, *$p < 0.05$).

TABLE 2 | Logistic regression analysis for severity in COVID-19 patients.

	Univariate OR (95% CI)	*p*-value	Adjusted OR* (95% CI)	*p*-value
apoA-1 (10^{-1}g/L)	0.617 (0.507, 0.751)	<0.001	0.651 (0.456, 0.929)	0.018
apoA-1 group				
Q1 (4–10.4)	Ref		Ref	
Q2 (10.5–13.8)	0.126 (0.036, 0.443)	0.001	0.538 (0.059, 4.882)	0.581
Q3 (14.0–21.7)	0.036 (0.009, 0.136)	<0.001	0.066 (0.005, 0.823)	0.034
apoA-1 group trend		<0.001		0.023
HDL-C (10^{-1}mmol/L)	0.709 (0.602, 0.835)	<0.001	0.643 (0.456, 0.906)	0.012
HDL-C group				
Q1 (3.1–9.1)	Ref		Ref	
Q2 (9.3–11.78)	0.400 (0.139, 1.147)	0.090	0.264 (0.028, 2.469)	0.242
Q3 (11.8–33.5)	0.103 (0.033, 0.316)	<0.001	0.065 (0.005, 0.778) 0.03093	0.031
HDL-C group trend		<0.001		0.029
Total cholesterol (mmol/L)	0.505 (0.328, 0.776)	0.002	0.866 (0.425, 1.766)	0.693
TC group				
Q1 (1.97–3.44)	Ref		Ref	
Q2 (3.49–4.54)	0.301 (0.106, 0.860)	0.025	0.497 (0.079, 3.146)	0.458
Q3 (4.56–7.38)	0.120 (0.040, 0.362)	<0.001	0.338 (0.048, 2.360)	0.274
TC group trend		<0.001		0.281
Triglycerides (mmol/L)	0.808 (0.538, 1.214)	0.304	0.808 (0.269, 2.423)	0.703
TG group				
Q1 (0.29–0.82)	Ref		Ref	
Q2 (0.84–1.43)	0.778 (0.295, 2.051)	0.611	1.212 (0.242, 6.079)	0.815
Q3 (1.44–5.35)	0.648 (0.244, 1.724)	0.385	1.006 (0.119, 8.472)	0.996
TG group trend		0.402		0.993
LDL-C (mmol/L)	0.588 (0.343, 1.007)	0.053	1.281 (0.508, 3.230)	0.599
LDL-C group				
Q1 (0.79–1.96)	Ref		Ref	
Q2 (2.01–2.68)	0.471 (0.174, 1.273)	0.137	1.614 (0.208, 12.524)	0.647
Q3 (2.70–4.93)	0.286 (0.104, 0.787)	0.015	1.709 (0.241, 12.144)	0.592
LDL-C group trend		0.01545		0.62094
apoB (g/L)	0.638 (0.147, 2.766)	0.548	3.908 (0.279, 54.710)	0.311
apoB group				
Q1 (0.43–0.76)	Ref		Ref	
Q2 (0.77–1.02)	0.300 (0.108, 0.830)	0.02	1.022 (0.147, 7.105)	0.982
Q3 (1.03–1.82)	0.444 (0.165, 1.194)	0.108	1.706 (0.270, 10.797)	0.57
apoB group trend		0.145		0.512

*Logistic regression was used to determine association between lipid profile with severity of COVID-19. *Adjusted for age and albumin, D-dimer, CRP, and IL-6 levels. LDL-C, low-densitylipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; apoA-1, apolipoproteinA-1; apoB, apolipoprotein B; TG, triglycerides; TC, total cholesterol.*

groups from hospital admission, mid-term hospitalization, and end of hospitalization. As illustrated in **Figures 4A,B**, throughout hospitalization, CRP and IL-6 levels were significantly and continuously high in the severe-surviving and mortality cases but showed low levels among mild cases. Notably, compared with that in the severe-surviving group, both CRP and IL-6 levels in mortality cases were significantly higher at the end of hospitalization ($p < 0.05$).

On admission, regardless of severity or outcome, most patients presented comparable TG and LDL-C levels (**Figures 4C,D**). By the end of hospitalization, however, TG levels displayed a slight upward trend in the mortality cases and were significantly higher than that in the severe survivors ($p = 0.013$); in addition, LDL-C levels were significantly lower in severe survivors and non-survivors compared to that in the mild cases (both $p < 0.01$). Levels of apoA-1 and HDL-C were inversely proportional to disease severity, with mortality cases showing continuously lower levels across hospitalization (**Figures 4E,F**). Of note, after a slight downward trend in mid-term apoA-1 levels, severe survivors showed a significant recovery in apoA-1 levels at the end of hospitalization (vs. mid-term apoA-1 levels, $p = 0.02$). By the end of hospitalization, the lowest apoA-1 levels were found in severe cases with a fatal outcome ($p < 0.01$). For TC and apoB, no significant differences

TABLE 3 | Diagnostic values of lipid profiles in assessment of COVID-19 severity.

	AUC (95% CI)	Best threshold	Specificity	Sensitivity	*p*-value
apoA-1	0.85 (0.76–0.91)	1.16	0.86	0.66	<0.001
HDL-C	0.78 (0.69–0.85)	1.00	0.76	0.68	<0.001
TC	0.71 (0.61–0.81)	3.24	0.94	0.42	<0.001
apoB	0.58 (0.46–0.68)	0.78	0.78	0.46	0.192
LDL-C	0.62 (0.52–0.76)	1.78	0.92	0.40	0.016
TG	0.59 (0.46–0.70)	1.13	0.61	0.62	0.126
apoA-1 + HDL-C	0.85 (0.77–0.92)	–	0.86	0.66	<0.001

AUC, area under the curve; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; apoA-1, apolipoprotein A-1; apoB, apolipoprotein B; TG, triglycerides; TC, total cholesterol.

TABLE 4 | Diagnostic values of lipid profiles in assessment of COVID-19 mortality.

	AUC (95% CI)	Best threshold	Specificity	Sensitivity	*p*-value
apoA-1	0.74 (0.61–0.88)	0.95	0.83	0.67	0.002
HDL-C	0.75 (0.61–0.88)	0.84	0.81	0.73	0.002
apoB	0.62 (0.43–0.79)	0.71	0.85	0.53	0.093
LDL-C	0.64 (0.46–0.80)	1.83	0.80	0.60	0.054
TG	0.44 (0.27–0.61)	1.01	0.58	0.53	0.444
TC	0.66 (0.51–0.80)	3.18	0.83	0.53	0.040
apoA-1 + HDL-C	0.77 (0.63–0.90)	–	0.83	0.67	0.002

AUC, area under the curve; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; apoA-1, apolipoprotein A-1; apoB, apolipoprotein B; TG, triglycerides; TC, total cholesterol.

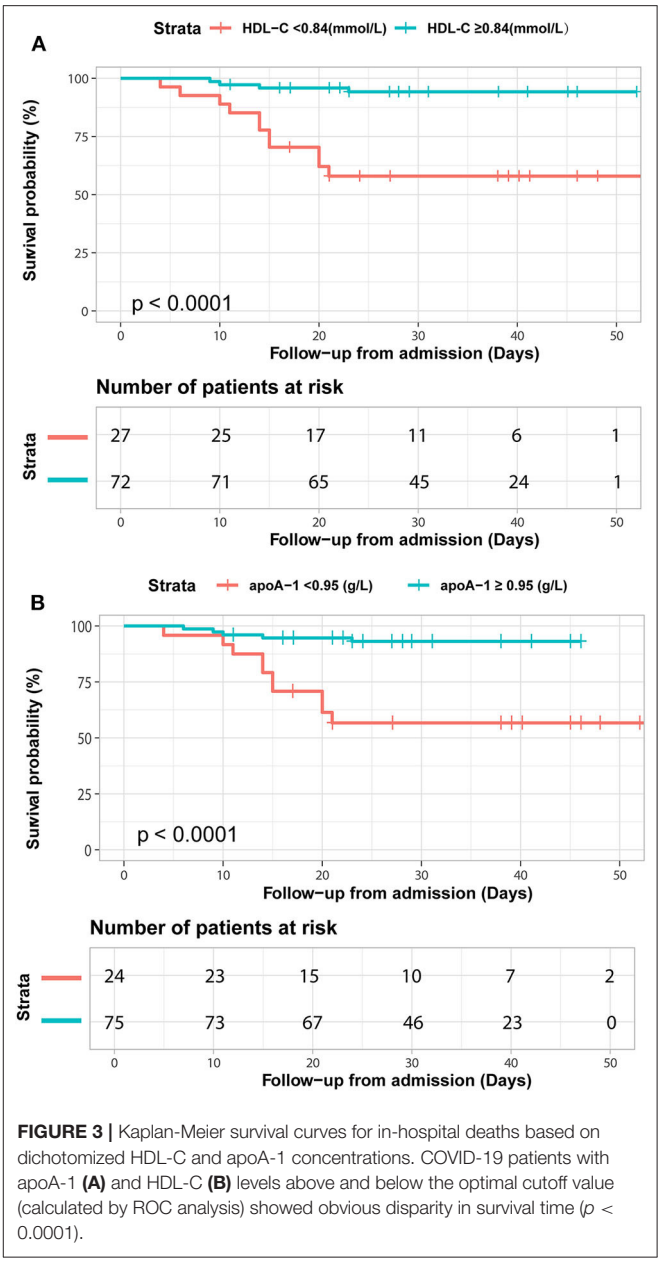

FIGURE 3 | Kaplan-Meier survival curves for in-hospital deaths based on dichotomized HDL-C and apoA-1 concentrations. COVID-19 patients with apoA-1 **(A)** and HDL-C **(B)** levels above and below the optimal cutoff value (calculated by ROC analysis) showed obvious disparity in survival time ($p < 0.0001$).

were observed among the three groups across the three time points (**Figures 4G,H**).

Correlation analysis was performed to detect potential factors related to lipid characteristics. As shown in **Figure 5A**, admission lipid profiles, especially apoA-1 and HDL-C, were negatively correlated with inflammatory factors, such as CRP and IL-6. Admission apoA-1 and HDL-C levels were inversely correlated with peak CRP and IL-6 concentrations during the clinical course of the disease (**Figure 5B**).

DISCUSSION

Our study highlighted an important association between lipid profiles and fatal clinical outcomes in COVID patients. The main findings are as follows: (1) COVID-19 patients in severe disease were characterized by decreased apoA-1 and HDL-C levels; (2) low apoA-1 and HDL-C levels on admission were able to predict COVID-19 severity and mortality during hospitalization; and (3) apoA-1 and HDL-C levels were strongly correlated with inflammatory indicators, and deviated markedly from the normal reference range in severe cases throughout the course of the disease.

Previous studies have shown that infection and sepsis are accompanied by a metabolic change in the lipid profile, featuring hypertriglyceridemia and reduced HDL-C levels in serum (4, 8). Lipid metabolism dysregulation has also been confirmed in septic patients secondary to both community and hospital-acquired pneumonia (9, 10). In the context of COVID-19, excessive cytokine activation in response to SARS-CoV-2 infection appears to contribute to multiple organ dysfunction. As a result, sepsis and septic shock are frequently observed complications in severe COVID-19 patients (11, 12). Therefore, it is not surprising that serum apoA-1 and HDL-C levels were lower in severely ill patients, especially non-survivors, compared to mild cases.

Both apoA-1 ($r = -0.55$; $p < 0.001$) and HDL-C ($r = -0.45$; $p < 0.001$) levels were negatively related to SOFA scores, a common diagnostic tool for identifying sepsis severity (13). Based on multivariate analyses, decreased apoA-1 and HDL-C levels

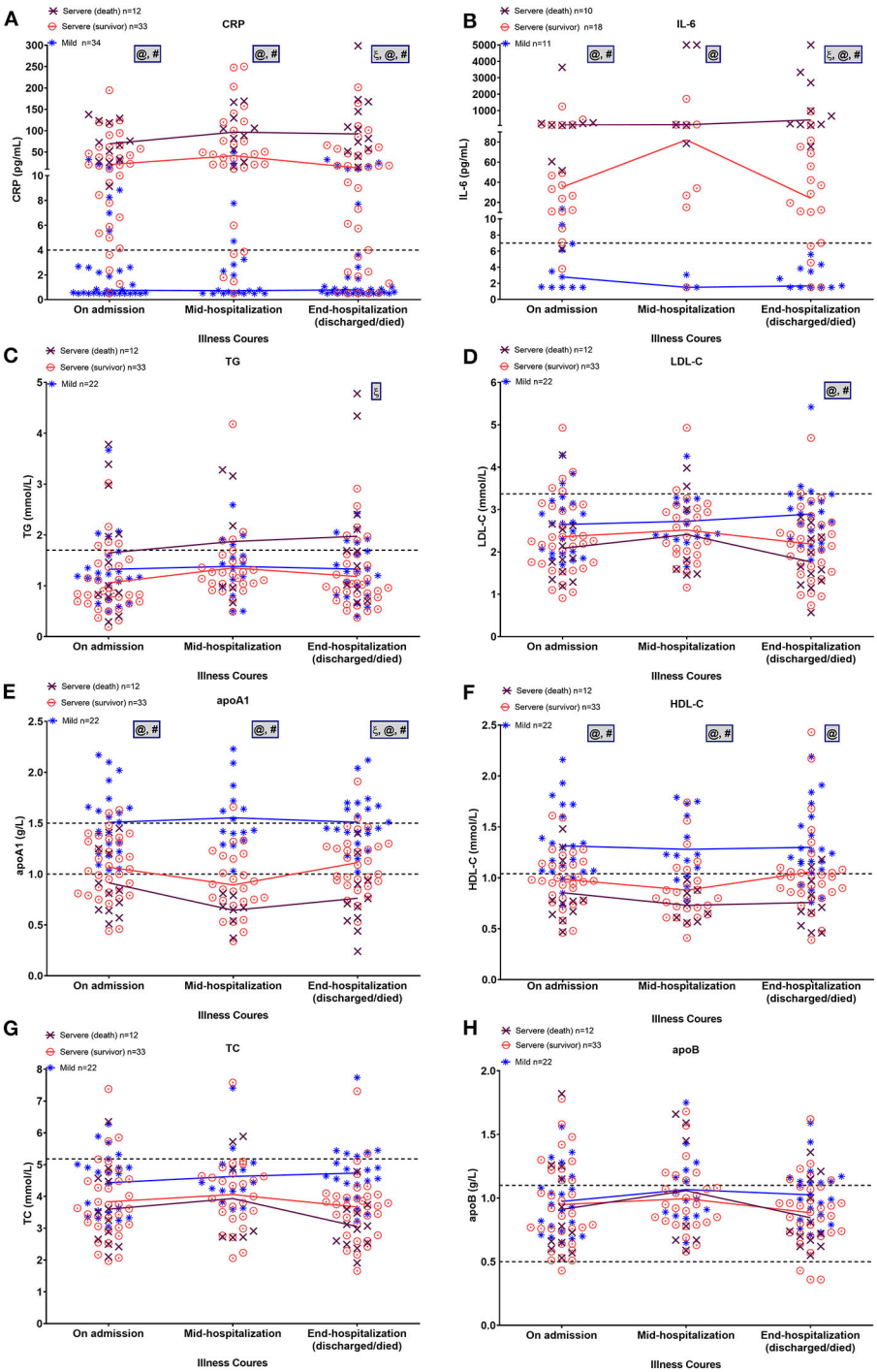

FIGURE 4 | Dynamic alterations in lipid and major laboratory markers from admission in COVID-19 patients. Temporal changes in CRP **(A)**, IL-6 **(B)**, TG **(C)**, LDL-C **(D)**, apoA-1 **(E)**, HDL-C **(F)**, TC **(G)**, and apoB **(H)** in a subset of COVID-19 patients with ≥2 longitudinal data across three time periods, including on admission, mid-hospitalization, and end of hospitalization. Horizontal dashed lines indicate normal reference range of factors. Mean values of normally distributed parameters (lipid metrics) and median values of non-normally distributed factors (CRP and IL-6) in each group at three time periods are linked by lines. Significant differences among three groups at each time point were compared using one-way ANOVA with Tukey's multiple comparisons test or Kruskal-Wallis test as appropriate. Statistical significance ($p < 0.05$) is indicated by ξ between severe (death) and severe (survivor) cases, @ between severe (death) and mild cases, and # between severe (survivor) and mild cases. IL-6, interleukin-6; CRP, C-reactive protein; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol, apoA-1, apolipoprotein A-1; apoB, apolipoprotein B.

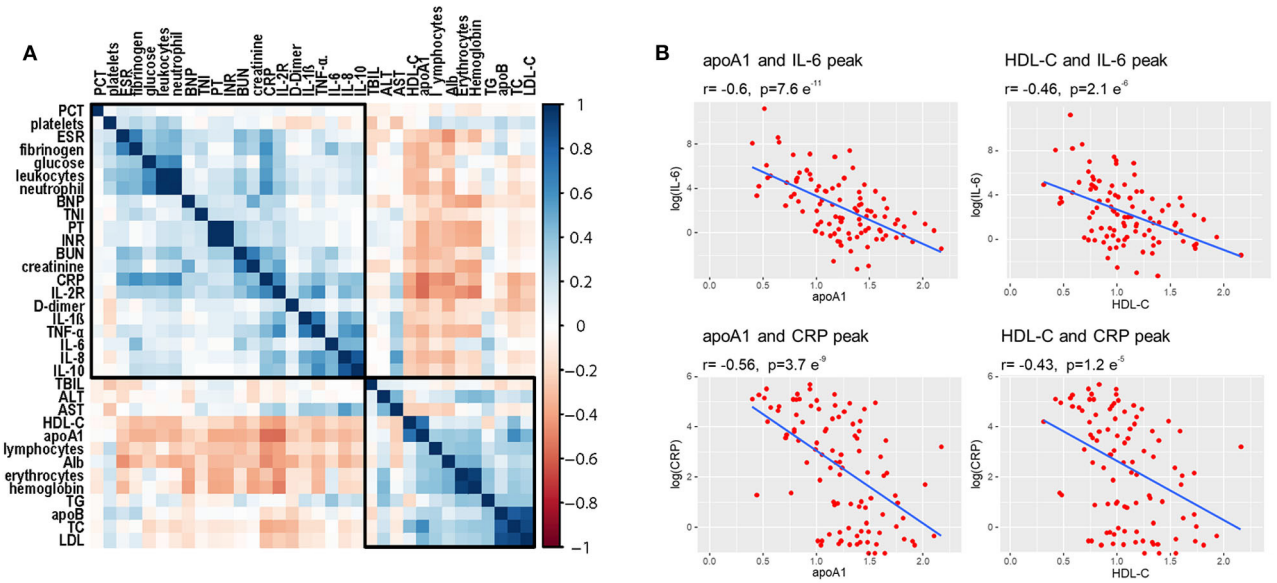

FIGURE 5 | Correlations among lipid profiles and laboratory parameters. **(A)** Heatmap values represent pairwise Spearman rank correlation coefficients. Blue indicates positive correlation, red indicates negative correlation. **(B)** Spearman correlation coefficient analysis shows that initial HDL-C and apoA-1 levels were significantly inversely correlated with peak values of CRP and IL-6 during disease course.

were independently associated with COVID-19 severity after adjusting for established indicators of severity, such as age, low albumin, and increased D-dimer, CRP, and IL-6 levels (14, 15). These covariates were included in the multivariate analysis due to their close association with sepsis development reported in previous studies (16, 17). In addition, ROC analysis illustrated that decreased apoA-1 and HDL-C levels were strong predictors of COVID-19 severity. In line with our findings, Groin et al. found that low serum HDL-C concentration on admission is a risk factor for the development of severe sepsis (18).

Our results also highlighted the predictive value of decreased HDL/apoA-1 levels on admission to in-hospital death in COVID-19 patients. Almost half of our research population developed into severe cases, with a relatively high mortality rate of 15.1%. This may be because Leishenshan Hospital was a designated hospital for treating complicated patients transferred from other local hospitals. Our study, for the first time, illustrated that in-hospital death increased significantly in patients with low serum apoA-1 (<0.95 g/L) or HDL-C (<0.84 mmol/L). In addition, ROC analysis verified the predictive value of HDL-C and apoA-1 levels for in-hospital death among COVID-19 patients. This is in agreement with previous study, which found that low apoA-1 concentration is independently associated with the 30-day mortality rate in septic patients (19). Interestingly, here, the temporal recording of lipid profiles showed that the initial decrease in apoA-1/HDL-C levels in survivors began to recover at the end of hospitalization. A similar tendency in HDL-C change has also been observed in patients recovering from sepsis (20). Here, however, apoA-1 rapidly deteriorated in non-survivors throughout the clinical course of the disease.

The underlying mechanisms of HDL-C reduction in severe COVID-19 patients and its association with increased mortality are not fully understood. HDL-C and its major structural protein (apoA-1) directly exert anti-inflammatory effects by neutralizing lipopolysaccharides (LPS), thus playing an important role in host resistance to bacterial, viral, and parasitic infection (21). The protective role of apoA-1 is also evidenced in acute lung injury and acute respiratory distress syndrome. Specifically, apoA-1-deficient mice exhibit enhanced recruitment of neutrophils and monocytes to airspace under LPS inhalation (22). However, both HDL-C and its beneficial effects can be disturbed by inflammation (23, 24). For example, pro-inflammatory cytokines like IL-6 and CRP directly inhibit apolipoprotein synthesis enzyme activity, resulting in reduced apoA-1 and HDL-C production (25). In our study, IL-6 and CRP concentrations were significantly higher in the severe group, and were negatively correlated with lipid indicators apoA-1 and HDL-C. We also found that serum amyloid A (SAA), an acute phase protein, was markedly increased in severe patients. SAA-enriched HDL is reported to clear more rapidly from circulation than normal HDL (26). Hence, the inflammatory-induced humoral innate response to scavenge lipoprotein from circulation may be another potential mechanism leading to low-HDL-C levels. As a result, a vicious cycle occurs in severely ill COVID-19 patients, with a deficiency in HDL-C resulting in cytokine overproduction and a further depletion of HDL-C.

In our study, TC and LDL-C levels in severe patients tended to follow a pattern similar to that of HDL-C. Low TC and LDL-C levels are considered as markers of malnutrition, as nutrition provides the basic substrate for cholesterol synthesis

(27). Furthermore, early enteral nutrition is reported to accelerate the recovery of TC levels (20). Consistently, the nutrition states of patients deteriorated in our study, as reflected by continuously decreased levels of albumin in the severe group. Like HDL-C, inflammatory mediators also participate in impaired LDL-C synthesis. Thus, hypocholesterolemia may reflect both malnutrition and an overactive inflammatory status in severe COVID-19 patients.

Although admission TG levels were comparable between mild and severe cases, TG levels were remarkably elevated in non-survivors. Serum TG frequently increases under a septic environment due to reduced TG hydrolysis. Inflammatory cytokines also contribute to inhibit LPL activity, overproduction of free fatty acid, and TG synthesis (26). Besides, after comparing the survival rates between four groups of patients stratified by TG and apoA-1 levels, we found that patients with lower apoA-1 levels and elevated TG levels displayed the unfavorable prognosis with the lowest survival rate (**Supplementary Figure 2**). Thus, we considered that elevated TG levels, together with persistently low lipoprotein cholesterol concentrations, might be a marker of uncontrolled inflammation and increased risk of death in COVID-19 patients. And further assessment in larger cohorts are required for validation.

STUDY LIMITATIONS

There are several limitations in our study. First, given the small sample size, to avoid overfitting, we only calculated the Kaplan-Meier survival curve to evaluate the prognostic values of apoA-1 and HDL-C but did not conduct multivariate cox regression to assess the independent prognostic values of these lipid metrics. Thus, further larger cohorts are warranted to verify our conclusions. Second, some patients were already in poor condition when transferred from the local hospital to Leishenshan Hospital, resulting in a higher rate of severe cases in our study. Further studies on outpatients and other mobile hospitals are required to provide a more complete picture of the relationship between lipid profiles and disease progression. Third, our study only focused on lipid concentrations rather than their quality. Therefore, whether lipid particle composition and functional alteration can affect COVID-19 outcomes deserves further investigation.

CONCLUSIONS

Lipid metabolism disorders, characterized by low HDL-C and apoA-1 levels, were found in severely ill COVID-19 patients. The altered HDL-C and apoA-1 levels were negatively correlated with inflammatory indicators. Low apoA-1 and HDL-C levels on admission exhibited predictive value in discriminating disease severity and mortality during hospitalization. Our study examined COVID-19 in regard to lipid metabolism, and thus provides new insights into the disease.

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 Commission of Renji Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JS and JP: conceived and designed the experiments. ZC, PN, HG, LS, FY, XQ, WW, MZ, XY, and YZ: collected and analyzed the data. JS, ZC, and JP: wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2020.584987/full#supplementary-material

REFERENCES

1. Verity R, Okell LC, Dorigatti I, Winskill P, Whittaker C, Imai N, et al. Estimates of the severity of coronavirus disease 2019: a model-based analysis. *Lancet Infect Dis.* (2020) 20:669–77. doi: 10.1016/S1473-3099(20)30243-7
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. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J Allergy Clin Immunol.* (2020) 146:110–18. doi: 10.1016/j.jaci.2020.04.006
4. Golucci A, Marson FAL, Ribeiro AF, Nogueira RJN. Lipid profile associated with the systemic inflammatory response syndrome and sepsis in critically ill patients. *Nutrition.* (2018) 55–6:7–14. doi: 10.1016/j.nut.2018.04.007
5. Shen B, Yi X, Sun Y, Bi X, Du J, Zhang C, et al. Proteomic and metabolomic characterization of COVID-19 patient sera. *Cell* (2020) 182:59–72.e15. doi: 10.1016/j.cell.2020.05.032

6. Sorokin AV, Karathanasis SK, Yang ZH, Freeman L, Kotani K, Remaley AT. Covid-19-associated dyslipidemia: implications for mechanism of impaired resolution and novel therapeutic approaches. *Faseb J.* (2020). doi: 10.1096/fj.202001451. [Epub ahead of print].
7. World Health Organization. *Clinical Management of Severe Acute Respiratory Infection When Novel Coronavirus (2019-nCoV) Infection is Suspected: Interim Guidance*. World Health Organization (2020). Available online at: https://apps.who.int/iris/handle/10665/330893
8. Tanaka S, Diallo D, Delbosc S, Genève C, Zappella N, Yong-Sang J, et al. High-density lipoprotein (HDL) particle size and concentration changes in septic shock patients. *Ann Intensive Care.* (2019) 9:68. doi: 10.1186/s13613-019-0541-8
9. Sharma NK, Ferreira BL, Tashima AK, Brunialti MKC, Torquato RJS, Bafi A, et al. Lipid metabolism impairment in patients with sepsis secondary to hospital acquired pneumonia, a proteomic analysis. *Clin Proteomics.* (2019) 16:29. doi: 10.1186/s12014-019-9252-2
10. Sharma NK, Tashima AK, Brunialti MKC, Ferreira ER, Torquato RJS, Mortara RA, et al. Proteomic study revealed cellular assembly and lipid metabolism dysregulation in sepsis secondary to community-acquired pneumonia. *Sci Rep.* (2017) 7:15606. doi: 10.1038/s41598-017-15755-1
11. 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). *Crit Care Med.* (2020) 48:e440–69. doi: 10.1097/CCM.0000000000004363
12. Li H, Liu L, Zhang D, Xu J, Dai H, Tang N, et al. SARS-CoV-2 and viral sepsis: observations and hypotheses. *Lancet.* (2020) 395:1517–20. doi: 10.1016/S0140-6736(20)30920-X
13. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA.* (2016) 315:801–10. doi: 10.1001/jama.2016.0287
14. 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) 201:1380–88. doi: 10.1164/rccm.202002-0445OC
15. 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:m1295. doi: 10.1136/bmj.m1091
16. Rodelo JR, De la Rosa G, Valencia ML, Ospina S, Arango CM, Gómez CI, et al. D-dimer is a significant prognostic factor in patients with suspected infection and sepsis. *Am J Emerg Med.* (2012) 30:1991–9. doi: 10.1016/j.ajem.2012.04.033
17. Pierrakos C, Vincent JL. Sepsis biomarkers: a review. *Crit Care.* (2010) 14:R15. doi: 10.1186/cc8872
18. Grion CM, Cardoso LT, Perazolo TF, Garcia AS, Barbosa DS, Morimoto HK, et al. Lipoproteins and CETP levels as risk factors for severe sepsis in hospitalized patients. *Eur J Clin Invest.* (2010) 40:330–8. doi: 10.1111/j.1365-2362.2010.02269.x
19. Chien JY, Jerng JS, Yu CJ, Yang PC. Low serum level of high-density lipoprotein cholesterol is a poor prognostic factor for severe sepsis. *Crit Care Med.* (2005) 33:1688–93. doi: 10.1097/01.CCM.0000171183.79525.6B
20. Marik PE. Dyslipidemia in the critically ill. *Crit Care Clin.* (2006) 22:151–9, viii. doi: 10.1016/j.ccc.2005.08.008
21. Tanaka S, Couret D, Tran-Dinh A, Duranteau J, Montravers P, Schwendeman A, et al. High-density lipoproteins during sepsis: from bench to bedside. *Crit Care.* (2020) 24:134. doi: 10.1186/s13054-020-02860-3
22. Gordon EM, Figueroa DM, Barochia AV, Yao X, Levine SJ. High-density lipoproteins and apolipoprotein a-i. Potential new players in the prevention and treatment of lung disease. *Front Pharmacol.* (2016) 7:323. doi: 10.3389/fphar.2016.00323
23. Jahangiri A, de Beer MC, Noffsinger V, Tannock LR, Ramaiah C, Webb NR, et al. HDL remodeling during the acute phase response. *Arterioscler Thromb Vasc Biol.* (2009) 29:261–7. doi: 10.1161/ATVBAHA.108.178681
24. Sun JT, Liu Y, Lu L, Liu HJ, Shen WF, Yang K, et al. Diabetes-invoked high-density lipoprotein and its association with coronary artery disease in patients with type 2 diabetes mellitus. *Am J Cardiol.* (2016) 118:1674–9. doi: 10.1016/j.amjcard.2016.08.044
25. Pirillo A, Catapano AL, Norata GD. HDL in infectious diseases and sepsis. *Handb Exp Pharmacol.* (2015) 224:483–508. doi: 10.1007/978-3-319-09665-0_15
26. Wendel M, Paul R, Heller AR. Lipoproteins in inflammation and sepsis. II. Clinical aspects. *Intensive Care Med.* (2007) 33:25–35. doi: 10.1007/s00134-006-0433-x
27. Chiarla C, Giovannini I, Giuliante F, Zadak Z, Vellone M, Ardito F, et al. Severe hypocholesterolemia in surgical patients, sepsis, and critical illness. *J Crit Care.* (2010) 25:361 e7–12. doi: 10.1016/j.jcrc.2009.08.006

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REVIEW
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The Role of MSC Therapy in Attenuating the Damaging Effects of the Cytokine Storm Induced by COVID-19 on the Heart and Cardiovascular System

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The global pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes coronavirus disease 2019 (COVID-19) has led to 47 m infected cases and 1. 2 m (2.6%) deaths. A hallmark of more severe cases of SARS-CoV-2 in patients with acute respiratory distress syndrome (ARDS) appears to be a virally-induced over-activation or unregulated response of the immune system, termed a "cytokine storm," featuring elevated levels of pro-inflammatory cytokines such as IL-2, IL-6, IL-7, IL-22, CXCL10, and TNFα. Whilst the lungs are the primary site of infection for SARS-CoV-2, in more severe cases its effects can be detected in multiple organ systems. Indeed, many COVID-19 positive patients develop cardiovascular complications, such as myocardial injury, myocarditis, cardiac arrhythmia, and thromboembolism, which are associated with higher mortality. Drug and cell therapies targeting immunosuppression have been suggested to help combat the cytokine storm. In particular, mesenchymal stromal cells (MSCs), owing to their powerful immunomodulatory ability, have shown promise in early clinical studies to avoid, prevent or attenuate the cytokine storm. In this review, we will discuss the mechanistic underpinnings of the cytokine storm on the cardiovascular system, and how MSCs potentially attenuate the damage caused by the cytokine storm induced by COVID-19. We will also address how MSC transplantation could alleviate the long-term complications seen in some COVID-19 patients, such as improving tissue repair and regeneration.

Keywords: COVID-19, mesenchymal stem cells, cytokine storm, cardiovascular, regeneration and repair

INTRODUCTION

As of 3rd November 2020, there are >47 million cases of the coronavirus 19 or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes coronavirus disease 2019 (COVID-19) in the World. There have been >1.2 million reported deaths due to COVID-19, and >34 million infected cases have recovered. As it stands, the infection and death rate due to COVID-19 is below that of previous pandemics. For example, the 1918 Spanish flu outbreak saw 500 million people infected throughout the World and 17–50 million people died over a 2 year span; with up to 25 million deaths in the first 25 weeks (1). Prior to the 1918 flu pandemic, influenza outbreaks had only killed juveniles and the elderly or already weakened patients. However, the Spanish flu was killing completely healthy young adults, while leaving children and those with weaker immune systems still alive (2). This high mortality was attributed to malnourishment, overcrowded medical camps and hospitals, and poor hygiene, all exacerbated by the recent war which promoted bacterial superinfection (3). The outcome of the COVID-19 pandemic is impossible to predict, however history shows that past pandemics have reshaped societies in profound ways. It is clear that COVID-19 has already changed the World and the way we live and work forever.

SARS-CoV-2 gains entry to human cells through the angiotensin-converting enzyme 2, or ACE2 receptor (4). ACE2-mediated viral entry is facilitated by serine proteases, most notably transmembrane protease serine 2 (TMPRSS2), which primes the SARS-CoV-2 spike glycoprotein (5). Initial infection of lung epithelia or alveoli allows SARS-CoV-2 to access the otherwise enclosed systemic circulation, subsequently predisposing multiple organs to potential infection. Multiple organs and tissues, such as the lungs, heart, kidneys, liver, and the vasculature, contain cells which co-express ACE2 and TMPRSS2, or other serine proteases (cathepsin B and cathepsin L1) (6–9).

Similar to other diseases caused by coronaviruses, the main transmission route of SARS-CoV-2 is *via* respiratory droplets and aerosolised particles (10) that are propelled into the air when a person speaks, coughs, shouts, sings, sneezes, or laughs. At the onset of the COVID-19 pandemic, the main symptoms were fever (98%), cough (76%), and myalgia or fatigue (44%) (11). Then, loss of sense of taste and smell, termed anosmia, became a symptom in March 2020 (12), with a large proportion of those reporting anosmia presenting with mild symptoms. Patients can then develop breathing difficulty within 1 week and the severely ill patients soon developed acute respiratory distress syndrome (ARDS), acute cardiac injury, secondary infections, or a combination, resulting in hospital admission and severe cases requiring mechanical ventilation in the ICU (11). Such patients typically exhibit an exaggerated immune response, or cytokine storm, that has become a hallmark of severe SARS-CoV-2 infection. Suppressing the pro-inflammatory nature of the disease is critical to improving patient morbidity and mortality rates and, therefore, developing and identifying viable therapeutic strategies is of urgent scientific importance. Transplantation of mesenchymal stem/stromal cells (MSCs) is one such potential therapy to combat COVID-19 induced inflammation and regeneration of damaged tissues.

The merits of MSCs are that they are multipotent stromal cells that can differentiate into a variety of cell types, including osteoblasts, chondrocytes, myocytes, and adipocytes that have their own characteristic structures and functions of specific tissues. They are typically found in the bone marrow, but have also been characterized in the adipose tissue, dental pulp, umbilical cord tissue, amniotic fluid, and heart (13). Mesenchymal stromal cells are easily accessible from various tissues, are free from ethical issues and have demonstrated no adverse outcomes in clinical trials. They have high proliferation rates, can be systemically administered, and possess key stem cell properties, such as multipotency (14, 15), in addition to being effective immunomodulators, collectively making MSCs a promising therapy in improving COVID-19 morbidity and mortality.

Old Age, Being Male and CVD Co-morbidity—Significant Risk Factors for Mortality

Severity and high mortality from COVID-19 has been linked to old age, being male, cardiovascular disease (CVD), hypertension, and cardiometabolic disease including diabetes and obesity. A retrospective, multicentre cohort study by Zhou et al. (16) examined 191 patients, of whom 137 were discharged and 54 died in hospital. Of these patients, 91 (48%) had a comorbidity, with hypertension being the most common [58 (30%) patients], followed by diabetes [36 (19%) patients] and coronary heart disease [15 (8%) patients]. Multivariable regression analysis showed increasing odds of in-hospital death associated with older age [odds ratio (OR) 1.10, 95% CI 1.03–1.17, per year increase; $p = 0.0043$], higher Sequential Organ Failure Assessment (SOFA) score (5.65, 2.61–12.23; $p < 0.0001$), and D-dimer >1 μg/mL (18.42, 2.64–128.55; $p = 0.0033$) on admission. In univariable analysis, odds of in-hospital death was higher in patients with diabetes or coronary heart disease. Age, lymphopenia, leucocytosis, and elevated ALT, lactate dehydrogenase, high-sensitivity cardiac troponin I, creatine kinase, D-dimer, serum ferritin, IL-6, prothrombin time, creatinine, and procalcitonin were also associated with death (16).

In a retrospective case series involving 1,591 critically ill COVID-19 patients admitted from February 20 to March 18, 2020 in Lombardy, Italy, who required treatment in the ICU, the median (IQR) age was 63 (56–70) years and 1,304 (82%) were male. Of the 1,043 patients with available data, 709 (68%) had at least one comorbidity and 509 (49%) had hypertension. The second most common comorbidities were CVD [223 patients, 21% (95% CI, 19–24)] and hypercholesterolemia [188 patients, 18% (95% CI, 16–20%)]. ICU mortality was higher in those who were older (≥64 years). The prevalence of hypertension was higher among patients who died in the ICU (63%, 195 of 309 patients) compared with those discharged from the ICU (40%, 84 of 212 patients) [difference, 23% (95% CI, 15–32); $P < 0.001$] (17).

Emerging evidence strongly implicates COVID-19 as a vascular disease, with many COVID-19 positive patients purportedly developing cardiovascular complications, such as myocardial injury (18), cardiac arrhythmia (19) and thromboembolism (20, 21). Interestingly, cardiovascular complications have also been reported in patients with no underlying pathology, for instance with acute viral myocarditis (22, 23). Cardiovascular (CV) system involvement is associated with higher mortality rates and is largely indicated by elevated inflammatory biomarkers, including D-dimer, cardiac troponin (cTn), ferritin, and interleukin (IL)-6 (24). For further insight, readers are directed to our review on Vascular Manifestations of COVID-19 (25) in this series.

Myocardial Damage: The Role of Cardiac Troponin and Other Relevant Markers

A number of studies show that a high proportion of COVID-19 patients exhibit elevated levels of cardiac damage biomarkers, such as cTn, with reports of up to 38% of patients testing positive for COVID-19 displaying high circulating levels of cTn (26). In comparison to COVID-19 patients with low cTn, those exhibiting high levels of cTn are hospitalized for longer requiring mechanical ventilation and admission to ICU, are at a significantly greater risk of developing ARDS and cardiac arrhythmias, and ultimately have a higher risk of mortality (27). In a study comparing clinical characteristics between survivors of COVID-19, and those who succumbed to the disease, researchers found that elevated levels of cTn were found in 77% of patients who subsequently died, compared to only 14% of patients who had survived (28). In addition, Guo et al. (29) showed that myocardial injury (elevated cTnT levels) was associated with worse outcome. Patients with underlying CVD are more likely to present with high cTn levels, with the poor prognosis for those with elevated levels further compounded if the patient had underlying CVD, compared to those without underlying CVD (69.4 vs. 37.5% mortality rate, respectively) (29). In the study by Zhou et al. (16) the highest OR for mortality in COVID-19 patients ($n = 191$) was for elevated cTn (>28 pg/mL, OR: 80.1) compared to other biomarkers, including circulating lymphocyte count (OR: 0.02) and D-dimer (OR: 20.04). It is also evident that throughout hospitalization, levels of cTn rise, and importantly, survivors showed no rise in this biomarker during the hospital stay, whereas patients with COVID-19 who died from complications, showed a steady upward rise in cTn until death (16). In another study, a significant predictor of mortality due to COVID-19 was the peak cTn during hospitalization, not the level measured upon admission (26), suggestive that risk stratification should include serial cTn measurements.

Besides cTn, other biomarkers, such as creatine kinase (CK), electrocardiographic (ECG) changes, and imaging might also reveal cardiac pathology in COVID-19 patients. Data acquired from multi-centers showed plasma lactate dehydrogenase and CK levels were correlated with COVID-19 severity and ICU admissions, reaching 26.1 and 70.5%, respectively (30). CK isoenzyme-MB (CK-MB), myohaemoglobin (MYO), and N-terminal pro-brain natriuretic peptide (NT-proBNP) are elevated above normal ranges in 3.7, 10.6, and 12.4% confirmed cases, respectively (31). When stratified by disease severity, patients with abnormal CK-MB, MYO, and NT-proBNP increased to 6.7, 26.7, and 33.3% respectively in the critical cases, underscoring underlying ischaemia and cardiac dysfunction. This is further supported by ECG findings characteristic of ischaemia, such as T-wave depression and inversion, ST depression, and presence of Q waves (18). In a case report, the presence of acute pulmonary embolism in COVID-19 was associated with right ventricular dilatation and dyskinesis on echocardiography, indicating that some patients develop ventricular hypertrophy (32).

Immune Response to COVID-19: Healthy vs. Hyperactive

The immune response to COVID-19 can be split into a healthy antiviral immune response or a defective/overactive immune response. The latter has been linked to damage to the lungs and other organs, resulting in onset of severe illness. Initially, SARS-CoV-2 infection and destruction of lung cells switches on antiviral defenses triggering a local immune response. This includes recruitment of macrophages and monocytes to respond to the infection, interferons and release of cytokines and chemokines and primed adaptive T and B cell immune responses. In most cases, this process is capable of resolving the infection. However, in some cases, a dysfunctional immune response occurs, resulting in severe lung and multi-system damage, and possible failure (33).

In the healthy immune response, the innate antiviral defenses fight against the virus and virus-specific T cells can later eliminate the infected cells before the virus spreads. Neutralizing antibodies in these individuals can block viral infection, and phagocytic cells such as alveolar macrophages recognize neutralized viruses and apoptotic cells and clear them by phagocytosis. Altogether, these processes lead to clearance of the virus with minimal lung and multi-system damage, resulting in recovery (33).

In a defective immune response, there is a hyperactivation of the immune cells, with excessive infiltration of monocytes, macrophages and T cells, in the lungs. This causes overproduction of pro-inflammatory cytokines, the so-called "cytokine storm" or "cytokine release syndrome," which eventually can lead to lung damage, pulmonary oedema and pneumonia. The resulting cytokine storm leads to widespread inflammation circulating to other organs, leading to multiple organ damage (33). Elucidating the mechanisms underlying the immune response to COVID-19 and the causes for the hyperactivation of the immune response are at the forefront of this exciting research area. Recently, Merad and Martin (34) reviewed how activated monocyte-derived macrophages leading to a dysregulated macrophage response contribute to the COVID-19 cytokine storm by releasing massive amounts of pro-inflammatory cytokines (34). Moreover, the biological and clinical consequences of the so-called cytokine storm are still largely unknown.

CYTOKINE STORM IN COVID-19

The term cytokine storm was first employed in describing the events modulating the onset of graft-vs.-host disease (35). Cytokine storms characterize a wide spectrum of infectious and non-infectious diseases. Since 2005, it was associated to the avian H5N1 influenza virus infection (36) and then infections with MERS and SARS, with an inflammatory milieu containing IL-1β, IL-6, and TNF-α being associated with worse disease outcomes (37). Now, severe COVID-19 disease caused by SARS-CoV-2 infection is also associated with a dysregulated and hyperactive systemic inflammatory response; a cytokine storm (38).

It was first reported that several pro-inflammatory cytokines and chemokines, including IL-2, IL-7, IL-10, CXCL10 (IP-10), CXCL8, CCL2 (MCP1), TNFα, and IFNγ were higher in the plasma of COVID-19 patients as compared to healthy controls. More importantly, among infected patients, IL-2, IL-7, IL-10, granulocyte colony- stimulating factor (G-CSF), macrophage inflammatory protein 1α (MIP1α), CXCL10, CCL2, and TNFα circulating concentrations (but not those of IFNγ) were found to be significantly higher in patients requiring admission to ICU and mechanical ventilation, compared to patients experiencing a less severe clinical course (11).

Chen et al. (39) characterized the immunological features of COVID-19 patients presenting with differing disease severity. Eleven patients with severe disease displayed significantly higher serum levels of IL-6, IL-10, and TNF-α and lower absolute numbers of T lymphocytes, $CD4^+$T cells, and $CD8^+$T cells as compared with 10 patients with moderate disease. Of note, severe cases were characterized by a lower expression of IFN-γ by $CD4^+$T cells as compared with moderate cases (39). Likewise, analysis from Liu et al. (40) demonstrated significant decreases in the counts of T cells, especially $CD8^+$ T cells, as well as increases in IL-6, IL-10, IL-2, and IFN-γ levels in the peripheral blood in the severe COVID-19 cases ($n = 13$) compared to those in the mild cases ($n = 27$), suggesting that disease severity is associated with significant lymphopenia and hyperinflammation.

Del Valle et al. (41) used a multiplex cytokine assay to measure serum IL-6, IL-8, TNF-α, and IL-1β in hospitalized COVID-19 patients ($n = 1{,}484$) upon admission to the Mount Sinai Health System in New York, USA. They showed that serum IL-6, IL-8, and TNFα levels at the time of hospitalization were strong and independent predictors of patient outcomes, with elevated inflammatory profile associated with reduced survival. Importantly, when adjusting for disease severity score, common laboratory inflammation markers, hypoxia and other vitals, demographics, and a range of comorbidities, IL-6 and TNF-α serum levels remained independent and significant predictors of disease severity and death (41).

In an elegant study, Lucas et al. (42) have identified that development of a maladaptive immune response profile was associated with severe COVID-19 outcome, and early immune signatures correlated with divergent disease trajectories. Through serially analyzing immune responses in peripheral blood in 113 COVID-19 patients with moderate (non-ICU) and severe (ICU) disease, they revealed an association between early, elevated cytokines and worse disease outcomes. Indeed, they observed a "core COVID-19 signature" shared by both moderate and severe groups of patients defined by the following inflammatory cytokines that positively correlated with each other; these included: IL-1α, IL-1β, IL-17A, IL-12 p70, and IFN-α. In severe patients, they observed an additional inflammatory cluster defined by: thyroid peroxidase (TPO), IL-33, IL-16, IL-21, IL-23, IFN-λ, eotaxin, and eotaxin 3. Interestingly, most of the cytokines linked to cytokine release syndrome, such as IL-1α, IL-1β, IL-6, IL-10, IL-18, and TNF-α, showed increased positive associations in severe patients. After day 10, in patients with moderate disease, these markers steadily declined. In contrast, severe patients maintained elevated levels of these core signature makers. Notably, additional correlations between cytokines emerged in patients with severe disease following day 10. Therefore, there were sharp differences in the expression of inflammatory markers along disease progression between patients who exhibit moderate vs. severe COVID-19 symptoms. Altogether, data showed a broad elevation of type-1, type-2, and type-3 signatures in severe cases of COVID-19, with distinct temporal dynamics and quantities between severe and moderate patients. Unsupervised clustering analysis of plasma and peripheral blood leukocyte data identified four immune signatures, representing (A) tissue repair growth factors, (B) type-2/3 cytokines, (C) mixed type-1/2/3 cytokines, and (D) chemokines involved in leukocyte trafficking that correlated with three distinct disease trajectories of patients. The immune profile of patients who recovered with moderate disease was enriched in tissue reparative growth factor signature (A), while the profile for those with worsened disease trajectory had elevated levels of all four signatures. Overall, results suggested that a multi-faceted inflammatory response is associated with late COVID-19 severity, which raises the possibility that early immunological interventions that target inflammatory markers predictive of worse disease outcome are preferred to blocking late-appearing cytokines.

Supporting the work of Lucas et al. (42) a recently published article has identified a core peripheral blood immune signature across 63 hospital-treated patients in London, UK with COVID-19. Specifically, among several changes in immune cells expressed at unusual levels in the blood of patients, the work identified a triad of IP-10 (CXCL10), IL-10, and IL-6 to correlate strongly with disease severity. Indeed, patients with COVID-19 who displayed measurably higher levels of IP-10 (CXCL10), IL-10, and IL-6 when first admitted to hospital went on to become more severely ill. The triad of cytokines was found to be a rigorous predictor of disease severity than commonly-used clinical indicators, including CRP, D-dimer, and ferritin (43).

As the COVID-19 cytokine storm is a multi-faceted inflammatory response, therapies that target this as a whole and those that enhance tissue repair (i.e., mesenchymal stem/stromal cells; MSCs) should be considered. Indeed, Lucas et al. (42) found IL-6 to be highly enriched in patients with severe disease. In fact, all ICU patients in their study, including the ones who succumbed to the disease, received Tocilizumab, an IL-6R blocking antibody. Positive outcomes have been reported with Tocilizumab treatment, including a reduction in an inflammatory-monocyte population associated with worse

outcomes (44). However, as patients still succumbed to COVID-19, this highlights the need for combination therapy to block other cytokines highly represented in severe COVID-19 cases, including inflammasome-dependent cytokines and type-2 cytokines (42).

THE EFFECTS OF THE COVID-19 CYTOKINE STORM

On the Lungs Leading to Acute Respiratory Distress Syndrome (ARDS)

Acute respiratory distress syndrome (ARDS) is a form of hypoxaemic respiratory failure that is characterized by severe impairment of gas exchange and lung mechanics, with a high case fatality rate. Acute respiratory distress syndrome can come about through the severe widespread inflammatory injury present throughout the lungs, leading to a loss of vascular barrier integrity and likely promoting pulmonary oedema, thereby causing inflammation of endothelial cells (endothelialitis). Acute respiratory distress syndrome is a prominent feature in patients with severe COVID-19 infection (45, 46) and is the leading cause of mortality (47).

The precise pathophysiological mechanisms underlying ARDS in COVID-19 patients are not fully understood. However, alveolar macrophages are central to mediating the inflammation associated with ARDS (48), with the initial inflammatory stage involving alveolar macrophages interacting with lymphocytes (49) and epithelial cells (50), thereby augmenting the inflammatory response and accentuating tissue damage (51). Following initial stimulation, neutrophils and circulating macrophages are recruited to the lungs (activated by the pro-inflammatory cytokines), thereby triggering further inflammatory responses (52) equating to a positive feedback loop. These cells may disrupt the air–blood barrier by causing collateral tissue damage, particularly to airway epithelial cells and vascular endothelial cells, which express the ACE2 entry receptor for SARS-CoV-2; the damage of vascular endothelial cells may account for thrombotic microangiopathies (53). Furthermore, severe infection of the lung alveoli allows the SARS-CoV-2 virus and pro-inflammatory cytokine overload to enter the systemic circulation where it can infiltrate multiple organs, particularly since cells in many of them co-express ACE2 and TMPRSS2 (7, 8, 54).

In addition to the marked lung damage observed in COVID-19 infection, clinical cohort studies have revealed involvement of the kidneys (11, 16, 19, 30, 55, 56), liver (11, 30, 57, 58), gastrointestinal tract (11, 30, 59, 60), central nervous system (61, 62), and CV system (16, 18, 19, 63).

Mitochondrial-Related Mechanisms

Mitochondria are essential for meeting the rise in energy demand required to fuel the immune system response and also for inducing immunomodulatory mechanisms, serving as a platform for host defense against RNA viruses such as SARS-CoV-2 (64, 65). The effects of SARS-CoV-2 infection upon mitochondrial respiratory capacity is a key consideration in the context of the host cytokine response. Mitochondrial respiratory capacity has been suggested to account for 10–30% of the variance in circulating leukocyte immune reaction across individuals, influencing the cytokine signature produced by leukocytes in response to lipopolysaccharide (LPS) administration (66). In particular, complex IV activity was positively correlated with LPS-stimulated IL-6 release (66). This is of particular interest in relation to SARS-CoV-2, whereby blood IL-6 has been identified as a predictor of patient fatality (47).

Aside from respiration, mitochondria are essential in host cell detection of RNA *via* pattern recognition receptors (PPRs), including cytosolic sensors retinoic acid-inducible gene 1 (RIG-1) and melanoma differentiation-associated protein 5 (MDA5) (67). These utilize the mitochondrial signaling protein MAVS (mitochondrial antiviral signaling protein), which recruits the E3 ligases TNF receptor associated factor 3 (TRAF3) and TRAF6, facilitating activation of interferon regulatory factors (IRFs) and NF-κB to induce antiviral genes. In this manner, MAVS activity coordinates the activation of a dominant antiviral mechanism, the type 1 interferon (IFN) pathway (64). SARS-CoV-2 open reading frame (Orf) 9b targets the translocase of outer mitochondrial membrane protein 70 (TOMM70), linking mitochondrial signaling to induction of the IFN pathway (68). The Orf9b of SARS-CoV-2 also localizes to the outer mitochondrial membrane, disrupting the MAVS signalosome (69) and impairing the host IFN response (69, 70). Other mitochondrial factors that may impact the IFN response include mitochondrial stress, whereby release of mtDNA into the cytosol is detected by the DNA sensor cGAS, which promotes STING-IRF3 signaling, potentiating IFN pathway signaling (71).

Inflammasomes, the multiprotein complexes providing a platform for the activation of pro-inflammatory caspase-1 culminating in cytokine release, are also mitochondrial-dependent. An example is NLRX1, a target of SARS-CoV-2 Orf9c (68). NLRX1 interacts with mitochondrial complex III, stimulating reactive oxygen species (ROS) production (72). ROS production from mitochondrial complexes I and III is known to mediate both innate and adaptive viral immune responses (73), impacting both MAVS and NF-κB signaling (72).

Pro-inflammatory cytokines are known to elicit metabolic alterations, with NF-κB and interleukin signaling impacting glucose control and glycolytic function. For instance, development of insulin resistance has been linked to IL-1 and IL-6 signaling in the context of type 2 diabetes mellitus (74). This is a key consideration in SARS-CoV-2, whereby poor blood glucose control has been associated with higher mortality in diabetic patients (75) and high glucose levels associated with viral replication in monocytes, with enhanced glycolytic capacity coinciding with raised IL-1β (76).

NF-κB mediated metabolic re-programming has been demonstrated in acute viral myocarditis (VM) (77, 78), a condition characterized by viral induced leukocyte infiltration and cardiac dysfunction. Case studies of acute VM have been reported in female COVID-19 patients (ages 21 and 43), resulting in substantial disruption to cardiac function in the absence of coronary artery disease (22, 23). Viral fulminant myocarditis, a syndrome on the clinical spectrum of acute myocarditis, has also

been associated with death in SARS-CoV-2 patients suffering from cardiac injury (79).

In human and mouse models of VM, cardiac inflammation indicated through cytokine mediated NF-κB activation was linked to impaired expression of genes related to oxidative metabolism. This included downregulation of genes encoding mitochondrial regulatory proteins associated with biogenesis (PGC-1α, PGC1-1β, Tfam, and NRF-1) alongside regulators of β-oxidation (e.g., PPAR-α), tricarboxylic acid cycle and electron transport chain (ETC) function. This coincided with a fall in high energy phosphates and NAD levels and a shift toward anaerobic glycolysis, indicated through increased expression of glucose and lactate transporters and glycolytic enzymes (77). Together, this indicates that the inflammatory response associated with acute VM initiates reprogramming of cardiomyocyte energy metabolism away from oxidative metabolism and toward glycolysis. This culminated in an energy-starved status of the heart, the extent to which likely contributed to impaired cardiac function. NF-κB signaling has also been linked to impaired insulin signaling by stimulating phosphorylation of insulin receptor substrate-1, in turn inducing insulin resistance and cardiac dysfunction associated with VM (78). The metabolic implications of VM onset and resulting impairment of myocardial function are thus vital considerations in the pathophysiology of SARS-CoV-2 infection.

On the Cardiovascular System

A number of case reports have demonstrated cardiac abnormalities in patients with COVID-19, including myocarditis, myo-pericarditis, electrocardiographic complications, cardiogenic shock, decompensated heart failure, and other histological/imaging complications, such as reduced left ventricular ejection fraction (LVEF) (80–85). Moreover, and as described previously, cross-sectional studies have consistently reported elevations in cardiac injury markers, such as cTn, NT-proBNP, and creatine kinase myocardial band (CK-MB) concentrations, with patients presenting with cardiac injury being at a higher risk of mortality, even after being adjusted for confounding variables such as age, pre-existing CVD, and ARDS (18). These data give strong evidence for cardiac complications associated with COVID-19, however, the mechanisms for these complications may not be solely the result of a direct viral infection of cardiac cells.

The CV system is also at high-risk as a result of indirect mechanisms, such as the cytokine storm. The cytokine storm is likely to induce cardiovascular damage through mechanisms related to endothelial dysfunction, atherosclerotic plaque instability/rupture, cardiomyocyte death, and myocarditis. The mechanisms of endothelial dysfunction within the COVID-19 population are not limited to elevations in pro-inflammatory cytokine concentrations and include direct viral infection of endothelial cells, angiotensin II (Ang II) hyperactivity, complement activation, and other elements of immune dysregulation, such as neutrophil extracellular trap (NET) formation. Indeed, evidence of SARS-CoV-2 viral structures have been observed in endothelial cells in various tissue beds (63), which may promote an imbalance between ACE2 and Ang II. Liu et al. (86) support this notion by demonstrating elevated plasma Ang II concentrations in patients with COVID-19. For a more in depth review of direct viral infection of endothelial cells, including Ang II hyperactivity, readers are directed to our recent review on the vascular manifestations of COVID-19 (25). Complement activation has been associated with microthrombosis in a small number of patients with COVID-19 (87) and NET formation has been correlated with COVID-19-associated ARDS (88). Both complement activation and NET formation are associated with pro-inflammatory responses. The complement system detects viral pathogens, thus contributing to the innate immune response to viral infections (89), whilst NETs have the ability to induce IL-1β secretion from macrophages and play a role in the development of atherosclerosis, causing endothelial damage and dysfunction (90, 91). Moreover, endothelial cells undergoing apoptosis have been shown to activate the complement system (92), which may further exacerbate cytokine secretion and promote microthrombosis. Therefore, it should be acknowledged that direct viral infection of endothelial cells, subsequent Ang II hyperactivity and the pro-inflammatory effects of complement activation and NET formation promote both direct and indirect perturbations to the cardiovascular system, whilst exacerbating the cytokine storm. Moving forward, the predominant focus of this section is to discuss the potential effects of the cytokine storm upon the cardiovascular system.

The cytokine storm is not only one of the predominant pathophysiological mechanisms of fulminant myocarditis (without evidence of viral infiltration) (93), which has been reported in patients with COVID-19, but inflammatory infiltration into endothelial cells has also been reported in histological studies (63, 94). Inflammatory infiltration into endothelial cells promotes endothelialitis, perturbing endothelial cell membrane function, loosening inter-endothelial junctions, and causing cell swelling (94, 95). Indeed, Varga et al. (63) showed endothelial cell death and dysfunction in patients infected with SARS-CoV-2, which facilitated the induction of endothelialitis in several organs, including cardiac tissue, as a direct consequence of viral involvement and of the host inflammatory response.

The presence of endothelialitis demonstrates the activation of endothelial cells, promoting the expression of cell-surface adhesion molecules and thus the binding of inflammatory cells to the endothelium (96, 97). These pathophysiological consequences promote vascular hyperpermeability. Disruption of inter-endothelial junctions cause endothelial cells to be "pulled apart," thus resulting in inter-endothelial gaps (95, 98), denoting cytoskeletal alterations to the endothelium. Moreover, this cytokine storm-induced endothelial dysfunction pre-disposes the CV system to a pro-coagulant state, promoting thromboembolic events, which has been linked to higher disease severity, and higher instances of mortality (99). Interestingly thrombin exposure, coupled with an elevation in the influx of Ca^{2+} promotes elevations in endothelial cell permeability which can be induced by an increase in TNF-α expression (100, 101).

Elevations in cytosolic Ca^{2+} influx into endothelial cells is a pivotal step in the disruption to inter-endothelial junctions and thus the progression to increased vascular permeability

(101, 102). A determinant of this increased Ca^{2+} influx is the upregulation of transient receptor potential channels, which is induced *via* TNF-α (100), causing a destabilization of microtubules (103). Evidence supports the notion of a cytokine-induced hyperpermeability response of the vasculature, with Tinsley et al. (104) demonstrating the role of cytokine (TNF-α, IL-1β, and IL-6) induced-vascular hyperpermeability through a protein kinase C (PKC) and myosin light chain kinase (MLCK) dependent mechanism in cultured rat heart microvascular endothelial cells. Moreover, the authors replicated these findings *in vivo* using a coronary ischemia/reperfusion (I/R) rodent model of heart failure, demonstrating TNF-α increases endothelial permeability in a PKC and MLCK dependent manner (104). Therefore, translating this to COVID-19 pathophysiology, cytokine storm induced Ca^{2+} influx into endothelial cells may be a contributing mechanism underpinning the disruption to inter-endothelial junctions and the promotion of vascular permeability. Furthermore, the cytokine-induced stimulation of PKC and MLCK may promote direct damage to cardiac tissue, which may pose significant deleterious effects upon patients with pre-existing CVD, a common comorbidity in the more severe COVID-19 population (105).

Histological studies in pulmonary vasculature have indicated endothelialitis, with unexpected observations of intussuseptive angiogenesis. In this study (94), the degree of intussuseptive angiogenesis was associated with the duration of hospitalization. Whilst hypoxia may be a contributing mechanism, the authors concluded the predominant mechanism was likely the presence of endothelialitis and thrombosis (94). Intussuseptive angiogenesis is the formation of intravascular vessel formation, through non-sprouting mechanisms, commonly observed as "pillar" formation within the vasculature (106), which can significantly alter the microcirculation, and can be triggered by extraluminal processes, including inflammation (107). Inflammatory-mediated intussuseptive angiogenesis has been demonstrated previously in murine models of colitis, suggesting this is an adaptive response to prolonged inflammation (108). This provides further evidence of the perturbations to the vasculature caused by the cytokine storm in COVID-19. The promotion of intussuseptive angiogenesis as an adaptive response to vascular damage, has also been shown to accelerate fibrotic neovascularisation (109).

Inflammatory environments also promote the generation of ROS which can result in damage and dysfunction of the vasculature. ROS act as signaling molecules to defend against oxidative stress by promoting the upregulation of antioxidant mechanisms, however, high concentrations of ROS can activate endothelial cells and inhibit normal endothelial functioning. Cytokines, such as TNF-α, have been shown to interact with the ETC and stimulate the release of mitochondrial-derived ROS, such as hydrogen peroxide (110) and superoxide (111). Moreover, in response to infections, inflammatory cytokines, such as TNF-α and IL-1β, coming into contact with endothelial cells induce NAD(P)H oxidase-derived ROS (112, 113). The generation of excessive ROS elevates superoxide anion production, which can degrade nitric oxide (NO), lead to the formation of other free radicals, such as peroxynitrite, and thus result in endothelial cell dysfunction and apoptosis (96, 114, 115). Therefore, it is likely that the cytokine storm experienced in patients with COVID-19 will promote the elevation in ROS and result in oxidative stress, which is a key mechanism of endothelial dysfunction in hypertension (116) and CVD (117). Elevations in ROS also act as secondary inflammatory signals, which has been shown to induce the secretion of pro-inflammatory cytokines, such as IL-1β, TNF-α, and IL-6 (118). Therefore, this creates a vicious cycle of cytokine-induced oxidative stress and ROS-induced pro-inflammatory cytokine signaling, secondary to the COVID-19 hyper-activation of the immune response.

Inflammatory cytokines do not just alter endothelial structure and function. Cytokines such as TNF-α, IL-1β, and IL-6 promote vascular smooth muscle cell (VSMC) proliferation from the media to the intima of the vasculature, which results in the secretion of extracellular matrix proteins within, and thus expanding the intima in pathological conditions, such as atherosclerosis (119). Moreover, in human coronary VSMCs, IL-1β has been shown to stimulate an upregulation in Rho-kinase, *via* a PKC-dependent mechanism, which may contribute to medial thickening and the atherogenic environment (120). Interestingly, this can also be stimulated by an upregulation in angiotensin II, which has been noted within the COVID-19 literature if infected cells experience a downregulation of ACE2 expression (121), which will also contribute to the pro-inflammatory environment experienced in patients with COVID-19. Activation of RhoA can also be stimulated by TNF-α which has been shown to promote endothelial cell permeability in cultured human umbilical vein endothelial cells (HUVECs) (122). These pathophysiological processes are shared with thrombosis, which is a common manifestation in patients with severe COVID-19 (99). Combined with damage to endothelial cells contributing to the apparent "COVID-19 coagulopathy" (123), VSMC proliferation, stimulated by various cytokines, may contribute to the high instance of coagulation derangements and thromboembolic events observed in patients with severe COVID-19.

Whilst the COVID-19 induced cytokine storm can predispose the CV system to damage and progression of pre-existing cardiovascular comorbidities, perturbations to vascular cells may also contribute to the overexpression of pro-inflammatory cytokines. Both endothelial cells and VSMCs secrete pro-inflammatory cytokines when either damaged or undergoing apoptosis. Expression of cell-surface adhesion molecules and certain cytokines, such as IL-8, on the surface of endothelial cells induce a pro-inflammatory phenotype and the recruitment of blood monocytes which induce the secretion of pro-inflammatory cytokines, such as TNF-α and IL-1β (124). Moreover, under atherogenic conditions, VSMCs have been shown to also adopt a pro-inflammatory phenotype, promoting the secretion of IL-6 and IL-8, along with cell-surface adhesion molecules, such as vascular cell adhesion molecule 1 (124, 125). Therefore, both endothelial cells and VSMCs, once damaged, may switch to a pro-inflammatory phenotype and thus propagate the expression of pro-inflammatory cytokines.

Whilst there is a plethora of evidence which suggests that the cytokine storm experienced in COVID-19 patients may promote

damage to the vasculature, sustained inflammation directly contributes to progressive cardiomyocyte apoptosis. Elevated TNF-α levels seen in a variety of clinical conditions including COVID-19, drives cardiomyocytes to apoptosis (126, 127). TNF-α can induce cardiomyocyte apoptosis directly, *via* the TNF receptor, or indirectly, through stimulation of NO production or ROS, which in turn is induced by pro-inflammatory cytokines such as IL-1, IL-6, TNF-α, and IFN-7 (128). High levels of cTn are reflective of cardiomyocyte death and injury, and as stated earlier, are associated with COVID-19 disease severity and mortality (16).

In the heart, the acute inflammatory response can expand tissue damage and prolonged inflammation leads to accentuated adverse remodeling. Indeed, pro-inflammatory cytokines and upregulated monocytes/macrophages can inhibit cardiac repair, which is dependent on timely suppression and resolution of pro-inflammatory signaling. Activation of IL-1 signaling induces cytokine expression, promotes matrix-degrading properties, suppresses fibroblast proliferation and inhibits transdifferentiation of fibroblasts into myofibroblasts, altogether delaying activation of a reparative response (129). Moreover, a severe or prolonged reparative response is associated with pathological scarring and fibrosis (130).

The full extent of cardiovascular cell dysfunction and death, induced by the cytokine storm in COVID-19, is yet to be fully elucidated. This section provides evidence of the potential effects and mechanisms of the COVID-19 cytokine storm on the cardiovascular system. It is likely that cardiomyocyte and vascular cell damage and dysfunction, as well as mitochondrial-related mechanisms play a role in the progression of COVID-19 and in the pathogenesis of cardiovascular injury in COVID-19. The induction of ROS generation and the ensuing oxidative stress, coupled with vascular cell secretion of pro-inflammatory cytokines further propagates the inflammatory environment and exaggerated immune response in patients with COVID-19, promoting disease progression and multi-organ dysfunction. Moreover, cardiac and vascular cell dysfunction pre-disposes the CV system to a pro-inflammatory and pro-atherogenic state and thus increases the risk of serious cardiac events. Therefore, suppression of the cytokine storm, is key for improving patient outcomes with COVID-19, whilst also protecting the CV system. One such therapy is transplantation of mesenchymal stem/stromal cells (MSCs).

MSCs AS A THERAPY FOR SEVERE COVID-19 PATIENTS

Immunomodulatory Role of MSCs

An important function of MSCs is that they have powerful immunomodulatory properties, possessing natural abilities to detect changes in their environment such as inflammation. Mesenchymal stromal cells can both directly and indirectly stimulate immunomodulation by interacting with immune cells and releasing various anti-inflammatory cytokines *via* paracrine effects, respectively (131). Functional alterations to dendritic cells, monocytes, macrophages, regulatory T-cells (Tregs), and B-cells underpin MSCs' immunomodulatory capacity, whilst also through cell-to-cell interaction mechanisms (13). Once systemically administered, a significant portion of MSCs accumulate within the lungs, which can promote anti-inflammatory effects, thus improving the lung microenvironment and potentially restoring vascular barrier integrity and reducing oedema; whilst also promoting endogenous repair and regeneration mechanisms to reduce (or prevent further) fibrosis of the lung (132, 133).

Animal models of ARDS lung injury due to influenza virus have shown that infection by this and related viruses causes ion channel transporter abnormalities which causes fluid secretion, a major cause of the pulmonary oedema in the lungs of infected individuals. In such animal models, MSCs prevent or reduce the secretory effect of influenza virus on lung alveolar cell ion channels, and when administered intravenously in aged animals have resulted in increased oxygenation, improved respiration, reduction in pro-inflammatory cytokines, and an increase in survival (134).

Mesenchymal stromal cells are well-known to respond to the inflammatory environment with multimodal activity resulting in sustained anti-inflammatory effects; conversion of Th17 cells to anti-inflammatory FOXP3 Treg cells by MSC-secreted transforming growth factor (TGF) β1 and the essential presence of CCL18 producing type-2 anti-inflammatory macrophages from differentiated pro-inflammatory monocytes (135). They are known to dampen the innate immune response to insult (such as acute lung injury, burn injuries) or infection *via* preventing neutrophil infiltration into injured/infected sites (136–139) or *via* shifting the phenotype of macrophages from an M1 to M2 anti-inflammatory phenotype (140). Specifically the MSCs appear to reduce inflammation *via* reducing macrophage secretion of neutrophil chemoattractant proteins CXCL1, CXCL2 (137, 141) as a result of activation of phosphorylation of p38 MAPK (141) and greater IL-10 release (137), dampened production of IL-6 and TNF-α (137, 138), and suppression of reactive oxygen species production by neutrophils (142, 143). Together this contributes toward a shift from a pro- to an anti-inflammatory environment and is an essential part of the immunomodulatory function of MSCs as this helps prevent against autoimmunity (13), as demonstrated in MSC-treated graft vs. host disease (144).

Mesenchymal stromal cells can also induce local and systemic immunomodulatory responses independently of the cytokine storm. For instance, MSCs can prevent the infiltration of cells of the innate immune system, thereby indirectly reducing the secretion of inflammatory cytokines. In a murine model, BM-MSCs reduced $CD45^+$ cells and neutrophil populations in the mucosa *via* release of tumor necrosis factor-induced protein 6 (TSG-6) (145). Both MSCs and TSG-6 induced the expansion of regulatory macrophages, expressing IL-10 and inducible nitric oxide synthase (NOS), and increased the population of $FOXP3CD45^+$ cells. Interestingly, TSG-6 was associated with MSC-mediated depletion of corneal, splenic, and peripheral blood $CD11b^+$ monocytes/macrophages in a model of inflammatory corneal neovascularization (146). In addition to TSG-6, MSCs can also release other bioactive molecules that promote protective responses in innate immune

cells, including kynurenic acid (147), spermine (148, 149), and lactate (150). Adaptive immune cells, such as T and B cells, are also direct targets of MSCs. Following transplantation, MSCs form aggregates with B and T cells, stimulating the production of FOXP3 and IL-10 (145). Mesenchymal stromal cells directly inhibit the activation of cytotoxic $CD8^+$ T-cells *via* downregulation of CD25, CD38, and CD69 (151). In B cells, MSCs downregulate chemotactic properties, with no effect on costimulatory molecules or cytokine production (152). Mesenchymal stromal cell-mediated indoleamine 2,3-dioxygenase signaling promotes the survival and proliferation of $CD5^+$ Bregs (153). There are also data to suggest that MSCs could act *via* extracellular vesicles and exosomes to modulate innate and adaptive immunity (154, 155). The immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory disease are reviewed in (156).

Consequently, on the basis of these and other studies with MSCs in animal models, clinical investigators have postulated that human MSCs should be effective in the pathology of human ARDS (157). Indeed in a report of allogeneic MSCs in ARDS patients, a single low dose of cells (2 million cells/kg/BW) achieved rapid reduction in inflammatory cytokines and efficacy in influenza-related ARDS which was otherwise refractory to conventional supportive therapy (158). For further insight on the therapeutic potential of cell therapy to treat ARDS readers are directed too (159, 160).

The systemic redistribution of MSCs have the ability to target other organs that are damaged. As multi-organ damage is a common manifestation in patients with severe COVID-19, this makes MSCs an attractive therapy to combat not only lung damage, but also damage observed in other organs, such as the heart. Therefore, the use of MSCs to modulate the immune response, avoiding, preventing or attenuating the cytokine storm leading to multi-organ failure may be the key for the treatment of COVID-19 infected patients.

Use of MSCs to Treat COVID-19

Table 1 summarizes the published clinical studies thus far using MSCs as a therapy to treat COVID-19. **Table 2** summarizes the ongoing, registered clinical trials using MSCs as a therapy to treat COVID-19. For review articles on the rationale and treatment of COVID-19-related ARDS using MSCs, readers are directed to Moll et al. (165) and Can and Coskun (166).

The first clinical study undertaken in China, showed that for seven patients with COVID-19-related pneumonia, transplantation of 1×10^6 MSCs/Kg/BW allogeneic MSCs was effective by restoring the balance of the immune system resulting in significant resolution of signs and symptoms of pulmonary disease (133). Before the transplantation, all patients had COVID-19-related pneumonia with symptoms of high fever, weakness, shortness of breath, and low oxygen saturation. Results showed that all symptoms had disappeared by 2–4 days after the transplantation. The oxygen saturations rose to $\geq$ 95% at rest, without or with oxygen treatment. This was not the case in the three placebo control patients. Among the MSC-treated patients, one severe and two mild patients were able to make a recovery and be discharged 10 days after treatment. The study found improvement was particularly dramatic for an elderly male patient in a severe critical condition (133). The improved recovery time with MSC treatment would lead to decreased hospitalization which would be vital for overwhelmed hospital wards and ICUs.

The transplanted MSCs significantly elevated IL-10 and reduced TNF-α concentrations in seven MSC transplanted patients with COVID-19-pneumonia compared to the three patients in the placebo control group receiving standard care. In the severe ($n = 4$) and critically severe ($n = 1$) patients, a significant elevation in Tregs and dendritic cells were observed after MSC administration, compared with the mild and control patients. Specifically, there was a switch from pro-inflammatory cytokine producing $CXCR3^+CD4^+$ T cells, $CXCR3^+CD8^+$ T cells, and $CXCR3^+$ NK cells to $CD14^+CD11c^+CD11b$ mid regulatory dendritic cell (DCreg) population, indicating improvement in immunomodulatory function. Furthermore, in the critically severe patient an over activation of T-cells and natural killer (NK) cells were evident, however, after MSC treatment, T-cells and NK cells were almost eradicated, with the CD14+CD11c+CD11b mid DCregs restored to normal levels (133). These findings demonstrate the ability of MSCs to induce their immunomodulatory benefits in a set of patients with COVID-19, restoring the balance of the immune response by attenuating the cytokine storm.

These findings have been further supported within the literature with a case study by Zhang et al. (162) demonstrating a regression of COVID-19 symptoms between 2 and 7 days post-Wharton's Jelly derived human umbilical cord MSCs administration, with a reduction in ground glass opacity and pneumonia infiltration within the lungs 6 days post-transplantation. Moreover, $CD3^+$, $CD4^+$, and $CD8^+$ T-cells were increased and CRP, IL-6, and TNF-α concentrations were reduced. Another case report of a patient with severe COVID-19 who experienced two cytokine storms, was treated with a synergistic use of convalescent plasma and umbilical cord MSCs. Treatment resulted in lymphocyte counts returning to normal after the fourth day following convalescent plasma administration and a reduction in inflammatory markers, with a steady elevation in PaO_2 following the administration of umbilical cord MSCs (167).

One limitation to MSC therapies for treating COVID-19 may be the expression of ACE2 and the predominant serine protease responsible for priming the SARS-CoV-2 spike glycoprotein, TMPRSS2, which may promote SARS-CoV-2 infection of transplanted cells and thus promote further spread and progression of COVID-19. However, Leng et al. (133) after performing 10x single cell RNA sequencing analysis, demonstrated transplanted MSCs are ACE2-negative and TMPRSS2-negative.

Taken together, *via* their immunomodulatory and reparative role these studies provide support to the rationale for MSC transplantation as a therapy to treat COVID-19. Moreover, whilst these studies demonstrate evidence for their use against lung damage, the suppression of pro-inflammatory markers will provide protection against damage or further damage to other organs. For example, with COVID-19 leading

TABLE 1 | Summarisation of clinical studies and ongoing clinical trials assessing the therapeutic benefit of MSC transplantation in patients with COVID-19, including studies assessing the therapeutic potential of MSCs in patients with acute respiratory distress syndrome (ARDS), without COVID-19.

Citation	*N*	Subjects	MSC source and dose	MSC timing	Recipient site	Results
Leng et al. (133)	MSC transplant: $n = 7$; CON: $n = 3$	COVID-19 pneumonia	Clinical grade ACE2$^-$ MSCs at 1×10^6 cells/kg	The time when symptoms and/or signs were still getting worse, even as the expectant treatments were being conducted	Systemic	- ↑ IL-10 vs. CON - ↓ TNF-α vs. CON - ↔ IP-10 - Trend for ↑ VEGF vs. CON - Inflammation, AAT, MYO and CK reduced in critically severe patient with a reduction in ground-glass opacity and pneumonia infiltration
Liang et al. (161)	Case study	Critical COVID-19	Allogenic hUCMSCs at 5×10^7 cells 3 times	Admitted 2 days after symptoms onset and MSCs were transplanted on the 9, 12, and 15th days after admission. In combination with antibiotics and thymosi*n* α1	Systemic	No side effects were observed. After 2nd administration: - ↓ Bilirubin, WBC and neutrophil count, CRP and ALT/AST - ↑ lymphocyte count - ↑ $CD3^+$, $CD4^+$, and $CD8^+$ T cells - Trachea cannula removed After 3rd administration: - Pneumonia relieved - Removed from ICU 2 days following - Negative throat swab
Zhang et al. (162)	Case study	COVID-19 pneumonia - History of diabetes	Wharton's jelly-derived hUCMSCs at 1 x 10^6 cells/kg	Admitted 5 days after symptoms onset and MSCs were transplanted on the 17th day of admission	Systemic	Post-transplant: - COVID-19 symptoms disappeared 2 to 7 days - ↓ Ground glass opacity and pneumonia infiltration day 6 - ↑ $CD3^+$, $CD4^+$ & $CD8^+$ T cells - ↓ CRP, IL-6 & TNF-α
Chen et al. (163)	MSC transplant: $n = 17$; CON: $n = 44$	H7N9-induced ARDS	Allogenic menstrual-blood-derived MSCs at 1×10^6 cells/kg	3 patients treated with 3 infusion at the early stage of infection; 6 patients were treated with 3 infusions at the late stage of infection; 8 patients accepted 4 infusions of at late stage of infection	Systemic	At admission: - No differences, except ↓ PCT vs. CON At discharge: - ↑ mortality rate of CON - ↓ PCT, ALT, sCr, CK, PT, and D-dimer vs. CON At follow-up (5 year; $n = 4$): - ↑ Hb - ↓ PT
Sengupta et al. (164)	$N = 23$	COVID-19: cohort a (mild COVID-19): $n = 1$; cohort b (hypoxaemia and COVID-19): $n = 20$; cohort c (intubated COVID-19): $n = 3$	Bone-marrow derived MSCs exosome agent—ExoFlow—15 mL	Not specified	Systemic	- 71% patients recovered and/or were discharged after 5.6 days post-infusion - 13% remained critically ill - 16% died - 80% improved PaO_2/FiO_2 ratio within 3 days - ↓ CRP, ferritin and D-dimer on day 5 -↑ $CD3^+$, $CD4^+$, and $CD8^+$ T cells on day 5

CON, control; ACE2, Angiotensin converting enzyme 2; IL-10, Interleukin-10; TNF-α, Tumor necrosis factor α; IP-10, Interferon gamma-induced protein 10; VEGF, Vascular endothelial growth factor; AST, Aspartate amino transferase; MYO, Myoglobin; CK, Creatine kinase; hUCMSC, human umbilical cord mesenchymal stem cells; WBC, white blood cell; CRP, C-reactive protein; ALT, Alanine aminotransferase; ICU, intensive care unit; ARDS, Acute respiratory distress syndrome; PCT, Procalcitonin; sCr, serum creatinine; PT, Prothrombin time.

TABLE 2 | List of registered, ongoing, clinical trials using mesenchymal stem/stromal cells (MSCs) as a therapy to treat COVID-19.

Clinical trials number	Participants	MSC source	Outcomes
NCT04371393 (USA)	Target: $N = 300$	MSCs (Remestemcel-L) at 2×10^6 cells/kg administered twice during first week (second infusion 4 days following first) plus standard care vs. placebo (Plasma-Lyte) (second infusion 4 days following first) plus standard care	- All-cause mortality - SAEs - No. of days off mechanical ventilation - Resolution/improvement of ARDS - Length of stay - Clinical improvement scale - Hs-CRP, IL-6, IL-8, TNF-α
NCT03042143 (Northern Ireland)—REALIST trial	Target: $N = 75$	Single infusion of human umbilical cord derived CD362 enriched MSCs at maximum tolerable dose from phase I (dose escalation pilot study) plus standard care vs. placebo (Plasma-Lyte) plus standard care	- Oxygenation index - SAEs - SOFA - Respiratory compliance - P/F ratio - Driving pressure - Extubation and reintubation - Ventilation free days - Length of ICU/hospital stay - Mortality
NCT04444271 (Pakistan)	Target: $N = 20$	Bone marrow derived MSCs at 2×10^6 cells/kg on day 1 and 7 plus standard care vs. saline injection plus standard care	- Survival - No. oxygen support days - Time to negative nCoV test - CT scan - No. days to discharge
NCT04416139 (Mexico)	Target: $N = 10$	Umbilical cord derived MSCs from De bank Laboratory at 1×10^6 cells/kg (no control group—data compared to controls treated in a previous trial)	- PaO_2/FiO_2 ratio - HR and RR - Body temperature - Leukocyte, lymphocyte, and platelet counts - PCT, fibrinogen, D-dimer, ferritin - CRP, TNF-α, IL-1, IL-10, IL-6, IL-17 - VEGF - T-cell analysis ($CD4^+$ and $CD8^+$) - NK and dendritic cells - SAEs - CT scan - nCoV-test
NCT04429763 (Colombia)—CELMA	Target: $N = 30$	Umbilical cord derived MSCs at 1×10^6 cells/kg plus standard care vs. placebo (not stated) plus standard care control	- NEWS scale - Time to hospital discharge - Respiratory function - Inflammatory markers - Hematological and renal assessments
NCT04315987 (Brazil)	Target: $N = 90$	NestaCell MSCs at 2×10^7 cells/kg on days 1, 3, 5, and 7 plus standard care vs. placebo (not stated) on days 1, 3, 5, and 7 plus standard care	- Change in clinical condition - Mortality - SpO_2 - PaO_2/FiO_2 ratio - T-cell analysis ($CD4^+$ and $CD8^+$) - SAEs - Blood count and cardiac, hepatic, and renal profiles
NCT04366323 (Spain)	Target: $N = 26$	Allogenic and expanded adipose tissue derived MSCs at 8×10^6 cells × 2 (no control group)	- Safety of administration (SAEs) - Efficacy of administration
NCT04456361 (Mexico)	Target: $N = 9$	Wharton's jelly derived MSCs at 1×10^8 cells/kg (no control group)	- SpO_2 - PaO_2/FiO_2 ratio - Ground glass opacity and pneumonia infiltration - LDH, CRP, D-dimer, and Ferritin
NCT04366271 (Spain)	Target: $N = 106$	Undifferentiated allogenic umbilical cord MSCs (dose not stated) vs. standard care	- Mortality due to lung involvement - All-cause mortality - Days without mechanical ventilation - Days without vasopressors - Negative nCoV-test - SAEs

(Continued)

TABLE 2 | Continued

Clinical trials number	Participants	MSC source	Outcomes
NCT04252118 (China)	Target: $N = 20$	MSCs (source not stated) at 3×10^7 cells at day 0, 3, and 6 vs. standard care	- CT scan - SAEs - Pneumonia evaluation - Mortality - T-cell analysis ($CD4^+$ and $CD8^+$) - AAT, CRP, and CK
NCT04313322 (Jordan)	Target: $N = 5$	Wharton's jelly derived MSCs at 1×10^6 cells/kg for 3 doses, spaced 3 days apart (No control group)	- Alleviations of symptoms - CT scan - Negative nCoV-test
NCT04336254 (China)	Target: $N = 20$	Allogenic human dental pulp MSCs at 3×10^7 cells at day 1, 4, and 7 vs. saline control at day 1, 4, and 7	- TTCI - CT scan - Immune function markers - Time for negative nCoV-test - Blood count and classification - SpO_2 - RR - Body temperature - SAEs - CRP
NCT04346368 (China)	Target: $N = 20$	Bone marrow derived MSCs at 1×10^6 cells/kg at day 1 vs. standard care	- PaO_2/FiO_2 ratio - SAEs - Clinical outcome - No. days in hospital - CT scan - Changes in viral load - T-cell analysis ($CD4^+$ and $CD8^+$) - Mortality - CRP
NCT04288102 (China)	Target: $N = 100$	Umbilical cord derived MSCs at 4×10^7 at day 0, 3, and 6 vs. saline control at day 0, 3, and 6	- Pneumonia evaluation - Time to clinical improvement - PaO_2/FiO_2 ratio - Days on oxygen therapy - SpO_2 - 6-min walk test - Lymphocyte counts - Cytokine/chemokine assessment - SAEs - All-course mortality
NCT04273646 (China)	Target: $N = 48$	Umbilical cord derived MSCs at 0.5×10^6 cells/kg at day 1, 3, 5, and 7 plus standard care vs. saline control at day 1, 3, 5, and 7 plus standard care	- Pneumonia evaluation - SAEs - Survival - Organ failure assessment - CRP and Procalcitonin - Lymphocyte count - T-cell analysis ($CD3^+$, $CD4^+$, and $CD8^+$) - $CD4^+/CD8^+$ ratio
NCT04339660 (China)	Target: $N = 30$	Umbilical cord derived MSCs at 1×10^6 cells/kg vs. saline control	- TNF-α, IL-1β, IL-6, TGF-β, IL-8, PCT, CRP - SpO_2 - Mortality - CT scan - Blood count recovery time - Duration of respiratory symptoms - Negative nCoV-test
NCT04382547 (Belarus)	Target: $N = 40$	Allogenic pooled olfactory mucosa derived MSCs (dose not stated) vs. standard care control	- nCoV-test - SAEs
NCT04457609 (Indonesia)	Target: $N = 40$	Umbilical cord derived MSCs at 1×10^6 cells/kg with Oseltamivir and Azithromycin vs. standard care with Oseltamivir and Azithromycin	- Clinical improvement markers - General laboratory outcomes - PCT, bilirubin, D-dimer, and fibrinogen - Troponin and NT-proBNP - LIF, IL-6, IL-10, ferritin, CXCR3 - T-cell analysis ($CD4^+$, $CD8^+$, and $CD56^+$)

(Continued)

TABLE 2 | Continued

Clinical trials number	Participants	MSC source	Outcomes
			- VEGF - CT scan
NCT04352803 (USA)	Target: $N = 20$	Autologous adipose derived MSCs at 0.5×10^6 cells/kg vs. standard care control	- SAEs - Progression and time to/on mechanical ventilation - Length of hospital stay - All-cause mortality
NCT04490486 (USA)	Target: $N = 21$	Umbilical cord derived MSCs at 1×10^8 cells on day 0 and 3 vs. 1% human serum albumin in Plasmalyte A on day 0 and 3	- SAEs - Inflammatory markers - COVID-19 viral load - SOFA score - Electrolyte levels - LDH - No. ICU discharges - Vasoactive agent use - Mortality - Immune markers - CT scan
NCT04522986 (Japan)	Target: $N = 6$	Adipose derived MSCs at 1×10^8 cells once a week for 4 weeks (no control group)	- SAEs
NCT04461925 (Ukraine)	Target: $N = 30$	Placenta derived MSCs at 1×10^6 cells/kg once every 3 days for 3 infusions vs. standard care control	- PaO_2/FiO_2 ratio - Length of hospital stay - Mortality - CRP - CT scan - Duration of respiratory symptoms - Blood count recovery time
NCT04362189 (USA)	Target: $N = 100$	Allogenic adipose tissue derived MSCs (Hope Biosciences) at 1×10^6 cells/dose at day 0, 3, 7, and 10 vs. saline control at day 0, 3, 7, and 10	- IL-6, CRP, TNF-α, and IL-10 - Oxygenation - RTRA - ECG assessment - Routine blood assessments - Cardiac, hepatic, and renal assessment - Blood count - Platelets, Prothrombin time, D-dimer, and INR - Immune markers - SAEs - Chest X-ray - CT scan - Negative nCoV-test
NCT04371601 (China)	Target: $N = 60$	Umbilical cord derived MSCs at 1×10^6 cells/kg once every 4 days for 4 infusions vs. standard care control	- PaO_2/FiO_2 ratio - TNF-α and IL-6 - Immune markers - CRP and calcitonin
NCT04348461 (Spain)	Target: $N = 100$	Allogenic expanded adipose tissue derived MSCs at 1.5×10^6 cells/kg vs. standard care control	- Efficacy of administration of MSCs - SAEs
NCT04452097 (USA)	Target: $N = 9$	Umbilical cord derived MSCs (3 groups): - Low dose: 0.5×10^6 cells/kg - Middle dose: 1×10^6 cells/kg - High dose: 1.5×10^6 cells/kg	- SAEs - TEAEs - Selection of appropriate dose for Phase II trial
NCT04494386 (USA)	Target: $N = 60$	Umbilical cord lining derived MSCs at 1×10^6 cells/dose vs. saline control—either a single dose or 2 doses separated by 48 h	- DLT - SAEs - Berlin definition of ARDS - SpO_2 and PaO_2/FiO_2 ratio - No. of VFDs - Blood count - Routine blood assessments - BUN and urinalysis - AAT
NCT04345601 (USA)	Target: $N = 30$	MSCs (source not specified) at 1×10^8 cells vs. standard care control	- SAEs - Change to clinical status

(Continued)

TABLE 2 | Continued

Clinical trials number	Participants	MSC source	Outcomes
NCT04377334 (Germany)	Target: $N = 40$	Allogenic bone marrow derived MSCs (dose not stated) vs. standard care control	- Lung injury score - D-dimer - Pro-resolving lipid mediators - Phenotype of immune cells - Cytokine and chemokine analysis - Survival - Extubation - Lymphocyte subpopulation - Complement molecules - SARS-CoV-2 specific antibody
NCT04390139 (Spain)	Target: $N = 30$	Wharton's jelly derived MSCs at 1×10^6 cells/kg on day 1 and 3 vs. placebo (not stated) on day 1 and 3	- All-cause mortality - SAEs - Need for mechanical ventilation - No. of VFDs - PaO_2/FiO_2 ratio - SOFA index - APACHE II score - Duration of hospitalization - Immune response - Feasibility of MSCs - nCoV-test - LDH, D-dimer, and ferritin - Subpopulations of lymphocytes and immunoglobins - *In vitro* response of receptor lymphocytes
NCT04392778 (Turkey)	Target: $N = 30$	MSCs (source not stated) at 3×10^6 cells/kg on day 0, 3, and 6 to COVID-19 patients with a ventilator vs. saline control on day 0, 3, and 6 to COVID-19 patients with a ventilator vs. standard care control to COVID-19 patients without a ventilator	- Clinical improvement - CT scan - Negative nCoV-test - Blood tests
NCT04467047 (Brazil)	Target: $N = 10$	MSCs (source not stated) at 1×10^6 cells/kg (safety and feasibility study)	- Survival - CRP - Length of hospital stay - PaO_2/FiO_2 ratio - Liao's score (2020) - CT scan - Negative nCoV-test
NCT04398303 (USA)	Target: $N = 70$	Allogenic umbilical cord derived MSCs at 1×10^6 cells/kg vs. MSC conditioned media at 100 ml vs. placebo (MEM-α) at 100 ml	- Mortality - No. of VFDs - No. of days on O_2 therapy - No. of ICU-free days - Pulmonary function - Berlin criteria score
NCT04437823 (USA)	Target: $N = 20$	Umbilical cord derived MSCs at 0.5×10^6 cells/kg on day 1, 3, and 5 vs. standard care control	- SAEs - CT scan - Negative nCoV-test - SOFA score - Mortality - Clinical respiratory changes
NCT04269525 (China)	Target: $N = 16$	Umbilical cord derived MSCs at 3.3×10^7 cells on day 1, 3, 5, and 7	- PaO_2/FiO_2 ratio - Mortality - Length of hospital stay - nCoV PCR and antibody-test - Lung imaging - WBC and lymphocyte count - PCT - IL-2, IL-4, IL-4, IL-6, IL-10, TNF-α, γ-IFN, and CRP - NK cells - T-cell analysis ($CD4^+$, $CD8^+$)

(Continued)

TABLE 2 | Continued

Clinical trials number	Participants	MSC source	Outcomes
NCT04447833 (Sweden)	Target: $N = 9$	Allogenic bone marrow derived MSCs at 1×10^6 cells/kg ($n = 3$) and 2×10^6 cells/kg ($n = 6$)	- SAEs - All-cause mortality - Leucocytes and thrombocytes - CRP - Prothrombin - Creatinine - AST and AAT - NT-proBNP - Blood pressure - Body temperature - Efficacy for MSC use - Lung function - 6-min walk test - Quality of life assessment - Blood biomarkers - Sensitisation test
NCT04491240 (Russia)	Target: $N = 90$	Inhalation of MSC exosomes at $0.5–2 \times 10^{10}$ nanoparticles for COVID-19 patients ($n = 30$) and SARS-CoV-2 pneumonia patients ($n = 30$) vs. inhalation of solution free placebo ($n = 30$)—inhalation twice a day for 10 days	- SAEs - TTCI - Blood gases - SpO_2 - Chest imaging
NCT04333368 (France)	Target: $N = 40$	Umbilical cord Wharton's jelly derived MSCs at 1×10^6 cells/kg at day 1, 3, and 5 vs. placebo (NaCl) control at day 1, 3, and 5	- PaO_2/FiO_2 ratio - Lung injury score - Mortality - No. of VFDs - Use of sedatives - Use of neuromuscular blocking agent - ICU-acquired weakness - SAEs - Quality of life at 1 year - Cytokine analysis - Anti-HLA antibodies
NCT04466098 (USA)	Target: $N = 30$	Thawed product containing MSCs (source not stated) at 300×10^6 cells 3 times separated by 48 h vs. placebo (dextran and human serum albumin) control 3 times separated by 48 h	- SAEs - Inflammatory markers - PaO_2/FiO_2 ratio - Mean airway, peak and plateau pressure - PEEP - Mortality - No. of ICU free days - No. of VFDs - Acute lung injury score - No. of days off O_2 therapy
NCT04445220 (USA)	Target: $N = 22$	Allogenic human MSCs at 2.5×10^6 cells (low dose) and 7.5×10^6 cells (high dose) vs. standard care control—patients with COVID-19 and acute kidney injury	- Safety and tolerability - SAEs
NCT04276987 (China)	Target: $N = 30$	Allogenic adipose tissue derived MSC exosomes inhaled at 2×10^8 nano-vesicles on 5 consecutive days	- SAEs - TTCI - No. of patients weaning from mechanical ventilation - Vasoactive agent use - No. of days on mechanical ventilation - Mortality - SOFA score - Lymphocyte count - CRP, LDH, and D-dimer - NT-proBNP - IL-1β, IL-2R, IL-6, and IL-8 - Chest imaging - Negative nCoV-test

(Continued)

TABLE 2 | Continued

Clinical trials number	Participants	MSC source	Outcomes
IRCT20140528017891N8 (Iran)	Target: $N = 10$	Umbilical cord derived MSCs at 0.5–1 million cells/kg at 1st, 3rd, and 6th day vs. saline injection at 1st, 3rd, and 6th day plus standard care	- Mortality - Pneumonia severity index and CT scan - SpO_2 supply - CRP and PCT - Lymphocyte count - T-cell analysis ($CD3^+$, $CD4^+$, and $CD8^+$)
NCT04355728 (USA)	Target: $N = 24$	Umbilical cord derived vs. standard care control	- Adverse events - 90 day survival post-infusion - No. of VFDs - Change in oxygenation index and plat-PEEP - SOFA and SIT scores - TnI, CRP, and D-dimer - WBC and platelet count - AA/EPA ratio - 25-Hydroxyl Vitamin D - Alloantibody levels
CHICTR2000030224 (China)	Target: $N = NA$	MSCs (source unknown): critical and severe group injected with MSCs vs. critical and severe control group injected with saline	- SpO_2 - CT scan - Temperature - Routine blood markers - Inflammatory markers - Hepatic and renal function
ChiCTR2000030173 (China)	Target: $N = NA$	Umbilical cord derived vs. standard care control	- Pulmonary function - nCoV pneumonic nucleic acid test - Pulmonary CT and chest radiography
CHICTR2000030138 (China)	Target: $N = NA$	Umbilical cord derived vs. standard care plus saline injection control	- Clinical index
ChiCTR2000030088 (China)	Target: $N = NA$	Umbilical cord Wharton's jelly derived MSCs at 1×10^6 cells/kg vs. standard care and saline injection control	- nCoV pneumonic nucleic acid test - CT scan of ground glass shadow
CHICTR2000029990; TARGET $N = NA$ (China)	Target: $N = NA$	MSCs (source unknown) vs. standard care and saline injection control	- Respiratory system function (O_2 saturation) recovery time
ChiCTR2000029817 (NA)	Target: $N = NA$	Umbilical cord derived MSCs and NK cells: - High dose group: NK cells and MSCs at $> 5 \times 10^9$; Once every 2 days, five times - Conventional dose group: NK cells and MSCs at $> 3 \times 10^9$; once every 2 days, three times - Preventive dose group: NK cells and MSCs at $> 3 \times 10^9$; one infusion	- Time to disease recovery and time to negative nCoV test - Clearance rate and time of main symptoms - Transfer to ICU time - Routine blood tests - Biochemical indicators - Immune indices
CHICTR2000029816 (NA)	Target: $N = NA$	Umbilical cord derived MSCs (dose not stated) vs. standard care control	- Time to disease recovery and time to negative nCoV test - Clearance rate and time of main symptoms - Transfer to ICU time - Routine blood tests - Biochemical indicators - Immune indices
ChiCTR2000029580 (China)	Target: $N = NA$	Ruxolitinib and MSCs (source and dose not stated) vs. standard care control	- Safety
CHICTR2000029569 (China)	Target: $N = NA$	Umbilical cord derived blood mononuclear cells conditioned medium vs. standard care control	- PSI, CT, and X-Ray - Arterial blood gas - Assisted breathing time - Mortality - Disease evolution - Hospitalization days - Safety outcome index
EUCTR2020-001450-22-ES (Spain)	Target: $N = NA$	Allogenic umbilical cord derived MSCs (dose not stated)	- Mortality - Mechanical ventilation incidence - Need for vasopressors - Safety profile of MSCs - Neutrophils, monocytes and NK cells

(Continued)

TABLE 2 | Continued

Clinical trials number	Participants	MSC source	Outcomes
			PCT, ferritin, D-dimer and hs-troponin - PCR test - B and T lymphocytes - Interleukins, Th1, 2&17, NLRP3, and HMGB1
IRCT20200421047150N1 (Iran)	Target: $N = NA$	Umbilical card Wharton's jelly derived: three injections at 0.5–1 million cells/kg at 1st, 3rd, and 6th day. Control receiving standard care plus saline injection at 1st, 3rd, and 6th day	- Not stated
ACTRN12620000612910 (Australia)	Target: $N = NA$	Mesenchymoangioblast derived MSCs (CYP-001) at 2×10^6 cells/kg twice vs. ICU standard care control	- Not stated
NCT04361942 (Spain)	Target: $N = 24$	Allogenic MSCs (source unknown) vs. placebo (not stated)	- Withdrawal of invasive mechanical ventilation - Mortality - Patients achieving a clinical response - Patients achieving a radiological response
EUCTR2020-001266-11-ES (Spain)	Target: $N = 100$	Allogenic adipose tissue MSCs	- Efficacy and safety of administration of MSCs - Survival - Temperature - Withdrawal of mechanical ventilation - Patients transitioning to O_2 therapy from mechanical ventilation - O_2 therapy duration - Days in ICU - Duration of hospitalization - PaO_2/FiO_2 - Chest radiology - Routine blood markers - Inflammatory markers - Coagulation markers - Immune markers

Source: https://clinicaltrials.gov/ct2/home and https://trialstreamer.robotreviewer.net/.

hs-CRP, high sensitivity C-reactive protein; IL-, Interleukin-; TNF-α, Tumor necrosis factor-α; SAE, Serious adverse event; HR, Heart rate; RR, Respiratory rate; PCT, Procalcitonin; VEGF, Vascular endothelial growth factor; RTRA, Return to room air; INR, International normalized ratio of blood coagulation; TEAE, treatment emergent serious adverse events; DLT, Dose limiting toxicity; VFD, Ventilator free days; BUN, Blood urea nitrogen; APACHE, Acute physiology and chronic health disease classification; AST, Aspartate aminotransferase; NEWS, National early warning score; LDH, Lactate dehydrogenase; AAT, Alanine aminotransferase; CK, Creatine kinase; TTCI, Time to clinical improvement; LIF, Leukemia inhibiting factor; PEEP, Positive end-expiratory pressure; SOFA, Sequential organ failure assessment; SIT, Small identification test; TnI, Troponin I; AA, Arachidonic acid; EPA, Eicosapentaenoic acid; nCoV, novel coronavirus; Polymerase chain reaction; NK, Natural killer; Th, T helper; NLRP3, NLR Family Pyrin Domain Containing 3; HMGB1, High mobility group box 1.

to myocardial injury, MSC transplantation could offer a cardioprotective role.

MSC TRANSPLANTATION COULD ATTENUATE DAMAGE AND FACILITATE REPAIR OF THE CARDIOVASCULAR SYSTEM SEEN WITH COVID-19

In addition to the potential for MSCs to modulate the immune response and subsequent tissue damage in COVID-19, there is prospect for MSCs to treat the cardiac and cardiovascular effects of the SARS-CoV-2 virus, which may be long-lasting (**Figure 1**). As previously discussed, in a large proportion of patients there is evidence of myocardial injury, as suggested by elevated cTnI and cTnT levels (16, 19, 168, 169), and ventricular dysfunction indicated by raised circulating NT-proBNP (29, 31). Elevated cardiac biomarkers are associated with more severe prognosis and mortality in COVID-19 patients (18, 26, 29, 169, 170), suggesting the cardiac effects of the virus can drive worsening prognosis for the patient. Moreover, there are a number of studies detailing the severe cardiac effects of the virus, such as the development of heart failure (HF) (28), as well as incidences of acute coronary syndromes (ACS) (171, 172), ischaemic stroke (173) and myocardial infarction (MI) (171, 172). Given the significant deleterious effect of the virus on the myocardium, treatment options to minimize or to alleviate the cardiovascular side effects of the infection and disease are needed.

Treatment with MSCs may offer a clinical benefit to patients due to their regenerative and reparative potential if there is significant myocardial injury and myocardial cell death. There have been a number of studies investigating the use of autologous (174–180) or allogeneic MSCs (178, 181–184) for the treatment of cardiomyopathies and post-MI. Although the use of MSCs to treat cardiovascular dysfunction and damage in COVID-19 patients has yet to be fully elucidated, the studies over the past decade provide good preliminary evidence for researchers and clinicians alike to further investigate the use of this cellular therapy in COVID-19 patient cohorts.

Several studies in pig, rat and mouse models of MI showed significant reduction in infarct size or fibrosis (185–194), and improvements in cardiac function (185–187, 189, 190, 195, 196).

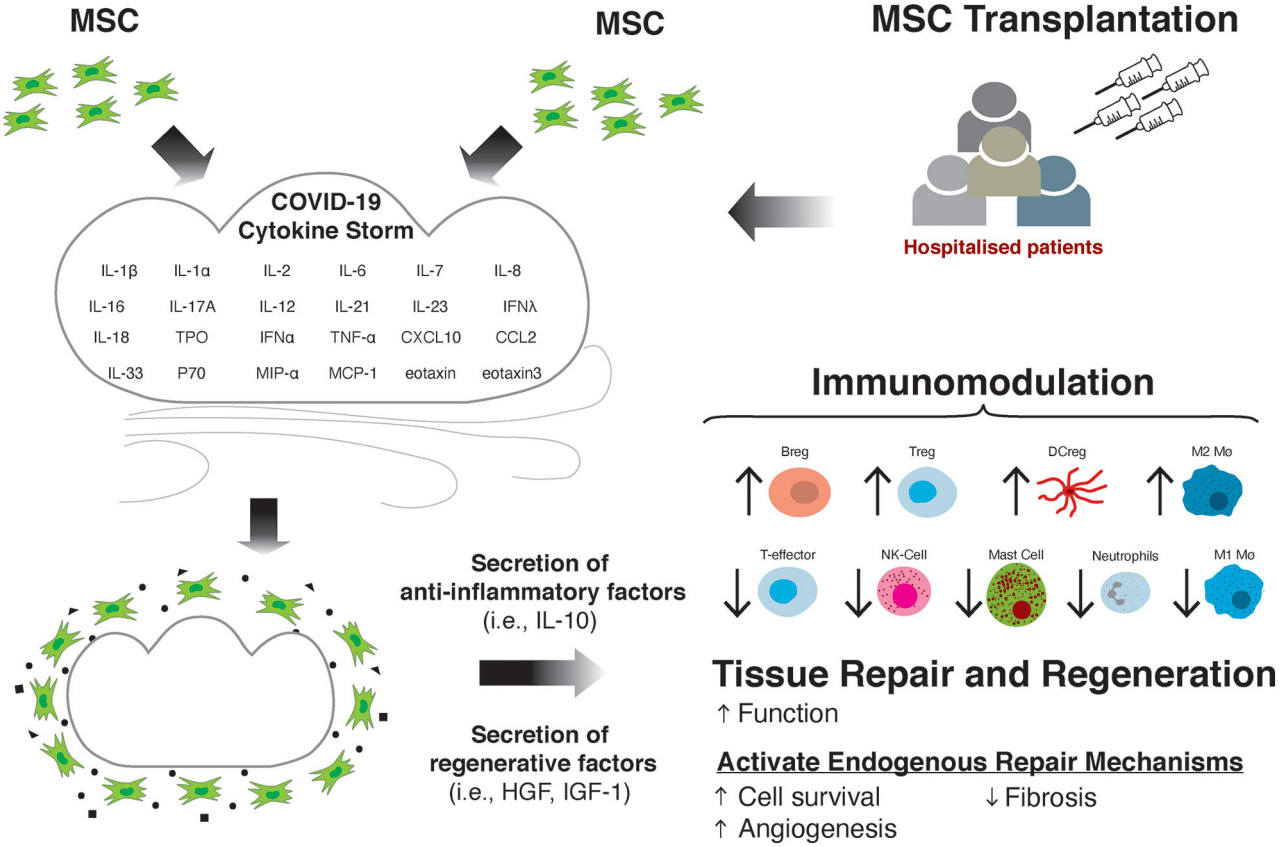

FIGURE 1 | MSC transplantation attenuates the damaging effects of the cytokine storm through immunomodulation and improving tissue repair and regeneration.

A meta-analysis of 52 pre-clinical animal studies of cell therapy for ischaemic heart disease reported that MSC therapy is safe and associated with significant ∼7.5% improvements in LVEF (197). In order to elicit increased efficacy, cell combination therapy has been investigated. In swine models of MI, human bone marrow-derived MSCs and cardiac-derived stromal MSC stem/progenitor cells from autologous or allogeneic sources were co-injected into the border zone of the infarct. Results showed that by combining the cell types there was greater therapeutic efficacy, improving cardiac repair/regeneration and LV functional recovery without adverse immunologic reaction (198, 199).

These promising findings have been followed by a number of human clinical trials. In a number of these human studies, the infusion and transplantation of MSCs have been deemed safe for treating MI patients (179, 200) as well as having been successful in improving some cardiac functional measures post-MI, such as LVEF (175, 177, 200–204), and improving global longitudinal strain measures (201). Penn et al. (204) showed in a phase I clinical trial in patients with first ST-elevation–myocardial infarction (STEMI), delivery of MSCs (MultiStem) using a coronary adventitial delivery system was well-tolerated and safe. In patients who exhibited significant myocardial damage, the delivery of ≥50 million MultiStem resulted in improved EF and stroke volume 4 months later (204). However, some of these studies, and others, found no difference between MSC treatment and no treatment/placebo on infarct size or perfusion changes in the months following the enrolment to the study (177, 205, 206). Additionally, several human studies fail to observe any clinical benefit for patients (179, 184, 205, 207). Inconsistent findings are likely due to the number and phenotype of MSCs being transplanted, their source, as well as mode and location of administration (myocardial, epicardial, or endocardial injection; systemic transplantation).

Despite mixed findings on the efficacy for improving cardiac function, MSCs can offer potential as regenerative cells for the CV system, where through a paracrine mechanism they activate endogenous repair mechanisms leading to blood vessel growth *via* angiogenesis, improved cardiomyocyte survival, reduced cardiomyocyte reactive hypertrophy, and fibrosis (**Figure 1**). We have clonally derived (from a single cell) a population of stromal cells with multipotent stem/progenitor cell properties from the adult mammalian heart, including human (208–210). These cells produce a repertoire of pro-survival and cardiovascular regenerative growth factors. We administered these cells intracoronary at differential doses (5×10^6, 5×10^7, and 1×10^8) in three groups of white Yorkshire female

pigs with MI, 30 min after coronary reperfusion. Pig serum was injected to six control pigs after MI. We found a high degree of cell engraftment in the damaged pig myocardium. By 3 weeks after MI and cell transplantation, there was increased new cardiomyocyte and capillary formation, which was not evident in the control hearts (194). Moreover, cell treatment preserved myocardial wall structure and attenuated remodeling by reducing cardiomyocyte hypertrophy, apoptosis, and scar formation (fibrosis) (211).

In mouse, rat and *in vitro* cell model studies, MSCs have been found to be potently angiogenic (192, 212–221). As outlined previously, MSCs most likely promote angiogenesis *via* paracrine means, such as secretion of angiogenic factors; vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor beta (TGF-β), and platelet-derived growth factor (PDGF) (222, 223), which are promoted under hypoxic conditions (224). Proteomic analysis of secreted exosomes, which carry lipids, proteins and genetic material to target tissues, from MSCs reveal several target pathways (225). These include inflammation and angiogenesis, of which, the angiogenesis pathway revealed specific interaction with NF-κ-B signaling. When these exosomes were cultured with HUVECs, a significant increase in endothelial tube formation was detected in a dose-dependent fashion (225). Zhang et al. (226) investigated the potential for MSC-derived exosomes to promote angiogenesis and cardiac repair post-MI in rats. Firstly, they observed that exosomes isolated from MSCs promoted tube formation of cardiac stem/progenitor cells *in vitro*. They subsequently transplanted cardiac stem/progenitor cells internalized with these exosomes into a rat model of MI, and observed an increased capillary density, which was followed by an improvement in LVEF, and reduction in fibrosis after 28 days post-implantation. Interestingly, the source of MSCs can significantly alter their pro-angiogenic potential. Du et al. (219) isolated MSCs from bone marrow, adipose tissue, umbilical cord and placenta and assessed their pro-angiogenic capacity using *in vitro* tube formation assays, as well as endothelial cell proliferation and assessment of angiogenic gene expression by RT-PCR. They found that MSCs isolated from the bone marrow and the placenta promoted angiogenesis *in vitro* to a greater extent than MSCs from adipose tissue and umbilical cord. In addition, they found that MSCs from these sources had a greater expression of VEGF mRNA and protein (219).

As well as promoting angiogenesis, MSCs may promote recovery from cardiac injury/insult by differentiating into mature cardiomyocytes, or by promoting resident cardiomyocyte proliferation. Mesenchymal stromal cells have a broad differentiation capacity, and have been shown to be able to differentiate into osteoblasts (227), neuronal cells (228) as well as upregulate cardiomyocyte markers, such as cardiac myosin heavy chain (229) and troponin T (229, 230). However, several studies have failed to observe significant trans-differentiation of MSCs into either endothelial cells or functional cardiomyocytes (189, 231, 232). Otherwise, MSCs have been found to promote cardiomyocyte DNA synthesis and proliferation, and signal cardiomyocyte gene upregulation (including VEGF, cyclin A2, and TGF-β2) (194, 233). Through their paracrine activity, they also prevent cardiomyocyte cell apoptosis (188, 221, 234–236) with several studies observing a reduced activation of the caspase-3 pathway in cardiomyocytes exposed to either MSC-derived exosomes (236) or conditioned media (237).

Other methods to maximize cellular function of cell therapies include "priming" which involves promoting expression of certain receptors, proteins and cytokines in the cells prior to transplantation or infusion. Mesenchymal stromal cells primed *in vitro*, prior to *in vivo* administration may offer opportunity to improve the efficacy of MSC treatment. Several studies have shown that by priming these cells *in vitro*, for example to highly express GATA-4 (MSC^{GATA-4}) (238), or CXCR4 (MSC^{CXCR4}) (233, 239) may improve the angiogenic paracrine activity of these cells. Mesenchymal stromal cells which were overexpressing GATA-4 contained more VEGF and IGF-1 protein, which, when blocked with neutralizing antibodies, attenuated the pro-angiogenic activity of MSC^{GATA-4} (238). Moreover, cardiac-derived stem/progenitor cells that express high levels of GATA-4 have shown to foster cardiomyocyte survival through IGF-1 paracrine signaling (240). MSC^{CXCR4} cells themselves were found to be highly angiogenic compared to un-primed MSCs, with greater expression of VEGF, which may partly explain the greater *in vitro* tube formation observed in a study by Zhang et al. (239). CXCR4 over-expression may be beneficial in promoting cell migration to ischaemic tissue due to the ligand stromal-derived factor-1 (SDF-1) (241), which is released in ischaemic tissue (242, 243). Thus, by selecting $CXCR4^{+}$ MSCs, or promoting CXCR4 expression *in vitro*, MSC migration to target infarct or damaged areas may be improved, subsequently allowing the cells to stimulate repair in the area required more efficiently.

Heart tissue damage post-MI, although largely due to ischaemic tissue injury and insult and associated cardiomyocyte loss, is also due to inflammation associated in the hours and days post-MI (244, 245). This inflammatory response is associated with further cardiac tissue damage and injury, as indicated by sustained and continual increases in cTnI and cTnT (246). Indeed MSC exosomes can regulate T-cell proliferation (215) as well as alter the balance between M1 and M2 macrophages in the infarcted heart (191), and the number of neutrophils and NK cells post-MI in the cardiac tissue (244) suggesting strong anti-inflammatory properties of the MSCs. In fact, a study by Luger et al. (244) found that MSC exosomes were able to reduce the number of NK cells in cardiac tissue post-MI, followed by a separate experiment whereby depleting NK cells 24 h prior to MI in mice, reduced the resulting infarct size. These findings infer that NK cells are involved in causing, or significantly contributing to, the cardiac damage resulting from an ischaemic challenge, and that MSCs could attenuate this inflammation. Taken together, it appears that MSCs also promote cardiac recovery *via* attenuating the ongoing inflammatory response, which is also a likely pathway for COVID-19-associated myocardial injury.

Although there is significant promise in the use of MSCs for cellular therapy to treat cardiovascular conditions, their efficacy for use in treating COVID-19-related cardiac dysfunction and injury is yet to be determined.

MSC TRANSPLANTATION IN COVID-19 PATIENTS COULD ALLEVIATE PULMONARY FIBROSIS

Fibrotic disorders in the lung, such as idiopathic pulmonary fibrosis (IPF), share similar comorbidities with COVID-19. Both conditions are progressive in nature, often because of worsening lung injury and fibrosis of alveolar walls. This underscores a common anti-fibrotic strategy.

Clinical trials with anti-fibrotic agents have shown promise in reversing progression of pulmonary fibrosis, as evidenced with nintedanib (247) and pirfenidone (248), which were approved by the FDA more than 6 years ago (249). This is supported by findings from pre-clinical animal models. An animal model of IPF with increased fibrosis and defective clearance of fibrocytes and myofibroblasts, was improved upon treatment with nintedanib (250). However, whether these agents will have clinical efficacy in COVID-19 remains unknown. Notably, commercial anti-fibrotic drugs, such as nintedanib and pirfenidone, are only available for oral delivery. This limits their use in COVID-19 patients, given that the population with fibrotic lung damage are usually hospitalized and intubated. Moreover, the hepatoxic side effects of both drugs and the contraindication of pirfenidone in renal dysfunction further limit their use, especially noting that SARS-CoV-2 is associated with development of both liver and kidney dysfunctions (58, 251). This highlights the need for better therapeutic strategies for lung fibrosis. Novel treatment options, such as cell-based therapy for replenishing lost functional capacity of resident stromal cells, have great potential for patients with COVID-19.

Cell-based therapy has been keenly investigated in the pre-clinical models using bleomycin-induced pulmonary fibrosis. Bleomycin-induced lung injury is a well-characterized model of human pulmonary fibrosis, with an initial phase of inflammatory activation and consequent fibrosis. In mice, intravenous injection of the primary human amniotic epithelial cells (hAECs) reduced lung inflammation and expression of the pro-fibrotic ligand TGF-β1 (252). Human amniotic epithelial cells transplantation also reduced the Ashcroft score, a validated marker of severity of lung fibrosis (253), likely due to increased degradation by matrix metalloproteinase (MMP)-2 and reduced expressions of tissue inhibitors of MMPs (TIMP)-1 and 2 (252). A pooled analysis of pre-clinical evidence demonstrated significantly better results on Ashcroft score and collagen contents for hAECs compared to placebo (254). Much akin to hAECs, MSCs have been shown to ameliorate pulmonary injury induced by bleomycin in experimental models (255). This has been demonstrated for bone marrow, umbilical cord, and amniotic fluid derived MSCs, respectively. The therapeutic efficacy of MSCs is also reported in other models of lung fibrosis. For example, adipose tissue-derived MSCs significantly attenuated lung function and fibrosis in a rodent model of silica-induced lung fibrosis (256). In summary, these data show that MSC-based therapy is a promising tool to address the pathophysiological consequences of COVID-19 in the lung. However, clinical translation would require more refined understanding of the anti-fibrotic mechanisms of MSCs.

Cumulative data show that MSCs protect against fibrosis *via* hepatocyte growth factor (HGF)-mediated mechanisms. Hepatocyte growth factor was originally identified as a mitogen for hepatocytes. It has now been shown to mediate mitogenic, anti-inflammatory, anti-apoptotic, and regenerative effects during tissue repair. In models of I/R lung injury, transplanted HGF-overexpressed MSCs resulted in lessened oxidative stress, inflammation, and attenuated lung injury (257). Hepatocyte growth factor also prolonged the survival of engrafted MSCs *via* increased expression of the anti-apoptotic protein Bcl-2 and repression of caspase-3 activation. In the context of fibrosis, there is evidence to suggest that HGF modulates pro-fibrotic pathways. For instance, microvesicles from human Wharton's Jelly MSCs inhibited apoptosis, fibrosis in pulmonary tissues, and activation of PI3K/AKT/mTOR pathway (258). These effects were blocked by using HGF-mRNA-deficient microvesicles or PI3K inhibitor. Hepatocyte growth factor also inhibits alveolar epithelial-to-mesenchymal transition and production of TGF-β1 independent of MSCs (259).

Other pathways have also been implicated in mediating the anti-fibrotic role of MSCs, including the activation of MMP-9 (260), programmed death (PD)-1/PD-L1 (261), and anti-apoptotic Bcl-2 (256, 257). MMP-9 is said to promote the degradation of collagen deposits, thereby facilitating the repair process following lung injury. On the other hand, MSC transplantation has been associated with repressed TGF-β1/SMAD3 (255), Wnt/β-catenin signaling (262), MyD88/TGF-β1 signaling (263), and N-methyl-d-aspartate receptor activity (264). Inhibition of Wnt/β-catenin signaling has a two-fold function. Firstly, it prevents downstream activation of pro-fibrotic genes and development of fibrosis; and, secondly, it rescues lung resident MSCs from differentiating to myofibroblasts (265).

Whether similar benefits will be seen in COVID-19 patients remains to be established. A single center, non-randomized, dose-escalation phase 1b trial of eight patients with moderate-to-severe IPF treated with intravenous bone marrow-derived MSC showed a good short-term safety profile (266). CT fibrosis score did not change 6 months after administration compared to baseline; however, there was no further worsening of fibrosis during follow-up. Similar findings were noted in a larger (randomized) trial of 20 IPF patients treated with high-dose bone marrow-derived MSCs (267). Subsequently, a trial of 61 patients with influenza A (H7N9)-induced ARDS showed significant reduction in the inflammatory marker CRP following menstrual-blood-derived MSC treatment, compared to placebo (163). While treated patients showed linear fibrosis, ground-glass opacity, and pleural thickening on chest CT at baseline, there was improvement in all patients after 24 weeks and up to 1 year after MSC treatment.

Our current understanding of the mechanisms of MSC-mediated improvement in lung (fibrotic) injury is incomplete, especially in the context of COVID-19. There are other important questions that will need to be addressed, too. For instance, would the MSCs need to be primed for improved efficacy? Previous studies have shown that pre-conditioning of MSCs with oncostatin M (268, 269), low-dose TGF-β1 (270), IL-6

(269), or ischaemia (271) improves the survival and therapeutic benefits. Obtaining the best MSCs for transplantation in terms of optimum immunomodulatory capacity and availability should be considered in COVID-19 studies. Primary MSCs, such as those obtained from bone marrow, umbilical cord, or adipose tissue, are limited by lack of available donors, many lack standardized preparations, with variations in quality, limited regenerative capacity, and finite lifespans. To overcome these limitations, a recent study investigated a novel hESC-derived MSC-like cell population, termed Immunity-and Matrix-Regulatory Cells (IMRCs) (272). Produced to good manufacturing standards, IMRCs demonstrated excellent safety and efficacy profiles in *in vivo* models of mice and monkeys. Additionally, IMRCs demonstrated superior immunomodulatory effects compared to umbilical cord-derived MSCs and the anti-fibrotic agent, pirfenidone (272).

CONCLUSION

Evidence now supports severe COVID-19 being associated with a dysregulated and hyperactive inflammatory systemic response; a cytokine storm. Older people (>60 years) and people with co-morbidities are more likely to develop a dysfunctional immune response, and resultant cytokine storm, that causes pathology and fails to successfully eradicate the pathogen. The exact reasons for this are unclear, although one reason may be a decline in immune function with age and chronic sterile inflammation due to the build-up of senescent cells and immunosenescence in aging humans (273).

The manifestations of elevated pro-inflammatory, sustained circulating factors due to the cytokine storm are not just confined to the lungs, with significant damage to the CV system and multi-organ damage and dysfunction. Interventions that target single cytokines (i.e., Tocilizumab targeting IL-6) do not seem efficacious in reducing mortality. Mesenchymal stromal cells owing to their powerful immunomodulatory function can holistically target and suppress the cytokine storm. At the same time, MSC transplantation is safe and has proven effective at activating endogenous repair mechanisms, leading to improved cardiac function, tissue regeneration and decreased fibrosis. Therefore, attenuating persistent organ dysfunction. Further mechanistic studies are required to investigate if MSC therapy can alleviate the cardiovascular consequences of COVID-19, and thus reduce cardiovascular risk in these patients. Work should also focus on determining the optimal dose, timing of injections (multiple dosing at different stages of the disease), systemic distribution of transplanted cells, type of MSCs used or use of exosomes, and the anti-viral effects of MSC transplantation.

AUTHOR CONTRIBUTIONS

LC put together the tables. TA put together the figure. GE-H oversaw the completion of the article. All authors contributed to writing the article.

REFERENCES

1. Rosenwald SM. History's deadliest pandemics, from ancient Rome to modern America | The Spokesman-Review. *The Spokesman-Review*. (2020) Available online at: https://www.spokesman.com/stories/2020/apr/15/historys-deadliest-pandemics-from-ancient-rome-to-/ (accessed August 23, 2020).
2. Gagnon A, Miller MS, Hallman SA, Bourbeau R, Herring DA, Earn DJD, et al. Age-specific mortality during the 1918 influenza pandemic: unravelling the mystery of high young adult mortality. *PLoS ONE.* (2013) 8:e69586. doi: 10.1371/journal.pone.0069586
3. Morens DM, Fauci AS. The 1918 influenza pandemic: insights for the 21st century. *J Infect Dis.* (2007) 195:1018–28. doi: 10.1086/511989
4. 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
5. Hoffmann M, Kleine-Weber H, Schroeder S, Mü MA, Drosten C, Pö S. 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
6. Aimes TR, Zijlstra A, Hooper DJ, Ogbourne MS, Sit M-L, Fuchs S, et al. Endothelial cell serine proteases expressed during vascular morphogenesis and angiogenesis. *Thombosis Haemost.* (2003) 89:561–72. doi: 10.1055/s-0037-1613388
7. Pan X-W, Xu D, Zhang H, Zhou W, Wang L-H, Cui X-G. Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: a study based on single-cell transcriptome analysis. *Intensive Care Med.* (2020) 46:1114–6. doi: 10.1007/s00134-020-06026-1
8. Sungnak W, Huang N, Bécavin C, Berg M, Queen R, Litvinukova M, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat Med.* (2020) 26:681–7. doi: 10.1038/s41591-020-0868-6
9. 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. *Eur Soc Cardiol.* (2020) 116:1097–100. doi: 10.1093/cvr/cvaa078
10. Meselson M. Droplets and aerosols in the transmission of SARS-CoV-2. *N Engl J Med.* (2020) 382:2063. doi: 10.1056/NEJMc2009324
11. 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
12. Kaye R, Chang CWD, Kazahaya K, Brereton J, Denneny JC. COVID-19 anosmia reporting tool: initial findings. *Otolaryngol Head Neck Surg (United States).* (2020) 163:132–4. doi: 10.1177/0194599820922992
13. Weiss ARR, Dahlke MH. Immunomodulation by mesenchymal stem cells (MSCs): mechanisms of action of living, apoptotic, and dead MSCs. *Front Immunol.* (2019) 10:1191. doi: 10.3389/fimmu.2019.01191
14. Golchin A, Seyedjafari E, Ardeshirylajimi A. Mesenchymal stem cell therapy for COVID-19: present or future. *Stem Cell Rev Rep.* (2020) 16:427–33. doi: 10.1007/s12015-020-09973-w
15. Golchin A, Farahany TZ, Khojasteh A, Soleimanifar F, Ardeshirylajimi A. The clinical trials of mesenchymal stem cell therapy in skin diseases: an update and concise review. *Curr Stem Cell Res Ther.* (2018) 14:22–33. doi: 10.2174/1574888x13666180913123424
16. 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
17. 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
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. 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
20. Léonard-Lorant I, Delabranche X, Séverac F, Helms J, Pauzet C, Collange O, et al. Acute pulmonary embolism in patients with COVID-19 at CT angiography and relationship to d-Dimer levels. *Radiology.* (2020) 296:E189–91. doi: 10.1148/radiol.2020201561
21. 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
22. 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
23. 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
24. 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
25. Roberts KA, Colley L, Agbaedeng TA, Ellison-Hughes GM, Ross MD. Vascular manifestations of COVID-19—thromboembolism and microvascular dysfunction. *Front Cardiovasc Med.* (2020) 7:598400. doi: 10.3389/fcvm.2020.598400
26. 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
27. 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.* (in press). doi: 10.1016/j.ajem.2020.04.052
28. 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
29. 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
30. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med.* (2020) 382:1708–20. doi: 10.1056/NEJMoa2002032
31. 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
32. Danzi GB, Loffi M, Galeazzi G, Gherbesi E. Acute pulmonary embolism and COVID-19 pneumonia: a random association? *Eur Heart J.* (2020) 41:1858. doi: 10.1093/eurheartj/ehaa254
33. 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
34. 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
35. Ferrara JLM, Abhyankar S, Gilliland DG. Cytokine storm of graft-versus-host disease: a critical effector role for interleukin-1. *Transpl Proc.* (1993) 56:1518–23. doi: 10.1097/00007890-199312000-00045
36. Yuen K, Wong S. Human infection by avian influenza A H5N1. *Hong Kong Med.* (2005) 11:189–199.
37. Noroozi R, Branicki W, Pyrc K, Łabaj PP, Pospiech E, Taheri M, et al. Altered cytokine levels and immune responses in patients with SARS-CoV-2 infection and related conditions. *Cytokine.* (2020) 133:155143. doi: 10.1016/j.cyto.2020.155143
38. Blanco-Melo D, Nilsson-Payant BE, Liu W-C, Lim JK, Albrecht RA, Tenoever BR. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell.* (2020) 181:1036–45. doi: 10.1016/j.cell.2020.04.026
39. 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
40. Liu J, Li S, Liu J, Liang B, Wang X, Wang H, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *EBioMedicine.* (2020) 55:102763. doi: 10.1016/j.ebiom.2020.102763
41. Del Valle DM, Kim-Schulze S, Hsin-Hui H, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature helps predict COVID-19 severity and death. *medRxiv Prepr Serv Heal Sci. [Preprint]* (2020). doi: 10.1101/2020.05.28.20115758
42. Lucas C, Wong P, Klein J, Castro TBR, Silva J, Sundaram M, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature.* (2020) 584:463. doi: 10.1038/s41586-020-2588-y
43. Laing AG, Lorenc A, Del Molino Del Barrio I, Das A, Fish M, Monin L, et al. A dynamic COVID-19 immune signature includes associations with poor prognosis. *Nat Med.* (2020) 26:1–13. doi: 10.1038/s41591-020-1038-6
44. Guo C, Li B, Ma H, Wang X, Cai P, Yu Q, et al. Single-cell analysis of two severe COVID-19 patients reveals a monocyte-associated and tocilizumab-responding cytokine storm. *Nat Commun.* (2020) 11:1–11. doi: 10.1038/s41467-020-17834-w
45. 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
46. Pedersen SF, Ho YC. SARS-CoV-2: a storm is raging. *J Clin Invest.* (2020) 130:2202–5. doi: 10.1172/JCI137647
47. 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
48. Aggarwal NR, King LS, D'Alessio FR. Diverse macrophage populations mediate acute lung inflammation and resolution. *Am J Physiol Lung Cell Mol Physiol.* (2014) 306:709–25. doi: 10.1152/ajplung.00341.2013
49. D'Alessio FR, Tsushima K, Aggarwal NR, West EE, Willett MH, Britos MF, et al. $CD4^+CD25^+Foxp3^+$ tregs resolve experimental lung injury in mice and are present in humans with acute lung injury. *J Clin Invest.* (2009) 119:2898–913. doi: 10.1172/JCI36498
50. Geiser T, Atabai K, Jarreau P-H, Ware BL, Pugin JR, Matthay AM. Pulmonary edema fluid from patients with acute lung injury augments *in vitro* alveolar epithelial repair by an IL-1b-dependent mechanism. *Am J Respir Crit Care Med.* (2001) 163:1384–8. doi: 10.1164/ajrccm.163.6.2006131
51. Han S, Mallampalli RK. The acute respiratory distress syndrome: from mechanism to translation. *J Immunol.* (2015) 194:855–60. doi: 10.4049/jimmunol.1402513
52. Hu X, Chakravarty SD, Ivashkiv LB. Regulation of interferon and toll-like receptor signaling during macrophage activation by opposing feedforward and feedback inhibition mechanisms. *Immunol Rev.* (2008) 226:41–56. doi: 10.1111/j.1600-065X.2008.00707.x
53. Risitano AM, Mastellos DC, Huber-Lang M, Yancopoulou D, Garlanda C, Ciceri F, et al. Complement as a target in COVID-19? *Nat Rev Immunol.* (2020) 20:343–4. doi: 10.1038/s41577-020-0320-7
54. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int J Oral Sci.* (2020) 12:1–5. doi: 10.1038/s41368-020-0074-x
55. 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
56. 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
57. Wong SH, Lui RNS, Sung JJY. Covid-19 and the digestive system. *J Gastroenterol Hepatol.* (2020) 35:744–8. doi: 10.1111/jgh.15047

58. Zhang C, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol.* (2020) 5:428–30. doi: 10.1016/S2468-1253(20)30057-1
59. Jin X, Lian JS, Hu JH, Gao J, Zheng L, Zhang YM, et al. Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. *Gut.* (2020) 69:1002–9. doi: 10.1136/gutjnl-2020-320926
60. Zhou Z, Zhao N, Shu Y, Han S, Chen B, Shu X. Effect of gastrointestinal symptoms in patients with COVID-19. *Gastroenterology.* (2020) 158:2294–7. doi: 10.1053/j.gastro.2020.03.020
61. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, et al. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. *JAMA Neurol.* (2020) 77:683–90. doi: 10.1001/jamaneurol.2020.1127
62. Varatharaj A, Thomas N, Ellul M, Davies NW, Pollak T, Tenorio EL, et al. UK-wide surveillance of neurological and neuropsychiatric complications of COVID-19: the first 153 patients. *SSRN Electron J [Preprint].* (2020). doi: 10.2139/ssrn.3601761
63. 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
64. Jacobs JL, Coyne CB. Mechanisms of MAVS regulation at the mitochondrial membrane. *J Mol Biol.* (2013) 425:5009–19. doi: 10.1016/j.jmb.2013.10.007
65. Rongvaux A. Innate immunity and tolerance toward mitochondria. *Mitochondrion.* (2018) 41:14–20. doi: 10.1016/j.mito.2017.10.007
66. Karan KR, Trumpff C, McGill MA, Thomas JE, Sturm G, Lauriola V, et al. Mitochondrial respiratory capacity modulates LPS-induced inflammatory signatures in human blood. *Brain Behav Immun Heal.* (2020) 5:1–12. doi: 10.1016/j.bbih.2020.100080
67. Kawai T, Akira S. Antiviral signaling through pattern recognition receptors. *J Biochem.* (2007) 141:137–45. doi: 10.1093/jb/mvm032
68. Gordon DE, Jang GM, Bouhaddou M, Xu J, Obernier K, White KM, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature.* (2020) 583:459–68. doi: 10.1038/s41586-020-2286-9
69. Shi C-S, Qi H-Y, Boularan C, Huang N-N, Abu-Asab M, Shelhamer JH, et al. SARS-coronavirus open reading frame-9b suppresses innate immunity by targeting mitochondria and the MAVS/TRAF3/TRAF6 signalosome. *J Immunol.* (2014) 193:3080–9. doi: 10.4049/jimmunol.1303196
70. Spiegel M, Pichlmair A, Martínez-Sobrido L, Cros J, García-Sastre A, Haller O, et al. Inhibition of beta interferon induction by severe acute respiratory syndrome coronavirus suggests a two-step model for activation of interferon regulatory factor 3. *J Virol.* (2005) 79:2079–86. doi: 10.1128/jvi.79.4.2079-2086.2005
71. West AP, Khoury-Hanold W, Staron M, Tal MC, Pineda CM, Lang SM, et al. Mitochondrial DNA stress primes the antiviral innate immune response. *Nature.* (2015) 520:553–7. doi: 10.1038/nature14156
72. Arnoult D, Soares F, Tattoli I, Castanier C, Philipott D, Girardi ES. An N-terminal addressing sequence targets NLRX1 to the mitochondrial matrix. *J Cell Sci.* (2009) 122:3161–8. doi: 10.1242/jcs.051193
73. Breda CN de S, Davanzo GG, Basso PJ, Saraiva Câmara NO, Moraes-Vieira PMM. Mitochondria as central hub of the immune system. *Redox Biol.* (2019) 26:101255. doi: 10.1016/j.redox.2019.101255
74. Fève B, Bastard J-P. The role of interleukins in insulin resistance and type 2 diabetes mellitus. *Nat Rev Endocrinol.* (2009) 5:305–11. doi: 10.1038/nrendo.2009.62
75. 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 Metab.* (2020) 31:1068–77.e3. doi: 10.1016/j.cmet.2020.04.021
76. Codo AC, Davanzo GG, Monteiro L de B, de Souza GF, Muraro SP, Virgilio-da-Silva JV, et al. Elevated glucose levels favor SARS-CoV-2 infection and monocyte response through a HIF-1α/glycolysis-dependent axis. *Cell Metab.* (2020) 32:437–46.e5. doi: 10.1016/j.cmet.2020.07.007
77. Remels AHV, Derks WJA, Cillero-Pastor B, Verhees KJP, Kelders MC, Heggermont W, et al. NF-κB-mediated metabolic remodelling in the inflamed heart in acute viral myocarditis. *Biochim Biophys Acta Mol Basis Dis.* (2018) 1864:2579–89. doi: 10.1016/j.bbadis.2018.04.022
78. Al-Huseini I, Harada M, Nishi K, Nguyen-Tien D, Kimura T, Ashida N. Improvement of insulin signalling rescues inflammatory cardiac dysfunction. *Sci Rep.* (2019) 9:1–13. doi: 10.1038/s41598-019-51304-8
79. 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
80. 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
81. He J, Wu B, Chen Y, Tang J, Liu Q, Zhou S, et al. Characteristic electrocardiographic manifestations in patients with COVID-19. *Can J Cardiol.* (2020) 36:966.e1–e4. doi: 10.1016/j.cjca.2020.03.028
82. Hu H, Ma F, Wei X, Fang Y. Coronavirus fulminant myocarditis treated with glucocorticoid and human immunoglobulin. *Eur Heart J.* (2020) ehaa190. doi: 10.1093/eurheartj/ehaa190
83. 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
84. 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
85. 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
86. 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
87. Magro C, Mulvey JJ, Berlin D, Nuovo G, Salvatore S, Harp J, et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. *Transl Res.* (2020) 220:1–13. doi: 10.1016/j.trsl.2020.04.007
88. Middleton EA, He 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
89. Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, et al. Coronavirus infections and immune responses. *J Med Virol.* (2020) 92:424–32. doi: 10.1002/jmv.25685
90. Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. *Science.* (2015) 349:316–20. doi: 10.1126/science.aaa8064
91. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, et al. Targeting potential drivers of COVID-19: neutrophil extracellular traps. *J Exp Med.* (2020) 217:e20200652. doi: 10.1084/jem.20200652
92. Mold C, Morris CA. Complement activation by apoptotic endothelial cells following hypoxia/reoxygenation. *Immunology.* (2001) 102:359–64. doi: 10.1046/j.1365-2567.2001.01192.x
93. Irabien-Ortiz Á, Carreras-Mora J, Sionis A, Pàmies J, Montiel J, Tauron M. Fulminant myocarditis due to COVID-19. *Rev Española Cardiol (English Ed).* (2020) 73:503–4. doi: 10.1016/j.rec.2020.04.005
94. 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
95. Teuwen L-A, 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
96. Incalza MA, Perrini S. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Vascul Pharmacol.* (2017) 100:1–19. doi: 10.1016/j.vph.2017.05.005
97. 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
98. Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. *Nat Rev Immunol.* (2007) 7:803–15. doi: 10.1038/nri2171
99. 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
100. Paria BC, Vogel SM, Ahmmed GU, Alamgir S, Shroff J, Malik AB, et al. Tumor necrosis factor-α-induced TRPC1 expression amplifies store-operated $Ca2^+$ influx and endothelial permeability. *Am J Physiol Lung Cell Mol Physiol.* (2004) 287:1303–13. doi: 10.1152/ajplung.00240.2004
101. Vandenbroucke E, Mehta D, Minshall R, Malik AB. Regulation of endothelial junctional permeability. *Ann N Y Acad Sci.* (2008) 1123:134–45. doi: 10.1196/annals.1420.016
102. Sandoval R, Malik AB, Minshall RD, Kouklis P, Ellis CA, Tiruppathi C. $Ca2^+$ signalling and PKCα activate increased endothelial permeability by disassembly of VE-cadherin junctions. *J Physiol.* (2001) 533:433–45. doi: 10.1111/j.1469-7793.2001.0433a.x
103. Petrache I, Birukova A, Ramirez SI, Garcia JGN, Verin AD. The role of the microtubules in tumor necrosis factor-induced endothelial cell permeability. *Am J Respir Cell Mol Biol.* (2003) 28:574–81. doi: 10.1165/rcmb.2002-0075OC
104. Tinsley JH, Hunter FA, Childs EW. PKC and MLCK-dependent, cytokine-induced rat coronary endothelial dysfunction. *J Surg Res.* (2009) 152:76–83. doi: 10.1016/j.jss.2008.02.022
105. 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
106. Styp-Rekowska B, Hlushchuk R, Pries AR, Djonov V. Intussusceptive angiogenesis: pillars against the blood flow. *Acta Physiol.* (2011) 202:213–23. doi: 10.1111/j.1748-1716.2011.02321.x
107. Mentzer, SJ, Konerding, MA. Intussusceptive angiogenesis: expansion and remodeling of microvascular networks. *Angiogenesis* (2014) 17:499-509. doi: 10.1007/s10456-014-9428-3
108. Konerding MA, Turhan A, Ravnic DJ, Lin M, Fuchs C, Secomb TW, et al. Inflammation-induced intussusceptive angiogenesis in murine colitis. *Anat Rec.* (2010) 293:849–57. doi: 10.1002/ar.21110
109. Ackermann M, Stark H, Neubert L, Schubert S, Borchert P, Linz F, et al. Morphomolecular motifs of pulmonary neoangiogenesis in interstitial lung diseases. *Eur Respir J.* (2020) 55:1900933. doi: 10.1183/13993003.00933-2019
110. García-Ruiz C, Colell A, Marí M, Morales A, Fernández-Checa JC. Direct effect of ceramide on the mitochondrial electron transport chain leads to generation of reactive oxygen species: role of mitochondrial glutathione. *J Biol Chem.* (1997) 272:11369–77. doi: 10.1074/jbc.272.17.11369
111. Zhang D, Yi F-X, Zou A-P, Li P-L. Role of ceramide in TNF-α-induced impairment of endothelium-dependent vasorelaxation in coronary arteries. *Am J Physiol Circ Physiol.* (2002) 283:H1785–94. doi: 10.1152/ajpheart.00318.2002
112. Frey RS, Rahman A, Kefer JC, Minshall RD, Malik AB. PKCζ regulates TNF-α-induced activation of NADPH oxidase in endothelial cells. *Circ Res.* (2002) 90:1012–9. doi: 10.1161/01.RES.0000017631.28815.8E
113. Wu F, Schuster DP, Tyml K, Wilson JX. Ascorbate inhibits NADPH oxidase subunit p47phox expression in microvascular endothelial cells. *Free Radic Biol Med.* (2007) 42:124–31. doi: 10.1016/j.freeradbiomed.2006.10.033
114. Liaudet L, Vassalli G, Pacher P. Role of peroxynitrite in the redox regulation of cell signal transduction pathways. *Front Biosci.* (2009) 14:4809–14. doi: 10.2741/3569
115. Radi R. *Nitric Oxide, Oxidants, and Protein Tyrosine Nitration.* (2004). Available online at: www.pnas.orgcgidoi10.1073pnas.0307446101 (accessed August 5, 2020).
116. Schulz E, Gori T, Münzel T. Oxidative stress and endothelial dysfunction in hypertension. *Hypertens Res.* (2011) 34:665–73. doi: 10.1038/hr.2011.39
117. Landmesser U, Spiekermann S, Dikalov S, Tatge H, Wilke R, Kohler C, et al. Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure. *Circulation.* (2002) 106:3073–8. doi: 10.1161/01.CIR.0000041431.57222.AF
118. Naik E, Dixit VM. Mitochondrial reactive oxygen species drive proinflammatory cytokine production. *J Exp Med.* (2011) 208:417–20. doi: 10.1084/jem.20110367
119. Browner NC, Sellak H, Lincoln TM. Downregulation of cGMP-dependent protein kinase expression by inflammatory cytokines in vascular smooth muscle cells. *Am J Physiol Cell Physiol.* (2004) 287:88–96. doi: 10.1152/ajpcell.00039.2004.-NO
120. Hiroki J, Shimokawa H, Higashi M, Morikawa K, Kandabashi T, Kawamura N, et al. Inflammatory stimuli upregulate Rho-kinase in human coronary vascular smooth muscle cells. *J Mol Cell Cardiol.* (2004) 37:537–46. doi: 10.1016/j.yjmcc.2004.05.008
121. Cheng H, Wang Y, Wang G. Organ-protective effect of angiotensin-converting enzyme 2 and its effect on the prognosis of COVID-19. *J Med Virol.* (2020) 92:726–30. doi: 10.1002/jmv.25785
122. Yan C, Yu H, Huang M, Li J, Zhang X, Han Y. Tumor necrosis factor-α promote permeability of human umbilical vein endothelial cells *via* activating RhoA-ERK1/2 pathway. *Zhonghua Xin Xue Guan Bing Za Zhi.* (2011) 39:531–7.
123. Goshua G, Pine AB, Meizlish ML, Chang C, Zhang H, Bahel P, et al. Articles Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. *Lancet Haematol.* (2020) 3026:1–8. doi: 10.1016/S2352-3026(20)30216-7
124. Orr AW, Hastings NE, Blackman BR, Wamhoff BR. Complex regulation and function of the inflammatory smooth muscle cell phenotype in atherosclerosis. *J Vasc Res.* (2010) 47:168–80. doi: 10.1159/000250095
125. Jung YD, Fan F, McConkey DJ, Jean ME, Liu W, Reinmuth N, et al. Role of P38 MAPK, AP-1, and NF-κb in interleukin-1β-induced IL-8 expression in human vascular smooth muscle cells. *Cytokine.* (2002) 18:206–13. doi: 10.1006/cyto.2002.1034
126. Krown KA, Page MT, Nguyen C, Zechner D, Gutierrez V, Comstock KL, et al. Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes: involvement of the sphingolipid signaling cascade in cardiac cell death. *J Clin Invest.* (1996) 98:2854–65. doi: 10.1172/JCI119114
127. Haudek SB, Taffet GE, Schneider MD, Mann DL. TNF provokes cardiomyocyte apoptosis and cardiac remodeling through activation of multiple cell death pathways. *J Clin Invest.* (2007) 117:2692–701. doi: 10.1172/JCI29134
128. Pulkki KJ. Cytokines and cardiomyocyte death. *Ann Med.* (1997) 29:339–43. doi: 10.3109/07853899708999358
129. Frangogiannis NG. Inflammation in cardiac injury, repair and regeneration. *Curr Opin Cardiol.* (2015) 30:240–5. doi: 10.1097/HCO.0000000000000158
130. Prabhu SD, Frangogiannis NG. The biological basis for cardiac repair after myocardial infarction. *Circ Res.* (2016) 119:91–112. doi: 10.1161/CIRCRESAHA.116.303577
131. Bernardo ME, Fibbe WE. Mesenchymal stromal cells: sensors and switchers of inflammation. *Cell Stem Cell.* (2013) 13:392–402. doi: 10.1016/j.stem.2013.09.006
132. de Witte SFH, Luk F, Sierra Parraga JM, Gargesha M, Merino A, Korevaar SS, et al. Immunomodulation by therapeutic mesenchymal stromal cells (MSC) is triggered through phagocytosis of MSC by monocytic cells. *Stem Cells.* (2018) 36:602–15. doi: 10.1002/stem.2779
133. Leng Z, Zhu R, Hou W, Feng Y, Yang Y, Han Q, et al. Transplantation of ACE2- mesenchymal stem cells improves the outcome of patients with covid-19 pneumonia. *Aging Dis.* (2020) 11:216–28. doi: 10.14336/AD.2020.0228
134. Chan MCW, Kuok DIT, Leung CYH, Hui KPY, Valkenburg SA, Lau EHY, et al. Human mesenchymal stromal cells reduce influenza A H5N1-associated acute lung injury *in vitro* and *in vivo*. *Proc Natl Acad Sci USA.* (2016) 113:3621–6. doi: 10.1073/pnas.1601911113
135. Melief SM, Schrama E, Brugman MH, Tiemessen MM, Hoogduijn MJ, Fibbe WE, et al. Multipotent stromal cells induce human regulatory T cells through a novel pathway involving skewing of monocytes toward anti-inflammatory macrophages. *Stem Cells.* (2013) 31:1980–91. doi: 10.1002/stem.1432
136. Huh JW, Kim WY, Park YY, Lim CM, Koh Y, Kim MJ, et al. Anti-inflammatory role of mesenchymal stem cells in an acute lung injury mouse model. *Acute Crit Care.* (2018) 33:154–61. doi: 10.4266/acc.2018.00619
137. Asami T, Ishii M, Namkoong H, Yagi K, Tasaka S, Asakura T, et al. Anti-inflammatory roles of mesenchymal stromal cells during acute Streptococcus pneumoniae pulmonary infection in mice. *Cytotherapy.* (2018) 20:302–13. doi: 10.1016/j.jcyt.2018.01.003
138. Lee SH, Jang AS, Kim YE, Cha JY, Kim TH, Jung S, et al. Modulation of cytokine and nitric oxide by mesenchymal stem cell transfer in lung injury/fibrosis. *Respir Res.* (2010) 11:16. doi: 10.1186/1465-9921-11-16

139. Khedoe PPSJ, de Kleijn S, van Oeveren-Rietdijk AM, Plomp JJ, de Boer HC, van Pel M, et al. Acute and chronic effects of treatment with mesenchymal stromal cells on LPS-induced pulmonary inflammation, emphysema and atherosclerosis development. *PLoS ONE.* (2017) 12:e0183741. doi: 10.1371/journal.pone.0183741
140. Geng Y, Zhang L, Fu B, Zhang J, Hong Q, Hu J, et al. Mesenchymal stem cells ameliorate rhabdomyolysis-induced acute kidney injury *via* the activation of M2 macrophages. *Stem Cell Res Ther.* (2014) 5:80. doi: 10.1186/scrt469
141. Li S, Zheng X, Li H, Zheng J, Chen X, Liu W, et al. Mesenchymal stem cells ameliorate hepatic ischemia/reperfusion injury *via* inhibition of neutrophil recruitment. *J Immunol Res.* (2018) 2018:1–10. doi: 10.1155/2018/7283703
142. Espinosa G, Plaza A, Schenffeldt A, Alarcón P, Gajardo G, Uberti B, et al. Equine bone marrow-derived mesenchymal stromal cells inhibit reactive oxygen species production by neutrophils. *Vet Immunol Immunopathol.* (2020) 221:109975. doi: 10.1016/j.vetimm.2019.109975
143. Jiang D, Muschhammer J, Qi Y, Kügler A, de Vries JC, Saffarzadeh M, et al. Suppression of neutrophil-mediated tissue damage-a novel skill of mesenchymal stem cells. *Stem Cells.* (2016) 34:2393–406. doi: 10.1002/stem.2417
144. Hashmi S, Ahmed M, Murad MH, Litzow MR, Adams RH, Ball LM, et al. Survival after mesenchymal stromal cell therapy in steroid-refractory acute graft-versus-host disease: systematic review and meta-analysis. *Lancet Haematol.* (2016) 3:e45–52. doi: 10.1016/S2352-3026(15)00224-0
145. Sala E, Genua M, Petti L, Anselmo A, Arena V, Cibella J, et al. Mesenchymal stem cells reduce colitis in mice *via* release of TSG6, independently of their localization to the intestine. *Gastroenterology.* (2015) 149:163–76.e20. doi: 10.1053/j.gastro.2015.03.013
146. Song HB, Park SY, Ko JH, Park JW, Yoon CH, Kim DH, et al. Mesenchymal stromal cells inhibit inflammatory lymphangiogenesis in the cornea by suppressing macrophage in a TSG-6-dependent manner. *Mol Ther.* (2018) 26:162–72. doi: 10.1016/j.ymthe.2017.09.026
147. Wang G, Cao K, Liu K, Xue Y, Roberts AI, Li F, et al. Kynurenic acid, an IDO metabolite, controls TSG-6-mediated immunosuppression of human mesenchymal stem cells. *Cell Death Differ.* (2018) 25:1209–23. doi: 10.1038/s41418-017-0006-2
148. Tjabringa GS, Zandieh-Doulabi B, Helder MN, Knippenberg M, Wuisman PIJM, Klein-Nulend J. The polymine spermine regulates osteogenic differentiation in adipose stem cells. *J Cell Mol Med.* (2008) 12:1710–7. doi: 10.1111/j.1582-4934.2008.00224.x
149. Yang Q, Zheng C, Cao J, Cao G, Shou P, Lin L, et al. Spermidine alleviates experimental autoimmune encephalomyelitis through inducing inhibitory macrophages. *Cell Death Differ.* (2016) 23:1850–61. doi: 10.1038/cdd.2016.71
150. Selleri S, Bifsha P, Civini S, Pacelli C, Dieng MM, Lemieux W, et al. Human mesenchymal stromal cell-secreted lactate induces M2-macrophage differentiation by metabolic reprogramming. *Oncotarget.* (2016) 7:30193–210. doi: 10.18632/oncotarget.8623
151. Groh ME, Maitra B, Szekely E, Koç ON. Human mesenchymal stem cells require monocyte-mediated activation to suppress alloreactive T cells. *Exp Hematol.* (2005) 33:928–34. doi: 10.1016/j.exphem.2005.05.002
152. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, et al. Human mesenchymal stem cells modulate B-cell functions. *Blood.* (2006) 107:367–72. doi: 10.1182/blood-2005-07-2657
153. Peng Y, Chen X, Liu Q, Zhang X, Huang K, Liu L, et al. Mesenchymal stromal cells infusions improve refractory chronic graft versus host disease through an increase of $CD5^+$ regulatory B cells producing interleukin 10. *Leukemia.* (2015) 29:636–46. doi: 10.1038/leu.2014.225
154. Zhu Y, Wang Y, Zhao B, Niu X, Hu B, Li Q, et al. Comparison of exosomes secreted by induced pluripotent stem cell-derived mesenchymal stem cells and synovial membrane-derived mesenchymal stem cells for the treatment of osteoarthritis. *Stem Cell Res The.r.* (2017) 8:64. doi: 10.1186/s13287-017-0510-9
155. Dabrowska S, Andrzejewska A, Strzemecki D, Muraca M, Janowski M, Lukomska B. Human bone marrow mesenchymal stem cell-derived extracellular vesicles attenuate neuroinflammation evoked by focal brain injury in rats. *J Neuroinflammation.* (2019) 16:1–15. doi: 10.1186/s12974-019-1602-5
156. Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C, et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. *Nat Rev Nephrol.* (2018) 14:493–507. doi: 10.1038/s41581-018-0023-5
157. Huppert LA, Matthay MA. Alveolar fluid clearance in pathologically relevant conditions: *in vitro* and *in vivo* models of acute respiratory distress syndrome. *Front Immunol.* (2017) 8:371. doi: 10.3389/fimmu.2017.00371
158. Simonson OE, Mougiakakos D, Heldring N, Bassi G, Johansson HJ, Dalén M, et al. *In vivo* effects of mesenchymal stromal cells in two patients with severe acute respiratory distress syndrome. *Stem Cells Transl Med.* (2015) 4:1199–213. doi: 10.5966/sctm.2015-0021
159. Horie S, Gonzalez HE, Laffey JG, Masterson CH. Cell therapy in acute respiratory distress syndrome. *J Thorac Dis.* (2018) 10:5607–20. doi: 10.21037/jtd.2018.08.28
160. Xiao K, Hou F, Huang X, Li B, Qian ZR, Xie L. Mesenchymal stem cells: current clinical progress in ARDS and COVID-19. *Stem Cell Res Ther.* (2020) 11:305. doi: 10.1186/s13287-020-01804-6
161. Liang B, Chen J, Li T, Wu H, Yang W, Li Y, Li J, Yu C, Nie F, Ma Z, et al. Clinical remission of a critically ill COVID-19 patient treated by human umbilical cord mesenchymal stem cells. *Medicine.* (2020) 99:e21429. doi: 10.1097/MD.0000000000021429
162. Zhang Y, Ding J, Ren S, Wang W, Yang Y, Li S, et al. Intravenous infusion of human umbilical cord Wharton's jelly-derived mesenchymal stem cells as a potential treatment for patients with COVID-19 pneumonia. *Stem Cell Res Ther.* (2020) 11:207. doi: 10.1186/s13287-020-01725-4
163. Chen J, Hu C, Chen L, Tang L, Zhu Y, Xu X, et al. Clinical study of mesenchymal stem cell treatment for acute respiratory distress syndrome induced by epidemic influenza A (H7N9) infection: a hint for COVID-19 treatment. *Engineering.* (in press). doi: 10.1016/j.eng.2020.02.006
164. Sengupta V, Sengupta S, Lazo A, Woods P, Nolan A, Bremer N. Exosomes derived from bone marrow mesenchymal stem cells as treatment for severe COVID-19. *Stem Cells Dev.* (2020) 29:747–54. doi: 10.1089/scd.2020.0080
165. Moll G, Drzeniek N, Kamhieh-Milz J, Geissler S, Volk H-D, Reinke P. MSC therapies for COVID-19: importance of patient coagulopathy, thromboprophylaxis, cell product quality and mode of delivery for treatment safety and efficacy. *Front Immunol.* (2020) 11:1091. doi: 10.3389/fimmu.2020.01091
166. Can A, Coskun H. The rationale of using mesenchymal stem cells in patients with COVID-19-related acute respiratory distress syndrome: what to expect. *Stem Cells Transl Med.* (2020) 9:sctm.20-0164. doi: 10.1002/sctm.20-0164
167. Peng H, Gong T, Huang X, Sun X, Luo H, Wang W, et al. A synergistic role of convalescent plasma and mesenchymal stem cells in the treatment of severely ill COVID-19 patients: a clinical case report. *Stem Cell Res Ther.* (2020) 291:1–6. doi: 10.1186/s13287-020-01802-8
168. 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
169. 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
170. 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 SARSCoV- 2: a prospective cohort study. *Eur Respir J.* (2020) 55:2000524. doi: 10.1183/13993003.00524-2020
171. 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
172. 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
173. 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
174. Zhu H, Song X, Jin LY, Jin P, Guan R, Liu X, et al. Comparison of intra-coronary cell transplantation after myocardial infarction: autologous skeletal myoblasts versus bone marrow mesenchymal stem cells. *J Int Med Res.* (2009) 37:298–307. doi: 10.1177/147323000903700203

175. Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardio.l.* (2004) 94:92–5. doi: 10.1016/j.amjcard.2004.03.034
176. Chin SP, Poey AC, Wong CY, Chang SK, Tan CS, Ng MT, et al. Intramyocardial and intracoronary autologous bone marrow-derived mesenchymal stromal cell treatment in chronic severe dilated cardiomyopathy. *Cytotherapy.* (2011) 13:814–21. doi: 10.3109/14653249.2011.574118
177. Lu M, Liu S, Zheng Z, Yin G, Song L, Chen H, et al. A pilot trial of autologous bone marrow mononuclear cell transplantation through grafting artery: a sub-study focused on segmental left ventricular function recovery and scar reduction. *Int J Cardiol.* (2013) 168:2221–7. doi: 10.1016/j.ijcard.2013.01.217
178. Premer C, Blum A, Bellio MA, Schulman IH, Hurwitz BE, Parker M, et al. Allogeneic mesenchymal stem cells restore endothelial function in heart failure by stimulating endothelial progenitor cells. *EBioMedicine.* (2015) 2:467–75. doi: 10.1016/j.ebiom.2015.03.020
179. Rodrigo SF, Van Ramshorst J, Hoogslag GE, Boden H, Velders MA, Cannegieter SC, et al. Intramyocardial injection of autologous bone marrow-derived *Ex vivo* expanded mesenchymal stem cells in acute myocardial infarction patients is feasible and safe up to 5 years of follow-up. *J Cardiovasc Transl Res.* (2013) 6:816–25. doi: 10.1007/s12265-013-9507-7
180. Heldman AW, DiFede DL, Fishman JE, Zambrano JP, Trachtenberg BH, Karantalis V, et al. Transendocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy: the TAC-HFT randomized trial. *JAMA.* (2014) 311:62–73. doi: 10.1001/jama.2013.282909
181. Anastasiadis K, Antonitsis P, Westaby S, Reginald A, Sultan S, Doumas A, et al. Implantation of a novel allogeneic mesenchymal precursor cell type in patients with ischemic cardiomyopathy undergoing coronary artery bypass grafting: an open label phase iia trial. *J Cardiovasc Transl Res.* (2016) 9:202–13. doi: 10.1007/s12265-016-9686-0
182. Florea V, Rieger AC, DiFede DL, El-Khorazaty J, Natsumeda M, Banerjee MN, et al. Dose comparison study of allogeneic mesenchymal stem cells in patients with ischemic cardiomyopathy (The TRIDENT study). *Circ Res.* (2017) 121:1279–90. doi: 10.1161/CIRCRESAHA.117.311827
183. Chullikana A, Majumdar A Sen, Gottipamula S, Krishnamurthy S, Kumar AS, Prakash VS, et al. Randomized, double-blind, phase I/II study of intravenous allogeneic mesenchymal stromal cells in acute myocardial infarction. *Cytotherapy.* (2015) 17:250–61. doi: 10.1016/j.jcyt.2014.10.009
184. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J Am Coll Cardiol.* (2009) 54:2277–86. doi: 10.1016/j.jacc.2009.06.055
185. Cai B, Wang G, Chen N, Liu Y, Yin K, Ning C, et al. Bone marrow mesenchymal stem cells protected post-infarcted myocardium against arrhythmias *via* reversing potassium channels remodelling. *J Cell Mol Med.* (2014) 18:1407–16. doi: 10.1111/jcmm.12287
186. Zhang S, Ge J, Sun A, Xu D, Qian J, Lin J, al. Comparison of various kinds of bone marrow stem cells for the repair of infarcted myocardium: single clonally purified non-hematopoietic mesenchymal stem cells serve as a superior source. *J Cell Biochem.* (2006) 99:1132–47. doi: 10.1002/jcb.20949
187. Haider HK, Jiang S, Idris NM, Ashraf M. IGF-1-overexpressing mesenchymal stem cells accelerate bone marrow stem cell mobilization *via* paracrine activation of SDF-1α/CXCR4 signaling to promote myocardial repair. *Circ Res.* (2008) 103:1300–8. doi: 10.1161/CIRCRESAHA.108.186742
188. Herrmann JL, Abarbanell AM, Weil BR, Wang Y, Poynter JA, Manukyan MC, et al. Postinfarct intramyocardial injection of mesenchymal stem cells pretreated with TGF-α improves acute myocardial function. *Am J Physiol Integr Comp Physiol.* (2010) 299:R371–8. doi: 10.1152/ajpregu.00084.2010
189. Beitnes JO, Øie E, Shahdadfar A, Karlsen T, Müller RMB, Aakhus S, et al. Intramyocardial injections of human mesenchymal stem cells following acute myocardial infarction modulate scar formation and improve left ventricular function. *Cell Transplant.* (2012) 21:1697–709. doi: 10.3727/096368911X627462
190. Chen L, Zhang Y, Tao L, Yang Z, Wang L. Mesenchymal stem cells with eNOS over-expression enhance cardiac repair in rats with myocardial infarction. *Cardiovasc Diagn Ther.* (2017) 31:9–18. doi: 10.1007/s10557-016-6704-z
191. Czapla J, Matuszczak S, Wiśniewska E, Jarosz-Biej M, Smolarczyk R, Cichoń T, et al. Human cardiac mesenchymal stromal cells with $CD105^{+}$ $CD34^{-}$ phenotype enhance the function of post-infarction heart in mice. *PLoS ONE.* (2016) 11:e0158745. doi: 10.1371/journal.pone.0158745
192. Shyu K-G, Wang B-W, Hung H-F, Chang C-C, Tzu-Bi Shih D. Mesenchymal stem cells are superior to angiogenic growth factor genes for improving myocardial performance in the mouse model of acute myocardial infarction. *J Biomed Sci.* (2006) 13:47–58. doi: 10.1007/s11373-005-9038-6
193. Zhang J, Wu Y, Chen A, Zhao Q. Mesenchymal stem cells promote cardiac muscle repair *via* enhanced neovascularization. *Cell Physiol Biochem.* (2015) 35:1219–29. doi: 10.1159/000373945
194. Ellison GM, Nadal-Ginard B, Torella D. Optimizing cardiac repair and regeneration through activation of the endogenous cardiac stem cell compartment. *J Cardiovasc Transl Res.* (2012) 5:667–77. doi: 10.1007/s12265-012-9384-5
195. Dai W, Hale SL, Kloner RA. Role of a paracrine action of mesenchymal stem cells in the improvement of left ventricular function after coronary artery occlusion in rats. *Regen Med.* (2007) 2:63–8. doi: 10.2217/17460751.2.1.63
196. De Macedo Braga LMG, Lacchini S, Schaan BDA, Rodrigues B, Rosa K, De Angelis K, et al. *In situ* delivery of bone marrow cells and mesenchymal stem cells improves cardiovascular function in hypertensive rats submitted to myocardial infarction. *J Biomed Sci.* (2008) 15:365–74. doi: 10.1007/s11373-008-9237-z
197. Van Der Spoel TIG, Jansen Of Lorkeers SJ, Agostoni P, Van Belle E, Gyongyosi M, Sluijter JPG, et al. Human relevance of pre-clinical studies in stem cell therapy: systematic review and meta-analysis of large animal models of ischaemic heart disease. *Cardiovasc Res.* (2011) 91:649–58. doi: 10.1093/cvr/cvr113
198. Natsumeda M, Florea V, Rieger AC, Tompkins BA, Banerjee MN, Golpanian S, et al. A combination of allogeneic stem cells promotes cardiac regeneration. *J Am Coll Cardiol.* (2017) 70:2504–15. doi: 10.1016/j.jacc.2017.09.036
199. Karantalis V, Suncion-Loescher VY, Bagno L, Golpanian S, Wolf A, Sanina C, et al. Synergistic effects of combined cell therapy for chronic ischemic cardiomyopathy. *J Am Coll Cardiol.* (2015) 66:1990–9. doi: 10.1016/j.jacc.2015.08.879
200. Lee JW, Lee SH, Youn YJ, Ahn MS, Kim JY, Yoo BS, et al. A randomized, open-label, multicenter trial for the safety and efficacy of adult mesenchymal stem cells after acute myocardial infarction. *J Korean Med Sci.* (2014) 29:23–31. doi: 10.3346/jkms.2014.29.1.23
201. Qi Z, Duan F, Liu S, Lv X, Wang H, Gao Y, et al. Effects of bone marrow mononuclear cells delivered through a graft vessel for patients with previous myocardial infarction and chronic heart failure: an echocardiographic study of left ventricular function. *Echocardiography.* (2015) 32:937–46. doi: 10.1111/echo.12787
202. Kim SH, Cho JH, Lee YH, Lee JH, Kim SS, Kim MY, et al. Improvement in left ventricular function with intracoronary mesenchymal stem cell therapy in a patient with anterior wall ST-segment elevation myocardial infarction. *Cardiovasc Drugs Ther.* (2018) 32:329–38. doi: 10.1007/s10557-018-6804-z
203. Chen S, Fang W, Qian J, YE F, Liu Y, Shan S, et al. Improvement of cardiac function after transplantation of autologous bone marrow mesenchymal stem cells in patients with acute myocardial infarction. *Chin Med J (Engl).* (2004) 117:1443–8.
204. Penn MS, Ellis S, Gandhi S, Greenbaum A, Hodes Z, Mendelsohn FO, et al. Adventitial delivery of an allogeneic bone marrow-derived adherent stem cell in acute myocardial infarction: phase i clinical study. *Circ Res.* (2012) 110:304–11. doi: 10.1161/CIRCRESAHA.111.253427
205. Wang X, Xi W-C, Wang F. The beneficial effects of intracoronary autologous bone marrow stem cell transfer as an adjunct to percutaneous coronary intervention in patients with acute myocardial infarction. *Biotechnol Lett.* (2014) 36:2163–8. doi: 10.1007/s10529-014-1589-z
206. Gao LR, Pei XT, Ding QA, Chen Y, Zhang NK, Chen HY, et al. A critical challenge: dosage-related efficacy and acute complication intracoronary injection of autologous bone marrow mesenchymal stem

cells in acute myocardial infarction. *Int J Cardiol.* (2013) 168:3191–9. doi: 10.1016/j.ijcard.2013.04.112

207. Yang Z, Zhang F, Ma W, Chen B, Zhou F, Xu Z, et al. A novel approach to transplanting bone marrow stem cells to repair human myocardial infarction: delivery *via* a noninfarct-relative artery. *Cardiovasc Ther.* (2010) 28:380–5. doi: 10.1111/j.1755-5922.2009.00116.x
208. Scalise M, Torella M, Marino F, Ravo M, Giurato G, Vicinanza C, et al. Atrial myxomas arise from multipotent cardiac stem cells. *Eur Heart J.* (2020) ehaa156. doi: 10.1093/eurheartj/ehaa156
209. Vicinanza C, Aquila I, Scalise M, Cristiano F, Marino F, Cianflone E, et al. Adult cardiac stem cells are multipotent and robustly myogenic: C-kit expression is necessary but not sufficient for their identification. *Cell Death Differ.* (2017) 24:2101–16. doi: 10.1038/cdd.2017.130
210. Lewis-McDougall FC, Ruchaya PJ, Domenjo-Vila E, Shin Teoh T, Prata L, Cottle BJ, et al. Aged-senescent cells contribute to impaired heart regeneration. *Aging Cell.* (2019) 18:1–15. doi: 10.1111/acel.12931
211. Ellison-Hughes GM, Madeddu P. Exploring pericyte and cardiac stem cell secretome unveils new tactics for drug discovery. *Pharmacol Ther.* (2017) 171:1–12. doi: 10.1016/j.pharmthera.2016.11.007
212. Zhu M, Chu Y, Shang Q, Zheng Z, Li Y, Cao L, et al. Mesenchymal stromal cells pretreated with pro-inflammatory cytokines promote skin wound healing through VEGFC-mediated angiogenesis. *Stem Cells Transl Med.* (2020) 9:1218–32. doi: 10.1002/sctm.19-0241
213. Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, Hao H, et al. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. *Nat Med.* (2006) 12:459–65. doi: 10.1038/nm1391
214. Qian D, Gong J, He Z, Hua J, Lin S, Xu C, et al. Bone marrow-derived mesenchymal stem cells repair necrotic pancreatic tissue and promote angiogenesis by secreting cellular growth factors involved in the SDF-1 /CXCR4 axis in rats. *Stem Cells Int.* (2015) 2015:1–20. doi: 10.1155/2015/306836
215. Teng X, Chen L, Chen W, Yang J, Yang Z, Shen Z. Mesenchymal stem cell-derived exosomes improve the microenvironment of infarcted myocardium contributing to angiogenesis and anti-inflammation. *Cell Physiol Biochem.* (2015) 37:2415–24. doi: 10.1159/000438594
216. Huang NF, Lam A, Fang Q, Sievers RE, Li S, Lee RJ. Bone marrow-derived mesenchymal stem cells in fibrin augment angiogenesis in the chronically infarcted myocardium. *Regen Med.* (2009) 4:527–38. doi: 10.2217/rme.09.32
217. Cai M, Ren L, Xiaoqin Y, Guo Z, Li Y, He T, et al. PET monitoring angiogenesis of infarcted myocardium after treatment with vascular endothelial growth factor and bone marrow mesenchymal stem cells. *Amino Acids.* (2016) 48:811–20. doi: 10.1007/s00726-015-2129-4
218. Carrion B, Kong YP, Kaigler D, Putnam AJ. Bone marrow-derived mesenchymal stem cells enhance angiogenesis *via* their α6β1 integrin receptor. *Exp Cell Res.* (2013) 319:2964–76. doi: 10.1016/j.yexcr.2013.09.007
219. Du WJ, Chi Y, Yang ZX, Li ZJ, Cui JJ, Song BQ, et al. Heterogeneity of proangiogenic features in mesenchymal stem cells derived from bone marrow, adipose tissue, umbilical cord, and placenta. *Stem Cell Res Ther.* (2016) 7:1–11. doi: 10.1186/s13287-016-0418-9
220. Gangadaran P, Rajendran RL, Lee HW, Kalimuthu S, Hong CM, Jeong SY, et al. Extracellular vesicles from mesenchymal stem cells activates VEGF receptors and accelerates recovery of hindlimb ischemia. *J Control Release.* (2017) 264:112–26. doi: 10.1016/j.jconrel.2017.08.022
221. Huang B, Qian J, Ma J, Huang Z, Shen Y, Chen X, et al. Myocardial transfection of hypoxia-inducible factor-1α and co-transplantation of mesenchymal stem cells enhance cardiac repair in rats with experimental myocardial infarction. *Stem Cell Res Ther.* (2014) 5:22. doi: 10.1186/scrt410
222. Kwon HM, Hur SM, Park KY, Kim CK, Kim YM, Kim HS, et al. Multiple paracrine factors secreted by mesenchymal stem cells contribute to angiogenesis. *Vascul Pharmacol.* (2014) 63:19–28. doi: 10.1016/j.vph.2014.06.004
223. Kehl D, Generali M, Mallone A, Heller M, Uldry AC, Cheng P, et al. Proteomic analysis of human mesenchymal stromal cell secretomes: a systematic comparison of the angiogenic potential. *npj Regen Med.* (2019) 4:1–13. doi: 10.1038/s41536-019-0070-y
224. Liu L, Gao J, Yuan Y, Chang Q, Liao Y, Lu F. Hypoxia preconditioned human adipose derived mesenchymal stem cells enhance angiogenic potential *via* secretion of increased VEGF and bFGF. *Cell Biol Int.* (2013) 37:551–60. doi: 10.1002/cbin.10097
225. Anderson JD, Johansson HJ, Graham CS, Vesterlund M, Pham MT, Bramlett CS, et al. Comprehensive proteomic analysis of mesenchymal stem cell exosomes reveals modulation of angiogenesis *via* nuclear factor-kappaB signaling. *Stem Cells.* (2016) 34:601–13. doi: 10.1002/stem.2298
226. Zhang Z, Yang J, Yan W, Li Y, Shen Z, Asahara T. Pretreatment of cardiac stem cells with exosomes derived from mesenchymal stem cells enhances myocardial repair. *J Am Heart Assoc.* (2016) 5:e002856. doi: 10.1161/JAHA.115.002856
227. Hanna H, Mir LM, Andre FM. *In vitro* osteoblastic differentiation of mesenchymal stem cells generates cell layers with distinct properties. *Stem Cell Res Ther.* (2018) 9:203. doi: 10.1186/s13287-018-0942-x
228. Takeda YS, Xu Q. Neuronal differentiation of human mesenchymal stem cells using exosomes derived from differentiating neuronal cells. *PLoS ONE.* (2015) 10:e0135111. doi: 10.1371/journal.pone.0135111
229. Xie X, Wang J, Cao J, Zhang X. Differentiation of bone marrow mesenchymal stem cells induced by myocardial medium under hypoxic conditions. *Acta Pharmacol Sin.* (2006) 27:1153–8. doi: 10.1111/j.1745-7254.2006.00436.x
230. Choi J-W, Kim K-E, Lee CY, Lee J, Seo H-H, Lim KH, et al. Alterations in cardiomyocyte differentiation-related proteins in rat mesenchymal stem cells exposed to hypoxia. *Cell Physiol Biochem.* (2016) 39:1595–607. doi: 10.1159/000447861
231. Noiseux N, Gnecchi M, Lopez-Ilasaca M, Zhang L, Solomon SD, Deb A, et al. Mesenchymal stem cells overexpressing Akt dramatically repair infarcted myocardium and improve cardiac function despite infrequent cellular fusion or differentiation. *Mol Ther.* (2006) 14:840–50. doi: 10.1016/j.ymthe.2006.05.016
232. Derval N, Barandon L, Dufourcq P, Leroux L, Lamazière J-MD, Daret D, et al. Epicardial deposition of endothelial progenitor and mesenchymal stem cells in a coated muscle patch after myocardial infarction in a murine model. *Eur J Cardio-Thoracic Surg.* (2008) 34:248–54. doi: 10.1016/j.ejcts.2008.03.058
233. Wu S-Z, Li Y-L, Huang W, Cai W-F, Liang J, Paul C, et al. Paracrine effect of CXCR4-overexpressing mesenchymal stem cells on ischemic heart injury. *Cell Biochem Funct.* (2017) 35:113–23. doi: 10.1002/cbf.3254
234. Yao Z, Liu H, Yang M, Bai Y, Zhang B, Wang C, et al. Bone marrow mesenchymal stem cell-derived endothelial cells increase capillary density and accelerate angiogenesis in mouse hindlimb ischemia model. *Stem Cell Res Ther.* (2020) 11:221. doi: 10.1186/s13287-020-01710-x
235. Nascimento DS, Mosqueira D, Sousa LM, Teixeira M, Filipe M, Resende TP, et al. Human umbilical cord tissue-derived mesenchymal stromal cells attenuate remodeling after myocardial infarction by proangiogenic, antiapoptotic, and endogenous cell-activation mechanisms. *Stem Cell Res The.r.* (2014) 5:1–14. doi: 10.1186/scrt394
236. Kang K, Ma R, Cai W, Huang W, Paul C, Liang J, et al. Exosomes secreted from CXCR4 overexpressing mesenchymal stem cells promote cardioprotection *via* akt signaling pathway following myocardial infarction. *Stem Cells Int.* (2015) 2015:1–14. doi: 10.1155/2015/659890
237. Li X, Xie X, Yu Z, Chen Y, Qu G, Yu H, et al. Bone marrow mesenchymal stem cells-derived conditioned medium protects cardiomyocytes from hypoxia/reoxygenation-induced injury through Notch2/mTOR/autophagy signaling. *J Cell Physiol.* (2019) 234:18906–16. doi: 10.1002/jcp.28530
238. Li H, Zuo S, He Z, Yang Y, Pasha Z, Wang Y, et al. Paracrine factors released by GATA-4 overexpressed mesenchymal stem cells increase angiogenesis and cell survival. *Am J Physiol Hear Circ Physiol.* (2010) 299:1772–81. doi: 10.1152/ajpheart.00557.2010.-Transplanted
239. Zhang D, Fan GC, Zhou X, Zhao T, Pasha Z, Xu M, et al. Over-expression of CXCR4 on mesenchymal stem cells augments myoangiogenesis in the infarcted myocardium. *J Mol Cell Cardiol.* (2008) 44:281–92. doi: 10.1016/j.yjmcc.2007.11.010
240. Kawaguchi N, Smith AJ, Waring CD, Hasan K, Miyamoto S, Matsuoka R, et al. c-kit pos GATA-4 high rat cardiac stem cells foster adult cardiomyocyte survival through IGF-1 paracrine signalling. *PLoS ONE.* (2010) 5:e14297. doi: 10.1371/journal.pone.0014297
241. Yamaguchi J-i, Kusano KF, Masuo O, Kawamoto A, Silver M, Murasawa S, et al. Stromal cell-derived factor-1 effects on *ex vivo* expanded endothelial progenitor cell recruitment for ischemic neovascularization. *Circulation.* (2003) 107:1322–8. doi: 10.1161/01.CIR.0000055313.77510.22

242. Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med.* (2004) 10:858–64. doi: 10.1038/nm1075
243. De Falco E, Porcelli D, Torella AR, Straino S, Iachininoto MG, Orlandi A, et al. SDF-1 involvement in endothelial phenotype and ischemia-induced recruitment of bone marrow progenitor cells. *Blood.* (2004) 104:3472–82. doi: 10.1182/blood-2003-12-4423
244. Luger D, Lipinski MJ, Westman PC, Glover DK, Dimastromatteo J, Frias JC, et al. Intravenously delivered mesenchymal stem cells. *Circ Res.* (2017) 120:1598–613. doi: 10.1161/CIRCRESAHA.117.310599
245. Yan X, Anzai A, Katsumata Y, Matsuhashi T, Ito K, Endo J, et al. Temporal dynamics of cardiac immune cell accumulation following acute myocardial infarction. *J Mol Cell Cardiol.* (2013) 62:24–35. doi: 10.1016/j.yjmcc.2013.04.023
246. Park KC, Gaze DC, Collinson PO, Marber MS. Cardiac troponins: from myocardial infarction to chronic disease. *Cardiovasc Res.* (2017) 113:1708–18. doi: 10.1093/cvr/cvx183
247. Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med.* (2014) 370:2071–82. doi: 10.1056/NEJMoa1402584
248. King TE, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *N Engl J Med.* (2014) 370:2083–92. doi: 10.1056/NEJMoa1402582
249. Karimi-Shah BA, Chowdhury BA. Forced vital capacity in idiopathic pulmonary fibrosis—FDA review of pirfenidone and nintedanib. *N Engl J Med.* (2015) 372:1189–91. doi: 10.1056/NEJMp1500526
250. Kasam RK, Reddy GB, Jegga AG, Madala SK. Dysregulation of mesenchymal cell survival pathways in severe fibrotic lung disease: the effect of nintedanib therapy. *Front Pharmacol.* (2019) 10:532. doi: 10.3389/fphar.2019.00532
251. Su H, Yang M, Wan C, Yi LX, Tang F, Zhu HY, et al. Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney Int.* (2020) 98:219–27. doi: 10.1016/j.kint.2020.04.003
252. Moodley Y, Ilancheran S, Samuel C, Vaghjiani V, Atienza D, Williams ED, et al. Human amnion epithelial cell transplantation abrogates lung fibrosis and augments repair. *Am J Respir Crit Care Med.* (2010) 182:643–51. doi: 10.1164/rccm.201001-0014OC
253. Ashcroft T, Simpson JM, Timbrell V. Simple method of estimating severity of pulmonary fibrosis on a numerical scale. *J Clin Pathol.* (1988) 41:467–70. doi: 10.1136/jcp.41.4.467
254. He F, Zhou A, Feng S. Use of human amniotic epithelial cells in mouse models of bleomycin-induced lung fibrosis: a systematic review and meta-analysis. *PLoS ONE.* (2018) 13:1–17. doi: 10.1371/journal.pone.0197658
255. Gad ES, Salama AAA, El-Shafie MF, Arafa HMM, Abdelsalam RM, Khattab M. The anti-fibrotic and anti-inflammatory potential of bone marrow–derived mesenchymal stem cells and nintedanib in bleomycin-induced lung fibrosis in rats. *Inflammation.* (2020) 43:123–34. doi: 10.1007/s10753-019-01101-2
256. Chen S, Cui G, Peng C, Lavin MF, Sun X, Zhang E, et al. Transplantation of adipose-derived mesenchymal stem cells attenuates pulmonary fibrosis of silicosis *via* anti-inflammatory and anti-apoptosis effects in rats. *Stem Cell Res Ther.* (2018) 9:110. doi: 10.1186/s13287-018-0846-9
257. Chen S, Chen X, Wu X, Wei S, Han W, Lin J, et al. Hepatocyte growth factor-modified mesenchymal stem cells improve ischemia/reperfusion-induced acute lung injury in rats. *Gene Ther.* (2017) 24:3–11. doi: 10.1038/gt.2016.64
258. Chen W, Wang S, Xiang H, Liu J, Zhang Y, Zhou S, et al. Microvesicles derived from human Wharton's Jelly mesenchymal stem cells ameliorate acute lung injury partly mediated by hepatocyte growth factor. *Int J Biochem Cell Biol.* (2019) 112:114–22. doi: 10.1016/j.biocel.2019.05.010
259. Gazdhar A, Temuri A, Knudsen L, Gugger M, Schmid RA, Ochs M, et al. Targeted gene transfer of hepatocyte growth factor to alveolar type II epithelial cells reduces lung fibrosis in rats. *Hum Gene Ther.* (2013) 24:105–16. doi: 10.1089/hum.2012.098
260. Zhao Y, Lan X, Wang Y, Xu X, Lu S, Li X, et al. Human endometrial regenerative cells attenuate bleomycin-induced pulmonary fibrosis in mice. *Stem Cells Int.* (2018) 2018:1–13. doi: 10.1155/2018/3475137
261. Ni K, Liu M, Zheng J, Wen L, Chen Q, Xiang Z, et al. PD-1/PD-L1 pathway mediates the alleviation of pulmonary fibrosis by human mesenchymal stem cells in humanized mice. *Am J Respir Cell Mol Biol.* (2018) 58:684–95. doi: 10.1165/rcmb.2017-0326OC
262. Zhang E, Yang Y, Chen S, Peng C, Lavin MF, Yeo AJ, al. Bone marrow mesenchymal stromal cells attenuate silica-induced pulmonary fibrosis potentially by attenuating Wnt/β-catenin signaling in rats. *Stem Cell Res Ther.* (2018) 9:1–14. doi: 10.1186/s13287-018-1045-4
263. Li F, Han F, Li H, Zhang J, Qiao X, Shi J, et al. Human placental mesenchymal stem cells of fetal origins-alleviated inflammation and fibrosis by attenuating MyD88 signaling in bleomycin-induced pulmonary fibrosis mice. *Mol Immunol.* (2017) 90:11–21. doi: 10.1016/j.molimm.2017.06.032
264. Li X, Li C, Tang Y, Huang Y, Cheng Q, Huang X, et al. NMDA receptor activation inhibits the antifibrotic effect of BM-MSCs on bleomycin-induced pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol.* (2018) 315:404–21. doi: 10.1152/ajplung.00002.2018.-Endogenous
265. Cao H, Wang C, Chen X, Hou J, Xiang Z, Shen Y, et al. Inhibition of Wnt/β-catenin signaling suppresses myofibroblast differentiation of lung resident mesenchymal stem cells and pulmonary fibrosis. *Sci Rep.* (2018) 8:13644. doi: 10.1038/s41598-018-28968-9
266. Chambers DC, Enever D, Ilic N, Sparks L, Whitelaw K, Ayres J, et al. A phase 1b study of placenta-derived mesenchymal stromal cells in patients with idiopathic pulmonary fibrosis. *Respirology.* (2014) 19:1013–18. doi: 10.1111/resp.12343
267. Averyanov A, Koroleva I, Konoplyannikov M, Revkova V, Lesnyak V, Kalsin V, et al. First-in-human high-cumulative-dose stem cell therapy in idiopathic pulmonary fibrosis with rapid lung function decline. *Stem Cells Transl Med.* (2020) 9:6–16. doi: 10.1002/sctm.19-0037
268. Lan Y-W, Theng S-M, Huang T-T, Choo K-B, Chen C-M, Kuo H-P, et al. Oncostatin M-preconditioned mesenchymal stem cells alleviate bleomycin-induced pulmonary fibrosis through paracrine effects of the hepatocyte growth factor. *Stem Cells Transl Med.* (2017) 6:1006–17. doi: 10.5966/sctm.2016-0054
269. Ayaub EA, Dubey A, Imani J, Botelho F, Kolb MRJ, Richards CD, et al. Overexpression of OSM and IL-6 impacts the polarization of pro-fibrotic macrophages and the development of bleomycin-induced lung fibrosis OPEN. *Sci Rep.* (2017) 7:1–16. doi: 10.1038/s41598-017-13511-z
270. Li D, Liu Q, Qi L, Dai X, Liu H, Wang Y. Low levels of TGF-β1 enhance human umbilical cord-derived mesenchymal stem cell fibronectin production and extend survival time in a rat model of lipopolysaccharide-induced acute lung injury. *Mol Med Rep.* (2016) 14:1681–92. doi: 10.3892/mmr.2016.5416
271. Chen S, Chen L, Wu X, Lin J, Fang J, Chen X, et al. Ischemia postconditioning and mesenchymal stem cells engraftment synergistically attenuate ischemia reperfusion-induced lung injury in rats. *J Surg Res.* (2012) 178:81–91. doi: 10.1016/j.jss.2012.01.039
272. Wu J, Song D, Li Z, Guo B, Xiao Y, Liu W, et al. Immunity-and-matrix-regulatory cells derived from human embryonic stem cells safely and effectively treat mouse lung injury and fibrosis. *Cell Res.* (2020) 30:1–16. doi: 10.1038/s41422-020-0354-1
273. Cunha LL, Perazzio SF, Azzi J, Cravedi P, Riella LV. Remodeling of the immune response with aging: immunosenescence and its potential impact on COVID-19 immune response. *Front Immunol.* (2020) 11:1748. doi: 10.3389/fimmu.2020.01748

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Telemedicine in Heart Failure During COVID-19: A Step Into the Future

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During the Coronavirus Disease 2019 worldwide pandemic, patients with heart failure are a high-risk group with potential higher mortality if infected. Although lockdown represents a solution to prevent viral spreading, it endangers regular follow-up visits and precludes direct medical assessment in order to detect heart failure progression and optimize treatment. Furthermore, lifestyle changes during quarantine may trigger heart failure decompensations. During the pandemic, a paradoxical reduction of heart failure hospitalization rates was observed, supposedly caused by patient reluctance to visit emergency departments and hospitals. This may result in an increased patient mortality and/or in more complicated heart failure admissions in the future. In this scenario, different telemedicine strategies can be implemented to ensure continuity of care to patients with heart failure. Patients at home can be monitored through dedicated apps, telephone calls, or devices. Virtual visits and forward triage screen the patients with signs or symptoms of decompensated heart failure. In-hospital care may benefit from remote communication platforms. After discharge, patients may undergo remote follow-up or telerehabilitation to prevent early readmissions. This review provides a comprehensive appraisal of the many possible applications of telemedicine for patients with heart failure during Coronavirus disease 2019 and elucidates practical limitations and challenges regarding specific telemedicine modalities.

Keywords: COVID-19, coronavirus, telemedicine, heart failure, remote monitoring, virtual visits, forward triage, telerehabilitation

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INTRODUCTION

The Coronavirus Disease 2019 (COVID-19) pandemic has caused considerable morbidity and mortality worldwide. Epidemiological data from China indicate that patients with concomitant cardiovascular disease are more likely to develop life-threatening complications from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (1–7). The risk of complications may be even higher in patients with heart failure (HF) because they are older and have more

comorbidities, but also due to the specific characteristics of this syndrome (8). Lockdown of social activities has allowed limiting the spreading of SARS-CoV-2, but it has also decreased medical contacts. For HF patients, this might have led to late recognition and treatment of episodes of decompensation and missed opportunities for optimization of medical and nonmedical therapy. In addition, lifestyle changes adopted during lockdown, such as dietary changes, increased alcohol consumption and decreased physical activity, may trigger HF decompensations (9, 10).

Telemedicine represents a useful tool to prevent negative direct and indirect consequences of SARS-CoV-2, and the present situation might be the right moment to implement a structured telemedicine program in clinical practice. Its main benefits include guiding the treatment of patients in primary care to minimize the risk of disease transmission during referral, continuing to provide optimal treatment to the patients with cardiovascular disease who are isolated at home or are discharged from the hospital to prevent clinical deterioration, monitoring early signs of new onset or worsening HF, and reducing unnecessary visits to the hospital to decrease the incidence of cluster infections (11).

In this review, we provide an overview of the many possible applications of telemedicine, its limitations and challenges, in patients with HF during COVID-19.

IMPACT OF COVID-19 ON THE MANAGEMENT OF HEART FAILURE

Already in the first months of the COVID-19 pandemic, the impact of cardiovascular comorbidities on disease course became clear in observational studies, indicating that patients with previous cardiovascular disease had higher COVID-19 disease severity and mortality (2, 6, 7). In addition, myocardial injury in COVID-19 has been broadly described (6, 7, 12, 13), which might further impair myocardial function and worsen prognosis in patients with known HF.

Patients with chronic HF represent a vulnerable group during a pandemic of infectious respiratory disease. Previous studies have shown that they are at increased risk for adverse consequences of seasonal influenza (14) and other causes of pneumonia (15). Furthermore, acute infections may trigger HF exacerbations (16).

The social and environmental effects of lockdown must also be mentioned. A significant decline in hospitalization rates for acute HF during the COVID-19 pandemic, compared to before the pandemic and each of the preceding 3 years, was described, which might be the consequence of fear for infection leading to reluctance to seek medical attention when needed (17). Notably, hospitalized patients had more severe symptoms on admission, possibly suggesting that patients have waited longer before presenting to the hospital or less severe cases did not come to the hospital at all. Further, lifestyle changes during lockdown, such as dietary changes, increased alcohol consumption and decreased physical activity, may trigger HF decompensations (9, 10).

Although lockdown represents a solution to prevent viral spreading, it may complicate regular follow-up visits, therefore encumbering optimization of medical therapy and limiting detection of development of complications or disease progression that may require a change in management.

For these reasons, the great challenge of patients with HF during COVID-19 is keeping them safe from infection risk, but equally continuing with strict monitoring in order to prevent hospitalizations. As a result, health systems have largely transitioned to noncontact care delivery methods for ambulatory care (9). In this setting, various strategies of telemedicine and remote monitoring were developed rapidly and implemented more widely in HF patients (**Table 1**, **Figure 1**).

TELEMEDICINE STRATEGIES DURING COVID-19

Home Monitoring

Several strategies can be applied to perform home monitoring of HF patients. Two small studies performed in Boston and New York City showed initial encouraging results of implantable hemodynamic monitoring in COVID-19 (18, 19). However, device and hemodynamic monitoring can only be performed in those patients, which had implanted a device or hemodynamic sensor before the lockdown, which are a minority of the HF population.

A new home monitoring system should be easy to install, be intuitive to users, and provide robust communication (20). Hence, structured telephone support (STS), defined as monitoring, self-care management, or both, delivered using telephone calls (21), may represent the most simple and affordable system for HF centers starting with telemedicine during COVID-19.

A recent study on 103 patients in an Italian tertiary referral center investigated whether a telemedicine service expressly set up during the COVID-19 outbreak changed HF outcomes compared with the same period of 2019 without telemedicine (22). Around 60% of patients accessed telemedicine services at least once, and half of contacts led to a clinical decision (e.g., adjustment of diuretic doses, change of blood pressure drugs, rate controls, and anticoagulant management). In this study, the telemedicine service reduced the composite of HF hospitalization and death compared to patients in the 2019 cohort, which is nevertheless to be interpreted cautiously in light of the previously mentioned reduction of HF hospitalizations during lockdown. In fact, new-established STS interventions are expected to give significant advantages only in the long term, since they could be influenced by a learning-to-care curve due to staff training (23). However, the main goal of telemonitoring during COVID-19 is not to provide superior care than standard, but to offer patients with HF a "health maintenance strategy" which provides an individualized target for each HF patient and adjusts treatment to maintain the monitored parameters as close as possible to ideal (20).

Besides HF patients in general, HF patients who suffer SARS-CoV-2 infection and are treated at home

TABLE 1 | Strengths and weaknesses of different telemedicine strategies for patients with heart failure during COVID-19.

Strategies	Definition	Objectives	Challenges
Home monitoring	Remote monitoring of vital parameters and transmission (via devices, telephone, apps) to a care center for interpretation and management	Individualized targets Therapy optimization Patients' empowerment Avoiding social disparities	Device delivery and patients' education Staff training Initial investment
Virtual visits	Remote visits with audiovisual telecommunication system or through an online portal	Assessment of symptoms Therapy optimization Maintain connection between patient and physician Seeing new HF patients	Adequate assessment of volume status or congestion Availability of stable internet connection and devices
Forward triage	Sorting of patients before presentation in the ED	Early assignation to the right path Protect patients from high-risk exposure	Logistic reorganization of ED triage models Software implementation
In-hospital telemedicine	Implementation of telemedicine in the in-hospital setting	Limiting unnecessary exposure to affected patients Favor communication and reduce social isolation	Staff training Hardware costs
Telerehabilitation	Delivery of rehabilitation services remotely	Allow cardiac rehabilitation during lockdown	Initial assessment Patients' compliance and motivation Costs and reimbursement

ED, emergency department.

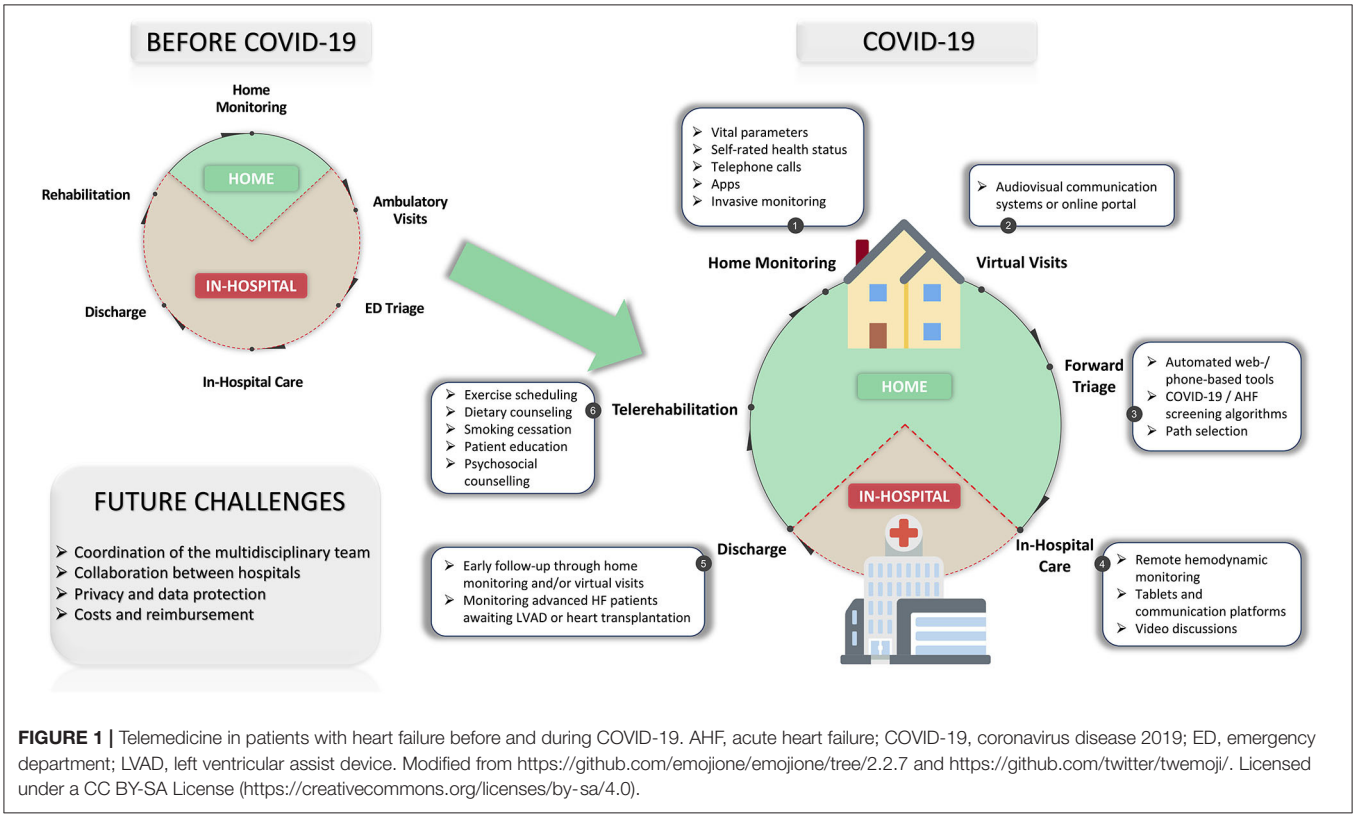

FIGURE 1 | Telemedicine in patients with heart failure before and during COVID-19. AHF, acute heart failure; COVID-19, coronavirus disease 2019; ED, emergency department; LVAD, left ventricular assist device. Modified from https://github.com/emojione/emojione/tree/2.2.7 and https://github.com/twitter/twemoji/. Licensed under a CC BY-SA License (https://creativecommons.org/licenses/by-sa/4.0).

could even more benefit from STS as they are at high risk for complications (8). Remote monitoring can also encourage patients to maintain home isolation and assist in correct timing of stopping the isolation precautions (24).

Virtual Visits

Virtual visits (VV) include remote visits, in which an audiovisual telecommunication system is used, and e-visits, which are communications between patients and providers through an online portal (9).

A recent statement from the Heart Failure Society of America provides information regarding platforms, workflows, and care models for VV in HF patients (25). Some institutions have already balanced the deferred or canceled face-to-face HF visits with rapid adoption of VV while employing several novel virtual health technologies with overall positive results (26). Specifically, the potential benefits of VV for HF patients are providing access to care and medical advice which would be otherwise difficult to obtain and reducing in-person exposure to SARS-CoV-2. Involvement of caregivers who may be present at home, but not in the outpatient clinic because of restrictions to hospital access, is an additional advantage of VV during the pandemic (25).

Hypothetically, this might represent also a smart working possibility for healthcare personnel, a class of workers for which this possibility is not usually considered or available.

VV may be best utilized for medication titration and optimization in stable patients with chronic HF. While substantial patient information can be gained from such visits, certain challenges remain, such as the adequate assessment of volume status or congestion (27). Thus, in-person visits should be reserved for recently hospitalized patients, patients approaching or with advanced HF, who are new post implantation of a left ventricular assist device (LVAD) or heart transplant, and those with new-onset HF (9).

Forward Triage

Respiratory symptoms, as well as functional decline and fatigue, may be early signs of both COVID-19 and of decompensated HF. Hence, stratification of patients before arriving in the emergency department (ED), the so-called *forward triage*, represents another potential strategy for health care surge control.

Before COVID-19, many EDs modified their triage model by allowing a remote provider to perform intake (28). In an emergency situation, web-conferencing software with a direct line from a triage room to a clinician can be rapidly implemented (29). An automated web- or phone-based tool could guide HF patients with concerning symptoms to determine the need for self-isolation, symptom monitoring, urgent VV, or presenting to the ED (30). Through a structured telemedicine program, detailed medical and exposure histories might be easily obtained. Screening algorithms can be integrated and local epidemiological information can be used to standardize screening and practice patterns across providers (29). The ultimate goal is to guide patients to the right diagnostic–therapeutic pathway while protecting them from unnecessary risk and exposure.

Patients with suspected COVID-19 are isolated immediately upon arrival to emergency departments. In several centers in the USA, telemedicine carts (i.e., systems that integrate displays, cameras, microphones, speakers, and network access) were already successfully deployed into COVID-19 isolation rooms. This initiative increased provider/patient communication and attention to staff safety, improved palliative care and patient support services, lowered consumption of personal protective equipment, increased patient comfort, and reduced the psychological toll of isolation (31).

In-Hospital Telemedicine

Certain principles of virtual medicine might be considered when approaching an HF patient seeking acute cardiac care during COVID-19. In this setting, telemedicine measures must aim at limiting unnecessary exposure to affected patients, utilizing remote hemodynamic monitoring and ICU flowcharts to evaluate patient progress and adjust medications (32). These data can be implemented with clinical assessments performed by a single bedside operator to generate operable conditions for safe, remote decision-making, using tools such as electronic stethoscopes and mobile ultrasound probes (32). Initial results of basic thoracic ultrasound programs in ICU are encouraging with rapid adoption of point-of-care ultrasound and commensurate reduction in formal imaging studies (26).

Importantly, COVID-19 has presented healthcare professionals with new and unusual barriers to effective communication between physician, patient, and family. As hospital visits are now frequently prohibited to patients' relatives, novel telecommunication and video options might be considered for patients to speak with loved ones, review treatment choices, and even discuss objectives of care (32). For this purpose, several hospitals introduced use of tablets and video calls with the ultimate goal to favor communication and reduce social isolation of hospitalized patients (33).

Telerehabilitation

Cardiovascular rehabilitation (CR) represents a cornerstone in the treatment of patients with HF. The term *telerehabilitation* has been used in much of the literature to date and is defined as the delivery of rehabilitation services via information and communication technologies (34). Before COVID-19, it has been shown to be a viable and effective alternative for individuals who are unable to access in-person healthcare services for the management of many conditions. During COVID-19, the reallocation of medical resources as well as the lockdown caused the cessation of all nonurgent medical services, including CR. Therefore, centers had to switch to alternative ways to deliver the core components of CR remotely.

A technology-driven CR model has been proposed, with the assistance of any form of technology (e.g., smartphones, mobile apps, internet, e-mail, webcams, and use of wearable sensors) (35). A recent survey about the implementation of cardiac telerehabilitation services during the COVID-19 pandemic in Belgium (36) showed that half of the answering centers switched to telerehabilitation during the pandemic, mainly for patients that were already undergoing CR. The most frequently used medium to deliver the CR components were online videos (71%) followed by website information (64%) and emails (64%). As the authors of this survey suggested, the remote delivery of CR can also play an important role after the reopening of the rehabilitation centers because of a reduced capacity due to social distancing measures (36). For this purpose, a recent call for action paper of the European Association of Preventive Cardiology provides a practical guide for the setup of a comprehensive cardiac telerehabilitation intervention during the COVID-19 pandemic, which could also be relevant to any cardiovascular

disease patient not able to visit CR centers regularly after the COVID-19 pandemic ceases (37).

Advanced Heart Failure

The evaluation of patients with advanced HF awaiting LVAD placement or heart transplantation may be interrupted during the pandemic, as traditional social work, nutrition, pharmacy referrals, and diagnostic procedures are delayed. Telemedicine offers a platform for these multidisciplinary assessments to occur serially or simultaneously without delay (10). Furthermore, heart transplant recipients on stable immunosuppression at low risk for allograft rejection and hemodynamically optimized LVAD patients may be managed remotely without exposing them to further unnecessary risks (9). A telemonitoring algorithm for patients with LVAD has been recently proposed (38), and it is potentially adaptable to every LVAD center, regardless of the number of LVAD patients or previous experiences.

Clinical Trials

Since the first wave of the pandemic, clinical trials unrelated to COVID-19 have been paused in most institutions. Telemedicine might avoid the loss of data during lockdown, which can jeopardize the entire research validity. In clinical trials, measurements and data collection are traditionally performed during patient visits. As stated by a recent document of the Heart Failure Association (39), endpoints like symptom status, quality of life questionnaires, or even vital signs could be assessed using home-based testing, with alternative methods such as telephone contacts, app-based self-assessments, or video links.

DISCUSSION

Practical Considerations and Limitations of Telemedicine

Although telemedicine provides numerous advantages in many fields, it currently still carries practical limitations and pitfalls, which must be taken into consideration.

First, the hardware required for telemonitoring (i.e., smartphones, tablets, as well as blood pressure machines, scales, etc.) and exercise equipment for telerehabilitation (i.e., treadmill, stationary bike, etc.) may represent a significant financial burden, so either patients must be able to afford this or their health insurance/national health service must provide or reimburse the equipment. Moreover, patients who are unable to utilize the required devices or participate in a telemedicine session unaided either because of old age, poor hearing, cognitive dysfunction, language barriers, or limited education which may require the assistance of a family member or caregiver, who may not be available (40, 41). Finally, the use of telemedicine may be technically limited by poor phone and internet connectivity in rural areas (42, 43).

Telephone support is the most readily applicable and can be performed competently by trained nurses. However, home monitoring creates a large amount of data which must be screened and interpreted by trained staff (44), a process that could be time-consuming. In addition, it requires a dedicated physician to act on critical laboratory abnormalities, all of which can be challenging for physicians managing their practices and possibly receiving limited reimbursement.

The care of a patient with HF requires a multidisciplinary collaboration among physicians, pharmacologists, nurses, physical therapists, nutritionists, and medical social workers. Hence, technology should be conjugated also to ensure communication between the team (e.g., virtual multidisciplinary meetings using video calling in times of social restrictions) (37). In addition, patients with HF often have several comorbidities and may be looked after by more than one hospital, thus requiring intensive collaboration between different specialists and clinics. Authors analyzing the impact of the first COVID-19 wave on patients with chronic diseases described a poor interconnection between telemedicine services operating at higher levels (i.e., secondary or tertiary care facilities) and those deployed in primary care clinics or community pharmacies, preventing to obtain the maximum benefit from these digital solutions (45). Future developments should encourage the collaboration between different professional figures, departments, hospitals, and care institutions.

Due to the fact that telemedicine involves the transmission of patients' confidential information, whether those data are processed and transferred via telephone calls, videoconference, mobile apps, or other platforms, their monitoring requires safe encrypted storage systems which only allow for authorized access to data and protect patient privacy. The interfaces used must be compliant with local regulations both regarding data protection (i.e., GDPR) and encryption (i.e., HIPAA requirements) (46, 47). Physicians implementing telemedicine in clinical practice during COVID-19 suggest using device management software for telehealth devices to create security settings and enforce encryption for devices given to patients (48).

The inclusion of new patients in a telerehabilitation program will be challenging during lockdown, especially with respect to the initial assessment (i.e., baseline stress test) and initial interview, a hurdle that may be overcome by a structured technology-based program with predefined remote assessment methods and audio-visual communication systems (35). However, not all patients could be comfortable with this mode of action, and the problem of financing and delivering technologies to the single patients still persists. An effective approach to reorganize CR could be to start a rehabilitation path in person and subsequently integrate this with a patient-tailored remote telerehabilitation program in order to optimize performance and extend patients' education.

Finally, telemedicine services are not yet included in the essential levels of care in many countries (9, 29, 45). During COVID-19, some efforts were already made by agencies like the US Food and Drug Administration, which is facilitating the use of remote monitoring devices, and Centers for Medicare and Medicaid Services, which is paying for telehealth services at the same rate they would have been paid, if provided in person (27). However, these costs were covered only due to the emergency situation. In order to continue after the pandemic, the shift to telemedicine should be done in parallel with developments in policymaking (27).

Future Perspectives

Evidence coming from observational studies on telemedicine during COVID-19 is of great importance. Centers having a dedicated HF unit should collect information regarding their own telemedicine approach, with the aim of defining strengths and weaknesses of each program and its impact on HF patients' care. This enormous amount of data provided during the pandemic should then be evaluated to be wisely implemented in daily clinical practice also after the crisis.

By evaluating results of telemedicine programs during COVID-19, one should keep in mind that in the particular setting of a pandemic, a system that is cost-efficient, user-friendly, and person-centered does not need to show that it improves outcome, but only that it is not inferior to traditional ways of delivering care and thus allows a safe maintenance of the *status quo* (20).

Although this pandemic has accelerated implementation of technology in the clinical setting, telemedicine should not be considered a cure-all for clinical scenarios. At its core, it remains a synergistic extension of the care team (49) and cannot entirely reproduce the bond-forming element of the traditional doctor–patient relationship based on direct face-to-face interactions (50).

CONCLUSIONS

COVID-19 represents a serious threat for the HF population due to both higher risk of severe disease and death and reduced availability of outpatient care. Telemedicine in all its different forms and possibilities can be adopted to ensure continued healthcare delivery to patients with HF. Thus, we are witnessing its rapid, large-scale implementation during the pandemic. However, there are still several limitations and issues that should be solved in order to continue providing high-quality telemedicine services in patients with HF also after COVID-19.

AUTHOR CONTRIBUTIONS

GT and DW drafted the manuscript. GMC, SG, MR, LB, GP, JD, PA, and MV critically reviewed the manuscript. All authors have participated in the work and have reviewed and agreed with the content of the article. None of the article contents are under consideration for publication in any other journal or have been published in any journal.

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REFERENCES

1. 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. doi: 10.1001/jama.2020.1585
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. 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
4. Guan W, Ni Z, Hu Y, Liang W-h, Qu C-q, He J-x, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. (2020) 382:1708–20. doi: 10.1056/NEJMoa2002032
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. 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
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) 5:811–18. doi: 10.1001/jamacardio.2020.1017
8. Zhang Y, Stewart Coats AJ, 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
9. DeFilippis EM, Reza N, Donald E, Givertz MM, Lindenfeld J, Jessup M. Considerations for heart failure care during the coronavirus disease 2019 (COVID-19) pandemic. *JACC Heart Fail*. (2020) 8:681–91. doi: 10.1016/j.jchf.2020.05.006
10. Reza N, DeFilippis EM, Jessup M. Secondary impact of the COVID-19 pandemic on patients with heart failure. *Circ Heart Fail*. (2020) 13:e007219. doi: 10.1161/CIRCHEARTFAILURE.120.007219
11. 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–e816. doi: 10.1161/CIRCULATIONAHA.120.047011
12. Tersalvi G, Vicenzi M, Calabretta D, Biasco L, Pedrazzini G, Winterton D. Elevated troponin in patients with coronavirus disease 2019: possible mechanisms. *J Card Fail*. (2020) 26:470–5. doi: 10.1016/j.cardfail.2020.04.009
13. Tersalvi G, Veronese G, Winterton D. Emerging evidence of myocardial injury in COVID-19: a path through the smoke. *Theranostics*. (2020) 10:9888–9. doi: 10.7150/thno.50788
14. Alon D, Stein GY, Korenfeld R, Fuchs S. Predictors and outcomes of infection-related hospital admissions of heart failure patients. *PLoS One*. (2013) 8:e72476. doi: 10.1371/journal.pone.0072476
15. Sandoval C, Walter SD, Krueger P, Smieja M, Smith A, Yusuf S, et al. Risk of hospitalization during influenza season among a cohort of patients with congestive heart failure. *Epidemiol Infect*. (2007) 135:574–82. doi: 10.1017/S095026880600714X
16. Kytömaa S, Hegde S, Claggett B, Udell JA, Rosamond W, Temte J, et al. Association of influenza-like illness activity with hospitalizations for heart failure: the atherosclerosis risk in communities study. *JAMA Cardiol*. (2019) 4:363. doi: 10.1001/jamacardio.2019.0549
17. 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
18. Almufleh A, Ahluwalia M, Givertz MM, Weintraub J, Young M, Cooper I, et al. Short-term outcomes in ambulatory heart failure during the COVID-19

pandemic: insights from pulmonary artery pressure monitoring. *J Card Fail.* (2020) 26:633–4. doi: 10.1016/j.cardfail.2020.05.021
19. Oliveros E, Mahmood K, Mitter S, Pinney SP, Lala A. Letter to the Editor: pulmonary artery pressure monitoring during the COVID-19 pandemic in New York city. *J Card Fail.* (2020) 26:900–1. doi: 10.1016/j.cardfail.2020.08.003
20. 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
21. Bui AL, Fonarow GC. Home monitoring for heart failure management. *J Am Coll Cardiol.* (2012) 59:97–104. doi: 10.1016/j.jacc.2011.09.044
22. Salzano A, D'Assante R, Stagnaro FM, Valente V, Crisci G, Giardino F, et al. Heart failure management during COVID-19 outbreak in Italy. Telemedicine experience from a heart failure university tertiary referral centre. *Eur J Heart Fail.* (2020) 22:1048–50. doi: 10.1002/ejhf.1911
23. Tersalvi G, Vicenzi M, Kirsch K, Gunold H, Thiele H, Lombardi F, et al. Structured telephone support programs in chronic heart failure may be affected by a learning curve. *J Cardiovasc Med.* (2020) 21:231–7. doi: 10.2459/JCM.0000000000000934
24. Razonable RR, Pennington KM, Meehan AM, Wilson JW, Froemming AT, Bennett CE, et al. A collaborative multidisciplinary approach to the management of coronavirus disease 2019 in the hospital setting. *Mayo Clin Proc.* (2020) 95:1467–81. doi: 10.1016/j.mayocp.2020.05.010
25. Gorodeski EZ, Goyal P, Cox ZL, Thibodeau JT, Reay RE, Rasmusson K, et al. Virtual visits for care of patients with heart failure in the era of COVID-19: a statement from the heart failure society of America. *J Card Fail.* (2020) 26:448–56. doi: 10.1016/j.cardfail.2020.04.008
26. Almufleh A, Givertz MM. Virtual health during a pandemic: redesigning care to protect our most vulnerable patients. *Circ Heart Fail.* (2020) 13:e007317. doi: 10.1161/CIRCHEARTFAILURE.120.007317
27. Abraham WT, Fiuzat M, Psotka MA, O'Connor CM. Heart failure collaboratory statement on heart failure remote monitoring in the landscape of COVID-19 and social distancing. *JACC Heart Fail.* (2020) 8:423–5. doi: 10.1016/j.jchf.2020.03.005
28. Joshi AU, Randolph FT, Chang AM, Slovis BH, Rising KL, Sabonjian M, et al. Impact of emergency department tele-intake on left without being seen and throughput metrics. *Acad Emerg Med.* (2020) 27:139–47. doi: 10.1111/acem.13890
29. Hollander JE, Carr BG. Virtually perfect? Telemedicine for Covid-19. *N Engl J Med.* (2020) 382:1679–81. doi: 10.1056/NEJMp2003539
30. Alwashmi MF. The use of digital health in the detection and management of COVID-19. *Int J Environ Res Public Health.* (2020) 17:2906. doi: 10.3390/ijerph17082906
31. Bains J, Greenwald PW, Mulcare MR, Leyden D, Kim J, Shemesh AJ, et al. Utilizing telemedicine in a novel approach to COVID-19 management and patient experience in the emergency department. *Telemed J E Health.* (2020). doi: 10.1089/tmj.2020.0162. [Epub ahead of print].
32. Katz JN, Sinha SS, Alviar CL, Dudzinski DM, Gage A, Brusca SB, et al. Disruptive modifications to cardiac critical care delivery during the Covid-19 pandemic: an international perspective. *J Am Coll Cardiol.* (2020) 76:72–84. doi: 10.1016/j.jacc.2020.04.029
33. Goulabchand R, Boclé H, Vignet R, Sotto A, Loubet P. Digital tablets to improve quality of life of COVID-19 older inpatients during lockdown. *Eur Geriatr Med.* (2020) 11:705–6. doi: 10.1007/s41999-020-00344-9
34. Brennan D, Tindall L, Theodoros D, Brown J, Campbell M, Christiana D, et al. A blueprint for telerehabilitation guidelines. *Int J Telerehab.* (2010) 2:31–4. doi: 10.5195/IJT.2010.6063
35. Babu AS, Arena R, Ozemek C, Lavie CJ. COVID-19: a time for alternate models in cardiac rehabilitation to take centre stage. *Can J Cardiol.* (2020) 36:792–4. doi: 10.1016/j.cjca.2020.04.023
36. Scherrenberg M, Frederix I, De Sutter J, Dendale P. Use of cardiac telerehabilitation during COVID-19 pandemic in Belgium. *Acta Cardiol.* (2020) :1-4. doi: 10.1080/00015385.2020.1786625
37. Scherrenberg M, Wilhelm M, Hansen D, Völler H, Cornelissen V, Frederix I, et al. The future is now: a call for action for cardiac telerehabilitation in the COVID-19 pandemic from the secondary prevention and rehabilitation section of the European Association of Preventive Cardiology. *Eur J Prev Cardiol.* (2020). doi: 10.1177/2047487320939671. [Epub ahead of print].
38. Mariani S, Hanke JS, Dogan G, Schmitto JD. Out of hospital management of LVAD patients during COVID-19 outbreak. *Artif Organs.* (2020) 44:873–6. doi: 10.1111/aor.13744
39. Anker SD, Butler J, Khan MS, Abraham WT, Bauersachs J, Bocchi E, et al. Conducting clinical trials in heart failure during (and after) the COVID-19 pandemic: an Expert Consensus Position Paper from the Heart Failure Association (HFA) of the European Society of Cardiology (ESC). *Eur Heart J.* (2020) 41:2109–17. doi: 10.1093/eurheartj/ehaa461
40. 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
41. Scott Kruse C, Karem P, Shifflett K, Vegi L, Ravi K, Brooks M. Evaluating barriers to adopting telemedicine worldwide: a systematic review. *J Telemed Telecare.* (2018) 24:4–12. doi: 10.1177/1357633X16674087
42. Hirko KA, Kerver JM, Ford S, Szafranski C, Beckett J, Kitchen C, et al. Telehealth in response to the Covid-19 Pandemic: implications for rural health disparities. *J Am Med Inform Assoc.* (2020) 27:1816–8. doi: 10.1093/jamia/ocaa156
43. Zachrison KS, Boggs KM, Hayden EM, Espinola JA, Camargo CA. Understanding barriers to telemedicine implementation in rural emergency departments. *Ann Emerg Med.* (2020) 75:392–9. doi: 10.1016/j.annemergmed.2019.06.026
44. Angermann CE, Störk S, Gelbrich G, Faller H, Jahns R, Frantz S, et al. Mode of action and effects of standardized collaborative disease management on mortality and morbidity in patients with systolic heart failure: the interdisciplinary network for heart failure (INH) study. *Circ Heart Fail.* (2012) 5:25–35. doi: 10.1161/CIRCHEARTFAILURE.111.962969
45. Omboni S. Telemedicine during the COVID-19 in Italy: a missed opportunity? *Telemed J E Health.* (2020) 26:973–5. doi: 10.1089/tmj.2020.0106
46. HealthITSecurity. *Healthcare Data Encryption not 'Required,' but Very Necessary.* HealthITSecurity (2017). Available online at: https://healthitsecurity.com/news/healthcare-data-encryption-not-required-but-very-necessary (accessed September 17, 2020).
47. HIPAA Encryption Requirements. HIPAA Journal. Available online at: https://www.hipaajournal.com/hipaa-encryption-requirements/ (accessed September 17, 2020).
48. Heslin SM, Nappi M, Kelly G, Crawford J, Morley EJ, Lingam V, et al. Rapid creation of an emergency department telehealth program during the COVID-19 pandemic. *J Telemed Telecare.* (2020). doi: 10.1177/1357633X20952632. [Epub ahead of print].
49. Czartoski T. *Commentary: Telehealth Holds Promise, but Human Touch Still Needed*. Articles, Abstracts, and Reports. 1278 (2019). Available online at: https://digitalcommons.psjhealth.org/publications/1278
50. Romanick-Schmiedl S, Raghu G. Telemedicine - maintaining quality during times of transition. *Nat Rev Dis Primer.* (2020) 6:45. doi: 10.1038/s41572-020-0199-4

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OPINION
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Women and COVID-19: A One-Man Show?

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Coronavirus disease 2019 (COVID-19) severity and mortality have consistently been higher in men compared to women. The possible biological and behavioral factors underlying this difference have recently been analyzed by Capuano et al. (1). The ideas raised by the authors define a clear need for a more adequate approach to sex differences in case fatality rate. The higher mortality rate in men has indeed been described extensively in literature (2–4). However, the impact of the current pandemic reaches far beyond mortality rates. To tackle this pandemic effectively, an integrated response is essential (5). That is why in this article, we would like to draw attention to some of the main structural, psychological, social and economic impacts this pandemic has on women, as observed by academics, practitioners and international organizations.

Although we acknowledge gender to be complex, social, and non-binary, we will mainly focus on the impact of the current pandemic on women and refer to other publications about the impact on transgender and non-binary populations (6–8).

THE CURRENT LACK OF SEX-DISAGGREGATED DATA

Sex- and gender-disaggregated data on COVID-19 confirmed cases are important in order to address gender disparities in COVID-19 health outcomes and ensure a gender-responsive approach. However, sex disaggregated data is lacking for most countries and gender disaggregated data is nearly absent. As of August 3, 2020, 18.07 million cases were reported worldwide. Data presented in **Figure 1** (n = 8,587,718 sex-disaggregated cases), therefore, represent only 47.5% of all reported cases, highlighting the current lack of these valuable data. Furthermore, a striking difference in the percentage of women among confirmed cases is seen, with 60% in countries such as Belgium, the United Kingdom, and Canada, to 20% in countries such as the Central African Republic, Uganda, and India. Indeed, recent data show that among all persons tested for COVID-19 in the Central African Republic, only 26% were women.

INFECTION RISK AMONG THE HEALTHCARE WORKFORCE

Women face a higher risk of becoming infected during a pandemic because of their position in society as reported by the United Nations (UN) and the World Health Organization (WHO) (9–11). As doctors, nurses, midwives, and community health workers, women are overrepresented at the frontlines, making up 70% of the global health and social workforce (11). Particular issues are the global lack of personal appropriate protective equipment (PPE) and the fact that most PPE are

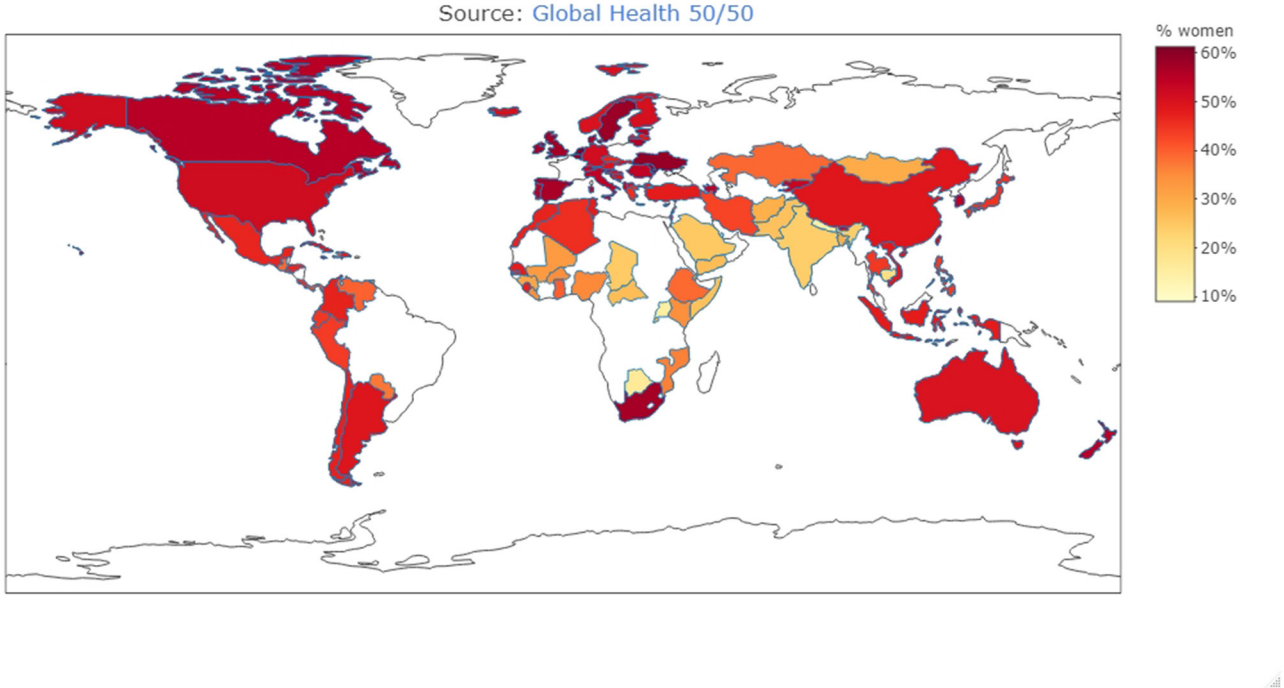

FIGURE 1 | Sex-disaggregated data (Source: Global Health 50/50, https://globalhealth5050.org/covid19/sex-disaggregated-data-tracker/, 15/08/2020) Data are reported from the date that sex-disaggregated data was last available. The map was created using R Statistical Software (version 4.0.2. 2020-06-22, Foundation for Statistical Computing, Vienna, Austria), "plotly" package.

based on a *"default man"* size providing a suboptimal barrier to most women and leaving them more exposed (12). Data from several outbreaks Ebola outbreaks and the SARS outbreak of 2003 demonstrate that nurses and other caretakers have been heavily infected in comparison to other groups in society (13).

SOCIAL IMPACT

As a result of traditional social roles and stereotypes, women still act as the primary caregiver in households, globally spending three to four times more time on unpaid domestic work than men [The International Labor Organization (ILO)] (14). The additional care burden associated with childcare and homeschooling during lockdowns and the care for sick family members can lead to considerable health impacts including e.g., psychological stress. Usual coping mechanisms are limited, given the reduced contact with peers and the disruption of supportive networks. This especially hits single-parent households, of which the majority are headed by women (21% of households with children in the United States compared to 4% by men) (15). Furthermore, as a result, having less time for education, paid work, and career advancement, women can experience increased social inequality during this pandemic (15, 16). Stay-at-home measures together with financial and security concerns can put considerable strain on families, which in some situations can lead to domestic abuse and sexual violence. UN-reports show that violence against women and girls has increased by 25% in several countries and even doubled in some countries since the outbreak of COVID-19 (17).

ECONOMIC IMPACT

Across the globe, women and girls earn less, have less access to educational opportunities, more often hold insecure jobs, and have limited access to financial resources and digital technology (18). Apart from deepening these existing inequalities, multiple studies show that the COVID-19 pandemic has a disproportionately large economic effect on women because the sectors in which they are most active are hard-hit (19). First of all, the manufacturing-and-retail industry has experienced large fallbacks in export and sales because of lockdown and distancing measures. The World Trade Organization (WTO) reports that female employees represent 80% of the workforce in ready-made garment production in Bangladesh, in which industry orders declined by 81% in April alone (20). Moreover, a larger share of women than men work in tourism and business travel which are highly disrupted by travel restrictions and will require a long recovery period (16, 18). Relying on face-to-face interactions, these occupations do not lend themselves to teleworking. Finally, this economic downturn will also be felt by female start-up entrepreneurs who are increasingly finding their way to micro, small and medium enterprises

TABLE 1 | Recommendations for a more gender-sensitive approach to pandemics.

Issue	Recommendation
Lack of sex-disaggregated data	States, their partners and research institutions should collect, report, and analyze data on confirmed COVID-19 cases and deaths that are disaggregated by sex and age (10). The WHO provides global and national surveillance guidelines (10).
Higher risk of infection	Employers should be aware of the higher risk women face in the health and social domain and provide safe and decent working conditions. This can be monitored by workplace representatives, trade unions, and mutual control between employers (12).
Social impact	There should be more social awareness about the social impacts of pandemics. (In)formal protection and support services should be in place together with innovative solutions such as online fora and hotlines (16). Core health and education services and systems should be maintained (26).
Economic impact	Apart from tackling existing economic inequalities, (financial) support measures for businesses should be provided to prevent an economic downfall (16). Moreover, the value of women's unpaid care work should be recognized by including it in the formal labor market and redistributing unpaid family care equally.
Human rights	Decision-makers should be aware that outbreaks affect groups differently and ensure a gender-responsive intersectional response to the COVID-19 pandemic (that recognizes the realities of different genders and addresses these) in policies, program development, implementation etc. Increased participation of women in decision-making will help establish adaptive responses to these realities (27). Inclusivity and diversity in decision-making should be ensured reflecting the population they represent. Existing women's and youth rights networks should be engaged to support connectivity and vital information flow (26).

(MSMEs) (21). MSMEs tend to be the first businesses impacted in times of recession. Given the long-term economic impact that COVID-19 will have, protecting female entrepreneurship should be on the priority list of governments in order to build a faster and more inclusive growth during the economic recovery period.

HUMAN RIGHTS

The Secretary General of the Council of Europe put it best: "While the virus is resulting in the tragic loss of life, we must nonetheless prevent it from destroying our way of life" (22). Human rights reflect the minimum standards necessary for people to live with dignity. While the COVID-19 crisis is fast becoming a socio-economic crisis it adds pressure on human rights. For women and girls, the problems identified form an undeniable increased threat to their right to life and right to health (23). Various international law instruments [e.g., The Universal Declaration of Human Rights, Art. 25 (24)] recognize the right to health as an inclusive right, encompassing a wide range of factors that help humans lead a healthy life (25). These factors include safe drinking water, safe food, sanitation, but also health-related education and information, the right to access to health care, and gender equality. As the UN state in their latest Policy Brief, the economic impact and prevalence of poverty among women, their experience of violence, their position in society, the limited power many women have over their sexual and reproductive lives, and their lack of influence in decision-making are social realities that adversely impact women's human rights and that should move to global action (9).

A WAY FORWARD

Prevention and response management is hindered when gendered impacts of outbreaks are ignored obscuring critical trends. In order to minimize these impacts, different steps should be undertaken. In **Table 1** we provide a list of important recommendations made by international organizations.

CONCLUSION

Gendered differences of COVID-19 are present not only at the biological level, but also at the psychological, social and societal level. Although literature shows that men are clearly predisposed to COVID-19 related mortality, women are just as well victimized, albeit in a different way. The current pandemic painfully highlights that gender inequality is still insufficiently addressed in our society. Public health should never be a predominantly men affair mainly focusing on the male body—a one-man show. In contrast, more gender-sensitive approaches that take into account different physical, mental, and social needs across the full gender spectrum are indispensable to guarantee optimal well-being of all.

AUTHOR CONTRIBUTIONS

JV and KD conceived and wrote the manuscript. KV and WO critically revised the manuscript and provided important intellectual contribution. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Capuano A, Rossi F, Paolisso G. Covid-19 kills more men than women: an overview of possible reasons. *Front Cardiovasc Med.* (2020) 7:131. doi: 10.3389/fcvm.2020.00131
2. Gagliardi MC, Tieri P, Ortona E, Ruggieri A. ACE2 expression and sex disparity in COVID-19. *Cell Death Discov.* (2020) 6:37. doi: 10.1038/s41420-020-0276-1
3. Elgendy IY, Pepine CJ. Why are women better protected from COVID-19: clues for men? Sex and COVID-19. *Int J Cardiol.* (2020) 315:105–06. doi: 10.1016/j.ijcard.2020.05.026

4. Agrawal H, Das N, Nathani S, Saha S, Saini S, Kakar SS, et al. An assessment on impact of COVID-19 infection in a gender specific manner. *Stem Cell Rev Rep*. (2020) 1–19. doi: 10.1007/s12015-020-10048-z
5. Wenham C, Smith J, Davies SE, Feng H, Grépin KA, Harman S, et al. Women are most affected by pandemics - lessons from past outbreaks. *Nature*. (2020) 583:194–8. doi: 10.1038/d41586-020-02006-z
6. Salerno JP, Williams ND, Gattamorta KA. LGBTQ populations: psychologically vulnerable communities in the COVID-19 pandemic. *Psychol Trauma*. (2020) 12:S239–42. doi: 10.1037/tra0000837
7. Wang Y, Pan B, Liu Y, Wilson A, Ou J, Chen R. Health care and mental health challenges for transgender individuals during the COVID-19 pandemic. *Lancet Diabetes Endocrinol*. (2020) 8:564–5. doi: 10.1016/S2213-8587(20)30182-0
8. Sevelius JM, Gutierrez-Mock L, Zamudio-Haas S, McCree B, Ngo A, Jackson A, et al. Research with marginalized communities: challenges to continuity during the COVID-19 pandemic. *AIDS Behav*. (2020) 24:2009–12. doi: 10.1007/s10461-020-02920-3
9. United Nations. *The Impact of COVID-19 on Women*. (2020). Available online at: https://www.unwomen.org/-/media/headquarters/attachments/sections/library/publications/2020/policy-brief-the-impact-of-covid-19-on-women-en.pdf?la=en&vs=1406 (accessed November 9, 2020).
10. World Health Organization? *Gender and COVID-19: Advocacy Brief* (2020). Available online at: https://apps.who.int/iris/handle/10665/332080 (accessed November 9, 2020).
11. World Health Organization. *Gender Equity in the Health Workforce: Analysis of 104 Countries*. (2019). Available online at: https://apps.who.int/iris/bitstream/handle/10665/311314/WHO-HIS-HWF-Gender-WP1-2019.1-eng.pdf?ua=1 (accessed August 16, 2020).
12. Trades Union Congress. *Personal Protective Equipment and Women*. (2017). Available online at: https://www.tuc.org.uk/sites/default/files/PPEandwomenguidance.pdf (accessed August 16, 2020).
13. World Health Organization. *Addressing Sex and Gender in Epidemic-Prone Infectious Diseases*. (2007). Available online at: https://www.who.int/csr/resources/publications/SexGenderInfectDis.pdf (accessed August 16, 2020).
14. International Labour Organization. *Care Work and Care Jobs for the Future of Decent Work*. (2018). Available online at: https://www.ilo.org/wcmsp5/groups/public/---dgreports/---dcomm/---publ/documents/publication/wcms_633135.pdf (accessed August 16, 2020).
15. Alon T, Doepke M, Olmstead-Rumsey J, Tertilt M. The impact of COVID-19 on Gender equality. In: *Covid Economics: Vetted and Real-Time Papers*. (2020) 4:62–85.
16. World Bank Group. *Gender Dimensions of the COVID-19 Pandemic*. (2020). Available online at: http://documents1.worldbank.org/curated/en/618731587147227244/pdf/Gender-Dimensions-of-the-COVID-19-Pandemic.pdf (accessed August 16, 2020).
17. UN Women. *COVID-19 and Ending Violence Against Women and Girls*. (2020). Available online at: https://prod.unwomen.org/-/media/headquarters/attachments/sections/library/publications/2020/issue-brief-covid-19-and-ending-violence-against-women-and-girls-en.pdf?la=es&vs=5006 (accessed August 16, 2020).
18. World Trade Organization. *The Economic Impact of COVID-19 on Women in Vulnerable Sectors and Economies*. (2020). Available online at: https://www.wto.org/english/news_e/news20_e/info_note_covid_05aug20_e.pdf?fbclid=IwAR131NFWHhdwPQIOM3GN6_jYpnwae5JTleO9pPqgFVo5sKubCi8NkNxOr6I (accessed August 16, 2020).
19. World Trade Organization. *Trade in Services in the Context of COVID-19*. (2020). Available online at: https://www.wto.org/english/tratop_e/covid19_e/services_report_e.pdf (accessed August 16, 2020).
20. Financial Express. *Bangladesh's RMG Export in April Declines Nearly 85 per cent*. (2020). Available online at: https://www.globaltimes.cn/content/1187514.shtml (accessed August 16, 2020).
21. World Trade Organization. *World Trade Report 2019 – The Future of Services Trade*. (2020). Available online at: https://www.wto.org/english/res_e/booksp_e/00_wtr19_e.pdf (accessed August 16, 2020).
22. Council of Europe. *Speeches 2020 - Saint Petersburg International Legal Forum*. (2020). Available online at: https://www.coe.int/en/web/secretary-general/-/saint-petersburg-international-legal-forum (accessed August 16, 2020).
23. United Nations. *COVID-19 and Human Rights – We are All in This Together*. (2020). Available online at: https://www.un.org/sites/un2.un.org/files/un_policy_brief_on_human_rights_and_covid_23_april_2020.pdf?fbclid=IwAR2ojuGQlNSdbBUOEfG-gsWWtc4FSI8f4KI7-DypyTpGBU_IiPO5R7cOSD0 (accessed August 16, 2020).
24. United Nations. *The Universal Declaration of Human Rights*. (1948). Available online at: https://www.ohchr.org/EN/UDHR/Documents/UDHR_Translations/eng.pdf (accessed August 16, 2020).
25. Office of the United Nations High Commissioner for Human Rights. *The Right to Health, Fact Sheet No. 31*. (2008). Available online at: https://www.ohchr.org/Documents/Publications/Factsheet31.pdf (accessed August 16, 2020).
26. UNICEF. *Five Actions for Gender Equality in the COVID-19 Response*. (2020). Available online at: https://www.unicef.org/media/66306/file/Five%20Actions%20for%20Gender%20Equality%20in%20the%20COVID-19%20Response:%20UNICEF%20Technical%20Note.pdf (accessed on November 9, 2020)
27. Bali S, Dhatt R, Lal A, Jama A, Van Daalen K, Sridhar D. Off the back burner: diverse and gender-inclusive decision-making for COVID-19 response and recovery. *BMJ Glob Health*. (2020) 5:e002595. doi: 10.1136/bmjgh-2020-002595

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ORIGINAL RESEARCH
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SARS-CoV-2 Infection in Asymptomatic Patients Hospitalized for Cardiac Emergencies: Implications for Patient Management

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Background: The coronavirus disease (COVID-19) pandemic imposed diverse challenges on the health care system. Morbidity and mortality of non-COVID-19 emergencies might also have changed because hospitals may not be able to provide optimal care due to restructured resources and uncertainties how to deal with potentially infected patients. It has been recommended to stratify treatment of cardiovascular emergencies according to cardiovascular risk. However, data on the prevalence of asymptomatic SARS-CoV-2 infection in patients presenting with cardiac emergencies remain scarce.

Methods: We retrospectively analyzed patients' data from a tertiary cardiology department between April 15 and May 31, 2020. All patients were screened on admission for COVID-19 symptoms using a questionnaire and body temperature measurements. All hospitalized patients were routinely screened using nasopharyngeal swab testing.

Results: In total, we counted 710 urgent and emergency admissions. Nasopharyngeal swab tests were available in 689 (97%) patients, 409 and 280 of which presented as urgent and emergency admissions, respectively. Among 280 emergency admissions, none tested positive for SARS-CoV-2.

Conclusion: In cardiac emergency patients which were screened negative for COVID-19 symptoms, the prevalence of SARS-CoV-2 infection in regions with a modest overall prevalence is low. This finding might be helpful to better determine timing of emergency procedures and reasonable usage of protective equipment during the COVID-19 crisis and the future.

Keywords: cardiac emergencies, SARS-CoV-2, COVID-19, personal protective equipment (PPE), screening

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INTRODUCTION

The coronavirus disease (COVID-19) pandemic imposed diverse challenges on health care providers and hospitals. For instance, hospitals needed to rapidly redistribute and reorganize resources to treat acutely ill COVID-19 patients while keeping up with other emergencies. At the same time outmost attention had to be spent on containing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to protect other patients and staff. Apart from rising numbers of COVID-19 patients, a change in the presentation pattern of non-COVID emergencies was observed. In that light, it was recently shown that hospital admissions for acute coronary syndrome (ACS) declined during the pandemic (1–4). Apart from a decrease in presentations, morbidity and mortality of non-COVID-19 emergencies might have changed because hospitals may not be able to provide optimal care due to restructured resources and uncertainties how to deal with potentially infected patients (5). Position papers consequently recommended to stratify treatment of cardiovascular emergencies according to cardiovascular risk: (1) only high-risk emergencies (e.g., ST elevation myocardial infarction) should be treated immediately with usage of personal protective equipment as in confirmed COVID-19 cases; (2) other emergencies and elective procedures should only be carried out after receiving results of SARS-CoV-2 testing (6–8). A better understanding of the prevalence of asymptomatic SARS-CoV-2 infections may help to guide timely management and reasonable usage of personal protective equipment without affecting the safety of staff and other patients.

We here sought to investigate the prevalence of SARS-CoV-2 infections in asymptomatic patients presenting with cardiac emergencies.

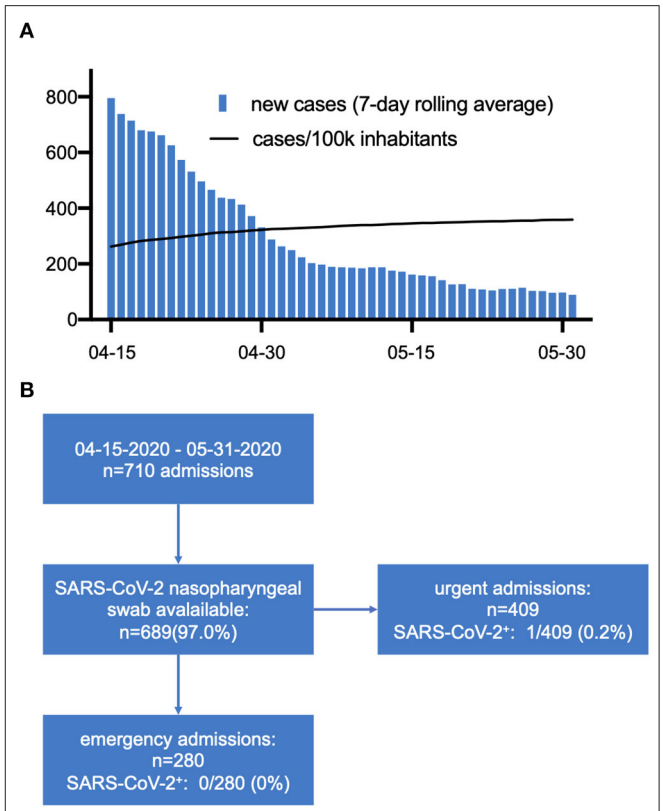

FIGURE 1 | (A) Daily reported cases (Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, accessed on 07-15-2020) and prevalence (Daily Situation Report of the Robert Koch Institute, accessed on 07-15-2020) of SARS-CoV-2 infections in the Free State of Bavaria during the study period. **(B)** Prevalence of asymptomatic SARS-CoV-2 infection.

METHODS

Study Cohort

The study protocol was approved by the institutional ethics committee (323/20 S) and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. We retrospectively analyzed patients' data from a tertiary cardiology department which provides 24/7 interventional cardiac care between April 15 and May 31, 2020 (i.e., at the peak of the pandemic's first wave in the region).

Screening of Patients

All patients were screened on admission for COVID-19 symptoms using a questionnaire and had their body temperature measured (ear thermometer). Patients were assigned as COVID-19 asymptomatic when none of the following criteria were met: body temperature $\geq$38.1°C, coughing, shortness of breath, runny nose, sore throat, or body aches. Additionally, patients were asked whether they had been in contact to a confirmed COVID-19 case or a patient suffering from fever and coughing without proven SARS-CoV-2 infection. Patients reporting shortness of breath were also regarded as COVID-19 asymptomatic if they did not report one of the other criteria. COVID-19 asymptomatic patients were required to wear standard surgical masks (no N95 or FFP2-3) throughout the entire stay. Hospital staff was also required to wear standard surgical masks at all times. These protective measurements were recently shown to reduce in particular the risk of SARS-CoV-2 infection for healthcare workers (9). N95 masks or FFP2-3 masks and further dedicated protective equipment were only used when treating SARS-CoV-2 confirmed or suspected patients.

SARS-CoV-2 Testing

All hospitalized patients were routinely screened for SARS-CoV-2 using nasopharyngeal swab testing (SARS-CoV-2 real-time polymerase chain reaction assay, Mikrogen Diagnostik, Neuried, Germany) since April 15, 2020. Patients without COVID-19 symptoms were only planned to be tested at admission. Repeated testing was performed if patients developed symptoms or if a more recent test result was required for transferal to other treatment facilities.

RESULTS

Until May 31, 2020, a total of 710 patients presented and were included this analysis. **Figure 1A** displays the number of daily infections in the Free State of Bavaria, Germany and the

TABLE 1 | Baseline characteristics and reasons of admission for the patients presenting with cardiac emergencies during the study period.

	Emergency admissions ***n* = 280**
Age, years ± *SD*	68.5 ± 15.0
Female gender, *n* (%)	103 (36.8)
Comorbidities	
COPD, *n* (%)	19 (6.8)
Diabetes, *n* (%)	60 (21.4)
Hypertension, *n* (%)	189 (67.5)
Coronary artery disease, *n* (%)	131 (46.8)
Peripheral artery disease, *n* (%)	26 (9.3)
Cerebrovascular disease, *n* (%)	34 (12.1)
Cancer, *n* (%)	30 (10.7)
Chronic renal dysfunction, *n* (%)	50 (17.9)
Immunodeficiency, *n* (%)	8 (2.9)
Reasons for admission	
Coronary, *n* (%)	97 (34.6)
Heart failure, *n* (%)	23 (8.2)
Structural, *n* (%)	7 (2.5)
Electrophysiology, *n* (%)	93 (33.2)
Other, *n* (%)	60 (21.4)

Coronary includes (suspected) acute coronary syndromes. Electrophysiology includes, e.g., tachycardia and bradycardia. Other includes, e.g., syncope, pulmonary embolism. COPD, chronic obstructive pulmonary disease; SD, standard deviation.

prevalence of SARS-CoV-2 infections per 100,000 inhabitants during the study period. Nasopharyngeal swab tests were available in 689 (97%) patients, 409 and 280 of which presented as urgent [reasons: coronary 116/409 (28.4%), structural 42/409 (10.3%), heart failure 8/409 (2%), electrophysiology 209/409 (51.1%), other 34/409 (8.3%)] and emergency admissions, respectively. As a suspected SARS-CoV-2 infection may have reduced the likelihood of presenting with a non-emergency leading to an underestimation of the actual prevalence, we focused on the 280 patients admitted as cardiac emergencies. Baseline characteristics and reasons for admission are displayed in **Table 1**. None of these COVID-19 asymptomatic patients tested positive for SARS-CoV-2. During the hospital stay, 27 (9.6%) of patients were repeatedly tested with no test revealing a positive result.

In the total cohort, only one patient was diagnosed to be SARS-CoV-2 positive (**Figure 1B**). The patient was sent in home quarantine and treatment was scheduled to be performed after 14 days of quarantine and two subsequent negative nasopharyngeal swabs. This patient remained asymptomatic and no further testing was performed during quarantine.

DISCUSSION

This result needs to be reviewed in the context of the overall SARS-CoV-2 prevalence in the respective region during the observation period. During the study period, ∼300 cases per 100,000 citizens were reported in the Free State of Bavaria. Thus, our data indicate that in cardiac emergency patients which were screened negative for COVID-19 symptoms, the prevalence of SARS-CoV-2 infection in regions with a modest overall prevalence is low. Under these circumstances, our findings indicate that a delay/deferral of emergency procedures due to waiting for SARS-CoV-2 test results may not be justified in emergency patients which are screened asymptomatic for COVID-19, but have an unclear SARS-CoV-2 infectious status. While our finding is in line with a recent report from Iceland, where in a random-sample screening of the population, 0.6% tested positive for SARS-CoV-2 (10), a study screening pregnant women admitted for delivery in New York City found that 13.5% of tested women were asymptomatic but tested positive (11).

In summary, the frequency of asymptomatic SARS-CoV-2 carriers among cardiac emergency patients is low when the overall prevalence of COVID-19 is modest. Consequently, emergency but also elective procedures may safely be carried out without delay and waiting for SARS-CoV-2 test results. Importantly, the safety of personnel and patients may be further increased by implementation of rapid or point-of-care tests which despite potential drawbacks [for an overview, see (12)] recently revealed promising results (13).

Our study was performed during a time period in which the prevalence of COVID-19 in Bavaria was rather low and our findings are therefore inherently not applicable in regions with higher prevalence. It was also previously shown that the highest sensitivity of SARS-CoV-2 was reached bronchoalveolar lavage fluid (14) and we may have missed SARS-CoV-2 infection due to only performing nasopharyngeal swab testing. Additional major limitations are the retrospective nature of this analysis and that the data are derived from a single center. The low prevalence of SARS-CoV-2 infection may therefore be due to chance and requires validation in further cohorts to draw definitive conclusions. Our data may nevertheless be helpful to better determine timing of emergency procedures and reasonable usage of protective equipment during the COVID-19 crisis and 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/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Technische Universität München. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AK and HBS designed the study. TK, JW, TG, and HS contributed data. TK, AK, and HBS drafted the manuscript. All authors were involved in critically revising the manuscript.

REFERENCES

1. 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
2. Piccolo R, Bruzzese D, Mauro C, Aloia A, Baldi C, Boccalatte M, et al. Population trends in rates of percutaneous coronary revascularization for acute coronary syndromes associated with the COVID-19 outbreak. *Circulation.* (2020) 133:916–8. doi: 10.1161/CIRCULATIONAHA.120.047457
3. 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
4. Kessler T, Graf T, Hilgendorf I, Rizas K, Martens E, zur Muhlen von C, et al. Hospital admissions with acute coronary syndromes during the COVID-19 pandemic in German cardiac care units. *Cardiovasc Res.* (2020) 116:1800–1. doi: 10.1093/cvr/cvaa192
5. Rosenbaum L. Facing covid-19 in Italy—ethics, logistics, and therapeutics on the epidemic's front line. *N Engl J Med.* (2020) 382:1873–5. doi: 10.1056/NEJMp2005492
6. 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
7. Welt FGP, 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
8. The European Society of Cardiology. *ESC Guidance for the Diagnosis and Management of CV Disease during the COVID-19 Pandemic*. (2020). Avaialble online at: https://www.escardio.org/Education/COVID-19-and-Cardiology/ESC-COVID-19-Guidance (accessed 10 June, 2020).
9. Wang X, Ferro EG, Zhou G, Hashimoto D, Bhatt DL. Association between universal masking in a health care system and SARS-CoV-2 positivity among health care workers. *JAMA.* (2020) 324:703–4. doi: 10.1001/jama.2020.12897
10. Gudbjartsson DF, Helgason A, Jonsson H, Magnusson OT, Melsted P, Norddahl GL, et al. Spread of SARS-CoV-2 in the icelandic population. *N Engl J Med.* (2020) 382:2302–15. doi: 10.1056/NEJMoa2006100
11. Sutton D, Fuchs K, D'Alton M, Goffman D. Universal screening for SARS-CoV-2 in women admitted for delivery. *N Engl J Med.* (2020) 382:2163–4. doi: 10.1056/NEJMc2009316
12. Guglielmi G. Fast coronavirus tests: what they can and can't do. *Nature.* (2020) 585:496–8. doi: 10.1038/d41586-020-02661-2
13. Gibani MM, Toumazou C, Sohbati M, Sahoo R, Karvela M, Hon T-K, et al. Assessing a novel, lab-free, point-of-care test for SARS-CoV-2 (CovidNudge): a diagnostic accuracy study. *Lancet Microbe.* (2020) 1:e300–7. doi: 10.1016/S2666-5247(20)30121-X
14. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA.* (2020) 323:1843–4. doi: 10.1001/jama.2020.3786

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OPINION
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Thromboinflammation and COVID-19: The Role of Exercise in the Prevention and Treatment

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic is currently the biggest public health concern across the globe. On a global scale, from December 2019 to September 2020, more than 34,114,000 people were infected with the disease, with 1,016,000 deaths recorded (1). Although the etiology of the disease has long been investigated, it is still a harsh challenge for the medical and scientific community.

COVID-19 infection is complex, and the risk factors are different from the known viral respiratory infections. People with chronic inflammatory diseases (such as obesity, hypertension, diabetes, and cardiovascular disorder) are at a huge risk of developing moderate to severe symptoms and being hospitalized in the intensive care unit (ICU) (2, 3). The most common phenomena among these conditions are chronic low-grade inflammation and increased cardiovascular complications. Several evidences have been put forward to support the association between COVID-19 and thromboinflammation (3, 4). Specifically, venous thrombosis has been found to be causally related to pulmonary embolism in many cases (5).

Exercise is well-known for having a prophylactic and therapeutic effect on chronic inflammatory diseases, with a high impact on the vascular system. Furthermore, it has been reported that exercise may decrease the severity of infectious diseases and number of days of disease symptoms (6). Consistent with this, it is speculated that regular exercise represents a protective factor against the severity of COVID-19 relating to thromboinflammation and its complications.

EXERCISE AS A TOOL FOR DECREASING CHRONIC INFLAMMATION AND IMPROVING ANGIOGENESIS AND IMMUNE RESPONSE

The vascular system is largely affected by COVID-19 infection. Although pulmonary failure is not directly related to the loss of pulmonary alveoli, lack of blood flow in this area can induce a collapse of the alveoli, as recently demonstrated by Ackermann et al. (7). Furthermore, kidneys are highly vascularized organs that also may be affected by this infection (2).

Venous thrombosis is usually found in coagulopathies and also observed in arterial thrombosis and stroke (7). Clinical markers of the coagulation cascade, such as D-dimer and fibrinogen, are elevated in those with moderate and severe forms of COVID-19 (8). Low innate antiviral defense and high inflammatory cytokine release contribute to the severity of COVID-19 (9), suggesting that it can be an important trigger for thrombotic complications. High amounts of pro-inflammatory cytokines contribute to the activation of thrombotic pathways. For instance, it was demonstrated that interleukin (IL)-6 induces thrombin generation and that IL-1 and tumor necrosis factor (TNF)-α inhibit anticoagulant pathways (8).

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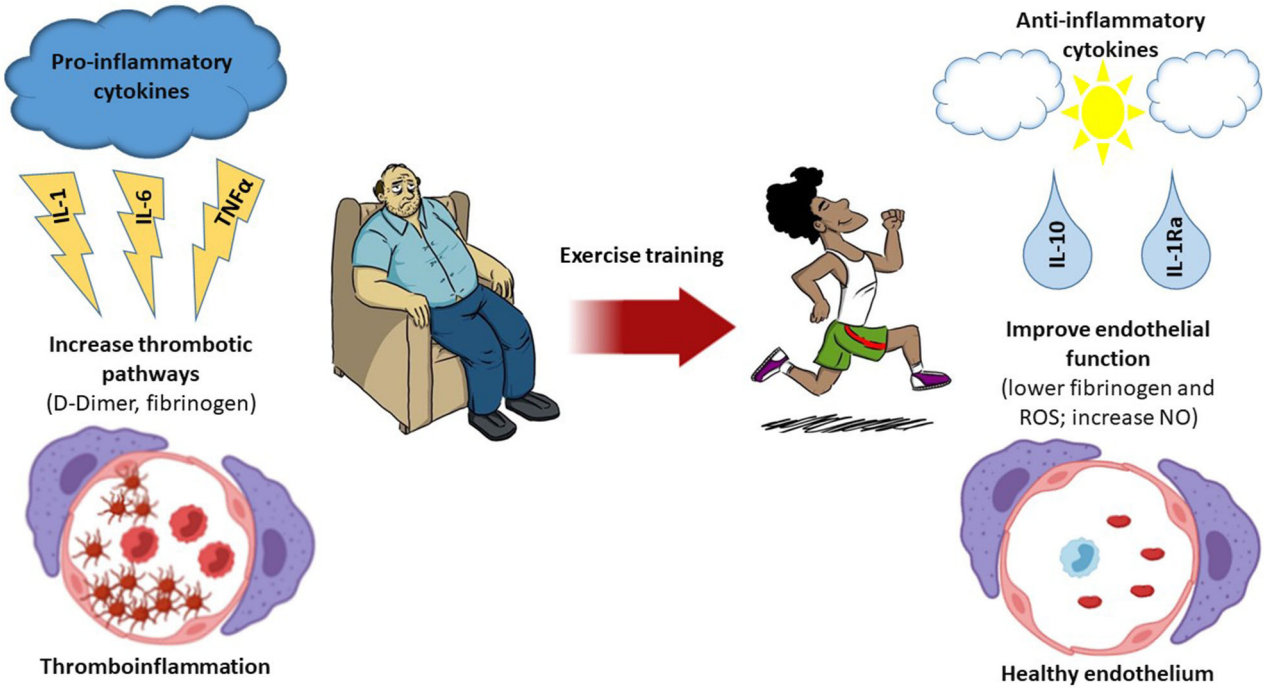

FIGURE 1 | Thromboinflammation and the effect of exercise. A sedentary lifestyle leads to an increase in the release of pro-inflammatory cytokines, which induce a low-grade chronic inflammation. These inflammatory mediators enhance the thrombotic pathways that facilitate thromboinflammation, which has been associated with poor prognosis in coronavirus disease 2019 (COVID-19) patients. Exercise decreases inflammation by many pathways, including the release of anti-inflammatory cytokines. Regular exercise is associated with lower levels of fibrinogen and reactive oxygen species and increased amounts of nitric oxide (NO) production, thus inducing a healthy endothelium environment.

Exercise, especially in the form of regular aerobic activities, have the potential of dampening chronic inflammation by stimulating anti-inflammatory pathways and associated improvement of cardiovascular functions. Accordingly, by decreasing the basal concentration of inflammatory cytokines and reducing the percentage of pro-inflammatory T effector memory CD45+ re-expressing T cells (T-EMRA cells), exercise indirectly prevents the activation of thrombotic pathways (10).

Exercise has been shown to directly affect coagulation. While acute and strenuous activities can culminate in pro-coagulative stimuli, regular activity has been shown to diminish platelet activation under resting conditions (11). Exercise reduces fibrinogen level and enhances the plasma volume without increasing the erythrocyte volume (11). Also, exercise was used as a treatment for deep venous post-thrombotic syndrome (12). Heart failure patients with reduced fraction of ejection, when treated with moderate endurance exercise, showed a reduction in vascular endothelial damage as well as suppression of inflammation and oxidative stress (13).

The intensity and duration of aerobic exercise are correlated with the increase in nitric oxide production and reduction of reactive oxygen species, which lead to an improvement in endothelial function. Moreover, aerobic exercise reduces hypertension on coronary arteries and vascular stiffness (14).

In parallel, regular exercise can enhance the innate and adaptive immune defense system, thus improving the response against viral infections. While it can only be speculated that exercise has a protective effect against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, regular activity has been shown to decrease the severity of infectious episodes and number of days of the symptom in other infectious diseases (6). Concerning influenza infection, exercise is associated with a lower excess risk of mortality (15). Similarly, in murine models, it was proven that moderate exercise reduces mortality in the initial days after an influenza virus infection (16). Moreover, moderate aerobic training has been shown to enhance T cell count, which is found to be decreased in the blood of SARS-CoV-2-infected patients (21), increase anti-inflammatory cytokines, improve endothelial function, and repair (**Figure 1**), enhance VO2peak, and have beneficial effects on clinical outcomes (22). A minimum of 150 min per week (30 min−5 days/week) of moderate aerobic exercise (5–7 on a scale of 0–10, where 0 is super easy and 10 is exhaustive) was recommended by the American College of Sports Medicine to achieve the health benefits of exercise. Moderate aerobic exercise is applied to improve immunity and metabolic complications that can reduce the poor prognosis of COVID-19 (23).

Therefore, we hypothesized that moderate intensity of aerobic training could be a protective factor against severe courses of

COVID-19 (17) (**Figure 1**). Therefore, we can draw the attention of physicians toward assessment of the fitness level of COVID-19 patients.

THE POTENTIAL ROLE OF EXERCISE IN THE RECOVERY OF THOSE INFECTED WITH CORONAVIRUS DISEASE 2019

In 2016, the WHO proposed "functioning" as a third clinical outcome indicator, such that diseases that are not fully cured are accompanied by some dysfunctions. Improving functional life while recovering from a disease is a key sign of medical effectiveness and overall health. Many patients who are recovering from COVID-19, especially those presenting severe symptoms during the infection phase, are not able to return to the normal life of caring for themselves after being discharged (18).

As discussed above, poor vascularization could cause alveoli collapse, thus leading to pulmonary failure. Several individuals infected by SARS-CoV-2 have presented respiratory problems with impairment of pulmonary ventilation function and air exchange in the alveoli, which lead to chest tightness, dyspnea, and pulmonary fibrosis (18). Pulmonary fibrosis is directly associated with high mortality rates. Furthermore, dyspnea, which is often associated with loss of skeletal muscle mass, is responsible for a decreased exercise capacity due to a reduction of daily leaving activities (19).

Several studies have investigated the role of exercise in the treatment of chronic lung disease and pulmonary fibrosis patients. A meta-analysis recently published stated that aerobic training significantly improves exercise capacity and health-related quality of life of patients with chronic respiratory disease and/or pulmonary fibrosis and that aerobic training improved the dyspnea scores when combined with breathing exercises (20).

It is important to remember that most of the benefits promoted by physical exercise in the rehabilitation of respiratory and cardiovascular diseases can be gradually lost if the patient does not continue to exercise in the long run (18). However, the practice of exercise for the improvement of medical conditions should be supervised. In conclusion, regular exercise could be an adjuvant for the prevention and treatment of COVID-19.

AUTHOR CONTRIBUTIONS

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

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REFERENCES

1. Johns Hopkins Coronavirus Resource Center. *Johns Hopkins Coronavirus Resource Center*. Available online at: https://coronavirus.jhu.edu/ (accessed June 19, 2020).
2. Gémes K, Talbäck M, Modig K, Ahlbom A, Berglund A, Feychting M, et al. Burden and prevalence of prognostic factors for severe COVID-19 in Sweden. *Eur J Epidemiol*. (2020) 35:401–9. doi: 10.1007/s10654-020-00646-z
3. Harenberg J, Favaloro E. COVID-19: progression of disease and intravascular coagulation—present status and future perspectives. *Clin Chem Lab Med*. (2020) 58:1029–36. doi: 10.1515/cclm-2020-0502
4. Lillicrap D. Disseminated intravascular coagulation in patients with 2019-nCoV pneumonia. *J Thromb Haemost*. (2020) 18:786–7. doi: 10.1111/jth.14781
5. 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
6. Grande AJ, Keogh J, Silva V, Scott AM. Exercise versus no exercise for the occurrence, severity, and duration of acute respiratory infections. *Cochrane Database Syst Rev*. (2020) 4:CD010596. doi: 10.1002/14651858.CD010596.pub3
7. 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
8. Levi M, Thachil J, Iba T, Levy JH. Coagulation abnormalities and thrombosis in patients with COVID-19. *Lancet Haematol*. (2020) 7:e438–40. doi: 10.1016/S2352-3026(20)30145-9
9. Blanco-Melo D, Nilsson-Payant BE, Liu W-C, Uhl S, Hoagland D, Møller R, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell*. (2020) 181:1036–45.e9. doi: 10.1016/j.cell.2020.04.026
10. Philippe M, Gatterer H, Burtscher M, Weinberger B, Keller M, Grubeck-Loebenstein B, et al. Concentric and eccentric endurance exercise reverse hallmarks of T-cell senescence in pre-diabetic subjects. *Front Physiol*. (2019) 10:684. doi: 10.3389/fphys.2019.00684
11. Heber S, Volf I. Effects of physical (in)activity on platelet function. *BioMed Res Int*. (2015) 2015:165078. doi: 10.1155/2015/165078
12. Kahn SR, Shrier I, Shapiro S, Houweling AH, Hirsch AM, Reid RD, et al. Six-month exercise training program to treat post-thrombotic syndrome: a randomized controlled two-centre trial. *CMAJ*. (2011) 183:37–44. doi: 10.1503/cmaj.100248
13. Hsu C-C, Fu T-C, Huang S-C, Wang J-S. High-intensity interval training recuperates capacity of endogenous thrombin generation in heart failure patients with reduced ejection fraction. *Thromb Res*. (2020) 187:159–65. doi: 10.1016/j.thromres.2020.01.013
14. Roque FR, Briones AM, García-Redondo AB, Galán M, Martínez-Revelles S, Avendaño MS, et al. Aerobic exercise reduces oxidative stress and improves vascular changes of small mesenteric and coronary arteries in hypertension. *Br J Pharmacol*. (2013) 168:686–703. doi: 10.1111/j.1476-5381.2012.02224.x
15. Wong C-M, Lai H-K, Ou C-Q, Ho S-Y, Chan K-P, Thach T-Q, et al. Is exercise protective against influenza-associated mortality? *PLoS ONE*. (2008) 3:e2108. doi: 10.1371/journal.pone.0002108
16. Lowder T, Padgett DA, Woods JA. Moderate exercise protects mice from death due to influenza virus. *Brain Behav Immun*. (2005) 19:377–80. doi: 10.1016/j.bbi.2005.04.002
17. Channappanavar R, Zhao J, Perlman S. T cell-mediated immune response to respiratory coronaviruses. *Immunol Res*. (2014) 59:118–28. doi: 10.1007/s12026-014-8534-z
18. Giallauria F, Piccioli L, Vitale G, Sarullo FM. Exercise training in patients with chronic heart failure: a new challenge for Cardiac Rehabilitation

Community. *Monaldi Arch Chest Dis Arch Monaldi Mal Torace*. (2018) 88:987. doi: 10.4081/monaldi.2018.987
19. Dixit S. Can moderate intensity aerobic exercise be an effective and valuable therapy in preventing and controlling the pandemic of COVID-19? *Med Hypotheses*. (2020) 143:109854. doi: 10.1016/j.mehy.2020.109854
20. Zbinden-Foncea H, Francaux M, Deldicque L, Hawley JA. Does high cardiorespiratory fitness confer some protection against pro-inflammatory responses after infection by SARS-CoV-2? *Obes Silver Spring Md*. (2020) 28:1378–81. doi: 10.1002/oby.22849
21. Li J. Rehabilitation management of patients with COVID-19. Lessons learned from the first experiences in China. *Eur J Phys Rehabil Med*. (2020) 24:9. doi: 10.23736/S1973-9087.20.06292-9
22. Dyspnea. Mechanisms, assessment, and management: a consensus statement. American Thoracic Society. *Am J Respir Crit Care Med*. (1999) 159:321–40. doi: 10.1164/ajrccm.159.1.ats898
23. Hanada M, Kasawara KT, Mathur S, Rozenberg D, Kozu R, Hassan SA, et al. Aerobic and breathing exercises improve dyspnea, exercise capacity and quality of life in idiopathic pulmonary fibrosis patients: systematic review and meta-analysis. *J Thorac Dis*. (2020) 12:1041–55. doi: 10.21037/jtd.2019.12.27

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Prevalence of Venous Thromboembolism in Critically Ill COVID-19 Patients: Systematic Review and Meta-Analysis

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Background: Recent studies revealed a high prevalence of venous thromboembolism (VTE) events in coronavirus disease 2019 (COVID-19) patients, especially in those who are critically ill. Available studies report varying prevalence rates. Hence, the exact prevalence remains uncertain. Moreover, there is an ongoing debate regarding the appropriate dosage of thromboprophylaxis.

Methods: We performed a systematic review and proportion meta-analysis following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. We searched PubMed and EMBASE for studies exploring the prevalence of VTE in critically ill COVID-19 patients till 25/07/2020. We pooled the proportion of VTE. Additionally, in a subgroup analysis, we pooled VTE events detected by systematic screening. Finally, in an exploratory analysis, we compared the odds of VTE in patients on prophylactic compared with therapeutic anticoagulation.

Results: The review comprised 24 studies and over 2,500 patients. The pooled proportion of VTE prevalence was 0.31 [95% confidence interval (CI) 0.24, 0.39; I^2 94%], of VTE utilizing systematic screening was 0.48 (95% CI 0.33, 0.63; I^2 91%), of deep venous thrombosis was 0.23 (95% CI 0.14, 0.32; I^2 96%), and of pulmonary embolism was 0.14 (95% CI 0.09, 0.20; I^2 90%). Exploratory analysis of few studies, utilizing systematic screening, VTE risk increased significantly with prophylactic, compared with therapeutic anticoagulation [odds ratio (OR) 5.45; 95% CI 1.90, 15.57; I^2 0%].

Discussion: Our review revealed a high prevalence of VTE in critically ill COVID-19 patients. Almost 50% of patients had VTE detected by systematic screening. Higher thromboprophylaxis dosages may reduce VTE burden in this patient's cohort compared with standard prophylactic anticoagulation; however, this is to be ascertained by ongoing randomized controlled trials.

Keywords: COVID-19, SARS-CoV-2, VTE, thrombosis, venous, ICU, DVT—deep vein thrombosis

INTRODUCTION

The pool of recent evidence suggests that coronavirus disease 2019 (COVID-19) is a thrombogenic condition. It leads to an increased incidence of both venous and arterial thromboembolic events (1). COVID-19 patients admitted to the intensive care units (ICU) seem to carry a higher risk (1). Venous thromboembolism (VTE) prevalence in the critically ill COVID-19 patients varied across individual studies. This is likely due to differences in screening methods (systematic vs. non-systematic screening), among other study-specific characteristics, leaving VTE's exact prevalence unknown. The prevalence of deep venous thrombosis (DVT) was considered low compared with pulmonary embolism (PE), which led researchers to consider microthrombosis as an additional mechanism of PE in COVID-19 patients (2).

VTE's heightened risk led to a wide chemoprophylaxis use for critically ill COVID-19 patients (3). Notwithstanding this, recent studies showed that even COVID-19 patients on chemoprophylaxis remain to carry a high risk of VTE compared with non-COVID-19 patients (4). As a result, guidance driven by expert opinions suggested utilizing higher doses of anticoagulation (1). However, this recommendation lacks robust, supporting systematic studies. Thus, we aimed to systematically review the literature and explore the pooled prevalence of VTE, PE, and DVT in critically ill COVID-19 patients. Additionally, we aimed to evaluate the yield of systematic VTE screening and its effect on the prevalence. Moreover, if data allow, we aimed to examine the odds of VTE in patients on prophylactic compared with therapeutic anticoagulation.

This review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (5). It is pre-registered at the International Prospective Register of Systematic Reviews (PROSPERO) (registration number: CRD42020185916).

ELIGIBILITY CRITERIA

We limited our review to observational studies (cohort, cross-sectional, retrospective, or case series), estimating the proportion of VTE events in critically ill COVID-19 adult (>18 years) patients (admitted to the ICU). To facilitate a timely review, we limited our inclusion to articles written in the English language only. We excluded studies where the proportion of VTE could not be ascertained or if the population of interest is not ICU patients.

INFORMATION SOURCES AND LITERATURE SEARCH

For a timely review, we performed the search in PubMed, MEDLINE, and EMBASE. We used free text, emtree, and MeSH terms in our search. There were no language or date limitations implied in the search. The last date of the formal search was the 10th of July 2020; however, we performed a scoping search till the 25th of July 2020. Example of a utilized search strategy was [("venous thromboembolism" OR "deep vein thrombosis" OR "lung embolism" OR "vein thrombosis"/exp/mj) AND [embase]/lim] AND [("covid 19" OR (coronavirus AND disease AND 2019) OR (sars AND cov AND 2) OR "covid 19"/exp/mj) AND [embase]/lim]. We also performed relevant citations and reference searches.

SCREENING AND DATA EXTRACTION

Two reviewers (MM and SM) conducted the screening in two stages. The first stage was screening the retrieved articles' titles and abstracts independently. Secondly, the articles' full text was retrieved and assessed for inclusion. When disagreement occurred, a third reviewer (LA) settled the disagreement guided by the protocol. We used pre-made excel sheets to collect relevant articles data. This included the last author name, publication date, study country, sample size, events number (DVT, PE, and VTE), baseline characteristics (median age, gender frequency, average BMI, and other comorbidities), intubation frequency, thromboprophylaxis frequency, and follow-up duration.

STUDY QUALITY AND RISK OF BIAS ASSESSMENT

We used a validated tool for assessing the risk of bias of prevalence studies. The tool was devised by Hoy et al. and is composed of 10 items summarizing four domains (6). We additionally generated funnel plots to examine the risk of publication bias in our review.

DATA ANALYSIS

A scoping review revealed heterogeneity of the method of VTE screening, reporting, and detection. Additionally, there were varying follow-ups given the nature of ICU admitted patients. Hence, neither the true incidence (different follow-up times and some patients may already have the event of interest before the study) nor the true prevalence (varying follow-up times and absence of unifying screening for all individuals at risk) could be accurately pooled. We instead decided *a priori* to pool a proportion of VTE with a 95% confidence interval (CI). This proportion represents the number of patients with the event of interest divided by the study population at risk during the study regardless of their follow-up duration. We felt that this would be a proxy or an estimate of the prevalence. We used the validated method of double arcsine transformation to stabilize the variance and confine the CI between 0 and 1 (7). We generated forest plots to display the results of the analysis. We used the Cochrane Q test and I^2 to examine heterogeneity. I^2 >60% indicates significant heterogeneity. Regardless of the heterogeneity, we would use the random-effects model (REM) in our analysis. We used MetaXl software for statistical analysis (version 5.3©, EpiGear International Pty Ltd., ABN 51 134 897 411, Sunrise Beach, Queensland, Australia, 2011–2016).

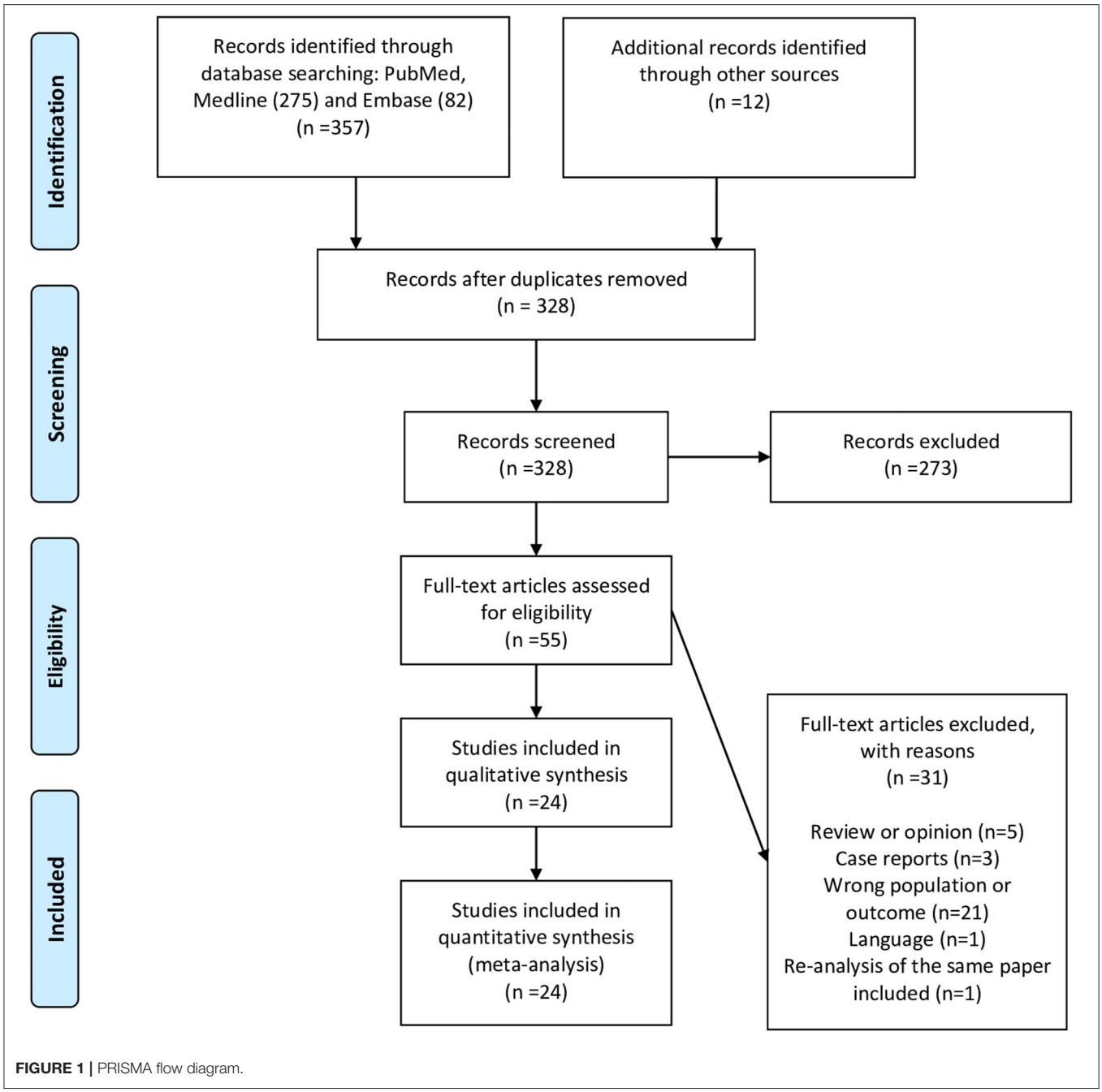

FIGURE 1 | PRISMA flow diagram.

SUBGROUP AND SENSITIVITY ANALYSES

We *a priori* decided to examine the proportion of DVT and PE. Additionally, we looked at the proportion of VTE in various populations (systematic screening vs. non-systematic screening, therapeutic vs. prophylactic anticoagulant dose). Moreover, we performed a sensitivity analysis to reflect the relative constituent studies' impact on the consistency of the pooled proportion of the primary endpoint.

RESULTS

Included Studies and Baseline Characteristics

Twenty-four studies describing a total of 2,570 patients were included in our final analysis (**Figure 1** shows the flow diagram) (4, 8–29). The studies were heterogeneous in terms of VTE events identification and screening (**Table 1**). In 10 studies, the screening for VTE was systematically done using lower and upper limb ultrasound (US) (systematic screening was only for

TABLE 1 | Summary of included studies.

Study (location)	Study design	Study duration in days	Total number	Age, mean, or median (males percentage %)	Intubated %	D-dimers (mean or median)	Pharmacologic prophylaxis %	Screening method	VTE proportion % (numbers)	Mortality %
Al-Samkari et al. (United States) (8)	Retrospective analysis	36 days (March–April 2020)	–	65 (males 64.7%)	–	–	98.6% (12.5% intermediate or full anticoagulation)	Clinical suspicion	10.4% (15/144)	18.8% (27/144)
Beun et al. (Netherlands) (19)	Retrospective analysis	24 days (March–April 2020)	75	–	–	–		Clinical suspicion	30.6% (23/75)	–
Bilaloglu et al. (United States)(23)	Retrospective analysis	48 days (March–April 2020)	829	–	–	–	Most patients (percentage not specified)	Clinical suspicion	13.6% (113/829)	54.4% (451/829)
Criel et al. (Belgium) (24)	Retrospective analysis	24 days (April 2020)	30	64.5 (males 67%)	70%	1,400 ng/ml	100% (intermediate prophylactic dose)	Systematic screening (Doppler US of upper and lower limbs)	13.3% (4/30)	13.3% (4/30)
Cui et al. (China) (25)	Retrospective analysis	53 days (Jan–March 2020)	81	59.9 (males 46%)	–	5,200 ng/ml	0%	Systematic screening (lower limb Doppler US)	24.6% (20/81)	10% (8/81)
Desborough et al. (United Kingdom) (26)	Retrospective analysis	31 days (March 2020)	66	59 (males 73%)	79%	1,200 ng/ml	100% (83% prophylactic, 17% therapeutic)	Clinical suspicion	16.6% (11/66)	30.3% (20/66)
Fraissé et al. (France) (27)	Retrospective analysis	–	92	61 (males 79%)	89%	2,400 ng/ml	100% (47% prophylactic, 53% therapeutic)	Clinical suspicion	33.6% (31/92)	–
Grandmaison et al. (Switzerland) (28)	Retrospective analysis	–	29	66 (males 64.7%)	–	8,760 ng/ml	93% (96% prophylactic, 4% therapeutic)	Systematic screening (Doppler US of upper and lower limbs)	58.6% (17/29)	–
Helms et al. (France) (29)	Retrospective analysis	29 days (March 2020)	150	63 (males 81%)	100%	2,270 ng/ml	100% (70% prophylactic, 30% therapeutic)	Clinical suspicion	18.6% (28/150)	8.70% (13/150)
Hippensteel et al. (United States) (9)	Retrospective analysis	28 days (March–April 2020)	91	55 (males 57%)	85%	1,071 ng/ml	54.3% therapeutic	Clinical suspicion	26.3% (24/91)	22% (22/91)
Klok et al. (Netherlands) (10)	Retrospective analysis	47 days (March–April 2020)	184	64 (males 76%)	–	–	100% (90.8% prophylactic, 9.2% therapeutic)	Clinical suspicion	36.9% (68/184)	22% (41/184)
Llitjos et al. (France) (4)	Retrospective analysis	24 days (March–April 2020)	26	68 (males 77%)	100%	1,750 ng/ml	100% (prophylactic 31%, therapeutic 69%)	Systematic screening (compression and Doppler US)	69.2% (18/26)	12% (3/26)
Lodigiani et al. (Italy) (11)	Retrospective analysis	58 days (February–April 2020)	48	61 (males 80.3%)	–	615 ng/ml	100% (40% weight adjusted or therapeutic)	Clinical suspicion	8.3% (4/48)	–

(Continued)

TABLE 1 | Continued

Study (location)	Study design	Study duration in days	Total number	Age, mean, or median (males percentage %)	Intubated %	D-dimers (mean or median)	Pharmacologic prophylaxis %	Screening method	VTE proportion % (numbers)	Mortality %
longchamp et al. (Switzerland) (12)	Retrospective analysis	26 days (March–April 2020)	25	68 (males 64%)	92%	2,071 ng/ml (953–3,606)	100% (prophylactic 23/25, therapeutic 2/25)	Systematic screening (proximal lower extremity DVT)	32% (8/25)	20% (5/25)
Maatman et al. (United States) (13)	Retrospective analysis	20 days (March 2020)	109	61 (males 57%)	94%	84,506 ng/ml	100% (prophylactic 102/109, therapeutic 7/109)	Clinical suspicion	28.4% (31/109)	25% (27/109)
Middeldorp et al. (Netherlands) (14)	Retrospective analysis	42 days (March–April 2020)	75	62 (males 58%)	100%	2,000 ng/ml	100%	Systematic screening (lower limb Doppler every 5 days)	46.6% (35/75)	
Moll et al. (United States) (15)	Retrospective analysis	38 days (March–April 2020)	102	64.61 (males 57.8%)	86.3%	3,964 ng/ml	97.1% (89.8% prophylactic, 10.1% therapeutic)	Clinical suspicion	8.8% (9/102)	27.5% (28/102)
Nahum et al. (France) (16)	Case series	Mid-March–April 2020	34	62.2 (males 78%)	100%	27,927 ng/ml	100% prophylactic anticoagulation	Systematic screening (lower limbs US for all patients)	79.4% (27/34)	Not mentioned
Pineton De Chambrun et al. (France) (17)	Retrospective analysis	26 days (March–April 2020)	25	47.7 (males 68%)	–	Highly elevated (NS)	100% therapeutic	Clinical suspicion	24% (6/25)	–
Poissy et al. (France) (18)	Retrospective analysis	34 days (February–March 2020)	107	57 (males 59%)	62.6%	–	100%	Clinical suspicion	22.4% (24/107)	14% (15/107)
Ren et al. (China) (22)	Cross-sectional	3 days (Feb–March)	48	70 (males 54.2%)	37.5%	3,480 ng/ml	97.9% prophylactic	Systematic screening (proximal and distal lower limbs compression US)	85.4% (41/48)	31.3% (15/48)
Stessel et al. (Belgium) (20)	Quasi-experimental	18 days (March 2020)	46	69.5 (males 73.9%)	–	970 ng/ml	100% Prophylactic standard dose	Systematic screening	41.3% (19/46)	39.13% (18/46)
Stessel et al. (Belgium) (20)	Quasi-experimental	21 days (March–April 2020)	26	62 (males 57.3%)	–	2,180 ng/ml	100% Intensive prophylactic dose	Systematic screening (Doppler US and compression US of the great veins in upper and lower limbs)	15.3% (4/26)	3.85% (1/26)
Thomas et al. (United Kingdom) (21)	Retrospective analysis	33 days (March–April 2020)	63	59 (males 69%)	83%	394 ng/ml	100% (prophylactic dose)	Clinical suspicion	9.5% (6/63)	8% (5/63)
Zhang et al. (China) (22)	Retrospective analysis	32 days (January February 2020)	65	–	–	–	–	Systematic screening (lower limbs US Doppler for DVT at proximal and distal levels)	66.1% (43/65)	–

(–) Refers to data unavailable for the ICU cohort.

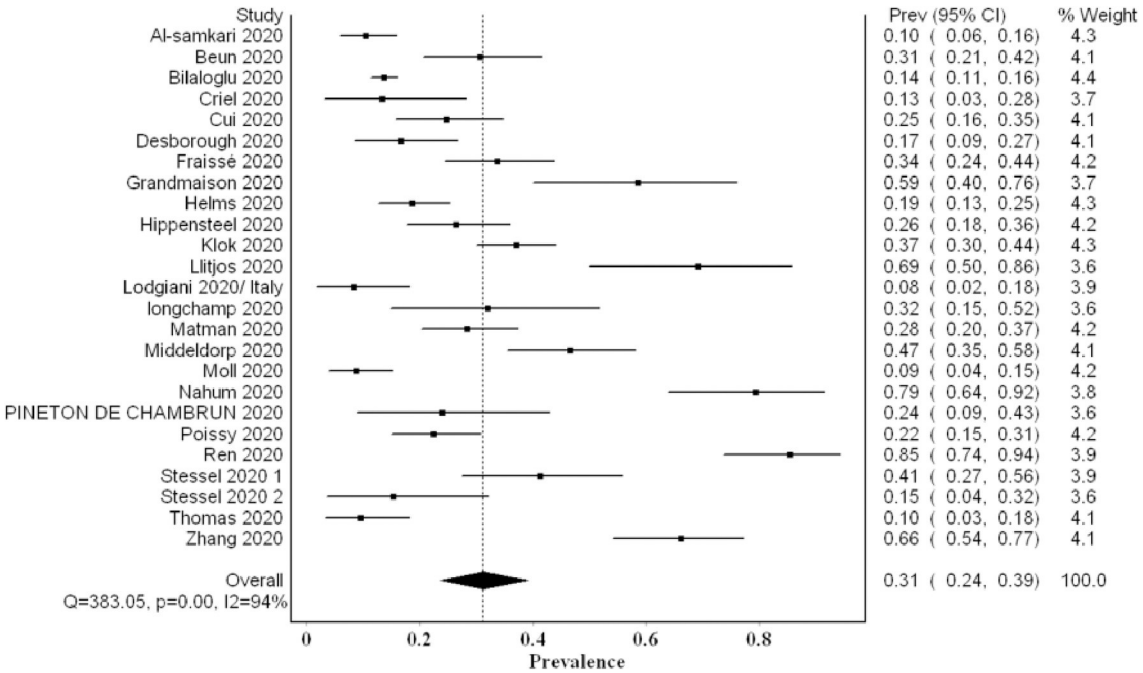

FIGURE 2 | Forest plot showing the overall pooled proportion of VTE events.

DVT and not PE). Fourteen studies evaluated for the presence of VTE based on clinical suspicion and further confirmation by imaging (non-systematic). Twenty-two studies reported the proportion of DVTs, and 17 studies reported the proportion of PE events. Out of the 10 studies where systematic screening was adopted, the screening was incomplete in one. In all studies but one (25), most patients were on thromboprophylaxis with varying doses.

THE PROPORTION OF VTE EVENTS

The overall pooled proportion of VTE from 24 studies examining a total of 2,570 was 0.31 (95% CI 0.24, 0.39; I^2 94%; Q 383) with significant heterogeneity (**Figure 2**). The funnel plot showed significant asymmetry suggestive of possible publication bias (**Supplementary 1**). The sensitivity analysis did not affect the final point estimate significantly (**Supplementary 2**).

THE PROPORTION OF VTE UTILIZING SYSTEMATIC SCREENING

Ten studies examining 478 patients using systematic screening revealed a higher VTE proportion of 0.48 (95% CI 0.33, 0.63; I^2 91%; Q 109) with significant heterogeneity (**Figure 3**). The funnel plot suggested a publication bias (**Supplementary 3**). The exclusion of Cui et al.'s study that did not utilize thromboprophylaxis resulted in a higher proportion of VTE events of 0.51. Additional sensitivity analyses revealed a lower VTE proportion with the exclusion of Ren et al.'s data (0.43); this proportion increased with the exclusion of Criel et al.'s study (0.52) (**Supplementary 4**). All the studies evaluated systematically for the presence of DVT events only (PE was not a primary aim). Hence, this pooled proportion represents the proportion of DVT events and may underestimate the overall VTE proportion.

THE PROPORTION OF VTE UTILIZING NON-SYSTEMATIC SCREENING

In most studies utilizing non-systematic screening, the authors addressed the high threshold for screening and imaging due to infection control implications. They stated that this might have underestimated the true prevalence. The analysis of 14 studies examining 2,085 patients revealed a pooled proportion of VTE of 0.20 (95% CI 0.15, 0.26; I^2 87%; Q 98.4) (**Figure 4**). The funnel plot suggested a publication bias (**Supplementary 5**). On sensitivity analysis, the final point estimate did not significantly change with the ordered exclusion of the constituent studies (**Supplementary 6**).

THE PROPORTION OF DVT EVENTS

The overall pooled proportion of DVT from 22 studies examining a total of 2,401 was 0.23 (95% CI 0.14, 0.32; I^2 96%; Q 531) with significant heterogeneity (**Figure 5**). The funnel plot suggested a publication bias (**Supplementary 7**), whereas the sensitivity analysis suggested a consistency of the final point estimate with ordered-single-study exclusion (**Supplementary 8**). The pooled proportion of DVT from studies utilizing non-systematic screening was 0.08 (95% CI 0.04, 0.12; I^2 87%; Q 85) (**Supplementary 9**).

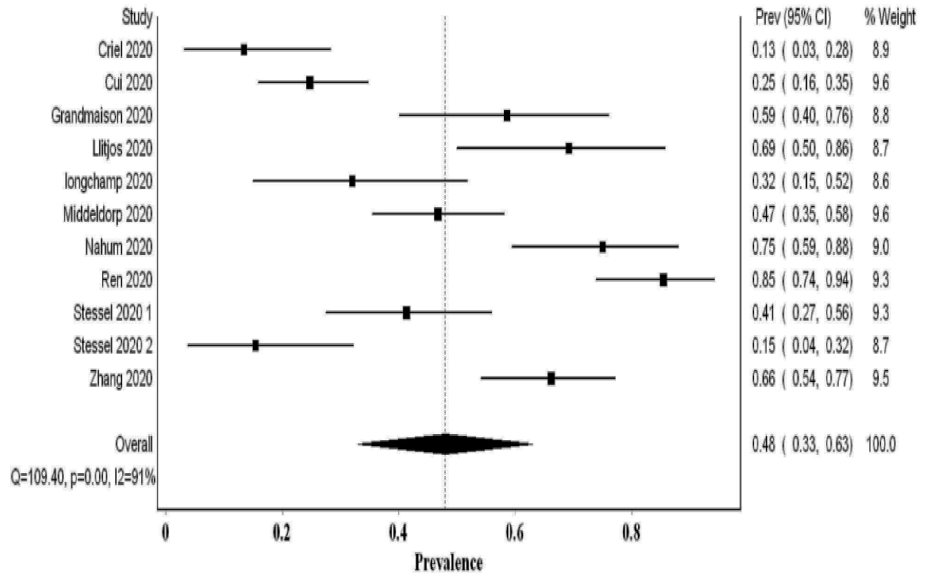

FIGURE 3 | Forest plot showing the pooled proportion of VTE events utilizing systematic screening methods.

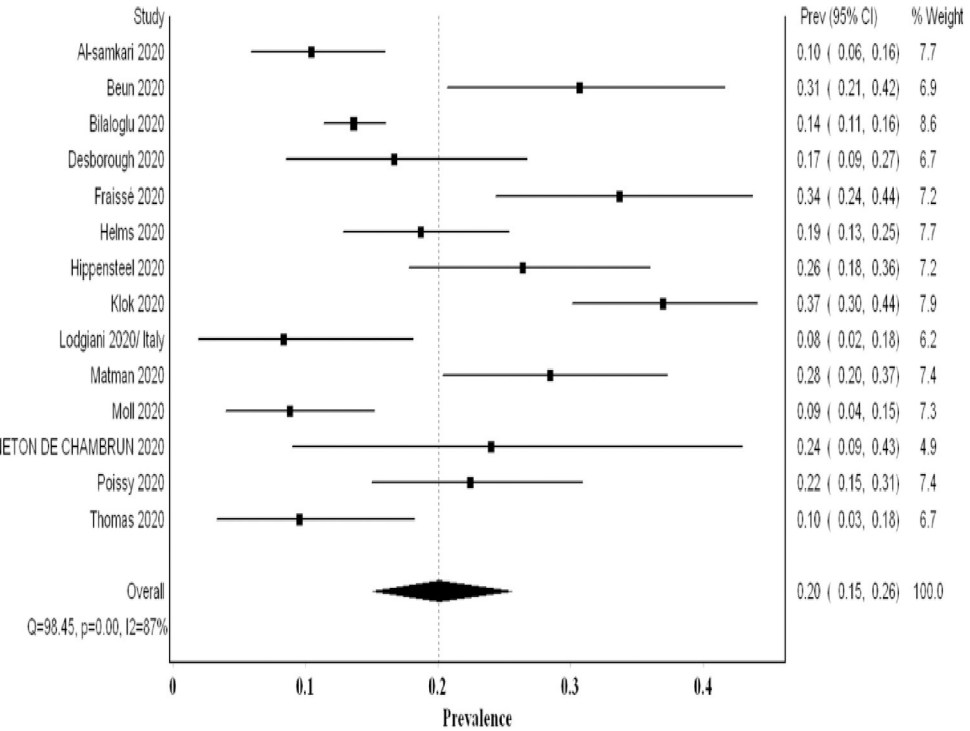

FIGURE 4 | Forest plot showing the pooled proportion of VTE events utilizing non-systematic screening methods.

THE PROPORTION OF PE EVENTS

PE was not screened systematically. The analysis of 2,096 patients (17 studies) revealed a pooled proportion of 0.14 (95% CI 0.09, 0.20; I^2 90%; Q 159) (**Figure 6**). The funnel plot revealed a major asymmetry suggestive of publication bias (**Supplementary 10**). Sensitivity analysis showed consistency of the results upon single-study-ordered exclusion.

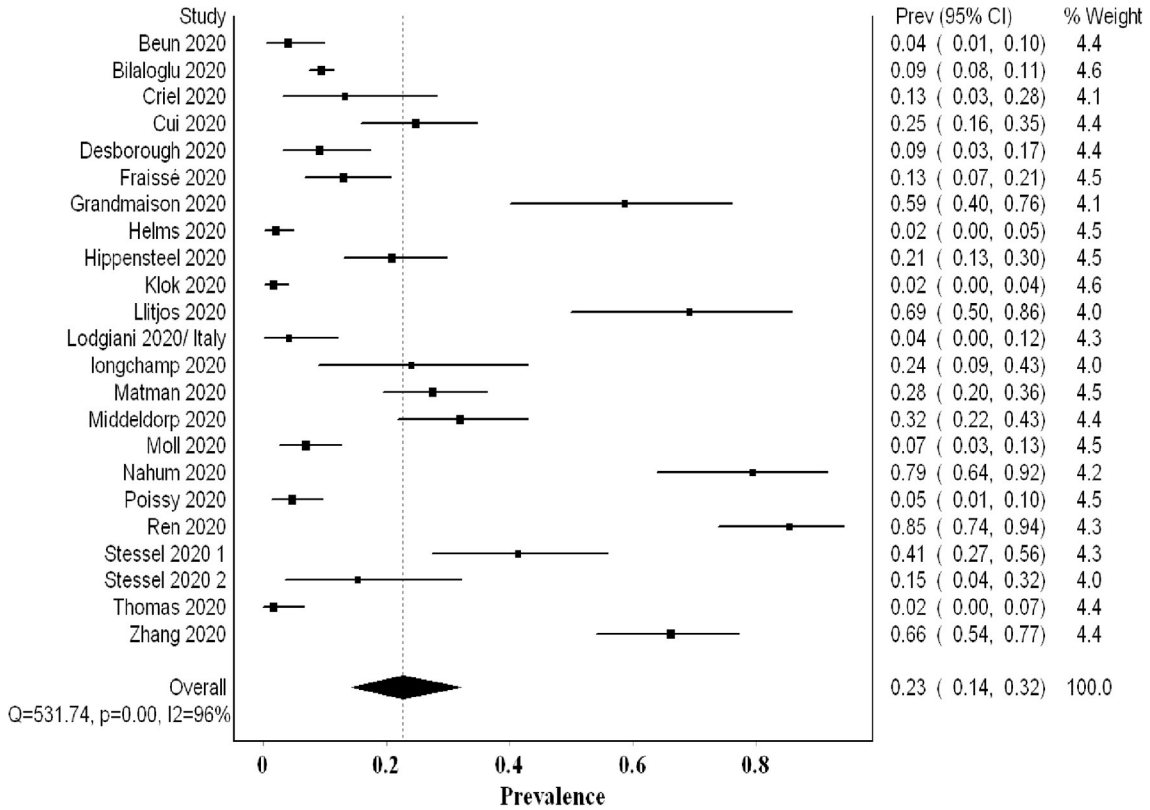

FIGURE 5 | Forest plot showing the overall pooled proportion of DVT.

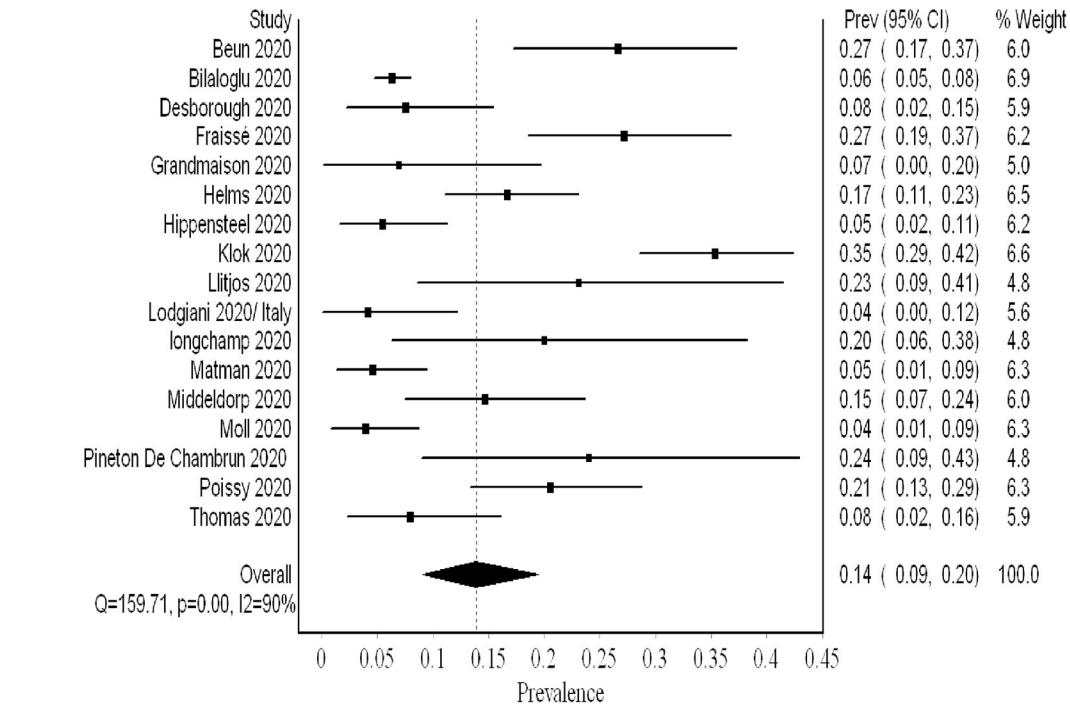

FIGURE 6 | Forest plot showing the overall pooled proportion of PE.

THROMBOPROPHYLAXIS STRATEGY

Six studies reported the number of VTE events in patients receiving prophylactic anticoagulation (479 patients) compared with therapeutic dosages (83 patients). The dosages and definitions varied across these studies. In one study (pre- and post-intervention), a higher prophylactic dosage of nadroparin with adjustment guided by factor X-a activity (labeled as semi-therapeutic) was compared with standard prophylactic dose (4, 14, 20). For synthesis, we considered this adjusted dosage therapeutic and analyzed it in the corresponding arm (due to the paucity of studies). The VTE odds ratio (OR) was increased in the prophylactic anticoagulation group with uncertainty in the final point estimate OR 2.34 (95% CI 0.77, 7.14; I^2 53%; Q 10). Three studies utilized systematic screening; hence, they provided a better estimate of the true VTE prevalence (20). In an exploratory analysis, we analyzed these studies separately, and the results showed significantly increased odds of VTE events with prophylactic dosing OR 5.45 (95% CI 1.90, 15.57; I^2 0%; Q 1.2), and there was no evidence of heterogeneity (**Figure 7**).

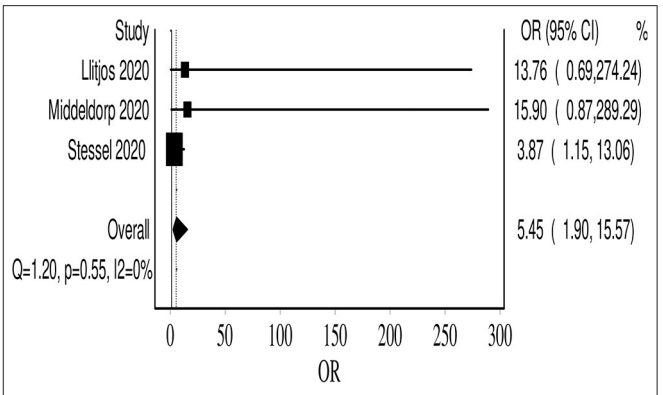

FIGURE 7 | Forest plot showing the VTE event odds in the prophylactic anticoagulation group, compared with therapeutic dosing.

QUALITY ASSESSMENT AND RISK OF BIAS ASSESSMENT

Most of the constituent studies had a moderate or unclear risk of bias (**Table 2**). Although the number of included studies is adequate, the funnel plot suggested publication bias (its value is limited in assessing prevalence studies publication bias). There was also reporting bias, as the reporting of distal DVT, PE, and VTE, method of diagnosis, and dosing of chemoprophylaxis varied across studies.

DISCUSSION

Our meta-analysis comprised over 2,500 patients and revealed a high VTE prevalence of 0.31 (95% CI 0.24, 0.39) in critically ill COVID-19 patients. This prevalence increased to 0.48 (95% CI 0.33, 0.63) when systematic screening was utilized, meaning that almost one in two critical COVID-19 patients suffers from VTE. Furthermore, this heightened prevalence of VTE when systematic screening was used did not include PE since it was not part of systematic screening. Hence, screening for PE systematically could have possibly further increased VTE prevalence. Even when non-systematic screening was utilized, VTE prevalence remained high at 0.20 (95% CI 0.15, 0.26). Regarding PE and DVT prevalence, the overall prevalence of DVT (0.23) was higher than that of PE (0.14). This concurs with finding a high prevalence of undiagnosed DVT in an autopsy evaluation of COVID-19 patients (31). Additionally, it may argue against the earlier literature suggesting that PE prevalence was much higher than DVT, proposing that PE events can originate in the lung's vasculature in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (32).

Our analysis revealed that approximately 40/100 additional DVTs are detected by systematic screening (0.48) compared with non-systematic screening (0.08). This is likely due to the fact that asymptomatic DVT can be overlooked in non-systematic screening. On the opposite side, PE is more likely to be associated with easily detected signs (sudden deterioration, unexplained tachycardia or sudden changes in the ventilator settings) especially in the context of the ICU.

A recent study by Zhang et al. evaluated the utility of bedside ultrasonography in the diagnosis of DVT. It revealed a significantly higher DVT prevalence in deceased patients than in surviving COVID-19 critically ill patients [94% (33/35) vs. 47% (22/46), $P < 0.001$] (30). Moreover, Wichmann et al. analyzed autopsies of 12 COVID-19 patients. They found that 7 (58%) had undiagnosed VTE, whereas in 4 (33.3%), massive PE was the direct cause of death (31). Based on these data, we understand that the high mortality reported by many studies may actually be attributed to undiagnosed fatal VTE events. Consequently, studies with high mortality will likely underestimate the true VTE prevalence when deceased patients are excluded from screening. We additionally understand the impact of prevention and early identification on patient's morbidity and mortality.

Tang et al. showed that prophylactic dosing of heparin in high-risk COVID-19 patients is associated with significantly lower mortality (33). This led the International Society on Thrombosis and Hemostasis (ISTH) among other societies to recommend a prophylactic dosage of pharmacological anticoagulants (LMWH or fondaparinux) for all hospitalized COVID-19 patients (3, 34). However, it seemed that prophylactic anticoagulation is not sufficient for severe COVID-19 patients. This was concluded in a study by Llitjos et al. where they found a higher prevalence of VTE in patients on a prophylactic dose of anticoagulation (100%) compared with therapeutic anticoagulation (56%) (4). More recently, Stessel et al. attempted the first quasi-experimental trial (pre- and post-intervention) comparing the mortality and incidence of VTE between conventional prophylaxis (once-daily nadroparin calcium 2,850 IU) compared with an individualized semi-therapeutic, prophylactic dosage guided by factor Xa activity (semi-therapeutic dosing). Both mortality (3.8 vs. 39.1%, $P < 0.001$) and VTE (15.3 vs. 41.3%, $P = 0.03$) were significantly lower in the aggressive thromboprophylaxis group (20). Emerging evidence showed that even in COVID-19 patients receiving therapeutic anticoagulation, there is a high incidence

TABLE 2 | Table summarizing the risk of bias assessment.

Study	1	2	3	4	5	6	7	8	9
Al-Samkari et al. (8)	?	+	+	−	−	−	−	−	−
Beun et al. (19)	+	?	+	−	−	+	−	−	−
Bilaloglu et al. (23)	+	+	+	?	−	+	−	−	−
Criel et al. (24)	?	?	−	+	+	?	−	−	−
Cui et al. (25)	?	?	−	?	+	?	+	+	−
Desborough et al. (26)	+	+	+	+	−	+	−	−	−
Fraissé et al. (27)	+	?	−	+	−	+	−	−	−
Grandmaison et al. (28)	+	+	−	+	+	+	+	+	−
Helms et al. (29)	+	+	−	+	−	+	−	−	−
Hippensteel et al. (9)	+	+	−	+	−	+	−	−	−
Klok et al. (10)	+	+	+	+	−	−	−	−	−
Llitjos et al. (4)	+	+	−	+	+	+	+	+	−
Lodigiani et al. (11)	+	+	+	+	−	+	−	−	−
longchamp et al. (12)	?	−	−	−	−	?	+	−	−
Maatman et al. (13)	+	+	−	+	−	+	−	−	−
Middeldorp et al. (14)	+	+	+	+	−	+	−	−	−
Moll et al. (15)	+	+	−	+	+	+	+	+	−
Nahum et al. (16)	+	+	+	+	−	−	−	−	−
Pineton de Chambrun et al. (17)	+	+	+	+	−	−	−	−	−
Poissy et al. (18)	+	+	+	+	−	−	−	−	−
Ren et al. (22)	+	+	+	+	+	+	+	+	−
Stessel et al. (20)	+	+	+	+	?	+	+	+	−
Stessel et al. (20)	+	+	+	+	+	+	+	+	−
Thomas et al. (21)	+	+	−	−	−	+	−	−	−
Zhang et al. (30)	?	+	+	−	?	+	?	+	−

+, low risk; −, high risk; ?, unclear risk assessment.

(1) Was the study's target population a close representation of the national population in relation to relevant variables, e.g., age, sex, occupation?; (2) was the sampling frame a true or close representation of the target population?; (3) was some form of random selection used to select the sample, OR, was a census undertaken?; (4) was the likelihood of non-response bias minimal?; (5) were data collected directly from the subjects (as opposed to a proxy)?; (6) was an acceptable case definition used in the study?; (7) was the study instrument that measured the parameter of interest (e.g., prevalence of low back pain) shown to have reliability and validity (if necessary)?; (8) was the same mode of data collection used for all subjects?; (9) were the numerator(s) and denominator r(s) for the parameter of interest appropriate?

of heparin resistance and sub-optimal peak in anti-Xa levels (19, 35). This may explain, in part, the high rate of VTE in patients on usual prophylactic doses and even in patients on therapeutic dosing (although relatively at a lower rate).

Our review also aimed to address the uncertainty of using higher vs. standard prophylactic doses. In an exploratory manner, we limited our analysis to studies that only used systematic screening and thus reduce the chances of missing fatal VTE events; we found that prophylactic dosing was associated with increased odds of VTE compared with therapeutic dosing (one study was counted in the therapeutic side although it used subtherapeutic dosing, due to limited studies) (20). The results

were homogenous. The reader should consider that the odds of VTE in the therapeutic arm were lower even in the likely event that those patients may have had VTE predisposing conditions, for which they were initiated on this therapeutic dosing (except Stessel et al.'s study, which was protocolized). This small exploratory unadjusted comparison suggests a value for a higher dosing or therapeutic chemoprophylaxis. Nonetheless, this will be ascertained by a number of ongoing trials aiming to address the efficacy and safety of various chemoprophylactic dosages (prophylactic, intermediates, weight-adjusted, or therapeutic); examples of such trials are IMPROVE (http://www.clinicaltrials.gov, NCT04367831), COVI-DOSE (http://www.clinicaltrials.gov, NCT04373707), and Hep-COVID (https:www.clinicaltrials.gov, NCT04401293). The safety of intensive thromboprophylaxis was not addressed in our review due to data paucity. Nonetheless, two recent observational studies suggested that this intensive thromboprophylaxis is safe in terms of inducing major bleeding events (36, 37). Thus, we believe that the intensive thromboprophylaxis protocol suggested by Stessel et al. seems promising as a chemoprophylaxis regimen until further data from ongoing randomized clinical trials (RCTs) become available (20).

Limitations of our review are the heterogeneity in the pooled prevalence in the constituent studies. This is likely due to varying detection methods (systematic vs. non-systematic, imaging modalities used, timing, etc.), screening threshold (many studies reported that the threshold was high due to infection control concerns), varying severity of illness, prophylaxis strategies, and dosage, missing VTE in deceased patients of fatal VTE events, and varying and insufficient follow-ups. Additionally, the inability to provide a mortality comparison between the VTE group and the non-VTE group due to data paucity (we contacted the primary authors; however, we could not get the data necessary for its computation) and limited conclusion provided by the comparison of VTE in the therapeutic vs. prophylactic anticoagulation groups (small number of studies, absence of adjustment, and varying doses between studies). Moreover, the retrospective nature of the included studies, inability to accurately compute the prevalence of PE (absence of systematic PE screening), and absence of autopsies to ascertain causes of death add to the limitations of our review.

Notwithstanding this, there are many strengths to our review that are worthy of mention. This is the most extensive review examining the prevalence of VTE exclusively in critically ill patients. Additionally, the review examines VTE prevalence based on the utilized screening method providing the readers with a better estimate of VTE prevalence. We also pooled a proportion that reflects the prevalence; nonetheless, we acknowledged its limited accuracy. Finally, the results of the limited comparison between lower and higher dosing of chemoprophylaxis may help inform therapeutic decisions until further data from RCTs become available.

Future research direction should evaluate the utility of systematic screening and early therapeutic anticoagulation dosage on outcomes (VTE progression, ICU stay, and mortality). The utility of systematic screening with US at regular intervals to ascertain the exact prevalence of VTE is needed. In these studies, patients with distal DVT should be temporally followed up and compared with a non-DVT cohort to determine the incidence of proximal DVT, PE, and mortality events. This will ascertain the exact need for therapy in these patients.

In conclusion, our review of critically ill COVID-19 patients revealed a high prevalence of VTE events. This prevalence is higher when systematic screening is utilized. Our review suggested a potential for higher prophylactic or therapeutic dosages in reducing VTE burden. Data from ongoing RCTs are awaited to further confirm the findings of our review.

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.

AUTHOR CONTRIBUTIONS

MM conceived the idea of the review and formed the team, performed the analysis, constructed the tables and figures, and wrote the initial draft. MM conducted the initial search and with SM conducted the screening. MM, KS, SA-S, SM, SI, MN, and LA extracted the data. The manuscript was then critically reviewed and revised by all the study authors. The final version was approved by all authors for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2020.598846/full#supplementary-material

REFERENCES

1. 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
2. McFadyen JD, Stevens H, Peter K. The emerging threat of (micro)thrombosis in COVID-19 and its therapeutic implications. *Circ Res.* (2020) 127:571–87. doi: 10.1161/circresaha.120.317447
3. 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
4. Llitjos JF, Chochois C, Monsallier JM, Ramakers M, Auvray M, Merouani K. High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. *J Thromb Haemost.* (2020) 18:1743–6. doi: 10.1111/jth.14869
5. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the

PRISMA statement. *BMJ*. (2009) 339:332–6. doi: 10.1136/bmj.b2535
6. Hoy D, Brooks P, Woolf A, Blyth F, March L, Bain C, et al. Assessing risk of bias in prevalence studies: Modification of an existing tool and evidence of interrater agreement. *J Clin Epidemiol.* (2012) 65:934–9. doi: 10.1016/j.jclinepi.2011.11.014
7. Barendregt JJ, Doi SA, Lee YY, Norman RE, Vos T. Meta-analysis of prevalence. *J Epidemiol Community Health.* (2013) 67:974–8. doi: 10.1136/jech-2013-203104
8. Al-Samkari H, Dzik WH, Carlson JC, Fogerty AE, Waheed A, Goodarzi K, et al. COVID and coagulation: bleeding and thrombotic manifestations of SARS-CoV2 infection. *Blood.* (2020) 136:489–500. doi: 10.1182/blood.2020006520
9. Hippensteel JA, Burnham EL, Jolley SE. Prevalence of venous thromboembolism in critically ill patients with COVID-19. *Br J Haematol.* (2020) 190:e134–7. doi: 10.1111/bjh.16908
10. Kloka FA, Kruipb MJHA, van der Meercd NJM, Arbouse MS, Gommersf D, Kant KM, et al. Confirmation of the high cumulative incidence of thrombotic complications in critically ill ICU patients with COVID-19: An updated analysis. *Thromb Res.* (2020) 191:148–50. doi: 10.1016/j.thromres.2020.04.041
11. 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
12. Longchamp A, Longchamp J, Manzocchi-Besson S, Whiting L, Haller C, Jeanneret S, et al. Venous thromboembolism in critically ill patients with Covid-19: results of a screening study for deep vein thrombosis. *Res Pr Thromb Haemost.* (2020) 4:842–847. doi: 10.1002/rth2.12376
13. Maatman TK, Feizpour C, Douglas A II, McGuire SP, Kinnaman G, Hartwell JL, et al. Routine venous thromboembolism prophylaxis may be inadequate in the hypercoagulable state of severe coronavirus disease 2019. *Crit Care Med.* (2020) 48:e78–90. doi: 10.1097/CCM.0000000000004466
14. Middeldorp S, Coppens M, van Haaps TF, Foppen M, Vlaar AP, Müller 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. Moll M, Zon RL, Sylvester KW, Chen EC, Cheng V, Connell NT, et al. Venous thromboembolism in COVID-19 ICU Patients. *Chest.* (2020) 158:2130–5. doi: 10.1016/j.chest.2020.07.031
16. Nahum J, Morichau-Beauchant T, Daviaud F, Echegut P, Fichet J, Maillet JM, et al. Venous thrombosis among critically ill patients with coronavirus disease 2019 (COVID-19). *JAMA Netw Open.* (2020) 3:e2010478. doi: 10.1001/jamanetworkopen.2020.10478
17. Pineton de Chambrun M, Frere C, Miyara M, Amoura Z, Martin-Toutain I, Mathian A, et al. High frequency of antiphospholipid antibodies in critically-ill COVID-19 patients: a link with hypercoagulability? *J Intern Med.* (2020). doi: 10.1111/joim.13126. [Epub ahead of print].
18. Poissy J, Goutay J, Caplan M, Parmentier E, Duburcq T, Lassalle F, et al. Pulmonary embolism in COVID-19 patients: awareness of an increased prevalence. *Circulation.* (2020) 142:184–6. doi: 10.1161/CIRCULATIONAHA.120.047430
19. Beun R, Kusadasi N, Sikma M, Westerink J, Huisman A. Thromboembolic events and apparent heparin resistance in patients infected with SARS-CoV-2. *Int J Lab Hematol.* (2020) (42 Suppl. 1) (Suppl. 1):19–20. doi: 10.1111/ijlh.13230
20. Stessel B, Vanvuchelen C, Bruckers L, Geebelen L, Callebaut I, Vandenbrande J, et al. impact of implementation of an individualised thromboprophylaxis protocol in critically ill ICU patients with COVID-19: a longitudinal controlled before-after study. *Thromb Res.* (2020) 194:209–15. doi: 10.1016/j.thromres.2020.07.038
21. Thomas W, Varley J, Johnston A, Symington E, Robinson M, Sheares K, et al. Thrombotic complications of patients admitted to intensive care with COVID-19 at a teaching hospital in the United Kingdom. *Thromb Res.* (2020) 191:76–7. doi: 10.1016/j.thromres.2020.04.028
22. 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
23. Bilaloglu S, Aphinyanaphongs Y, Jones S, Iturrate E, Hochman J, Berger JS. Thrombosis in hospitalized patients with COVID-19 in a New York City Health System. *J Am Med Assoc.* (2020) 324:799–801. doi: 10.1001/jama.2020.13372
24. Criel M, Falter M, Jaeken J, Van Kerrebroeck M, Lefere I, Meylaerts L, et al. Venous thromboembolism in SARS-CoV-2 patients: only a problem in ventilated ICU patients, or is there more to it? *Eur Respir J.* (2020) 56. doi: 10.1183/13993003.01201-2020
25. 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
26. Desborough MJR, Doyle AJ, Griffiths A, Retter A, Breen KA, Hunt BJ. Image-proven thromboembolism in patients with severe COVID-19 in a tertiary critical care unit in the United Kingdom. *Thromb Res.* (2020) 193:1–4. doi: 10.1016/j.thromres.2020.05.049
27. Fraissé M, Logre E, Pajot O, Mentec H, Plantefève G, Contou D. Thrombotic and hemorrhagic events in critically ill COVID-19 patients: a French monocenter retrospective study. *Crit Care.* (2020) 24:275. doi: 10.1186/s13054-020-03025-y
28. Grandmaison G, Andrey A, Périard D, Engelberger RP, Carrel G, Doll S, et al. Systematic screening for venous thromboembolic events in COVID-19 pneumonia. *TH Open.* (2020) 4:e113–5. doi: 10.1055/s-0040-1713167
29. 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) 1089–98. doi: 10.1007/s00134-020-06062-x
30. Zhang P, Qu Y, Tu J, Cao W, Hai N, Li S, et al. Applicability of bedside ultrasonography for the diagnosis of deep venous thrombosis in patients with COVID-19 and treatment with low molecular weight heparin. *J Clin Ultrasound.* (2020) 48:522–6. doi: 10.1002/jcu.22898
31. 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) 173:268–77. doi: 10.7326/m20-2003
32. Spyropoulos AC, Weitz JI. Hospitalized COVID-19 patients and venous thromboembolism: a perfect storm. *Circulation.* (2020) 142:129–32. doi: 10.1161/CIRCULATIONAHA.120.048020
33. 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
34. 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
35. White D, MacDonald S, Bull T, Hayman M, de Monteverde-Robb R, Sapsford D, et al. Heparin resistance in COVID-19 patients in the intensive care unit. *J Thromb Thrombolysis.* (2020) 50:287–91. doi: 10.1007/s11239-020-02145-0
36. Mattioli M, Benfaremo D, Mancini M, Mucci L, Mainquà P, Polenta A, et al. Safety of intermediate dose of low molecular weight heparin in COVID-19 patients. *J Thromb Thrombolysis.* (2020). doi: 10.1007/s11239-020-02243-z. [Epub ahead of print].
37. Kessler C, Stricker H, Demundo D, Elzi L, Monotti R, Bianchi G, et al. Bleeding prevalence in COVID-19 patients receiving intensive antithrombotic prophylaxis. *J Thromb Thrombolysis.* (2020) 50:833–6. doi: 10.1007/s11239-020-02244-y

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Pre-existing Health Conditions and Epicardial Adipose Tissue Volume: Potential Risk Factors for Myocardial Injury in COVID-19 Patients

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Background: Myocardial injury is a life-threatening complication of coronavirus disease 2019 (COVID-19). Pre-existing health conditions and early morphological alterations may precipitate cardiac injury and dysfunction after contracting the virus. The current study aimed at assessing potential risk factors for COVID-19 cardiac complications in patients with pre-existing conditions and imaging predictors.

Methods and Results: The multi-center, retrospective cohort study consecutively enrolled 400 patients with lab-confirmed COVID-19 in six Chinese hospitals remote to the Wuhan epicenter. Patients were diagnosed with or without the complication of myocardial injury by history and cardiac biomarker Troponin I/T (TnI/T) elevation above the 99th percentile upper reference limit. The majority of COVID-19 patients with myocardial injury exhibited pre-existing health conditions, such as hypertension, diabetes, hypercholesterolemia, and coronary disease. They had increased levels of

the inflammatory cytokine interleukin-6 and more in-hospital adverse events (admission to an intensive care unit, invasive mechanical ventilation, or death). Chest CT scan on admission demonstrated that COVID-19 patients with myocardial injury had higher epicardial adipose tissue volume ([EATV] 139.1 (83.8–195.9) vs. 92.6 (76.2–134.4) cm^2; $P = 0.036$). The optimal EATV cut-off value (137.1 cm^2) served as a useful factor for assessing myocardial injury, which yielded sensitivity and specificity of 55.0% (95%CI, 32.0–76.2%) and 77.4% (95%CI, 71.6–82.3%) in adverse cardiac events, respectively. Multivariate logistic regression analysis showed that EATV over 137.1 cm^2 was a strong independent predictor for myocardial injury in patients with COVID-19 [OR 3.058, (95%CI, 1.032–9.063); $P = 0.044$].

Conclusions: Augmented EATV on admission chest CT scan, together with the pre-existing health conditions (hypertension, diabetes, and hyperlipidemia) and inflammatory cytokine production, is associated with increased myocardial injury and mortality in COVID-19 patients. Assessment of pre-existing conditions and chest CT scan EATV on admission may provide a threshold point potentially useful for predicting cardiovascular complications of COVID-19.

Keywords: COVID-19, SARS-CoV-2, pandemic (COVID-19), CT imaging findings, cardiac complication

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a highly contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since its first breakout in Wuhan, China, the COVID-19 pandemic has triggered a worldwide health crisis. According to WHO, globally, as of September 20, 2020, COVID-19 has caused nearly one million deaths (1). SARS-CoV-2 mainly attacks the respiratory system, clinically characterized by rapidly progressive pneumonia and acute respiratory distress syndrome (ARDS) (2). However, the virus may damage other tissues and organs directly or indirectly, in particular, the cardiovascular system. Indeed, individuals with pre-existing health conditions are highly vulnerable to the pathological insults from the viral infection (3, 4). COVID-19 patients display not only the manifestations of pulmonary injury but also multiple organ damage and dysfunction. The viral injury to various tissue or organs constitutes a complex clinical syndrome with a broad spectrum of pathophysiological characteristics, which contribute to the severity and mortality of COVID-19 (5–8).

Currently, COVID-19 patients with myocardial injury are diagnosed when the serum levels of troponin I/T (TnI/T) increase above the 99th percentile upper reference limit, after excluding TnI/T elevation and other evidence related to pre-existing obstructive coronary artery disease. Thus, the abnormal levels of myocardial biomarkers constitute the main criteria to identify COVID-19 patients with myocardial injury. However, TnI/T changes may occur in other pathological conditions, such as infection, hypoxia, and renal insufficiency, commonly observed during the development of COVID-19. Hence, assessment of myocardial injury should be performed using a comprehensive approach, including non-invasive imaging, electrocardiography, and laboratory examination for proper clinical judgment in patients with abnormal TnI/T levels. Regarding cardiac morphological examination or image analysis, echocardiography or cardiovascular magnetic resonance (CMR) is not routine examination for COVID-19 patients, and generates non-specific images that may be lagging in early detection of myocardial injury (9). Conversely, chest computed tomography (CT) is routinely performed in patients suspected for COVID-19, usually as soon as hospital admission, to evaluate the severity of pneumonia. Therefore, an early imaging indicator based on chest CT is valuable for timely assessment and diagnosis of myocardial injury morphologically. Epicardial adipose tissue volume (EATV) has been used to evaluate the adipose tissue between the epicardial surface and pericardium, and reportedly associated with heart inflammation (10). In this multi-center, retrospective study, we explored the pre-existing health conditions and chest CT EATV as potential risk factors for myocardial injury in COVID-19 patients.

METHODS

Study Design, Participants, and Data Recording

The current multi-centered, retrospective study of laboratory-confirmed COVID-19 patients was conducted in six independent hospitals, located in the Eastern, Southern, Northern, and Central regions of China. All the cases of COVID-19 were confirmed positively in SARS-CoV-2 detection of respiratory specimens by real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR), according to the guidelines of the World Health Organization and the National Health Commission of China (11, 12). A total of 549 consecutive patients with confirmed COVID-19 were admitted from January 3 to February 26, 2020.

Except for 43 patients who remain hospitalized and 106 patients with no record of TnI/T, all other 400 patients were enrolled in the final analysis.

The epidemiological, demographic, clinical, laboratory, imaging, treatment, and outcome data of enrolled patients were collected by experienced local clinicians, and entered into a computerized database and cross-checked. The time from the onset of symptoms to hospital admission was 5 (3–7) days. All the patients underwent at least one TnI/T test, 285/400 (96.3%) patients had TnI/T data available within the first 24 h of hospital admission, and 373/400 (93.3%) patients had more than one test result of TnI/T during hospitalization. Myocardial injury was diagnosed and confirmed according to the highest level of TnI/T during hospitalization.

Study Definitions

Myocardial injury was diagnosed when the highest level of Troponin I/T (TnI/T) was above the 99th percentile upper reference limit (reference range of each hospital is available at **Supplementary Table 1**), after excluding the possibility of acute coronary syndrome (13). Fever was defined as an axillary temperature of 37.3°C or higher. Hypertension was defined as systolic blood pressure over 140 mmHg or diastolic blood pressure over 90 mmHg. In-hospital adverse events included admission to an intensive care unit (ICU), the use of invasive mechanical ventilation, or death (14, 15). The injury was further confirmed by reviewing admission logs and histories from electronic medical care records.

Analysis of Epicardial Adipose Tissue Volume (EATV) by CT Scan

Chest CT scan was performed within the first 24 h of hospital admission in accordance with the guidance for COVID-19 from the Chinese National Health Commission (12). Chest CT images were collected, and measured using breath-hold electrocardiogram-gated CT scanners with 256 or 64 detector rows (uCT 760, uMI 780 scanners, United Imaging, Shanghai, China; Precision 32, CAMPO Imaging, Shenyang, China; NeuViz 64 In/En, Neusoft, Liaoning, China; SOMATOM Emotion 16, Siemens, Germany; SOMATOM definition AS, Siemens, Germany; Optima CT680, GE Healthcare, USA). The scan conditions were set as 120–140 kV, 300–320 mA, 512 × 512 matrix, and the field of view was 240 mm with a slice thickness of 1–3 mm. Images were reconstructed using a soft-tissue algorithm. EATV was calculated and established from mediastinal window images according to the standardized operation protocol by trained radiologists blinded to the study protocol. The baseline characteristics of patients with and without EATV were roughly the same (**Table 1**). Epicardial adipose tissue was identified on the CT scan as a hypodense rim surrounding the myocardium and limited to the pericardium. The visceral pericardium was traced manually from the aortic arch to the left ventricular apex, and all extra-pericardial tissue was excluded. The individual EATV measurement within the manually traced epicardium in each slice was detected by assigning a threshold CT value of −200 and −30 HU and then was automatically summed with the software of Siemens Syngo.via (Siemens, Germany) to determine the total EATV.

Statistical Analyses

Data were presented as mean ± standard deviation (*SD*) or median with quartiles for continuous variables and number (%) for categorical variables. Differences between patients with and without myocardial injury were assessed with the two-tail *t*-test or Wilcoxon rank-sum test for continuous variables and Chi-square or Fisher's exact test for categorical variables. The receiver operating characteristic (ROC) curve analysis was used to select a cut-off value for EATV, and sensitivity and specificity for predicting myocardial injury incidence were calculated. Multivariate logistic regression analysis was applied to control confounding factors that might be associated with EATV (age, weight, history of hyperlipidemia, and coronary heart disease) when identifying the predicting value of EATV for the incidence of myocardial injury. Multivariate logistic regression analysis was also applied to control baseline confounders (age, history of hypertension, diabetes, and coronary heart disease) when exploring the association of myocardial injury with severe COVID-19. The consistency of the results was confirmed in patients with EATV value in subgroup analysis. Tests were two-sided with significance set at $\alpha < 0.05$. SPSS for Windows (Version 22.0, IBM) and Graphpad Prism 8.0 software were used for statistical analysis.

RESULTS

Baseline Characteristics and Pre-existing Health Conditions

The current cohort study enrolled 549 patients consecutively who suffered from laboratory-confirmed COVID-19 and admitted to six hospitals outside of the Wuhan epicenter as of March 8, 2020. Among them, there were 43 patients remained hospitalized and 106 patients with no record of TnI/T and thereby excluded from the study. All other 400 patients were entering into the final analysis, and the enrolling process was shown in **Figure 1**. There were 46 hospitalized COVID-19 patients were diagnosed suffering from myocardial injury. COVID-19 patients with myocardial injury were slightly older than those without [52.5 (42.8–68.0) vs. 49.0 (36.0–60.0) years]. The incidence of myocardial injury was much higher in patients with pre-existing health conditions, such as hypertension [12/46 (26.1%) vs. 50/354 (14.1%); $P = 0.035$], hyperlipidemia [4/46 (8.7%) vs. 7/354 (2.0%); $P = 0.028$], and chronic kidney disease [3/46 (6.5%) vs. 2/354 (0.6%); $P = 0.012$] as compared with non-myocardial injury COVID-19 patients.

There were no differences in the percentage of patients having the signs and symptoms between the myocardial and non-myocardial injury groups, except for fatigue and dyspnea (**Table 2**). Although no significant difference in pulse was found on admission, the incidence of tachycardia during hospitalization was significantly increased in patients undergoing myocardial injury.

TABLE 1 | Baseline comparison between general population ($n = 400$) and patients with EATV value ($n = 272$).

	Patients with EATV value	Patients without EATV value	*P*-value[a]
Age (yrs)	48.7 ± 15.4	48.0 ± 16.6	0.666
Female	137/272 (50.4%)	54/128 (42.2%)	0.127
Hypertension	45/272 (16.5%)	18/128 (14.1%)	0.525
Diabetes	26/272 (9.6%)	11/128 (8.6%)	0.756
White blood cells, $\times 10^9$/L	5.0 (4.0–5.7)	5.0 (4.0–6.4)	0.553
Platelets, $\times 10^9$/L ($n = 388$)	176.0 ± 60.6	167.7 ± 56.0	0.209
Alanine aminotransferase, U/L ($n = 392$)	24.0 (15.0–38.0)	23.0 (15.0–41.0)	0.922
Creatinine, μmol/L ($n = 391$)	54.9 (46.0–67.1)	64.0 (55.0–79.0)	<0.001
C-reactive protein, mg/L ($n = 393$)	17.0 (4.1–69.0)	23.0 (5.7–49.5)	0.082

[a]*Significant difference ($p < 0.05$) was determined between patients with EATV value and patients without EATV value.*

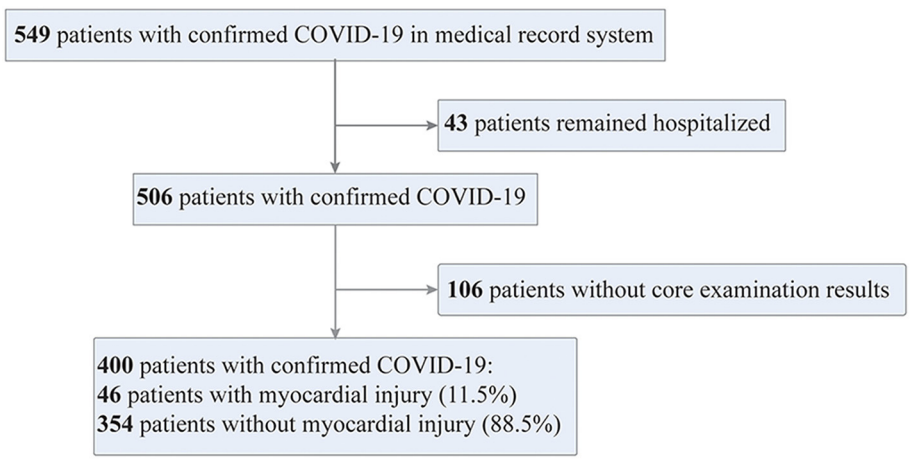

FIGURE 1 | Flowchart of patient recruitment. COVID-19 cases confirmed by RT-PCR assays were enrolled in this cohort study from six hospitals or regional medical centers remote to the Wuhan epicenter from January 3 to February 26, 2020.

Laboratory and Electrocardiographic Findings Showing Cardiac Dysfunction

COVID-19 patients with myocardial injury showed markedly increased levels of interleukin-6 [6.5 (5.2–17.9) vs. 2.3 (1.5–6.3) pg/mL; $P < 0.001$]. However, there was no significant difference on the levels of CRP, an acute phase protein known to arise during inflammation, between the cardiac injury and non-injury groups. We observed that patients with elevated TnI/T also had increased blood levels of other types of biomarkers for cardiac injury and dysfunction [e.g., lactate dehydrogenase, creatine kinase, and *N*-terminal pro-B-type natriuretic peptide (NT-proBNP)]. Compared with non-myocardial injury patients, the abnormality of lipid metabolites in peripheral blood occurred at a higher frequency in myocardial injury patients, with raising levels of total cholesterol (4.7 ± 1.1 vs. 4.0 ± 2.5 mmol/L; $P = 0.029$), low-density lipoprotein (2.8 ± 1.0 vs. 2.2 ± 0.7 mmol/L; $P = 0.001$), and triglycerides [2.7 (1.5–4.1) vs. 1.1 (0.9–1.9) mmol/L; $P < 0.001$] (**Table 3**).

Of 106 patients with the electrocardiogram records, 20 (18.9%) patients developed ST-T changes. However, the distribution was not significantly different between patients with and without elevated cTnI/T levels.

COVID-19 patients with myocardial injury showed no change in the pH values of arterial blood while having a higher prevalence of hypoxia (SaPO2 < 95%) than those without cardiac injury (**Table 3**), implying increased severity of COVID-19 injury toward the respiratory system in patients with myocardial injury.

Chest CT Scan Assessment of EATV Predicating Myocardial Injury

The chest CT scan performed on admission showed that EATV in patients with myocardial injury was significantly larger than the non- injury patients [139.1 (83.8–195.9) vs. 92.6 (76.2–134.4) cm^2; $P = 0.036$]. **Figure 2** illustrates chest CT images in COVID-19 cases with and without myocardial injury. Using the receiver operating characteristic (ROC) curve analysis, we found that a cut-off value of 137.1 cm^2 in EATV had predicted the occurrence of myocardial injury at 55% sensitivity, 77% specificity, and the area under the curve of 0.642. The positive likelihood ratio is 0.193, while the negative likelihood ratio is 0.046. Patients with EATV over 137.1 cm^2 on admission were more commonly diagnosed with myocardial injury than those not [11/68 (16.2%) vs. 9/204 (4.4%); $P = 0.001$]. In the univariable logistic analysis, odds of myocardial injury were greater in patients with EATV

TABLE 2 | Comparison in demographic and clinical characteristics of COVID-19 patients with and without myocardial injury.

	Total	Myocardial injury	Non-myocardial injury	*P*-value[a]
	(*n* = 400)	(*n* = 46)	(*n* = 354)	
Age (yrs)	49.0 (37.0–61.0)	52.5 (42.8–68.0)	49.0 (36.0–60.0)	0.046
Female	191 (47.8%)	20 (43.5%)	171 (48.3%)	0.538
Hypertension	62 (15.5%)	12 (26.1%)	51 (14.1%)	0.035
Diabetes	37 (9.3%)	8 (17.4%)	29 (8.2%)	0.056
Hyperlipidemia	11 (2.8%)	4 (8.7%)	7 (2.0%)	0.028
Liver Disease	7 (1.8%)	2 (4.3%)	5 (1.4%)	0.187
Kidney disease	5 (1.3%)	3 (6.5%)	2 (0.6%)	0.012
Signs and symptoms				
Fever	334 (83.5%)	40 (87.0%)	294 (83.1%)	0.502
Cough	295 (73.8%)	33 (71.7%)	262 (74.0%)	0.724
Fatigue	91 (22.8%)	16 (34.8%)	75 (21.2%)	0.039
Abdominal discomfort/ diarrhea/vomiting	43 (10.8%)	6 (13.0%)	37 (10.5%)	0.612
Sore throat	35 (8.8%)	7 (15.2%)	28 (7.9%)	0.102
Weight (Kg)	65.0 (57.0–72.0)	65.0 (57.0–75.0)	65.0 (57.0–71.8)	0.889
Respiratory rate >20 breaths/min	162 (40.5%)	19 (42.2%)	143 (40.5%)	0.826
Pulse rate, median (bpm)	83.6 ± 12.9	86.3 ± 10.6	83.3 ± 13.1	0.171
Peak pulse rate, (bpm)	97.3 ± 11.9	103.2 ± 14.0	96.8 ± 11.6	0.012

[a]*Significant difference ($p < 0.05$) was determined between the myocardial and non-myocardial injury groups.*

TABLE 3 | Laboratory and electrocardiographic findings of COVID-19 patients with or without myocardial injury.

	Total	Myocardial injury	Non-myocardial injury	*P*-value[a]
	(*n* = 400)	(*n* = 46)	(*n* = 354)	
Laboratory findings				
White blood cells, mean, $\times 10^9$/L	5.0 (4.0–5.8)	5.3 (3.8–6.7)	5.0 (4.0–5.8)	0.455
Neutrophils, mean, $\times 10^9$/L	3.2 (2.2–4.2)	3.3 (2.3–5.2)	3.2 (2.2–4.2)	0.567
Lymphocytes, mean, $\times 10^9$/L	1.0 (0.9–1.5)	1.0 (0.8–1.3)	1.0 (0.9–1.5)	0.058
Platelets, median, $\times 10^9$/L (*n* = 388)	173.5 ± 59.3	166.8 ± 61.4	174.4 ± 59.1	0.420
Alanine aminotransferase, U/L (*n* = 392)	24.0 (15.0–39.5)	20.0 (14.8–28.3)	24.0 (15.0–41.0)	0.130
Aspartate aminotransferase, U/L (*n* = 392)	26.0 (19.0–34.0)	27.5 (21.0–34.2)	26.0 (19.0–34.0)	0.259
Creatinine, μmol/L (*n* = 391)	57.6 (46.9–71.3)	66.4 (51.8–76.4)	56.9(46.1–70.0)	0.007
Creatine kinase, U/L (*n* = 391)	61.0 (41.0–100.0)	84.0 (54.6–150.8)	60.0 (39.5–92.9)	0.002
Lactate dehydrogenase, U/L (*n* = 391)	191.0 (154.0–263.0)	227.0 (167.5.0–311.5)	188.0 (152.5–256.0)	0.015
Interleukin-6, pg/mL (*n* = 103)	5.2 (1.5–7.2)	6.5 (5.2–17.9)	2.3 (1.5–6.3)	<0.001
C-reactive protein, mg/L (*n* = 393)	18.5 (4.6–38.8)	20.7 (5.8–43.3)	18.4 (4.1–37.8)	0.709
NT-Pro-BNP, pg/mL (*n* = 80)	68.0 (27.3–330.5)	663.6 (103.8–2450.5)	51 (24.2–179.8)	<0.001
Total cholesterol, mmol/L (*n* = 211)	4.1 ± 2.4	4.7 ± 1.1	4.0 ± 2.5	0.029
Low-density lipoprotein, mmol/L (*n* = 210)	2.2 ± 0.8	2.8 ± 1.0	2.2 ± 0.7	0.001
Triglycerides, mmol/L (*n* = 211)	1.1 (0.9–2.2)	2.7 (1.5–4.1)	1.1 (0.9–1.9)	<0.001
Arterial pH, (*n* = 80)	7.46 ± 0.05	7.45 ± 0.05	7.46 ± 0.05	0.322
$SaPO_2$ <95%	28/167 (16.8%)	7/18(38.9%)	21/149(14.1%)	0.015
Electrocardiographic findings				
ST-T change	20/106 (18.9%)	1/16(6.3%)	19/90(21.1%)	0.296
Left bundle branch block	3/106 (2.8%)	0/16 (0.0%)	3/90(3.3%)	>0.999

[a]*Significant difference ($p < 0.05$) was determined between the myocardial and non-myocardial injury groups.*

on admission over 137.1 cm^2. Age, and pre-existing health conditions, such as diabetes, hyperlipidemia, and coronary heart disease, were also significantly associated with myocardial injury. In a multivariable logistic regression model which included 265 patients with necessary data (20 with myocardial injury and 245 without myocardial injury), we found that EATV

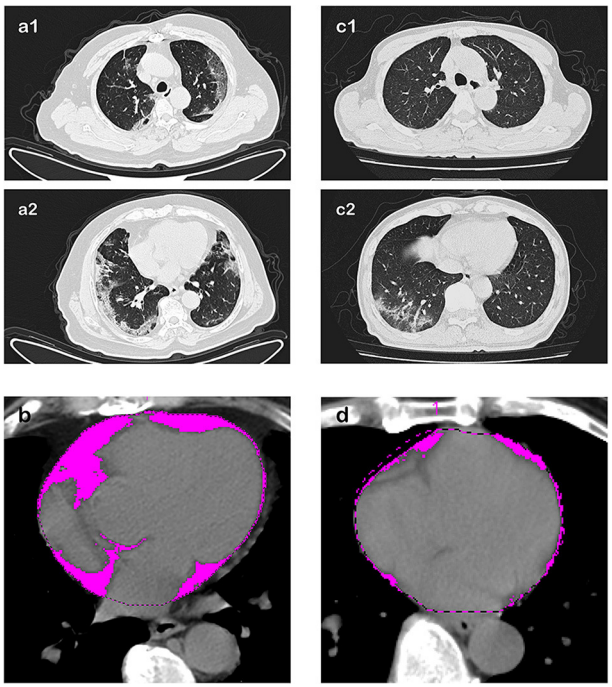

FIGURE 2 | Chest CT scan images and EATV assessment in COVID-19 patients with and without myocardial injury. Transverse chest CT images at two different positions were collected during the first 24 h of hospital admission. Epicardial adipose tissue volume was assessed and calculated in the pink area. Case A, in the age range of 60–70 years, who presented myocardial injury during hospitalization, representative chest CT scan image at two different pulmonary and cardiac positions: **(a1)** Upper position of heart and lung, and **(a2)** middle-lower position of heart and lung), showing subpleural strip-like ground-glass opacities and patchy consolidation scattering in the left upper lobe, right middle lobe, and bilateral lower lobes, the typical imaging findings of lung involvement in COVID-19. **(b)** The epicardial adipose tissue volume was 221 cm^3, presented as the pink area. Case B, also in the age range of 60–70 years but did not present myocardial injury during hospitalization. **(c1,c2)** Representative CT scan images at the lung window showing similar images of lung injury characteristic of COVID-19: subpleural irregular patchy and grid-like hyperdense shallows with blurred edges in the right lower lobe. **(d)** The epicardial adipose tissue volume or EATV was 86.5 cm^3 in this case. The volume of hypodense tissue surrounding the myocardium appearing much smaller than that shown in case A.

on admission over 137.1 cm^2 was associated with the higher incidence of myocardial injury [adjusted odds ratio (OR) 3.058, (95%CI, 1.032–9.063); $P = 0.044$], after adjusting the influence of age, body weight, the history of coronary heart disease and hyperlipidemia. Age and the history of hyperlipidemia also remained significant in this model (**Table 4**).

Therapeutic Approaches and Outcomes in COVID-19 Patients With and Without Myocardial Injury

Almost all the enrolled patients received various antiviral treatments. No differences in therapeutics were found between the myocardial injury and non-myocardial injury groups, except for the usage of corticosteroids [17/46 (37.0%) vs. 83/354 (23.4%); $P = 0.047$] (**Table 5**). In myocardial injury patients, corticosteroid therapies had markedly decreased the blood levels of IL-6 [6.0 (4.9–7.6) vs. 15.4 (5.8–34.9) pg/mL; $P = 0.03$] as well as the incidence of in-hospital adverse events [1/17 (5.9%) vs. 11/29 (37.9%); $P = 0.034$].

In-hospital adverse events (admission to an ICU, invasive mechanical ventilation, or death) occurred in 47 patients (11.8%), including 40 (10.0%) of whom were admitted to ICU, 5 (1.3%) underwent invasive mechanical ventilation, and 8 (2.0%) died (**Table 5**). Compared with those without myocardial injury, myocardial injury patients underwent more in-hospital adverse events [12/46 (26.1%) vs. 35/354 (9.9%); $P = 0.001$], while the incidence of death and ICU admission were higher too.

In the multivariable logistic regression model including all 400 patients (46 patients with myocardial injury and 354 without myocardial injury), the myocardial injury was independently associated with the risk of in-hospital adverse events [adjusted OR 2.607 (95%CI: 1.166–5.830); $P = 0.020$] after adjusting for age, sex, history of hypertension, diabetes and coronary heart disease. Age also remained significant in this model, indicating that it also contributes to in-hospital adverse events (**Supplementary Table 2**). This association remained stable in patients with EATV value ($n = 272$) in subgroup analysis (**Supplementary Table 3**).

DISCUSSION

In the current cohort study we investigated and compared the clinical characteristics between COVID-19 patients with and without myocardial injury, who were admitted to six hospitals and regional medical centers outside of the epicenter of Wuhan. This group of patients demonstrated certain pathophysiological characteristics, to a certain degree, different from those hospitalized and treated in Wuhan or other epicenters of COVID-19 around the world. We observed that many of the patients had pre-existing health conditions and increased values of EATV on admission which might be predisposed to the pathogenesis of myocardial injury. The average age of patients with myocardial injury appears higher than those without myocardial injury, but the age gap only marginable, suggesting that in this cohort, pre-existing health conditions, rather than age, might serve as the major risk factors for the development of myocardial injury. Pre-existing cardiovascular and metabolic comorbidities were more commonly observed in COVID-19 patients with myocardial injury, along with abnormal levels of metabolic indicators, indicating COVID-19 patients with underlying cardiovascular conditions, especially abnormal lipid metabolism, are exposed to an increased risk for myocardial injury. Myocardial injury serves as a contributor to the severity and mortality of COVID-19, with reported hazard ratio ranging from 2.1 to 8.9 (9, 16, 17), and odds ratio from 6.6 to 26.9 (18–20) in different studies. Our logistic regression analysis also suggests that myocardial injury is an independent adverse event, which precipitates poor prognosis. Thus, it is of great importance to timely detect and treat patients with a high risk of myocardial injury and to offer a special care to avoid relevant adverse events.

TABLE 4 | Predictors for the incidence of myocardial injury ($n = 265$).

	Univariable OR (95% CI)	*P*-value[a]	Multivariable OR (95% CI)	*P*-value[b]
Age, 10 years	1.225 (1.004–1.495)	0.045	1.602 (1.035–2.477)	0.034
Male sex (vs female)	1.215 (0.654–2.256)	0.538	–	–
Hyperlipidemia (*vs* not present)	4.721 (1.326–16.803)	0.017	5.247 (1.122–24.551)	0.035
Coronary heart disease (*vs* not present)	8.000 (1.099–58.224)	0.040	8.273 (0.742–92.187)	0.086
Epicardial adipose tissue volume on admission >137.1 cm^2 (vs. not present)	4.181 (1.651–10.588)	0.003	3.058 (1.032–9.063)	0.044
Weight	0.997 (0.972–1.023)	0.814	1.021 (0.973–1.072)	0.402

OR, odd ratio; CI, confidence intervals.
[a]*Significant difference ($p < 0.05$) was determined using univariable logistic regression model.*
[b]*Significant difference ($p < 0.05$) was determined using multivariable logistic regression model.*

TABLE 5 | Therapeutics received and outcomes of COVID-19 patients with or without myocardial injury.

	Total ($n = 400$)	Myocardial injury ($n = 46$)	Non-myocardial injury ($n = 354$)	*P*-value[a]
Treatment				
Oxygen therapy	199 (49.8%)	24 (52.2%)	175 (49.7%)	0.754
Invasive mechanic ventilation	5 (1.3%)	1 (2.2%)	4 (1.1%)	0.459
Non-invasive mechanic ventilation	25 (6.3%)	2/46 (4.3%)	23 (6.5%)	0.459
Lopinavir/ritonavir	236 (59.0%)	29 (63.0%)	207 (58.5%)	0.553
Arbidol	160 (40.0%)	22 (47.8%)	138 (39.0%)	0.266
Oseltamivir	94 (23.5%)	14 (30.4%)	80 (22.6%)	0.238
Antibiotics	282 (70.6%)	31 (67.4%)	251 (71.1%)	0.608
Corticosteroids	100 (25.0%)	17 (37.0%)	83 (23.4%)	0.047
Outcomes				
ICU admission	40 (10.0%)	11 (23.9%)	29 (8.2%)	0.003
Death	8 (2.0%)	5 (10.9%)	3 (0.8%)	0.001

ICU, intensive care unit.
[a]*Significant difference ($p < 0.05$) was determined between the myocardial and non-myocardial injury groups.*

Patients with the deadly contagious disease COVID-19 often receive medical attention in ICU or emergency room. Upon admission, less likely, they will have a comprehensive imaging assessment of cardiac complications, including echocardiography and CMR. Moreover, echocardiographic findings in patients with myocardial injury are mostly non-specific (9). Slight injury may not lead to functional or structural changes, and often it is undetectable by echocardiography and cardiac magnetic reasoning imaging. Only 20% of COVID-19 patients with myocardial injury showed abnormality on echocardiogram, left others with normal performance (21). CMR is reportedly helpful in revealing the cardiac involvement of COVID-19 in recovered patients, but its predicting value in COVID-19 patients is doubtful (22).

In the current cohort study, we explored the feasibility of using cardiac images from routine chest CT scan as a potential index of myocardial injury. Our findings demonstrate the correlation between EATV on admission and the occurrence of myocardial injury. First, the mean value of EATV is significantly larger in COVID-19 patients with myocardial injury than those without myocardial injury. Second, 137.1 cm^2 is the optimal cut-off point of EATV for predicting in-hospital myocardial injury on ROC analysis. Third, EATV over 137.1 cm^2 is the strong independent indicator for myocardial injury in general COVID-19 patients, with a valuable negative predictive value.

For the diagnosis and assessment of pneumonia, the predominant manifestation of COVID-19, patients are routinely examined by chest CT scan. Strictly speaking, EATV is a measurement of not mere fat tissue expansion but also peri- or epicardiac soft tissue (perhaps consisting of both fat and inflammatory connective tissues) enlargement with inflammatory responses (10, 23). It is exquisitely sensitive to the adjacent inflammatory states associated with coronary atherosclerotic plaque, atrial fibrillation, and systemic inflammatory disorders (24).

To date, the precise mechanisms that cause myocardial injury in COVID-19 patients are not entirely understood. The cytokine storm (i.e., excessive and uncontrollable cytokine production in response to SARS-CoV-2 infection, may be one of the main contributors to the pathogenic injury of myocardium). There have been plenty of studies indicating that serum levels of cytokines are significantly increased in COVID-19 patients (3, 4). Moreover, cytokine levels were associated with disease mortality and the incidence of myocardial injury (2, 25, 26), indicating the contributing role of cytokine storm in COVID-19 associated myocardial injury. In our population, compared with

patients without myocardial injury, IL-6 levels were significantly higher in myocardial injury patients, implying the possible pathogenic role of the cytokine storm in the development of myocardial injury. CRP levels were increased too, but statistically no significance was found between the groups of COVID-19 patients. Myocardial injury patients treated with corticosteroids had markedly decreased levels of IL-6. This observation may partially explain the improved outcome in myocardial injury patients treated with the steroids.

Epicardial fat may represent a transducer that mediates the detrimental impacts of systemic inflammation on the adjacent myocardium (27). We observed the significantly enlarged EATV in COVID-19 patients with myocardial injury, which may be due to inflammatory cell infiltration and temporary edema related to systemic cytokine storm and pericarditis and micro-myocarditis.

Increased EATV has been shown in obese individuals with increased chest and abdominal obesity, a possible risk factor for myocardial injury. Abdominal obesity is proved to be the major risk factor for disease progression and mortality in COVID-19 patients, independent of obesity-related comorbidities (28, 29). So high body mass index (BMI) and waist-hip ratio indicate a high risk of hospitalization (30). As a reflection of total visceral fatness, EATV is associated with BMI and waist circumference (31, 32), so the strong association between high EATV and myocardial injury may reveal the possible contributing role of overall and abdominal obesity to the development of myocardial injury. In this study, we observed hyperlipidemia in COVID-19 patients with myocardial injury. The elevation of EATV values in COVID-19 patients may also reflect this pathological condition.

Taken together, observations from the current study clearly document that EATV enlargement may serve as a potentially important parameter or predictor for the development of myocardial injury. Although the exact mechanism behind the association of high EATV and in-hospital myocardial injury remains unclear, it is recommendable to employ the CT scan measurement of EATV as an early risk evaluation for myocardial injury in COVID-19, in combination with other imaging methods.

Study Limitations

First, given the retrospective nature of this study, some parameters were not available in all the patients enrolled in the study. There were 128 enrolled patients who lacked the mediastinal window images, so the predicting value of EATV was analyzed based on data from the other 272 patients. Systemic bias might be introduced, though the baseline characteristics between patients with and without EATV values were roughly the same. Second, the inconsistency of troponin type between study centers deters us from clarifying the correlation between EATV and the severity of myocardial injury, which may offer a more comprehensive picture for EATV study in COVID-19 patients with cardiovascular complications. Third, myocardial injury was identified by a combination of biomarkers and clinical symptoms, primarily the abnormal levels of TnI/T during hospitalization. However, TnI/T levels could be affected by other determinants, such as the infection status, hypoxia, and renal insufficiency, which might lead to the false-positive diagnosis. On the other hand, false positive diagnosis might exist as some patients approaching to stable conditions might have a decreased likelihood of myocardial injury identification. This could cause a systematic bias when assessing the relationship between myocardial injury and disease severity. Fourth, echocardiographic data were not available in enrolled patients. A comprehensive assessment of the heart function using electrocardiography, imaging, and laboratory testing would help a deeper understanding of clinical profiles of myocardial injury.

Furthermore, we only account for weight in logistic regression, instead of other better indicator for obesity like BMI or waist-hip ratio. So as an early predictor for myocardial injury, EATV may not be independent of obesity. Whether simple anthropometric data is a predictor for myocardial injury will be explored in our further study. The role of abdominal obesity in myocardial injury development is also worthy of being investigated in the future, leveraging specific indicators like adiponectin. And finally, the cohort is relatively smaller and restricted to the Han Chinese COVID-19 patients. Thus, the conclusion should be further confirmed by large-scale prospective cohort studies in ethnically diverse cohorts.

CONCLUSIONS

Myocardial injury is the major in-hospital adverse event that contributes to the mortality of COVID-19 patients. Pre-existing health conditions, inflammatory cytokine production, and augmented EATV on admission may serve as potentially independent risk factors for the development of myocardial injury in COVID-19 patients. EATV at less than the threshold 137.1 cm^2 or so in a chest CT scan on admission may predict a better outcome for COVID-19 patients with increased risks of myocardial injury.

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 committees of Yichang Central People's Hospital, the First Affiliated Hospital of University of Science and Technology of China, Daye Chinese Medicine Hospital, Anqing Hospital, Baoding No.1 Central Hospital and Fifth Affiliated Hospital of Sun Yat-sen University. Written informed consent for participation was not provided by the participants' legal guardians/next of kin because: Written informed consent was

waived due to the rapid emergence of this infectious disease, and data analysis were performed anonymously.

AUTHOR CONTRIBUTIONS

Z-YW, H-YQ, W-XY, and Y-JG designed the study. Z-YW, H-YQ, and Y-JG drafted the manuscript. RQ, JC, W-JW, HY, JX, HW, CW, and C-HG acquired and analyzed the data. Z-YW and YW contributed to the statistical analysis. JH, ML, CL, JY, H-MD, M-JL, K-WL, and H-FS made technical support. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2020.585220/full#supplementary-material

REFERENCES

1. World Health Organization. *Coronavirus disease (COVID-19) outbreak*. Available online at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (accessed February 8, 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. Geng YJ, Wei ZY, Qian HY, Huang J, Lodato R, Castriotta RJ. Pathophysiological characteristics and therapeutic approaches for pulmonary injury and cardiovascular complications of coronavirus disease 2019. *Cardiovasc Pathol.* (2020) 47:107228. doi: 10.1016/j.carpath.2020.107228
4. Wei ZY, Geng YJ, Huang J, Qian HY. Pathogenesis and management of myocardial injury in coronavirus disease 2019. *Eur J Heart Fail.* (2020) 22:1994–2006. doi: 10.1002/ejhf.1967
5. 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
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. 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
8. Du Y, Tu L, Zhu P, Mu M, Wang R, Yang P, et al. Clinical features of 85 fatal cases of COVID-19 from wuhan: a retrospective observational study. *Am J Respir Crit Care Med.* (2020) 201:1372–79. doi: 10.1164/rccm.202003-0543OC
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. Braha A, Timar B, Diaconu L, Lupusoru R, Vasiluta L, Sima A, et al. Dynamics of epicardiac fat and heart function in type 2 diabetic patients initiated with SGLT-2 inhibitors. *Diabetes Metab Syndr Obes.* (2019) 12:2559–66. doi: 10.2147/DMSO.S223629
11. World Health Organization. *Clinical Management of Severe Acute Respiratory Infection when Novel Coronavirus (nCoV) Infection is Suspected: Interim Guidance*. Available online at: https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected (accessed January 28, 2020).
12. National Health Commission. *Notification for the Practice Guideline of the 2019 Novel Coronavirus Diease (version seventh)* Available online at: http://www.gov.cn/zhengce/zhengceku/2020-03/04/content_5486705.htm (accessed March 04, 2020).
13. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth universal definition of myocardial infarction 2018. *Circulation.* (2019) 138:e618–51. doi: 10.1161/CIR.0000000000000617
14. 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
15. 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: 10.1183/13993003.01227-2020
16. 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
17. 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
18. Chen C, Chen C, Yan JT, Zhou N, Zhao JP, Wang DW. Analysis of myocardial injury in patients with COVID-19 and association between concomitant cardiovascular diseases and severity of COVID-19. *Zhonghua xin xue guan bing za zhi.* (2020) 48:567–71. doi: 10.3760/cma.j.cn112148-20200225-00123
19. Ni W, Yang X, Liu J, Bao J, Li R, Xu Y, et al. Acute myocardial injury at hospital admission is associated with all-cause mortality in COVID-19. *J Am Coll Cardiol.* (2020) 76:124–5. doi: 10.1016/j.jacc.2020.05.007
20. Wei J-F, Huang F-Y, Xiong T-Y, 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
21. 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
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. Wang J, Chen D, Cheng XM, Zhang QG, Peng YP, Wang LJ, et al. Influence of phenotype conversion of epicardial adipocytes on the coronary atherosclerosis and its potential molecular mechanism. *Am J Transl Res.* (2015) 7:1712–23.

24. Wang J, Chen D, Cheng XM, Zhang QG, Peng YP, Wang LJ, et al. Epicardial adipose tissue may mediate deleterious effects of obesity and inflammation on the myocardium. *J Am Coll Cardiol.* (2018) 71:2360–72. doi: 10.1016/j.jacc.2018.03.509
25. 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
26. Song Y, Gao P, Ran T, Qian H, Guo F, Chang L, et al. High inflammatory burden: a potential cause of myocardial injury in critically Ill patients with COVID-19. *Front Cardiovasc Med.* (2020) 7:128. doi: 10.3389/fcvm.2020.00128
27. Patel VB, Shah S, Verma S, Oudit GY. Epicardial adipose tissue as a metabolic transducer: role in heart failure and coronary artery disease. *Heart Fail Rev.* (2017) 22:889–902. doi: 10.1007/s10741-017-9644-1
28. Tartof S, Qian L, Hong V, Wei R, Nadjafi R, Fischer H, et al. Obesity and mortality among patients diagnosed with COVID-19: results from an integrated health care organization. *Ann Intern Med.* (2020) 173:773–81. doi: 10.7326/M20-3742
29. Sales-Peres S, de Azevedo-Silva L, Bonato R, Sales-Peres M, Pinto A, Santiago Junior JJOr, et al. Coronavirus (SARS-CoV-2) and the risk of obesity for critically illness and ICU admitted: Meta-analysis of the epidemiological evidence. *Obes Res Clin Pract.* (2020) 14:389–97. doi: 10.2139/ssrn.3612053
30. Hamer M, Gale C, Kivimäki M, Batty G. Overweight, obesity, and risk of hospitalization for COVID-19: a community-based cohort study of adults in the United Kingdom. *Proc Natl Acad Sci USA.* (2020) 117:21011–3. doi: 10.1073/pnas.2011086117
31. Alexopoulos N, McLean DS, Janik M, Arepalli CD, Stillman AE, Raggi P. Epicardial adipose tissue and coronary artery plaque characteristics. *Atherosclerosis.* (2010) 210:150–4. doi: 10.1016/j.atherosclerosis.2009.11.020
32. de Vos AM, Prokop M, Roos CJ, Meijs MFL, van der Schouw YT, Rutten A, et al. Peri-coronary epicardial adipose tissue is related to cardiovascular risk factors and coronary artery calcification in post-menopausal women. *Eur Heart J.* (2008) 29:777–83. doi: 10.1093/eurheartj/ehm564

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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

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative virus for the current global pandemic known as coronavirus disease 2019 (COVID-19). SARS-CoV-2 belongs to the family of single-stranded RNA viruses known as coronaviruses, including the MERS-CoV and SARS-CoV that cause Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS), respectively. These coronaviruses are associated in the way that they cause mild to severe upper respiratory tract illness. This study has used an unbiased analysis of publicly available gene expression datasets from Gene Expression Omnibus to understand the shared and unique transcriptional signatures of human lung epithelial cells infected with SARS-CoV-2 relative to MERS-CoV or SARS-CoV. A major goal was to discover unique cellular responses to SARS-CoV-2 among these three coronaviruses. Analyzing differentially expressed genes (DEGs) shared by the three datasets led to a set of 17 genes, suggesting the lower expression of genes related to acute inflammatory response (TNF, IL32, IL1A, CXCL1, and CXCL3) in SARS-CoV-2. This subdued transcriptional response to SARS-CoV-2 may cause prolonged viral replication, leading to severe lung damage. Downstream analysis of unique DEGs of SARS-CoV-2 infection revealed changes in genes related to apoptosis (NRP1, FOXO1, TP53INP1, CSF2, and NLRP1), coagulation (F3, PROS1, ITGB3, and TFPI2), and vascular function (VAV3, TYMP, TCF4, and NR2F2), which may contribute to more systemic cardiovascular complications of COVID-19 than MERS and SARS. The study has uncovered a novel

set of transcriptomic signatures unique to SARS-CoV-2 infection and shared by three coronaviruses, which may guide the initial efforts in the development of prognostic or therapeutic tools for COVID-19.

Keywords: SARS-CoV-2, SARS-CoV, MERS-CoV, COVID-19 and transcriptome analysis, cardiovasclar disease

INTRODUCTION

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the Coronaviridae family of viruses (coronaviruses) and is responsible for the coronavirus disease 2019 (COVID-19) pandemic (1). Along with its other accomplices, Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV), SARS-CoV-2 can jump species barrier followed by human-to-human transmission via droplet infection. In late December 2019, initial reports suggested the origin of SARS-CoV-2 in a seafood and wild animal trading market in Wuhan, China (2). To date, the pandemic has caused more than 83 million infections and more than 1.8 million deaths worldwide (https://www.worldometers.info/coronavirus/). SARS-CoV-2 leads to more cardiovascular complications than do MERS-CoV and SARS-CoV; however, what causes these major differences remains obscure (3, 4).

The initial genome identification of SARS-CoV-2 suggested that it has a ∼80% similarity with SARS-CoV and 96% identical to a bat coronavirus; however, there are differences in its pathogenicity and host response (2). The virus nucleic acid shedding patterns in both symptomatic and asymptomatic patients of SARS-CoV-2 are similar, which explains the transmission potential of otherwise asymptomatic carriers (5). In contrast, the viral burden in the upper respiratory tract in SARS-CoV infection peaks at around 10 days after the initial exposure (6). On the contrary to SARS-CoV-2, viral load in MERS-CoV–infected individuals peak at week 2 of the onset of infection (7). This suggests the difference in the virulence and host response of these three strains.

Upon entry, next steps are viral replication, amplification, and spread in the host, which largely depend on similarities and/or uniquenesses in transcriptional signature of these viruses. Patients with SARS-CoV-2 manifest a few different but aggravated symptoms, particularly major cardiovascular complications, from SARS-CoV and MERS-CoV, which may be attributable to the difference in their transcriptional signatures. Reports of aggravated blood coagulation in COVID-19 patients suggested the mechanism of prominent elevation of D-dimer and fibrin/fibrinogen degradation products (8). Higher mortality rate is reported in COVID-19 patients with thromboembolic events (9), and treatment with anticoagulant-heparin has improved prognosis (10).

The present comparative analysis has determined key differences in transcriptional changes in lung epithelial cells induced by these virus strains. To further examine transcriptional responses of SARS-CoV-2 and other two coronaviruses, we analyzed a comprehensive map of lung epithelial cells infected with these three coronaviruses and explored pathological host responses unique to SARS-CoV-2. Our findings may help to understand potential mechanisms by which SARS-CoV-2 causes more cardiovascular complications than do two other coronaviruses and to establish molecular bases for the development of therapies against COVID-19.

METHODS

RNA Sequencing and Microarray Analysis of Gene Expression Omnibus Datasets

Figure 1A depicts the workflow of gene expression analysis. For differential gene expression analysis of SARS-CoV-2 infection, raw expression counts were downloaded from Gene Expression Omnibus (GEO) accession number GSE147507 (11). RNA sequencing (RNAseq) dataset was generated on Illumina Nextseq 500 platform. The raw read counts were normalized by log2 transformation, before and after normalization box plot; principal component analysis (PCA) and density plot are shown in **Supplementary Figure 1**. Using INMEX tool that employs DESeq (12, 13), differential expression analysis was performed and differentially expressed genes (DEGs) were characterized for each sample with adjusted $p < 0.05$ [false discovery rate (FDR) corrected by Benjamini–Hochberg method]. GSE81909, the dataset we used for analysis of MERS-CoV, was generated on Agilent-Whole Human Genome Microarray 4x44K G4112. After downloading the raw read counts from GEO, we normalized the dataset using variance-stabilizing normalization followed by quantile normalization (14). Before and after normalization box plot, PCA and density plot are shown in **Supplementary Figure 2**. Similarly, we downloaded raw read counts of GSE17400 (15) for analysis of SARS-CoV infection. This dataset was generated on Affymetrix Human Genome U133 plus 2.0 Array. After normalization of dataset using variance-stabilizing normalization followed by quantile normalization (**Supplementary Figure 3**), both microarray datasets, GSE81909 and GSE17400, were subjected to DEGs analysis using LIMMA algorithm (16). DEGs were characterized for each sample with adjusted $p < 0.05$ (FDR corrected by Benjamini–Hochberg method). Heatmap visualization of a subset of 25 overexpressed and underexpressed genes was constructed using heatmap.2 from the gplot package in R. Volcano plots were constructed using custom scripts in R, and PCA was performed on log2 fold-change values using PMA package in R (17) (**Supplementary Figure 4**). It is worthwhile mentioning that all three datasets used in this study are collected from different laboratories and using different cell lines, as well as experimental techniques (e.g., microarrays, RNAseq). We selected RNAseq dataset for SARS-CoV-2 as there were especially no microarray datasets on SARS-CoV-2 in humans. Therefore, we did take the present analysis strategy

of comparing each dataset with its internal control to call the DEGs for each coronavirus infection. Further, we compared the three sets of DEGs to find the shared and unique genes between coronavirus infections; we applied this strategy to minimize data variabilities (e.g., operator and platform biases). GSE147507 was generated in primary human lung epithelium (NHBE); GSE81909 was generated in human airway epithelial cells, whereas GSE17400 was generated in human bronchial epithelial cells. **Supplementary Table 1** provides detailed information of each dataset and sequencing/microarray platform used.

Functional Gene Set Enrichment Analysis of DEGs

To discern the implication of DEGs called from transcriptome analysis of coronavirus infection in lung epithelial cells, we performed a functional analysis using the EnrichR platform (18). This web-based software product evaluates significantly enriched pathways/terms in an input gene list with the help of its extensive gene set libraries, which includes Gene Ontology (GO) (19) and various pathway analysis libraries such as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, Reactome pathway, wikipathway, Panther, and Biocarta. We retrieved tables of enriched pathways from each database and prepared a comprehensive table of most significant pathways for each coronavirus infection based on the adjusted *p* value (ranking derived from Fisher exact test for gene sets) significance.

Common DEGs Analysis Between Coronaviruses

We created a coronavirus–gene network for better visualization of the shared genes between the coronaviruses using Cytoscape software (20). The network was generated by utilizing the list of DEGs from three coronaviruses studies in which coronaviruses are the source nodes; genes are the target nodes, and the connections between them are the edges in the network. The network core represents the coronaviruses, whereas the inner-circle genes in the network are the shared ones, and outer-circle genes are unique to each coronavirus (**Figure 2C**). A Venn diagram representing the shared and unique DEG portion between three coronaviruses was generated using VENNY 2.1 tool (https://bioinfogp.cnb.csic.es/tools/venny/index.html). A heatmap represents the expression profiles for common DEGs between coronaviruses. Clustering of selected genes on the heatmap was performed by hierarchical clustering algorithm utilizing Euclidean distance measure.

Pathway Clustering and Network-Based Hub Gene Analysis

For visualization and interpretation of the biological relevance of unique DEGs to SARS-CoV-2 DEGs, Cytoscape v3.1 plug-in was used for analysis. Biological pathway clustering analysis was done using BinGO (21). BinGO analyzes GO terms and functional groups association within the biological networks. We performed biological pathway clustering analysis to see collective function of these genes. The size of a node is proportional to the number of targets in the biological process category. The color represents enrichment significance—the deeper the color on a color scale, the higher the enrichment significance. Hub gene network analysis was performed using NetworkAnalyst (13), which created a protein–protein interaction (PPI) network by integrating the InnateDB interactome with the original seed of 221 DEGs. This tool supports integrative analysis of gene expression data through statistical, visual, and network-based analysis approaches by taking the advantage of common functions for network topology and module analysis approaches. Briefly, the complete list of unique DEGs from SARS-CoV-2 was uploaded into the web-based server of NetworkAnalyst. Network construction was restricted to contain all the original seed proteins in order to visualize the connections. To help identify highly interconnected hub nodes, topological measures (e.g., degree and betweenness centrality) were used. Expression of the genes was considered as the network feature, where red-colored nodes are genes with increased expression, green nodes are genes with decreased expression, and gray nodes are genes not expressed in our data.

Expression2Kinases Analysis of Regulatory Gene Networks

ChEA is a comprehensive databases of kinases and transcription factors (22), and it is used in background of Expression2Kinases (X2K) (23), the tool we used to understand the upstream regulatory molecules of DEGs in SARS-CoV-2 infection. The 10 most significant transcription factors and kinases were extracted based on Fisher exact test *p* value enrichment scoring. We downloaded the ".graphml" file generated from the analysis to create and visualize regulatory network on Cytoscape environment. This ensures that the protein network obtained during network expansion is properly connected by automatically increasing the path length, so that there are more intermediate proteins used to connect the transcription factors. In the network, a yellow node represents intermediate proteins in the PPI regulatory network. Node size represents the significance of protein based on adjusted *p* value; the bigger the nodes size, the higher the significance value.

Statistical Analyses

For differential expression analysis of SARS-CoV-2 dataset GSE147507, read counts were subjected to differential expression analysis using INMEX, which utilizes the Rpackage DESeq (13). Genes with adjusted $p < 0.05$ were considered significant. The *p* value adjustment for multiple comparisons was done by the Benjamini–Hochberg method. For MERS-CoV-GSE81909 and SARS-CoV-GSE17400, differential expression analysis was performed with LIMMA algorithm for each dataset, independently using adjusted $p < 0.05$, based on the FDR using the Benjamini–Hochberg method and moderated *t* test. Significantly enriched GO terms were identified using hypergeometric tests, and $p \leq 0.05$ was applied as a cutoff for statistical significance.

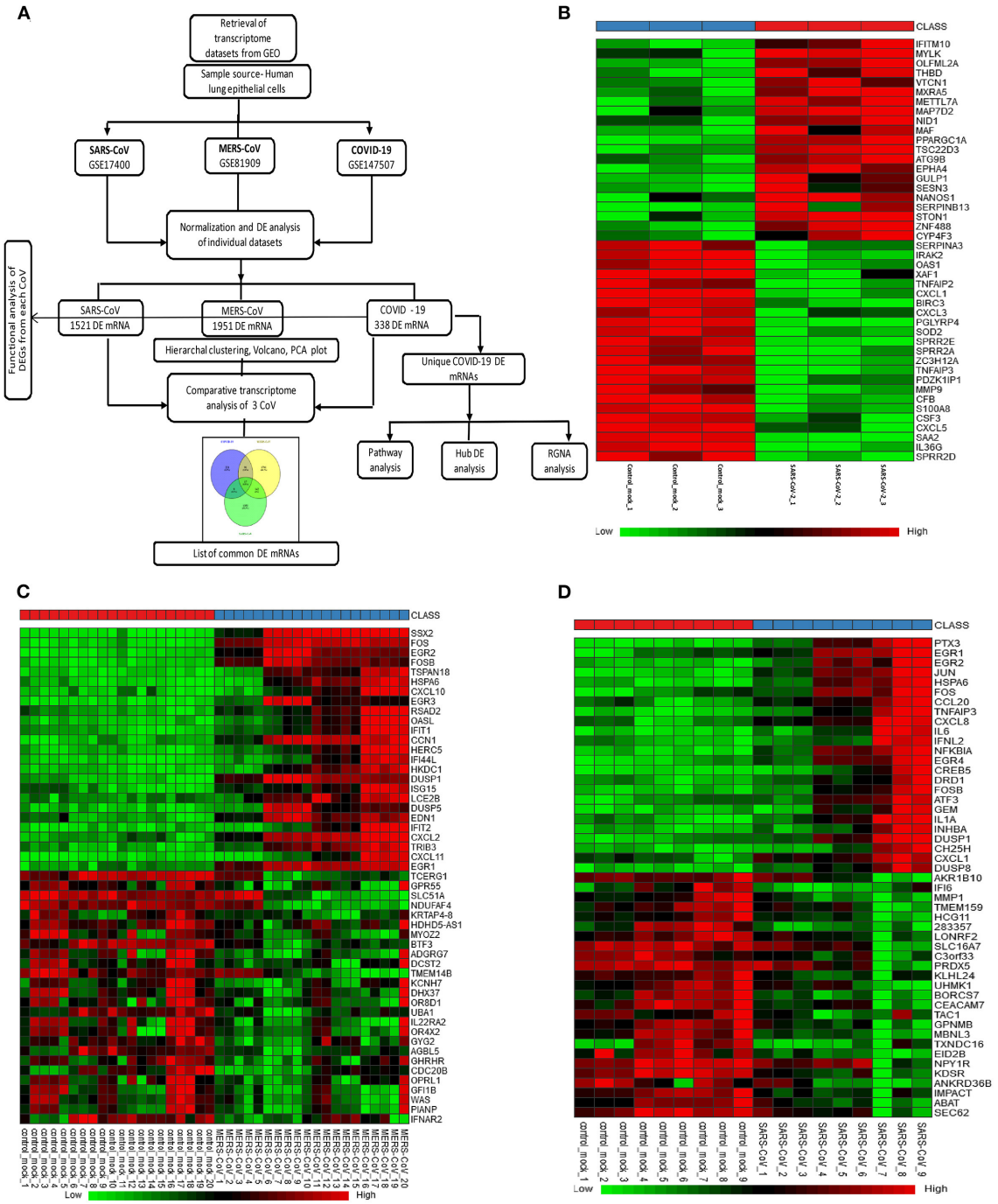

FIGURE 1 | Gene expression profiles of the differentially expressed genes (DEGs) in human lung epithelial cells infected with coronaviruses. **(A)** Workflow of gene expression analysis. Selection process of eligible datasets for transcriptome analysis was based on datasets generated from infection of human lung epithelial cell in culture with SARS-CoV-2, MERS-CoV, or SARS-CoV. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CoV, coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus and GEO, Gene Expression Omnibus. **(B–D)** Heatmaps of expression profiles for the top 25 increased and 25 decreased DEGs obtained from RNA-seq data analysis. Clustering of selected genes on the heatmap was performed by hierarchical clustering algorithm using Euclidean distance measure. **(B)** SARS-CoV-2, **(C)** MERS-CoV, and **(D)** SARS-CoV.

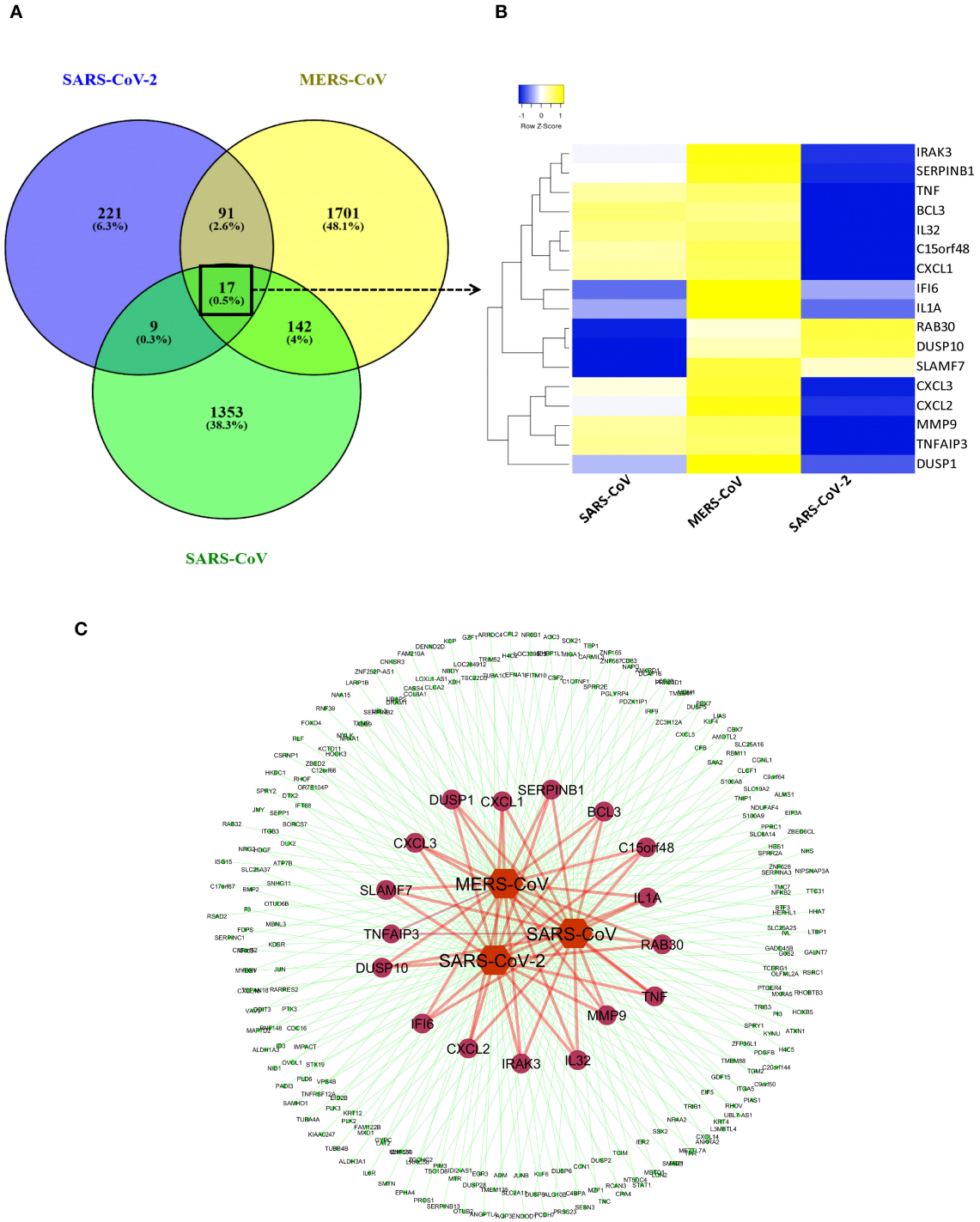

FIGURE 2 | Shared transcriptional signatures between the three coronaviruses. **(A)** Venn diagram representing the shared and unique DEGs portion between three coronaviruses. **(B)** Heatmap representation of expression profiles for the common DEGs between coronaviruses. Clustering of selected genes on the heatmap was performed by hierarchical clustering algorithm using Euclidean distance measure. Expression scale: blue (low expression) to yellow (high expression). **(C)** coronavirus–gene network representing the shared and unique DEGs. Network was created between coronaviruses and their top 100 DEGs on cytoscape platform. Network core represents the coronaviruses (source nodes) and gene (target nodes). Inner circles of genes in the network are the shared ones, while outer circle genes are unique to each coronavirus.

RESULTS

Selection of Eligible Gene Expression Datasets for Coronavirus Infection in Human Lung Epithelial Cells

We selected three studies from the GEO accession numbers: GSE147507 for SARS-CoV-2, GSE81909 for MERS-CoV, and GSE17400 for SARS-CoV. The search was limited to transcriptome data generated in human lung epithelial cells. **Figure 1A** depicts the overall workflow of the analysis in this study. A total of 3/3, 20/20, and 9/9 control/infected cell culture replicates SARS-CoV-2, MERS-CoV, and SARS-CoV, respectively, were used in this analysis. GSE147507 dataset was RNAseq data and generated on Illumina Nextseq 500, and we utilized only six samples (GSM4432378, GSM4432379, GSM4432380, GSM4432381, GSM4432382, and GSM4432383); these were independent biological triplicates of primary human lung epithelium (NHBE), which were mock treated or infected with SARS-CoV-2 (USA-WA1/2020). Of note, the other two datasets were generated by microarray using Affymetrix Human Genome U133A series (GSE17400-SARS-CoV) and Agilent-014850 Whole Human Genome Microarray 4x44K G4112F (GSE81909-MERS-CoV). Sample sources of all three datasets were of human lung epithelial cells and primary lung cells infected with coronaviruses. **Supplementary Table 1** provides detailed information of each dataset and sequencing/microarray platform used.

Analysis of Differentially Expressed Genes (DEGs) in the SARS-CoV-2 Dataset Led to Perturbation of Inflammatory, Coagulation, and Apoptotic Pathways

In SARS-CoV-2–infected dataset (GSE147507), we identified a total of 338 DEGs with adjusted $p < 0.05$ (**Supplementary Dataset 1**). Among these 338 DEGs, 92 genes increased, and 246 decreased. **Figure 1B** depicts the heatmap of expression of top significant DEGs among the samples. **Table 1** lists the top 20 increased and decreased DEGs from our analysis of SARS-CoV-2 infection. Interferon (IFN)–induced transmembrane protein 10 (*IFITM10*), C-X-C motif chemokine ligand 14 (*CXCL14*), and myosin light chain kinase (*MYLK*) were among the most significantly increased genes, while small proline-rich protein 2D (*SPRR2D*), interleukin 36 gamma (*IL36G*), and serum amyloid A2 (*SAA2*) were the most decreased genes in our analysis of SARS-CoV-2–infected lung epithelial cells compared to mock controls. When these DEGs were subjected to the analysis of overrepresented biological pathways and enriched terms, several pathways related to inflammation, apoptosis, blood coagulation, and lung fibrosis were enriched (**Table 2**). Enriched terms and biological pathways were significantly overrepresented in the gene list if they showed an adjusted $p < 0.05$. DEGs from SARS-CoV-2 infection were associated with the KEGG pathways such as IL-17 signaling pathway (hsa04657) with database overlap of 21/93 (which means of 93 genes associated with this pathway reported in KEGG, 21 are present among our DEGs) and adjusted $p =$ 1.24E-15 and TNF signaling pathway (hsa04668) with database overlap of 19/110 and adjusted $p =$ 4.76E-12. Besides, other databases resulted in enrichment of pathways including blood coagulation (P00011) with overlap of 7/38 and adjusted $p =$ 1.67E-04, apoptosis signaling pathway (P00006) with overlap of 7/102 and adjusted $p = 0.038822$, and lung fibrosis (WP3624) with overlap of 11/63 and adjusted $p =$ 4.53E-07, among others.

Identification of DEG Signature in MERS-CoV– or SARS-CoV–Infected Human Lung Epithelial Cells

In the case of MERS-CoV dataset, GSE81909, there are a total of 1,951 DEGs with adjusted $p < 0.05$ (**Supplementary Dataset 2**). Among these 1,951 DEGs, 1,120 genes increased, and 831 decreased. The microarray analysis of GSE17400 for SARS-CoV infection resulted in a total of 1,521 DEGs with adjusted $p <$ 0.05 (**Supplementary Dataset 3**). Among these 1,521 DEGs, 475 increased, and 1,046 decreased. **Figures 1C,D** depict the heatmap of expression of top significant DEGs among the samples for MERS-CoV and SARS-CoV, respectively. As shown in **Supplementary Table 2**, SSX family member 2 (*SSX2*), fos proto-oncogene, AP-1 transcription factor subunit (*FOS*), and early growth response 1 (*EGR1*) were among the most significantly increased genes, whereas transcription elongation regulator 1 (*TCERG1*), G protein–coupled receptor 55 (*GPR55*), and casein kappa (*CSN3*) were the most decreased genes in our analysis of MERS-CoV–infected lung epithelial cells compared to controls. Similarly, **Supplementary Table 3** shows that pentraxin 3 (*PTX3*), early growth response 1 (*EGR1*), and EGR2 were among the most significantly increased genes, whereas aldo-keto reductase family 1 member B10 (*AKR1B10*), IFN-α-inducible protein 6 (*IFI6*), and matrix metallopeptidase 1 (*MMP1*) are the most decreased genes in our analysis of SARS-CoV–infected lung epithelial cells compared to mock controls. When these DEGs from both MERS-CoV and SARS-CoV were subjected to the analysis of overrepresented biological pathways and enriched terms, several pathways related to inflammation and apoptosis were commonly enriched (**Supplementary Tables 4**, **5**).

Muted Expression of Acute Inflammatory Genes Was Observed in the SARS-CoV-2 When Compared to MERS-CoV-2 and SARS-CoV

Previous studies revealed that lung epithelial cells, dendritic cells, and macrophages all express cytokines to some extent during major viral infection causing cytokine storm. However, little is known about the situation in COVID-19. Earlier studies showed IFN-γ-related cytokine storm in SARS-CoV infection, whereas MERS-CoV infection had delayed induction of proinflammatory cytokines and suppression of innate antiviral response. It is crucial to identify the primary source of the cytokine storm in response to SARS-CoV-2 infection and the underlying virological mechanisms. Our analysis of shared DEGs between three coronaviruses resulted in 17 shared DEGs (**Figure 2A**), among which most genes are related to acute inflammation. **Figure 2B** indicates that the

TABLE 1 | Top 20 DEGs identified in the SARS-CoV-2 analysis.

Gene ID	EntrezID	Gene Name	baseMean	P.Value	adj.P.Val	logFC
Increased DEGs						
IFITM10	402778	Interferon-induced transmembrane protein 10	194.45	9.90E-13	2.60E-10	1.0377
CXCL14	9547	C-X-C motif chemokine ligand 14	100.64	4.25E-08	6.47E-06	0.8338
MYLK	4638	Myosin light chain kinase	90.676	4.45E-08	6.71E-06	0.83025
OLFML2A	169611	Olfactomedin like 2A	413.24	4.58E-12	1.16E-09	0.79462
THBD	7056	Thrombomodulin	404.62	8.33E-11	1.96E-08	0.79168
VTCN1	79679	V-set domain containing T cell activation inhibitor 1	182.21	4.56E-08	6.79E-06	0.76269
MXRA5	25878	Matrix remodeling associated 5	721.64	1.21E-11	2.96E-09	0.76162
METTL7A	25840	Methyltransferase like 7A	149.49	9.17E-08	1.34E-05	0.74126
MAP7D2	256714	MAP7 domain containing 2	54.277	5.84E-06	0.000547	0.69934
GPNMB	10457	Glycoprotein nmb	2057.8	8.18E-11	1.96E-08	0.68713
Decreased DEGs						
SPRR2D	6703	Small proline rich protein 2D	365.31	1.79E-53	8.02E-50	−2.1217
IL36G	56300	Interleukin 36 gamma	271.41	6.98E-57	4.68E-53	−2.0691
SAA2	6289	Serum amyloid A2	575.93	3.57E-81	4.79E-77	−2.0679
CXCL5	6374	C-X-C motif chemokine ligand 5	104.68	2.18E-35	2.44E-32	−1.8864
MX1	4599	MX dynamin like GTPase 1	427.77	4.07E-37	4.96E-34	−1.7731
CSF3	1440	Colony-stimulating factor 3	68.054	2.84E-28	1.81E-25	−1.6959
S100A8	6279	S100 calcium-binding protein A8	1707.4	4.04E-52	1.35E-48	−1.6127
ICAM1	3383	Intercellular adhesion molecule 1	1885	8.73E-45	1.95E-41	−1.5713
CFB	629	Complement factor B	789.4	2.86E-44	5.47E-41	−1.5634
MMP9	4318	Matrix metallopeptidase 9	318.36	6.38E-26	3.42E-23	−1.5215

Genes were ranked based on the log fold change and adjusted p value (<0.05). The corresponding p values are adjusted, based on the false discovery rate using the Benjamini–Hochberg procedure.

expression levels of these genes were lower in SARS-CoV-2 when compared with the other two coronaviruses. Our identification of a muted transcriptional response to SARS-CoV-2 supports a model in which initial failure to rapidly respond to infection results in prolonged viral replication and subsequent recruitment of proinflammatory cells as the infection progresses to induce alveolar damage in COVID-19. **Table 3** depicts the expression value of DEGs shared in all three coronaviruses. It is evident that the expression of critical acute inflammatory genes including TNF-α-induced protein 3 (*TNFAIP3*), C-X-C motif chemokine ligand 1 (*CXCL1*), and *TNF* was lower in the SARS-CoV-2 dataset compared to two other coronaviruses. These results may suggest that lung epithelial cells do not directly contribute to the cytokine storm during COVID-19 and that other immune cells appear to participate in this process.

SARS-CoV-2 Elicits Suppressed Type I IFN Response and Activation of Apoptotic Gene Signature

Dissecting the DEGs involved in IFN response to coronavirus infection in primary human lung epithelial cells revealed that SARS-CoV-2 elicits a muted response that lacks robust induction of a subset of cytokines including the type I IFN compared to the response to MERS-CoV and SARS-CoV (**Figure 3A**). Furthermore, our analysis revealed that in desperate bid to control the viral propagation, SARS-CoV-2 infection

TABLE 2 | Top enriched terms identified by functional analysis of the DEGs from SARS-CoV-2–infected human lung epithelial cells.

Enrichment terms	Pathway/term ID	Overlap	GSEA library	Adjusted *p* value
IL-17 signaling pathway	hsa04657	21/93	KEGG	1.24E-15
TNF signaling pathway	hsa04668	19/110	KEGG	4.76E-12
Signal transduction through IL1R	h il1rPathway	06/36	Biocarta	0.006706
NF-κB activation by nontypeable *Haemophilus influenzae*	h nthiPathway	05/29	Biocarta	0.013491
Plasminogen-activating cascade	P00050	07/15	Panther	2.37E-07
Blood coagulation	P00011	07/38	Panther	1.67E-04
Apoptosis signaling pathway	P00006	07/102	Panther	0.038822
Hemostasis	R-HSA-109582	27/552	Reactome	1.91E-04
Platelet degranulation	R-HSA-114608	11/105	Reactome	2.81E-04
Lung fibrosis	WP3624	11/63	Wikipathway	4.53E-07

Overlap: indicates the number of hits from the meta-analysis compared to each curated gene set library. Gene set functional analysis was performed using extended libraries of the EnrichR tool. Enriched terms and pathways were ranked based on the p value. KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology Biological Process; GSEA, Gene Set Enrichment Analysis.

induced several apoptosis-related genes in human lung epithelial cells compared to responses to MERS-CoV and SARS-CoV (**Figure 3B**).

Downstream Analysis of DEGs Unique to SARS-CoV-2 and Network-Based Meta-Analysis Led to Pathways and Hub Genes Related to Inflammation and Vascular Dysfunction

A set of 221 DEGs unique to SARS-CoV-2 underwent downstream analysis to understand the enriched pathways and associated hub genes. Using BinGO enrichment clusters of biological process (GO terms) associated with unique DEGs of SARS-CoV-2 was generated, which revealed enriched pathway clusters associated with immune responses/chemotaxis, blood coagulation, apoptosis, vascular remodeling, and vascular cell proliferation (**Figure 4A**). **Supplementary Dataset 4** compiles the complete list of GO: terms enriched in DEGs associated with SARS-CoV-2 infection. We generated a PPI network by integrating the InnateDB interactome with the original seed of 221 DEGs. An expanded PPI network was generated with 2,542 nodes representing the proteins and 4,457 edges representing the interaction between these proteins. Network-based hub DEG analysis (**Figure 4B**) identified ribosomal protein L9 (*RPL9*) and SMAD family member 3 (*SMAD3*) to be the most highly ranked hub genes that increased and decreased among the DEGs, respectively, based on betweenness centrality and degree score. The list of top 15 hub genes based on network topology scores is shown in **Table 4**.

Identification of the Transcription Factors and Regulatory Kinases Network Upstream to the Unique DEGs Obtained From SARS-CoV-2

To understand what lies to the upstream of the unique DEGs identified from the SARS-CoV-2 infection, we used X2K bioinformatics tool. The regulatory gene network analysis resulted in identification of transcription factors and kinases related to the DEGs. Network in **Figure 4C** shows the top kinases and transcription factors related to our DEGs. The list of top 10 ranked transcription factors and protein kinases is shown in **Supplementary Table 4**. This analysis revealed the most important regulatory gene candidates that may be involved in the formation of regulatory complexes. Mitogen-activated protein kinase 1 (*MAPK1*) and MAPK3 are among the top kinases, whereas SMAD3 and SMAD2 are among the top transcription factors associated with the unique DEGs from lung epithelial cells infected with SARS-CoV-2.

Distinct Pathways and Gene Signatures Associated With SARS-CoV-2

After we removed the DEGs shared by three coronaviruses (17 DEGs) and those shared between SARS-CoV-2 and MERS-CoV (91 DEGs); and SARS-CoV-2 and SARS-CoV (9 DEGs), a total of 221 DEGs remained that was specific to SARS-CoV-2 (**Figure 2A** and **Supplementary Dataset 1**). Our interest was to identify the distinct pathways that may participate in the pathogenesis of COVID-19. Using the list of SARS-CoV-2–specific DEGs, we conducted biological process (GO) analysis on the unique set of DEGs using a Cytoscape plugin, BinGO tool. **Table 5** depicts the most distinct pathways and their associated representative genes with its known function and expression fold change that may have implication on the disease pathogenesis. In the analysis, the following pathways were enriched. In the apoptosis-related pathway, the expression of neuropilin 1 (*NRP1*), forkhead box O1 (*FOXO1*), and tumor protein p53 inducible nuclear protein 1 (*TP53INP1*) is increased, whereas that of colony-stimulating factor 2 (*CSF2*) and NLR family pyrin domain containing 1 (*NLRP1*) is decreased. Acute inflammation–related genes included IL-6 receptor (*IL-6R*) that increased and serpin family A member 3 (*SERPINA3*), complement component 1s (*C1S*), serum amyloid A2 (*SAA2*), and complement factor B (*CFB*) that decreased. Among vascular dysfunction–related genes, vav guanine nucleotide exchange factor 3 (*VAV3*) and transcription factor 4 (TCF4) are increased, while thymidine

TABLE 3 | Shared DEGs between coronaviruses.

		COVID-19		MERS-CoV		SARS-CoV	
Symbols	**Name**	**logFC**	**adj.P.Val**	**logFC**	**adj.P.Val**	**logFC**	**adj.P.Val**
TNFAIP3	TNF-α-induced protein 3	−1.4397	6.31E-48	1.3062	8.28E-07	1.0224	0.010541
CXCL1	C-X-C motif chemokine ligand 1	−1.2721	1.39E-38	0.88054	5.05E-07	0.54891	0.005485
C15orf48	chromosome 15 open reading frame 48	−1.1055	3.28E-28	0.62913	0.024618	0.27721	0.048513
IL32	Interleukin 32	−1.0607	1.44E-21	0.55427	6.13E-06	0.4451	0.021275
CXCL2	C-X-C motif chemokine ligand 2	−1.1072	1.87E-16	1.9732	6.51E-13	0.31032	0.025261
CXCL3	C-X-C motif chemokine ligand 3	−1.2936	4.47E-15	1.5534	1.66E-07	0.49265	0.006207
IL1A	Interleukin 1 alpha	−0.90381	3.18E-13	0.79049	3.99E-05	−0.63885	0.029208
SERPINB1	Serpin family B member 1	−0.65532	1.12E-09	0.2583	0.001145	−0.16797	0.034799
DUSP10	Dual specificity phosphatase 10	0.49169	0.000102	0.43159	0.017097	0.24686	0.014842
TNF	Tumor necrosis factor	−0.72256	0.000217	0.32484	0.003589	0.19034	0.039241
BCL3	BCL3 transcription coactivator	−0.57674	0.0004	0.45737	0.001256	0.53122	0.007329
IFI6	Interferon α-inducible protein 6	−0.49295	0.009535	0.4471	0.02091	−0.63885	0.029208
RAB30	"RAB30, member RAS oncogene family"	0.4949	0.023868	0.38751	0.000627	0.1745	0.048354
IRAK3	Interleukin 1 receptor associated kinase 3	−0.50297	0.024857	0.27287	0.034926	−0.14418	0.034042
SLAMF7	SLAM family member 7	0.44487	0.02517	0.72071	1.59E-07	−0.23004	0.028654
DUSP1	Dual specificity phosphatase 1	−0.34689	0.025301	2.1214	1.58E-20	0.24686	0.014842

Expression values of the shared genes from each analysis. The corresponding p values are adjusted, based on the false discovery rate using the Benjamini–Hochberg procedure.

phosphorylase (*TYMP*) and nuclear receptor subfamily 2 group F member 2 (*NR2F2*) are decreased. Genes related to blood coagulation included coagulation factor III/tissue factor (*F3*) and protein S (*PROS1*) are increased, whereas IFN-γ receptor 1 (*IFNGR1*), integrin subunit β3 (*ITGB3*), and tissue factor pathway inhibitor 2 (*TFPI2*) are decreased. Several pathways associated with cardiovascular dysfunction were enriched by the unique set of DEGs specific to SARS-CoV-2 infection. **Table 6** summarizes the pathways and their associated genes that might play a role in cardiovascular complications.

DISCUSSION

In the present study, we focus on defining transcriptional responses to SARS-CoV-2 relative to MERS-CoV and SARS-CoV. A major goal was to discover unique cellular responses to SARS-CoV-2 among these three coronaviruses. We specifically selected datasets generated in cultured human lung epithelial cells infected with each of these three coronaviruses as this cell type is the major interface between the environment and the host and defends the lung against foreign substances and pathogens. In general, our data show that overall transcriptional footprints to SARS-CoV-2 infection were distinct from those to the other two coronaviruses. Despite the decreased expression of acute inflammatory and type I IFN genes in response to SARS-CoV-2, we observed increased expression of several genes associated with interleukin signaling, complement pathways, and chemokines. This finding echoes with the previously published study, which conducted RNAseq analysis to understand host transcriptional response to influenza A virus and SARS-CoV-2 in primary human bronchial epithelial cells (11). We used a publicly available subset of RNAseq data (GSE147507) from this study to compare it with independent datasets for SARS-CoV and MERS-CoV. It is worth mentioning that the list of DEGs unique to SARS-CoV-2 infection generated in our analysis was associated with coagulation and vascular function, which may explain why COVID-19 causes more systemic cardiovascular complications than do MERS and SARS (4, 8).

By analyzing RNAseq dataset of lung epithelial cells infected with SARS-CoV-2, we defined transcriptional signatures of 338 DEGs, including 92 increased and 246 decreased genes across the datasets. Among the top 10 increased DEGs, *IFITM10*, *CXCL14*, and *MYLK* are the most significantly increased genes. CXCL14 is a cytokine involved in immunoregulatory and inflammatory

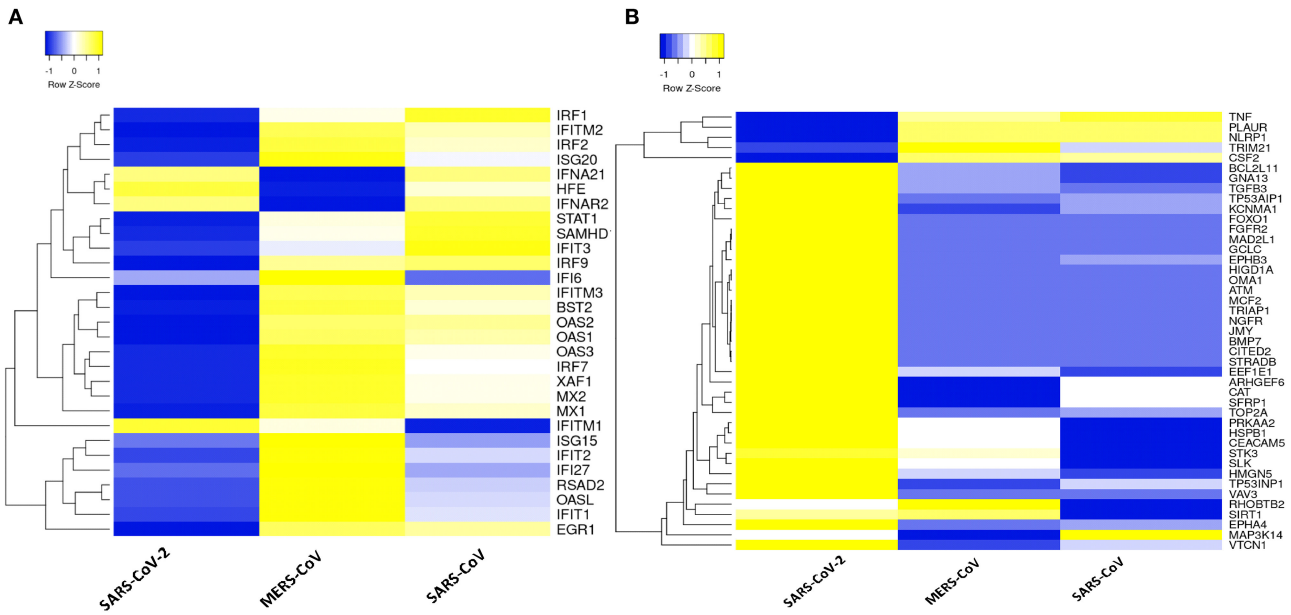

FIGURE 3 | Type 1 IFN response and apoptotic genes signature in three coronaviruses. **(A)** Heatmap representation of expression profiles for the type 1 IFN response genes (GO:0060337) between coronaviruses. **(B)** Heatmap representation of expression profiles for the apoptotic gene signatures (GO:0042981) between coronaviruses. Clustering of selected genes on the heatmap was performed by hierarchical clustering algorithm using Euclidean distance measure. Expression scale: blue (low expression) to yellow (high expression).

TABLE 4 | Network-based hub genes of SARS-CoV-2 specific DEGs.

Label	Degree	Betweenness	Expression
SMAD3	330	705,502.9	−0.32876
STAT1	223	437,249.9	−0.4737
SH3KBP1	178	358,520.9	−0.32778
HDGF	177	330,006.3	−0.38192
TUBB	173	263,526.3	−0.34572
NFKB2	138	213,825.4	−0.86093
ETS1	133	255,847.5	−0.43368
TUFM	116	174,412.6	−0.27641
UBC	106	115,3510	0
TRAF3	104	1,793,40.7	−0.43528
CCT5	99	147,216.3	−0.31476
RPL9	98	114,815.3	0.38872
TUBB4B	86	79,259.32	−0.38926
CSNK1E	84	158,782.5	−0.3193
S100A9	83	123,102.9	−1.0222

Top 15 genes prioritized based on topological parameters are shown. Expression levels are incorporated in the table from the transcriptome analysis result.

processes by mediating the chemotactic activity for monocytes and therefore can be implicated in the immune cell infiltration in the lung during SARS-CoV-2 infection (24). IFITM proteins family inhibit the entry of a large number of viruses; however, the exact role of IFITM10 as an antiviral agent remains unknown (25). SAA2 is the most significantly decreased gene in our analysis. SAA2 is a useful inflammatory marker in acute viral infections such as influenza, but its decreased expression in our analysis is consistent with the aberrant inflammatory response of SARS-CoV-2 infection (26). Viral infection is marked by the activation of immune system, which is evident from the enrichment of several pathways, including IL-17, TNF, and apoptosis signaling pathways among others (27). Patients who are infected with COVID-19 may develop pneumonia and progress to severe respiratory failure termed acute respiratory distress syndrome, which may result in the development of lung fibrosis

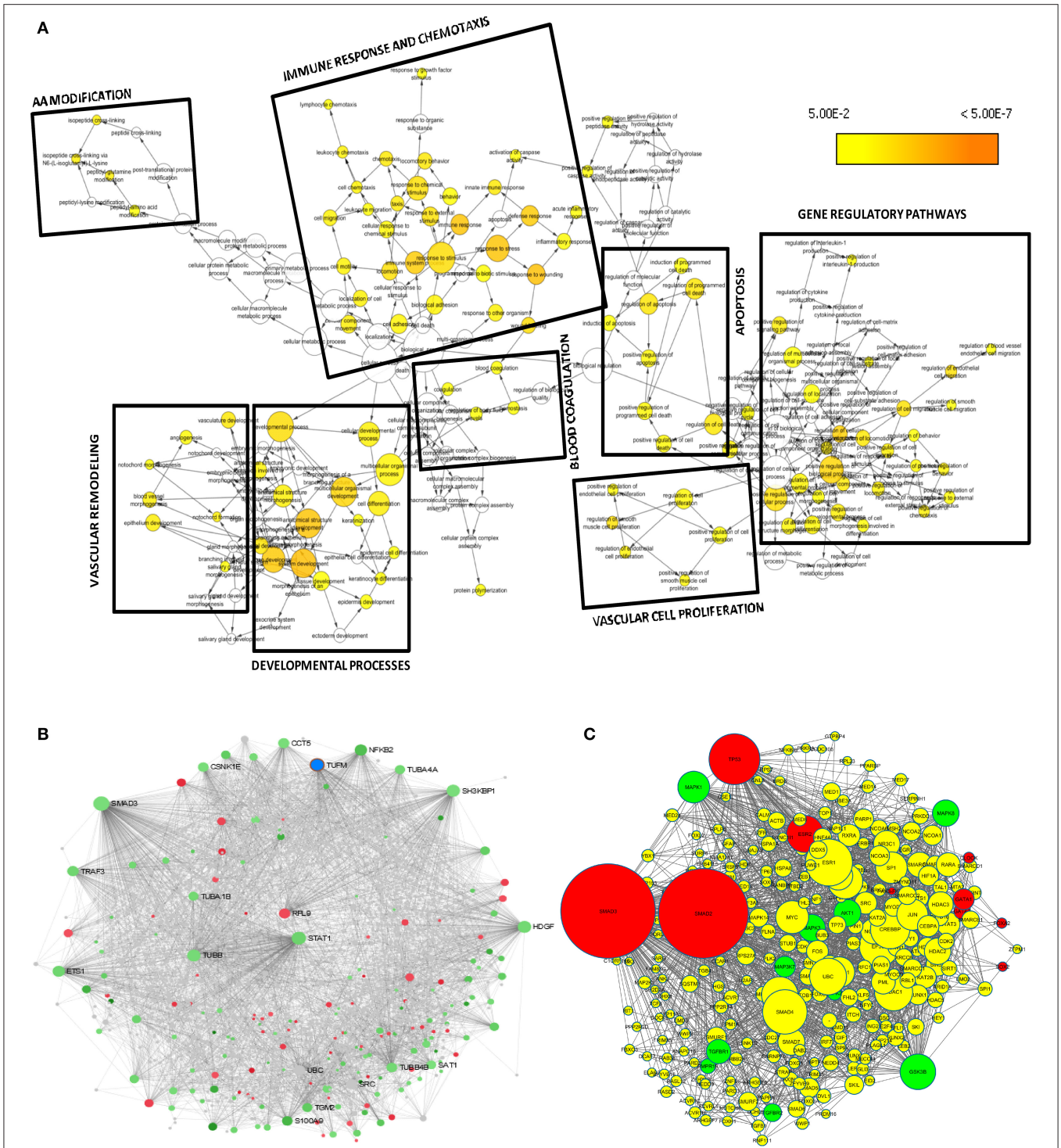

FIGURE 4 | Downstream analysis of SARS-CoV-2–specific DEGs. **(A)** Overrepresentation of pathways and Gene Ontology categories in biological networks identified from DEGs unique to SARS-CoV-2. Significantly overrepresented biological processes based on GO terms were visualized in Cytoscape. The size of a node is proportional to the number of targets in the GO category. The color represents enrichment significance—the deeper the color on a color scale, the higher the enrichment significance. *p* values were adjusted using a Benjamini and Hochberg FDR correction. Analysis revealed the enriched pathways associated with immune responses and chemotaxis, blood coagulation, apoptosis signaling pathway, vasculature remodeling, and vascular cell proliferation. **(B)** Network-based analysis of hub DEGs. Interaction network of SARS-CoV-2 unique genes; red nodes represent increased and green nodes represent decreased DEGs. **(C)** Regulatory gene network analysis of the top 10 enriched kinases (green) and transcription factors (red) using SARS-CoV-2 specific DEGs. Yellow nodes represent the intermediate proteins in the regulatory network. Node size represents the significance of protein based on *p* value; the bigger the node size, the higher the significance value.

TABLE 5 | Distinct pathways and related genes associated with SARS-CoV-2.

Gene	Gene name	Role	logFold change	Adj p-val
Apoptosis-related genes in COVID-19–infected lung epithelial cells				
NRP1	Neuropilin 1	Regulation of apoptotic pathways	0.332	0.023326
FOXO1	Forkhead box O1	Important regulator of cell death acting downstream of several signaling pathways including CDK1, PKB/AKT1 and STK4/MST1	0.432	0.014544
TP53INP1	Tumor Protein P53 inducible nuclear protein 1	Induce cell death by an autophagy and caspase-dependent mechanism	0.467	0.002053
CSF2	Colony-stimulating factor 2	Inhibits induction of apoptosis in several cell types	−1.136	2.92E-11
NLRP1	NLR family pyrin domain containing 1	Can induce pyroptosis, an inflammatory form of programmed cell death	−0.302	0.032517
Acute inflammation–related genes in COVID-19–infected lung epithelial cells				
SERPINA3	Serpin family A member 3	Is a typical acute-phase protein secreted into the circulation during acute and chronic inflammation	−1.194	1.26E-20
C1S	Complement C1s	Subunit of first component of the classical pathway of the complement system, released in acute inflammatory response	−0.525	0.01783
SAA2	Serum amyloid A2	SAA2 encode acute phase proteins (ASAA) that are released in response to inflammatory stimuli	−2.067	4.79E-77
IL6R	Interleukin 6 receptor	Regulation of the immune response, acute-phase reactions and hematopoiesis	0.498	0.000642
CFB	Complement factor B	Important component of complement system and inflammatory response	−1.563	5.47E-41
Vascular dysfunction–related genes in COVID-19–infected lung epithelial cells				
VAV3	Vav Guanine Nucleotide Exchange Factor 3	Vav3-induced cytoskeletal dynamics contribute to heterotypic properties of endothelial barriers, thus important in vascular stability	0.531	0.000426
TYMP	Thymidine phosphorylase	Role in maintaining the integrity of the blood vessels and angiogenesis	−0.755	6.23E-06
TCF4	Transcription factor 4	No known direct role	0.472	0.00286
NR2F2	Nuclear receptor subfamily 2 group F member 2	Suppression of COUP-TFII in venous ECs switched its phenotype toward proatherogenic by up-regulating the expression of inflammatory genes and down-regulating antithrombotic genes	−0.370	0.007664
Blood coagulation–related genes in COVID-19–infected lung epithelial cells				
F3	Coagulation factor III, tissue factor	Enables cells to initiate the blood coagulation cascades, and it functions as the high-affinity receptor for the coagulation factor VII	0.384	0.0002
PROS1	Protein S	Anticoagulant plasma protein, which helps to prevent coagulation and stimulating fibrinolysis	0.541	0.000358
IFNGR1	Interferon γ receptor 1	No known direct role	−0.365	0.01711
ITGB3	Integrin subunit beta 3	Rapid platelet aggregation, which physically plugs ruptured endothelial surface	−0.747	6.52E-05
TFPI2	Tissue factor pathway inhibitor 2	Protein can inhibit a variety of serine proteases including factor VIIa/tissue factor, thus suppress coagulation	−0.316	0.022707

List of differentially expressed pathways and genes associated with them. Possible roles were extracted from STRING database, and the expression values were added from the RNA-seq analysis results.

(28). Consistent with this report, several genes such as colony-stimulating factor 3 (*CSF3*), endothelin 1 (*EDN1*), plasminogen activator, urokinase (*PLAU*), and *MMP9* were reported to be differentially expressed in SARS-CoV-2 infection.

Previous studies revealed that lung epithelial cells, macrophages, and dendritic cells express cytokines to some extent during major viral infection causing cytokine storm. Evidence for molecular mechanisms of cytokine storm in

TABLE 6 | Cardiovascular dysfunction–related pathways and genes in SARS-CoV-2 infection.

Cardiovascular dysfunction–related pathway/terms	GO-ID	*p* value	Adjusted *p* value	Overlap	Associated genes
Vasculature development	1944	4.77E-05	3.35E-03	13/273	VAV3, NRP1, ITGB3, NR2F2, FOXO1, TYMP, ZC3H12A, COL8A1, TCF4, ITGA5, EPHA, TGM2, S100A7
Blood vessel morphogenesis	48514	1.20E-04	6.14E-03	11/220	VAV3, NRP1, ITGB3, ZC3H12A, COL8A1, NR2F2, ITGA5, EPHA2, TYMP, TGM2, S100A7
Leukocyte chemotaxis	30595	1.84E-04	7.54E-03	5/41	PDGFB, SAA2, IL6R, S100A9, CXCL16
Cell adhesion	7155	2.79E-04	9.71E-03	21/710	NRP1, DST, ITGB3, PCDH7, COL12A1,TNC, NPNT, NID1, F3, MTSS1, FLRT3, FEZ1, CDH10, LY6D, CLCA2, COL8A1, FAT2, NRCAM, FAT4, ITGA5, DSG3
Regulation of endothelial cell proliferation	1936	3.15E-04	1.01E-02	5/51	BMP2, ITGB3, PDGFB, NR2F2, F3
Regulation of chemotaxis	50920	4.63E-04	1.37E-02	5/51	SMAD3, PDGFB, F3, IL6R, S100A7
Positive regulation of cell death	10942	6.32E-04	1.69E-02	15/449	VAV3, NRP1, SMAD3, STAT1, TUBB, IGFBP3, ETS1, ALDH1A3, BMP2, TRAF3, KCNMA1, TP53INP1, NLRP1, BID, TGM2
Positive regulation of endothelial cell proliferation	1938	6.92E-04	1.80E-02	4/32	BMP2, ITGB3, PDGFB, F3
Angiogenesis	1525	7.31E-04	1.85E-02	8/152	VAV3, NRP1, ITGB3, ZC3H12A, COL8A1, ITGA5, TYMP, S100A7
Leukocyte migration	50900	1.14E-03	2.67E-02	5/62	PDGFB, SAA2, IL6R, S100A9, CXCL16
Regulation of smooth muscle cell migration	14910	1.97E-03	3.86E-02	3/20	IGFBP3, PDGFB, F3
Lymphocyte chemotaxis	48247	2.34E-03	4.23E-02	2/6	SAA2, CXCL16
Regulation of blood vessel endothelial cell migration	43535	2.62E-03	4.50E-02	3/22	EFNA1, PDGFB, EPHA2

The corresponding p values are adjusted, based on the false discovery rate using the Benjamini–Hochberg procedure.

COVID-19 remains limited. Earlier studies have shown IFN-γ-related cytokine storm in SARS patients (29), while delayed induction of proinflammatory cytokines and suppression of innate antiviral response by the MERS-CoV (30). It is crucial to identify the primary source of the cytokine storm in response to SARS-CoV-2 infection and the virological mechanisms behind the cytokine storm. Consistent to this model, our analysis of shared DEGs between three coronaviruses resulted in 17 DEGs, among which most molecules are related to acute inflammation. It is evident that important acute inflammatory genes (e.g., *TNFAIP3*, *CXCL1*, and *TNF*) are decreased in SARS-CoV-2–infected cells compared to other coronaviruses. These results may suggest that the major source of cytokine storm in COVID-19 is not lung epithelial cells, but possibly immune cell types. These aberrant transcriptional responses to SARS-CoV-2 may indicate low responses to infection, resulting in prolonged viral replication and serious lung damage in COVID-19 (31).

Our study linked DEGs unique to SARS-CoV-2 infection with pathway clusters related to immune responses, blood coagulation, apoptosis, and vascular remodeling. Apoptosis, which is a defense mechanism of hosts against the viral infection, depends on the rapid programmed cell death to curtail viral spread. Previous studies reported that SARS-CoV has evolved sophisticated molecular strategies to trigger host cell apoptotic defenses (32, 33). In our data, SARS-CoV-2 infection increased the expression of *NRP1* and *FOXO1*. NRP1 has a regulatory role of apoptotic pathways, whereas FOXO1 is an important regulator of cell death acting downstream of

several signaling pathways, including CDK1, PKB/AKT1, and STK4/MST1 (34).

Evidence suggests elevation of D-dimer and fibrin/fibrinogen degradation products in patients with COVID-19, highlighting aggravated blood coagulation (8). A recent clinical study examined seven lungs obtained during autopsy from patients with COVID-19 (35). The study observed vascular endothelial injury and widespread thrombus formation in pulmonary vessels. Immunohistochemical staining of pulmonary vasculature of COVID-19 showed alveolar capillary microthrombi were nine times as prevalent in patients with COVID-19 compared with influenza patients. In consistent with these reports, we identified coagulation-related genes in SARS-CoV-2 infection, including tissue factor that initiates the external coagulation cascades. In contrast, SARS-CoV-2 suppressed the expression of antithrombotic gene TFPI2, which inhibits a variety of serine proteases including factor VIIa/tissue factor complex.

Despite the clinical impact, the information on the mechanisms of COVID-19 and its cardiovascular complications remains limited. A systems approach, involving unbiased bioinformatics and network analysis, may help to identify causative genes and integrated pathways as drug targets for the improvement in disease management (36). Network-based analysis of hub genes in the DEGs dataset unique to SARS-CoV-2 infection resulted in prioritization of RPL9 as the most highly ranked DEG that had increased expression, based on betweenness centrality and degree score. The increased expression of RPL9, a ribosomal protein, can be attributed to the fact that virus hijacks the translational machinery of the host for its survival by the mechanisms such as ribosome shunting and phosphorylation of ribosomal proteins (37, 38).

Regulatory gene network analysis helps to understand what lies upstream of the DEGs in cells infected with SARS-CoV-2. It is important to find out the regulatory kinases and transcription factors as they participate in the pathogenesis and the progression of the virus infection (39). Among several kinases regulating the expression of DEGs expression in our analysis, genes involved in MAPK cascades (*MAPK1*, *MAPK2*, *MAPK8*, and *MAP3K7*) have roles in host response to viral infection (40). SMAD3, an effector molecule in the transforming growth factor-β signaling pathway, is also an interesting candidate in our analysis as it was the top hub gene in our network analysis and also the most significant transcription factor in our regulatory gene analysis. In the present study, SARS-CoV-2 reduced SMAD3 expression, which is consistent with previous findings on the decreased expression of SMAD3 during viral infection to overtake the host innate antiviral mechanism (41, 42).

In conclusion, our study provides the snapshot of transcriptional host responses to SARS-CoV-2 infection, in which expression of various inflammatory genes is decreased. These results may explain why many infected individuals show no symptoms. Furthermore, our study revealed that SARS-CoV-2 elicits muted antiviral type I IFN response, which may result in prolonged viral replication. These findings may also explain why SARS-CoV-2 infection has a longer incubation period than other coronavirus infections. Our analysis revealed expression of several genes related to apoptosis, coagulation, and vascular function, which may contribute to cardiovascular complications. Furthermore, our study has identified a novel set of candidate transcriptomic signatures unique to SARS-CoV-2 infection, which may guide the initial efforts in the development of diagnostic or therapeutic tools for 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

PJ, AV, and MA conceived, coordinated, and designed the study. PJ and AV retrieved the datasets, did the analysis, and wrote the manuscript. MA did the editing and result interpretation. AH and SU edited and provided technical advices on the study. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2020.623012/full#supplementary-material

REFERENCES

1. Thao TTN, Labroussaa F, Ebert N, V'kovski P, Stalder H, Portmann J, et al. Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform. *Nature.* (2020) 582:561–5. doi: 10.1038/s41586-020-2294-9
2. Zhou P, Yang X-L, Wang X-G, 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. 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
4. 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
5. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N Engl J Med.* (2020) 382:1177–9. doi: 10.1056/NEJMc2001737

6. Peiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, Poon LL, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet.* (2003) 361:1767–72. doi: 10.1016/S0140-6736(03)13412-5
7. Oh M-d, Park WB, Choe PG, Choi S-J, Kim J-I, Chae J, et al. Viral load kinetics of MERS coronavirus infection. *N Engl J Med.* (2016) 375:1303–5. doi: 10.1056/NEJMc1511695
8. Connors JM, Levy JH. COVID-19 and its implications for thrombosis and anticoagulation. *Blood.* (2020) 135:2033–40. doi: 10.1182/blood.2020006000
9. Middeldorp S, Coppens M, van Haaps TF, Foppen M, Vlaar AP, Müller MCA, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. *J Thromb Haemost.* (2020) 18:1995–2002. doi: 10.20944/preprints202004.0345.v1
10. 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
11. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Moller R, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell.* (2020) 181:1036–45.e9. doi: 10.1016/j.cell.2020.04.026
12. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* (2014) 15:550. doi: 10.1186/s13059-014-0550-8
13. Xia J, Benner MJ, Hancock RE. NetworkAnalyst–integrative approaches for protein-protein interaction network analysis and visual exploration. *Nucleic Acids Res.* (2014) 42:W167–74. doi: 10.1093/nar/gku443
14. Lin SM, Du P, Huber W, Kibbe WA. Model-based variance-stabilizing transformation for Illumina microarray data. *Nucleic Acids Res.* (2008) 36:e11. doi: 10.1093/nar/gkm1075
15. Yoshikawa T, Hill TE, Yoshikawa N, Popov VL, Galindo CL, Garner HR, et al. Dynamic innate immune responses of human bronchial epithelial cells to severe acute respiratory syndrome-associated coronavirus infection. *PLoS ONE.* (2010) 5:e8729. doi: 10.1371/journal.pone.0008729
16. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* (2015) 43:e47. doi: 10.1093/nar/gkv007
17. Witten DM, Tibshirani R, Hastie T. A penalized matrix decomposition, with applications to sparse principal components and canonical correlation analysis. *Biostatistics.* (2009) 10:515–34. doi: 10.1093/biostatistics/kxp008
18. Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinform.* (2013) 14:128. doi: 10.1186/1471-2105-14-128
19. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet.* (2000) 25:25–9. doi: 10.1038/75556
20. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* (2003) 13:2498–504. doi: 10.1101/gr.1239303
21. Maere S, Heymans K, Kuiper M. BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics.* (2005) 21:3448–9. doi: 10.1093/bioinformatics/bti551
22. Lachmann A, Xu H, Krishnan J, Berger SI, Mazloom AR, Ma'ayan A. ChEA: transcription factor regulation inferred from integrating genome-wide ChIP-X experiments. *Bioinformatics.* (2010) 26:2438–44. doi: 10.1093/bioinformatics/btq466
23. Chen EY, Xu H, Gordonov S, Lim MP, Perkins MH, Ma'ayan A. Expression2Kinases: mRNA profiling linked to multiple upstream regulatory layers. *Bioinformatics.* (2012) 28:105–11. doi: 10.1093/bioinformatics/btr625
24. Schett G, Sticherling M, Neurath MF. COVID-19: risk for cytokine targeting in chronic inflammatory diseases? *Nat Rev Immunol.* (2020) 20:271–2. doi: 10.1038/s41577-020-0312-7
25. Qian J, Le Duff Y, Wang Y, Pan Q, Ding S, Zheng YM, et al. Primate lentiviruses are differentially inhibited by interferon-induced transmembrane proteins. *Virology.* (2015) 474:10–8. doi: 10.1016/j.virol.2014.10.015
26. Vollmer AH, Gebre MS, Barnard DL. Serum amyloid A (SAA) is an early biomarker of influenza virus disease in BALB/c, C57BL/2, Swiss-Webster, and DBA.2 mice. *Antiviral Res.* (2016) 133:196–207. doi: 10.1016/j.antiviral.2016.08.011
27. Mukherjee S, Lindell DM, Berlin AA, Morris SB, Shanley TP, Hershenson MB, et al. IL-17-induced pulmonary pathogenesis during respiratory viral infection and exacerbation of allergic disease. *Am J Pathol.* (2011) 179:248–58. doi: 10.1016/j.ajpath.2011.03.003
28. George PM, Wells AU, Jenkins RG. Pulmonary fibrosis and COVID-19: the potential role for antifibrotic therapy. *Lancet Respir Med.* (2020) 8:807–15. doi: 10.1016/S2213-2600(20)30225-3
29. Huang KJ, Su IJ, Theron M, Wu YC, Lai SK, Liu CC, et al. An interferon-gamma-related cytokine storm in SARS patients. *J Med Virol.* (2005) 75:185–94. doi: 10.1002/jmv.20255
30. Lau SKP, Lau CCY, Chan KH, Li CPY, Chen H, Jin DY, et al. Delayed induction of proinflammatory cytokines and suppression of innate antiviral response by the novel Middle East respiratory syndrome coronavirus: implications for pathogenesis and treatment. *J Gen Virol.* (2013) 94 (Pt 12):2679–90. doi: 10.1099/vir.0.055533-0
31. Blanco-Melo D, Nilsson-Payant BE, Liu W-C, Møller R, Panis M, Sachs D, et al. SARS-CoV-2 launches a unique transcriptional signature from *in vitro*, *ex vivo*, and *in vivo* systems. bioRxiv. (2020). doi: 10.1101/2020.03.24.004655
32. Krahling V, Stein DA, Spiegel M, Weber F, Muhlberger E. Severe acute respiratory syndrome coronavirus triggers apoptosis via protein kinase R but is resistant to its antiviral activity. *J Virol.* (2009) 83:2298–309. doi: 10.1128/JVI.01245-08
33. Tan YX, Tan TH, Lee MJ, Tham PY, Gunalan V, Druce J, et al. Induction of apoptosis by the severe acute respiratory syndrome coronavirus 7a protein is dependent on its interaction with the Bcl-XL protein. *J Virol.* (2007) 81:6346–55. doi: 10.1128/JVI.00090-07
34. Fu Z, Tindall DJ. FOXOs, cancer and regulation of apoptosis. *Oncogene.* (2008) 27:2312–9. doi: 10.1038/onc.2008.24
35. 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
36. Barabasi AL, Gulbahce N, Loscalzo J. Network medicine: a network-based approach to human disease. *Nat Rev Genet.* (2011) 12:56–68. doi: 10.1038/nrg2918
37. Hertz MI, Landry DM, Willis AE, Luo G, Thompson SR. Ribosomal protein S25 dependency reveals a common mechanism for diverse internal ribosome entry sites and ribosome shunting. *Mol Cell Biol.* (2013) 33:1016–26. doi: 10.1128/MCB.00879-12
38. Meyuhas O. Physiological roles of ribosomal protein S6: one of its kind. *Int Rev Cell Mol Biol.* (2008) 268:1–37. doi: 10.1016/S1937-6448(08)00801-0
39. Ramezani A, Nahad MP, Faghihloo E. The role of Nrf2 transcription factor in viral infection. *J Cell Biochem.* (2018) 119:6366–82. doi: 10.1002/jcb.26897
40. Chu WM, Ostertag D, Li ZW, Chang L, Chen Y, Hu Y, et al. JNK2 and IKKbeta are required for activating the innate response to viral infection. *Immunity.* (1999) 11:721–31. doi: 10.1016/S1074-7613(00)80146-6
41. Gough NR. Enhancing and inhibiting TGF-β signaling in infection. *Sci Signal.* (2015) 8:ec9-ec. doi: 10.1126/scisignal.aaa6549
42. Nie Y, Cui D, Pan Z, Deng J, Huang Q, Wu K. HSV-1 infection suppresses TGF-beta1 and SMAD3 expression in human corneal epithelial cells. *Mol Vis.* (2008) 14:1631–8.

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Biventricular Longitudinal Strain Predict Mortality in COVID-19 Patients

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Background: Biventricular longitudinal strain has been recently demonstrated to be predictive of poor outcomes in various cardiovascular settings. Therefore, this study sought to investigate the prognostic implications of biventricular longitudinal strain in patients with coronavirus disease 2019 (COVID-19).

Methods: We enrolled 132 consecutive patients with COVID-19. Left ventricular global longitudinal strain from the apical four-chamber views (LV GLS_{4CH}) and right ventricular free wall longitudinal strain (RV FWLS) were obtained using two-dimensional speckle-tracking echocardiography.

Results: Compared with patients without cardiac injury, those with cardiac injury had higher levels of coagulopathy and inflammatory biomarkers, higher incidence of complications, more mechanical ventilation therapy, and higher mortality. Patients with cardiac injury displayed decreased LV GLS_{4CH} and RV FWLS, elevated pulmonary artery systolic pressure, and higher proportion of pericardial effusion. Higher biomarkers levels of inflammation and cardiac injury, and the presence of pericardial effusion were correlated with decreases in LV GLS_{4CH} and RV FWLS. During hospitalization, 19 patients died. Compared with survivors, LV GLS_{4CH} and RV FWLS were impaired in non-survivors. At a 3-month follow-up after discharge, significant improvements were observed in LV GLS_{4CH} and RV FWLS. Multivariate Cox analysis revealed that LV GLS_{4CH} [hazard ratio: 1.41; 95% confidence interval [CI]: 1.08 to 1.84; $P = 0.011$] and RV FWLS (HR: 1.29; 95% CI: 1.09–1.52; $P = 0.003$) were independent predictors of higher mortality in patients with COVID-19.

Conclusions: LV GLS_{4CH} and RV FWLS are independent and strong predictors of higher mortality in COVID-19 patients and can track improvement during the convalescent phase of their illness. Therefore, biventricular longitudinal strain may be crucial for risk stratification and serial follow-up in patients with COVID-19.

Keywords: COVID-19, speckle tracking echocardiography, strain, left ventricular function, right ventricular function

INTRODUCTION

Coronavirus disease 2019 (COVID-19), which is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a pandemic health crisis. Although, there is increasing awareness of the cardiovascular involvement in COVID-19 disease and its adverse impact on prognosis (1, 2), there is limited data regarding cardiac abnormalities due to SARS-CoV- 2 infection. Echocardiography remains the mainstay imaging modality for assessing cardiac function in clinical practice. Recently, left ventricular (LV) and right ventricular (RV) longitudinal strain measured by two-dimensional speckle-tracking echocardiography (2D-STE) has been proposed as more accurate and sensitive indicators of cardiac function in a variety of cardiovascular diseases (3–5). Furthermore, a number of studies confirmed the prognostic value of biventricular longitudinal strain in various clinical settings (6–8). However, the prognostic implications of biventricular longitudinal strain in COVID-19 patients has not been well-established. Accordingly, our study aimed to investigate whether biventricular longitudinal strain were independently predictive of higher mortality in patients with COVID-19 and explore their utility in the follow-up in these patients.

METHODS

Study Population

This single-center, prospective study was performed at the west branch of Union Hospital, Huazhong University of Science and Technology, China, which was a designated hospital to treat patients with COVID-19. We enrolled 169 consecutive adult patients who were diagnosed with COVID-19 according to interim guidance of World Health Organization, from February 11 to March 16, 2020. Considering the presence of cardiac involvement in COVID-19 patients, bedside echocardiography was performed in all patients from three wards managed by the investigators for evaluation of cardiac function. The median time from admission to echocardiographic assessment was 7 days [interquartile range [IQR] 3–11]. Among these patients, three had dilated cardiomyopathy, four had old myocardial infarction, and 30 did not have images of sufficient quality for STE analysis. Finally, 132 patients were recruited in our analysis.

This study was approved by Union Hospital, Tongji Medical College, Huazhong University of Science and Technology Ethics Committee (KY-2020-02.06). Written informed consent was waived for all participants with emerging infectious diseases. Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research.

Data Collection

Demographic characteristics, comorbidities, laboratory findings, medical history, complications, and outcomes for patients during hospitalization were independently reviewed by a team of trained physicians from electronic medical records. The timing of laboratory measurements were within 3 days of echocardiographic examinations with a mean interval of 1 days (IQR: 1–2). Acute cardiac injury was defined as serum levels of cardiac high-sensitivity troponin I (hs-TNI) above the 99th-percentile upper reference limit. The outcome was defined as in-hospital death. The final date of follow-up outcome were April 9, 2020.

Transthoracic Echocardiography

Bedside transthoracic echocardiographic examinations were performed using an EPIQ7C machine (Philips Medical Systems, Andover, MA, USA) at the designated COVID-19 isolation wards or intensive care units (ICU). Forty-six survivors underwent follow-up echocardiographic examinations at 3 months after discharge. All scans were conducted by trained individuals in full personal protective equipment. All echocardiographic images were stored in digital format and analyzed by two independent observers (C.M. and Y.Z.) who were blinded to epidemiological and clinical characteristics, laboratory findings, treatment, and outcomes.

Conventional Echocardiographic Analysis

Left ventricular (LV) and right ventricular (RV) structural and functional parameters were measured based on the guidelines of the American Society of Echocardiography (9). LV mass was assessed by the Devereux's formula. LV volumes and ejection fraction (EF) were obtained using Simpson's biplane method. LV diastolic function was assessed by the ratio of peak early-diastolic transmitral inflow velocity (E) to late-diastolic inflow velocity (A), and the ratio of transmitral E to the peak early-diastolic mitral annual velocity (e′). We also measured the deceleration time (DT) of the E-wave.

Tricuspid annular plane systolic excursion (TAPSE) was measured on M-mode echocardiography. RV fractional area change (RVFAC) was calculate as (RV end-diastolic area -RV end-systolic area)/end-diastolic area ×100%. Tricuspid lateral annular systolic velocity (S′) was assessed by tissue Doppler imaging from the apical 4-chamber view. Pulmonary artery systolic pressure (PASP) was evaluated using the simplified Bernoulli equation and right atrial pressure assessed on the basis of the size and collapsibility of the inferior vena cava.

STE Analysis

STE analyses were performed using commercially available AutoStrain software (Qlab13, Philips Healthcare, Andover, MA, USA). LV global longitudinal strain (GLS) was calculated by averaging the values obtained in the apical 4-chamber, 3-chamber and 2-chamber views. LV GLS_{4CH} was defined as the mean of the strain values in the six segments of left ventricle from the apical 4-chamber view. LV GLS and GLS_{4CH} were obtained from the standard two-dimensional gray-scale image with a frame rate of 50∼70 frames/s. The LV endocardial border was automatically traced at end diastole. Subsequently, the software tracked the endocardial layer throughout the cardiac cycle. The operator could manually adjust the endocardial border if necessary. Right ventricular free wall longitudinal strain (RV FWLS) was obtained from the standard two-dimensional gray-scale image of the RV-focused apical four-chamber view with a frame rate of 50∼70 frames/s. The RV endocardial border was automatically traced at end diastole. The software tracked automatically the endocardial

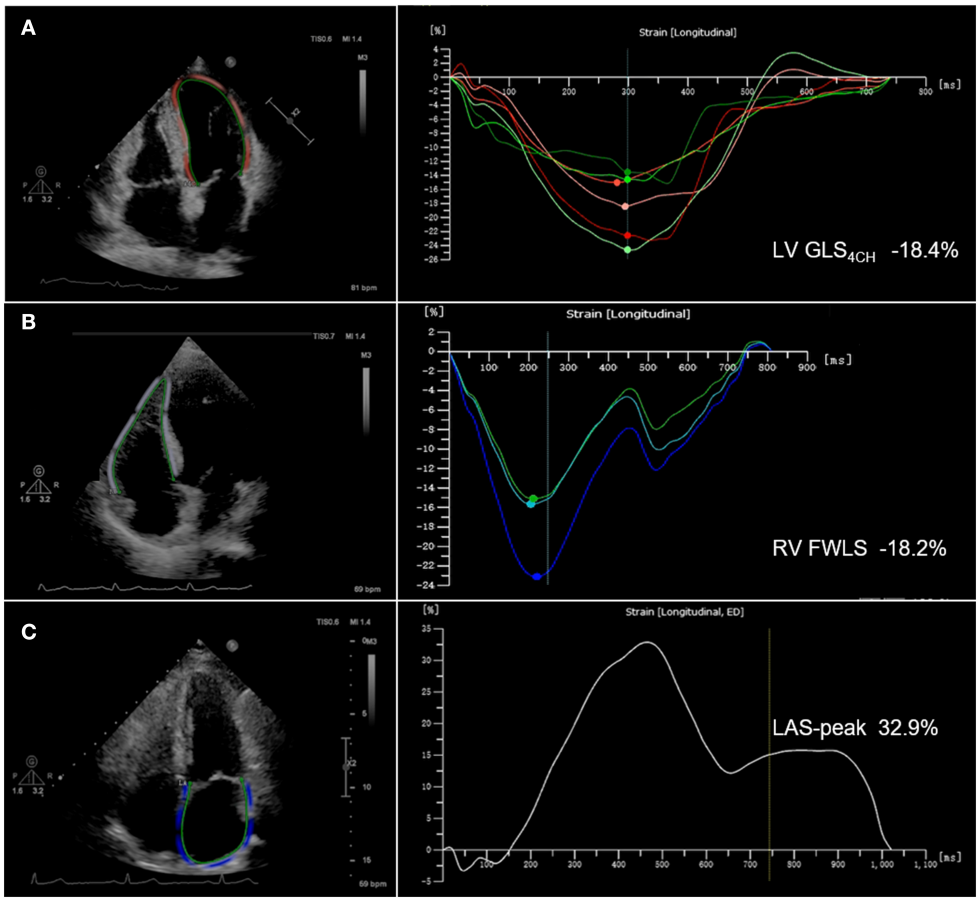


FIGURE 1 | Biventricular and left atrial longitudinal strain obtained from two-dimensional speckle-tracking echocardiography in COVID-19 patients. **(A)** Representative image with left ventricular global longitudinal strain from the apical 4-chamber view (LV GLS_{4CH}); **(B)** Representative image with right ventricular free wall longitudinal strain (RV FWLS); **(C)** Representative image with peak left atrial strain (LAS-peak).

layer throughout the cardiac cycle and the observer may manually adjust the endocardial border if necessary. RV FWLS was calculated as the average of the basal, mid and apical RV free wall segments. Left atrial (LA) endocardial contours were drawn in the apical 4-chamber view with a frame rate of 50∼70 frames/s at end systole. The appendage and pulmonary veins were not included. The endocardial border was automatically tracked by software throughout the cardiac cycle. Manual adjustments were performed when tracking was suboptimal. Peak left atrial strain (LAS-peak) was automatically generated from the software. Patients with two or more inadequately tracked segments were removed from analysis. Representative images with LV GLS_{4CH}, RV FWLS and LAS-peak are shown in **Figure 1**. Absolute values of LV GLS, GLS_{4CH} and RV FWLS were presented in this study for a simpler interpretation, as LV GLS, GLS_{4CH} and RV FWLS were negative values.

Interobserver and Intraobserver Reproducibility

Intraobserver and interobserver variability of LV GLS_{4CH}, RV FWLS and LAS-peak were estimated in 20 randomly selected subjects and evaluated by intra-class correlation coefficient (ICC) and Bland-Altman analysis. Intraobserver variability was evaluated by having one observer remeasure after 4 weeks. Interobserver variability was assessed by a second observer who was blinded to the first observer's measurements.

Statistical Analysis

Continuous numeric variables were expressed as mean ± SD or medians (IQR) and compared using a two-sample Student's *t*-test and one-way analysis of variance for normally distributed data, or Mann-Whitney test and Kruskal-Wallis test for non-normally distributed data. Categorical variables were expressed as frequency (percentage), and compared using the χ^2 test or Fisher's exact test. Spearman's correlation coefficient were used to evaluate the association between biventricular strain and laboratory findings. Univariate and multivariate Cox regression models were used to assess the predictors of higher mortality. All potential predictors of higher mortality were included into univariate analyses: age, gender, comorbidities, complications, laboratory findings and echocardiographic parameters. Variables with $P < 0.05$ at

TABLE 1 | Baseline clinical characteristics of patients with COVID-19 according to acute cardiac injury.

Variables	All patients ($n = 132$)	Without cardiac injury ($n = 92$)	With cardiac injury ($n = 40$)	*P*-value
Clinical characteristics				
Age, years	61 ± 13	60 ± 13	63 ± 12	0.176
Male, *n* (%)	68 (51.5%)	43 (46.7%)	25 (62.5%)	0.096
Body mass index, kg/m^2	23.6 ± 2.9	23.6 ± 2.9	23.8 ± 3.0	0.653
Heart rate, beats/min	86 (80,102)	86 (80,100)	90 (80,107)	0.143
Respiratory rate, breaths/min	23 (20,30)	23 (20,30)	24 (20,29)	0.966
SBP, mm Hg	132 (120,144)	132 (121,144)	131 (115,146)	0.735
DBP, mm Hg	80 (73,87)	80 (75,89)	80 (72,85)	0.235
Smoker, *n* (%)	6 (4.5%)	4 (4.3%)	2 (5.0%)	0.591
Comorbidities				
Hypertension, *n* (%)	58 (43.9%)	38 (41.3%)	20 (50.0%)	0.355
Diabetes mellitus, *n* (%)	15 (11.4%)	12 (13.0%)	3 (7.5%)	0.533
Obesity, *n* (%)	20 (15.2%)	15 (16.3%)	5 (12.5%)	0.767
COPD, *n* (%)	5 (3.8%)	3 (3.3%)	2 (5.0%)	0.639
Coronary artery disease, *n* (%)	19 (14.4%)	10 (10.9%)	9 (22.5%)	0.080
Chronic kidney disease, *n* (%)	1 (0.8%)	1 (1.1%)	0 (0)	1.000
Chronic liver disease, *n* (%)	5 (3.8%)	3 (3.3%)	2 (5.0%)	0.639
Arrhythmia, *n* (%)	9 (6.8%)	6 (6.5%)	3 (7.5%)	1.000
Malignancy, *n* (%)	9 (6.8%)	6 (6.5%)	3 (7.5%)	1.000
Laboratory findings				
Lymphocyte count, × 10^9/l	1.0 (0.6,1.5)	1.1 (0.7,1.6)	0.6 (0.4,1.1)	0.001
D-dimer, mg/l	1.1 (0.3, 3.0)	1.0 (0.4, 2.8)	1.6 (0.2, 4.3)	0.789
PT, s	13.7 (12.5, 15.0)	13.2 (12.4, 14.3)	13.9 (13.2, 15.3)	0.021
APTT, s	37.7 (33.1, 44.7)	36.8 (32.6, 42.1)	39.5 (36.7, 45.7)	0.013
CK-MB, U/l	10 (6, 15)	9 (5, 13)	14 (9, 30)	<0.001
hs-TNI, ng/l	4.1 (2.0, 30.2)	3.0 (1.5, 4.8)	85.6 (51.8, 262.1)	<0.001
BNP, pg/ml	62.4 (31.5, 164.2)	53.4 (29.3, 120.5)	130.5 (41.2, 449.0)	0.019
PaO_2/FIO_2, mm Hg	233.3 (153.5, 270.7)	236.4 (156.0, 272.4)	221.5 (144.7, 274.0)	0.559
CRP, mg/l	26.5 (3.8, 68.0)	15.8 (3.0, 52.6)	54.0 (18.4, 128.4)	0.001
PCT, ng/ml	0.09 (0.05, 0.21)	0.06 (0.04, 0.14)	0.23 (0.07, 0.39)	<0.001
IL-6, pg/ml	4.1 (2.0, 21.0)	3.9 (1.2, 7.8)	11.2 (2.9, 23.4)	0.039
Treatments				
Antiviral therapy, *n* (%)	122 (92.4%)	86 (93.5%)	36 (90.0%)	0.737
Antibiotic therapy, *n* (%)	98 (74.2%)	64 (69.6%)	34 (85.0%)	0.062
Glucocorticoid therapy, *n* (%)	57 (43.5%)	30 (32.6%)	27 (67.5%)	<0.001
Intravenous immune globulin, *n* (%)	49 (37.1%)	30 (32.6%)	19 (47.5%)	0.104
Anticoagulant therapy, *n* (%)	62 (47.0%)	41 (44.5%)	21 (52.5%)	0.401
Diuretics, *n* (%)	35 (26.5%)	21 (22.8%)	14 (35.9%)	0.145
Beta-blockers, *n* (%)	26 (19.7%)	19 (20.7%)	7 (17.5%)	0.676
Alpha-blockers, *n* (%)	2 (1.5%)	1 (1.1%)	1 (2.5%)	0.516
Calcium channel blockers, *n* (%)	40 (30.3%)	28 (30.4%)	12 (30.0%)	0.960
ACE inhibitor/ARB, *n* (%)	9 (6.9%)	7 (7.8%)	2 (5.0%)	0.840
Oxygen therapy, *n* (%)	117 (88.6%)	80 (87.0%)	37 (92.5%)	0.523
High-flow oxygen, *n* (%)	72 (55.0%)	45 (49.5%)	27 (67.5%)	0.056
Mechanical ventilation, *n* (%)	32 (24.2%)	15 (16.3%)	17 (42.5%)	0.001
IMV, *n* (%)	22 (16.7%)	10 (10.9%)	12 (30.0%)	0.007
NIMV, *n* (%)	10 (7.6%)	5 (5.4%)	5 (12.5%)	0.170
ICU admission, *n* (%)	25 (18.9%)	13 (14.1%)	12 (30.0%)	0.032
Complications				
Acute kidney injury, *n* (%)	20 (15.2%)	6 (6.5%)	14 (35.0%)	<0.001
ARDS, *n* (%)	49 (37.1%)	28 (30.4%)	21 (52.5%)	0.016

(Continued)

TABLE 1 | Continued

Variables	All patients ($n = 132$)	Without cardiac injury ($n = 92$)	With cardiac injury ($n = 40$)	*P*-value
Shock, *n* (%)	1 (0.8%)	0 (0)	1 (2.5%)	0.303
Prognosis				
Discharge, *n* (%)	113 (85.6%)	88 (95.7%)	25 (62.5%)	<0.001
Death, *n* (%)	19 (14.4%)	4 (4.3%)	15 (37.5%)	<0.001

Values are mean ± SD, n (%), median (interquartile range). ACE, angiotensin-converting enzyme; APTT, activated partial thromboplastin time; ARB, angiotensin II receptor blocker; ARDS, acute respiratory distress syndrome; BNP, B-type natriuretic peptide; CK-MB, creatine kinase muscle-brain; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; DBP, diastolic blood pressure; FIO_2, fraction of inspiration oxygen; ICU, intensive care unit; IL-6, interleukin-6; IMV, invasive mechanical ventilation; hs-TNI, high-sensitivity troponin I; NIMV, non-invasive mechanical ventilation; PaO_2, partial pressure of oxygen; PCT, procalcitonin; PT, prothrombin time; SBP, systolic blood pressure.

TABLE 2 | Echocardiographic characteristics of patients with COVID-19 according to acute cardiac injury.

Variables	All patients ($n = 132$)	Without cardiac injury ($n = 92$)	With cardiac injury ($n = 40$)	*P*-value
Left heart				
LA dimension, mm	34.3 ± 5.4	34.4 ± 5.3	34.1 ± 5.7	0.728
LV dimension, mm	45.5 ± 4.9	45.6 ± 4.8	45.3 ± 4.9	0.715
IVS, mm	9.6 ± 1.3	9.5 ± 1.4	9.9 ± 1.1	0.183
PW, mm	9.2 (8.3, 9.9)	9.0 (8.1, 9.8)	9.6 (8.9, 10.4)	0.018
LVM, g	143.2 (116.0, 168.5)	143.1 (117.9, 168.6)	154.4 (114.9, 168.6)	0.707
DT, ms	204.4 ± 53.7	203 ± 54.6	207 ± 50.5	0.686
E/A ratio	0.8 (0.7,1.1)	0.8 (0.7,1.1)	0.8 (0.7,1.1)	0.884
E/e′ ratio	8.4 (6.8,10.6)	8.9 ± 3.1	9.4 ± 3.4	0.453
LVEDVI, ml/m^2	52.2 ± 16.1	53.3 ± 14.8	49.5 ± 19.4	0.349
LVESVI, ml/m^2	19.6 ± 7.5	20.2 ± 7.0	18.1 ± 8.8	0.256
LVEF, %	62.8 ± 6.9	62.7 ± 7.4	63.2 ± 5.8	0.735
LV GLS_{4CH}, %	18.9 (16.8, 20.9)	19.1 (17.1, 20.9)	17.3 (15.8, 20.4)	0.017
LAS-peak, %	33.7 ± 7.6	34.1 ± 8.0	33.0 ± 6.7	0.489
Moderate-severe MR, *n* (%)	2 (1.5%)	0 (0)	2 (5.0%)	0.090
Right heart				
RA dimension, mm	35.5 ± 4.6	35.2 ± 4.4	36.6 ± 5.1	0.126
RV dimension, mm	33.9 ± 4.4	33.6 ± 4.3	34.7 ± 4.7	0.250
TAPSE, mm	22.2 ± 3.8	22.8 ± 3.8	20.8 ± 3.2	0.005
RVFAC, %	46.9 ± 6.6	47.6 ± 6.3	45.3 ± 7.1	0.066
S′, cm/s	13.3 (11.9,15.0)	14.0 (12.0,15.0)	13.0 (11.0,15.0)	0.361
RV FWLS, %	22.8 ± 4.9	23.5 ± 5.2	21.1 ± 3.8	0.009
Moderate-severe TR, *n* (%)	4 (3.0%)	2 (2.2%)	2 (5.0%)	0.584
PASP, mm Hg	33 (24,47)	28 (23,43)	41 (30,54)	0.007
Pericardial effusion, *n* (%)	11 (8.3%)	4 (4.3%)	7 (17.5%)	0.030

Values are mean ± SD, n (%), median (interquartile range). COVID-19, coronavirus disease 2019; DT, peak E deceleration time of mitral inflow; IVS., interventricular septum; LA, left atrial; LAS, left atrial strain; LV, left ventricular; LV GLS_{4CH}, left ventricular global longitudinal strain derived from the apical four-chamber view; LVEDVI, left ventricular end diastolic volume index; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end systolic volume index; LVM, left ventricular mass; MR, mitral regurgitation; PASP, pulmonary artery systolic pressure; PW, posterior wall of left ventricle; RA, right atrial; RV, right ventricular; RV FWLS, right ventricular free wall longitudinal strain; RVFAC, RV fractional area change; SBP, systolic blood pressure; TAPSE, tricuspid annular plane systolic excursion; TR, tricuspid regurgitation. LV GLS_{4CH} and RV FWLS values are absolute values.

univariate analysis were entered into multivariate Cox regression models. Owing to smaller patients with endpoints, there may exist an over-fitting issue. Therefore, to avoid problems of overfitting the data, a separate Cox proportional hazard model including clinical variables and each of biventricular function parameters (LV GLS_{4CH}, TAPSE, RVFAC, and RV FWLS), was used to determine the independent predictors of higher mortality. The model performance was assessed by Akaike Information Criterion (AIC). Receiver operator characteristic (ROC) curves were used to determine the optimal cutoff value of LA, LV and RV function parameters for detecting poor outcomes. Kaplan-Meier survival curves were plotted and compared using the log-rank test. All statistical analyses were performed using a SPSS version 20.0 (SPSS Inc., Chicago, Illinois), and a two-sided value of $P < 0.05$ was considered as statistically significant.

RESULTS

Clinical Characteristics

Clinical characteristics of patients with COVID-19 are presented in **Table 1**. The mean age of patients was 61 ± 13 years, and 68 (51.5%) were male. Of the 132 patients, 40 (30.3%) patients displayed acute cardiac injury. Compared with patients without cardiac injury, those with cardiac injury had lower lymphocyte count, and higher levels of coagulopathy and inflammatory biomarkers [prothrombin time (PT), activated partial thromboplastin time (APTT), C-reactive protein (CRP), procalcitonin (PCT) and interleukin 6 (IL-6)]. The levels of creatine kinase muscle-brain (CK-MB) and B-type natriuretic peptide levels were also higher in patients with cardiac injury than those without. Additionally, patients with cardiac injury were more likely to develop acute kidney injury and

TABLE 3 | Clinical and echocardiographic characteristics of patients with COVID-19 according to disease severity.

Variables	All patients ($n = 132$)	Disease severity			p-value
		Moderate ($n = 50$)	Severe ($n = 35$)	Critical ($n = 47$)	
Clinical characteristics					
Age, years	61 ± 13	59 ± 12	62 ± 15	63 ± 12	0.241
Male, *n* (%)	68 (51.5%)	20 (40.0%)	19 (54.3%)	29 (61.7%)	0.093
Body mass index, kg/m^2	23.6 ± 2.9	23.6 ± 2.6	23.4 ± 3.0	23.9 ± 3.2	0.810
Heart rate, beats/min	91 ± 17	90 ± 16	88 ± 15	94 ± 19	0.216
Respiratory rate, breaths/min	25 ± 6	24 ± 6	24 ± 5	25 ± 7	0.707
SBP, mm Hg	134 ± 18	134 ± 17	133 ± 20	131 ± 17	0.742
DBP, mm Hg	81 ± 12	82 ± 10	81 ± 14	80 ± 12	0.767
Left heart					
LA dimension, mm	34.3 ± 5.4	34.3 ± 4.9	35.2 ± 4.7	34.2 ± 6.5	0.466
LV dimension, mm	45.5 ± 4.9	44.9 ± 4.6	46.4 ± 4.8	45.8 ± 5.1	0.526
IVS, mm	9.6 ± 1.3	9.7 ± 1.1	9.6 ± 1.9	9.6 ± 1.0	0.629
PW, mm	8.8 ± 1.9	9.2 ± 1.1	8.2 ± 2.7*	9.0 ± 1.7	0.049
LVM, g	144.3 ± 36.1	143.7 ± 35.6	148.0 ± 40.9	144.7 ± 33.6	0.861
DT, ms	204.4 ± 53.7	209.3 ± 60.4	190.0 ± 48.8	209.8 ± 46.5	0.176
E/A ratio	0.8 (0.7, 1.1)	0.8 (0.7, 1.1)	0.9 (0.7, 1.3)	0.8 (0.7, 1.0)	0.515
E/e′ ratio	8.4 (6.8, 10.6)	8.4 (6.5, 10.0)	8.8 (6.3, 10.6)	8.0 (6.9, 10.9)	0.891
LVEDVI, ml/m^2	52.2 ± 16.1	55.0 ± 15.6	53.0 ± 17.5	48.9 ± 14.4	0.321
LVESVI, ml/m^2	17.5 (15.2, 22.9)	17.5 (15.2, 22.8)	18.6 (15.8, 24.2)	16.2 (13.8, 23.1)	0.724
LVEF, %	62.8 ± 6.9	63.4 ± 7.7	62.5 ± 7.4	62.9 ± 5.8	0.882
LV GLS_{4CH}, %	18.9 (16.8, 20.9)	20.1 (18.2, 22.0)	19.0 (17.3, 21.9)	17.0 (15.7, 18.6)*#	<0.001
LAS-peak, %	33.7 ± 7.6	31.9 ± 7.9	35.8 ± 8.1	32.0 ± 10.2	0.146
Moderate-severe MR, *n* (%)	2 (1.5%)	0 (0)	0 (0)	2 (4.3%)	0.090
Right heart					
RA dimension, mm	35.5 ± 4.6	34.7 ± 4.9	36.4 ± 3.4	35.9 ± 5.1	0.209
RV dimension, mm	34.0 (30.6, 36.4)	33.3 (29.8, 35.8)	34.4 (32.4, 36.6)	34.0 (30.7, 36.6)	0.485
TAPSE, mm	22.2 ± 3.8	22.8 ± 3.8	22.6 ± 3.5	20.8 ± 3.7*#	0.019
RVFAC, %	47.2 (41.6, 51.2)	49.3 (42.0, 52.2)	45.5 (40.8, 51.2)	46.9 (40.9, 50.3)	0.255
S′, cm/s	13.3 (11.9, 15.0)	14.0 (12.0, 15.0)	14.0 (12.0, 16.4)	12.9 (11.0, 15.0)	0.301
RV FWLS, %	22.7 (19.2, 25.6)	23.9 (20.1, 26.2)	23.6 (19.8, 26.1)	20.2 (18.1, 24.0)*#	0.015
Moderate-severe TR, *n* (%)	4 (3.0%)	0 (0)	0 (0)	4 (8.5%)	0.016
PASP, mm Hg	36 ± 14	30 ± 11	39 ± 14*	39 ± 15*	0.037
Prognosis					
Discharge, *n* (%)	113 (85.6%)	50 (100.0%)	33 (97.1%)	30 (62.5%)*#	<0.001
Death, *n* (%)	19 (14.4%)	0 (0)	1 (2.9%)	18 (37.5%)*#	<0.001

*Values are mean ± SD, n (%), median (interquartile range). *P < 0.05, severe or critical vs. moderate; #P < 0.05, critical vs. severe. COVID-19, coronavirus disease 2019; DBP, diastolic blood pressure; DT, peak E deceleration time of mitral inflow; IVS., interventricular septum; LA, left atrial; LAS, left atrial strain; LV, left ventricular; LV GLS_{4CH}, left ventricular global longitudinal strain derived from the apical four-chamber view; LVEDVI, left ventricular end diastolic volume index; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end systolic volume index; LVM, left ventricular mass; MR, mitral regurgitation; PASP, pulmonary artery systolic pressure; PW, posterior wall of left ventricle; RA, right atrial; RV, right ventricular; RV FWLS, right ventricular free wall longitudinal strain; RVFAC, RV fractional area change; SBP, systolic blood pressure; TAPSE, tricuspid annular plane systolic excursion; TR, tricuspid regurgitation. LV GLS_{4CH} and RV FWLS values are absolute values.*

acute respiratory distress syndrome (ARDS), and be admitted to ICU. And they were more likely to receive treatment with high-flow oxygen and mechanical ventilation, and had higher mortality.

Echocardiographic Characteristics

LV GLS_{4CH} measurements were obtained in all patients. LV GLS measurements were feasible in 99 patients. LV GLS_{4CH} was strongly correlated with LV GLS ($r = 0.93$, $P < 0.001$). Furthermore, No significant difference between LV GLS_{4CH} and LV GLS was observed in our study (19.1 ± 2.9% vs. 19.1 ± 2.7%, $P = 0.885$) (**Supplementary Figure 1**). Therefore, we used LV GLS_{4CH} to assess the LV GLS in 132 patients with COVID-19 to obtain larger sample size. We consider it is reasonable to use LV GLS_{4CH} as a surrogate for LV GLS during the epidemic of COVID-19 to allow rapid image acquisition, improve feasibility in LV strain analysis and reduce contagion exposure duration to healthcare worker. Echocardiographic characteristics of COVID-19 patients are described in **Table 2**. Eleven patients had pericardial effusion. Patients with cardiac injury displayed lower TAPSE, LV $GLS_{4CH,}$ and RV FWLS, higher PASP, and higher proportion of pericardial effusion than those without cardiac injury. However, there was no significant difference in left and right heart size, LAS-peak, LV volumes, mass and diastolic function, LVEF, and moderate–severe MR and TR between these two groups. In addition, LV GLS_{4CH} and RV FWLS was lower in patients with ARDS than those without (18.1 ± 2.7% vs. 19.7 ± 3.3%, $P = 0.004$; 21.0 ± 4.9% vs. 23.7 ± 4.7%, $P = 0.003$;

TABLE 4 | Clinical and echocardiographic characteristics of survivors and non-survivors with COVID-19.

Variables	All patients ($n = 132$)	Survivor ($n = 113$)	Non-survivor ($n = 19$)	*P*-value
Clinical characteristics				
Age, years	61 ± 13	61 ± 13	64 ± 13	0.556
Male, *n* (%)	68 (51.5%)	54 (47.8%)	14 (73.7%)	0.037
Body mass index, kg/m^2	23.6 ± 2.9	23.6 ± 2.9	23.7 ± 3.5	0.701
Heart rate, beats/min	91 ± 17	90 ± 16	96 ± 22	0.092
Respiratory rate, breaths/min	25 ± 6	24 ± 5	28 ± 8	0.059
SBP, mm Hg	134 ± 18	133 ± 18	134 ± 16	0.914
DBP, mm Hg	81 ± 12	81 ± 12	78 ± 14	0.245
Left heart				
LA dimension, mm	34.2 (31.7, 37.0)	34.2 (31.6, 36.9)	35.7 (31.7, 37.7)	0.602
LV dimension, mm	45.8 (42.3, 49.0)	45.9 (42.3, 49.3)	45.8 (41.7, 48.4)	0.751
IVS, mm	9.6 (8.9, 10.4)	9.6 (9.0, 10.4)	9.6 (9.2, 10.1)	0.962
PW, mm	9.2 (8.3, 9.9)	9.0 (8.1, 9.9)	9.4 (8.9, 9.7)	0.350
LVM, g	143.2 (116.0, 168.5)	143.0 (121.5, 169.3)	148.7 (114.1, 170.4)	0.824
DT, ms	202.0 (163.9, 235.0)	206.5 (162.5, 239.0)	195.0 (164.0, 222.0)	0.488
E/A ratio	0.8 (0.7, 1.1)	0.8 (0.7, 1.1)	0.9 (0.7, 1.4)	0.218
E/e′ ratio	8.4 (6.8, 10.6)	8.1 (6.8, 10.1)	8.9 (7.1, 11.7)	0.274
LVEDVI, ml/m^2	49.8 (39.2, 59.3)	51.1 (41.1, 63.0)	38.9 (33.1, 38.9)	0.043
LVESVI, ml/m^2	17.5 (15.2, 22.9)	18.2 (15.5, 23.3)	14.2 (10.6, 23.5)	0.077
LVEF, %	63.2 (59.1, 68.0)	63.3 (59.0, 68.0)	63.4 (59.9, 67.8)	0.635
LV GLS_{4CH}, %	18.9 (16.8, 20.9)	19.3 (17.3, 21.6)	16.0 (14.7, 16.9)	<0.001
LAS-peak, %	33.7 (27.6, 37.9)	33.4 (27.0, 39.5)	30.2 (27.1, 36.7)	0.155
Moderate-severe MR, *n* (%)	2 (1.5%)	0 (0)	2 (10.5%)	0.020
Right heart				
RA dimension, mm	35.7 (32.4, 38.2)	35.1 (32.1, 37.7)	37.6 (34.2, 39.1)	0.030
RV dimension, mm	34.0 (30.6, 36.4)	33.2 (30.3, 35.7)	35.8 (32.0, 41.0)	0.022
TAPSE, mm	22.2 (19.1, 25.2)	22.3 (20.2, 25.4)	19.0 (17.1, 21.1)	0.001
RVFAC, %	47.2 (41.6, 51.2)	48.2 (42.0, 52.0)	43.2 (37.8, 49.0)	0.008
S′, cm/s	13.3 (11.9, 15.0)	14.0 (12.0, 15.7)	11.7 (10.0, 14.7)	0.017
RV FWLS, %	22.7 (19.2, 25.6)	23.6 (20.0, 26.1)	18.0 (17.3, 20.6)	<0.001
Moderate-severe TR, *n* (%)	4 (3.0%)	2 (1.8%)	3 (15.8%)	0.018
PASP, mm Hg	33 (24, 47)	31 (24, 47)	47 (32, 60)	0.041

Values are mean ± SD, n (%), median (interquartile range). COVID-19, coronavirus disease 2019; DBP, diastolic blood pressure; DT, peak E deceleration time of mitral inflow; IVS., interventricular septum; LA, left atrial; LAS, left atrial strain; LV, left ventricular; LV GLS_{4CH}, left ventricular global longitudinal strain derived from the apical four-chamber view; LVEDVI, left ventricular end diastolic volume index; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end systolic volume index; LVM, left ventricular mass; MR, mitral regurgitation; PASP, pulmonary artery systolic pressure; PW, posterior wall of left ventricle; RA, right atrial; RV, right ventricular; RV FWLS, right ventricular free wall longitudinal strain; RVFAC, RV fractional area change; SBP, systolic blood pressure; TAPSE, tricuspid annular plane systolic excursion; TR, tricuspid regurgitation. LV GLS_{4CH} and RV FWLS values are absolute values.

respectively), whereas LAS-peak did not differ between patients with ARDS and without (33.0 ± 8.2 % vs. 33.4 ± 8.2%, $P = 0.793$).

According to the seventh version of the guidelines on the Diagnosis and Treatment of COVID-19 by the National Health Commission, COVID-19 severity is classified as mild, moderate, severe and critical types (10). There were 50 moderate, 35 severe, and 47 critical patients in our study. Our results revealed that critical group had decreased LV GLS_{4CH}, RV FWLS, and TAPSE, elevated PASP, higher proportion of moderate-severe TR, and higher mortality compared with moderate and severe groups. There was no significant difference in LVEF, LAS-peak, RVFAC and S′ among the moderate, severe, and critical groups (**Table 3**).

At the time of the echocardiographic examinations, 32 patients were intubated. 117 (88.6%) patients were in oxygen therapy. 72 (55.0%) patients were treated with high-flow oxygen. Compared with patients who did not require mechanical ventilation, those who required mechanical ventilation had impaired LV GLS_{4CH}, RV FWLS, TAPSE and RVFAC, and elevated PASP, whereas LVEF and LAS-peak were not different between these two groups (**Supplemental Table 1**).

During hospitalization, 19 patients died. Compared with survivors, non-survivors displayed dilated right heart chamber, impaired TAPSE, RVFAC, S′, RV FWLS and LV GLS_{4CH}, higher proportion of moderate–severe MR and TR, and higher PASP. In contrast, left heart chamber dimension, LAS-peak, LV wall thickness, mass and diastolic function, and LVEF were similar between survivors and non-survivors (**Table 4**).

Follow-Up Study in COVID-19 Patients Who Were Alive

Forty-six survivors were followed up at 3 months after discharge (**Table 5**). We observed significant improvements in LV GLS_{4CH}, RV FWLS, and LAS-peak (**Figure 2**), and a decrease in PASP in recovered patients, whereas LVEF and conventional RV function parameters (TAPSE, S′ and RVFAC) were not different from the baseline values ($P > 0.05$).

Correlation of Biventricular Function With Cardiac Injury and Inflammatory Marker

A decrease in LV GLS_{4CH} weakly correlated with decreased lymphocyte count (r = 0.37, $P < 0.001$), and elevated levels of CRP (r = −0.39, $P < 0.001$), PCT (r = −0.31, $P = 0.001$), IL-6 (r = −0.28, $P = 0.041$), CK-MB (r = −0.17, $P = 0.044$), hs-TNI (r = −0.30, $P = 0.001$), D-dimer (r = −0.24, $P = 0.012$) and APTT (r = −0.26, $P = 0.003$) (**Supplementary Figure 2**). A reduction in RV FWLS had weak correlations with higher levels of CRP (r = −0.29, $P = 0.001$), PCT (r = −0.33, $P = 0.001$), CK-MB (r = −0.21, $P = 0.018$), hs-TNI (r = −0.43, $P < 0.001$), APTT (r = −0.26, $P = 0.003$), and PT (r = −0.30, $P = 0.001$) (**Supplementary Figure 3**). Additionally, decreased LV GLS_{4CH} and RV FWLS were also related to the presence of pericardial effusion (r = −0.217, $P = 0.012$; r = −0.339, $P < 0.001$, respectively). In contrast, LAS-peak and LVEF had no significant correlation with biomarkers levels of inflammation, coagulopathy, and cardiac injury ($P > 0.05$ for all).

TABLE 5 | Clinical and echocardiographic characteristics of patients with COVID-19 three months after discharge.

Variables	Baseline ($n = 46$)	3 months after discharge ($n = 46$)	*P*-value
Clinical characteristics			
Age, years	59 ± 13		
Male, *n* (%)	18 (39.1%)		
Body mass index, kg/m^2	23.3 ± 3.0		
Heart rate, beats/min	94 ± 18	80 ± 12	<0.001
Respiratory rate, breaths/min	24 ± 5	21 ± 2	0.010
SBP, mm Hg	137 ± 19	134 ± 16	0.509
DBP, mm Hg	82 ± 10	86 ± 10	0.075
Laboratory findings			
Lymphocyte count, × 10^9/l	1.0 (0.5, 1.4)	1.9 (1.5, 2.5)	<0.001
D-dimer, mg/l	0.59 (0.16, 1.92)	0.34 (0.24, 0.55)	0.096
hs-TNI, ng/l	4.4 (1.7, 60.8)	1.4 (0.3, 2.6)	0.001
CRP, mg/l	35.8 (4.3, 75.1)	1.0 (0.6, 3.1)	<0.001
Left heart			
LA dimension, mm	36.7 ± 4.4	36.5 ± 5.2	0.864
LV dimension, mm	46.4 ± 4.7	46.1 ± 3.6	0.786
IVS, mm	9.5 ± 1.2	8.8 ± 1.2	0.007
PW, mm	8.7 ± 1.6	8.6 ± 2.1	0.813
LVM, g	136.1 ± 37.7	134.2 ± 34.9	0.811
DT, ms	195 ± 52	199 ± 45	0.751
E/A ratio	0.9 ± 0.4	1.0 ± 0.7	0.255
E/e′ ratio	8.8 ± 2.9	8.1 ± 3.6	0.360
LVEDVI, ml/m^2	56.5 ± 18.9	56.0 ± 17.1	0.916
LVESVI, ml/m^2	21.6 ± 8.9	20.3 ± 7.3	0.571
LVEF, %	62.1 ± 8.2	63.1 ± 8.0	0.613
LV GLS_{4CH}, %	19.4 ± 2.7	26.6 ± 4.4	<0.001
LAS-peak, %	31.4 ± 7.5	38.9 ± 7.3	<0.001
Right heart			
RA dimension, mm	35.7 ± 3.5	33.9 ± 3.4	0.023
RV dimension, mm	33.5 ± 3.2	33.3 ± 3.4	0.804
TAPSE, mm	22.8 ± 3.6	23.5 ± 8.3	0.636
RVFAC, %	48.8 ± 7.1	49.6 ± 10.0	0.699
S′, cm/s	14.0 ± 2.6	13.8 ± 2.4	0.722
RV FWLS, %	24.1 ± 4.7	29.1 ± 5.2	<0.001
PASP, mm Hg	36 ± 10	27 ± 7	0.026

Values are mean ± SD, n (%), median (interquartile range). COVID-19, coronavirus disease 2019; DBP, diastolic blood pressure; DT, peak E deceleration time of mitral inflow; IVS., interventricular septum; LA, left atrial; LAS, left atrial strain; LV, left ventricular; LV GLS_{4CH}, left ventricular global longitudinal strain derived from the apical four-chamber view; LVEDVI, left ventricular end diastolic volume index; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end systolic volume index; LVM, left ventricular mass; PASP, pulmonary artery systolic pressure; PW, posterior wall of left ventricle; RA, right atrial; RV, right ventricular; RV FWLS, right ventricular free wall longitudinal strain; RVFAC, RV fractional area change; SBP, systolic blood pressure; TAPSE, tricuspid annular plane systolic excursion. LV GLS_{4CH} and RV FWLS values are absolute values.

Predictors of Mortality in Patients With COVID-19

A univariate Cox regression analysis showed that elevated level of hs-TNI, ARDS, LV GLS_{4CH}, RV FWLS, TAPSE, and RVFAC were

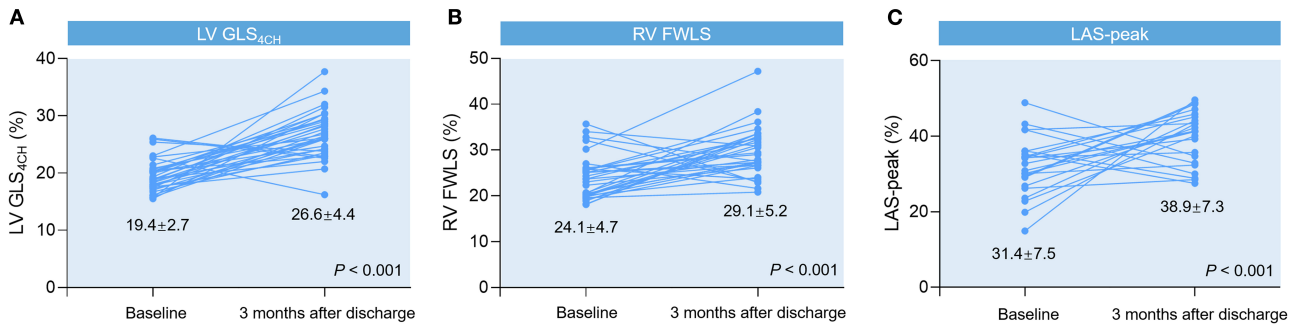


FIGURE 2 | Spaghetti plots of LV GLS_{4CH} **(A)**, RV FWLS **(B)**, and LAS-peak **(C)** in patients with COVID-19 at 3-month follow-up after discharge compared with baseline values.

associated with higher risk of mortality (**Table 6**). Whereas, LAS-peak, LVEF and S′ were not predictive of death. The multivariate Cox analysis models revealed that hs-TNI elevation and ARDS continued to be of prognostic significance. LV GLS_{4CH} [hazard ratio [HR]: 1.41, 95% confidence intervals [CI]: 1.08-1.84; $P = 0.011$], RV FWLS (HR: 1.29, 95% CI: 1.09-1.52; $P = 0.003$), TAPSE (HR: 0.82, 95% CI: 0.69-0.98; $P = 0.031$), and RVFAC (HR: 0.92, 95% CI: 0.85-0.99; $P = 0.032$) were independent predictive of higher risk of death. The Cox models using LV GLS_{4CH} (AIC = 131) or RV FWLS (AIC = 122) were observed to predict higher mortality more accurately than that with TAPSE (AIC = 134), RVFAC (AIC = 134) or traditional risk model (AIC = 138) (**Table 6**).

LAS-peak, LV GLS_{4CH}, RV FWLS, conventional RV function parameters and LVEF were entered into ROC analysis to estimate probability of in-hospital death. Impaired LV GLS_{4CH} and RV FWLS were associated with higher mortality (**Figure 3**). Areas under the curve were 0.85 for LV GLS_{4CH} and 0.80 for RV FWLS. The optimal cutoff value of LV GLS_{4CH} for detection of increased mortality was −17.9% with sensitivity of 94.7% and specificity of 65.8%. The best cutoff value of RV FWLS for identification of death was −22.9% (sensitivity, 94.4%; specificity, 55.7%).

Kaplan-Meier survival curves of biventricular longitudinal strain for mortality are presented in **Figure 4**. When stratified by cutoff values, LV GLS_{4CH} lower than 17.9 % or RV FWLS lower than 22.9% were associated with higher mortality ($P < 0.001$) (**Figures 4A,B**). Patients with below cutoff LV GLS_{4CH} and RV FWLS had the worst prognosis compared those with above cutoff LV GLS_{4CH} and RV FWLS (**Figure 4C**). To determine the relationship between levels of hs-TNI, cardiac function parameters and mortality, a contour plot was performed. Our findings revealed that decreased LV GLS_{4CH}, RV FWLS, RVFAC, and TAPSE were associated with increased death, which was pronounced in patients with higher levels of hs-TNI (**Figure 5**).

Reproducibility

The intraobserver and interobserver reproducibility of LV GLS_{4CH}, RV FWLS and LAS-peak are summarized in **Supplemental Table 2**. The intraobserver and interobserver reproducibility of LV GLS_{4CH}, RV FWLS, and LAS-peak were high.

DISCUSSION

To the best of our knowledge, our study is the first to systematically assess cardiac structure and function in COVID-19 patients using both conventional echocardiography and 2D-STE. This study demonstrates that patients with cardiac injury had higher levels of coagulopathy and inflammatory biomarkers, higher incidence of complications, more treatment with mechanical ventilation, higher mortality, and lower LV GLS_{4CH} and RV FWLS than those without cardiac injury. Compared with survivors, non-survivors displayed reduced biventricular longitudinal strain, and comparable LVEF. At a 3-month follow-up after discharge, we identify that biventricular longitudinal strain can track clinical improvement in the convalescent phase. Importantly, LV GLS_{4CH} and RV FWLS are powerful predictors of higher mortality in patients with COVID-19. Therefore, biventricular longitudinal strain may be essential for risk stratification and serial follow-up in patients with COVID-19.

Biventricular Function in Patients With COVID-19

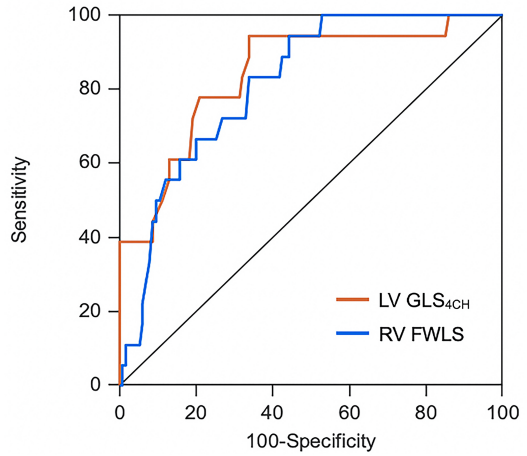
SARS-CoV-2 are known to result in the acute and chronic damage of the cardiovascular system (11, 12). Although several recent studies have demonstrated that 5.2%-23% patients with COVID-19 suffered myocardial injury from the infection (12–14), there are limited echocardiographic data regarding the cardiac abnormalities. Prior report highlights the significance of assessing cardiac function of hospitalized COVID-19 patients (15).

Despite the importance and extensive use of LVEF in routine clinical practice, there are several limitations of its application. First, it depends on geometric assumptions and loading conditions. Moreover, it could not reflect myocardial contractility (16). Finally, LVEF may have considerable inter- and intra-observer variability. Accordingly, LVEF may not be an optimal index to detect myocardial impairment. Novel, more sensitive indices for cardiac dysfunction at an earlier stage are required. Recently, LV and RV longitudinal strain have been recommended as sensitive and early indicators of

TABLE 6 | Predictors of mortality in patients with COVID-19 by cox proportional hazard model.

	Univariate Cox regression		Model 1 ARDS + hs-TNI		Model 2 ARDS + hs-TNI + LV GLS_{4CH}		Model 3 ARDS + hs-TNI + TAPSE		Model 4 ARDS + hs-TNI + RVFAC		Model 5 ARDS + hs-TNI + RV FWLS	
	HR (95% CI)	***p*-value**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
Age, years	1.02 (0.99, 1.06)	0.354										
Male (yes vs. no)	3.06 (1.01, 9.22)	0.048										
Hypertension (yes vs. no)	2.58 (0.98, 6.79)	0.055										
Diabetes mellitus (yes vs. no)	0.38 (0.05, 2.86)	0.349										
Obesity, *n* (%)	0.88 (0.25, 3.04)	0.837										
Coronary artery disease (yes vs. no)	1.49 (0.53, 4.18)	0.447										
Malignancy (yes vs. no)	1.99 (0.46, 8.65)	0.359										
Arrhythmia (yes vs. no)	1.09 (0.25, 4.79)	0.909										
ARDS (yes vs. no)	7.50 (2.18, 25.80)	0.001	5.52 (1.59, 19.22)	0.007	4.27 (1.20, 15.21)	0.025	5.43 (1.50, 19.65)	0.010	5.90 (1.68, 20.71)	0.006	3.77 (1.04, 13.67)	0.044
Elevated CK-MB (yes vs. no)	0.20 (0.03, 1.49)	0.116										
Elevated hs-TNI (yes vs. no)	8.13 (2.69, 24.51)	<0.001	6.23 (2.04, 19.00)	0.001	3.53 (1.06, 11.80)	0.041	3.70 (1.12, 12.23)	0.032	4.36 (1.36, 13.95)	0.013	4.28 (1.36, 13.47)	0.013
Elevated BNP (yes vs. no)	0.70 (0.57, 4.17)	0.397										
PaO_2:FIO_2, mmHg	1.00 (0.98, 1.01)	0.599										
Mechanical ventilation (yes vs. no)	2.20 (0.89, 5.42)	0.088										
ACE inhibitor/ARB (yes vs. no)	0.59 (0.08, 4.45)	0.610										
Pericardial effusion (yes vs. no)	1.93 (0.56, 6.68)	0.299										
E/e′ ratio	1.02 (0.90, 1.16)	0.794										
LVEDVI, ml/m^2	0.95 (0.90, 1.00)	0.070										
LVESVI, ml/m^2	0.90 (0.84, 1.03)	0.163										
LVM, g	1.00 (0.99, 1.01)	0.771										
LVEF, %	1.02 (0.95, 1.10)	0.607										
LV GLS_{4CH}, %	1.70 (1.30, 2.23)	<0.001			1.41 (1.08, 1.84)	0.011						
LAS-peak, %	0.96 (0.90, 1.03)	0.217										
TAPSE, mm	0.81 (0.70, 0.93)	0.003					0.82 (0.69, 0.98)	0.031				
RVFAC, %	0.89 (0.82, 0.97)	0.007							0.92 (0.85, 0.99)	0.032		
S′, cm/s	0.83 (0.68, 1.01)	0.058										
RV FWLS, %	1.32 (1.15, 1.50)	<0.001									1.29 (1.09, 1.52)	0.003
AIC	/	/	138		131		134		134		122	

ACE, angiotensin-converting enzyme; AIC, Akaike Information Criterion; ARB, angiotensin II receptor blockers; ARDS, acute respiratory distress syndrome; BNP, B-type natriuretic peptide; CI, confidence interval; CK-MB, creatine kinase muscle-brain; COVID-19, coronavirus disease 2019; FIO_2, fraction of inspiration oxygen; HR, hazard ratio; hs-TNI, high-sensitivity troponin I; LAS, left atrial strain; LV GLS_{4CH}, left ventricular global longitudinal strain derived from the apical four-chamber view; LVEDVI, left ventricular end diastolic volume index; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end systolic volume index; LVM, left ventricular mass; PaO_2, partial pressure of oxygen; RV FWLS, right ventricular free wall longitudinal strain; RVFAC, right ventricular fractional area change; TAPSE, tricuspid annular plane systolic excursion. CK-MB ≥ 25 U/l was defined as elevated CK-MB; hs-TNI ≥ 26.2 ng/ml was defined as elevated hs-TNI; BNP ≥ 100 pg/ml was defined as elevated BNP.



Variables	AUC (95% CI)	*P* value	Sensitivity (%)	Specificity (%)
RV FWLS (%)	0.80(0.71, 0.88)	< 0.001	94.4	55.7
LV GLS_{4CH} (%)	0.85(0.78, 0.90)	< 0.001	94.7	65.8

FIGURE 3 | ROC curves of biventricular longitudinal strain for adverse clinical outcome. LV GLS_{4CH}, left ventricular global longitudinal strain from the apical four-chamber view; RV FWLS, Right ventricular free wall longitudinal strain.

subclinical cardiac dysfunction (17). They are measurements of myocardial deformation, and objective parameters with excellent reproducibility and high feasibility. We previously found that COVID-19 patients had impaired RV FWLS (18). However, there are no data regarding the use of LV GLS_{4CH} in patients with COVID-19. In the present study, we identified that COVID-19 patients exhibited significantly impaired LV GLS_{4CH} and RV FWLS, while no difference was found in LVEF. Moreover, impaired biventricular longitudinal strain appeared to be worse in critically ill patients or those who required mechanical ventilation therapy. These findings are in agreement with the study of SARS, which revealed that the diminished LV performance was worse in patients who needed treatment with mechanical ventilation (19). In a study of 28 patients with acute myocarditis, reduced LV GLS correlated with the amount of oedema, and added important information on the diagnosis and degree of myocardial dysfunction, especially in patients with preserved LVEF (20). Recently, there are increasing data regarding the cardiac impairment in patients diagnosed with COVID-19 infection (21–24). The mechanisms of cardiac injury are uncertain but likely involve direct viral injury, aggravation of a systemic inflammatory response, hypoxemia, destabilized coronary plaques and microthrombogenesis (25). Consistent with this postulation, the correlations of diminished LV GLS_{4CH} and RV FWLS with elevated biomarkers levels of inflammation, coagulopathy, and cardiac injury were observed in our study. Besides, we found that patients with cardiac injury displayed higher proportion of pericardial effusion than those without cardiac injury. Moreover, decreased LV GLS_{4CH} and RV FWLS were also correlated with the presence of pericardial effusion, suggesting that the presence of pericardial effusion or pericarditis have a major influence on the biventricualr strain values.

In addition to myocardial injury, RV function was predisposed to impairment owing to increased RV afterload from ARDS, hypoxic pulmonary vasoconstriction, pulmonary microthrombi, and endothelial and microvascular injury (26). RV dilation and dysfunction may also affect the LV function and aggravate LV dysfunction by ventricular interdependence and paradoxical septum. The reductions in LV GLS_{4CH} and RV FWLS are important in COVID-19 patients, as owing to overlapping symptoms of dyspnea, the diagnosis of myocardial involvement may be challenging. These findings are also particularly significant to the majority of COVID-19 patients with a normal LVEF.

The Utility of Biventricular Longitudinal Strain During the Follow-Up Study

At 3-month follow-up after discharge, significant improvements in biventricular longitudinal strain were identified in our study, indicating that depressed LV and RV performance may be reversible on disease recovery when the acute inflammatory response waned. Consistent with our results, Li et al. showed that impaired LV function appeared to be reversible at 30-day follow-up study in 46 patients with SARS (19). In another follow-up observation of 11 COVID-19 patients with LV dysfunction, Dr. Churchill and colleagues demonstrated resolution of LV abnormalities after a median of 14 days (27). However, LVEF and conventional RV function parameters did not show significant improvements with therapy in our study. These findings suggests biventricular longitudinal strain may be more sensitive to detect subtle myocardial improvement compared to other standard echocardiographic parameters. Our results demonstrate the superiority of biventricular longitudinal strain over conventional echocardiographic indices during the follow-up in patients with COVID-19.

The Prognostic Value of Biventricular Longitudinal Strain in COVID-19 Patients

To the best of our knowledge, this may be the first study to investigate whether biventricular longitudinal strain were associated with fatal outcomes in COVID-19 patients. Indeed, in the present study, patients with diminished LV GLS_{4CH} and RV FWLS were at higher risk of death. Our findings reveal that biventricular longitudinal strain serve as novel imaging biomarkers that predicts higher mortality in patients with COVID-19. Consistent with these results, our study previously revealed that RV FWLS was an independent predictor of poor outcomes in COVID-19 patients (18). Similarly, Argulian et al. showed that RV dilation was predictive of in-hospital mortality in patients with COVID-19. (28) Another observation was reported by Szekely et al. (21), which demonstrated increased RV end diastolic area was significantly associated with mortality.

In addition, LV GLS has presented additional prognostic significance over LVEF in a range of cardiovascular disorders (6, 29). However, the prognostic implication of LV GLS_{4CH} in COVID-19 patients remained unknown. Our findings showed that LV GLS_{4CH} was predictive of higher mortality in COVID-19 patients, whereas LVEF was not. This is in

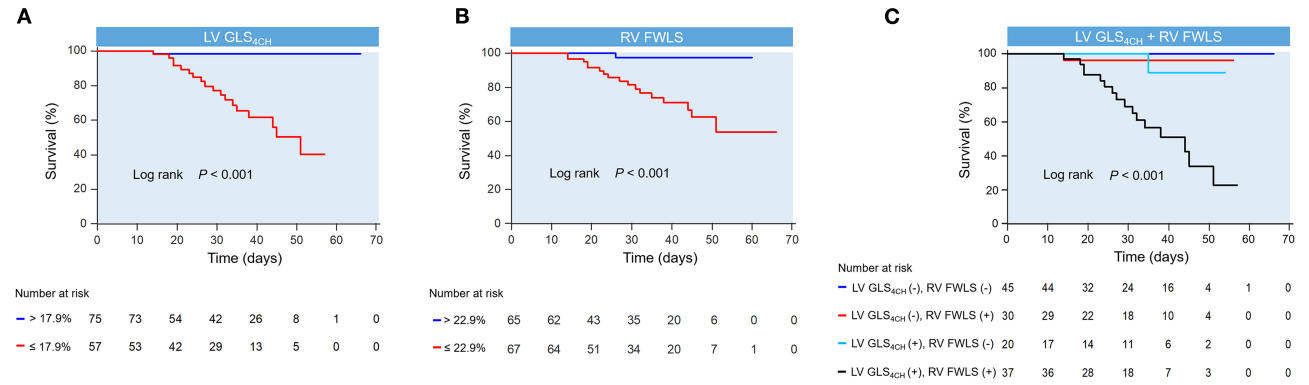


FIGURE 4 | Kaplan-meier survival curves showing the association of biventricular longitudinal strain and higher mortality. Kaplan-Meier curves in COVID-19 patients stratified by the cutoff value of LV GLS_{4CH} **(A)** and RV FWLS **(B)**. **(C)** Kaplan-Meier curves reveal that COVID-19 patients below cutoff LV GLS_{4CH} and RV FWLS have the highest mortality. LV GLS_{4CH} and RV FWLS values are absolute values. LV GLS_{4CH} (+), below cutoff LV GLS_{4CH}; LV GLS_{4CH} (–), above cutoff LV GLS_{4CH}; RV FWLS (+), below cutoff RV FWLS; RV FWLS (–), above cutoff RV FWLS.

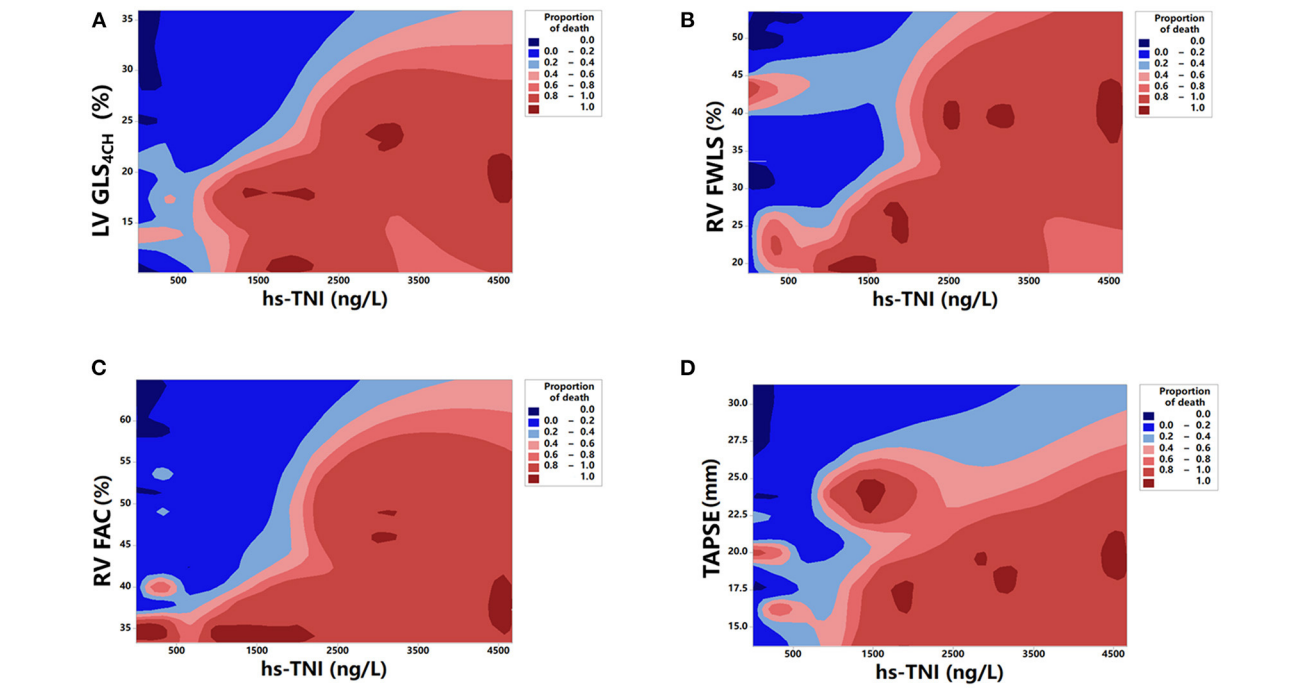


FIGURE 5 | Contour plot of survival probability in hospitalized patients with COVID-19. Decreased LV GLS_{4CH} **(A)**, RV FWLS **(B)**, RV FAC **(C)**, and TAPSE **(D)** are associated with higher mortality, which is pronounced in patients with higher levels of hs-TNI.

contradistinction to a recent study in patients with COVID-19 in Israel, which reported that lower LVEF was associated with mortality (21). However, in the previous study (21), patients were older, and have higher rate of male, hypertension, diabetes mellitus, and obesity. The current data indicates that LV GLS_{4CH} and RV FWLS are not only more sensitive markers of subclinical myocardial impairment, but also powerful and independent predictors of higher mortality. Therefore, biventricular longitudinal strain could help risk stratification of COVID-19 patients.

Clinical Implications

LVEF is a key determinant in clinical decision-making in various diseases. However, it is relatively indiscriminant within the normal range. Novel biventricular longitudinal strain may be of particular clinical significance in COVID-19 patients with relatively normal LVEF. Our data showed LV GLS_{4CH} and RV FWLS, rather than LVEF, were strong predictors of higher risk of mortality. Furthermore, biventricular longitudinal strain can provide highly useful and clinically relevant information during the follow-up in patients with COVID-19. The present

study revealed the important clinical implication of biventricular longitudinal strain, as measurements of LV GLS_{4CH} and RV FWLS are fast and non-invasive methods that can be easily obtained from bedside echocardiography. More importantly, they can identify subclinical myocardial impairment, help detect in higher risk of COVID-19 patients and serially follow patients.

Limitations

Our study has several limitations that should be mentioned. First, as 2D-STE depends on image quality, severe and critically ill patients with inadequate echocardiographic images might have been underrepresented. Furthermore, 2D-STE analysis was performed using Qlab software in our study, so the results in the present study may not be apply to other software algorithms because 2D-STE parameters are hampered by inter-vendor variability. Although our study exclude dilated cardiomyopathy and old myocardial infarction that may significant lead to impaired biventricular longitudinal strain, patients had hypertension or coronary artery diseases, who had underlying medical condition that could have affected strain values. In addition, our study used the LV GLS_{4CH} rather than the LV GLS to estimate LV myocardial longitudinal function during the epidemic of COVID-19 to allow rapid image acquisition and reduce contagion exposure duration to healthcare worker. Another limitation was that only a small proportion of COVID-19 patients had follow-up echocardiographic data, though improvement in biventricular longitudinal strain was noted. Finally, the study was a single-center study with a relatively limited sample size. Therefore, further large multi-center studies are needed to confirm the results in the present study.

CONCLUSIONS

Our study demonstrates that LV GLS_{4CH} and RV FWLS are independently predicative of higher mortality, providing incremental prognostic implications over conventional echocardiographic parameters in patients with COVID-19. We also identify that biventricular longitudinal strain provide highly relevant information regarding the recovery of cardiac function when the acute inflammatory response subsided. Therefore, biventricular longitudinal strain are valuable non-invasive parameters in risk stratification and serial follow-up of patients with 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 Medical Ethics Committee of 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.

AUTHOR CONTRIBUTIONS

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2020.632434/full#supplementary-material

REFERENCES

1. 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
2. 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
3. Potter E, Marwick TH. Assessment of left ventricular function by echocardiography: the case for routinely adding global longitudinal strain to ejection fraction. *JACC Cardiovasc Imaging.* (2018) 11:260–74. doi: 10.1016/j.jcmg.2017.11.017
4. 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
5. Xie M, Li Y, Cheng TO, Wang X, Dong N, Nie X, et al. The effect of right ventricular myocardial remodeling on ventricular function as assessed by two-dimensional speckle tracking echocardiography in patients with tetralogy of fallot: a single center experience from China. *Int J Cardiol.* (2015) 178:300–7. doi: 10.1016/j.ijcard.2014.10.027
6. Kim HM, Cho GY, Hwang IC, Choi HM, Park JB, Yoon YE, et al. Myocardial strain in prediction of outcomes after surgery for severe mitral regurgitation. *JACC Cardiovasc Imaging.* (2018) 11:1235–44. doi: 10.1016/j.jcmg.2018.03.016
7. Li Y, Wang T, Haines P, Li M, Wu W, Liu M, et al. Prognostic value of right ventricular two-dimensional and three-dimensional speckle-tracking strain in pulmonary arterial hypertension: superiority of longitudinal strain over circumferential and radial strain. *J Am Soc Echocardiogr.* (2020) 33:985–94. doi: 10.1016/j.echo.2020.03.015
8. Mast TP, Taha K, Cramer MJ, Lumens J, van der Heijden JF, Bouma BJ, et al. The prognostic value of right ventricular deformation imaging in early arrhythmogenic right ventricular cardiomyopathy. *JACC Cardiovasc. Imaging.* (2019) 12:446–55. doi: 10.1016/j.jcmg.2018.01.012
9. 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. *Eur Heart J Cardiovasc Imaging*. (2015) 28:1–39.e14. doi: 10.1016/j.echo.2014.10.003
10. Guideline for the Diagnosis and Treatment of (2019). N*ovel Coronavirus (2019-nCoV) Infected Pneumonia*. Available online at: http://news.cyol.com/app/2020-02/05/content_18353703.htm (accessed March 4, 2020).
11. 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
12. 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
13. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARSCoV-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
14. 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
15. Gackowski A, Lipczyńska M, Lipiec P, Szymański P. Expert opinion of the working group on echocardiography of the polish cardiac society on performing echocardiographic examinations during COVID-19 pandemic. *Kardiol Pol.* (2020) 78:357–63. doi: 10.33963/KP.15265
16. Patel RB, Vaduganathan M, Greene SJ, Butler J. Nomenclature in heart failure: a call for objective, reproducible, biologically-driven terminology. *Eur J Heart Fail*. (2018) 20:1379–81. doi: 10.1002/ejhf.1231
17. Nauta JF, Jin X, Hummel YM, Voors AA. Markers of left ventricular systolic dysfunction when left ventricular ejection fraction is normal. *Eur J Heart Fail*. (2018) 20:1636–8. doi: 10.1002/ejhf.1326
18. 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
19. Li SS, Cheng CW, Fu CL, Chan YH, Lee MP, Chan JW, et al. Left ventricular performance in patients with severe acute respiratory syndrome. *Circulation*. (2003) 108:1798–803. doi: 10.1161/01.CIR.0000094737.21775.32
20. Løgstrup BB, Nielsen JM, Kim WY, Poulsen SH. Myocardial oedema in acute myocarditis detected by echocardiographic 2D myocardial deformation analysis. *Eur Heart J Cardiovasc Imaging*. (2016) 17:1018–26. doi: 10.1093/ehjci/jev302
21. Szekely Y, Lichter Y, Taieb P, Banai A, Hochstadt A, Merdler I, et al. The spectrum of cardiac manifestations in coronavirus disease 2019 (COVID-19) - a systematic echocardiographic study. *Circulation*. (2020) 142:342–53. doi: 10.1161/CIRCULATIONAHA.120.047971
22. Sud K, Vogel B, Bohra C, Garg V, Talebi S, Lerakis S, et al. Echocardiographic findings in COVID-19 patients with significant myocardial injury. *J Am Soc Echocardiogr*. (2020) 33:1054–5. doi: 10.1016/j.echo.2020.05.030
23. Zhang L, Wang B, Zhou J, Kirkpatrick J, Xie M, Johri AM. Bedside focused cardiac ultrasound in COVID-19 infection from the wuhan epicenter: the role of cardiac point of care ultrasound (POCUS), limited transthoracic echocardiography and critical care echocardiography. *J Am Soc Echocardiogr*. (2020) 33:676–82. doi: 10.1016/j.echo.2020.04.004
24. Jain SS, Liu Q, Raikhelkar J, Fried J, Elias P, Poterucha TJ. Indications and findings on transthoracic echocardiogram in COVID-19. *J Am Soc Echocardiogr*. (2020) 33:1278–84. doi: 10.1016/j.echo.2020.06.009
25. 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
26. Park JF, Banerjee S, Umar S. In the eye of the storm: the right ventricle in COVID-19. *Pulm Circ*. (2020) 10:2045894020936660. doi: 10.1177/2045894020936660
27. 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
28. 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
29. Caspar T, Fichot M, Ohana M, El Ghannudi S, Morel O, Ohlmann P. Late detection of left ventricular dysfunction using two-dimensional and three-dimensional speckle-tracking echocardiography in patients with history of nonsevere acute myocarditis. *J Am Soc Echocardiogr*. (2017) 30:756–62. doi: 10.1016/j.echo.2017.04.002

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REVIEW
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Thrombosis and Coagulopathy in COVID-19: Current Understanding and Implications for Antithrombotic Treatment in Patients Treated With Percutaneous Coronary Intervention

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Coronavirus disease 2019 (COVID-19), a respiratory syndrome, is a global pandemic. Therefore, there is an urgent need to explore mechanisms implicated in the pathogenesis of the disease. Clinical and autopsy studies show a complex chain of events preceding COVID-19-related death. The disease is characterized by endothelial dysfunction, platelet activation, thrombosis, coagulopathy, and multiple organ failure. Globally, millions of patients with coronary heart disease undergo percutaneous coronary intervention (PCI) each year. These patients undergo high-intensity antithrombotic therapy during hospitalization and dual antiplatelet therapy (DAPT) for at least 6 months post PCI. COVID-19 is characterized by changes in platelet counts. Treatment of ischemic events that occur during stent implantation is associated with bleeding complications in patients following PCI complicated by COVID-19. This review summarizes recent progress in activation status and levels of COVID-19-related platelet changes. These findings will provide information on the effectiveness of antithrombotic therapy for the management of platelet changes in COVID-19 patients.

Keywords: COVID-19, thrombosis, coagulopathy, antithrombotic treatment, percutaneous coronary intervention

INTRODUCTION

Coronavirus disease 2019 (COVID-19), a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, is a worldwide pandemic. In November 2020, the World Health Organization reported over 50 million confirmed cases of COVID-19 and 1.3 million deaths globally (1). COVID-19 is associated with pneumonia and a wide range of effects on the cardiovascular system, thus, it is a health and economic burden worldwide (2–5).

SARS-CoV-2 enters host cells through coupling of viral spike protein and angiotensin-converting enzyme 2 (ACE-2) on the surface of host cells (6, 7), in a similar way as observed during SARS-CoV infection (8). Previous autopsy evaluations of SARS-infected patients (9) and recent clinical trials on COVID-19 patients (10, 11) show that diffuse alveolar injury and development of acute respiratory distress syndrome (ARDS) are the main pulmonary pathological manifestations. Cardiovascular effects, especially venous thromboembolic disease (12, 13) and

ischemic complications in arterial system, such as ischemic stroke (14), have been reported in COVID-19 patients.

A recent study reports significant changes in platelet gene expression and function in COVID-19 patients. These changes result in platelet activation and aggregation, which are potential novel mechanisms for management of COVID-19-associated thrombosis and coagulopathy (15). Notably, severe COVID-19 cases present with thrombocytopenia (16), which is associated with platelet depletion and a high risk of bleeding. Approximately 5 million percutaneous coronary interventions (PCIs) are performed each year worldwide (17). Therefore, COVID-19 patients requiring antithrombotic therapy have a high risk of thrombotic events and bleeding complications (16). Hence, in this review, we explored recent studies reporting relationships between changes in platelet function and coagulopathy in COVID-19 patients. The findings of this study will provide a mechanistic basis for designing new treatment approaches for thrombosis and coagulopathy in COVID-19 patients. Further, this study provides information for the development of personalized antithrombotic therapy regimen for COVID-19 patients treated with PCI.

CLINICAL CHARACTERISTICS OF COVID-19 PATIENTS

A previous clinical trial reports that the prevalence of hypertension, diabetes, and coronary heart disease among COVID-19 patients in the first 2 months of the outbreak was 15, 7.4, and 2.5%, respectively (18). Prevalence of hypertension, diabetes, and coronary heart disease significantly increased to 35.8, 26.9, and 9.0%, respectively, for patients who were admitted in intensive care units receiving mechanical ventilation or patients who succumbed to the disease (18). In a study carried out at Mount Sinai Hospital, comorbidity with hypertension (62.7%), diabetes mellitus (40.3%), coronary artery disease (31.3%), chronic kidney disease (26.7%), and asthma (17.9%) was higher in patients who succumbed to COVID-19 compared with that of survivors (19). Notably, thrombocytopenia (defined as a platelet count of $<$150,000/μl) was observed in 36.2% patients on admission, mainly in patients with severe cases (18). Moreover, prolonged prothrombin time and elevated D-dimer level, which indicated coagulopathy associated with COVID-19, were mainly reported in severe cases (3, 18, 20).

SARS-CoV-2 is a new coronavirus strain that belongs to the same class with SARS reported in 2003 (21). Clinical studies on SARS patients reported an increase in activated partial thromboplastin time (42.8%), thrombocytopenia (44.8%), and elevated D-dimer (45.0%) (22). In addition, a previous study reports thrombocytopenia in SARS patients (55%), increase in activated partial thromboplastin time (63%) and disseminated intravascular coagulation (DIC, 2.5%) (23). Clinical manifestations observed in SARS-CoV- and SARS-CoV-2-infected patients indicate a high risk of DIC. Therefore, World Health Organization interim guidance statement recommends prophylactic administration of low-molecular-weight heparin daily or subcutaneous administration of unfractionated heparin 2 times in a day (24). In addition, American College of Cardiology recommends that patients should receive all scheduled doses of venous thromboembolism prophylaxis (25). Administration of low-molecular-weight heparin daily is preferred over unfractionated heparin, as it reduces personal protective equipment use and exposure of health care workers (25).

Abbreviations: ACS, acute coronary syndrome; ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; DAPT, dual antiplatelet therapy; DIC, disseminated intravascular coagulation; IL-6, interleukin-6; MOF, multiple organ failure; PCI, percutaneous coronary intervention; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; STEMI, ST-segment elevation myocardial infarction.

BRIEF SUMMARY OF VIRAL PNEUMONIA PATHOLOGY

Viral pneumonia accounts for one third of adult community-acquired pneumonia. Most viral pneumonia cases are caused by influenza, rhinovirus, and coronavirus infections (26). Viral pneumonia is characterized by histopathological changes including interstitial pneumonitis with lymphocytic infiltrations. Other manifestations such as necrotizing bronchiolitis, diffuse alveolar injury with alveolar hemorrhage, alveolar septal edema, and hyaline-membrane formation may be present depending on conditions associated with co-infection and underlying disease (26).

Lung Pathology of Severe Acute Respiratory Syndrome

During 2002 and 2003, the SARS-CoV caused severe respiratory infection in more than 8,000 people and led to 774 deaths, with a mortality rate of 9.6% (27). The typical pathological change in SARS-infected lungs was diffuse hemorrhage on the lung surface and serous, fibrinous, and hemorrhagic inflammation in most pulmonary alveoli (9). In addition to diffuse alveolar hemorrhage, other commonly observed findings were the presence of intra-artery fibrin thrombi (5/8) and intra-alveolar hemorrhage (6/8) (28).

Lung Pathology and Multiple Organ Failure in Coronavirus Disease 2019

The early pulmonary pathological changes in SARS-CoV2-infected lungs included edema, proteinaceous exudate, and focal reactive hyperplasia of pneumocytes with patchy inflammatory cellular infiltration, whereas hyaline membranes were not prominent (29). The key features of lung pathology from severe COVID-19 patients were bilateral diffuse alveolar injury with cellular fibromyxoid exudates, as well as hyaline membrane formation (11). Other pathological findings included the presence of inflammatory lesions (gray-white lesions), dark red bleeding lesions, and sticky secretions in the lung tissue (10) and severe alveolar edema and hemorrhagic necrosis in both lungs, along with extensive pulmonary interstitial fibrosis and partial hyaline degeneration (30). These findings provided clear evidence for diffuse alveolar injury in severe COVID-19 cases.

There have been accumulating pathologic findings of COVID-19 outside China (12, 31–34). Diffuse alveolar injury, endothelial injury, thromboembolism, and viral particles within renal cells were reported in various COVID-19 cases (12). A case series from Washington State showed that coronavirus-like particles were detected in the respiratory system, kidney, and gastrointestinal tract (32). Additionally, in one patient complicated by myocarditis, the viral RNA could be detected in the heart as well (32). More recent findings suggest that COVID-19 may be a complex infection associated with extensive vascular endotheliitis (33, 34), manifesting an imbalance between the coagulation and immune functions in the body, which would pose the infected individual at risk for developing multiple organ failure (MOF).

It should be noted that the above pathological findings are mainly the direct and indirect consequences of lung tissue destruction induced by intracellular viral proliferation. In the rapid progressive and life-threatening form of viral pneumonia, the underlying pathological process is often diffuse alveolar injury, coagulopathy, and MOF (26). Collectively, these findings revealed a mechanistic link between virus infection, proliferation, and diffuse alveolar injury (35): the pathologic change evolves from alveolar capillary dysfunction and platelet activation, followed by intravascular fibrin and micro-thrombus formation; if left uncontrolled, these alterations would trigger systemic dissemination and secondary fibrinolysis and result in platelet and coagulation factor depletion and consequently lead to DIC, even MOF.

NEW MECHANISMS UNDERLYING THROMBOSIS AND COAGULOPATHY IN COVID-19

Replication and dissemination of SARS-CoV-2 in systemic circulation lead to extrapulmonary manifestations, which play key roles in disease progression (2, 34, 36). A previous German prospective cohort study reports a high incidence of deep venous thrombosis (58%) and diffuse alveolar injury (67%) (12). These manifestations are associated with enhanced inflammatory state and hypercoagulable state, resulting in higher rates of venous and arterial thrombosis (12, 37). Moreover, increased severe bleeding rates are reported in critically ill patients following preventive or therapeutic anticoagulant and antiplatelet therapy (37). Subsequent sections of this review will summarize mechanisms involved in the pathogenesis of thrombosis and coagulopathy in COVID-19 patients as a complex chain of pathophysiological events preceding COVID-19-related death (summarized in **Figure 1**).

Endothelial Dysfunction

Endothelial dysfunction induces inflammation and vascular remodeling (38), which are associated with severe COVID-19. Endotheliopathy or endothelial dysfunction, including endothelial activation, endotheliitis, and thrombotic events, is an indicator of coagulopathy in COVID-19 patients (33, 34, 39).

SARS-CoV-2 enters host cells by binding to ACE-2 on pulmonary epithelial cells resulting in lung damage (6, 7). Moreover, vascular endothelial cells of multiple organs, including kidney, heart, and small bowel are infected by SARS-CoV-2 directly. Infection induces apoptosis and pyroptosis, which result in diffuse endothelial inflammation (40). Upregulation of vascular endothelial growth factor and downregulation of E-cadherin expression enhance the permeability of endothelial cells in COVID-19 patients (41). In addition, biopsy of lung tissues of COVID-19 patients shows upregulation of interleukin-6 (IL-6), tumor necrosis factor-α, intercellular adhesion molecule-1, and caspase-1 expression (39). Further, quantitative analysis showed a significant increase in expression levels of von Willebrand factor antigen and soluble P-selectin, which are markers of endothelial cell and platelet activation, in COVID-19 patients admitted to intensive care units (34). Notably, increased expression levels of von Willebrand factor antigen and soluble P-selectin are correlated with mortality (34). Increases in expression levels of these markers imply that endotheliopathy is implicated in the pathogenesis of COVID-19.

Platelet Activation and Depletion

A previous study reports that lungs have a high hematopoietic potential, thus they contribute to terminal platelet production (nearly 50%) (42). Therefore, the platelet-related response in COVID-19 patients may be more rapid and severe during the initial stage of pulmonary infection. In addition to thrombosis and hemostasis, previous studies report a putative role of platelets in host defense against infections (43–45). A previous study using a mouse model reports that platelets migrate to the microvasculature (46). Migratory phenotype contributes to mechano-scavenging and bundling of bacteria and boosts innate immunity in a mouse model of severe bacteremia (46). Moreover, human and murine platelets are induced by a range of antimicrobial compounds, especially platelet microbicidal proteins to exert direct microbicidal activity (43). These findings make it challenging to interpret the impact of platelet activation and depletion in COVID-19 patients. Reports from observational studies during the early days of the outbreak in China and other countries show a significant change of platelet response (from excessive activation to depletion) during the progression of severe COVID-19 cases (15, 47).

At the early phase of infection, SARS-CoV-2 invades the lung tissue of the host, which may activate platelets through changes in gene expression (15, 42). Platelets detect invading pathogens through a broad array of receptors and elicit interaction with immune cells (neutrophils, monocytes, and lymphocytes) (15). Activated platelets then exhibit an augmented aggregation capacity by upregulating membrane P-selectin level, which enhances interactions and aggregation with neutrophils, monocytes, and T cells, through mitogen-activated protein kinase (MAPK) pathway activation and thromboxane generation (15).

Lungs and bone marrow are the main sources of compensatory production of platelet consumption in a variety of thromboembolic disorders (42). During infection, neutrophil extracellular trap formation, platelet aggregation,

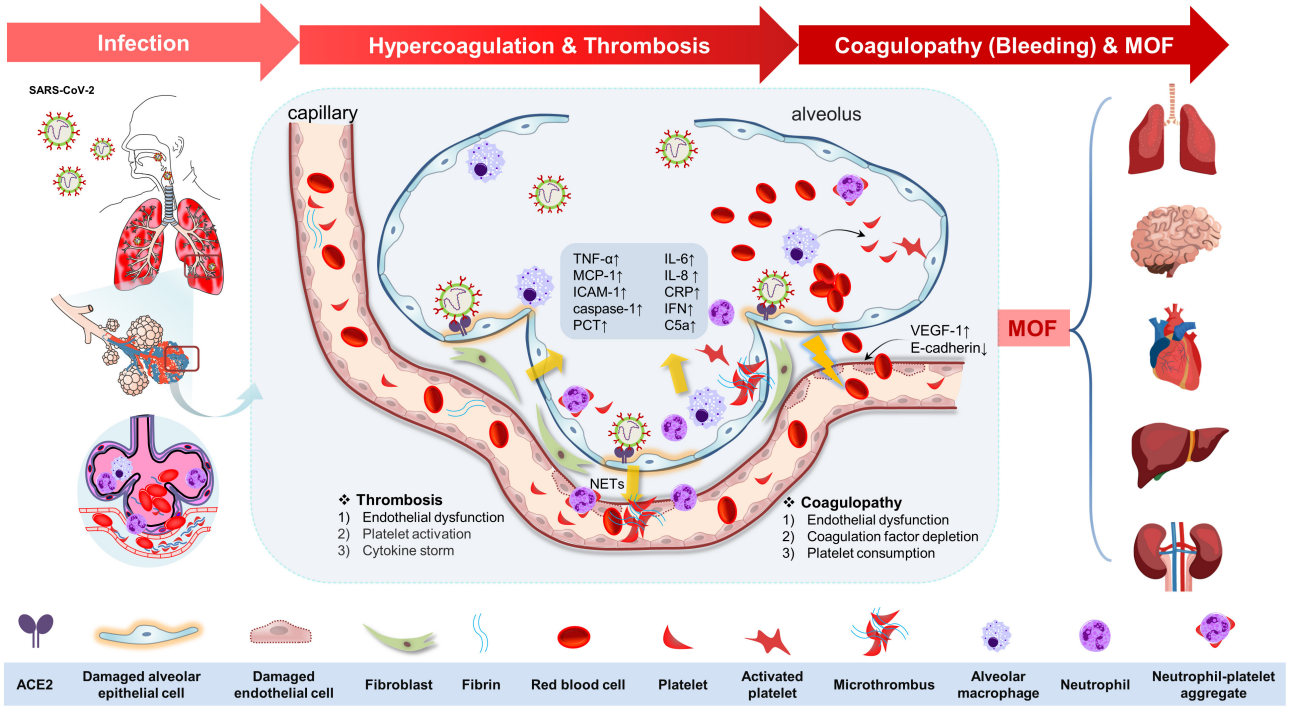

FIGURE 1 | The potential pathophysiological evolutions underlying severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, linking pulmonary inflammation, multiple organ failure, and thrombosis and coagulopathy. The potential pathophysiological evolutions underling SARS-CoV-2 infection was summarized as the following three stages: infection, hypercoagulation, and thrombosis, coagulopathy (bleeding tendency), and multiple organ failure. First, the SARS-CoV-2 could transmit through the respiratory tract in the infected host by angiotensin-converting enzyme 2 (ACE-2) in the epithelial cells in the trachea or lung tissues. The viral proliferation and dissemination within the lung tissue lead to *in situ* endothelial cell injury and platelet and immune system activation. Second, endothelial dysfunction [upregulation of vascular endothelial growth factor receptor 1 (VEGF-1) and downregulation of E-cadherin] elicits the inflammation response and platelet activation to seal the damaged endothelium. Platelet activation was mediated by mitogen-activated protein kinase (MAPK) pathway activation and thromboxane generation, which further induces neutrophil extracellular trap (NET) formation, contributing to the microthrombus with fibrin. Additionally, immune defense and cytokine storm also participate in this process by means of immunothrombosis. This process is characterized by upregulation of tumor necrosis factor-α (TNF-α), monocyte chemotactic protein 1 (MCP-1), intercellular cell adhesion molecule-1 (ICAM-1), caspase-1, interleukin-6 (IL-6), IL-8, C-reactive protein (CRP), interferon (IFN), C5a, procalcitonin (PCT), etc. Antiplatelet therapy is of therapeutic potential at this stage. Third, coagulopathy (bleeding tendency) was associated with endothelial dysfunction, coagulation factor depletion (including fibrinogen and others), and platelet consumption. Finally, all of these changes in COVID-19 drive the progress of multiple organ failure (MOF), including brain, heart, lung, liver, and kidney.

and microthrombus formation reduce tissue perfusion and aggravate inflammation and endothelial injury by activating leukocyte signaling (48). A previous study showed that a high bleeding rate in critically ill COVID-19 patients (7.6 vs. 3.1%) was positively correlated with peak D-dimer levels and negatively correlated with platelet counts (49). Replenishment of circulating platelets is a fine-tuned process determined by the dynamic balance between platelet consumption and production. In severe pulmonary inflammatory response, such as SARS and COVID-19 cases, virus proliferation and dissemination within the lung tissue may directly contribute to *in situ* activation of lung megakaryocyte-derived platelets or have a direct impact on lung megakaryocytes. This may lead to changes in gene expression profile of platelets as observed in COVID-19 patients (15). If this response is not resolved within the lung tissue, platelet activation and ensuring microthrombus formation in lung vasculature would further aggravate pulmonary inflammation. Systemic endothelitis caused by dissemination of SARS-CoV-2 then elicits a second wave of platelet activation in extrapulmonary organs. The second wave leads to a more severe form of platelet activation resulting in consumptive thrombocytopenia (18). Therefore, there is a critical transition in which the beneficial effect of antiplatelet therapy at an early stage of COVID-19 can be attenuated or may have severe effects by aggravating bleeding when clinically significant thrombocytopenia develops.

Immune Defense and Cytokine Storms

Activation of the immune system, which includes production of cytokines, immune complements, and various immune cells, plays an important role in fighting SARS-CoV-2 infection (41). Immune response is always a double-edged sword. Under inflammatory conditions in COVID-19 patients, immunothrombosis, which contains invading pathogens driven by platelets, neutrophils, and the coagulation cascade, is a central pathogenic factor linking respiratory failure and systemic hypercoagulation (50). In addition, immunothrombosis leads to vessel occlusion and tissue hypoxia, which may enhance

the inflammatory response. COVID-19 patients with loss-of-function variants of Toll-like receptor 7, which mediates type I interferon and interferon-γ production, show poor prognosis and subtle subsegmental pulmonary embolisms (51). These findings imply that the immune system is closely associated with thrombosis during SARS-CoV-2 infection.

Recent studies report that the serum of COVID-19 patients showed elevated cytokine levels (C-reactive protein, IL-6, IL-8, and monocyte chemotactic protein-1) (41), high complement levels (C5a) (52), and reduced lymphocyte counts (41). The negative correlation between high levels of IL-6 or IL-8 and low lymphocyte counts indicates underlying mechanisms that link these characteristics in severe disease, including immunothrombosis. This finding is consistent with reports that treatment of COVID-19 patients with tocilizumab, which blocks IL-6-mediated signaling, restored circulating levels of lymphocytes to levels close to normal ranges (53). Enhanced host immune response plays a pivotal role in inducing MOF. Therefore, the efficacy of dexamethasone, a nonspecific immunosuppressant, was evaluated in a large randomized clinical trial and a meta-analysis in patients hospitalized with COVID-19 (54, 55). The results showed approximately 30% reduction in mortality for patients under respiratory support. Therefore, these findings imply that excessively activated host immune response aggravates COVID-19-associated MOF.

ANTITHROMBOTIC THERAPY IN COVID-19 PATIENTS TREATED WITH PERCUTANEOUS CORONARY INTERVENTION

During the COVID-19 global pandemic lockdown period, the number of patients presenting with acute coronary syndrome (ACS) and emergency coronary procedures reduced significantly in Europe (56–58), the USA (59, 60), and Asia (61). However, ACS patients were the main target population among patients with coronary heart disease in cardiology departments during the COVID-19 epidemic compared with patients with chronic coronary syndrome (62). At the beginning of the COVID-19 outbreak, the number of admitted ACS patients in most world regions significantly reduced. Clinical management of ACS during this period was characterized by a decrease in hospitalization rate [−48.4% in Italy (57)], a decrease in PCI rate [−24% in China (61), −43% in Hubei (61), −32% in Italy (63)], and an increase in thrombolytic rate [+66% in China (61), +378% in Hubei (61)]. A recent study in UK showed that a reduction in ACS hospitalization by 40% from the initial days of the COVID-19 outbreak was gradually decreasing to a 16% reduction in May 2020 (64). The number of PCI procedures decreased in both ST-segment elevation myocardial infarction (STEMI) and non-STEMI patients (−21 and −37%, respectively) (64). Furthermore, STEMI patients with COVID-19 showed a higher thrombus load, with 17.9% of these patients presenting with multiple thrombus formation (65). In addition to ensuring timely and effective revascularization of ACS patients (especially STEMI patients), the control of COVID-19 infection in ACS patients is important. Different countries have different views on treatment approaches of ACS patients coinfected with COVID-19 (66–73). Therefore, there is a need to explore appropriate treatment measures for ACS patients during the COVID-19 epidemic.

Coronavirus Disease 2019-Related Delay: The Dilemma for Pre-hospital Management of Acute Coronary Syndrome

Early diagnosis and timely management are critical in reducing morbidity and mortality related to ACS. Ischemic time duration is a major determinant of infarct size in patients with STEMI. Current delays in COVID-19 testing, termed as "COVID-19-related delay," may contribute to total ischemia time (74). Tam et al. (75) reported that median pre-hospital delay increased from 82.5 to 318 min and door to device time increased from 84.5 to 110 min.

Primary Percutaneous Coronary Intervention or Thrombolysis: The Choice of Optimal In-hospital Treatment of Acute Coronary Syndrome

Recent studies showed that patients with STEMI presenting with concurrent COVID-19 present with unique findings during coronary angiography (65, 76). A study carried out in Italy reports that 11 patients (39.3%) out of 28 COVID-19 patients admitted for STEMI showed no obstructive coronary artery disease (76). Another single-center study from UK comprising 115 consecutive STEMI patients with confirmed concurrent COVID-19 reported significantly higher rates of multivessel thrombosis, stent thrombosis, and glycoprotein IIb/IIIa inhibitor use (65). Notably, these findings were based on small observational studies. However, angiographic manifestations require a dedicated diagnostic approach and a modified antithrombotic regimen for this special population.

While primary PCI remains the treatment of choice for STEMI, the balance between exposure risk of medical staff and benefit of patient from thrombolysis should be considered in certain circumstances. Strategic Reperfusion Early After Myocardial Infarction study demonstrated that even a single hour of delay may affect the effectiveness of primary PCI compared with thrombolysis (77).

In China and Iran, thrombolytic therapy is recommended over primary PCI for STEMI management if COVID-19 was confirmed or could not be excluded within a short time. On the other hand, SARS-CoV-2 infection is excluded first for non-STEMI and unstable angina pectoris approaches (66–69). Conversely, organizations from the United States (72), Europe (73), Australia, and New Zealand (70) recommend the use of existing primary PCI protocols for STEMI patients except for confirmed COVID-19 patients and persons under investigation or cases in which primary PCI could not be performed within required time frames. Moreover, previous studies recommend that coronary angiography should be performed prior to discharge after the patient has stabilized from COVID-19 (78).

Periprocedural Anticoagulant Therapy: Intensified and Prolonged

A high risk of thrombotic complications in patients with COVID-19 complicates the dosage of anticoagulation in hospitalized patients with COVID-19 (25). Anticoagulation is recommended for patients with thrombotic complications in addition to antiplatelet therapy during primary PCI (79, 80). In addition, routine use of unfractionated heparin (I, C) and enoxaparin intravenous (IIa, A) should be considered (79). In patients with heparin-induced thrombocytopenia, bivalirudin is recommended as an anticoagulant agent during primary PCI (I, C) (79). The optimal dosage of anticoagulants (conservative or radical) in COVID-19 patients should be personalized based on inflammatory state and a hypercoagulable state of the patients.

The 2018 European Society of Cardiology guideline does not give guidelines on routine post-procedural anticoagulant therapy after primary PCI (79). STEMI patients should receive at least 48 h of anticoagulation therapy after intravenous thrombolysis (80). Introduction of post-procedural anticoagulation and prolongation of anticoagulation therapy is required to counterbalance the COVID-19-related systemic hypercoagulability after primary PCI and intravenous thrombolysis for COVID-19 patients. Notably, these therapy approaches may increase the risk of heparin-induced thrombocytopenia (81).

Dual Antiplatelet Therapy: The Choice of Optimal $P2Y_{12}$ Inhibitor

In a previous prospective study, our group summarized reports on thrombotic and bleeding incidence from early findings of COVID-19 outbreak and reported on the pros and cons of antithrombotic treatment for patients following PCI (16). The findings from this review add more information on the use of antithrombotic treatment in COVID-19 patients. Notably, the time of initiating an antithrombotic regimen should be considered. In the early phase of COVID-19, platelet inhibition by dual antiplatelet therapy (DAPT) may suppress the hyperactivation state of platelets probably through inhibition of *in situ* platelet activation in lung vasculature (15, 42). Antiplatelet agents used at this stage affect intravascular fibrin and thrombus formation, thereby preventing secondary fibrinolysis and coagulation factor depletion. Notably, observational studies report that pre-hospitalization aspirin use is associated with lower mortality in patients with community-acquired pneumonia (100 mg) (82) and ARDS (75–300 mg) (83). On the contrary, findings from a randomized clinical trial show that aspirin administration after admission (325 mg loading followed by 81 mg daily for 7 days) does not prevent the development of ARDS (84). In addition, the choice of antiplatelet agents with different intensity modulates the effectiveness of antithrombotic treatment. An observational study showed that pre-hospital exposure to clopidogrel is associated with an increased risk for community-acquired pneumonia (85). Most patients treated with clopidogrel would receive aspirin. Therefore, it is difficult to draw a conclusion that $P2Y_{12}$ inhibitor is harmful in terms of pneumonia prevention. However, higher intensity of platelet inhibition may lead to the suppression of antimicrobial effect of platelets. Furthermore, discontinuation of aspirin 1 to 3 months after PCI with continued $P2Y_{12}$ inhibitor monotherapy significantly reduces the risk of major bleeding by 40∼50%, with no increased risk of major adverse cardiovascular events, compared with traditional DAPT (86). Therefore, continued $P2Y_{12}$ inhibitor monotherapy may be relatively safe after PCI in COVID-19 patients with a higher risk of bleeding. Ticagrelor, a unique $P2Y_{12}$ inhibitor, has an additional target of inhibition, the equilibrative nucleoside transporter 1; therefore, it results in higher antiplatelet effects and antibacterial activity (87). Moreover, a clinical benefit of ticagrelor in the management of pneumonia by preventing sepsis complications and reducing lung injury was reported in the recent XANTHIPPE (Targeting Platelet-Leukocyte Aggregates in Pneumonia With Ticagrelor) trial (88) and PLATO study (89). Furthermore, clinicians should carefully evaluate platelet counts and levels of other hematological parameters when describing antiplatelet agents. Both primary (idiopathic thrombocytopenic purpura) and secondary thrombocytopenia (enhanced consumption) are associated with an increased risk of infection (including pneumonia) (90), poor outcomes associated with pneumonia (91, 92), and increased mortality for ARDS (93). Individuals who are thrombocytopenic lose the ability to deposit fibrinogen and fail to seal damaged pulmonary vasculature (94). Therefore, platelets are potential therapeutic targets to help predict the onset of ARDS. Currently, there are no available studies on prolongation and intensified antiplatelet therapy in reducing COVID-19-related thrombosis and MOF; therefore, antiplatelet therapy, especially ticagrelor, following PCI should be maintained. However, a study carried out on East Asian populations showed a significantly higher incidence of clinical bleeding in the ticagrelor group compared with that in the clopidogrel group (11.7 vs. 5.3%; hazard ratio, 2.26; 95% confidence interval, 1.34–3.79; $P = 0.002$) (95). A recent study from the SWEDEHEART Registry reports that ticagrelor use among elderly ACS patients is associated with a higher risk of bleeding (hazard ratio, 1.48; 95% confidence interval, 1.25–1.76) and death (hazard ratio, 1.17; 95% confidence interval, 1.03–1.32) compared with the use of clopidogrel (96). Therefore, ticagrelor should not be prescribed to the elderly and East Asian populations. Moreover, the balance between platelet consumption and production, host immune response, and the fact that the clinical benefit of DAPT in the context of COVID-19 is dependent on the severity of the disease should be considered during treatment.

Prognosis of Acute Coronary Syndrome Patients Presenting With Concurrent Coronavirus Disease 2019

Currently, the long-term impact of COVID-19-related endothelial activation, hypercoagulability, microvascular thrombosis, and myocardial injury is not well-known (97). Previous studies report that multiple imaging techniques can accurately assess cardiovascular conditions in COVID-19 patients (98–101). Cardiac nuclear magnetic resonance in

patients recovering from COVID-19 infection shows myocardial involvement (mainly myocarditis, including myocardial edema, fibrosis, and impaired right ventricular function) (99–101). Interestingly, a 12-year follow-up survey of 25 patients who recovered from SARS-CoV infection showed that 68% of these patients had hyperlipidemia, 44% had cardiovascular system abnormalities, and 60% had glucose metabolism disorders (102). SARS-CoV-2 and SARS-CoV mechanism of infection and systemic involvements are similar; therefore, long-term prognosis of COVID-19 patients should be explored.

Zhang et al. (103) reported that in-hospital use of statins among 13,981 cases of COVID-19 was significantly associated with a lower risk of death (5.2 vs. 9.4%, adjusted hazard ratio of 0.58) and less inflammatory response during the entire hospitalization period compared with non-statin use. This finding implies that statin plays a protective role in the acute management of COVID-19 by protecting vascular endothelium and regulating immunity (104).

CONCLUSION

Findings from clinical observations and autopsy studies show a complex chain of events preceding COVID-19-related death. The adverse event chain starts with viral infection and proliferation, followed by endothelial dysfunction induced by local and systemic viral dissemination. Further, platelet activation, thrombosis, and platelet and coagulation factor depletion occur leading to MOF and life-threatening bleeding. Patients treated with PCI and patients on antithrombotic treatment should undergo post-procedural anticoagulation and prolonged anticoagulation following intravenous thrombolysis and standard DAPT treatment to reduce the risk of thrombotic complications during early-to-mid stages of COVID-19 progression. Pros and cons of these antithrombotic treatment regimens should be evaluated in an individualized manner in cases of clinical thrombocytopenia (induced either by platelet consumption or by heparin) and/or bleeding complications.

AUTHOR CONTRIBUTIONS

HL and ZW performed the manuscript writing and illustration drawing. HS performed part of literature collecting. TT and YL provided critical scientific input and discussions. XZ and QY conceived, designed, and supervised all studies and the drafting and editing of the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Word Health Organization. *WHO Coronavirus Disease (COVID-19) Dashboard*. WHO (2020). Available online at: https://covid19.who.int/
2. 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
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) 323:1061–9. doi: 10.1001/jama.2020.1585
4. 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
5. Goyal P, Choi JJ, Pinheiro LC, Schenck EJ, Chen R, Jabri A, et al. Clinical characteristics of Covid-19 in New York city. *N Engl J Med.* (2020) 382:2372–4. doi: 10.1056/NEJMc2010419
6. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet.* (2020) 395:565–74. doi: 10.1016/S0140-6736(20)30251-8
7. 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.e278. doi: 10.1016/j.cell.2020.02.052
8. Li F, Li W, Farzan M, Harrison SC. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science.* (2005) 309:1864–8. doi: 10.1126/science.1116480
9. Lang ZW, Zhang LJ, Zhang SJ, Meng X, Li JQ, Song CZ, et al. A clinicopathological study of three cases of severe acute respiratory syndrome (SARS). *Pathology.* (2003) 35:526–31. doi: 10.1080/00313020310001619118
10. Liu Q, Wang RS, Qu GQ, Wang YY, Liu P, Zhu YZ, et al. Gross examination report of a COVID-19 death autopsy. *Fa Yi Xue Za Zhi.* (2020) 36:21–3. doi: 10.12116/j.issn.1004-5619.2020.01.005
11. 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
12. Wichmann D, Sperhake JP, 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
13. 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
14. Li Y, Li M, Wang M, Zhou Y, Chang J, Xian Y, et al. Acute cerebrovascular disease following COVID-19: a single center, retrospective, observational study. *Stroke Vasc Neurol.* (2020) 5:279–84. doi: 10.1136/svn-2020-000431
15. 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
16. Zhou X, Li Y, Yang Q. Antiplatelet therapy after percutaneous coronary intervention in patients with COVID-19: implications from clinical features to pathologic findings. *Circulation.* (2020) 141:1736–8. doi: 10.1161/CIRCULATIONAHA.120.046988
17. Medmarket diligence. *Global Dynamics of Surgical and Interventional Cardiovascular Procedures 2015-2022.* Medmarket diligence (2018).
18. 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
19. Bryce C, Grimes Z, Pujadas E, Ahuja S, Beasley MB, Albrecht R, et al. Pathophysiology of SARS-CoV-2: targeting of endothelial cells renders a complex disease with thrombotic microangiopathy and aberrant immune

response. The mount sinai COVID-19 autopsy experience. *medRxiv [Preprint]*. (2020). doi: 10.1101/2020.05.18.20099960
20. 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
21. Cui J, Li F Shi ZL. Origin and evolution of pathogenic coronaviruses. *Nat Rev Microbiol.* (2019) 17:181–92. doi: 10.1038/s41579-018-0118-9
22. Lee N, Hui D, Wu A, Chan P, Cameron P, Joynt GM, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. *N Engl J Med.* (2003) 348:1986–94. doi: 10.1056/NEJMoa030685
23. Wong RS, Wu A, To KF, Lee N, Lam CW, Wong CK, et al. Haematological manifestations in patients with severe acute respiratory syndrome: retrospective analysis. *BMJ.* (2003) 326:1358–62. doi: 10.1136/bmj.326.7403.1358
24. World Health Organization. *Clinical Management of Severe Acute Respiratory Infection When Novel Coronavirus (2019-nCoV) Infection is Suspected.* WHO (2020). Available online at: https://apps.who.int/iris/handle/10665/332299
25. 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
26. Radigan KA, Wunderink RG. Epidemic viral pneumonia and other emerging pathogens. *Clin Chest Med.* (2011) 32:451–67. doi: 10.1016/j.ccm.2011.05.010
27. Word Health Organization. *Summary of Probable Sars Cases With Onset of Illness From 1 November 2002 to 31 July* 2003. WHO (2020). Available online at: https://www.who.int/csr/sars/country/table2004_04_21/en/
28. Franks TJ, Chong PY, Chui P, Galvin JR, Lourens RM, Reid AH, et al. Lung pathology of severe acute respiratory syndrome (SARS): a study of 8 autopsy cases from Singapore. *Hum Pathol.* (2003) 34:743–8. doi: 10.1016/S0046-8177(03)00367-8
29. Tian S, Hu W, Niu L, Liu H, Xu H Xiao SY. Pulmonary pathology of early-phase 2019. Novel coronavirus (COVID-19) pneumonia in two patients with lung cancer. *J Thorac Oncol.* (2020) 15:700–4. doi: 10.1016/j.jtho.2020.02.010
30. Luo W, Yu H, Guo J. Clinical pathology of critical patient with novel coronavirus pneumonia (covid-19). *Preprints [Preprint].* (2020). doi: 10.1097/TP.0000000000003412
31. 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
32. 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
33. 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
34. Goshua G, Pine AB, Meizlish ML, Chang CH, Zhang H, Bahel P, et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. *Lancet Haematol.* (2020) 7:e575–82. doi: 10.1016/S2352-3026(20)30216-7
35. Taubenberger JK, Morens DM. The pathology of influenza virus infections. *Annu Rev Pathol.* (2008) 3:499–522. doi: 10.1146/annurev.pathmechdis.3.121806.154316
36. 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
37. Chan NC, Weitz JI. COVID-19 coagulopathy, thrombosis, and bleeding. *Blood.* (2020) 136:381–3. doi: 10.1182/blood.2020007335
38. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation.* (2007) 115:1285–95. doi: 10.1161/CIRCULATIONAHA.106.652859
39. Nagashima S, Mendes MC, Camargo Martins AP, Borges NH, Godoy TM, Miggiolaro A, et al. Endothelial dysfunction and thrombosis in patients with COVID-19. *Arterioscler Thromb Vasc Biol.* (2020) 40:2404–7. doi: 10.1161/ATVBAHA.120.314860
40. 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
41. Moore JB, June CH. Cytokine release syndrome in severe COVID-19. *Science.* (2020) 368:473–4. doi: 10.1126/science.abb8925
42. 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
43. Nicolai L, Gaertner F, Massberg S. Platelets in host defense: experimental and clinical insights. *Trends Immunol.* (2019) 40:922–38. doi: 10.1016/j.it.2019.08.004
44. Ho-Tin-Noé B, Boulaftali Y, Camerer E. Platelets and vascular integrity: how platelets prevent bleeding in inflammation. *Blood.* (2018) 131:277–88. doi: 10.1182/blood-2017-06-742676
45. Elzey BD, Tian J, Jensen RJ, Swanson AK, Lees JR, Lentz SR, et al. Platelet-mediated modulation of adaptive immunity. A communication link between innate and adaptive immune compartments. *Immunity.* (2003) 19:9–19. doi: 10.1016/S1074-7613(03)00177-8
46. Gaertner F, Ahmad Z, Rosenberger G, Fan S, Nicolai L, Busch B, et al. Migrating platelets are mechano-scavengers that collect and bundle bacteria. *Cell.* (2017) 171:1368–1382.e1323. doi: 10.1016/j.cell.2017.11.001
47. 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
48. Guo L, Rondina MT. The era of thromboinflammation: platelets are dynamic sensors and effector cells during infectious diseases. *Front Immunol.* (2019) 10:2204. doi: 10.3389/fimmu.2019.02204
49. 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
50. Nicolai L, Leunig A, Brambs S, Kaiser R, Weinberger T, Weigand M, 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
51. van der Made CI, Simons A, Schuurs-Hoeijmakers J, van den Heuvel G, Mantere T, Kersten S, et al. Presence of genetic variants among young men with severe COVID-19. *JAMA.* (2020) 324:1–11. doi: 10.1001/jama.2020.13719
52. Shen B, Yi X, Sun Y, Bi X, Du J, Zhang C, et al. Proteomic and metabolomic characterization of COVID-19 patient sera. *Cell.* (2020) 182:59–72.e15. doi: 10.1016/j.cell.2020.05.032
53. 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.e1003. doi: 10.1016/j.chom.2020.04.009
54. Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, et al. Dexamethasone in hospitalized patients with Covid-19 - preliminary report. *N Engl J Med.* (2020). doi: 10.1056/NEJMoa2021436. [Epub ahead of print].
55. Siemieniuk RA, Bartoszko JJ, Ge L, Zeraatkar D, Izcovich A, Pardo-Hernandez H, et al. Drug treatments for covid-19: living systematic review and network meta-analysis. *BMJ.* (2020) 370:m2980. doi: 10.1136/bmj.m2980
56. Metzler B, Siostrzonek P, Binder RK, Bauer A, Reinstadler SJ. Decline of acute coronary syndrome admissions in Austria since the outbreak of COVID-19: the pandemic response causes cardiac collateral damage. *Eur Heart J.* (2020) 41:1852–3. doi: 10.1093/eurheartj/ehaa314
57. 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/ehaa610
58. 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

59. 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
60. 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
61. 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
62. Ferreira E, Alves TS, Mourilhe-Rocha R, Lacerda ALI, Albuquerque FN, Spineti PPM, et al. Safety of interventional cardiology procedures in chronic coronary syndrome during the COVID-19 pandemic. *Arq Bras Cardiol.* (2020) 115:712–6. doi: 10.36660/abc.20200704
63. Piccolo R, Bruzzese D, Mauro C, Aloia A, Baldi C, Boccalatte M, et al. Population Trends in rates of percutaneous coronary revascularization for acute coronary syndromes associated with the COVID-19 outbreak. *Circulation.* (2020) 141:2035–7. doi: 10.1161/CIRCULATIONAHA.120.047457
64. 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) 396:381–389. doi: 10.1016/S0140-6736(20)31356-8
65. 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
66. 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
67. 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
68. Xiang D, Huo Y, Ge J. Expert consensus on operating procedures at chest pain centers in China during the coronavirus infectious disease-19 epidemic. *Cardiology Plus.* (2020) 5:21–32. doi: 10.4103/cp.cp_5_20
69. Sadeghipour P, Talasaz AH, Eslami V, Geraiely B, Vojdanparast M, Sedaghat M, et al. Management of ST-segment-elevation myocardial infarction during the coronavirus disease (2019). (COVID-19) outbreak: Iranian "247" national committee's position paper on primary percutaneous coronary intervention. *Catheter Cardiovasc Interv*. (2020). doi: 10.1002/ccd.28889. [Epub ahead of print].
70. Zaman S, MacIsaac AI, Jennings GL, Schlaich MP, Inglis SC, Arnold R, et al. Cardiovascular disease and COVID-19: Australian and New Zealand consensus statement. *Med J Aust*. (2020) 213:182–7. doi: 10.5694/mja2.50714
71. 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
72. 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
73. 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
74. 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
75. Tam CF, 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
76. Stefanini GG, Montorfano M, Trabattoni D, Andreini D, Ferrante G, Ancona M, et al. ST-elevation myocardial infarction in patients with COVID-19: clinical and angiographic outcomes. *Circulation.* (2020) 141:2113–6. doi: 10.1161/CIRCULATIONAHA.120.047525
77. Armstrong PW, Gershlick AH, Goldstein P, Wilcox R, Danays T, Lambert Y, et al. Fibrinolysis or primary PCI in ST-segment elevation myocardial infarction. *N Engl J Med.* (2013) 368:1379–87. doi: 10.1056/NEJMoa1301092
78. Nijjer SS, Petraco R, Sen S. Optimal management of acute coronary syndromes in the era of COVID-19. *Heart.* (2020) 106:1609–16. doi: 10.1136/heartjnl-2020-317143
79. 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: 10.5603/KP.2018.0041
80. Chinese Society of Cardiology of Chinese Medical Association, Editorial Board of Chinese Journal of Cardiology. 2019 Chinese society of cardiology (CSC) guidelines for the diagnosis and management of patients with ST-segment elevation myocardial infarction. *Chin J Cardiol*. (2019) 47:766–83. doi: 10.3760/cma.j.issn.0253-3758.2019.10.003
81. Daviet F, Guervilly C, Baldesi O, Bernard-Guervilly F, Pilarczyk E, Genin A, et al. Heparin-induced thrombocytopenia in severe COVID-19. *Circulation.* (2020) 142:1875–7. doi: 10.1161/CIRCULATIONAHA.120.049015
82. Falcone M, Russo A, Cangemi R, Farcomeni A, Calvieri C, Barillà F, et al. Lower mortality rate in elderly patients with community-onset pneumonia on treatment with aspirin. *J Am Heart Assoc.* (2015) 4:e001595. doi: 10.1161/JAHA.114.001595
83. Boyle AJ, Di Gangi S, Hamid UI, Mottram LJ, McNamee L, White G, et al. Aspirin therapy in patients with acute respiratory distress syndrome (ARDS) is associated with reduced intensive care unit mortality: a prospective analysis. *Crit Care.* (2015) 19:109. doi: 10.1186/s13054-015-0846-4
84. Kor DJ, Carter RE, Park PK, Festic E, Banner-Goodspeed VM, Hinds R, et al. Effect of aspirin on development of ARDS in at-risk patients presenting to the emergency department: the LIPS-A randomized clinical trial. *JAMA.* (2016) 315:2406–14. doi: 10.1001/jama.2016.6330
85. Gross AK, Dunn SP, Feola DJ, Martin CA, Charnigo R, Li Z, et al. Clopidogrel treatment and the incidence and severity of community acquired pneumonia in a cohort study and meta-analysis of antiplatelet therapy in pneumonia and critical illness. *J Thromb Thrombolysis.* (2013) 35:147–54. doi: 10.1007/s11239-012-0833-4
86. O'Donoghue ML, Murphy SA, Sabatine MS. The safety and efficacy of aspirin discontinuation on a background of a P2Y(12) inhibitor in patients after percutaneous coronary intervention: a systematic review and meta-analysis. *Circulation.* (2020) 142:538–45. doi: 10.1161/CIRCULATIONAHA.120.046251
87. Lancellotti P, Musumeci L, Jacques N, Servais L, Goffin E, Pirotte B, et al. Antibacterial activity of ticagrelor in conventional antiplatelet dosages against antibiotic-resistant gram-positive bacteria. *JAMA Cardiol.* (2019) 4:596–9. doi: 10.1001/jamacardio.2019.1189
88. Sexton TR, Zhang G, Macaulay TE, Callahan LA, Charnigo R, Vsevolozhskaya OA, et al. Ticagrelor reduces thromboinflammatory markers in patients with pneumonia. *JACC Basic Transl Sci.* (2018) 3:435–49. doi: 10.1016/j.jacbts.2018.05.005
89. Cannon CP, Harrington RA, James S, Ardissino D, Becker RC, Emanuelsson H, et al. Comparison of ticagrelor with clopidogrel in patients with a planned invasive strategy for acute coronary syndromes (PLATO): a randomised double-blind study. *Lancet.* (2010) 375:283–293. doi: 10.1016/S0140-6736(09)62191-7
90. Ekstrand C, Linder M, Cherif H, Kieler H Bahmanyar S. Increased susceptibility to infections before the diagnosis of immune thrombocytopenia. *J Thromb Haemost.* (2016) 14:807–14. doi: 10.1111/jth.13267

91. Gorelik O, Izhakian S, Barchel D, Almoznino-Sarafian D, Tzur I, Swarka M, et al. Prognostic significance of platelet count changes during hospitalization for community-acquired pneumonia. *Platelets.* (2017) 28:380–6. doi: 10.1080/09537104.2016.1219032
92. Brogly N, Devos P, Boussekey N, Georges H, Chiche A, Leroy O. Impact of thrombocytopenia on outcome of patients admitted to ICU for severe community-acquired pneumonia. *J Infect.* (2007) 55:136–40. doi: 10.1016/j.jinf.2007.01.011
93. Tejera P, Christiani DC. Deconstructing ARDS variability: platelet count, an ARDS intermediate phenotype and novel mediator of genetic effects in ARDS. *Semin Respir Crit Care Med.* (2019) 40:12–8. doi: 10.1055/s-0039-1683891
94. Washington AV, Esponda O, Gibson A. Platelet biology of the rapidly failing lung. *Br J Haematol.* (2020) 188:641–51. doi: 10.1111/bjh.16315
95. Park DW, Kwon O, Jang JS, Yun SC, Park H, Kang DY, et al. Clinically significant bleeding with ticagrelor versus clopidogrel in korean patients with acute coronary syndromes intended for invasive management: a randomized clinical trial. *Circulation.* (2019) 140:1865–77. doi: 10.1161/CIRCULATIONAHA.119.041766
96. Szummer K, Montez-Rath ME, Alfredsson J, Erlinge D, Lindahl B, Hofmann R, et al. Comparison between ticagrelor and clopidogrel in elderly patients with an acute coronary syndrome: insights from the SWEDEHEART registry. *Circulation.* (2020) 142:1700–8. doi: 10.1161/CIRCULATIONAHA.120.050645
97. Giustino G, Pinney SP, Lala A, Reddy VY, Johnston-Cox HA, Mechanick JI, et al. Coronavirus and cardiovascular disease, myocardial injury, and arrhythmia: JACC focus seminar. *J Am Coll Cardiol.* (2020) 76:2011–23. doi: 10.1016/j.jacc.2020.08.059
98. Rudski L, Januzzi JL, Rigolin VH, Bohula EA, Blankstein R, Patel AR, et al. Multimodality imaging in evaluation of cardiovascular complications in patients with COVID-19: JACC scientific expert panel. *J Am Coll Cardiol.* (2020) 76:1345–57. doi: 10.1016/j.jacc.2020.06.080
99. 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
100. 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. [Epub ahead of print].
101. 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
102. Wu Q, Zhou L, Sun X, Yan Z, Hu C, Wu J, et al. Altered lipid metabolism in recovered SARS patients twelve years after infection. *Sci Rep.* (2017) 7:9110. doi: 10.1038/s41598-017-09536-z
103. 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) 32:176–87.e174. doi: 10.1016/j.cmet.2020.06.015
104. Hermida N, Balligand JL. Low-density lipoprotein-cholesterol-induced endothelial dysfunction and oxidative stress: the role of statins. *Antioxid Redox Signal.* (2014) 20:1216–37. doi: 10.1089/ars.2013.5537

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frontiers in Cardiovascular Medicine

SYSTEMATIC REVIEW
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COVID-19 and the Heart: A Systematic Review of Cardiac Autopsies

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Importance: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-associated cardiac injury has been postulated secondary to several mechanisms. While tissue diagnosis is limited during the acute illness, postmortem studies can help boost our understanding and guide management.

Objective: To report the cardiac tissue autopsy findings in coronavirus disease 2019 (COVID-19) decedents.

Evidence Review: Articles published in PubMed and Embase reporting postmortem cardiac pathology of COVID-19 decedents till September 2020. We included adult studies excluding preprints. The Joanna Briggs Institute Critical Appraisal Checklist for Case Reports was used to assess quality. We extracted gross and histology data as well as the incidence of myocarditis, cardiac ischemia, thrombosis, and dilatation. We also looked at the reported cause of death (PROSPERO registration CRD42020190898).

Findings: Forty-one relevant studies identified including 316 cases. The deceased were mostly male (62%) and elderly (median age, 75; range, 22–97 years). The most common comorbidities were hypertension (48%) and coronary artery disease (33%). Cardiac pathologies contributed to the death of 15 cases. Besides chronic cardiac pathologies, postmortem examination demonstrated cardiac dilatation (20%), acute ischemia (8%), intracardiac thrombi (2.5%), pericardial effusion (2.5%), and myocarditis (1.5%). SARS-CoV-2 was detected within the myocardium of 47% of studied hearts.

Conclusions and Relevance: SARS-CoV-2 can invade the heart, but a minority of cases were found to have myocarditis. Cardiac dilatation, ischemia, mural, and microthrombi were the most frequent findings. The systematic review was limited by the small number of cases and the quality of the studies, and there is a need to standardize the cardiac postmortem protocols.

Keywords: COVID-19, SARS-CoV-2, post-mortem, cardiac injury, autopsy

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KEY POINTS

- **Question**: What are the pathological cardiac findings in postmortem autopsies of COVID-19 patients?
- **Findings**: The systematic review included 41 studies and 316 cases. Apart from chronic pathological findings, postmortem examination demonstrated cardiac dilatation (20%), acute ischemia (8%), intracardiac thrombi (2.5%), pericardial effusion (2.5%), and myocarditis (1.5%). SARS-CoV-2 was detected within the myocardium of 47% of studied hearts.
- **Meaning**: The main pathological findings in patients dying during the acute COVID-19 illness were cardiac dilatation, ischemia, and (micro)thrombosis. Myocarditis was a rare finding in this cohort of patients.

INTRODUCTION

While coronavirus disease 2019 (COVID-19) primarily affects the lungs, it is increasingly recognized as a multiorgan disease. The underlying mechanism may be direct viral invasion or secondary to the systematic effect of the infection (e.g., hypoperfusion, hypoxia, massive inflammatory response/cytokine storm).

Cardiac comorbidity and standard coronary risk factors (e.g., obesity, diabetes, and hypertension) are associated with adverse outcomes among patients with COVID-19 (1). COVID-19 is also associated with release of the highly specific marker of myocardial cell death—Troponin. Where this is tested in all hospitalized patients, the prevalence of elevated Troponin has been reported in up to 71% and is a predictor of outcome (40% mortality vs. 8% in those without myocardial injury) (2). A recent meta-analysis of published retrospective observational studies identified a positive troponin in 27% of 1,550 patients, with a similar impact on increased mortality and increased probability of needing intensive care (3).

Acute setting cardiac imaging (mainly echocardiography), while a valuable tool to assess the cardiac function and structure, suffers many limitations (4). Endomyocardial biopsies (EMBs) are rarely performed due to logistics and infection control reasons.

Postmortem examination (PM) is a valuable resource to understand the pathophysiology, cause of death, and the extent of organ involvement. Lessons from previous infectious diseases [e.g., human immunodeficiency virus (HIV)] have demonstrated the benefit of PMs (5).

To date, single case reports to modest-sized autopsy series have failed to clarify the nature of cardiac involvement. Histological findings vary from interstitial edema with or without myocarditis (6), lymphocytic endothelialitis (7), microvascular microthrombi and venous thrombosis (8), to extensive interstitial fibrosis with no endothelialitis (9), and no evidence of myocarditis (10). Optimal management depends on knowledge of the mechanism of myocardial injury, as the treatment and required follow-up will differ among the various pathologies outlined above.

To gain a better understanding of the prevalent cardiac findings in patients dying of COVID-19—we undertook a systematic review of all reported autopsies that included cardiac findings.

METHODOLOGY

A protocol of a systematic review was registered on PROSPERO database (CRD42020190898) on the 23rd June 2020. The aim was to investigate autopsy findings for patients who died from a confirmed COVID-19 infection (https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=190898).

An initial systematic search was conducted through the *NHS Healthcare Databases Advanced Search* tool (HDAS) on 7th of June 2020 for published articles in PubMed and Embase databases. The search strategy is shown in **Table 1**. An electronic search alert was set to identify any new study on the EMBASE database through Healthcare Databases Advanced Search (HDAS) (option not available for PubMed) till the 21st of September 2020. The search was done by AR and included the period from 1st January 2019 to the search date. AR screened the references for additional articles. We identified 88 articles that reported PM tissue pathology. AR reviewed the full-text to retrieve articles which reported PM cardiac pathology. We reviewed only published articles in journals (excluding pre-prints) in the English language and included humans since 2019 (**Figure 1**: PRISMA diagram). Articles or cases with duplicate reporting have been excluded to the best of our knowledge. AR assessed the quality of the case series studies using the Joanna Briggs Institute Critical Appraisal Checklist for Case Reports (12) (**Supplementary Table 2**). SZ and AR extracted the data from the included studies. Any conflict was resolved by discussion and mutual agreement.

Patient, Intervention, Comparison, and Outcome (PICO) Statement

Patient

Adult patients (≥18 years old) who died and had a laboratory confirmation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

Intervention

None.

Comparison

None or other patients who died from another cause.

Outcome

Pathological description of PM cardiac involvement.

RESULTS

Search Strategy

The search resulted in 226 titles. After duplicate removal of and title screening, we screened the full text of 108 articles (52 from weekly alerts) that yielded 88 articles reporting PM tissue pathology. Among those, 41 studies reported PM heart examination and included 336 cases (**Figure 1**). Studies were mostly case reports ($n = 13$) or case series ($n = 24$),

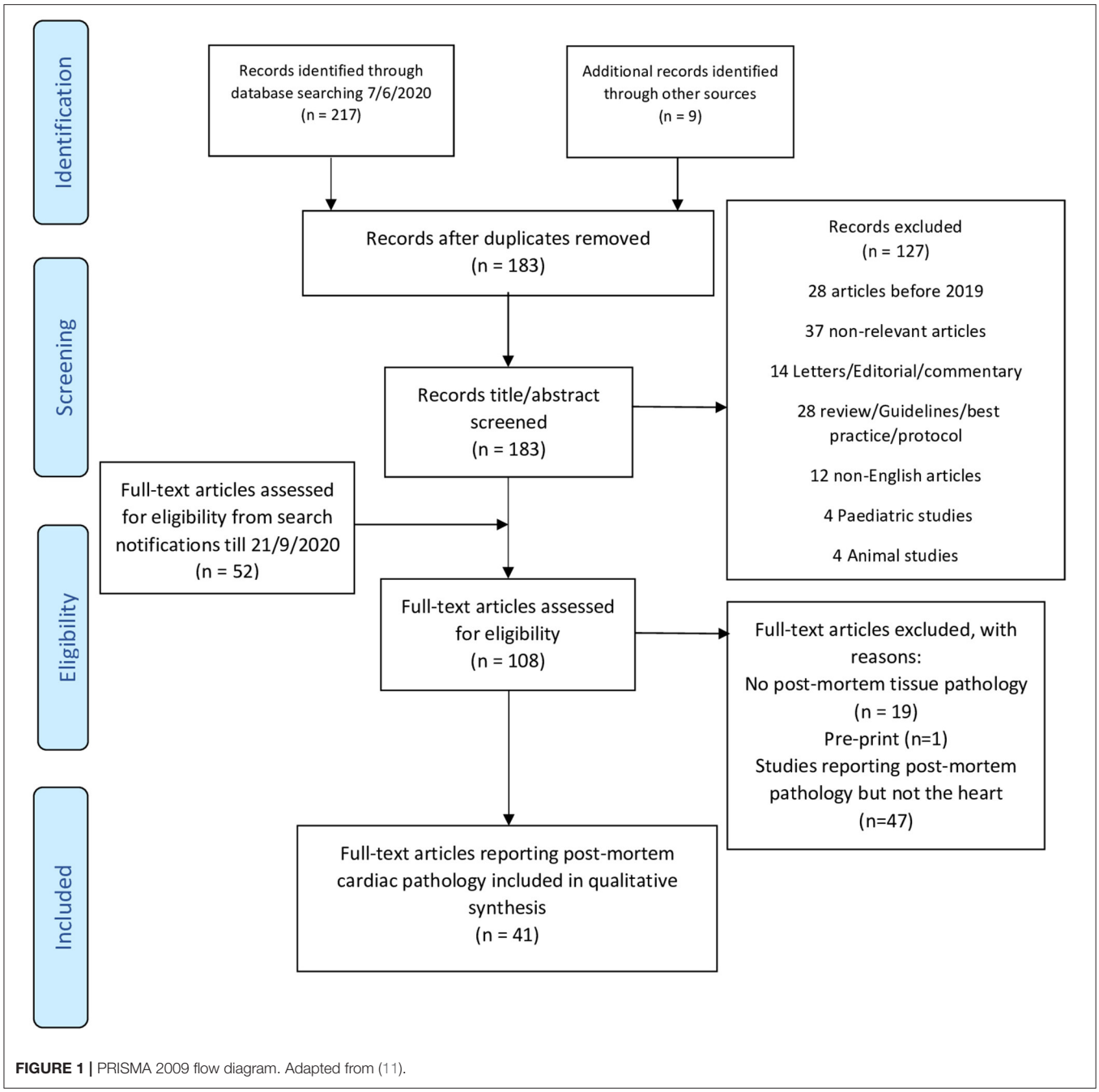

FIGURE 1 | PRISMA 2009 flow diagram. Adapted from (11).

while three studies compared cases to controls (6–10, 13–48) (**Table 2**, **Figure 2**). Authors reported cases from 14 countries, mostly developed westernized ones (**Supplementary Table 1**). Two studies reported on the same population, with one mainly focusing on PM cardiac examination (22, 23). The quality of the included studies was mostly moderate (**Supplementary Table 2**).

We analyzed the PM cardiac histopathology for 316 cases [after excluding cases unconfirmed as COVID-19 ($n = 6$) or with no PM cardiac tissue examination ($n = 14$)].

General Characteristics of the Studies

Study characteristics and pathological findings are detailed in **Supplementary Tables 1**, **2**, respectively. Cases were predominantly male (172/275, 62%). The deceased were mostly elderly [median: 75 years; interquartile range (IQR), 63–84 years; range, 22–97 years, $n = 228$] and overweight [body mass index (BMI): median, 27; IQR, 22.9–34.7 kg/m^2; range = 15.4–61.2 kg/m^2, $n = 148$).

TABLE 1 | Search strategy.

Search	Query
PubMed	
#1	(COVID*).ti,ab
#2	(SARS-CoV-2).ti,ab
#3	(Coronavirus 2019).ti,ab
#4	(nCOV 19).ti,ab
#5	(1 OR 2 OR 3 OR 4)
#6	(autopsy).ti,ab
#7	(necropsy).ti,ab
#8	(post-mort*).ti,ab
#9	(postmort*).ti,ab
#10	(histolog*).ti,ab
#11	(6 OR 7 OR 8 OR 9 OR 10)
#12	(5 AND 11)
Embase	
#13	(COVID*).ti,ab
#14	(SARS-CoV-2).ti,ab
#15	(Coronavirus 2019).ti,ab
#16	(nCOV 19).ti,ab
#17	(13 OR 14 OR 15 OR 16)
#18	(autopsy).ti,ab
#19	(necropsy).ti,ab
#20	(post-mort*).ti,ab
#22	(postmort*).ti,ab
#23	(histolog*).ti,ab
#24	(18 OR 19 OR 20 OR 21 OR 22)
#25	(17 AND 23) [DT 2019–2020] [English language] [Human age groups Adult 18–64 years OR Aged 65+ years] [Humans]

Comorbidities

Cardiovascular comorbidities were prevalent, most commonly hypertension ($n = 152$, 48.1%), coronary artery disease (CAD) ($n = 105$, 33.2%), cardiomyopathy and heart failure ($n = 68$, 21.5%), and atrial fibrillation (AF) ($n = 35$, 11.1%). Other comorbidities included chronic respiratory diseases ($n = 91$, 28.7%), diabetes mellitus ($n = 81$, 25.6%), chronic kidney disease (CKD) ($n = 53$, 16.7%), dementia ($n = 40$, 12.7%), and cancer ($n = 39$, 12.3%).

Timing

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, $n = 98$). The median time interval between death to PM autopsy was 1.2 days ($n = 31$).

Pathological Findings

Cardiac abnormalities either on gross pathology or histology were identified in almost all cases. Most autopsies demonstrated chronic cardiac pathologies [hypertrophy ($n = 85$), fibrosis ($n = 72$), and amyloidosis ($n = 11$)], which may have contributed to the increased heart weight where this was reported (median, 455 g; IQR, 399–576 g; range, 250–1,070 g, exceeded normal range in 39/44 (normal reference: male, 270–360 g; female, 200–280 g)] (47).

While myocardial fibrosis was identified in only 72 cases, in a series where this was specifically reported, the prevalence was high (9, 10). Myocyte and ventricular wall hypertrophy were reported in 85 cases, again highly prevalent where specifically reported (18). Significant cardiac dilatation/cardiomegaly was described in 66 cases (10, 14, 15, 21, 24, 27, 30).

Overall changes consistent with cardiac ischemia and thrombosis were the most frequently reported acute findings. Acute myocardial ischemia was evident in 25 cases either in the form of acute myocardial infarction (MI) ($n = 11$) or microscopic evidence of acute or early ischemia ($n = 14$). Moreover, fibrin microvascular thrombi were identifiable in 27 cases (6, 8, 35, 36, 42, 47, 48). Thrombi in cardiac veins were described in three cases (8, 10). Lastly, there was eight cases with mural thrombi including the heart valves ($n = 3$) and the right atrium (RA) ($n = 1$) (10, 35, 47, 48).

Viral Invasion of Myocardium

Twelve studies explored the presence of SARS-CoV-2 within the myocardium using different techniques (**Table 2**) (8, 9, 17, 19, 23, 24, 29, 30, 39, 41, 44, 48). In those studies, SARS-CoV-2 was detected in 50 of 105 hearts (47%). However, clear myocarditis meeting the Dallas criteria was described in only five cases (6, 9, 17, 22). In an additional 35 cases, minimal lymphocytic ($n = 33$) or mononuclear infiltration ($n = 2$) not meeting the criteria for myocarditis was identified (13, 15, 27, 28). In three cases, authors attributed those changes as consistent with ischemic damage response (28). Overall, lymphocytic infiltration was scarce but can be detected in any of the pericardium, myocardium, epicardium, or endothelium. Lastly, pericardial affection was described in the form of pericardial effusion ($n = 8$) and pericarditis ($n = 5$, one had chronic pericarditis).

Cause of Death

The cause of death was reported for 190 cases and, for the majority of these, was respiratory in origin (**Supplementary Tables 1**, **2**). However, cardiac contribution to death was mentioned for 15 cases while pulmonary embolism (PE) was mentioned in eight cases.

DISCUSSION

Our review confirms that among patients dying from COVID-19, cardiac abnormalities are prevalent, but that specific changes of acute myocarditis are uncommon (1.5% of cases). Myocardial ischemia, thrombosis, and cardiac dilatation were the most dominant acute findings (**Figure 2**). Prevalence of the non-specific myocardial edema (ME) was 100% in the six studies reporting it (6, 15, 19, 35, 44, 45). The highly prevalent chronic cardiac pathologies not only reflect the impact of cardiac comorbidities but also complicated the histopathological interpretation.

TABLE 2 | Postmortem pathology findings in the included studies.

References	Number of cases	Autopsy technique	Time from death to autopsy	Postmortem pathology					Cause of death
				Gross pathology/heart weight	Histology and microscopy	Tissue SARS-CoV-2	Myocarditis (*n*)	Acute ischemia (*n*)	
Duarte-Neto et al. (6)*	10	Ultrasound-guided minimally invasive autopsy	N/A	N/A	**Related to comorbidities: (*n*)** • Cardiomyocytes hypertrophy (9) • Myocardial fibrosis (9) • Previous MI (4) **Acute:** • Mild lymphomononuclear myocarditis (2) • Fibrin microthrombi (2) • Interstitial edema (9)	N/A	2	0	N/A
Schaller et al. (13)	10	Autopsy	N/A	N/A	4/10 mild lymphocytic myocarditis (no true myocarditis) 2/10 epicarditis	N/A	0	0	N/A
Buja et al. (14)	3	Autopsy	N/A	**P1**: • Weight 420 g • CA: patent with minimal atherosclerosis • LV wall thickness: 1.1 cm • RV wall thickness: 0.2–0.3 cm **P2**: • Weight: 1,070 g • 4-chamber hypertrophy and dilatation • CA: patent with minimal atherosclerosis • LV wall thickness: 1.5–1.6 cm • RV wall thickness: 0.5 cm **P3**: • Weight: 670 g. • CA: minimal atherosclerosis, widely patent • Both ventricles were dilated • Thickness of LV free wall and IVS was 1.6 cm and that of the RV was 0.3 cm	**P1**: Microscopy: • Cardiomyocytes with moderately enlarged hyperchromatic nuclei • Individual cardiomyocytes with vacuolar degenerative change • No evidence of inflammatory infiltrate indicative of myocarditis **P2**: **Histology:** • Epicardial lymphocytic infiltrates • Cardiomyocyte hypertrophy • Multifocal interstitial and replacement fibrosis • Scattered damaged individual cardiomyocytes • No inflammatory foci indicative of myocarditis **P3**: • Multifocal lymphocytic infiltrates in epicardium • CMC -enlarged hyperchromatic nuclei • Individual CMC—changes of acute injury • No inflammatory cellular infiltrates found • Prominent foci of CMC disarray—superior portion of the IVS • Intramural coronary arteries—intimal and medical thickening with luminal narrowing • Both diagnostic features of hypertrophic cardiomyopathy • Random sections—sinoatrial and atrioventricular conduction system—no abnormalities	N/A	0	0	N/A
Yan et al. (15)	1	Autopsy	18 h after death	Heart weight: 410 g **Gross:** • Streaking of right atrial wall myocardial tissue: thin myocardial trabecula alternating with areas of epicardium lacking underlying myocardial tissue • No CAD	• LV: No focal lesions suggestive of acute or chronic hypoxic injury • RV: dilated • Mild myxoid edema • Mild myocyte hypertrophy • Rare foci of lymphocytes in myocardium No evidence of viral myocarditis	N/A	0	0	N/A
Lax et al. (10)	11	Autopsy	N/A	**P1**: Myocardial hypertrophy, myocardial fibrosis, endocardial thrombi LV **P2**: Myocardial hypertrophy, coronary small vessel disease myocardial fibrosis **P3**: Myocardial hypertrophy, coronary small vessel disease myocardial fibrosis, thrombosis of a myocardial vein **P4**: Myocardial hypertrophy, coronary small vessel disease myocardial fibrosis **P5**: Myocardial hypertrophy, myocardial fibrosis **P6**: Myocardial hypertrophy, myocardial fibrosis **P7**: Myocardial hypertrophy, coronary small vessel disease myocardial fibrosis **P8**: Myocardial hypertrophy, coronary small vessel disease myocardial fibrosis **P9**: Myocardial hypertrophy **P10**: Myocardial hypertrophy, coronary small vessel disease myocardial fibrosis, amyloidosis **P11**: Myocardial hypertrophy, myocardial fibrosis, Focal lymphocytic infiltrate • Myocardial hypertrophy 11/11 • Coronary small vessel disease 6/11 • Myocardial fibrosis 10/11 • No viral myocarditis In 10 patients, both ventricles were massively dilated In 1 patient, intraventricular endocardial mural thrombi without ischemic changes of adjacent myocardium No acute myocardial necrosis or inflammatory changes found except 1 patient with focus of fragmented cardiomyocytes with lymphocytic and granulocytic reaction		N/A	0	0 (1 venous thrombus with no ischemia)	N/A

(Continued)

TABLE 2 | Continued

References	Number of cases	Autopsy technique	Time from death to autopsy	Postmortem pathology					Cause of death
				Gross pathology/heart weight	Histology and microscopy	Tissue SARS-CoV-2	Myocarditis (*n*)	Acute ischemia (*n*)	
Lacy et al. (16)	1	Autopsy with minor modifications	N/A	• Weight: 438 g • Moderate coronary atherosclerosis in each of the main coronary distributions, no occlusions or critical stenoses • Myocardium: no obvious infarct, firm texture, and red-brown color. • LV thickness: 1.2–1.4 cm • Cardiac valves: normal	• Myocyte hypertrophy • No acute ischemic changes • Interstitial and perivascular fibrous tissue • No viral myocarditis • Moderate infrarenal aortic atherosclerosis	N/A	0	0	**Autopsy:** ARDS due to viral pneumonia due to COVID-19
Wichmann et al. (17)	12	Complete autopsy	P1: 1 day P2: 1 day P3: 2 days P4: 1 day P5: 2 days P6: 1 day P7: 4 days P8: 1 day P9: 4 day P10: 5 days P11: 2 days P12: 3 days	Mean heart weight: 503 g (median, 513 g) **P1:** 660 g, eccentric hypertrophy of both ventricles **P2**: 515 g, CAD with stenting, post-MI, cardiac aneurysm **P3:** 510 g, biventricular hypertrophy, moderate CAD **P4**: 605 g, LVH **P5**: 360 g, CAD, post-MI **P6**: 250 g, normal **P7**: 415 g, CAD, moderate hypertrophy, mitral ring calcification, post MI, pacemaker, lipomatous cordis **P8**: 575 g, CAD, post bypass surgery, post-MI cardiac aneurysm, global hypertrophy **P9**: 355 g, left atrial dilatation, CAD, post-MI **P10:** 390 g, CAD, post-MI **P11:** 650 g, CAD, post aortic valve replacement, biventricular hypertrophy **P12:** 745 g, CAD, hypertrophy	Lymphocytic myocarditis: 1/12	In 5 of the patients, viral RNA detected in other tissues (heart, liver, or kidney) in concentrations exceeding viremia	1	0	N/A
Menter et al. (18)	21	Full body autopsy in 17 cases Partial autopsy in some (?) *in-corpore technique*	Mean PMI from death to autopsy: 33.3 h (11–84.5 h)	• Hypertrophy: 15/21 • Senile cardiac amyloidosis: 6/21 • Peracute myocyte cell necrosis: 3/21 (sequelae of shock) • Acute MI−1/21		N/A	0	1 (acute MI) 3 peracute myocyte cell necrosis	N/A
Varga et al. (7)	3 (1 excluded as still alive)	Autopsy	N/A		• No lymphocytic myocarditis • Endotheliitis **P1**: • Inflammatory cells associated with endothelium and apoptotic bodies **P2**: • Lymphocytic endotheliitis • Acute posterior myocardial infarction • No viral lymphocytic myocarditis	N/A	0	1 (acute posterior MI)	N/A
Tian et al. (19)	4 (2 heart biopsies)	Needle core biopsies of lung, liver, and heart	N/A		Heart biopsies obtained from P 1 and 4 **Both:** • Focal mild edema • Interstitial fibrosis • Myocardial hypertrophy • No inflammatory cellular infiltration • Endocardia and myocardia—no inflammatory cellular infiltration • Focally, myocardium irregular in shape with darkened cytoplasm—not sufficient for acute myocardial injury • Focal interstitial fibrosis, and myocardial hypertrophy	RT-PCR assay for SARS-COV-2: Positive for P1 and negative for P4	0	0	N/A
Barton et al. (20)	2	Autopsy	N/A	**P1**: • Heart weight: 402 g • No adhesions, effusions, or thrombi • CAD: marked 2 vessels **P2**: • Heart weight: 372 g • No adhesions, effusions, or thrombi • CAD: mild • Aorta intimal fatty streaking	**P1**: Microscopic: acute ischemic injury Abdominal aorta atherosclerosis no evidence of myocarditis **P2**: No myocarditis	N/A	0	1 (microscopic acute injury)	**Autopsy:** **P1**: COVID-19, with CAD listed under "other contributing factors." **P2**: complications of hepatic cirrhosis," with muscular dystrophy, aspiration pneumonia, and COVID-19 listed as other significant conditions

(Continued)

TABLE 2 | Continued

References	Number of cases	Autopsy technique	Time from death to autopsy	Postmortem pathology					Cause of death
				Gross pathology/heart weight	Histology and microscopy	Tissue SARS-CoV-2	Myocarditis (*n*)	Acute ischemia (*n*)	
Conde et al. (21)	1	Autopsy	N/A	• Mild stenosis of aortic valve • Slight increase LV thickness • Dilatation of both ventricles		N/A	0	0	Severe bilateral CAP
Edler et al. and Lindner et al. (22, 23)	80 (74 pre-mortem and 6 post-mortem)	Full autopsy	**Days: n** 0d: 3 1 day: 9 2 days: 19 3 days:14 4 days: 12 5 days: 7 6 days: 1 7 days: 1 8 days: 3 9 days: 3 12 days: 2 15 days: 1 41 days: 1 n/a: 4	**P39:** MI + cardiac tamponade in 1 case *(despite COVID positive, authors noted death not related to COVID)*	**P4**: A small lymphocytic infiltrate in RV as a sign of myocarditis Chronic diseases changes—scarring in the myocardium	SARS-CoV-2 RNA in the myocardium: 24/39 • Viral load: >1,000 copies per μg RNA: 16/24 • <1,000 copies per μg RNA: 8/24 • Virus replication: 5/16 (among those with high viral load of SARS-CoV-2) *(sub-analysis in subsequent study)***	1 (RV)	1	See **Supplementary Table 1**
Sekulic et al. (24)	2	Autopsy (P1 autopsy sine brain and spinal cord) (P2 chest and abdomen only per family request)	**P1**: autopsy 29 h after death **P2**: 39 h after death	**P1**: • Heart enlarged • Weight: 620 g • Chronic IHD: severe stenosis native CA (left anterior descending, left circumflex, and right main CA), patent graft vessels • Moderately extensive replacement-type interstitial fibrosis **P2**: • Heart enlarged • Weight: 560 g • LV hypertrophy, • Mild calcified atherosclerotic CAD	• **P1**: no significant findings • **P2**: none described	Lower levels of SARS-CoV-2 RNA detected in the heart of P1	0	0	**P1**: RF due to SARS-CoV-2 **P2**: SARS-CoV-2 infection leading to respiratory and multiorgan system failure
Suess et al. (25)	1	Autopsy	N/A	Accumulation of serous fluids in pericardial cavity (30 ml)	• Patchy non-specific pericardial infiltration including lymphocytes and plasma cells • No neutrophils/granulomas seen • No inflammatory infiltrate/substantial damage in the myocardium	N/A	0	0	ARDS due to severe DAD as a result of severe infection with SARS CoV-2.
Aguiar et al. (26)	1	Autopsy	N/A	• Heart weight: normal for BMI (460 g) • LV and IVS wall thickness: 1.3 cm • RV wall thickness: 0.3 cm • Fatty streaks: anterior interventricular branch of left CA	No signs of cardiac hypertrophy	N/A	0	0	**Pathology:** Pulmonary changes related to SARS-CoV-2 and high fever without secondary bacterial infection
Fox et al. (27)	10 (African American)	Autopsy (cardiac examination in 9 cases)	N/A	**P2**: 420 g **P3**: 550 g **P4**: 540 g **P5**: 480 g **P6**: 370 g **P7**: 420 g **P8**: 450 g **P9**: 340 g **P10**:600 g • Myocardium: firm, red-brown, and free of significant lesions in all patients • Mild to moderate serosanguinous pericardial and pleural effusions ($n = ?$) • CA: no significant stenosis or acute thrombus formation • Most significant was cardiomegaly and RV dilatation. In several patients, massive dilatation could be seen; for example, in one case, RV cavity was 3.6 cm in diameter and the LV was 3.4 cm at its greatest diameter	**Microscopic examination:** • Myocardium: no large/confluent areas of myocyte necrosis • Scattered individual myocyte necrosis • In rare areas, lymphocytes adjacent to, but not surrounding, degenerating myocytes • May be early manifestation of viral myocarditis, but no significant brisk lymphocytic inflammatory infiltrate suggestive of viral myocarditis	LM: No viral cytopathic effect, but direct viral myocardial infection cannot be ruled out by this limited examination	0	0	COVID-19 (Withdrawal of care)

(Continued)

TABLE 2 | Continued

References	Number of cases	Autopsy technique	Time from death to autopsy	Postmortem pathology					Cause of death
				Gross pathology/heart weight	Histology and microscopy	Tissue SARS-CoV-2	Myocarditis (*n*)	Acute ischemia (*n*)	
Beigmohammadi et al. (28)	7 (5 with cardiac tissues)	Core needle biopsies	n/a		**P1**: • Few scattered lymphocytes and mastocytes without evidence of myocyte necrosis or degeneration • No myocarditis **P3**: • All inflammatory cells positive for CD68; but none stained with CD3 • No myocarditis • No evidence of myocyte necrosis • Ischemic process of cardiac muscle highly suggested **P5**: • Severe interstitial infiltration of LCA-positive inflammatory cells with predominance of CD68 positive macrophages and focal aggregation of CD3 positive T cells • Histologic evidence of myocyte necrosis including hyper-eosinophilia and enucleation • Ischemic necrosis of myocardium should be considered **P6**: • No interstitial inflammation **P7**: • Majority of inflammatory cells showed immunoreactivity for CD68 and rare cells positive for CD3. • No myocarditis • No evidence of myocyte necrosis • item Ischemic process of cardiac muscle highly suggested	N/A	0	3 (suggested)	N/A
Wang et al. (29)	2	Autopsy	**P1**: 6 h **P2**: 9 h	No obvious gross abnormalities	• Multifocal myocardial degeneration and myocardial atrophy and interstitial fibrous tissue hyperplasia Few scattered CD20-positive B cells and CD3-positive T cells	No obvious viral infection in parenchymal cells using IHC with antibodies against Rp3-NP.	0	0	Respiratory and circulatory failure in both
Rapkiewicz et al. (8)	7 vs. 9 controls died from ARDS from other cause	Autopsy + Tissue+ IHC + EM	N/A		In all cases, megakaryocytes associated with fibrin microthrombi within the cardiac microvasculature Venous thrombosis in 2 hearts of P3 and P7 **P4**: • Focal inflammatory infiltrate composed of lymphocytes, mixture of Band T cells as per CD20 and CD3, with CD4 in greater number than CD8 • Associated myocardial necrosis in epi-myocardial region • Localized infiltrate • Diffuse, transmural pallor of the LV. Platelet microthrombi in the region of inflammation identified using CD 61 • No granulomas • Staining for complement (C4d) negative in all tested cases **P7**: • Intramyocardial venous thrombosis with septal MI despite only minimal coronary atherosclerosis • Elevated levels of antiphospholipid IgM Ab detected postmortem	No viral inclusions on EM of the heart in any of 4 cases analyzed (P 2, 4, 6, and 7)	0	MI 1/7 venous thrombi 2/7 (both high Troponin but only 1 with septal MI on gross examination) Early ischemic changes 3/7 Mural fibrin thrombi 2/7	N/A

(Continued)

TABLE 2 | Continued

References	Number of cases	Autopsy technique	Time from death to autopsy	Postmortem pathology					Cause of death
				Gross pathology/heart weight	Histology and microscopy	Tissue SARS-CoV-2	Myocarditis (*n*)	Acute ischemia (*n*)	
Bösmüller et al. (30)	4	Autopsy Tissue for virology and EM (4 cases)	Autopsy after 48 h for patient 1 and within 24 h for P 2, 3, and 4	**P1**: • Increased weight: 520 g • Biventricular dilatation • Coronary arteries: no sclerosis or signs of ischemia • Hyperplastic myocardium **P2**: weight 527 g **P3**: weight 411 g **P4**: weight 590 g		Significant levels of SARS-CoV-2 RNA in the lungs of all patients by qRT-PCR, but not in the hearts	0	0	**Clinical** P1: Pneumonia (Pathology: acute cardiac failure was considered the likely cause of death.) P2: ARDS, liver failure, shock P3: ARDS, liver failure, shock P4: ARDS, multiorgan failure
Schweitzer et al. (31)	1 (and 1 control)	Autopsy	N/A	• Weight: 340 g • CA: atherosclerosis with pre-existing narrowing to 50% of the lumen of both the left anterior descending and right coronary arteries • No macroscopic signs of myocardial ischemia	No relevant histological findings (such as contraction band necroses, infarction, or inflammation) noted	N/A	0	0	N/A ? severe ARDS
Xu et al. (32)	1	PM biopsy samples	N/A		No obvious histological changes seen in heart tissue	N/A	0	0	N/A
Youd et al. (33)	3	Autopsy	**P1**: 5 days **P2**: 8 days **P3**: 10 days	**P1**: • Minimal CA atheroma **P2**: • Enlarged heart • Weight: 592 g • CA: minimal atheroma **P3**: • Enlarged heart • Weight: 582 g • CA: focal significant stenosis by atheroma • Old myocardial scarring	No myocarditis	N/A	0	0	N/A
Bradley et al. (9)	14	Standard autopsy for 7 cases *In situ* dissection for 7 cases (3 cases: fresh tissue collection)	**n/a**	No endotheliitis and scarce microthrombi (focal pulmonary microthrombi were identified in five patients) **P1**: Interstitial fibrosis, myocyte hypertrophy **P2**: Interstitial fibrosis, myocyte hypertrophy, replacement fibrosis **P3**: Interstitial fibrosis, myocyte hypertrophy **P4**: Interstitial fibrosis, myocyte hypertrophy, replacement fibrosis **P5**: Interstitial fibrosis, myocyte hypertrophy **P6**: Interstitial fibrosis, myocyte hypertrophy **P7**: Interstitial fibrosis, myocyte hypertrophy, vascular predominant amyloid **P8**: Interstitial fibrosis, myocyte hypertrophy, replacement fibrosis Myocarditis (aggregates of lymphocytes surrounding necrotic myocyte. SARS-CoV-2 S protein immunohistochemistry was negative) **P9**: Interstitial fibrosis, myocyte hypertrophy **P10**: Interstitial fibrosis, myocyte hypertrophy, replacement fibrosis, subsegmental pulmonary embolus **P11**: Interstitial fibrosis **P12**: Interstitial fibrosis, myocyte hypertrophy, replacement fibrosis, subsegmental pulmonary emboli **P13**: Interstitial fibrosis, myocyte hypertrophy, replacement fibrosis, myocardial amyloid **P14**: Interstitial fibrosis, myocyte hypertrophy, replacement fibrosis		Viral RNA detected in the liver, heart, and blood for P8 and P13	1	0	See **Supplementary Table 1**
Ducloyer et al. (34)	1	Autopsy PMCT IHC	48 h	• Heart weight: 470 g • Moderate RV dilatation • No increase in myocardial wall thickness • Nonobstructive atherosclerotic plaques in CAs and aortic bifurcation	• Mild coronary artery atherosclerosis • No myocarditis • Scattered wavy fibers	Not done	0	0	

(Continued)

TABLE 2 | Continued

References	Number of cases	Autopsy technique	Time from death to autopsy	Postmortem pathology					Cause of death
				Gross pathology/heart weight	Histology and microscopy	Tissue SARS-CoV-2	Myocarditis (*n*)	Acute ischemia (*n*)	
Cirstea et al. (35)	1	Autopsy IHC	N/A	Cardiomegaly with dilation of the RV and blood clots in the heart	• Recent intracardiac thrombosis • Vascular leukostasis with thrombi formation mainly in the small subepicardium vessels • Massive interstitial edema (obliterated the intercalated disks in between the myocardial cells) • Occasional scant mononuclear inflammatory cells and petechial hemorrhages	N/A	0	0	
Nicolai et al. (36)	1 (5 cases and 5 controls but only 1 with heart tissue)	Autopsy IHC	N/A	N/A	Inflammatory microthrombi. Neutrophil extracellular trap-like structures in heart specimens associated with fibrin deposition (1/1 patient)	N/A	0	0	
Grosse et al. (37)	14	Autopsy	N/A	• Myocardial hypertrophy (heart weight range, 385–750 g): 13/14	• Acute MI in 3/14 • Focal myocardial fibrosis 3/14 • Previous MI in 6 (42.9%) • Cardiac amyloidosis in 1 • Mild to severe CA atherosclerosis in 14/14: ➢ 2: mild 1-vessel coronary artery disease with 25% lumen stenosis, ➢ 6: 2-vessel coronary artery disease (25% lumen stenosis: $n = 1$; 25–50% lumen stenosis: $n = 4$; >75% lumen stenosis: $n = 1$), ➢ 6: moderate to severe 3-vessel coronary artery disease (25–50% lumen stenosis: $n = 1$; 50% lumen stenosis: $n = 1$; >75% lumen stenosis: $n = 4$ • All patients: some mononuclear inflammatory cells in myocardial interstitium, mainly CD3-positive T-lymphocytes (ranging in density from 2 to 4 lymphocytes/HPF)	N/A	0	3	
Schwenson et al. (38)	1	Autopsy	4 days	Heart enlarged weight: 380 g. RV: normal thickness (3 mm) LV: concentrically hyperplastic (23 mm)	Tissue samples normal No evidence of microthrombosis	N/A	0	0	
Remmelink et al. (39)	17	Autopsy	<5 days	• Cardiomegaly: 14/17 • Pericardial effusion: 2/17 • Atheromatosis: 8/17 (2- severe)	• Chronic ischemic cardiomyopathy: 15/17 • Acute MI: 2/17 • No evidence of contraction bands or myocarditis • Cardiac fibrosis: 5/17 • Chronic pericarditis: 1/17 • Abdominal aortic aneurysm: 1/17	Viral RNA detected by RT-PCR in heart tissue of 14/17	0	2	
Okudela et al. (40)	1	Autopsy	13 h	N/A	No remarkable changes	N/A	0	0	
Adachi et al. (41)	1	Autopsy	5 h	Heart weight: 420 g RV dilatation, with 10 ml of cardiac effusion	No notable changes	Not detected in heart	0	0	
Nadakarni et al. (42)	26 (focus on thromboembolism)	Autopsy	N/A	N/A	Microthrombi in heart: 4/26	N/A	0	0	
Dalahmah et al. (43)	1	Autopsy	3 h	N/A	The heart showed LVH, focal subendocardial fibrosis, but no myocarditis or ischemia	N/A	0	0	

(Continued)

TABLE 2 | Continued

References	Number of cases	Autopsy technique	Time from death to autopsy	Postmortem pathology					Cause of death
				Gross pathology/heart weight	Histology and microscopy	Tissue SARS-CoV-2	Myocarditis (*n*)	Acute ischemia (*n*)	
Oprinca and Muja (44)	3	**P2**: full autopsy **P1 and P3**: thoraco-abdomino-pelvic autopsies	**P1**: 24 h **P2**: N/A **P3:** N/A	**P1**: Weight: 355 g Dilated cardiomyopathy, LVH, RA, and RV dilatation. Coronary atherosclerosis but preserved luminal permeability. Aortic atherosclerosis **P2**: Weight: 342 g RA and RV dilatation No morphological abnormalities of the myocardium, CA, or aorta **P3**: Weight: 412 g Ischemic cardiomyopathy LVH RA and RV dilatation. Severe coronary atherosclerosis. Aorto-coronary bypass. Complicated atherosclerosis	**P1:** • Mild to moderate perivascular edema • Vascular congestion • Areas of small contraction band-like lesions • Small number of scattered lymphocytes between the myocardial fibers **P2**: • Small vessel thrombosis • Marked vascular congestion • Mild edema between the muscle fibers • Myocardial fibers tend to form contraction bands **P3**: • Myocardosclerosis • Myocardial fibrosis due to old MI • Mild edema • Marked vascular congestion • Acute circulatory disorders ***Overall (P1–3)*** Small areas of contraction bands and scattered lymphocytes No signs of myocarditis *P2, P3: Pulmonary emdotheliitis (mild vasculitic reaction: lymphocytic invasion of pulmonary vascular wall with no fibrinoid necrosis)*	No microscopic signs of viral infection of myocardium	0	0	
Wang et al. (45)	1	Percutaneous biopsies (heart tissue in 1 patient among 3)	N/A		• Old MI • Hypertrophic myocytes • Fatty infiltration • Nuclear pyknosis • Interstitial edema and fibrosis • No viral myocarditis	N/A	0	0	
Jensen et al. (46)	2	Autopsy	9 days	**P1**: Foramen ovale fully closed. Aorta and its branches: mild atheroma **P2**: Foramen ovale was probe patent		N/A	N/A	0	
Elsoukkaryet al. (47)	30	Autopsy	5–382 h (median: 43)	Normal weight 2/30: Mean 350 g Cardiomegaly 28/30: Mean 490 g Heart Intramyocardial small vessel thrombi: 6/30 Valve-associated thrombi 2/30 Thrombosis and co-existing infarction: 1/30	• Atherosclerosis (>50% stenosis): 17/30 • Myocyte hypertrophy: 24/30 • Myocyte ischemia: 5/30 (1 with acute MI due to thrombosis into atherosclerotic plaque) • Interstitial fibrosis: 20/30	N/A	0	5 (1 acute MI)	
Hanley et al. (48)	10	9 full autopsies + 1 limited biopsy	Median: 6 days	• Median weight was high (450 g; IQR, 315–535 g) • LVH: 4/9 • RA thrombus: 1 • Pericardial effusion: 3 • Pericarditis: 2 (1 acute pericarditis + P5 showed florid fibrinous pericarditis containing fungal hyphae) • **P5**: Non-bacterial thrombotic (marantic) endocarditis (no known history or autopsy findings consistent with malignancy or chronic disorder associated with non-bacterial thrombotic (marantic) endocarditis). Disseminated mucormycosis and numerous other thrombotic features • **P8**: Cardiac amyloidosis and RA thrombosis • Macroscopic acute coronary thrombosis in right CA: 1/9	• Fibrinous pericarditis • with fungal hyphae • Non-bacterial thrombotic endocarditis • Thrombi in the microcirculation of the heart: 5/9 • CAD: negligible, 3/9; mild, 4/9; moderate, 2/9 • Acute myocardial ischemic damage (<24 h) noted in patient with acute coronary thrombus • **P2**: mottled myocardium and subendocardial contraction band necrosis; uncertain whether the contraction band necrosis was related to ischemia or inotropic medication in the ICU	PCR of viral E gene: 3/5 (P1,P2,P4) Sub-genomic viral RNA transcripts: 2/5 (P1, P2)	0	1 (± 1 with band necrosis of unknown etiology)	

**Among 10 cases, one COVID-19 diagnosis based on radiological and pathological findings.*

***A subanalysis of cardiac tissue histopathology had been subsequently published (37).*

Ab, antibodies; ARDS, acute respiratory distress syndrome; BMI, body mass index; CA, coronary artery; CAD, coronary artery disease; CAP, community-acquired pneumonia; CMC, cardiomyocytes; DAD, diffuse alveolar damage; EM, electron microscopy; ICU, intensive care unit; IHC, immunohistochemistry; IVS, interventricular septum; LM, light microscopy; LV, left ventricle; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; MI, myocardial infarction; MR, mitral regurgitation; P, patient; PAD, peripheral arterial disease, PE, pulmonary embolism; PM, postmortem; PMI, postmortem interval; RA, right atrium; RF, respiratory failure; RV, right ventricle.

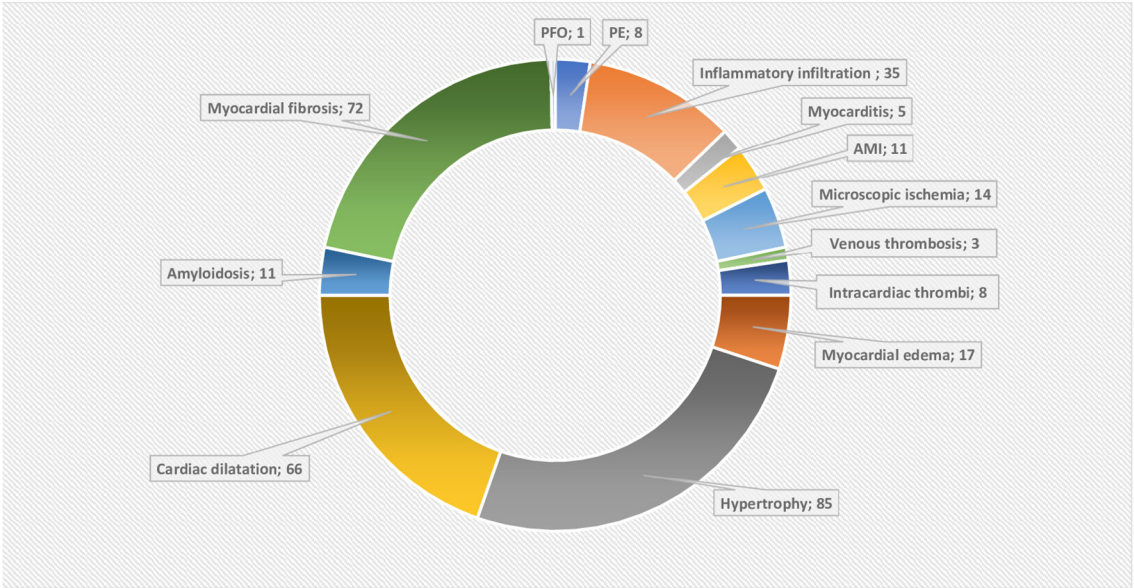

FIGURE 2 | Doughnut chart showing the reported post-mortem acute and chronic pathologies. AMI, Acute myocardial infarction; PE, pericardial effusion; PFO, patent foramen ovale. The data labels show the number of reported acute or chronic pathologies, note that they can overlap in the single patient. Chronic pathologies include hypertrophy and amyloidosis, while myocardial fibrosis, pericardial effusion and dilatation can be acute or chronic. The rest are considered as acute pathologies.

Role of Ischemia, Endotheliitis, and Hypercoagulability

The most alarming finding is the intracardiac, coronary arterial, and venous thrombosis, which may be in part explained by the COVID-19-associated coagulopathy (CAC). Myocardial ischemia can be further aggravated by the frequent pre-existing CAD and myocardial supply–demand mismatch.

By means of its receptor, SARS-CoV-2 can directly invade the endothelium leading to endothelial cell (EC) inflammation (i.e., endothelialitis), dysfunction, and death (49). Endothelial dysfunction can also result from an inappropriate immune and cytokine response. Endothelialitis, and hence EC dysfunction, subsequently induces a procoagulant state (CAC), loss of barrier function, inflammatory tissue infiltration, edema, and injury (49, 50). Cardiovascular comorbidities are usually associated with chronic EC dysfunction, which can explain the worse outcome when further acute insult is superadded.

However, endothelialitis was not a consistent finding in our reviewed studies but, when detected, was associated with microthrombi and had multiorgan distribution. Varga et al. showed multiorgan endotheliitis in all three studied cases (7). Ackermann et al. showed widespread endotheliitis and capillary thrombosis in COVID-19-affected lungs in a much more common prevalence than in non-COVID acute respiratory distress syndrome (ARDS) lungs (51). In contrast, Bradley et al. concluded not only no evidence of endothelialitis but also little evidence of cardiac microthrombi (9). Rapkiewics et al. noted no endothelial abnormalities but a platelet-rich microthrombi in all seven hearts examined, despite anticoagulation (8). It appears that alternate mechanisms of ischemia overlap, and while anticoagulation may be highly relevant in limiting pulmonary thrombosis, this may be less likely to significantly ameliorate any cardiac contribution to poor outcomes. Nicolai et al. highlighted thrombi to be rich in platelets, fibrin, and neutrophil extracellular traps (NETs), while Jensen et al. described platelet-rich cerebral microangiopathy (36, 46). The role of NET and platelets may be significant and could support other potential therapies (e.g., antiplatelet therapy).

Chamber Dilatation and Myocardial Edema

Heart weight exceeded the normal range in 90% of cases reflecting a combination of chronic pathologies (e.g., hypertrophy), myocardial edema (marker of injury), and chambers dilatation. The observed cardiac dilatation (especially of the right heart) may be long standing or acute and hence relate to preload or afterload (pulmonary hypertension) changes occurring during the acute illness and its treatment.

ME reflects myocardial tissue response to most types of injury and hence its nonspecificity. Ischemia, septic cardiomyopathy, viral, or inflammatory infiltration can all contribute to it. Schmittinger et al. showed ME in 90% of PM septic hearts in a patchy distribution (median of 25% of tissue sections) (52). Of note, ME can reflect an early tissue change after insult (as early as 3 min in the setting of ischemia due to the disruption of the Na+/K+ pump) (53). Detecting ME has therapeutic implications, as it causes less energetic efficiency, arrhythmias, and reduced cardiac wall compliance. All of these are expected to impair systolic and diastolic function and can ultimately lead to fibrosis (53, 54). While cardiac MRI (CMR) can detect it *in vivo*, histological diagnosis remains technically challenging (53). This challenge, combined with the lack of standardized protocol

guidelines for PM cardiac pathology reporting, may mean that ME was overlooked in many of the published reports.

Myocardial Fibrosis

Myocardial fibrosis was reported in nearly a quarter of cases. It is the end result of cardiac injury arising from different acute or chronic mechanisms. Cytokines were also implicated in cardiac fibroblast activation (55, 56).

The interpretation in COVID-19 is difficult and depends on many factors. It can reflect a chronic or a *de novo* subacute process. Aging and many reported comorbidities are strongly associated with fibrosis (56). Of note, amyloidosis (a pathology associated with fibrosis) was described in 11 cases and was significantly more prevalent when compared to a historical age-matched cohort (18, 37, 48).

Myocardial fibrosis can be divided into two types: interstitial fibrosis and replacement fibrosis, with considerable overlap between the two (55). While interstitial fibrosis is considered reactive and potentially reversible, replacement fibrosis is not (55). Interstitial fibrosis was previously detected in 100% of PM septic hearts but in a patchy nature (52). Such focal nature means that an extensive PM cardiac pathological examination is necessary. In fact, CMR may be superior as a diagnostic modality despite the difficultly to perform in unstable patients (55, 57).

Myocardial fibrosis represents the structural equivalent of heart failure. While ME is expected in the "reversible" septic cardiomyopathy, increased fibrous deposition (i.e., replacement fibrosis) would not be a likely finding in such reversible pathology (52, 57, 58).

Viral Invasion, Inflammatory Infiltrate, and Myocarditis

Studies investigating the presence of SARS-CoV-2 within the myocardium were positive in about half the cases. In 1986, The Dallas criteria were proposed for the histopathological categorization and diagnosis of myocarditis based on endomyocardial biopsies. The "Dallas criteria" defines acute myocarditis as "an inflammatory infiltrate associated with myocyte necrosis or damage not characteristic of myocardial ischemia." Borderline myocarditis requires a less intense inflammatory infiltrate with no light microscopic signs of myocyte destruction (59). In COVID-19 PM studies, inflammatory infiltrate (mainly lymphocytic) was observed in a minor proportion (about 10%) and was limited in extent for the majority of cases. As such, when interstitial edema and inflammatory infiltrate were observed, they did not meet the diagnostic criteria of myocarditis, except in five cases. In fact, some authors attributed such inflammatory infiltrate to an ischemic process (28). This suggests that contrary to early conjectures, acute and fulminant myocarditis are rare during the acute illness.

Clinical and Imaging Correlation

Correlating the histopathological data to the clinical, imaging, and investigational data can provide more insights into the likely mechanisms of cardiac involvement in COVID-19. Clinical presentation varies from ST elevation MI due to thrombotic occlusion of epicardial coronaries, to ischemia and/or infarction without obstructive coronary disease, through to tachy and brady arrhythmias, depressed left and right ventricular function, and occasional pericardial involvement (60). A review of published literature suggests that elevated Troponin and heart failure dominate the clinical presentations (61).

Echocardiography is readily performed in the acute setting but provides limited insights into the cause when compared to CMR. In a large multinational survey, Dweck et al. reported the echocardiographic findings in 1,216 studies performed over 17 days (62). Fifty-five percent of scans were abnormal. Impaired LV function or dilation (39%) followed by RV abnormalities (33%) dominated. These findings are non-specific, but clear wall motion abnormalities suggesting infarction were rare (3%). The RV abnormalities most likely relate to increased afterload given the high prevalence of pulmonary thromboembolism and extensive lung damage associated with COVID-19 infection (63). The LV abnormalities are non-specific but provide further evidence of the high prevalence of cardiac damage.

CMR-based studies have focused on patients post recovery (too late for confirmation of myocarditis) but have shown a high prevalence of abnormalities. The largest to date is a German study of relatively young patients (mean age, 49 years), largely managed at home (67 of 100), studied a median of 71 days post infection. Seventy-eight percent were reported to show abnormalities, including reduction in LV function, elevated T1 and T2 (the latter suggesting ME) and late gadolinium enhancement (LGE) (non-ischemic pattern in 20, ischemic in 12). Three patients with very elevated T2 were referred for endomyocardial biopsy and typical features of myocarditis reported. The T1 and T2 abnormalities suggest ongoing myocardial edema, and the LGE enhancement suggests fibrosis—both of which are common in the autopsy data (64).

The second CMR-based study included only patients in whom Troponin had been elevated during hospital admission. Fifty-one patients were studied 27 days post hospital discharge. In 22 patients, pulmonary embolism and/or coronary ischemia were identified before scanning as the most likely cause of troponin leakage. Among 29 patients (mean age, 64 years) with no clinically identified cause for myocardial injury, an ischemic pattern injury (LGE) was identified in 5, dual pathology (ischemic and non-ischemic) in 4, and non-ischemic in 11. Intriguingly, T1 and T2 were not abnormal in this study. This study thus also supports the histological finding of significant myocardial fibrosis but suggests that edema clears fairly quickly in those that recover (2). Again, Rajpal et al. performed CMR on 26 athletes with a history of mild COVID-19 infection. Four of them (15%) had criteria of myocarditis despite mild or no symptoms, and 30% showed signs of previous cardiac injury (65).

What Can We Conclude From Integrating All Available Data?

Merging the clinical, investigational, and autopsy data, we are presented with a picture that demonstrates a high prevalence of cardiac abnormalities, in part due to exacerbation of underlying cardiac pathology and partly coagulation disorders

affecting the pulmonary and coronary vessels. Direct cardiac involvement mainly takes the form of non-coronary myocyte death, myocyte dysfunction, and interstitial fibrosis without substantial inflammatory infiltration or clear ischemia.

The role of direct viral cellular damage remains to be fully explored, and if this is the driving force, it is intriguing that the inflammatory response appears muted. However, it is possible that while the virus is rarely causing a fulminant or acute myocarditis, it can cause a persistent chronic myocardial inflammation with significant long-term implications. It is also important to note the reporting of a delayed immune response in the form of Kawasaki's disease in pediatric patients supporting the issue of long-term sequelae of the SARS-CoV-2 infection (66). Whether immunosuppressive treatment (e.g., dexamethasone and Tocilizumab) during the acute illness is of benefit or causes more harm to the heart should await randomized controlled studies including long-term follow-up.

Thus, on balance, the data strongly suggest significant viral replication in the myocardium without true acute myocarditis in most instances, with frequent non-MI pattern fibrosis—consistent with microvascular ischemia/thrombi and, in some cases, endothelial inflammation. Given the frequent presence of fibrosis associated with cell death, it is likely that complete recovery is unlikely—a clear distinction from septic cardiomyopathy. In addition, the exacerbation of underlying disease would appear to frequently unmask coronary disease, further increasing the benefit of careful cardiological follow-up.

As the vast majority of studied patients in this review died during the acute illness and cardiac abnormality was prevalent in the population studied, we can conclude that myocarditis was not a dominant cause of cardiac dysfunction identified premortem in COVID-19 patients, while the role of endothelialitis needs further clarification.

Limitations

Our work delineates the importance of PM to guide the understanding of COVID-19. However, the small number of published PM cases in a disease, which has caused more than 1 million fatalities, highlights a hugely missed opportunity. Cardiac pathological changes are more likely to be focal in nature and hence easily missed if the heart is not examined in its entirety. Furthermore, the high prevalence of myocardial fibrosis, myocyte damage, or viral RNA in some studies but not others suggest a need to standardize histological reporting to establish common ground between pathologists and clinicians. There is also a genuine need for an international case register to gather the largest possible data in the shortest interval.

While our work is limited by the quality and small number of cases per study, we think it can contribute to a better understanding of COVID-19-associated cardiac injury. Other limits include the probable selection and reporting bias. PM is performed for patients who died during the acute illness and for certain subgroups of patients due to clinical or legal reasons. The longest duration of illness in our cohort is 52 days, which means that the long-term evolution or complications of the disease cannot be covered by this review.

CONCLUSIONS

To conclude, our review confirmed the high prevalence of cardiac pathological findings in COVID-19 patients. Cardiac dilatation, ischemia, and thrombosis were the most prevalent findings. SARS-CoV-2 was present in nearly half of the examined hearts, but true myocarditis was evident in just 1.5% of the deceased patients.

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

AR: conceptualization and design, registration of the protocol, conduct of the search, quality assessment, data extraction, data interpretation, and manuscript drafting. SZ: conceptualization, design, and writing of the protocol, extraction and interpretation of the data, and manuscript drafting. HF: data analysis and interpretation and writing and revising the manuscript. JC: data analysis and interpretation and writing and reviewing the manuscript. All authors: contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2020.626975/full#supplementary-material

REFERENCES

1. 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
2. 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
3. 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].
4. Roshdy A. Echodynamics: interpretation, limitations, and clinical integration! *J Intensive Care Med.* (2018) 33:439–46. doi: 10.1177/0885066617734151
5. Schwartz DA, Herman CJ. The importance of the autopsy in emerging and reemerging infectious diseases. *Clin Infect Dis.* (1996) 23:248–54. doi: 10.1093/clinids/23.2.248
6. Duarte-Neto AN, Monteiro RAA, da Silva LFF, Malheiros DMAC, de Oliveira EP, Theodoro-Filho J, et al. Pulmonary and systemic involvement of COVID-19 assessed by ultrasound-guided minimally invasive autopsy. *Histopathology.* (2020) 77:186–97. doi: 10.1111/his.14160
7. 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
8. 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
9. 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
10. 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
11. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* (2009) 6:e1000097. doi: 10.1371/journal.pmed1000097
12. Moola S, Munn Z, Tufanaru C, Aromataris E, Sears K, Sfetcu R, et al. Chapter 7: systematic reviews of etiology and risk. In: Aromataris E, Munn Z, editors. *JBI Manual for Evidence Synthesis*. JBI (2020) 248–61. Available online at: https://synthesismanual.jbi.global. doi: 10.46658/JBIMES-20-08
13. 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
14. 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
15. Yan L, Mir M, Sanchez P, Beg M, Peters J, Enriquez O, et al. COVID-19 in a Hispanic Woman. *Arch Pathol Lab Med.* (2020) 144:1041–7. doi: 10.5858/arpa.2020-0217-SA
16. Lacy JM, Brooks EG, Akers J, Armstrong D, Decker L, Gonzalez A, et al. COVID-19: postmortem diagnostic and biosafety considerations. *Am J Forensic Med Pathol.* (2020) 41:143–51. doi: 10.1097/PAF.0000000000000567
17. 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
18. 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
19. Tian S, Xiong Y, Liu H, Niu L, Guo J, Liao M, et al. Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. *Mod Pathol.* (2020) 33:1007–14. doi: 10.1038/s41379-020-0536-x
20. 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
21. Navarro Conde P, Alemany Monraval P, Medina Medina C, Jiménez Sánchez A, Andrés Teruel JC, Ferrando Marco J, et al. Autopsy findings from the first known death from Severe Acute Respiratory Syndrome SARS-CoV-2 in Spain. *Revista Española de Patol.* (2020) 53:188–92 doi: 10.1016/j.patol.2020.04.002
22. Edler C, Schröder AS, Aepfelbacher M, Fitzek A, Heinemann A, Heinrich F, et al. Dying with SARS-CoV-2 infection-an autopsy study of the first consecutive 80 cases in Hamburg, Germany. *Int J Legal Med.* (2020) 134:1275–84. doi: 10.1007/s00414-020-02317-w
23. 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
24. Sekulic M, Harper H, Nezami BG, Shen DL, Sekulic SP, Koeth AT, et al. Molecular detection of SARS-CoV-2 infection in FFPE samples and histopathologic findings in fatal SARS-CoV-2 cases. *Am J Clin Pathol.* (2020) 154:190–200. doi: 10.1093/ajcp/aqaa091
25. Suess C, Hausmann R. Gross and histopathological pulmonary findings in a COVID-19 associated death during self-isolation. *Int J Legal Med.* (2020) 134:1285–90. doi: 10.1007/s00414-020-02319-8
26. Aguiar D, Lobrinus JA, Schibler M, Fracasso T, Lardi C. Inside the lungs of COVID-19 disease. *Int J Legal Med.* (2020) 134:1271–4. doi: 10.1007/s00414-020-02318-9
27. 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
28. Beigmohammadi MT, Jahanbin B, Safaei M, Amoozadeh L, Khoshavi M, Mehrtash V, et al. Pathological findings of postmortem biopsies from lung, heart, and liver of 7 deceased COVID-19 patients. *Int J Surg Pathol.* (2020) 1–11. doi: 10.1177/1066896920935195. [Epub ahead of print].
29. Wang C, Xie J, Zhao L, Fei X, Zhang H, Tan Y, et al. Alveolar macrophage dysfunction and cytokine storm in the pathogenesis of two severe COVID-19 patients. *EBioMedicine.* (2020) 57:102833. doi: 10.1016/j.ebiom.2020.102833
30. Bösmüller H, Traxler S, Bitzer M, Häberle H, Raiser W, Nann D, et al. The evolution of pulmonary pathology in fatal COVID-19 disease: an autopsy study with clinical correlation. *Virchows Arch.* (2020) 477:349–57. doi: 10.1007/s00428-020-02881-x
31. Schweitzer W, Ruder T, Baumeister R, Bolliger S, Thali M, Meixner E, et al. Implications for forensic death investigations from first Swiss post-mortem CT in a case of non-hospital treatment with COVID-19. *Forensic Imaging.* (2020) 21:200378. doi: 10.1016/j.fri.2020.200378
32. 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
33. Youd E, Moore L. COVID-19 autopsy in people who died in community settings: the first series. *J Clin Pathol.* (2020) 73:840–4. doi: 10.1136/jclinpath-2020-206710
34. Ducloyer M, Gaborit B, Toquet C, Castain L, Bal A, Arrigoni PP, et al. Complete post-mortem data in a fatal case of COVID-19: clinical, radiological and pathological correlations. *Int J Legal Med.* (2020) 134:2209–14. doi: 10.1007/s00414-020-02390-1
35. Cîrstea AE, Buzulică RL, Pirici D, Ceaușu MC, Iman RV, Gheorghe OM, et al. Histopathological findings in the advanced natural evolution of the SARS-CoV-2 infection. *Rom J Morphol Embryol.* (2020) 61:209–18. doi: 10.47162/RJME.61.1.23
36. Nicolai L, Leunig A, Brambs S, Kaiser R, Weinberger T, Weigand M, 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
37. 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
38. Schwensen HF, Borreschmidt LK, Storgaard M, Redsted S, Christensen S, Madsen LB. Fatal pulmonary fibrosis: a post-COVID-19 autopsy case. *J Clin Pathol.* (2020). doi: 10.1136/jclinpath-2020-206879. [Epub ahead of print].
39. Remmelink M, De Mendonça R, D'Haene N, De Clercq S, Verocq C, Lebrun L, et al. Unspecific post-mortem findings despite multiorgan viral spread in COVID-19 patients. *Crit Care.* (2020) 24:495. doi: 10.1186/s13054-020-03218-5

40. Okudela K, Hayashi H, Yoshimura Y, Sasaki H, Horiuchi H, Miyata N, et al. A Japanese case of COVID-19: an autopsy report. *Pathol Int*. (2020) 70:820–4. doi: 10.1111/pin.13002
41. Adachi T, Chong JM, Nakajima N, Sano M, Yamazaki J, Miyamoto I, et al. Clinicopathologic and immunohistochemical findings from autopsy of patient with COVID-19, Japan. *Emerg Infect Dis.* (2020) 26:2157–61. doi: 10.3201/eid2609.201353
42. Nadkarni GN, Lala A, Bagiella E, Chang HL, Moreno PR, Pujadas E, et al. Anticoagulation, bleeding, mortality, and pathology in hospitalized patients With COVID-19. *J Am Coll Cardiol.* (2020) 76:1815–26. doi: 10.1016/j.jacc.2020.08.041
43. Al-Dalahmah O, Thakur KT, Nordvig AS, Prust ML, Roth W, Lignelli A, et al. Neuronophagia and microglial nodules in a SARS-CoV-2 patient with cerebellar hemorrhage. *Acta Neuropathol Commun.* (2020) 8:147. doi: 10.1186/s40478-020-01024-2
44. Oprinca GC, Muja LA. Postmortem examination of three SARS-CoV-2-positive autopsies including histopathologic and immunohistochemical analysis. *Int J Legal Med.* (2020) 135:329–39. doi: 10.1007/s00414-020-02406-w
45. Wang XX, Shao C, Huang XJ, Sun L, Meng LJ, Liu H, et al. Histopathological features of multiorgan percutaneous tissue core biopsy in patients with COVID-19. *J Clin Pathol.* (2020). doi: 10.1136/jclinpath-2020-206623. [Epub ahead of print].
46. Jensen MP, Le Quesne J, Officer-Jones L, Teodòsio A, Thaventhiran J, Ficken C, et al. Neuropathological findings in two patients with fatal COVID-19. *Neuropathol Appl Neurobiol.* (2020). doi: 10.1111/nan.12662. [Epub ahead of print].
47. Elsoukkary SS, Mostyka M, Dillard A, Berman DR, Ma LX, Chadburn A, et al. Autopsy findings in 32 patients with COVID-19: a single-institution experience. *Pathobiology.* (2020) 1–13. doi: 10.1159/000511325. [Epub ahead of print].
48. 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
49. Teuwen LA, Geldhof V, Pasut A, Carmeliet P. COVID-19: the vasculature unleashed. *Nat Rev Immunol.* (2020) 7:389–91. doi: 10.1038/s41577-020-0343-0
50. Iba T, Connors JM, Levy JH. The coagulopathy, endotheliopathy, and vasculitis of COVID-19. *Inflamm Res.* (2020) 69:11819. doi: 10.1007/s00011-020-01401-6
51. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid19. *N Engl J Med.* (2020) 383:120–8. doi: 10.1056/NEJMoa2015432
52. Schmittinger CA, Dünser MW, Torgersen C, Luckner G, Lorenz I, Schmid S, et al. Histologic pathologies of the myocardium in septic shock: a prospective observational study. *Shock.* (2013) 39:329–35. doi: 10.1097/SHK.0b013e318289376b
53. Friedrich MG. Myocardial edema–a new clinical entity? *Nat Rev Cardiol.* (2010) 7:292–6. doi: 10.1038/nrcardio.2010.28
54. Vasques-Nóvoa F, Laundos TL, Madureira A, Bettencourt N, Nunes JPL, Carneiro F, et al. Myocardial Edema: an Overlooked Mechanism of Septic Cardiomyopathy? *Shock.* (2020) 53:616–9. doi: 10.1097/SHK.0000000000001395
55. Bing R, Dweck MR. Myocardial fibrosis: why image, how to image and clinical implications. *Heart.* (2019) 105:1832–40. doi: 10.1136/heartjnl-2019-315560
56. Hinderer S, Schenke-Layland K. Cardiac fibrosis - a short review of causes and therapeutic strategies. *Adv Drug Deliv Rev.* (2019) 146:77–82. doi: 10.1016/j.addr.2019.05.011
57. Siddiqui Y, Crouser ED, Raman SV. Nonischemic myocardial changes detected by cardiac magnetic resonance in critical care patients with sepsis. *Am J Respir Crit Care Med.* (2013) 188:1037–9. doi: 10.1164/rccm.201304-0744LE
58. Aneman A, Vieillard-Baron A. Cardiac dysfunction in sepsis. *Intensive Care Med.* (2016) 42:2073–6. doi: 10.1007/s00134-016-4503-4
59. Aretz HT. Myocarditis: the Dallas criteria. *Hum Pathol.* (1987) 18:619–24. doi: 10.1016/S0046-8177(87)80363-5
60. Hendren NS, Drazner MH, Bozkurt B, Cooper LT Jr. Description and proposed management of the acute COVID-19 cardiovascular syndrome. *Circulation.* (2020) 141:1903–14. doi: 10.1161/CIRCULATIONAHA.120.047349
61. Singh R, Kashyap R, Hutton A, Sharma M, Surani S. a review of cardiac complications in coronavirus disease 2019. *Cureus.* (2020) 12:e8034. doi: 10.7759/cureus.8034
62. 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
63. 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
64. 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
65. 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) e204916. doi: 10.1001/jamacardio.2020.4916. [Epub ahead of print].
66. Cogan E, Foulon P, Cappeliez O, Dolle N, Vanfraechem G, De Backer D. Multisystem inflammatory syndrome with complete kawasaki disease features associated with SARS-CoV-2 infection in a young adult. a case report. *Front Med.* (2020) 7:428. doi: 10.3389/fmed.2020.00428

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REVIEW
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COVID-19 and Acute Coronary Syndromes: Current Data and Future Implications

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Coronavirus disease-2019 (COVID-19) pandemic is a global healthcare burden, characterized by high mortality and morbidity rates all over the world. During the outbreak period, the topic of acute coronary syndromes (ACS) has raised several clinical issues, due to the risks of COVID-19 induced myocardial injury and to the uncertainties about the management of these cardiologic emergency conditions, which should be organized optimizing the diagnostic and therapeutic resources and ensuring the maximum protection to healthcare personnel and hospital environment. COVID-19 status should be assessed as soon as possible. Moreover, considerably lower rates of hospitalization for ACS have been reported all over the world, due to patients' hesitations to refer to hospital and to missed diagnosis. As a result, short- and long-term complications of myocardial infarction are expected in the near future; therefore, great efforts of healthcare providers will be required to limit the effects of this issue. In the present review we discuss the impact of COVID-19 pandemic on ACS diagnosis and management, with possible incoming consequences, providing an overview of the available evidence and suggesting future changes in social and clinical approach to ACS.

Keywords: SARS-CoV2, myocardial injury, NSTEMI, STEMI, acute coronary syndromes, COVID-19

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INTRODUCTION

Background

Coronavirus-2019 (COVID-19) outbreak is currently the most discussed public health issue, caused by the highly infectious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 was declared a pandemic by the World Health Organization in early March 2020 and it was characterized by an exponential rise in contagions worldwide, with continuously increasing number of victims (1). The typical clinical spectrum of COVID-19 includes fever, cough, myalgia, dyspnea (2), with frequent progression to pneumonia, which in one third of the cases eventually leads to acute respiratory distress syndrome (ARDS), of which another third warrant critical care (3). Therefore, prevention and treatment of COVID-19 are currently the primary focus of clinical and scientific debates. However, acute coronary syndromes (ACS) management during this emergency period is gaining growing interest, yielding many scientific researches as well as national and international societies consensus documents, stimulated by four major concerns:

- an increase in short-term risk of myocardial injury and infarction has been reported, particularly for patients with underlying CAD and/or pro-inflammatory cardiovascular risk factors (such as diabetes mellitus, hypertension, and obesity);
- differential diagnosis between non-COVID ACS and COVID-19 induced acute myocardial injury (COVID-AMI), and within COVID-AMI, among myocardial infarction (MI), acute viral myocarditis, stress cardiomyopathy, is currently challenging, due also to the restricted availability of diagnostic tools;
- a sensible reduction of the rates of ACS has been recorded all over the world (4), probably not only as a consequence of lower patients' referral to the emergency department (ED), but also of misdiagnosis;
- lack of preparation and standardized protocols to balance between timely management of ACS and protection of healthcare personnel and hospital environment has provoked delays in the treatment of high-risk ACS; this fact, in conjunction with the previous point, has led to an increased incidence of short-term MI complications and estimated higher long-term MI complications, which will probably require changes in public health resources and system.

Aims

In the present review we sought to address these four important issues, discussing the earliest evidence and recommendations present in literature, and providing hints and previsions for the future, in order to prepare clinicians and solve their uncertainties on the matter of ACS during and after COVID-19 pandemic.

ACUTE MYOCARDIAL INJURY TRIGGERED BY COVID-19

The development of myocardial injury is not uncommon among patients with COVID-19 and correlates with disease severity. In fact, a meta-analysis involving 1,527 COVID-19 patients revealed that at least 8% of the patients had acute myocardial injury and that the risk of myocardial injury is 13-fold higher in patients with severe clinical presentation (5).

COVID-AMI has been defined as the elevation of high-sensitivity cardiac troponin (hs-cTn) above the 99th percentile of its upper limit of normal or evidence of new electrocardiographic (ECG) or echocardiographic abnormalities (6). In fact, the presence of increased levels of hs-cTn was found to be an independent predictor of disease severity and mortality rate in COVID-19 (7) even after adjustment for baseline characteristics and medical comorbidities, also showing an association with the need for intensive care unit (ICU) admittance (RR 13.48, 95%CI 3.60 to 50.47, $p = 0.0001$) (5).

DIFFERENTIAL DIAGNOSIS

There are different potential etiologies of COVID-AMI: ACS due to plaque rupture or thrombosis (type I MI) or to supply-demand mismatch (type II MI), myocardial injury due to disseminated intravascular coagulation (DIC), and non-ischemic injury (myocarditis, stress-induced cardiomyopathy, cytokine release syndrome, acute pulmonary embolism). Each one is the result of a direct or indirect effect of severe viral infection, as explained in **Table 1**. It is essential to recognize ACS and ACS-mimicker in order to provide an adequate treatment and avoid additional risks (e.g., fibrinolysis in case of myocarditis or stress-cardiomyopathy would expose patients to bleeding risk and eventual invasive coronary angiography (ICA) for unresolved ST-elevation rather than being beneficial) (6).

Differential Diagnosis: First Contact With Patients

The distinction between primary ACS and COVID-AMI for outpatients referring to ED would be crucial for the subsequent patient management, not only for treatment but also for the safety measures to employ (i.e., isolation, use of adequate personal protective equipment [PPE]). In accordance to the European Association of Percutaneous Cardiovascular Interventions (EAPCI) recommendations (27), for patients with suspected ACS, the likelihood of COVID-19 status should be assessed through accurate clinical interview, investigating the presence of typical symptoms (e.g., fever, cough, dyspnea, cold) or contacts with COVID-19 infected, together with the execution of nasal and/or oropharyngeal swab for SARS-CoV2 Nucleic Acid test as soon as the patient arrives in the ED, if possible. Fast-track pathways for the exclusion of COVID status would expedite the management of these patients. Until the result of the swab is ready, each patient should be considered as COVID infected; this is also valid for STEMI patients who are transferred to the catheterization laboratory (Cath-lab) before having the results. Healthcare workers and patients must always wear at least droplets PPE (i.e., surgical mask, gloves, cup, goggles, and single-use gown for clinicians, surgical mask and gloves for patient). Moreover,

- in case of patients with asymptomatic/negative anamnesis and negative SARS-CoV2 Nucleic Acid test the *common ACS-pathway* should be followed;
- in case of patients with symptomatic/positive anamnesis and negative SARS-CoV2 Nucleic Acid test, the *swab should be repeated;*
- in case of positive SARS-CoV2 Nucleic Acid test, patients are considered as COVID infected, healthcare professionals must wear total-protection PPE (i.e., cup, facial protection, waterproof single-use gown and gloves) and filtering face piece class 3 (FFP3) or N95 mask.

Based on our clinical experience, we suggest that it could be reasonable, while awaiting swab results, prioritize timely treatment in high-risk patients, considering them as COVID-19 infected in order to provide timely treatment and perform ICA, whenever indicated, using airborne PPE (coverall or disposable gown, gloves, headcover, eye shield, FFP3/N99 respirators masks, and shoe covers); then, after revascularization, assess COVID-19 status in order to organize hospitalization in a dedicated ward or isolation in coronary care unit, and subsequent healthcare workers' use of different types of PPE.

TABLE 1 | Different etiologies and hypothesized mechanism of COVID-induced myocardial injury.

Type of myocardial injury	Possible mechanism	Clinical consequences	Available evidence
Type 1 myocardial infarction	*Systemic inflammatory response syndrome*: ↑risk of plaque rupture and thrombus formation **Cytokine storm** due to imbalanced TH1/TH2 response ⇒**DIC** [*71.4% non-survivors vs. 0.6% survivors* (8)]: MOF	STEMI or NSTEMI (9) **Thrombosis** of coronary epi- and subepicardial arteries ⇒focal myocardial necrosis and dysfunction (10)	Bangalore et al. (11) Xhuan et al. (12) Tang et al. (8) Sugiura et al. (10)
Type 2 myocardial infarction	**Myocardial oxygen imbalance** (↑demand for sepsis state, not satisfiable for COVID-19 induced hypoxaemia and vasoconstriction)	Severe myocardial ischaemia, ++ in patients with underlying CAD	Li et al. (5) Shi et al. (13) Guo et al. (14)
Venous thromboembolism	Hypercoagulable status + active inflammation + propensity for DIC + prolonged immobilization + oxidative stress + endothelial dysfunction + increased platelet reactivity + mechanical ventilation + liver dysfunction + central venous catheters + nutritional deficit	↑D-dimer *(>1μg/mL on admission ⇒↑ in-hospital death)*, FDP, fibrinogen Pulmonary embolism or deep venous thrombosis *[22.7% non-ICU and 27% in ICU patients* (15)]	Tang et al. (10) Han et al. (15) Klok et al. (16)
Acute myocarditis	*Indirect mechanism:* innate immunity activation ⇒inflammatory cascade and exaggerated cytokine release *Direct mechanism*: ACE2 receptor (used by SARS-CoV2 for binding, overexpressed in diseased hearts)	STEMI-like presentation with myocardial degenerative changes and necrosis	Zhou et al. (17) Yao et al. (18) Beri et al. (19) Tavazzi et al. (20) Hu et al. (21) Zeng et al. (22) Sala et al. (23)
Stress cardiomyopathy	Infective +/- emotional trigger ⇒catecholamine induced myocardial stunning or macro- and micro-vascular spasm	Tako-tsubo syndrome	Moderato et al. (24) Meyer et al. (25) Chadha et al. (26)

ACE2, angiotensin-converting enzyme-2; CAD, coronary artery disease; DIC, disseminated intravascular coagulation; ICU, intensive care unit; MOF, multi-organ failure; NSTEMI, non ST-elevation myocardial infarction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; STEMI, ST-elevation myocardial infarction; TH1, T-helper lymphocytes 1; TH2, T-helper lymphocytes 2; VTE, venous thromboembolism.
+, plus; ++, above all; ↑, higher.

Differential Diagnosis in COVID-19 Patient

Differential diagnosis of COVID-AMI really became a challenge for clinicians. Commonly, a rise and/or fall of hs-cTn is not sufficient to ensure the diagnosis of myocardial infarction, but it should also be corroborated with clinical judgment, symptom and signs, ECG changes, and imaging studies (28). As recent documents of the European Association of Cardiovascular Imaging (EACVI) and the American College of Cardiology (ACC) highlighted, this is especially valid in case of COVID-19, considering that cardiac enzymes elevation could either be secondary to non-specific raise during COVID infection or to other acute pathologic complications (e.g., sepsis, acute kidney injury, stroke) (29, 30). Moreover, as troponin elevation in patients with COVID-19 infection seems to be lower than in most cases of ACS or acute myocarditis, EAPCI suggests considering marked elevation (e.g., >5 times the upper normal limit) in a patient who is not critically ill to suspect COVID-AMI (27).

As a matter of fact, the access to diagnostic resources is currently limited since, considering the high infective power of SARS-CoV2, performing unnecessary imaging tests should be avoided in order to limit healthcare personnel and devices exposure to the risk of contamination (31).

Sometimes, COVID-19 presentation could entail cardiovascular symptoms rather than fever, cough, dyspnoea, as shown in a small Italian report with 81% of patients presenting ST-elevation MI (STEMI) as COVID-19 first manifestation, of whom 78.6% referring to ED with acute chest pain. Interestingly, only 39.3% demonstrated absence of obstructive coronary artery disease (32). In fact, the EACVI recommendations on the use of cardiac imaging during COVID-19 pandemic suggest considering the optimization of computed tomography (CT), often used to confirm of COVID-pneumonia, with the addition of coronary CT methods to exclude ACS in case of raised troponin (30). Similarly, the use of CT completed with contrast enhanced sequences has been proposed by Hendren et al. to exclude acute myocarditis avoiding the additional use of cardiac magnetic resonance (CMR) and invasive endomyocardial biopsy, since patterns of delayed myocardial enhancement consistent with acute myocarditis revealed by cardiac CT have also been described (33).

As regards patients hospitalized for COVID-19 with suspected ACS, EACVI recommends to evaluate the pre-test probability (PTP) based on symptoms, ECG signs, age, sex, previous history, and cardiovascular risk factors, to use coronary CT angiography

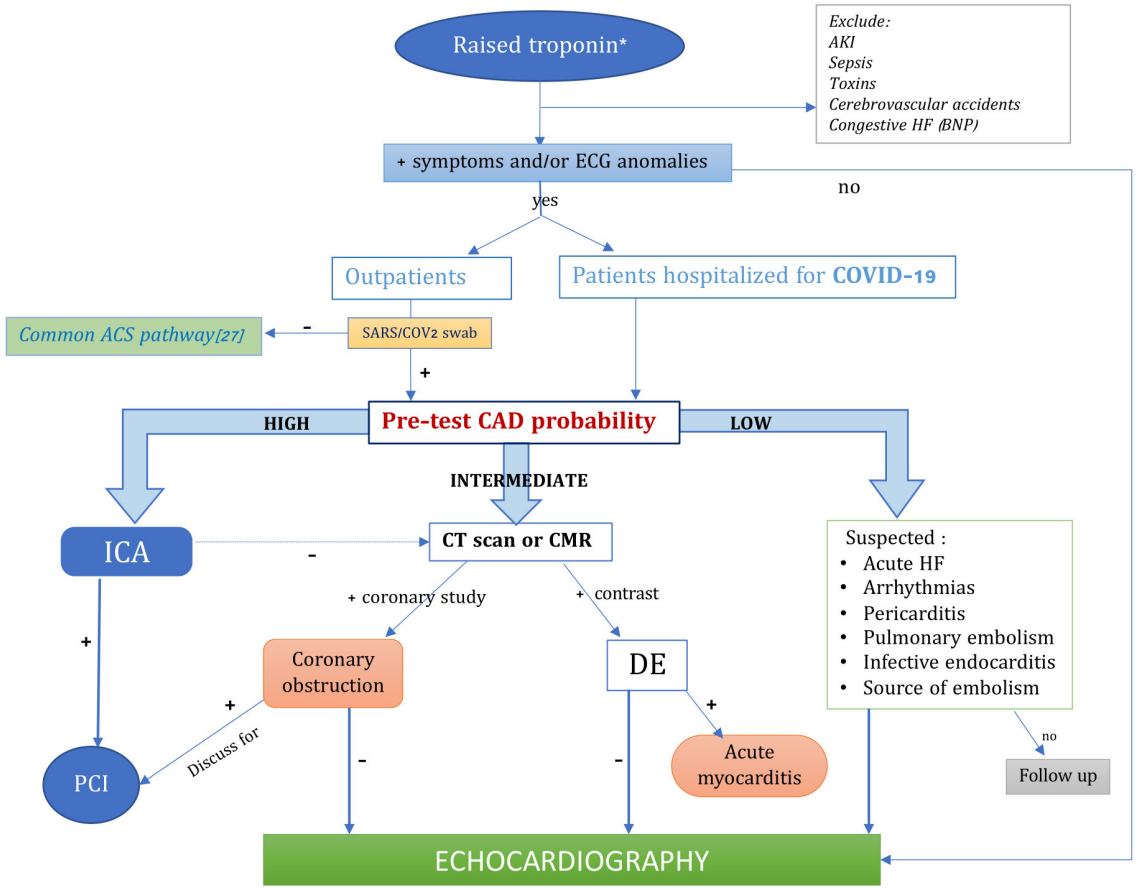

FIGURE 1 | Algorithm for the diagnosis of COVID-induced acute myocardial injury optimizing the available imaging techniques. *hs-cTn>99th percentile of its upper normal limit, or >5 times the upper normal limit in COVID patients. ACS, acute coronary syndromes; AKI, acute kidney injury; BNP, brain natriuretic peptide; CMR, cardiac magnetic resonance; CT, cardiac tomography; DE, delayed enhancement; HF, heart failure; ICA, invasive coronary angiography; PCI, percutaneous coronary intervention.

for intermediate PTP, and to reserve ICA only for cases with very high PTP or STEMI, high-risk non-STEMI (NSTEMI) or crescendo angina (34).

A schematic representation of the suggested pathway for differential diagnosis of COVID-AMI preventing from wasting unnecessary diagnostic resources is available in **Figure 1**. In that regard, two important messages deriving from the international societies' recommendations (27, 29, 30, 34), both for outpatients referring to ED and for hospitalized patients, should be highlighted:

- **ICA** should be performed only in patients with suspected type 1 MI (27) and who are expected to derive meaningful changes in outcome from invasive management; therefore, patients with high level of comorbidities, poor quality of life, and frailty should be early assigned to medical therapy, since additional investigations are futile;
- the use of **echocardiography**, which has always been regarded as a "gatekeeper" for differential diagnosis of cardiovascular disease, should be reconsidered in this emergency period. Transthoracic echocardiography should not be routinely performed if patients are asymptomatic and stable, but it remains the first line approach in patients with high suspicion of COVID-AMI, in order to address diagnosis (35). Given its high aerosol-generating procedure, the use of transoesophageal echocardiography should be restricted to the selected cases of poorly feasible or informative transthoracic echocardiography, and when it would lead to change and optimization of the patient's management; when necessary, this procedure must be performed with FFP3 or N95 equipment.

Bearing all these recommendations and the possible poor availability of advanced imaging methods in some center, also due to the overwhelming requests of CT scan, for the purpose to determine the presence of an atypical COVID presentation with ACS, we would like to highlight the importance of performing accurate anamnesis, investigating symptoms occurrence and timing; a thorough ECG observation, seeking for ischemic abnormalities corresponding to coronary regions; rely on the dosage of troponin, after excluding troponin-affecting comorbidities which could act as confounders. In cases of

extreme uncertainties, echocardiography should be applied with the use of appropriate PPE (**Figure 1**).

In-hospital ACS Management During COVID-19

Outpatients

The best therapeutic strategy for patients with ACS during the pandemic has been extensively discussed. Even though in early Chinese algorithms primary PCI was sacrificed in favor of the protection of healthcare personnel from contagion, opting for rapid testing for COVID-19 infection and immediate fibrinolysis, European societies recommend a halfway approach (34, 36). Accordingly, as stated in the EAPCI document on invasive management of ACS during COVID-19 (27), the COVID-19 infective danger should not change the first-line therapeutic approach to STEMI. Primary percutaneous coronary intervention (PCI) remains the standard of care for STEMI patients referred to Hub centers or transferred rapidly from non-PCI centers within 120 min from the first medical contact. For patients in whom a rapid reperfusion with primary PCI is not feasible, initial fibrinolysis is recommended, followed by consideration of transfer to a PCI center. More specifically, the consensus statement from the Society for Cardiovascular Angiography and Interventions (SCAI), ACC and the American College of Emergency Physicians (ACEP) suggests that for *STEMI patients with positive SARS-CoV2 swab* referred to a Spoke center, the transfer to a PCI center should be discussed, possibly preferring to perform fibrinolysis within 30 min of STEMI diagnosis, and eventually transfer to Hub Center for rescue PCI if needed (37), where this should be performed by experienced operators equipped with high-level PPE in dedicated rooms.

For NSTEMI management an approach based on individual risk is recommended (27):

- *very high risk NSTEMI* patients should follow a similar management of STEMI;
- *high risk NSTEMI* patients should follow medical treatment while waiting for SARS-CoV2 test results and planning an early invasive therapy, possibly < 24 h; in case of positive test, the patients should undergo ICA in a COVID-19 hospital;
- *low risk NSTEMI* could be firstly evaluated non-invasively, in order to exclude alternative etiology to type 1 MI, using coronary CT, if possible; if low risk is confirmed, they should follow conservative strategy.

Table 2 summarizes the criteria for risk stratification of NSTEMI patients based on the newest European Society of Cardiology (ESC) guidelines (38).

In case of necessary ICA approach, preventive strategies are of outmost importance to ensure protection to healthcare personnel and their relatives, hospital environment, and also other patients.

As regards high-risk patients whose COVID status is unknown, as soon as the patient arrives in the Cath-lab, vital signs should be assessed (with particular attention to body temperature and arterial oxygen saturation). Furthermore, blood gas analysis and biologic specimens (swab) collection for COVID-19 test should be performed using the necessary PPE according to the severity of respiratory symptoms (39):

- Low COVID-19 risk: surgical mask.
- High COVID-19 risk: PPE with FFP2 or FFP3 mask, depending on the gravity of respiratory impairment of the individual patient.

Operators should follow precise protocols of dressing/undressing (40) and, after the procedure, in patients with unknown or positive SARS-CoV2 Nucleic Acid test a sanitization of the Cath-lab is mandatory.

TABLE 2 | Risk stratification for non-ST-elevation myocardial infarction (NSTEMI) treatment (38).

Very high risk	High risk	Low risk
- Hemodynamic instability - Cardiogenic shock - Recurrent/refractory chest pain despite medical treatment - Life-threatening arrhythmias - Mechanical complications of myocardial infarction - Acute HF related to NSTEMI - ST-segment depression > 1 mm in 6 leads + ST-segment elevation in aVr and/or V1	- NSTEMI diagnosis already established - Symptomatic/ asymptomatic - dynamic new (or presumably new) contiguous ST-T segment changes - Resuscitated cardiac arrest without ST-segment elevation or cardiogenic shock - GRACE risk score > 140	No recurrence of symptoms and none of the *very high* or *high-risk* criteria. Also includes patients with: - History of revascularization - Early post-infarction angina - LVEF<40% or congestive HF - GRACE risk score 109–140 - Diabetes mellitus Ruled out based on troponin levels

HF, heart failure, NSTEMI; LVEF, left ventricular ejection fraction; non-ST-elevation myocardial infarction.

Inpatients

As for patients already hospitalized in a *COVID-Unit* with *suspected STEMI*, the risk and benefits of a possible coronary revascularization should be evaluated, weighting the individual patients' clinical conditions and comorbidities and the risks related to the transport in the Cath-lab. In case of risks overweight, fibrinolysis could be considered as an alternative to PCI (41, 42). However, the increased hemorrhagic and DIC risk in COVID-19 patients, especially those with severe conditions, should be considered.

Fibrinolytic Strategy

Even if bigger evidence is required in this field, the use of fibrinolysis as an alternative to PCI seemed to reach comparable results for in-hospital and 30-day clinical outcome (all-cause death, cardiac death, stroke, re-infarction/coronary re-occlusion, and revascularization) in patients during the COVID-19 pandemic with absence of major bleeding (43) and was proposed by several authors as a reasonable alternative to PCI, providing spare of medical resources (e.g., PPE and workflow) and of healthcare professionals exposure to the risk of contagion (41, 44, 45). However, we suggest that (1) the well-known superiority of PCI to definitely restore blood flow and in reducing

mortality, re-infarction, or stroke (46); (2) the risk of early re-thrombosis of the culprit lesion requiring rescue PCI if sufficient anticoagulation is not reached after the fibrinolytic treatment, resulting in longer hospitalization and possible complications; (3) the fatal/non-fatal bleeding risk of fibrinolysis itself (particularly if performed in patient with "STEMI-mimicker") should be taken into account both in COVID-19 and non-COVID-19 patients; therefore, in our view, fibrinolysis-lone strategy should be considered only in case of higher risks connected to patients' transfer to PCI-center or to the Cath lab outweighing incremental benefits of PCI, or in case of impossibility to provide timely PCI. Importantly, the bleeding risk of the single patient should be evaluated in the decision-making between primary PCI and fibrinolysis.

ACS METAMORPHOSIS IN COVID-19 ERA

Now that the control of COVID-19 contagion and management is improved, with resulting lower rates of morbidity, it is time for clinicians to look beyond COVID-19 and to care about the cardiovascular consequences of the pandemic. A serious concern regarding ACS is currently affecting global healthcare services: a downward trend in ACS incidence has been registered all over the world, awakening the interest of the scientific community. First, the Italian society of Cardiology multicenter register, which compared acute MI incidence in a week with the equivalent period in 2019, observed a drastic reduction of 48.4% ($p<0.001$), which was significant for both STEMI (26.5%, higher for women: 41.2% vs. 17.8%) and NSTEMI (65.1%) and was similar throughout Italy (52.1% Northern vs. 59.3% Central vs. 52.1% Southern). Importantly, they have also registered a substantial increase in STEMI fatality rate [risk ratio (RR) = 3.3, 1.7–6.6; $p< 0.001$] and complications (RR = 1.8; 1.1–2.8; $p = 0.009$) during the pandemic, compared to 2019 (46).

Then, Metzler et al. conducted an Austrian nationwide retrospective survey involving 17 primary PCI centers for 27 days during COVID-19 outbreak, founding a relative reduction from the beginning to the end of this period of 39.4% in admission for all subtypes of ACS (47). Huet et al. reported almost halved numbers of admission for acute MI or heart failure in 9 French ICU centers comparing 14 days periods before and after containment (4.8±1.6 vs. 2.6±1.5 patients per day, $p = 0.0006$) (48).

Furthermore, the impact of the pandemic on interventional cardiology procedures has been assessed by Garcia et al., who quantified STEMI activations in 9 high-volume (>100 PCI/year) United States cardiac Cath-labs from January 1, 2019, to March 31, 2020, and observed a 38% reduction in Cath-lab STEMI activations in the after-COVID period (49), similar to the 40% registered in Spain (50). Moreover, an analysis of the Italian Society of Interventional Cardiology (GISE) reported a decrease in interventional coronary and structural procedures of 48.5% for ICA, 45.7% for PCI, 84.7% for transcatheter aortic valve replacement, and 50% for Mitraclip in Piedmont (Italy), during the COVID-19 period (51).

In our experience, we have observed not only a reduction of hospitalization for AMI but also a dramatic increase of hospitalization for subacute myocardial infarction >72 h, with cases of malignant arrhythmias and severe heart failure resistant to conventional therapy and often requiring inotropic support; this unavoidably resulted in poor prognosis for patients and challenges for clinicians to select the best therapeutic strategy, due to the doubtful benefits of a late revascularization and the difficult selection of patients for the allocation of advanced therapeutic resources (such as mechanical assist devices).

Causative Factors

Altogether, these data depict a picture of almost half of patients with ACS not reaching the hospital and not receiving timely treatment. The embraceable opinion is that this worrisome phenomenon could be multifactorial:

- **Patient-related factors:** to start with, there was a reduced referral to ED of patients with chest discomfort or unclear ACS symptoms due to their fear of catching SARS-CoV-2 in the hospital, encouraged by in-hospital contagion described by the media and by the strict instructions to stay at home. These have led patients to underestimate their symptoms, such as in a case-report by Masroor et al. regarding a 48-year-old man who referred to the ED for chest pain started 2 days earlier, but not seeking attention until later, due to his reluctance to access the hospital for dreaded COVID-19 contagion. ECG clearly showed STEMI and he underwent ICA with successful PCI on the occluded right coronary artery; few hours later, he developed cardiogenic shock for postinfarction ventricular septal defect of 2 cm, initially treated with intra-aortic balloon pump to let the myocardium heal, and then with surgical repair using a pericardial patch (52). Other patient-related features explaining the reduction in hospital admissions for ACS during the COVID-19 era are a negative psychological response, emotional distress, distrust/avoidance behaviors, and reluctance to activate pre–hospital networks.
- **Healthcare-related factors**: during this period, the emergency services have focused on COVID-19, with most healthcare resources relocated to manage the pandemic and with possible fails in identification of MI, which could have led to an artificial decreasing of ACS diagnoses. First, the priority given to COVID-19 suspected or known patients could have finally distracted from cardiovascular emergencies. Then, it seems that, for patients presenting symptoms consistent with COVID-19, all the resources and clinical attention have been dedicated to excluding SARS-CoV-2 infection, with consequent overlooking of acute cardiovascular conditions, causing misdiagnosis and/or delayed treatment. A clear example was described by Yousefzai et al. in a case-report of a 56-year-old patient with cardiovascular risk factors presenting exertional dyspnea and left bundle branch block at ECG who at first hesitated to refer to the ED and was then misdiagnosed with COVID-19-induced acute myocarditis, though presenting STEMI. Meanwhile, he developed acute respiratory distress syndrome

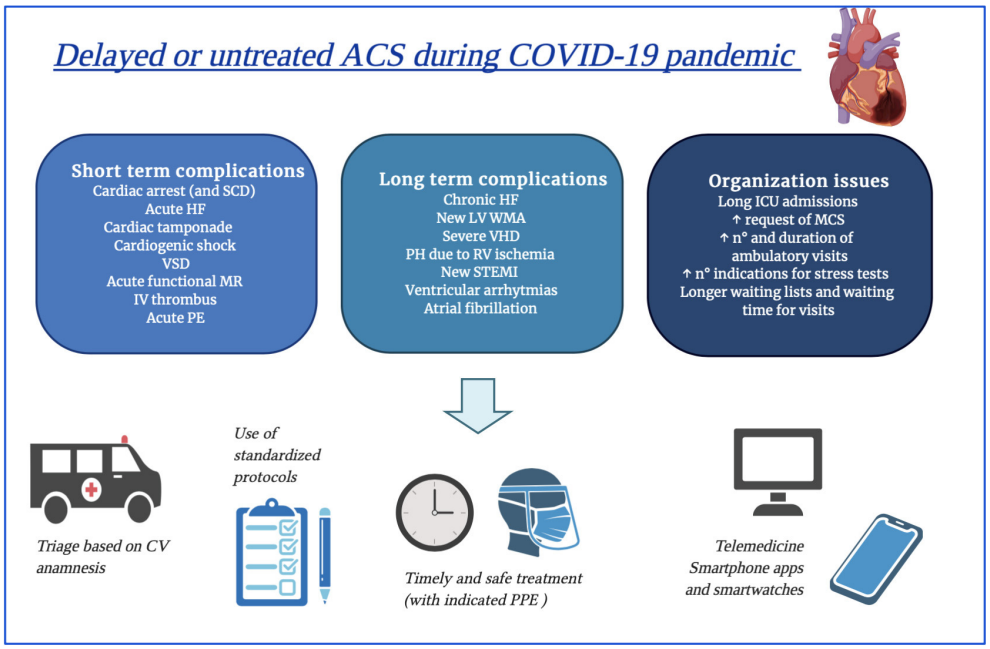

FIGURE 2 | Possible complications deriving from late or untreated acute coronary syndromes during COVID-19 pandemic and figurative hints for limiting them. ACS; acute coronary syndromes; HF, heart failure; ICU, intensive care unit; IV, intraventricular; LV, left ventricular; MCS, mechanical circulatory support; NSTEMI, non-ST elevation myocardial infarction; PE, pulmonary embolism; PH, pulmonary hypertension; PPE, personal protective equipment; RV, right ventricular; SCD, sudden cardiac death; STEMI, ST elevation myocardial infarction; VHD, valvular heart disease; VSD, ventricular septum defect; WMA, wall motion abnormalities.

requiring ventricular assistance and underwent late ICA with evidence of 99% left anterior descending coronary stenosis, 60% proximal circumflex artery stenosis, and moderate disease on right coronary artery; therefore, the clinicians opted not to perform revascularization. Then, after this completed anterior MI, he remained in ICU waiting for recovery or definitive ventricular assistance therapy (53).

Short- and Long-Term Consequences

The delay among symptoms presentation and revascularization could result in dramatic effects. Noteworthy, conjunction of the longer time from symptoms onset to first medical contact due to patients' reticence and waiting times for triaging, COVID-19 testing (since not all the healthcare facilities are equipped with ultra-rapid tests) and personnel precautions, would result in further delay for a needed PCI. This should represent an alarm for clinicians and public health, since the paramount importance of the timing of primary revascularization to save myocardial structure and function is well-known (53). In fact, in a recent study by Trabattoni et al., despite a regional optimization of the STEMI network through a re-structured Hub-Spoke model in Lombardy (Italy), a significant delay (> 24 h) in patients' referral to ED was present in 41% of STEMI patients in 2020, compared to 20% in 2019, resulting in in-hospital mortality rates of 38 vs. 10%, respectively (54). Similar results were shown by a Chinese group in an observational study on 149 patients with MI before *(group 1, n = 85 patients)* and after *(group 2, n = 64 patients)* COVID-19 emergency measures; the second group not only had longer symptom-to-first medical contact time and higher presentation rates out of the PCI window (33 vs. 27.8%) but also showed a more elevated incidence of the composite outcome measure including in-hospital death, cardiogenic shock, sustained ventricular tachycardia/fibrillation, and use of mechanical circulatory support (29.7, vs. 14.1%, $p = 0.02$) (55).

These data, together with those previously mentioned (34), suggest that an increase in the incidence of late presenting MIs with chronic heart failure and sudden cardiac death is the most expectable eventuality in the near future, together with raised early and late morbidity and mortality. Short term-complications would require prolonged hospitalization in ICU, which could represent a serious concern in these times of poor resources. Over the long-term, suboptimal revascularization and large infarct size will result in maladaptive ventricular remodeling and dysfunction (56). Short and long-term complications and their impact on healthcare services are presented in **Figure 2**.

The earliest reports referred to cases with initially mild symptoms who experienced sudden cardiac death at home while in quarantine (57). Moreover, Baldi et al. described an increased incidence of out-of-hospital cardiac arrest during 40 days of COVID-19 pandemic in Italy compared to the same period in 2019, which such cumulative increased incidence being strongly associated with the diffusion of COVID-19 (58). Similarly, a

4.97-fold increase in out-of-hospital sudden cardiac arrest and a doubling of pronounced deaths on the scene was reported in New York City during the surge of pandemic, compared with the same period (March 20–April 22) of 2019 (59). These data could reflect the eventual consequences of medical care avoidance or distraction.

Possible Solutions

As the ESC guidance for the diagnosis and management of cardiovascular disease during the COVID-19 pandemic illustrates (60), it would be rational to triage patients with suspected or known COVID-19 according to the presence of underlying cardiovascular risk factors and co-morbidities, as well as to evidence of myocardial injury, in order to select those who deserve prioritized treatment and even more aggressive therapeutic strategies.

Organization of healthcare facilities should be improved with dedicated pathways and rapid SARS-CoV-2 testing, if available, allowing a timely supply of diagnostic and interventional procedures. ACS patients with highly suspected COVID-19 should be isolated and undergo necessary laboratory and imaging tests, with all healthcare workers wearing the appropriate PPE (34).

Besides, the most important issue is to educate the general population about the early recognition of high-risk ACS symptoms with promptly referral to ED (or at least to contact a physician) in such cases. This could be reached by social media, television, and journals. Interestingly, following this rationale, the Italian Society of Cardiology promoted a national campaign to raise public awareness about MI symptoms during the outbreak, showing encouraging results in terms of subsequent fall in the time from symptoms to ED admission (50).

Social education should emphasize the concept of an outweigh of untreated-MI consequences, rather than of COVID-19 in-hospital infection, since hospitals are now equipped with appropriate PPE and follow the preventive protocols to minimize the risk of contagion. The use of telemedicine and/or telemonitoring in doubtful cases would allow to obtain a close follow-up of patients' symptoms and clinical conditions and, sometimes, to perform some kinds of triaging in order to avoid unrecognized MI on one hand, and to optimize resources allocation on the other hand. More compliant patients could also be engaged in the use of smartwatches and smartphone apps, achieving rapid medical screening and/or self-monitoring.

CONCLUSIONS

During the COVID-19 pandemic, the topic of ACS has been widely discussed. Even if there is paucity of randomized data on the best methods for management, expert consensus and international society recommendation could help us in adopting a standardized approach. First of all, it is important to distinguish between primary ACS or COVID-AMI and, for the latter, discriminate the actual etiology and provide the optimal treatment. This should be done balancing timeliness of screening and conscious use of diagnostic resources and protective measures, in order to ensure safety conditions to all patients and healthcare professionals. COVID status should be assessed as soon as possible. Each primary PCI center should evaluate the feasibility of a timely primary PCI, based on staff, PPE and Cath-lab availability, and the need for additional testing. Otherwise, a first approach with fibrinolysis should be considered. The other important concern is the global registration of lower rates of admitted (and therefore treated) patients with ACS. This could lead to a substantial increase in early and late infarct-related morbidity and mortality. To face the possible collateral cardiac damage caused by COVID-19, every attempt should be done by the clinicians in means of avoiding delayed or missed diagnosis, re-organization of healthcare tools, and social education.

AUTHOR CONTRIBUTIONS

MC, MCP, GM, FD'A, PC, GP, GB, and MF performed the data search and drafted the manuscript. MC, FF, GP, SM, and SV critically revised the draft. All Authors contributed to the conception of this work and approved the final version of the manuscript.

REFERENCES

1. *COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU)*. Available online at: https://coronavirus.jhu.edu/map.html (accessed July 3, 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. 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
4. 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
5. Li B, Yang J, Zhao F, Zhi L, Wang X, Liu L, et al. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. *Clin Res Cardiol*. (2020) 109:531–8. doi: 10.1007/s00392-020-01626-9
6. 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
7. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol*. (2020) 17:259–260. doi: 10.1038/s41569-020-0360-5
8. 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
9. Warren-Gash C, Hayward AC, Hemingway H, Denaxas S, Thomas SL, Timmis AD, et al. Influenza infection and risk of acute myocardial infarction in England and Wales: a CALIBER self-controlled case series study. *J Infect Dis*. (2012) 206:1652–9. doi: 10.1093/infdis/jis597

10. Sugiura M, Hiraoka K, Ohkawa S, Ueda K, Matsuda T. A clinicopathological study on cardiac lesions in 64 cases of disseminated intravascular coagulation. *Jpn Heart J.* (1977) 18:57–69. doi: 10.1536/ihj.18.57
11. 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
12. Xuan TM, Wang XX, Pu XY, Han WL, Guo XG. Primary percutaneous coronary intervention in a COVID-19 patient with ST-segment elevation myocardial infarction after lung transplantation: a case report. *J Zhejiang Univ Sci B.* (2020) 21:411–5. doi: 10.1631/jzus.B2000182
13. 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
14. 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) 5:1–8. doi: 10.1001/jamacardio.2020.1017
15. Han H, Yang L, Liu R, Liu F, Wu KL, Li J, et al. Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. *Clin Chem Lab Med.* (2020). 58:1116–20. doi: 10.1515/cclm-2020-0188
16. 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
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. Yao XH, Li TY, He ZC, Ping YF, Liu HW, Yu SC, et al. [A pathological report of three COVID-19 cases by minimal invasive autopsies]. *Zhonghua Bing Li Xue Za Zhi.* (2020) 49:411–7. doi: 10.3760/cma.j.cn112151-20200312-00193
19. Beri A, Kotak K. Cardiac injury, Arrhythmia and Sudden death in a COVID-19 patient. *HeartRhythm Case Rep.* (2020) 6:367–9. doi: 10.1016/j.hrcr.2020.05.001
20. 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
21. Hu H, Ma F, Wei X, Fang Y. Coronavirus fulminant myocarditis saved with glucocorticoid and human immunoglobulin. *Eur Heart J.* (2020). doi: 10.1093/eurheartj/ehaa190. [Epub ahead of print].
22. 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) 48:1–5. doi: 10.1007/s15010-020-01424-5
23. 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
24. Moderato L, Monello A, Lazzeroni D, Binno S, Giacalone R, Ferraro S, et al. [Takotsubo syndrome during SARS-CoV-2 pneumonia: a possible cardiovascular complication]. *G Ital Cardiol.* (2020) 21:417–20. doi: 10.1714/3359.33323
25. Meyer P, Degrauwe S, Van Delden C, Ghadri JR, Templin C. Typical takotsubo syndrome triggered by SARS-CoV-2 infection. *Eur Heart J.* (2020) 41:1860. doi: 10.1093/eurheartj/ehaa306
26. Chadha S. 'COVID-19 pandemic' anxiety-induced Takotsubo cardiomyopathy. *QJM.* (2020) 113:488–90. doi: 10.1093/qjmed/hcaa135
27. 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
28. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, et al. ESC Scientific Document Group. 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
29. American College of Cardiology. *Troponin and BNP Use in COVID-19*. Available online at: https://www.acc.org/latest-in-cardiology/articles/2020/03/18/15/25/troponin-and-bnp-use-in-covid19 (accessed July 3, 2020).
30. Skulstad H, Cosyns B, Popescu BA, Galderisi M, Salvo GD, Donal E, et al. COVID-19 pandemic and cardiac imaging: EACVI recommendations on precautions, indications, prioritization, and protection for patients and healthcare personnel. *Eur Heart J Cardiovasc Imaging.* (2020) 21:592–8. doi: 10.1093/ehjci/jeaa072
31. Cameli M, Pastore MC, Henein M, Aboumarie HS, Mandoli GE, D'Ascenzi F, et al. Safe performance of echocardiography during the COVID-19 pandemic: a practical guide. *Rev Cardiovasc Med.* (2020) 21:217–23. doi: 10.31083/j.rcm.2020.02.90
32. Stefanini GG, Montorfano M, Trabattoni D, Andreini D, Ferrante G, Ancona M, et al. ST-elevation myocardial infarction in patients with COVID-19: clinical and angiographic outcomes. *Circulation.* (2020) 141:2113–6. doi: 10.1161/CIRCULATIONAHA.120.047525
33. Hendren NS, Drazner MH, Bozkurt B, Cooper LT Jr. Description and proposed management of the acute COVID-19 cardiovascular syndrome. *Circulation.* (2020) 141:1903–14. doi: 10.1161/CIRCULATIONAHA.120.047349
34. 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
35. 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
36. 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
37. Mahmud E, Dauerman HL, Welt FG, Messenger JC, Rao SV, Grines C, et al. Management of acute myocardial infarction during the COVID-19 pandemic. *J Am Coll Cardiol.* (2020) 96:336–45. S0735-1097(20)35026-9. doi: 10.1016/j.jacc.2020.04.039
38. Collet J, Thiele H, Barbato E, Barthélémy O, Bauersachs J, Bhatt DL, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur Heart J.* (2020) 1–79. doi: 10.1093/eurheartj/ehaa575. [Epub ahead of print].
39. Valente S, Anselmi F, Cameli M. Acute coronary syndromes during COVID-19. *Eur Heart J.* (2020) 41:2047–49. doi: 10.1093/eurheartj/ehaa457
40. Italian Society of Interventional Cardiology (GISE). *Management of catheterization lab and interventional cardiology during COVID-19 emergency*. (2020). 41. Available online at: https://gise.it/Uploads/EasyCms/GM%20CF%20per%20PD%20gestione%20covid-19%20-_14892.pdf (accessed July 3, 2020).
41. 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
42. Welt FGP, Shah PB, Aronow HD, Bortnick AE, Henry TD, Sherwood MW, et al. American College of Cardiology's Interventional Council and the Society for Cardiovascular Angiography and Interventions. 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.2020.03021
43. Wang N, Zhang M, Su H, Huang Z, Lin Y, Zhang M. Fibrinolysis is a reasonable alternative for STEMI care during the COVID-19 pandemic. *J Int Med Res.* (2020) 48:300060520966151. doi: 10.1177/03000605209 66151
44. Zhang L, Fan Y, Lu Z. Experiences and lesson strategies for cardiology from the COVID-19 outbreak in Wuhan, China, by 'on the scene' cardiologists. *Eur Heart J.* (2020) 41:1788–90. doi: 10.1093/eurheartj/ehaa266
45. Keeley EC, Boura JA, Grines CL. Primary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomised trials. *Lancet.* (2003) 361:13–20. doi: 10.1016/S0140-6736(03)12113-7
46. 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/ehaa610
47. Metzler B, Siostrzonek P, Binder RK, Bauer A, Reinstadler SJ. Decline of acute coronary syndrome admissions in Austria since the outbreak of COVID-19: the pandemic response causes cardiac collateral damage. *Eur Heart J*. (2020) 41:1852–3. doi: 10.1093/eurheartj/ehaa314
48. Huet F, Prieur C, Schurtz G, Gerbaud E, Manzo-Silberman S, Vanzetto G, et al. One train may hide another: acute cardiovascular diseases could be neglected because of the COVID-19 pandemic. *Arch Cardiovasc Dis*. (2020) 113:303–7. doi: 10.1016/j.acvd.2020.04.002
49. 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
50. Rodriguez-Leor O, Cid-Alvarez B. ST-segment elevation myocardial infarction care during COVID-19: losing sight of the forest for the trees. *JACC Case Rep*. (2020) 2:1625–7. doi: 10.1016/j.jaccas.2020.04.011
51. Quadri G, Rognoni A, Cerrato E, Baralis G, Boccuzzi G, Brsic E, et al. Catheterization laboratory activity before and during COVID-19 spread: a comparative analysis in Piedmont, Italy, by the Italian Society of Interventional Cardiology (GISE). *Int J Cardiol*. (2020) 323:288–91. doi: 10.1016/j.ijcard.2020.08.072
52. Masroor S. Collateral damage of COVID-19 pandemic: delayed medical care. *J Card Surg*. (2020) 35:1345–7. doi: 10.1111/jocs.14638
53. Scott IA. "Time is muscle" in reperfusing occluded coronary arteries in acute myocardial infarction. *Med J Aust*. (2010) 193:493–5. doi: 10.5694/j.1326-5377.2010.tb04030.x
54. Trabattoni D, Montorsi P, Merlino L. Late STEMI and NSTEMI patients' emergency calling in COVID-19 outbreak. *Can J Cardiol*. (2020) 36:1161.e7–1161.e8. doi: 10.1016/j.cjca.2020.05.003
55. Tam CF, Cheung KS, Lam S, Wong A, Yung A, Sze M, et al. Impact of coronavirus disease 2019 (COVID-19) outbreak on outcome of myocardial infarction in Hong Kong, China. *Catheter Cardiovasc Interv*. (2020) 13:e006631. doi: 10.1002/ccd28943
56. Yousefzai R, Bhimaraj A. Misdiagnosis in the COVID-19 Era: when zebras are everywhere, don't forget the horses. *JACC Case Rep*. (2020) 2:1614–9. doi: 10.1016/j.jaccas.2020.04.018
57. 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
58. Baldi E, Sechi GM, Mare C, Canevari F, Brancaglione A, Primi R, et al. Lombardia CARe researchers. Out-of-hospital cardiac arrest during the Covid-19 outbreak in Italy. *N Engl J Med*. (2020) 383:496–8. doi: 10.1056/NEJMc2010418
59. Mountantonakis SE, Saleh M, Coleman K, Kuvin J, Singh V, Jauhar R, et al. Out-of-hospital cardiac arrest and acute coronary syndrome hospitalizations during the COVID-19 surge. *J Am Coll Cardiol*. (2020) 76:1271–3. doi: 10.1016/j.jacc.2020.07.021
60. The European Society for Cardiology. *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 June 10, 2020).

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Electrocardiographic Risk Stratification in COVID-19 Patients

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Background: The COVID-19 pandemic has resulted in worldwide morbidity at unprecedented scale. Troponin elevation is a frequent laboratory finding in hospitalized patients with the disease, and may reflect direct vascular injury or non-specific supply-demand imbalance. In this work, we assessed the correlation between different ranges of Troponin elevation, Electrocardiographic (ECG) abnormalities, and mortality.

Methods: We retrospectively studied 204 consecutive patients hospitalized at NYU Langone Health with COVID-19. Serial ECG tracings were evaluated in conjunction with laboratory data including Troponin. Mortality was analyzed in respect to the degree of Troponin elevation and the presence of ECG changes including ST elevation, ST depression or T wave inversion.

Results: Mortality increased in parallel with increase in Troponin elevation groups and reached 60% when Troponin was >1 ng/ml. In patients with mild Troponin rise (0.05–1.00 ng/ml) the presence of ECG abnormality and particularly T wave inversions resulted in significantly greater mortality.

Conclusion: ECG repolarization abnormalities may represent a marker of clinical severity in patients with mild elevation in Troponin values. This finding can be used to enhance risk stratification in patients hospitalized with COVID-19.

Keywords: predictors, mortality, troponin, COVID−19, ECG

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INTRODUCTION

Coronavirus Disease (COVID19) pandemic, induced by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is reaching now historical magnitude as one of the deadliest outbreaks in modern history (1). As of December 30 2020, over 80 million individuals were reported to be infected by SARS-CoV-2, with more than 1.8 million deaths (2). Recent reports (3, 4) revealed that cardiac complications are common (≈20–25%) in COVID19 infection and are associated with increased mortality. However, in those reports, "cardiac complications" were defined according to clinical and laboratory parameters (troponin levels) without systematic electrocardiographic (ECG) evaluation. It is unknown if elevations in troponin levels are reflective of a primary myocardial infarction, supply-demand inequity, or non-ischemic direct myocardial injury. The ECG is an attractive diagnostic tool as it is widely available and can be rapidly performed without inducing significant exposure of caregivers to SARS-CoV-2. ECG has been demonstrated to aid

with prognostication in population-based studies (5, 6) and thus offers a particularly appealing modality during the current pandemic. We thus sought to determine whether findings on the first presenting ECG provide prognostic information and provide insights on myocardial injury. We reviewed ECGs of consecutive patients with COVID19 infection requiring hospitalization. We examined our findings stratified by troponin levels and clinical condition.

METHODS

This is a retrospective study performed at NYU Langone Medical Center, New York, USA. We included 204 consecutive adult patients hospitalized at NYU Langone Medical Center with COVID19 disease. Medical records were reviewed to obtain baseline characteristics, laboratory data, and ECGs. Troponin I concentrations were assessed via the Abbott Architect method (Abbott, Abbott Park, Illinois) wherein the 99th percentile for a normal population is 0.05 ng/mL and the maximal Troponin level was recorded. Descriptive analyses were performed by troponin levels stratified into normal (0.00–0.05 ng/mL), mildly elevated (0.05–1 ng/mL), and significantly elevated (>1 ng/mL). The first, presenting ECGs were reviewed and interpreted by five senior cardiologists who were blinded to the clinical status of the patients. Data reviewed from each ECG included heart rate, rhythm categorized as normal sinus rhythm or atrial fibrillation/flutter (AF), atrioventricular block (AVB), right bundle branch block (RBBB), left bundle branch block (LBBB), a non-specific intraventricular conduction block (QRS duration >120 ms), the presence of ST segment or T-wave changes (localized ST elevation, localized T-wave inversion, or other non-specific repolarization abnormalities). The closing date of follow-up was April 15th 2020. Collected data on the closing date included arrhythmic events and mortality. The study was reviewed and approved by the NYU Institutional Review Board and Quality Improvement initiative in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments, with a waiver of informed consent.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 26, and figures were constructed using GraphPad Prism 8. Continuous variables are expressed as mean ± standard deviation or median (25th−75th percentile), and categorical variables are expressed as count (percentages). Normality of data samples was assessed using Shapiro-Wilk test. Two sample hypothesis testing for continuous variables was performed using Student's *t*-test if samples had normal distributions and Mann-Whitney U test if samples did not have normal distributions. Hypothesis testing for categorical variables was performed using Fisher's exact test. Significance testing for Kaplan-Meier curves was performed using log-rank test. For predictors of mortality, univariate analysis was performed using Cox proportional hazards regression, and significant univariate predictors were included in the multivariate analysis.

TABLE 1 | Baseline characteristics of patients with COVID-19.

	Overall (*n* = 204)	ECG changes (*n* = 36)	No ECG changes (*n* = 168)	*p*
Age (years)	64 ± 13	67 ± 13	63 ± 13	0.14
Gender (% male)	156 (76%)	25 (69%)	131 (78%)	0.28
Race				0.08
African-American	10 (5%)	4 (11%)	6 (4%)	
Non-AA	191 (95%)	31 (89%)	160 (96%)	
Weight (kg)	86.6 ± 17.6	83.7 ± 16.4	87.3 ± 17.8	0.25
CAD	25 (12%)	7 (19%)	18 (11%)	0.16
HTN	114 (56%)	20 (56%)	94 (56%)	1
CKD	17 (8%)	6 (17%)	11 (7%)	0.09
DM	61 (30%)	18 (50%)	43 (26%)	<0.01
COPD	13 (6%)	4 (11%)	9 (5%)	0.25
CHF	7 (3%)	2 (6%)	5 (3%)	0.61
Initial creatinine (mg/dL)	1.3 ± 1.0	1.6 ± 1.4	1.2 ± 0.9	0.57
Abnormal LFTs	48 (25%)	10 (29%)	38 (24%)	0.51
Initial troponin (ng/mL)	0.02 (0.01 - 0.04)	0.02 (0.01 - 0.08)	0.02 (0.01 - 0.04)	0.17
Maximum troponin	0.04 (0.01 - 0.15)	0.12 (0.02 - 0.47)	0.03 (0.01 - 0.11)	0.01
Troponin group				<0.01
≤0.05	120 (59%)	14 (39%)	106 (63%)	
0.05–1.00	64 (31%)	14 (39%)	50 (30%)	
>1.00	20 (10%)	8 (22%)	12 (7%)	
Mortality	50 (23%)	13 (36%)	37 (22%)	0.09

RESULTS

We included 204 patients in our cohort with a mean follow up time of 24.2 ± 7.4 days. The clinical and epidemiological characteristics stratified by ECG abnormalities are presented in **Table 1**. The mean age was 64 ± 13 years and 76% were male. Comorbidities were common: 30% of patients had diabetes mellitus, 56% had hypertension, 12% had coronary artery disease, 3% had heart failure, and 6% had chronic obstructive pulmonary disease (COPD). Baseline electrocardiographic characteristics revealed mean HR of 89 ± 16 bpm and mean Bazett-corrected QT interval of 444 ± 26 ms. The vast majority were in normal sinus rhythm (95%), while 5% of patients had AF. Atrioventricular block was rare: 9 (4%) patients had a first degree AV block and no patients had second or third degree AV block. Abnormal intraventricular conduction was found in 11% (with RBBB in 8%, LBBB in 3%). Repolarization abnormalities (ST elevation, ST depression, or T wave inversion) were common (36 patients, 17.6%): one patient (0.5%) had localized ST elevation, 12 (5.9%) had ST depression, and 28 (13.7%) had localized T-wave inversion. Patients with repolarization abnormalities demonstrated higher troponin levels and a trend toward higher

Abbreviations: ECG, Electrocardiography; COPD, Chronic obstructive pulmonary disease; CAD, Coronary artery disease; CHF, Congestive heart failure; CKD, Chronic kidney disease; HTN, Hypertension; DM, Diabetes mellitus; LFTs, Liver function tests.

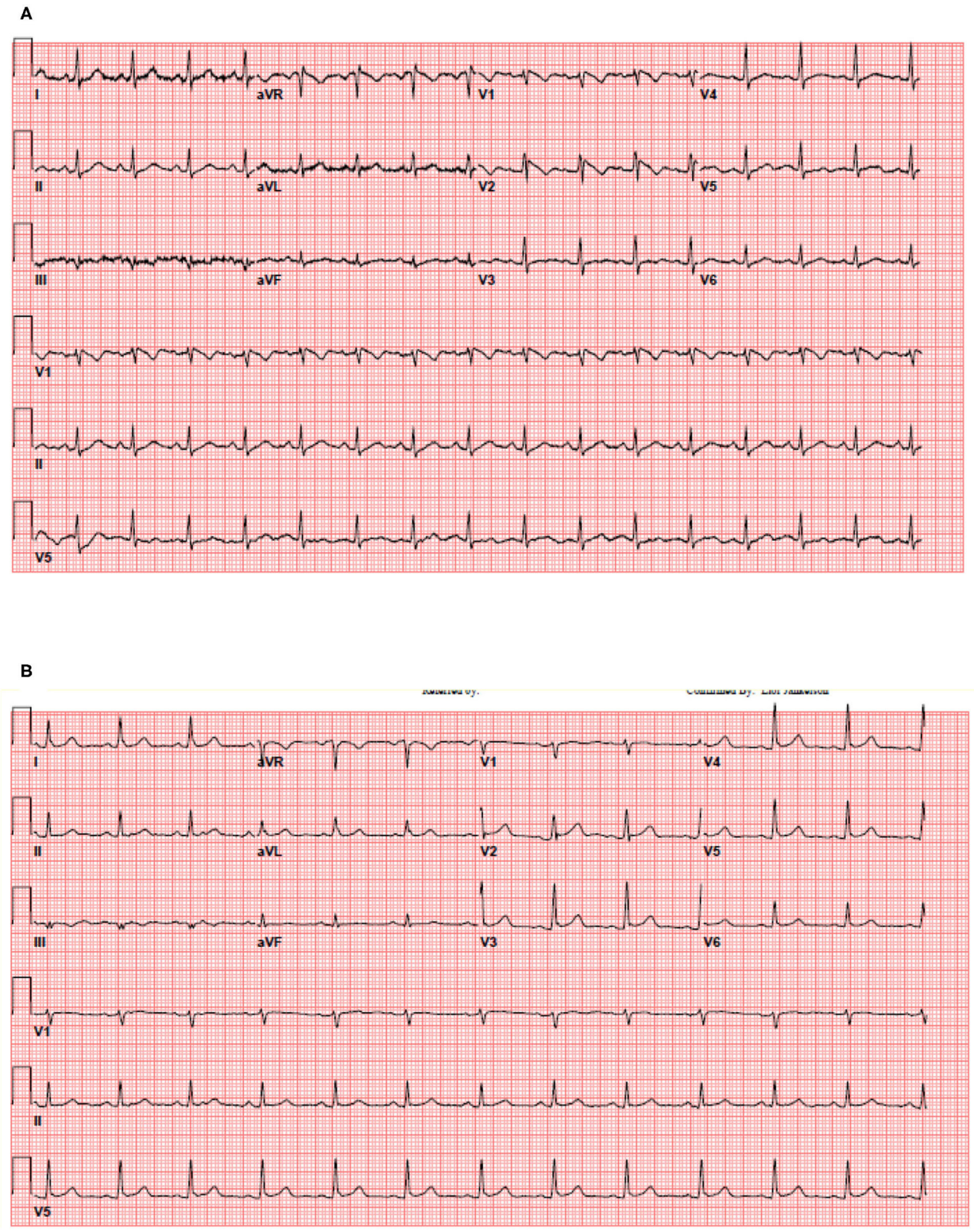

FIGURE 1 | Thirty five year old female patient without significant medical history presented with a fever of 103.1 F. **(A)** The patient's initial 12-lead electrocardiogram in the emergency department. **(B)** The patient's repeat 12-lead electrocardiogram with resolution of fever.

TABLE 2 | Predictors of mortality in patients with COVID-19.

Variable	HR	95% CI	*p*-value
UNIVARIATE REGRESSIONS			
Age (years)	1.08	1.05–1.1	<0.01
Gender (% male)	1.09	0.56–2.13	0.80
RACE			
African-American	0.8	0.2–3.3	0.76
Weight (kg)	0.99	0.97–1.01	0.18
CAD	1.08	0.49–2.41	0.84
HTN	1.39	0.78–2.48	0.26
CKD	2.4	1.12–5.11	0.02
DM	1.77	1.01–3.1	0.05
COPD	1.46	0.53–4.07	0.47
CHF	2.09	0.65–6.73	0.22
Initial creatinine (mg/dL)	1.24	1.08–1.43	<0.01
Abnormal LFTs	0.84	0.42–1.7	0.64
Initial troponin (ng/mL)	0.99	0.84–1.16	0.91
Maximum troponin	1.01	1.0–1.02	0.02
Positive troponin (>0.05)	4.24	2.28–7.86	<0.01
MULTIVARIATE REGRESSION			
Age (years)	1.06	1.04–1.1	<0.01
CKD	1.53	0.65–3.6	0.33
DM	0.99	0.53–1.86	0.98
Positive troponin (>0.05)	3.22	1.71–6.05	<0.01

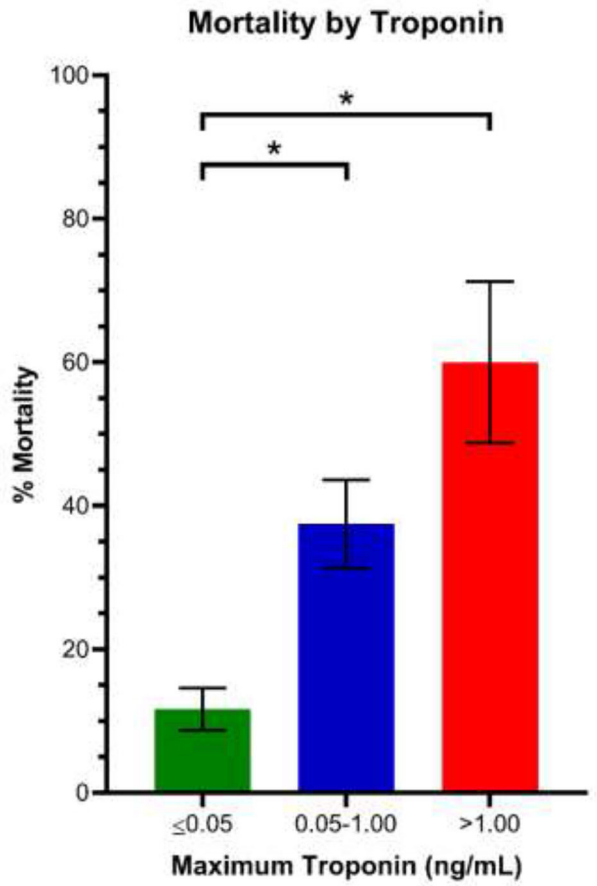

FIGURE 2 | Mortality rate stratified by Troponin elevation groups. *$P < 0.01$.

mortality (**Table 1**). One patient presented with a fever of 103.1 F which unmasked a previously unknown type I Brugada pattern (**Figure 1**). Fifty (25%) patients died of respiratory or multi-organ failure. In univariate and multivariate Cox regression analyses, clinical predictors of death included age and elevated Troponin (**Table 2**), but did not include gender, race or cardiovascular comorbidities (CAD, CHF, HTN). The mortality rate increased with incrementally higher troponin group: 14/120 [11.7%] for patients with negative troponin, 24/64 [37.5%] for patients with mildly elevated troponin, and 12/20 [60%], for patients with significantly elevated troponin ($p < 0.01$; **Figure 2**). The presence of an abnormal ECG finding resulted in significantly lower survival in the intermediate Troponin elevation group (0.05–1 ng/ml) but not in the low (<0.05 ng/ml) or high (> 1 ng/ml) Troponin elevation groups (**Figure 3**). In multivariate regression analysis, T wave inversion but not ST depression remained a significant predictor of mortality (HR 2.71, 95% CI 1.01–7.25, $p = 0.04$) in the intermediate Troponin group.

DISCUSSION

Multiple mechanisms have been shown to explain the frequent COVID-19 induced cardiovascular injury. These include direct injury to the myocardium induced by a cytokine storm resulting from a hyperinflammatory state, microvascular damage resulting from abnormal activation of the coagulation cascade including disseminated intravascular coagulation and thrombosis, supply-demand mismatch resulting from respiratory induced tissue hypoxia in conjunction with increase in metabolic demand of infection and inflammation, and myocardial injury by direct entry of SARS-CoV-2 into cardiomyocytes expressing the ACE2 receptor (7–9). In this retrospective cohort study we further assess the interaction of ECG abnormalities and Troponin elevation. We demonstrate that (1) myocardial injury defined by elevated Troponin is indeed prevalent in patients hospitalized with COVID-19 but is more often mild, associated with low-level elevation in troponin concentration. (2) more significant myocardial injury, as evident by increased Troponin level may be associated with higher risk of mortality. (3) In the group of patients with mild Troponin elevation (0.05–1 ng/ml), ECG abnormalities, and particularly T wave inversions are associated with significantly increased mortality. Consistent with our findings, a recent study had demonstrated that T wave inversion is highly frequent finding in patients with COVID-19, conferring increased risk for mortality and particularly when accompanied by Troponin elevation (10).

Though troponin elevation above the 99th percentile of the upper reference limit is considered the central marker of "myocardial injury," mild elevation between 0.05 and 1 is often non-specific and associated with non-vascular

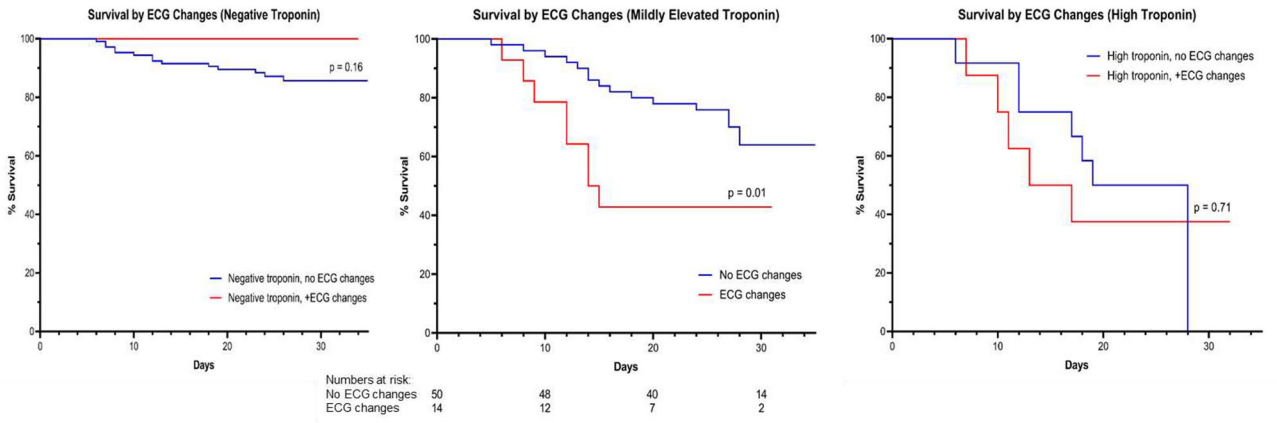

FIGURE 3 | KM survival according to ECG changes stratified by Troponin elevation group.

etiologies such as strain, myocyte necrosis and increased cell membrane permeability (11–13). Indeed, mild Troponin elevation was a frequent finding in our cohort, present in 31% of patients with COVID-19. In this regard, our data suggests that assessment for the presence of ECG abnormalities can be used to enhance inpatient risk stratification in those patients with mild Troponin elevation, with potentially intensification of monitoring and therapy. Finally, as persistent fever is a frequent clinical feature of COVID-19, as well as potential side effect of the novel vaccines, caregivers should be familiar with the phenomena of fever induced Brugada pattern and not mistake it for ST elevation myocardial infarction. For patients who present with transient, fever induced Brugada pattern, elective challenge with sodium channel blocking agent (Procainamide, Flecainide, Ajmalin) after resolution of the acute illness can establish the diagnosis of Brugada.

LIMITATIONS

Our study has several limitations. This is an observational, retrospective study. Because of its retrospective nature, the study is subject to selection bias, and its results imply association, not cause and effect. Relatively short follow-up time after was available. The study was not aimed at providing mechanistic insight for the cause of repolarization changes and Troponin elevation. We did not asses structural information from echo due to limited number of tests performed. However, this study was directed at assessing the presenting ECG as a readily available tool for risk stratification in combination with Troponin, a simple blood test.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Data sharing will be considered pending request. Requests to access these datasets should be directed to lior.jankelson@nyumc.org.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Quality Control Act With Accordance to the NYU Langone Health IRB. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

LJ and EC: concept, data collection, and manuscript. MD and EK: statistics. LW, ES, CN-R, RK, and RB-C: data collection. CB, AA, DH, SB, MS, DP, and LC: manuscript writing and review. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, 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
2. WHO. *Coronavirus Disease (COVID-19) Pandemic.* Geneva: World Health Organization (2020). Available online at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019
3. 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
4. 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
5. Rautaharju PM, Kooperberg C, Larson JC, LaCroix A. Electrocardiographic predictors of incident congestive heart failure and all-cause mortality in post-menopausal women: the Women's Health Initiative. *Circulation.* (2006) 113:481–9. doi: 10.1161/CIRCULATIONAHA.105.537415

6. Daviglus ML, Liao Y, Greenland P, Dyer AR, Liu K, Xie X, et al. Association of nonspecific minor ST-T abnormalities with cardiovascular mortality: the Chicago Western electric study. *JAMA*. (1999) 282:530–6. doi: 10.1001/jama.281.6.530
7. Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A, et al. Coronavirus disease 2019 (COVID-19) and cardiovascular disease. *Circulation*. (2020) 140:1648–55. doi: 10.1161/CIRCULATIONAHA.120.046941
8. Patel AB, Verma A. COVID-19 and angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: what is the evidence? *JAMA*. (2020) 323:1769–1770. doi: 10.1001/jama.2020.4812
9. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of coronaviruses on the cardiovascular system: a review. *JAMA Cardiology*. (2020) 5:831–40. doi: 10.1001/jamacardio.2020.1286
10. Korff S, Katus HA, Giannitsis E. Differential diagnosis of elevated troponins. *Heart*. (2006) 92:987–93. doi: 10.1136/hrt.2005.071282
11. Romero J, Alviz I, Parides M, Diaz JC, Briceno D, Gabr M, et al. T-wave inversion as a manifestation of COVID-19 infection: a case series. *J Interv Card Electrophysiol*. (2020) 59:485–93. doi: 10.1007/s10840-020-00896-7
12. Januzzi JL Jr, McCarthy CP. Trivializing an elevated troponin: adding insult to injury? *J Am Coll Cardiol*. (2019) 73:10–2. doi: 10.1016/j.jacc.2018.10.042
13. Park KC, Gaze DC, Collinson PO, Marber MS. Cardiac troponins: from myocardial infarction to chronic disease. *Cardiovasc Res*. (2017) 113:1708–18.e13. doi: 10.1093/cvr/cvx183

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Prognostic Value of Right Ventricular Ejection Fraction Assessed by 3D Echocardiography in COVID-19 Patients

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Background: RVEF (right ventricular ejection fraction) measured by three-dimensional echocardiography (3DE) has been used in evaluating right ventricular (RV) function and can provide useful prognostic information in other various cardiovascular diseases. However, the prognostic value of 3D-RVEF in coronavirus disease 2019 (COVID-19) remains unknown. We aimed to investigate whether 3D-RVEF can predict the mortality of COVID-19 patients.

Methods: A cohort of 128 COVID-19-confirmed patients who had undergone echocardiography were studied. Thirty-one healthy volunteers were also enrolled as controls. COVID-19 patients were divided into three subgroups (general, severe, and critical) according to COVID-19 severity-of-illness. Conventional RV structure and function parameters, RV free wall longitudinal strain (FWLS) and 3D-RVEF were acquired. RVFWLS was measured by two-dimensional speckle tracking echocardiography. RVEF was acquired by 3DE.

Results: Compared with controls, 2D-RVFWLS and 3D-RVEF were both significantly decreased in COVID-19 patients (−27.2 ± 4.4% vs. −22.9 ± 4.8%, $P < 0.001$; 53.7 ± 4.5% vs. 48.5 ± 5.8%, $P < 0.001$). Critical patients were more likely to have a higher incidence of acute cardiac injury and acute respiratory distress syndrome (ARDS), and worse prognosis than general and severe patients. The critical patients exhibited larger right-heart chambers, worse RV fractional area change (RVFAC), 2D-RVFWLS, and 3D-RVEF and higher proportion of pulmonary hypertension than general and severe patients. Eighteen patients died during a median follow-up of 91 days. The multivariate Cox regression analysis revealed the acute cardiac injury, ARDS, RVFAC, RVFWLS, and 3D-RVEF were independent predictors of death. 3D-RVEF (chi-square to improve 18.3; $P < 0.001$), RVFAC (chi-square to improve 4.5; $P = 0.034$) and 2D-RVFWLS (chi-square to improve 5.1; $P = 0.024$) all provided additional prognostic value of higher mortality over clinical risk factors. Moreover, the incremental predictive value of 3D-RVEF was significantly ($P < 0.05$) higher than RVFAC and RVFWLS.

Conclusion: 3D-RVEF was the most robust independent predictor of mortality in COVID-19 patients and provided a higher predictive value over conventional RV function parameters and RVFWLS, which may be helpful to identify COVID-19 patients at a higher risk of death.

Keywords: three-dimensional echocardiography, right ventricular function, Coronavirus disease 2019, myocardial strain, prognosis

INTRODUCTION

Cardiac injury was a prevalent complication and was associated with worse prognosis in COVID-19 patients, with an incidence ranging from 7.2 to 27.8% (1–5). The increased cardiac workload resulting from respiratory failure and hypoxemia is a common mechanism of cardiac injury and the right ventricle may bear the brunt of its impact (3). Echocardiography is a convenient and widely available imaging tool for assessing cardiac function. Although both left ventricular (LV) dysfunction and right ventricular (RV) dysfunction are noted in hospitalized COVID-19 patients, the incidence of the latter is higher and the worse RV function is associated with clinical deterioration (i.e., hemodynamic instability, cardiac deterioration, and respiratory deterioration) (6–8). Furthermore, right ventricular free wall longitudinal strain (RVFWLS) derived from two-dimensional speckle tracking echocardiography (2D-STE) has been proven to be a more effective factor to predict mortality than conventional RV function parameters in COVID-19 patients (9). However, 2D-STE has the intrinsic limitation of losing speckles from out-of-plane cardiac motion. Additionally, given the complex structure of the RV and the three–dimensional (3D) motion of heart, 3D analysis could potentially provide better and more accurate assessment compared to 2D analysis. Previous studies have proved that three–dimensional right ventricular ejection fraction (3D-RVEF) can provide valuable prognostic information in various cardiovascular diseases (10–12). However, the prognostic value of 3D-RVEF in COVID-19 patients has not been studied. Accordingly, this study aimed to assess RV structure and function in COVID-19 patients with different severity of illness and to explore whether 3D-RVEF provides incremental prognostic value with regards to fatal outcomes in COVID-19 patients.

METHODS

Study Population

This study was performed at Union Hospital in Wuhan, China. We enrolled a total of 172 consecutive patients confirmed with COVID-19 according to the WHO interim guidance (13) from January 29 to March 4, 2020. Bedside echocardiogram was performed in all patients for assessment of cardiac structure and function. The median time from admission to echocardiography examination was 5 days [interquartile range (IQR) 3–10 days]. A total of 44 patients were excluded because of dilated cardiomyopathy ($n = 2$), old myocardial infarction ($n = 4$), insufficient image quality for echocardiographic analysis ($n = 32$), arrhythmia during examination ($n = 6$), the remaining 128 patients were divided into three subgroups according to the guideline on the diagnosis and treatment of COVID-19 by the National Health Commission (version 7.0) (14): general ($n = 41$), severe ($n = 58$) and critical ($n = 29$) groups. Additionally, thirty-one healthy volunteers having no cardiopulmonary disease based on physical examinations, biochemical tests, electrocardiography, chest X-ray and echocardiogram were enrolled as the control group.

This study was 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.

Clinical Data

The demographic characteristics and clinical data (vital signs, comorbidities, major laboratory findings, treatment, complications, and prognosis during hospitalization) were extracted from electronic medical records by two researchers. The timing of laboratory measurements was within 3 days of echocardiogram with a median interval of 1 day (Interquartile Range, IQR: 1–2 days). Patients clinical outcomes were followed up to May 18, 2020. Acute cardiac injury was defined as serum plasma levels of high-sensitivity troponin I (hs-TNI) above the 99th percentile of the upper limit of reference (4). Acute respiratory distress syndrome (ARDS) was defined according to the Berlin Definition (15). The criteria for COVID-19 severity-of-illness was defined by the Chinese management guideline for COVID-19 (version 7.0) as follows: (1) general: fever and respiratory symptoms, with evidence of pneumonia on radiological imaging; (2) severe: patients with any of the following symptoms and signs: respiratory distress with respiratory rate $\geq$30 breaths/min; SpO2 $\leq$ 93% at rest; and PaO2/FiO2 $\leq$ 300 mmHg (1 mm Hg = 0.133 kPa); and (3) critical: patients with any of the following conditions: respiratory failure requiring mechanical ventilation, shock, and/or other organ failure requiring admission to the intensive care unit (ICU) (14). The criteria for RV dysfunction is based on published reference, and the COVID-19 patients were divided into three subgroups:

Abbreviations: 2D, Two-dimensional; 3D, Three-dimensional; 3DE, Three-dimensional echocardiography; A, Late diastolic inflow velocity; COVID-19, Coronavirus disease 2019; E, Early diastolic inflow velocity; e', Early diastolic tissue velocity; FAC, Fractional area change; hs-TNI, high-sensitivity troponin I; ICC, intra-class correlation coefficient; IQR, interquartile range; PH, Pulmonary hypertension; RVFWLS, right ventricular free wall longitudinal strain; RVEDVI, Right ventricular end-diastolic volume index; RVESVI, Right ventricular end-systolic volume index; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; STE, Speckle-tracking echocardiography; S', Tricuspid lateral annular systolic velocity; TAPSE, Tricuspid annular plane systolic excursion; TR, Tricuspid regurgitation.

3DRVEF > 45%, 40% < 3DRVEF ≤ 45%, and 30% < 3DRVEF ≤ 40% (16).

Conventional Echocardiography

Bedside echocardiography was performed using a commercially available system (EPIQ 7C, Philips Medical Systems, Andover, USA). 2D and Doppler echocardiography examinations were performed based on the recommendations of the American Society of Echocardiography (17). And all 2D echocardiographic parameters were acquired according to the published guidelines (18, 19).

The left atrial volume, left ventricular end-diastolic and end-systolic volumes, left ventricular ejection fraction (LVEF) were measured by the biplane Simpson's method in apical two- and four-chamber views and volumes were indexed to body surface area (BSA) (18). Doppler mitral and tricuspid peak early (E) and late (A) diastolic velocities, and E/A velocity ratios were measured from the LV and RV inflow velocities on apical four-chamber view. RV transverse diameter at the base was measured from the RV-focused apical four-chamber view, and the minor right atrial (RA) transverse diameter was measured from the middle level of RA on apical four-chamber view. Tricuspid lateral annular systolic velocity (S'), tricuspid annular plane systolic excursion (TAPSE) and RV fractional area change (FAC) were measured according to the established guidelines (19). Systolic pulmonary arterial pressure (PASP) was calculated by the Bernoulli simplified equation on tricuspid regurgitation (TR) maximum jet velocity sum of estimated RA pressure. Pulmonary hypertension (PH) was defined as PASP > 40 mm Hg (19).

The off-line 2D-STE analysis was performed with the vendor-independent software TomTec (2D Cardiac Performance Analysis 1.2 for 2D-STE; TomTec Imaging Systems, Unterschleissheim, Germany) to acquire the RV strain in the RV focused apical four-chamber view with frame rate of 50–70 MHz, according to the published recommendations (20, 21). The workstation automatically performed a contour tracking of RV endocardium, and a manual adjustment was performed in case of unsatisfactory tacking. Finally, the time-strain curve of RVFWLS was generated automatically. RVFWLS was defined as the mean longitudinal peak systolic strain of three segments of the RV free wall. RVFWLS was performed 3 times during the regular heartbeats and the average was used for analysis.

3DE Imaging and Analysis

A wide-angled, single-beat, high frame rate (HeartModel mode) 3D full-volume images data sets were acquired from the apical 4-chamber RV-focused view. The 3DE datasets were stored digitally for offline analysis. The 3D full-volume RV images were analyzed by an experienced echocardiographer. RV-focused one-beat 3D full-volume images were analyzed with a novel, full automated RV quantification software (3D Auto RV, Phillips Medical Systems) that detect RV endocardial contours using artificial intelligence, which consists of knowledge-based identification of initial global shape and RV chamber orientation, followed by 3D speckle tracking analysis throughout a cardiac cycle (22, 23). The software initially identified LV and RV long-axis landmarks in end-diastole in the apical two- and four-chamber views. Based on that, the RV-focused four-chamber view and a short-axis view. Then RV endocardial surfaces were full automatically defined and tracked throughout the cardiac cycle, and a quick minimal manual adjustment was performed in case of unsatisfactory outcomes. Finally, a 3D RV cast, RV volume curves were provided, from which the RV end-diastolic volume (RVEDV), RV end-systolic volume (RVESV), and RVEF were determined (**Figures 1A–C**).

Interobserver and Intraobserver Reproducibility

Intraobserver and interobserver variability in measurement of 2D-RVFWLS and 3D-RVEF were analyzed in 20 randomly selected subjects. Intraobserver variability was assessed by the same observer 2 weeks later. Interobserver variability was assessed by a second observer in the same 20 patients.

Statistical Analysis

Continuous variables were expressed as mean ± SD, or median (IQR). The normality of distribution was tested by the Shapiro–Wilk test. Comparisons between groups were made by two-sample student *t*-test or one-way analysis of variance for normally distributed variables; and Mann–Whitney *U*-test or Kruskal–Wallis test for non-normal distribution of data. The *post-hoc* pairwise comparisons with Bonferroni correction was used for continuous variables. Categorical data were expressed as percentages and were compared by the χ^2 test or Fisher exact test, when appropriate. The correlation between 3D-RVEF and 2D-RVFWLS was examined using Pearson's Correlation coefficients.

Univariate and multivariable Cox proportional hazards models were performed to identify the independent risk factors of mortality in COVID-19 patients. Variables with $P < 0.05$ at univariate analysis were included in stepwise multivariable analysis. To avoid overfitting and collinearity issues, four separate multivariable Cox proportional hazard models were constructed to determine the independent predictors of higher mortality. To assess the potential additive prognostic value of 3D-RVEF and the other RV parameters, we evaluated the additional increment of the chi-square statistics of the combined models over the baseline model. Receiver operator characteristic curves (ROC) were used to calculate the sensitivity and specificity for predicting death by RV function index and to determine the optimal prognostic cutoff value (Youden method). The Hanley and McNeil methods were applied for comparison of area under the curves (AUCs) of RV function parameters (24). Survival curves were obtained using the Kaplan–Meier method and compared by the log-rank test. The reproducibility of 2D- RVFWLS and 3D-RVEF was assessed using intra-class correlation coefficients (ICC) and Bland-Altman analyses.

All statistical analyses were performed using SPSS version 23.0 (Statistical Package for the Social Sciences, Chicago, IL, USA), STATA software version 10 (StataCorp, Texas, USA) and R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). All tests were 2-tailed; $P < 0.05$ was considered statistically significant.

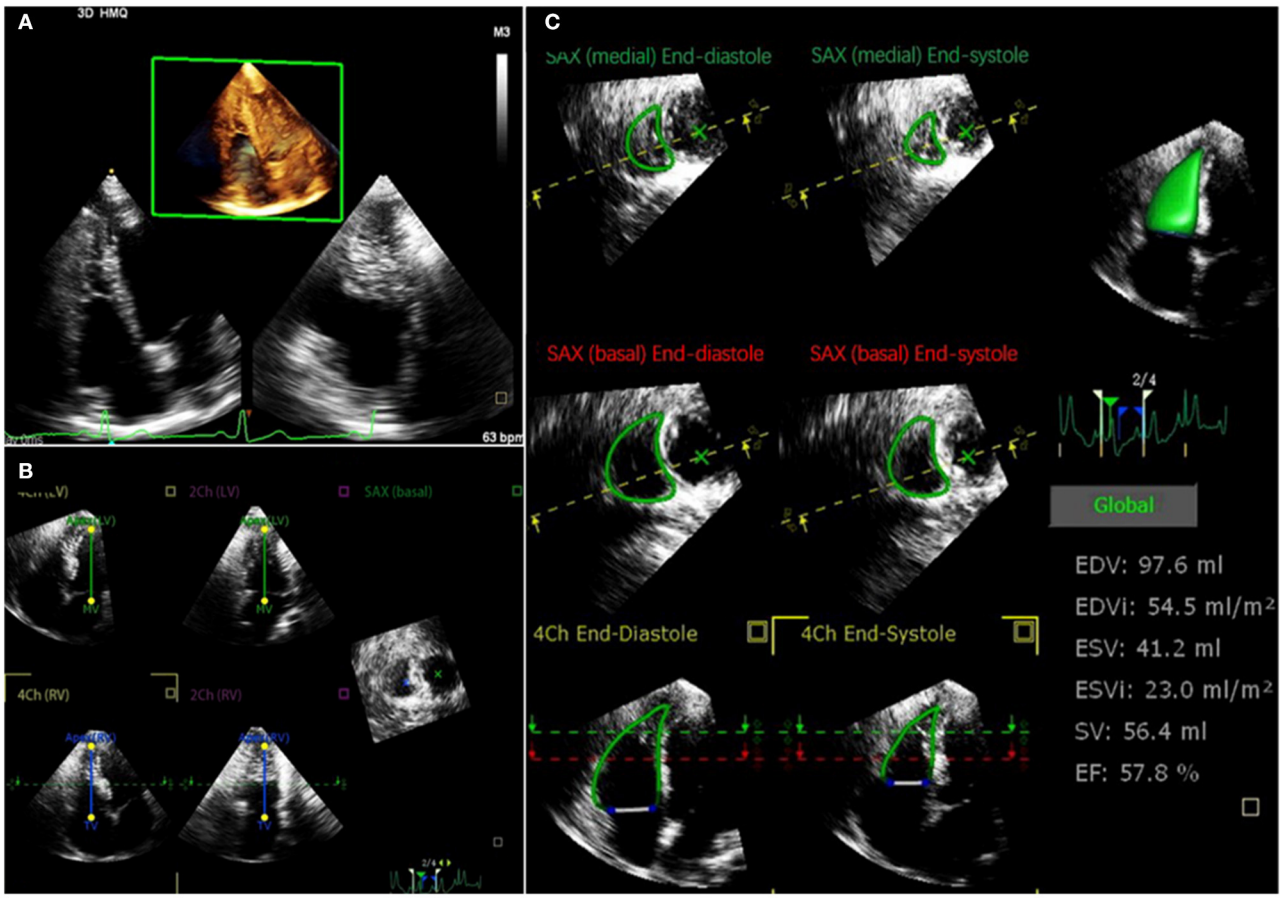

FIGURE 1 | Representative 3DE analysis in a COVID-19 patient. **(A)** Retrieving 3DE datasets aimed for the RV full-volume images from apical 4-chamber RV-focused view; **(B)** LV and RV long-axis landmarks in end-diastole in the apical 2- and 4-chamber views were identified by software initially; then, RV focused 4-chamber view and a short-axis view are derived; **(C)** RV endocardial surfaces were full automatically defined and tracked throughout the cardiac cycle; finally, RV end-diastolic volume, RV end-systolic volume, and RVEF were determined. 3DE, three-dimensional echocardiography; COVID-19, coronavirus disease 2019; LV, left ventricular; RV, right ventricular; RVEF, right ventricular ejection fraction.

RESULTS

Clinical Characteristics

The clinical characteristics of the 128 COVID-19 patients were shown in **Table 1**. The mean age was 61.3 ± 13.1 years and 61 (47.7%) patients were men. Of 128 patients, 7 (5.5%) had chronic obstructive pulmonary disease, 18 (14.1%) had cardiac disease including 14 with known coronary heart disease in the absence of abnormal wall motion by routine echocardiography and 4 with occasional arrhythmia (atrial and ventricular extrasystole) by the recording of a long-term electrocardiograph. Compared with general and severe patients, critical patients were older, predominantly male and had higher heart rates (HR) and lower oxygenation index.

In addition, compared with general and severe patients, critical patients were more likely to have underlying cardiac disease, lower levels of lymphocyte counts, higher levels of C-reactive protein and procalcitonin. They were also more prone to receive high-flow oxygen and invasive mechanical ventilation therapy, and were more likely to develop acute cardiac injury, ARDS. More often than not they got admitted to ICU, and had higher mortality.

Echocardiographic Characteristics

Table 2 revealed the echocardiographic characteristics of the subjects. Compared with healthy controls, COVID-19 patients had thickened interventricular septum thickness (IVST), decreased mitral and tricuspid E/A, lower LVEF and FAC, and higher left ventricular end systolic volume index (LVESVI). 2D-RVFWLS and 3D-RVEF were both significantly lower in COVID-19 patients than in controls (−22.9 ± 4.8% vs. −27.2 ± 4.4%, $P < 0.001$; 48.5 ± 5.8% vs. 53.7 ± 4.5%, $P < 0.001$). Moreover, 3D-RVEF correlated significantly with 2D-RVFWLS in COVID-19 patients ($r = -0.59$, $P < 0.001$) and in controls ($r = -0.64$, $P < 0.001$). Furthermore, critical patients exhibited significantly higher mitral E/e′, larger RA, RV and pulmonary artery (PA) diameter, worse FAC, 2D-RVFWLS, and 3D-RVEF. Moreover, a higher proportion of critical patients had PH. Additionally, **Table 2**

TABLE 1 | Clinical characteristics of COVID-19 patients according to severity of illness.

Variables	Total (n = 128)	General (n = 41)	Severe (n = 58)	Critical (n = 29)	P-value
Clinical characteristics					
Age (years)	61.3 ± 13.1	58.6 ± 16.0	60.9 ± 11.7	66.0 ± 9.8	0.06
Male, *n* (%)	61 (47.7)	15 (36.6)	26 (44.8)	20 (69.0)	0.024
Heart rate, beats/min	86 (80, 99)	84.0 (80, 95)	89 (80, 101)	90 (80, 99)	0.494
Respiratory rate, times/min	23 (20, 30)	20 (20, 23)	25 (20, 30)	26 (20, 33)	<0.001
SBP, mmHg	130 (120, 140)	132 (122, 146)	125 (116, 138)	134 (120, 146)	0.195
DBP, mmHg	80 (73, 88)	81 (75, 90)	78 (72, 87)	80 (74, 87)	0.325
OI, mmHg	286.0 (200.0, 337.9)	340.7 (317.2, 392.0)	250.7 (205.5, 301.7)	173.0 (141.7, 248.4)	<0.001
Comorbidities					
Hypertension, *n* (%)	52 (40.6)	17 (41.5)	20 (34.5)	15 (51.7)	0.301
Diabetes, *n* (%)	18 (14.1)	7 (17.1)	8 (13.8)	3 (10.3)	0.720
Cardiac disease, *n* (%)	18 (14.1)	4 (9.8)	5 (8.6)	9 (31.0)	0.028
COPD, *n* (%)	7 (5.5)	3 (7.3)	2 (3.4)	2 (6.9)	0.684
Chronic liver diseases, *n* (%)	4 (3.1)	1 (2.4)	3 (5.2)	0 (0.0)	0.559
Chronic kidney disease, *n* (%)	1 (0.8)	0 (0.0)	1 (1.7)	0 (0.0)	1.000
Malignancy, *n* (%)	9 (7.0)	2 (4.9)	5 (8.6)	2 (6.9)	0.904
Smoker, *n* (%)	7 (5.5)	4 (9.8)	3 (5.2)	0 (0.0)	0.235
Laboratory findings					
White blood cell × 10^9/L	6.6 (4.9, 9.4)	6.2 (4.5, 9.3)	6.0 (4.8, 8.7)	8.4 (6.2, 10.8)	0.010
Lymphocyte count × 10^9/L	1.01 (0.61, 1.44)	1.28 (1.00, 1.63)	0.97 (0.67, 1.36)	0.60 (0.28, 1.02)	<0.001
CRP, mg/L	26.3 (3.6, 63.3)	3.7 (1.0, 32.8)	24.0 (8.4, 53.3)	77.6 (49.0, 124.5)	<0.001
PCT, ng/ml	0.08 (0.05, 0.20)	0.06 (0.04, 0.17)	0.08 (0.05, 0.18)	0.15 (0.07, 0.32)	0.015
D-dimer, mg/L	1.4 (0.5, 5.8)	1.0 (0.2, 4.2)	1.2 (0.5, 5.8)	2.5 (1.0, 8.0)	0.006
hs-TNI, ng/mL	3.9 (1.8, 19.9)	2.7 (1.3, 12.1)	3.4 (1.6, 8.7)	29.3 (4.7, 74.8)	<0.001
CK-MB, U/L	12.0 (9.0, 20.8)	10.0 (7.0, 15.5)	12.0 (9.0, 16.0)	26.0 (11.0, 31.0)	0.001
BNP, pg/ml	51.8 (13.6, 140.9)	36.6 (10.0, 119.6)	33.5 (12.7, 83.1)	199.4 (102.5, 348.7)	<0.001
Serum creatinine (μmol/L)	65.1 (53.4, 80.5)	62.7 (49.3, 80.6)	63.3 (53.9, 82.0)	72.0 (59.3, 78.1)	0.235
Treatments					
Antiviral therapy, *n* (%)	120 (93.8)	34 (82.9)	57 (98.3)	29 (100.0)	0.004
Antibiotic therapy, *n* (%)	92 (71.9)	25 (61.0)	40 (69.0)	27 (93.1)	0.010
Glucocorticoid therapy, *n* (%)	50 (39.1)	11 (26.8)	19 (32.8)	20 (69.0)	0.001
ACE-I/ARB, *n* (%)	12 (9.4)	4 (9.8)	6 (10.3)	2 (6.9)	0.929
High-flow oxygen, *n* (%)	66 (51.6)	4 (9.8)	34 (58.6)	28 (96.6)	<0.001
Mechanical ventilation, *n* (%)	26 (20.3)	1 (2.4)	5 (8.6)	20 (60.9)	<0.001
IMV, *n* (%)	17 (13.3)	0 (0.0)	4 (6.9)	13 (44.8)	<0.001
NIMV, *n* (%)	9 (7.0)	1 (2.4)	1 (1.7)	7 (24.1)	0.001
ICU admission, *n* (%)	19 (14.8)	0 (0.0)	4 (6.9)	15 (51.7)	<0.001
Complications					
ARDS, *n* (%)	48 (37.5)	0 (0.0)	19 (32.8)	29 (100.0)	<0.001
Acute cardiac injury, *n* (%)	27 (21.1)	7 (17.1)	7 (12.1)	13 (44.8)	0.001
Acute kidney injury, *n* (%)	15 (11.7)	4 (9.8)	5 (8.6)	6 (20.7)	0.260
Coagulation dysfunction, *n* (%)	33 (25.8)	5 (12.2)	13 (22.4)	15 (51.7)	0.001
Prognosis					<0.001
Discharge, *n* (%)	110 (85.9)	41 (100.0)	56 (96.6)	13 (44.8)	
Death, *n* (%)	18 (14.1)	0 (0.0)	2 (3.4)	16 (55.2)	

Data were n (%), mean ± SD or median (IQR). ARDS, acute respiratory distress syndrome; ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; BNP, B-type natriuretic peptide; CK-MB, creatine kinase muscle-brain; COVID-19, coronavirus disease 2019; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; DBP, diastolic blood pressure; hs-TNI, high-sensitivity troponin I; ICU, intensive care unit; IMV, invasive mechanical ventilation; IQR; interquartile range; NIMV, non-invasive mechanical ventilation; OI, oxygenation index; PCT, procalcitonin; SBP, systolic blood pressure; SD, standard deviation.

showed that age, HR and systolic blood pressure (SBP) were significantly different between controls and COVID-19 patients. The echocardiographic parameters were further compared between controls and COVID-19 patients after making statistical adjustment of age, HR, and SBP in **Table 3**. After the adjustment, the differences between COVID-19 patients

TABLE 2 | Comparisons of baseline characteristics and echocardiographic characteristics in healthy controls and COVID-19 patients.

				COVID-19 patients			
Variables	**Control ($n = 31$)**	**All patients ($n = 128$)**	***P*-value**	**General ($n = 41$)**	**Severe ($n = 58$)**	**Critical ($n = 29$)**	***P*-value**
Age (years)	51.5 ± 8.4	61.3 ± 13.1	<0.001	58.6 ± 16.0	60.9 ± 11.7	66.0 ± 9.8	0.060
Male, *n* (%)	18 (58.1)	61 (47.7)	0.298	15 (36.6)	26 (44.8)*	20 (69.0)*#	0.024
Body surface area, m^2	1.69 ± 0.13	1.67 ± 0.15	0.589	1.66 ± 0.15	1.65 ± 0.15	1.76 ± 0.14*#	0.003
Heart rate, beats/min	66.0 (58.0, 73.0)	86.0 (80.0, 99.0)	<0.001	84.0 (80.0, 95.0)	89.0 (79.8, 101.3)	90.0 (80.0, 98.5)	0.494
SBP, mmHg	120.0 (114.0, 123.0)	130.0 (120.0, 140.0)	0.001	132 (122.5, 145.5)	124.5 (115.8, 138.0)	134.0 (120.0, 145.5)	0.195
DBP, mmHg	78.0 (70.0, 86.0)	80.0 (73.0, 87.8)	0.336	81.0 (74.5, 89.5)	77.5 (71.5, 87.3)	80.0 (73.5, 87.0)	0.325
Left chamber							
LA, mm	33.7 ± 3.3	34.3 ± 4.7	0.639	33.3 ± 4.9	34.2 ± 4.3	35.7 ± 5.1	0.087
LV, mm	46.9 ± 3.2	45.8 ± 4.3	0.101	44.8 ± 4.4	46.3 ± 3.8	46.2 ± 5.0	0.198
IVST, mm	8.9 ± 0.7	9.6 ± 1.2	0.001	9.6 ± 1.5	9.7 ± 1.0	9.5 ± 1.2	0.821
Mitral valve							
E/A	1.15 ± 0.31	0.93 ± 0.33	<0.001	0.9 ± 0.3	1.0 ± 0.4	0.9 ± 0.3	0.229
E/e'	7.9 ± 1.6	9.0 ± 3.0	0.144	8.1 ± 3.0	9.2 ± 3.0	9.8 ± 2.8*	0.004
LAVI, mL/m^2	32.6 ± 9.1	34.2 ± 10.3	0.407	33.0 ± 10.8	35.5 ± 10.0	32.9 ± 10.7	0.313
LVEDVI, mL/m^2	52.2 ± 12.4	54.5 ± 15.8	0.577	51.0 ± 17.5	57.8 ± 15.3*	52.4 ± 13.2	0.043
LVESVI, mL/m^2	16.6 ± 4.0	20.0 ± 7.2	0.027	18.3 ± 7.3	21.8 ± 7.5*	18.8 ± 5.9	0.030
LVEF, %	68.1 ± 4.0	63.4 ± 6.2	<0.001	64.3 ± 4.8	62.5 ± 7.0	64.1 ± 6.3	0.295
Right chamber							
RA, mm	36.3 ± 3.9	35.3 ± 4.3	0.136	34.5 ± 3.5	34.3 ± 3.7	38.1 ± 5.1*#	<0.001
RV, mm	33.3 ± 3.5	33.9 ± 3.9	0.437	33.3 ± 3.4	33.3 ± 3.8	36.1 ± 4.2*#	0.004
PA, mm	23.3 ± 2.5	23.4 ± 2.7	0.752	22.1 ± 2.4	23.4 ± 2.5	25.1 ± 2.8*	<0.001
Tricuspid valve							
E/A	1.3 ± 0.2	1.0 ± 0.3	<0.001	1.0 ± 0.3	1.0 ± 0.3	0.9 ± 0.3	0.416
E/e'	5.1 ± 2.0	5.2 ± 1.8	0.343	5.2 ± 1.7	4.9 ± 1.5	5.8 ± 2.0	0.077
TAPSE, mm	24.0 ± 2.4	22.9 ± 3.8	0.169	22.9 ± 4.0	23.1 ± 3.5	22.3 ± 4.1	0.652
S', cm/s	12.8 ± 2.0	14.1 ± 2.9	0.019	13.2 ± 2.1	14.2 ± 2.6	15.1 ± 3.9	0.117
FAC, %	51.2 ± 4.3	47.4 ± 5.7	<0.001	48.1 ± 5.2	46.8 ± 5.5	43.1 ± 5.0*#	0.001
PASP, mmHg	/	33.3 ± 12.8	/	27.0 ± 6.5	30.1 ± 8.9	45.3 ± 15.3*#	<0.001
PH, n (%)	0 (0)	18 (14.1)	0.025	1 (2.4)	4 (6.9)	13 (44.8) *#	<0.001
2D-STE parameter							
RVFWLS, %	−27.2 ± 4.4	−22.9 ± 4.8	<0.001	−23.9 ± 3.9	−24.2 ± 4.8	−19.1 ± 4.1*#	<0.001
3DE parameters							
RVEDVI, mL/m^2	60.5 ± 12.9	61.8 ± 11.5	0.445	59.2 ± 10.9	61.2 ± 11.7	66.7 ± 10.8*#	0.036
RVESVI, mL/m^2	28.0 ± 7.0	32.0 ± 7.6	0.005	28.9 ± 6.8	31.3 ± 6.5	37.8 ± 8.0*	<0.001
RVEF, %	53.7 ± 4.5	48.5 ± 5.8	<0.001	51.3 ± 5.6	48.9 ± 4.1	43.5 ± 5.8*#	<0.001

*Data were n (%), mean ± SD or median (IQR). *P < 0.05, vs. general group; #P <0.05, vs. severe groups. DBP, diastolic blood pressure; RVFAC, right ventricular fractional area change; IVST, interventricular septum thickness; LA, left atrial diameter; LAVI, left atrial volume index; LV, left ventricular anteroposterior diameter; LVEDVI, left ventricular end-diastolic volume index; LVESVI, left ventricular end-systolic volume index; EF, ejection fraction; PA, pulmonary artery diameter; PASP, pulmonary artery systolic pressure; PH, pulmonary hypertension; RA, right atrial diameter; RV, right ventricular diameter; RVEDVI, right ventricular end-diastolic volume index; RVESVI, right ventricular end-systolic volume index; RVFWLS, right ventricular free wall longitudinal strain; S', pulsed doppler peak velocity at the tricuspid lateral annulus; SBP, systolic blood pressure; STE, two-dimensional speckle tracking echocardiography; TAPSE, tricuspid annular plane systolic excursion; 2D, two-dimensional; 3DE, three-dimensional echocardiography.*

and controls persisted for the IVST, tricuspid E/A, LVESVI, LVEF, RVFAC, 2D-RVFWLS, and 3D-RVEF. Likewise, sex and BSA were significantly different among the general, severe and critical groups. So, the echocardiographic parameters among the three groups were further compared after statistical adjustment of sex and BSA in **Table 3**. Larger right heart chambers, worse RVFAC, 2D-RVFWLS, and 3D-RVEF remained statistically significant in critical patients than general and severe patients (**Table 3**).

During a median follow-up of 91 days (IQR: 74–93 days), 18 (14.1%) patients died. Non-survivors were more often male. They had lower oxygenation index than the survivors. The prevalence of comorbidities was similar between the two groups. Compared with non-survivors, survivors presented with more abnormal laboratory findings including lower lymphocyte, higher inflammation-related indices (white blood cell counts, C-reactive protein, procalcitonin, D-dimer) and elevated cardiac indices. There were no differences between the survivors and

TABLE 3 | Adjusted comparisons of echocardiographic characteristics in healthy controls and COVID-19 patients.

				COVID-19 patients			
Variables	**Control ($n = 31$)**	**All patients ($n = 128$)**	***P*-value**	**General ($n = 41$)**	**Severe ($n = 58$)**	**Critical ($n = 29$)**	***P*-value**
Left chamber							
LA, mm	34.1 (32.2, 35.9)	34.2(33.4, 35.0)	0.890	33.6 (32.1, 35.0)	34.4 (33.2, 35.6)	35.2 (33.4, 36.9)	0.378
LV, mm	46.4 (44.7, 48.1)	45.9 (45.2, 46.7)	0.651	45.0 (43.7, 46.3)	46.6 (45.5, 47.6)	45.3 (43.8, 46.8)	0.122
IVST, mm	9.0 (8.6, 9.5)	9.6 (9.4, 9.8)	0.036	9.7 (9.3, 10.1)	9.7 (9.4, 10.1)	9.3 (8.9, 9.8)	0.319
Mitral valve							
E/A	1.0 (0.9, 1.1)	1.0 (0.9, 1.0)	0.785	0.8 (0.7, 1.0)	1.0 (0.9, 1.1)	0.9 (0.8, 1.1)	0.199
E/e'	9.0 (7.8, 10.2)	8.7 (8.2, 9.2)	0.623	7.7 (6.8, 8.6)	9.2 (8.5, 10.0)*	10.0 (8.8, 11.1)*	0.007
LAVI, mL/m^2	30.8 (26.5, 35.1)	34.6 (32.7, 36.6)	0.128	33.0 (29.5, 36.5)	35.6 (32.8, 38.4)	32.7 (28.6, 36.9)	0.396
LVEDVI, mL/m^2	49.3 (42.9, 55.8)	55.2 (52.3, 58.1)	0.124	51.3 (46.3, 56.3)	57.1 (53.0, 61.2)	53.4 (47.3, 59.5)	0.193
LVESVI, mL/m^2	15.1 (12.3, 18.0)	20.4 (19.2, 21.7)	0.002	18.3 (16.0, 20.6)	21.5 (19.6, 23.4)	19.4 (16.7, 22.2)	0.097
LVEF, %	68.6 (66.1, 71.1)	63.3 (62.2, 64.4)	<0.001	64.4 (62.3, 66.4)	62.6 (60.9, 64.3)	63.8 (61.3, 66.3)	0.386
Right chamber							
RA, mm	36.1 (34.4, 37.9)	35.3 (34.5, 36.1)	0.430	34.8 (33.6, 36.0)	34.4 (33.3, 35.4)	37.6 (36.1, 39.1)*#	0.003
RV, mm	32.5(30.9, 34.1)	34.1 (33.4, 34.8)	0.081	33.5 (32.4, 34.7)	33.4 (32.4, 34.4)	35.6 (34.2, 37.1)#	0.031
PA, mm	23.6 (22.5, 24.8)	23.3 (22.8, 23.8)	0.618	22.1 (21.3, 22.9)	23.6 (22.9, 24.2)*	24.8 (23.8, 25.8)*	<0.001
Tricuspid valve							
E/A	1.2 (1.1, 1.4)	1.0 (1.0, 1.1)	0.011	1.0 (0.9, 1.1)	1.0 (1.0, 1.1)	0.9 (0.8, 1.1)	0.371
E/e'	5.2 (4.4, 6.0)	5.2 (4.8, 5.6)	0.906	5.3 (4.6, 5.9)	4.9 (4.4, 5.4)	5.7 (5.0, 6.4)	0.199
TAPSE, mm	23.6 (22.1, 25.1)	23.0 (22.3, 23.6)	0.467	23.0 (21.8, 24.2)	23.1 (22.1,24.1)	22.1 (20.7, 23.6)	0.544
S′, cm/s	13.4 (12.2, 14.5)	13.9 (13.4, 14.4)	0.438	13.1 (12.2, 14.0)	14.2 (13.5,15.0)	15.1 (14.0, 16.2)*	0.019
RVFAC, %	50.1 (47.9, 52.3)	46.7 (45.7, 47.7)	0.010	48.2 (46.5, 49.9)	46.9 (45.5,48.3)	43.0 (40.9, 45.0)*#	0.001
PASP, mmHg	/	33.3 (30.1, 36.4)	/	27.0 (22.3, 31.8)	31.1 (27.2,35.0)	43.6 (38.5, 48.7)*#	<0.001
PH, n (%)	/	18 (14.1)	/	1 (2.4)	4 (6.9)	13 (44.8)*#	<0.001
2D-STE parameter							
RVFWLS, %	−26.0 (−24.0, −28.0)	−23.2 (−22.4, −24.1)	0.021	−23.9 (−22.5, −25.2)	−24.2 (−23.1, −25.4)	−19.1 (−17.4, −20.8)*#	<0.001
3DE parameters							
RVEDVI, mL/m^2	57.6 (53.7, 62.5)	62.5 (60.4, 64.6)	0.092	59.6 (56.1, 63.0)	60.7 (57.9, 63.6)	67.1 (62.9, 71.3)*#	0.019
RVESVI, mL/m^2	27.1 (23.9,30.2)	32.2 (30.9, 33.6)	0.006	29.2 (27.1, 31.3)	31.0 (29.3, 32.8)	37.9 (35.3, 40.5)*#	<0.001
RVEF, %	52.7 (50.3, 55.0)	48.7 (47.7, 49.7)	0.004	51.2 (49.7, 52.8)	48.9 (47.6, 50.2)	43.6 (41.7, 45.5)*#	<0.001

*Data were mean (95% CI). *P < 0.05, vs. general group; #P < 0.05, vs. severe group. FAC, right ventricular fractional area change; IVST, interventricular septum thickness; LA, left atrial diameter; LAVI, left atrial volume index; LV, left ventricular anteroposterior diameter; LVEDVI, left ventricular end-diastolic volume index; LVESVI, left ventricular end-systolic volume index; EF, ejection fraction; PA, pulmonary artery diameter; PASP, pulmonary artery systolic pressure; PH, pulmonary hypertension; RA, right atrial diameter; RV, right ventricular diameter; RVEDVI, right ventricular end-diastolic volume index; RVESVI, right ventricular end-systolic volume index; RVFWLS, right ventricular free wall longitudinal strain; S′, pulsed doppler peak velocity at the tricuspid lateral annulus; STE, speckle tracking echocardiography; TAPSE, tricuspid annular plane systolic excursion; 2D, two-dimensional; 3DE, three-dimensional echocardiography. Comparison of COVID-19 patients and controls adjusted for age, heart rate and systolic blood pressure. Comparison of COVID-19 patients with different severity of illness adjusted for sex and body surface area.*

non-survivors in left heart chamber size and LV function parameters. However, the non-survivors showed larger RA, RV and PA diameters, lower tricuspid E/A, RVFAC, 2D-RVFWLS and 3D-RVEF than survivors. Moreover, a higher proportion of non-survivors presented PH than survivors (**Table 4**).

Prediction of the Death

Conventional RV function parameters including RVFAC, TAPSE and S′, 2D-RVFWLS and 3D-RVEF were analyzed by ROC for predicting mortality in COVID-19 patients. The ROC analyses showed only RVFAC, 2D-RVFWLS, and 3D-RVEF were associated with mortality (**Figure 2**). Moreover, the AUC of 3D-RVEF was greater than that of RVFAC (0.93 vs. 0.79, $P = 0.039$) and RVFWLS (0.93 vs. 0.83, $P = 0.032$). The best cutoff value to predict mortality was 42.7% for RVFAC (AUC, 0.79, $P < 0.001$; sensitivity, 72%; specificity, 78%), −18.9% for 2D-FWLS (AUC, 0.83, $P < 0.001$; sensitivity, 72%; specificity, 85%), and 42.5% for 3D-RVEF (AUC, 0.93, $P < 0.001$; sensitivity, 83%; specificity, 96%).

Kaplan–Meier survival curves showed lower survival rates for the groups with decreased RVFAC (≤42.7%), 2D-RVFWLS (> −18.9%), and 3D-RVEF (≤42.5%) that was classified by cutoff values of the above RV functional parameters (**Figures 3A–C**). In addition, decreased RVFAC, 2D-RVFWLS, and 3D-RVEF occurred in 37 (28.9%) patients, 29 (22.7%) patients and 19 (14.8%) patients, respectively. The incidence rate of mortality in these patients was significantly higher than in patients whose RVFAC (>42.7%), 2D-RVFWLS (≤−18.9%), and 3D-RVEF

TABLE 4 | Clinical and echocardiographic characteristics in COVID-19 survivors and non-survivors.

Variables	Survivors ($n = 110$)	Non-survivors ($n = 18$)	*P*-value
Clinical characteristics			
Age (years)	61 ± 13	66 ± 12	0.106
Male, *n* (%)	48 (43.6)	13 (72.2)	0.024
Heart rate, beats/min	86 (80, 99)	90 (79, 114)	0.541
Respiratory rate, times/min	22 (20, 30)	30 (22, 36)	0.009
SBP, mmHg	130 (120, 140)	131 (119, 151)	0.790
DBP, mmHg	80 (73, 89)	79 (72, 81)	0.296
OI, mmHg	300.0 (217.4, 340.0)	195.1 (160.6, 240.2)	<0.001
Comorbidities			
Hypertension, *n* (%)	42 (38.2)	10 (55.6)	0.164
Diabetes, *n* (%)	16 (14.5)	2 (11.1)	1.000
Cardiac disease, *n* (%)	13 (11.8)	5 (27.8)	0.134
COPD, *n* (%)	5 (4.5)	2 (11.1)	0.255
Chronic liver diseases, *n* (%)	4 (3.6)	0 (0.0)	1.000
Chronic kidney disease, *n* (%)	1 (0.9)	0 (0.0)	1.000
Malignancy, *n* (%)	7 (6.4)	2 (11.1)	0.613
Smoker, *n* (%)	6 (5.5)	1 (5.6)	1.000
Laboratory findings			
White blood cell × 10^9/L	6.2 (4.8, 8.9)	10.1 (7.2, 11.2)	0.001
Lymphocyte count × 10^9/L	1.07 (0.70, 1.47)	0.45 (0.25, 0.69)	<0.001
CRP, mg/L	20.9 (2.9, 53.1)	79.1 (49.0, 129.9)	<0.001
PCT, ng/ml	0.07 (0.04, 0.16)	0.22 (0.09, 0.44)	0.001
D-dimer, mg/L	1.4 (0.5, 5.6)	2.2 (0.9, 8.0)	0.067
hs-TNI, ng/mL	3.3 (1.6, 8.7)	40.2 (17.4, 464.2)	<0.001
CK-MB, U/L	11.0 (8.0, 18.0)	21.0 (11.8, 35.3)	0.005
BNP, pg/ml	35.0 (10.0, 107.2)	207.4 (110.4, 525.2)	<0.001
Serum creatinine (μmol/L)	63.8 (53.5, 79.9)	72.7 (52.9, 87.0)	0.196
Echocardiographic characteristics			
Left chamber			
LA, mm	34.2 ± 4.5	35.1 ± 5.9	0.536
LV, mm	45.9 ± 4.3	45.0 ± 4.0	0.399
IVST, mm	9.7 ± 1.2	9.5 ± 1.1	0.592
Mitral valve			
E/A	0.9 ± 0.3	1.0 ± 0.4	0.663
E/e'	8.9 ± 3.0	9.6 ± 2.8	0.198
LAVI, mL/m^2	34.3 ± 10.2	33.5 ± 11.7	0.696
LVEDVI, mL/m^2	54.9 ± 16.4	51.6 ± 11.9	0.500
LVESVI, mL/m^2	20.3 ± 7.4	18.3 ± 6.2	0.349
LVEF, %	63.1 ± 6.1	64.9 ± 6.8	0.280
Right chamber			
RA, mm	34.8 ± 3.6	38.2 ± 6.3	0.039
RV, mm	33.6 ± 3.7	36.3 ± 4.8	0.022
PA, mm	23.1 ± 2.6	25.0 ± 2.9	0.010
Tricuspid valve			
E/A	1.0 ± 0.3	0.9 ± 0.3	0.039
E/e'	5.2 ± 1.8	5.5 ± 1.5	0.173
TAPSE, mm	22.9 ± 3.8	22.3 ± 3.8	0.534
S', cm/s	13.9 ± 2.6	15.1 ± 4.3	0.394
RVFAC, %	47.2 ± 5.2	41.6 ± 5.3	<0.001
PASP, mmHg	30.3 ± 9.6	45.7 ± 16.7	0.003
PH, *n* (%)	9 (8.2)	9 (50.0)	<0.001
RVFWLS, %	−23.7 ± 4.6	−18.3 ± 3.5	<0.001
RVEDVI, mL/m^2	61.1 ± 11.3	66.4 ± 12.2	0.070
RVESVI, mL/m^2	30.7 ± 6.6	39.8 ± 8.9	<0.001
RVEF, %	49.8 ± 4.8	40.4 ± 4.7	<0.001

Data were mean ± SD, or n (%). BNP, B-type natriuretic peptide; CK-MB, creatine kinase muscle-brain; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; DBP, diastolic blood pressure; FAC, right ventricular fractional area change; hs-TNI, high-sensitivity troponin I; IVST, interventricular septum thickness; LA, left atrial diameter; LAVI, left atrial volume index; LV, left ventricular anteroposterior diameter; LVEDVI, left ventricular end-diastolic volume index; LVESVI, left ventricular end-systolic volume index; EF, ejection fraction; PA, pulmonary artery diameter; OI, oxygenation index; PASP, pulmonary artery systolic pressure; PCT, procalcitonin; PH, pulmonary hypertension; RA, right atrial diameter; RV, right ventricular diameter; RVEDVI, right ventricular end-diastolic volume index; RVESVI, right ventricular end-systolic volume index; RVFWLS, right ventricular free wall longitudinal strain; S', pulsed doppler peak velocity at the tricuspid lateral annulus; SBP, systolic blood pressure; STE, speckle tracking echocardiography; TAPSE, tricuspid annular plane systolic excursion; 2D, two-dimensional; 3DE, three-dimensional echocardiography.

(>42.5%) were not decreased (**Figures 3D–F**; $P < 0.001$ for all). In addition, we further divided the COVID-19 patients into three subgroups: 3DRVEF > 45% ($n = 107$), 40% < 3DRVEF ≤ 45% ($n = 15$), 30% < 3DRVEF ≤ 40% ($n = 6$). The Kaplan–Meier survival curves showed that the three groups had significantly different survival rates ($P < 0.001$), with the group of 30% < 3DRVEF ≤ 40% having the lowest survival rate (**Supplementary Figure 1**).

In univariate analysis (**Table 5**), sex, acute cardiac injury, ARDS, RVFAC, 2D-RVFWLS, and 3D-RVEF were significantly associated with higher mortality in COVID-19 patients. In stepwise multivariate analysis, acute cardiac injury and ARDS were used to construct the baseline model for predicting death in COVID-19 patients. Separated models using RVFAC, 2D-RVFWLS, and 3D-RVEF were found to have significant additional prognostic value for mortality over the baseline model (**Table 4**, **Figure 4**). Notably, the incremental predictive value of 3D-RVEF (chi-square to improve 18.3; $P < 0.001$) was significantly higher ($P < 0.05$) than RVFAC (chi-square to improve 4.5; $P = 0.034$) and 2D-RVFWLS (chi-square to improve 5.1; $P = 0.024$).

Variability of 2D-STE and 3DE Measurements

The intraobserver and interobserver variability for RVFWLS were 0.3 ± 4.3% and 0.6 ± 5.8%, 3D-RVEF were 0.3 ± 3.1% and 0.5 ± 3.9%. The intraobserver and interobserver ICC for RVFWLS were 0.95 and 0.90, 3D-RVEF were 0.95 and 0.91.

DISCUSSION

To our knowledge, this is the first study to comprehensively depict the conventional, 2D strain and 3DE characteristics of RV in COVID-19 patients with different severity of illness and to explore the prognostic value of 3D-RVEF in COVID-19 patients by directly comparing its utility with that derived from conventional echocardiography and 2D-STE. The major findings were as follows: (1) critical COVID-19 patients were more prone to have larger right heart chamber size, more impaired RV function and a higher prevalence of PH; (2) RVFAC, 2D-RVFWLS, and 3D-RVEF were all significant predictors for mortality in COVID-19 patients; and (3) 3D-RVEF could provide incremental value over 2D-RVFWLS and conventional echocardiographic parameters for predicting mortality in COVID-19 patients.

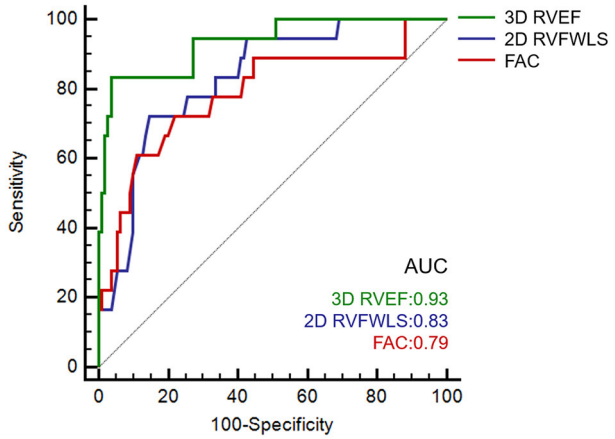

FIGURE 2 | Receiver operating characteristic curves in predicting the death of COVIID-19 patients. COVID-19, coronavirus disease 2019.

RV Size and Function in COVID-19 Patients

Accumulating studies revealed that acute cardiac injury was a common complication and was associated with fatal outcomes in COVID-19 patients (1, 2, 5). We found 27 (21.1%) patients in this cohort had acute cardiac injury as determined by plasma hs-TNI levels. The increased cardiac stress due to respiratory failure and hypoxemia may contribute to cardiac injury and the RV may bear the brunt of its impact (3, 25). Therefore, assessment of RV structure and function could be imperative and significant for COVID-19 patients. There are certain limitations for the assessment of RV size and function by 2D echocardiography due to its complex geometrical anatomy. 3D analysis has the advantage of full-volume acquisition of the entire RV, which may overcome the limitations of 2D analysis. In this study, we assessed RV size and function by the novel,

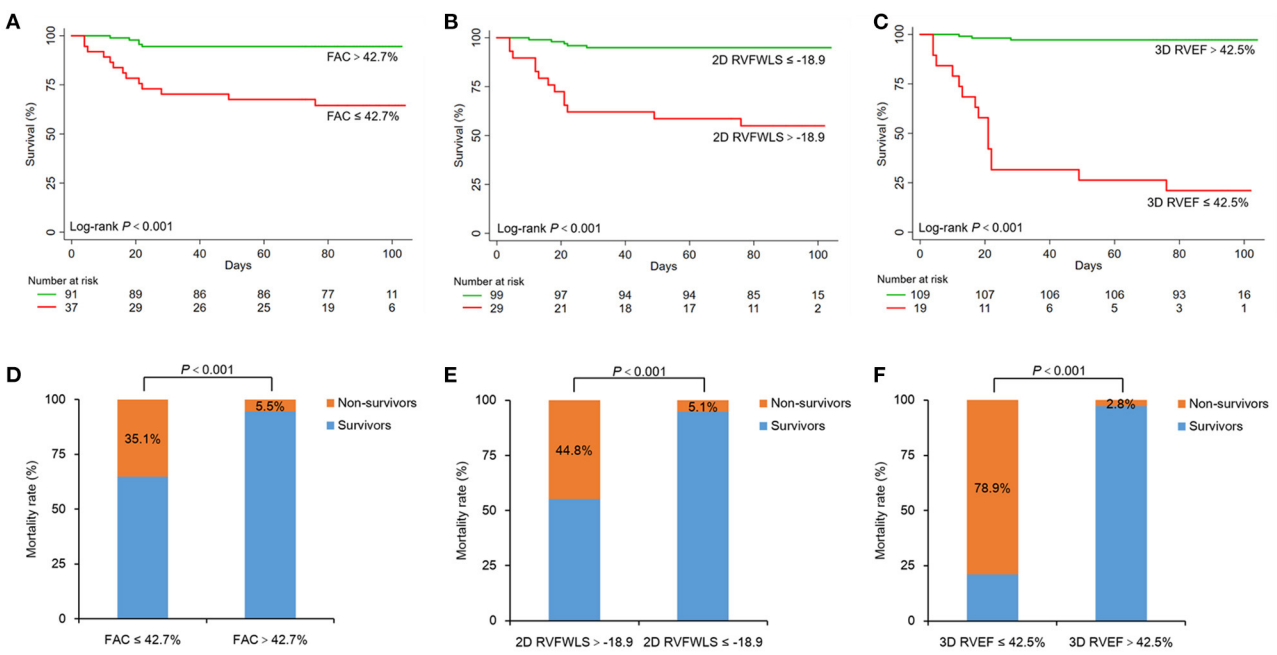

FIGURE 3 | Kaplan–Meier survival curves and percent bar graph of mortality rate in COVID-19 patients. Top, cumulative percentage survival free from death according to FAC **(A)**, 2D-RVFWLS **(B)**, and 3D-RVEF **(C)**. Bottom, mortality rates of different groups dividing by the cut-off value of FAC **(D)**, 2D-RVFWLS **(E)**, and 3D-RVEF **(F)**. COVID-19, coronavirus disease 2019; 2D-RVFWLS, two-dimensional right ventricular free wall longitudinal strain; 3D-RVEF, three-dimensional right ventricular ejection fraction; FAC, fractional area change.

TABLE 5 | Univariate and multivariate COX proportional hazard models for predicting death of COVID-19 patients.

	Univariate analysis		Multivariate analysis							
			Baseline model 1		Model 2 with RVFAC		Model 3 with RVFWLS		Model 4 with 3DRVEF	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age > 65 years	1.862 (0.735, 4.719)	0.190								
Male	3.164 (1.128, 8.877)	0.029								
Hypertension	1.883 (0.743, 4.771)	0.182								
Diabetes mellitus	0.722 (0.166, 3.142	0.665								
Cardiac disease	2.578 (0.919, 7.234)	0.072								
COPD	2.573 (0.591, 11.199)	0.208								
Malignancy	1.789 (0.411, 7.786)	0.438								
D-dimer, mg/L	1.106 (0.961, 1.272)	0.159								
Acute cardiac injury	7.119 (2.756, 18.387)	< 0.001	5.410 (2.084, 14.047)	0.001	3.981 (1.472, 10.765)	0.006	3.209 (1.129, 9.120)	0.029	3.223 (1.230, 8.446)	0.017
ARDS	33.437 (4.446, 251.447)	< 0.001	28.102 (3.721, 212.250)	0.001	17.994 (2.302, 140.660)	0.006	17.550 (2.229, 138.179)	0.007	9.404 (1.119, 79.064)	0.039
LVEF, %*	1.045 (0.964, 1.133)	0.288								
TAPSE, mm*	0.959 (0.849, 1.083)	0.498								
S', cm/s*	1.130 (0.973, 1.313)	0.108								
PH	7.564 (2.990, 19.136)	< 0.001								
RVFAC, %*	0.794 (0.710, 0.889)	< 0.001			0.874 (0.768, 0.996)	0.043				
RVFWLS, %*	1.401 (1.202, 1.633)	< 0.001					1.180 (1.008, 1.381)	0.039		
RVEF, %*	0.761 (0.705, 0.822)	< 0.001							0.809 (0.735, 0.889)	< 0.001

**Per 1 unit increase. ARDS, acute respiratory distress syndrome; CI, confidence interval; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; LVEF, left ventricular ejection fraction; HR, hazard ratio; PH, pulmonary hypertension; TAPSE, tricuspid annular plane systolic excursion; RVFAC, right ventricular fractional area change; RVFWLS, right ventricular free wall longitudinal strain; RVEF, right ventricular ejection fraction.*

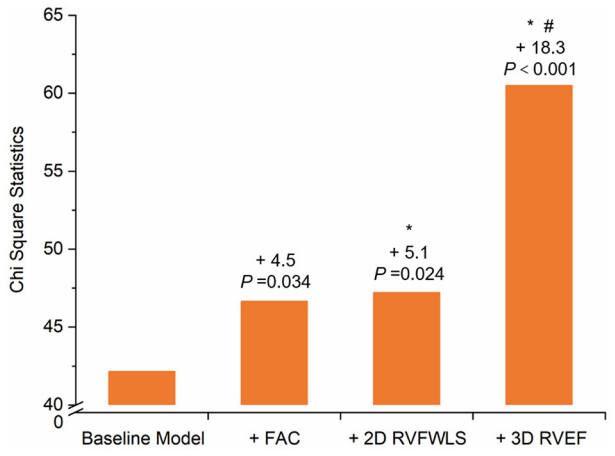

FIGURE 4 | Chi-Square Statistic of Models. Chi-square statistic of the baseline model and different RV functional tests value to predict mortality over the baseline model. Values indicate the additional chi-square value of the different models. $^{*}P < 0.05$ vs. FAC and $^{\#}P < 0.001$ vs. 2D-RVFWLS. Statistical comparisons by likelihood ratio tests. 2D-RVFWLS, two-dimensional right ventricular free wall longitudinal strain; 3D-RVEF, three-dimensional right ventricular ejection fraction; FAC, fractional area change; RV, right ventricular.

fully automated 3D RV quantification software based on new machine learning algorithm, which provided reasonably accurate RV function measurements are available for clinical use with excellent reproducibility and reliability, as well as less analysis time (10).

Our study showed that COVID-19 patients and the controls had similar size of right heart chambers, which was consistent with a previous study (9). We further depicted the right heart chamber size in COVID-19 patients with different severity of illness and found that the critical groups had larger right heart chambers than general and severe groups. Worse RVFAC, RVFWLS, and 3D-RVEF were also noted in COVID-19 patients than in controls. Moreover, decreased RV systolic function was more marked in critical patients and less pronounced in general and severe groups. A previous study has pointed out that severe COVID-19 patients might progress to ARDS more quickly (26). ARDS might cause a rise in RV afterload by increased vascular resistance and hypoxemia (3). The proportion of ARDS in critical groups was significantly higher than general and severe groups in our study, which may explain why critical groups were more likely to had the larger right heart chambers and RV dysfunction. It is suggested that clinicians should be alert to RV dysfunction in critically ill patients and take prompt treatments to improve patient outcomes.

Prognosis of RV Function in COVID-19 Patients

Previously, the prognostic value of RVFWLS and conventional RV function parameters in COVID-19 patients have been reported (9). 3D-RVEF also has been demonstrated as a strong prognostic value in other various cardiovascular diseases (11, 12, 27, 28), while its prognostic value in COVID-19 patients has not been validated yet. In our study, univariate and multivariate regression models revealed 3D-RVEF, RVFWLS, and FAC all were independent predictors for mortality after adjustment for gender, ARDS, and acute cardiac injury. The S′ and TAPSE were not predictors of mortality in our patients, possibly because they are angle-dependent and only reflect the longitudinal function of the basal portion of the RV free wall. RVFAC [cut-off value of 39% by Houard et al. (29) 40% by Amano et al. (30)], RVFWLS [cut-off value of −19% by Houard et al. (29) 22% by Gavazzoni et al. (31)] and 3D-RVEF [cut-off value of 43% by Jone et al. (28)] have been proven to be independent predictors of adverse outcomes in other various cardiovascular diseases. Moreover, a recent study suggested that a 43.5% threshold of RVFAC could help identify COVID-19 patients at higher risks of mortality (9). The prognostic value of RVFAC, RVFWLS, and 3D-RVEF to predict mortality was also noted in our study, with the best cut-off value of 42.7% for RVFAC, −18.9% for RVFWLS, and 42.5% for 3D-RVEF. More notably, we found the multivariate regression model with 3D-RVEF showed an incremental prognostic value of higher mortality over that with RVFWLS and FAC, which was in line with the previous study that reported 3D-RVEF was superior to RVFWLS and conventional echocardiographic parameters in predicting adverse clinical events in PH (28). Additionally, the COVID-19 patients were divided into three subgroups based on the published reference: (16) 3DRVEF > 45%, 40% < 3DRVEF ≤ 45%, and 30% < 3DRVEF ≤ 40%. The Kaplan–Meier survival curves showed that the three groups had significantly different prognosis ($P < 0.001$), with the group of 30% < 3DRVEF ≤ 40% having the lowest survival rate. RVFAC was measured by planimetry of the RV cavity and its measurement variability was limited by the accurate identification of the RV endocardial border. RVFWLS is mainly based on longitudinal myocardium deformation of RV outflow portions, neglecting the contributions of myocardium deformation in other directions (32). The study by Bleakley et al. reported that RVFWLS was not sensitive in identifying RV dysfunction, because severe COVID-19 is associated with a specific phenotype of RV radial impairment with sparing of longitudinal function (33). However, 3D-RVEF can comprehensively evaluate the different parts of the RV (including the inflow, apical, and outflow) and is not limited to longitudinal myocardial function (34, 35). Our study demonstrated that 3D-RVEF as a more robust prognostic indicator for mortality and could provide incremental prognostic value over RVFWLS and conventional echocardiography in COVID-19 patients.

Clinical Implications

Our findings emphasized that the significance of evaluating RV function and validated its predictive value in COVID-19 patients. Critical COVID-19 patients were more likely to suffer from RV dysfunction. This study offered the first evidence about the prognostic value of RVEF measured by 3DE in COVID-19 patients. 3D-RVEF is theoretically superior to conventional echocardiographic parameters and RVFWLS derived from 2D-STE in assessing RV function due to the complex anatomy of RV. Therefore, we demonstrated that 3D-RVEF could provide an incremental predictive value of death over the RVFWLS and conventional echocardiographic parameters in COVID-19

patients, which may help identify COVID-19 patients at higher risks of adverse outcomes.

Limitation

Our study did have some limitations. First, as both 3DE and 2D-STE analyses were dependent on good image quality, we excluded 38 (22.1%) patients with insufficient image quality or arrhythmia during examination, which may cause some selection bias. As a result, our findings were not applicable to COVID-19 patients with arrhythmia or unsatisfactory image quality. Moreover, part of subjects (78/128) in our study were included in the previous work (9), which was focus on the prognostic value of RV free wall longitudinal strain (RVFWLS) in COVID-19 patients. Second, this was a single-center study with a relatively small sample of hospitalized COVID-19 patients at different disease status, further studies with multi-center and larger sample size should be performed to validate our findings. Third, the cutoff values reported in this study may not be applicable to other software due to inter-vendor variability. Finally, the current fully automated 3D RV software does not provide 3D RV strain values yet, and hence the evidence of the prognostic value of 3D RV strain in COVID-19 patients was lacking in our study. Future studies should be performed to determine the prognostic superiority of 3D RV strain.

Conclusions

Our study emphasized that 3D-RVEF was an independent predictor of mortality in COVID-19 patients and provided an incremental prognostic value superior to RVFWLS and conventional echocardiographic parameters.

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 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. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.641088/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. Akhmerov A, Marban E. COVID-19 and the heart. *Circ Res.* (2020) 126:1443–55. doi: 10.1161/CIRCRESAHA.120.317055
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. *The Lancet*. (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
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. 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
7. Li Y, Li H, Li M, Zhang L, Xie M. The prevalence, risk factors and outcome of cardiac dysfunction in hospitalized patients with COVID-19. *Intensive Care Med.* (2020) 46:2096–8. doi: 10.1007/s00134-020-06205-0
8. Zhang L, Wang B, Zhou J, Kirkpatrick J, Xie M, Johri A M. 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 Echocardiogr.* (2020) 33:676–82. doi: 10.1016/j.echo.2020.04.004
9. 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
10. Muraru D, Spadotto V, Cecchetto A, Romeo G, Aruta P, Ermacora D, et al. New speckle-tracking algorithm for right ventricular volume analysis from three-dimensional echocardiographic data sets: validation with cardiac magnetic resonance and comparison with the previous analysis tool. *Eur Heart J Cardiovasc Imaging.* (2016) 17:1279–89. doi: 10.1093/ehjci/jev309
11. Moceri P, Duchateau N, Baudouy D, Schouver ED, Leroy S, Squara F, et al. Three-dimensional right-ventricular regional deformation and survival in pulmonary hypertension. *Eur Heart J Cardiovasc Imaging.* (2018) 19:450–8. doi: 10.1093/ehjci/jex163

12. Surkova E, Muraru D, Genovese D, Aruta P, Palermo C, Badano LP. Relative prognostic importance of left and right ventricular ejection fraction in patients with cardiac diseases. *J Am Soc Echocardiogr*. (2019) 32:1407–15.e3. doi: 10.1016/j.echo.2019.06.009
13. World Health Organization. *Clinical Management of Severe Acute Respiratory Infection When Novel Coronavirus (nCoV) Infection Is Suspected: Interim Guidance*. World Health Organization. (2020). Available online at: https://apps.who.int/iris/handle/10665/330854 (accessed January 25, 2020)
14. *Guideline for the Diagnosis and Treatment of 2019 Novel Coronavirus (2019-nCoV) in-fected Pneumonia. (2020).* Available online at: http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989/files/ce3e6945832a438eaae415350a8ce964.pdf
15. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, et al. Acute respiratory distress syndrome: the Berlin definition. *JAMA*. (2012) 307:2526–33. doi: 10.1001/jama.2012.5669
16. Muraru D, Badano LP, Nagata Y, Surkova E, Nabeshima Y, Genovese D, et al. Development and prognostic validation of partition values to grade right ventricular dysfunction severity using 3D echocardiography. *Eur Heart J Cardiovasc Imaging*. (2020) 21:10–21. doi: 10.1093/ehjci/jez233
17. Mitchell C, Rahko PS, Blauwet LA, Canaday B, Finstuen JA, Foster MC, et al. Guidelines for performing a comprehensive transthoracic echocardiographic examination in adults: recommendations from the American society of echocardiography. *J Am Soc Echocardiogr*. (2019) 32:1–64. doi: 10.1016/j.echo.2018.06.004
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. *Eur Heart J Cardiovasc Imaging*. (2015) 16:233–70. doi: 10.1093/ehjci/jev014
19. 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) 23:685–788. doi: 10.1016/j.echo.2010.05.010
20. Badano LP, Kolias TJ, Muraru D, Abraham TP, Aurigemma G, Edvardsen T, et al. Standardization of left atrial, right ventricular, and right atrial deformation imaging using two-dimensional speckle tracking echocardiography: a consensus document of the EACVI/ASE/industry task force to standardize deformation imaging. *Eur Heart J Cardiovasc Imaging*. (2018) 19:591–600. doi: 10.1093/ehjci/jey042
21. Badano LP, Muraru D, Parati G, Haugaa K, Voigt JU. How to do right ventricular strain. *Eur Heart J Cardiovasc Imaging*. (2020) 21:825–27. doi: 10.1093/ehjci/jeaa126
22. Genovese D, Rashedi N, Weinert L, Narang A, Addetia K, Patel AR, et al. Machine Learning-based three-dimensional echocardiographic quantification of right ventricular size and function: validation against cardiac magnetic resonance. *J Am Soc Echocardiogr*. (2019) 32:969–77. doi: 10.1016/j.echo.2019.04.001
23. Otani K, Nabeshima Y, Kitano T, Takeuchi M. Accuracy of fully automated right ventricular quantification software with 3D echocardiography: direct comparison with cardiac magnetic resonance and semi-automated quantification software. *Eur Heart J Cardiovasc Imaging*. (2019) 21:787–95. doi: 10.1093/ehjci/jez236
24. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology*. (1983) 148:839–43. doi: 10.1148/radiology.148.3.6878708
25. 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
26. 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
27. Nagata Y, Wu VC, Kado Y, Otani K, Lin FC, Otsuji Y, et al. Prognostic value of right ventricular ejection fraction assessed by transthoracic 3D echocardiography. *Circ Cardiovasc Imaging*. (2017) 10:e005384. doi: 10.1161/CIRCIMAGING.116.005384
28. Jone PN, Schäfer M, Pan Z, Bremen C, Ivy DD. 3D echocardiographic evaluation of right ventricular function and strain: a prognostic study in paediatric pulmonary hypertension. *Eur Heart J Cardiovasc Imaging*. (2018) 19:1026–33. doi: 10.1093/ehjci/jex205
29. Houard L, Benaets M-B, de Meester de Ravenstein C, Rousseau MF, Ahn SA, Amzulescu M-S, et al. Additional prognostic value of 2D right ventricular speckle-tracking strain for prediction of survival in heart failure and reduced ejection fraction. *JACC Cardiovasc Imaging*. (2019) 12:2373–85. doi: 10.1016/j.jcmg.2018.11.028
30. Amano M, Izumi C, Baba M, Abe R, Matsutani H, Inao T, et al. Progression of right ventricular dysfunction and predictors of mortality in patients with idiopathic interstitial pneumonias. *J Cardiol*. (2020) 75:242–9. doi: 10.1016/j.jjcc.2019.08.010
31. Gavazzoni M, Badano LP, Vizzardi E, Raddino R, Genovese D, Taramasso M, et al. Prognostic value of right ventricular free wall longitudinal strain in a large cohort of outpatients with left-side heart disease. *Eur Heart J Cardiovasc Imaging*. (2019) 21:1013–21. doi: 10.1093/ehjci/jez246
32. Cheung YF. The role of 3D wall motion tracking in heart failure. *Nat Rev Cardiol*. (2012) 11:644–57. doi: 10.1038/nrcardio.2012.128
33. Bleakley C, Singh S, Garfield B, Morosin M, Surkova E, Mandalia MS, et al. Right ventricular dysfunction in critically ill COVID-19 ARDS. *Int J Cardiol*. (2020) 23. doi: 10.1016/j.ijcard.2020.11.043
34. Ishizu T, Seo Y, Atsumi A, Tanaka YO, Yamamoto M, Machino-Ohtsuka T, et al. Global and regional right ventricular function assessed by novel three-dimensional speckle-tracking echocardiography. *J Am Soc Echocardiogr*. (2017) 30:1203–13.doi: 10.1016/j.echo.2017.08.007
35. Zaidi A, Knight DS, Augustine DX, Harkness A, Oxborough D, Pearce K, et al. Echocardiographic assessment of the right heart in adults: a practical guideline from the British Society of Echocardiography. *Echo Res Pract*. (2020) 7:G19–41. doi: 10.1530/ERP-19-0051

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POLICY AND PRACTICE REVIEWS
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Overview of Current International Recommendations for Echocardiography Exams During the Covid-19 Pandemic and Its Local Implementation in Austria

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Since its first appearance in December 2019, the novel Coronavirus SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) has spread throughout the world at rapid pace causing the *coronavirus disease 2019* (Covid-19). Originating in the Chinese province Hubei, more than 91.8 million people globally have now been infected with the coronavirus and more than 1.966.000 patients have died thus far from Covid-19 (as of January 13th 2021). The virus spreads primarily by droplet infection as well as via aerosols during close physical contact. Particularly in medical examinations with close physical contact between examiner and patient, like echocardiography, the risk of contracting the virus is increased. Therefore, the use of personal protective equipment is recommended for the protection of patients and medical personnel alike. In this article, the current recommendations of international professional associations on the use of personal protective equipment and their local implementation are presented.

Keywords: COVID-19, echocardiography, protective gear, cardiovascular imaging, SARS–CoV−2

INTRODUCTION

Close physical contact with patients suffering from *coronavirus disease 19* (Covid-19), may result in a significantly increased risk of transmission by droplet infection or via aerosols (1–3). Transthoracic echocardiography (TTE) examinations involve close patient contact over a long period of time (i.e., estimated 15–30 min). In addition to the exam length, transesophageal echocardiography (TEE) examinations in particular can result in aerosol formation. Although hard data on the extent of aerosol formation in TEE are lacking (4), some mathematical models have been proposed to explain virus transmission by aerosols even in patients with mild or asymptomatic Covid-19 (5). Special protection of patients and especially of medical staff is therefore necessary during TEE and TTE examinations. The procedure described here is based on current guidelines of the American Society of Echocardiography (6), the British Society of Echocardiography (7), the Italian Society of Echocardiography (8), the Japanese Society of Echocardiography (9), the Cardiological Society of India (10), collection of experience reports and recommendations of the European Society of Cardiology (ESC) (11, 12), as well as the recommendations of the local crisis management team of the Salzburg State hospitals.

First, the different collectives of patients must be distinguished, defined as patients with proven Covid-19 disease, patients with negative testing for SARS-CoV-2 infection, patients suspected with infection or those in which SARS-CoV-2 infection has not been excluded, as this has a decisive influence on the indication for imaging studies and on respective protective measures.

INDICATIONS

A strict indication is of primary importance within the context of a pandemic. This applies to standard cardiological examinations in cardiac patients infected with Covid-19 as well as in non-cardiac patients with Covid-19 to evaluate possible Covid-19-associated cardiac involvement (13). Only examinations that are clearly necessary for diagnosis and that have a further therapeutic consequence should be performed in patients with suspected or confirmed Covid-19 infection, this is also of utmost importance in TEE studies with increased risk of aerosol generation (10, 14). Instead of stress echocardiography, alternative forms for testing should be considered (9).

IMPLEMENTATION

Prior to performing an echocardiography examination during a pandemic, the patient's infection status should be determined in order to assess examination risk constellation. Depending on the status, appropriate protective equipment should be selected [see Section Use of Personal Protective Equipment (PPEs), **Table 1, Figure 1**]. Testing for SARS-CoV-2 would be desirable, especially before a TEE examination, however, in an outpatient setting this is not always feasible (no test available, or test result pending), therefore use of extensive personal protective equipment is recommended in this situation (see **Table 1**, "Suspected Covid-19 infection") (15). Patients with suspected or confirmed Covid-19 infection should be examined with a mobile echocardiography device if possible to avoid virus spread by transport. This is recommended especially in designated local Covid-19 wards and Covid-19 intensive care units. The examinations should only be performed by experienced personnel to keep examination time to a minimum. Examiners > age 60, pregnant women, persons with chronic conditions (i.e., hypertension, diabetes mellitus, adipositas, COPD, and pulmonary diseases) or immunosuppressed/immunocompromised individuals should avoid contact with patients with suspected Covid-19 and those with confirmed infections. Teaching or device training should not take place when examining patients with Covid-19. Authors also recommend to use limited echocardiography protocols focusing only on the most important cardiac views in order to further reduce scan time (16). During the current pandemic, it is recommend that internet-based training and education (online lectures, webinars, and use of simulators) should replace bedside training (17, 18). In the case of suspected Covid-19 infection and where test results are pending, examinations should be delayed with the exception of urgent cases. If possible, only one examiner should perform the echocardiography on one patient per room; several examiners in the same space should be avoided (11). It is also recommended that the patient should be ideally positioned, lying on their left side facing away from the examiner. A drape covering the patient should be used to reduce physical contact if only standard protective equipment for the examiner is available. Where available, transparent drapes should be used to cover the echocardiography machine (16). The preferred examination position by the individual examiner should be maintained to ensure that the quality of the test is not compromised, as this would potentially result in re-imaging and longer examination time. The advantages and disadvantages of performing an exam must be weighed carefully. If possible, only loop recordings should be captured directly on the device and measurements and findings should be made subsequently on the computer.

Where available, handheld echocardiography devices, such as tablet-based systems, to further reduce scan time and limit the risk of exposure of personnel could be used as alternatives to conventional echocardiography, at least for screening examinations (19, 20).

After use, the device, probes and examination table should be thoroughly cleansed using disinfection towels. TEE probes should be cleaned, disinfected and sterilized according to the manufacturer's instructions. Additionally, the use of virucidal disinfectants for probe reprocessing and the use of a disposable protective covering for the TEE probe are recommended for hygienic reasons [see recommendations proposed by Jain (21)]. As a substitute to intraoperative TEE, epicardial echocardiography using a sterile sleeve has been proposed by Senniappan et al. (22). Also during cleaning procedures, personnel should wear PPE (8).

USE OF PERSONAL PROTECTIVE EQUIPMENT (PPEs)

When performing TTE or TEE in patients with suspected Covid-19 or confirmed infection, comprehensive protective equipment should be used by the examiner, consisting of examination gloves, a protective coat, a FFP-2/3 mask, safety glasses/goggles or face shield, and a surgical cap (17, 23). Local hospital recommendations and SOPs regarding correct use of personal protective equipment should be followed.

Use of comprehensive personal protection equipment (PPE) is also recommended when testing patients without proven SARS-CoV-2 infection but who have not been tested. After thorough hand disinfection, this includes use of examination gloves and a surgical mask; see **Table 1**, **Figure 1**). A new standard requirement is for patients to also wear a facemask in order to reduce patient-physician transmission risk. In addition, regular and thorough hand disinfection is recommended for both the examiner and the patient, as this is an essential element to prevent spread of the disease (24).

Special care with respect to PPEs should also be given during TEE examinations, as higher aerosol formation is to be expected (3). In the case of positive Covid-19 detection, the use of examination gloves, a protective coat, an FFP-3 mask, safety glasses/goggles or face shield and a surgical cap

TABLE 1 | Personal protective equipment for echocardiography in Covid-19.

	Hand disinfection	Gloves	Protective coat	Surgical mask	FFP 2/3 mask	Protective glasses/face shield	Surgical cap
TTE Standard procedure Non-Covid 19 patients	X	X	*	X	FFP 2 mask, if available		
TTE Covid 19 patients General ward, Intensive care unit (suspected and diagnosed infection)	X	X	X		X (FFP-3 in Intensive Care Unit)	X	X
TEE Covid 19 patients General ward, Intensive care unit (suspected and diagnosed infection)	X	X	X		X (FFP-3)	X	X
TEE No suspicion of Covid 19 but test result not available or inconclusive (delay TEE preferable)	X	X	X		X (FFP-2)	X	X
TEE Negative Covid 19 test result (increase protective measures if indicated)	X	X	If needed	X FFP 2 mask, if available	If needed	If needed	If needed

Personal protection recommendations during echocardiographic examinations [adapted from the American Society of Echocardiography, the British Society of Echocardiography, the European Association of Cardiovascular Imaging recommendations and the Japanese Society of Echocardiography (6, 7, 9, 12)].
**Use a protective drape to cover the patient if the examination position is to the right of the patient.*

are recommended. If the Covid-19 test results are inconclusive, a swab PCR test should be taken before TEE examination if possible, with the exception of urgent indications. It is generally preferable to obtain results of Covid-19 tests prior to examination to save personal protective equipment resources. In the case of negative Covid-19 results or in those patients hospitalized for longer period of time without proven infection or symptoms, then standard protective measures, such as face mask and gloves may be applied (see Table and Figure). However, if a patient shows any clinical signs or laboratory signs of florid infection (i.e., fever, elevated CRP, coughing, and loss of taste), extensive personal protective equipment is recommended due to increased aerosol exposure during TEE examinations and possible false negative results. In case of unclear Covid-19 findings and urgent indication for TEE, extensive protective equipment should also be used (**Table 1**).

USE OF PPEs IN A PEDIATRIC SETTING

The American Society of Echocardiography and the Working Group on Congenital Heart Disease of the Italian Society of Cardiology have offered specific Covid-19 examination recommendations for children and infants (25, 26). While children as an entire group appear to be at lower risk of severe infection when compared to adults, certain subgroups of children may be more susceptible to severe disease courses and have the need for frequent examinations by means of echocardiography, e.g., children with congenital heart disease. Even though higher case-fatality rates in patients with cardiovascular diseases were initially assumed, most patients with congenital heart disease experience mild COVID-19 symptoms, though data on children remain scarce (27). Most children who are infected with SARs-CoV-2 have mild symptoms or are asymptomatic, which creates a special challenge to protect healthcare staff from exposure. TTE and TEE should only be performed if they are expected to provide clinical benefit. Given the higher risk of transmission in asymptomatic children, most centers are performing SARs-CoV-2 testing in all new pediatric admissions. If possible, imaging should be performed and images saved by a single experienced staff member and retrieved at a later time for evaluation. Prolonged scanning should be avoided. In infants and children in whom Covid-19 has not been ruled out, infection should be assumed and appropriate PPEs as well as meticulous and frequent handwashing are required. One single caregiver should accompany the child during the exam to facilitate the cooperation of an active child and should be fitted with a mask as well. Protective coverings on devices and disinfection should be done per standard protocols.

In patients with documented negative Covid-19 testing within 72 h arriving for examination risk of infection is low and standard gloves, face mask and eye protection is recommended. In patients with known Covid-19 infection or in which infection with SARS-CoV-2 cannot be ruled out, strict protocols for PPE use must be followed. If possible, staff members with risk

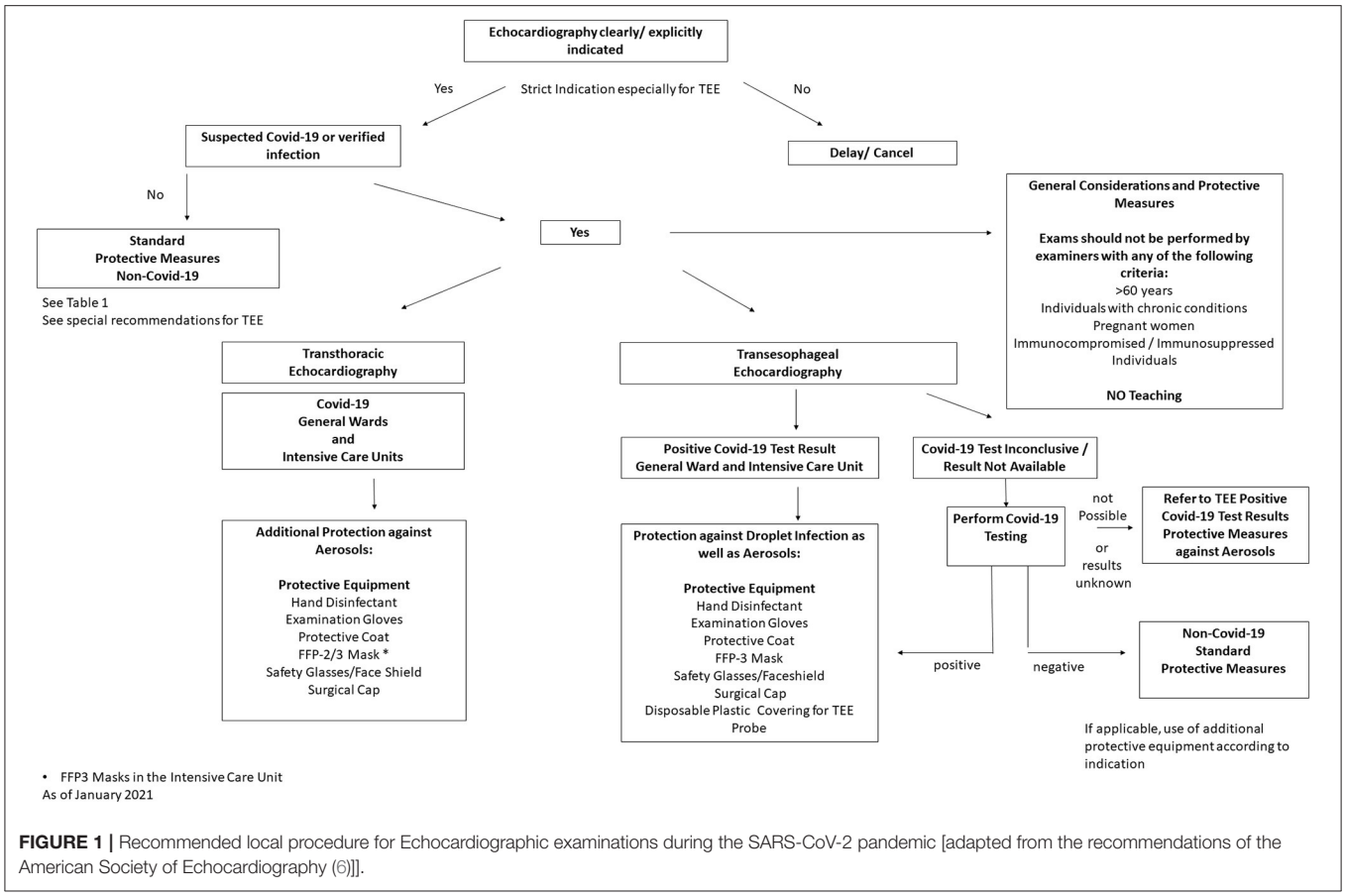

FIGURE 1 | Recommended local procedure for Echocardiographic examinations during the SARS-CoV-2 pandemic [adapted from the recommendations of the American Society of Echocardiography (6)]].

factors (> 60 years, chronic illness, immunocompromised and pregnancy) should not perform echocardiography exams. As a general rule, children below the age of six are exempt of wearing a face mask, whereas wearing a mask in older children should be mandatory during examinations.

CONCLUSION

In summary, in the context of the SARS-CoV-2 pandemic, a concise indication warranting echocardiography examination is essential to minimize transmission risk and limit use of personal protective equipment resources. It is important to emphasize that necessary echocardiographic examinations should not be postponed to the detriment of the cardiac patient collective due to heightened protection requirements during the current pandemic. In the case of proven and not explicitly excluded SARS-CoV-2 infections, personal protective equipment should be used during the examination to protect the medical staff and other patients.

This pandemic represents a very challenging situation for healthcare workers and hospitals. Apart from adapting daily clinical routines to adequately meet patient needs while protecting healthcare workers, also concepts for echocardiography teaching and education require customization for patient and staff protection alike (18). As the status of this worldwide pandemic represents an ever-changing situation and knowledge regarding protective measures is expanding on a monthly basis we sought to provide a state of the art overview on current recommendations and novel concepts for cardiovascular imaging.

AUTHOR CONTRIBUTIONS

ML prepared the article. EP, CG, and BW provided additional informationen. KK revised the manuscript. AD and UH supervised the preparation of the article. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Cook TM. Personal protective equipment during the coronavirus disease (covid) 2019 pandemic - a narrative review. *Anaesthesia.* (2020) 75:920–7. doi: 10.1111/anae.15071
2. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and surface stability of sars-cov-2 as compared with sars-cov-1. *N Engl J Med.* (2020) 382:1564–7. doi: 10.1056/NEJMc2004973
3. Dhama K, Khan S, Tiwari R, Sircar S, Bhat S, Malik YS, et al. Coronavirus disease 2019-covid-19. *Clin Microbiol Rev.* (2020) 33:e00028–20. doi: 10.1128/CMR.00028-20
4. Tran K, Cimon K, Severn M, Pessoa-Silva CL, Conly J. Aerosol generating procedures and risk of transmission of acute respiratory

infections to healthcare workers: a systematic review. *PLoS ONE.* (2012) 7:e35797. doi: 10.1371/journal.pone.0035797
5. Riediker M, Tsai DH. Estimation of viral aerosol emissions from simulated individuals with asymptomatic to moderate coronavirus disease 2019. *JAMA Netw Open.* (2020) 3:e2013807. doi: 10.1001/jamanetworkopen.2020.13807
6. 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
7. British Society of Echocardiography. *Clinical Guidance Regarding Provision of Echocardiography During the Covid-19 Pandemic*. London (2020).
8. Antonini-Canterin F, Pepi M, Monte IP, Trocino G, Barbieri A, Barchitta A, et al. Document addressed to cardiovascular echography operators at the time of covid-19: a document by the "società italiana di ecocardiografia e cardiovascular imaging" board 2019-2021. *J Cardiovasc Echogr.* (2020) 30:2–4. doi: 10.4103/jcecho.jcecho_27_20
9. Seo Y, Daimon M, Yamada H, Kagiyama N, Ohta M, Izumi C, et al. Review of the efforts of the japanese society of echocardiography for coronavirus disease 2019 (covid-19) during the initial outbreak in japan. *J Echocardiogr.* (2020) 18:226–33. doi: 10.1007/s12574-020-00487-5
10. Gupta R, Das MK, Mohanan PP, Deb PK, Parashar SK, Chopra HK, et al. Cardiological society of india document on safety measure during echo evaluation of cardiovascular disease in the time of covid-19. *Indian Heart J.* (2020) 72:145–50. doi: 10.1016/j.ihj.2020.05.016
11. ESC. *Protecting Cardiologists During the Covid-19 Epidemic – Lessons From Wuhan, China*. ESC. (2020).
12. Skulstad H, Cosyns B, Popescu BA, Galderisi M, Salvo GD, Donal E, et al. Covid-19 pandemic and cardiac imaging: eacvi recommendations on precautions, indications, prioritization, and protection for patients and healthcare personnel. *Eur Heart J Cardiovasc Imaging.* (2020) 21:592–8. doi: 10.1093/ehjci/jeaa072
13. 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
14. Cameli M, Pastore MC, Henein M, Aboumarie HS, Mandoli GE, D'Ascenzi F, et al. Safe performance of echocardiography during the covid-19 pandemic: a practical guide. *Rev Cardiovasc Med.* (2020) 21:217–23. doi: 10.31083/j.rcm.2020.02.90
15. Viéitez Flórez JM, Barrios Alonso V, Fernández-Gofín C. The day after tomorrow: echocardiography laboratories after the covid-19 outbreak. *Eur Heart J Cardiovasc Imaging.* (2020) 21:1057. doi: 10.1093/ehjci/jeaa207
16. Goldberg AB, Kyung S, Swearingen S, Rao A. Expecting the unexpected: echo laboratory preparedness in the time of covid-19. *Echocardiography.* (2020) 37:1272–7. doi: 10.1111/echo.14763
17. Augoustides JG. Perioperative echocardiography: key considerations during the coronavirus pandemic. *J Cardiothorac Vasc Anesth.* (2020) 34:1416–8. doi: 10.1053/j.jvca.2020.03.046
18. Madrazo JA. New challenges and opportunities for echocardiographic education during the covid-19 pandemic: a call to focus on competency and pathology. *J Am Soc Echocardiogr.* (2020) 33:1048–9. doi: 10.1016/j.echo.2020.05.011
19. McMahon SR, De Francis G, Schwartz S, Duvall WL, Arora B, Silverman DI. Tablet-based limited echocardiography to reduce sonographer scan and decontamination time during the covid-19 pandemic. *J Am Soc Echocardiogr.* (2020) 33:895–9. doi: 10.1016/j.echo.2020.05.005
20. Jenkins S, Garg P. Prime time for handheld echocardiography in covid-19 pandemic. *Clin Med.* (2020) 20:e132. doi: 10.7861/clinmed.Let.20.4.3
21. Jain A. Preventing contamination during transesophageal echocardiography in the face of the covid-19 pandemic. *J Cardiothorac Vasc Anesth.* (2020) 34:2849–51. doi: 10.1053/j.jvca.2020.04.011
22. Senniappan K, Damodaran S, Kanchi M. Epicardial echocardiography-a plausible alternative cardiac imaging technique in covid-19 pandemic. *J Cardiothorac Vasc Anesth.* (2021) 35:684–6. doi: 10.1053/j.jvca.2020.06.049
23. Sayburn A. Covid-19: phe upgrades ppe advice for all patient contacts with risk of infection. *BMJ.* (2020) 369:m1391. doi: 10.1136/bmj.m1391
24. Pradhan D, Biswasroy P, Kumar Naik P, Ghosh G, Rath G. A review of current interventions for covid-19 prevention. *Arch Med Res.* (2020) 51:363–74. doi: 10.1016/j.arcmed.2020.04.020
25. Barker PCA, Lewin MB, Donofrio MT, Altman CA, Ensing GJ, Arya B, et al. Specific considerations for pediatric, fetal, and congenital heart disease patients and echocardiography service providers during the 2019 novel coronavirus outbreak: council on pediatric and congenital heart disease supplement to the statement of the american society of echocardiography: endorsed by the society of pediatric echocardiography and the fetal heart society. *J Am Soc Echocardiogr.* (2020) 33:658–65. doi: 10.1016/j.echo.2020.04.005
26. Sirico D, Castaldi B, Ciliberti P, Sabatino J, Cazzoli I, Secinaro A, et al. Cardiac imaging in congenital heart disease during the coronavirus disease-2019 pandemic: recommendations from the working group on congenital heart disease of the italian society of cardiology. *J Cardiovasc Med (Hagerstown).* (2020) 21:467–71. doi: 10.2459/JCM.0000000000000990
27. Sabatino J, Ferrero P, Chessa M, Bianco F, Ciliberti P, Secinaro A, et al. Covid-19 and congenital heart disease: results from a nationwide survey. *J Clin Med.* (2020) 9:1774. doi: 10.3390/jcm9061774

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ORIGINAL RESEARCH
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Potential Relationship Between Lifestyle Changes and Incidence of Hospital Admissions for Acute Coronary Syndrome During the COVID-19 Lockdown

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Aims: To evaluate the impact of lockdown during the COVID-19 pandemic on lifestyle changes of the general population, and on admissions for acute coronary syndrome (ACS).

Methods and Results: All ACS admissions during the COVID-19 lockdown (10 March to 4 May, 2020), in 3 municipalities (3 spoke, and 1 hub hospital), in Southwestern Greece (411,576 inhabitants), were prospectively recorded and compared to the equivalent periods during 2018, and 2019. A telephone survey of 1014 participants was conducted to explore the lifestyle habits of citizens aged ≥35-years-old before and during lockdown. The median ACS incidence rate decreased from 19.0 cases per week in 2018 and 21.5 in 2019 down to 13.0 in 2020 (RR: 0.66 during the Covid-19 lockdown; 95%CI: 0.53–0.82; $P = 0.0002$). This was driven by a significant reduction of admissions for Non-ST elevation myocardial infarction (NSTEMI) (RR: 0.68; 95%CI: 0.52–0.88; $P = 0.0037$), mainly in patients with a lower burden of cardiovascular risk factors, as we noticed an inverse association between the reduction of the incidence of ACS during the Covid-19 lockdown period and the number of registered patient risk factors. There was no difference in the rates of STEMI and population-based all-cause mortality across the examined time periods. The telephone survey demonstrated reduction of passive smoking, working hours, alcohol, junk food and salt consumption, and an increase in sleeping hours, mainly in participants with a lower burden of cardiovascular risk factors.

Conclusions: A significant decline in ACS admissions during the COVID-19 lockdown was noted, affecting mainly NSTEMI patients with a lower burden of cardiovascular risk factors. This was accompanied by significant lifestyle changes. Thus, it is tempting to speculate that to some extend the latter might be associated with the observed decline in ACS admissions.

Keywords: ACS, COVID-19, lifestyle—related disease, way of life, stabilization of atherosclerotic plaque

INTRODUCTION

The coronavirus disease (Covid-19) pandemic has become a major cause of mortality worldwide. This has led to the adoption of social distancing measures, or even a complete lockdown policy over various timeframes, which have been ordered by many administrations in Europe, and the USA in an effort to restrict virus transmission. The impact of this extremely unique situation on a population's lifestyle habits has not been studied. During the above lockdown periods a decrease in hospital admissions for acute coronary syndrome (ACS) has been observed (1–6). The prevailing explanatory theory on this observation is that patients may have avoided seeking medical help through fear of the pandemic, thus causing a false decrease in the rate of ACS. However, we cannot exclude a real decrease in ACS incidence due to lifestyle changes associated with the enforced quarantine, especially in countries where the cases of COVID-19, and the resulting number of deaths were kept quite low, without significantly stressing of the health system. Greece is such a country, where strict quarantine and major lockdown measures were instituted at the very beginning of the outbreak.

The aim of the present study was (1) to compare the prospectively recorded rates of hospital admissions for ACS during the lockdown time interval (Covid-19 era, 10 March to 4 May 2020) with those during the same interval in the years 2018 and 2019 (pre-Covid-19 era, over a large network consisting of 1 hub, and 3 spoke hospitals in southwestern Greece (411,576 inhabitants); and, (2) to determine via a telephone interview survey any changes in citizens' basic lifestyle habits (exercise, sleep, smoking, diet, etc.), during and before the lockdown, that may have contributed to cardiovascular risk modification and a potential reduction in ACS incidence.

METHODS

The first CoVid-19 case in Greece was reported on 20 February 2020; strict social distancing was instituted by 10 March 2020; on 11 March 2020 the World Health Organization declared the outbreak a pandemic; and lockdown was imposed in Greece on 13 March 2020 lasting until 4 May 2020. This research was confined to southwestern Greece, and included 3 large municipalities with 411,576 inhabitants according to the last national census of 2011. Our hospital is the only hospital with a hemodynamic laboratory in southwestern Greece, and offers a primary percutaneous intervention (PCI) service on a 24/7 basis, being the hub hospital for 3 large general district hospitals (spoke hospitals). We prospectively recorded admissions for ACS in all hospitals during the period of strict social distancing and lockdown in Greece (10 March to 4 May, 2020), and searched all hospitals' databases for admissions with ACS [ST-elevation myocardial infarction (STEMI) and Non-ST-elevation MI (NSTEMI)], during the corresponding period in 2018 and 2019. Total population all-cause death rate was collected by the three large municipalities for the corresponding period over the last 3 years (2018–2020).

A telephone survey was conducted between 13 and 30 April, 2020, by a certified to implement a Quality Management System company (DATA RC SA, ISO 9001:2015 & Information Security ISO 27001:2013), to explore the lifestyle habits of citizens during and before quarantine. The survey sample was designed to represent the general population of the region of southwestern Greece aged $\geq$35 years old, in terms of geographical criteria (3 regional units). Data were collected via telephone interviews using CATI (Computer Assisted Telephone Interviewing) technology, conducted by experienced interviewers who read and completed the survey questionnaire remotely. Each respondent was asked to give his/ hers explicit consent in order to participate in the survey, in accordance with General Data Protection Regulation (GDPR) rules. Additionally, 20% of the questionnaires were cross-checked by the field manager who monitored the call. The duration of the interview was about 8 min and it was based on a strictly structured questionnaire that included a brief medical history, lifestyle data (smoking, alcohol consumption, hours of sleep, and work, type of diet, exercise), as well as self-evaluation of anxiety related to the pandemic, and depressive feelings during and before the application of lockdown.

Statistical Analysis

Patient characteristics were extracted from electronic medical records and defined according to international guidelines and standards of good practice. We calculated weekly counts of ACS admissions and ensuing urgent coronary revascularization across the regional hub-and-spoke referral network. We also looked separately into weekly counts of the types of ascertained ACS sub-diagnosis (NSTEMI vs. STEMI) and type of revascularization [percutaneous coronary intervention (PCI) vs. coronary artery bypass grafting (CABG)]. Population-based mortality rates were obtained from local municipal archives. Counts of events were stratified by year and by pre-CoVid-19 (years, 2018 and 2019) and Covid-19 period (year 2020). The same time interval (10 March to 04 May) was examined in each year to account for seasonal variations. Missing data (<0.5%) were filled by multivariate imputation with chained equations (Missing-At-Random principle). To compare patient covariates during the 3 years (2018, 2019, and 2020), one-way ANOVA was used for continuous variables and the chi square test was used for explanatory categorical variables. Stratified analyses were also performed for various patient risk factors as reported in the results section and respective tables. Generalized linear models were applied to investigate the associations between response and explanatory variables. A Poisson log-likelihood function was fitted to regress weekly counts of ACS admissions. Overdispersion was excluded by comparing residual deviance to degrees of freedom. Multicollinearity was excluded by calculation

Abbreviations: ACS, acute coronary syndromes; COVID-19, coronavirus disease; USA, United States of America; DATA RC SA, Data Research and Consulting Société Anonyme; ISO, International Organization for Standardization; STEMI, ST elevation myocardial infarction; NSTEMI, Non-ST elevation myocardial infarction; PCI, Percutaneous Coronary Intervention; CABG, Coronary Artery Bypass Grafting; CATI, Computer Assisted Telephone Interviewing; GDPR, General Data Protection Regulation; ANOVA, Analysis of Variance; SPSS, Statistical Package for the Social Sciences; CAD, coronary artery disease; FMC, First Medical Contact.

of variance inflation factors. We generated plots of the weekly incidence of ACS and studied subgroups, as well as of different patient strata to demonstrate the observed effect of the pandemic lockdown. All variables recorded in the telephone survey were classified as categorical and tested with the chi-square test. Logistic regression models were fitted to regress lifestyle habit changes collected by the telephone survey. To address the familywise error rate arising from multiple testing, we generously adjusted the level of type I error to $\alpha = 0.1\%$ by the stringent Bonferroni method (i.e., statistical significance was assumed for $p < 0.001$). All statistical analyses were performed in the R language environment (version 3.6.3).

TABLE 1 | Acute coronary syndrome (ACS), events and patient characteristics.

	2018	2019	2020	*P* value
ACS cases	$n = 160$	$n = 175$	$n = 111$	Chi^2
Male gender	127 (79.4%)	132 (75.4%)	81 (73.0%)	0.45
Age (years)	64.3 ± 13.6	65.3 ± 13.7	65.3 ± 12.3	0.49
Smoking	70 (43.8%)	74 (42.2%)	54 (48.6)	0.56
Hypertension	74 (46.3%)	95 (54.2%)	55 (49.5%)	0.34
Diabetes	34 (21.3%)	39 (22.2%)	28 (25.2%)	0.74
Dyslipidemia	48 (30.0%)	64 (36.6%)	47 (42.3%)	0.11
Renal disease	7 (4.4%)	3 (1.7%)	7 (6.3%)	0.13
Peripheral arterial disease	9 (5.6%)	15 (8.6%)	6 (5.4%)	0.46
Familial history	12 (7.5%)	32 (18.2%)	15 (13.5%)	0.01
CAD history	39 (24.4%)	36 (20.6%)	23 (20.7%)	0.66
Atrial fibrillation	12 (7.5%)	6 (3.4%)	2 (1.8%)	0.06

ACS, Acute Coronary Syndrome; CAD, coronary artery disease.

RESULTS

During the period of interest, a total of 160 ACS (34.4% STEMI) admissions were recorded in 2018, 175 (33.7% STEMI) in 2019, and 111(32.4%STEMI) in 2020. Age, gender, and all other CAD risk factors (smoking, diabetes, hypertension, hyperlipidemia), with the exception of familial history of CAD, did not differ among the admitted ACS patients (**Table 1**). The Median ACS incidence rate decreased from 19.0 cases per week in 2018 and 21.5 in 2019 down to 13.0 in 2020 (RR:0.66 during the lockdown; 95%CI: 0.53–0.82; $P = 0.0002$). The Median rates of coronary artery revascularizations decreased significantly from 12.5 per week in 2018 and 15.0 in 2019 down to 8.0 in 2020 (RR: 0.59 during the lockdown; 95%CI: 0.45–0.77; $P = 0.0001$). The observed decline in ACS admissions was driven by a significant reduction in patients with NSTEMI (RR: 0.68; 95%CI: 0.52–0.88; $P = 0.0037$); and correspondingly the decline in revascularizations was driven by a significant reduction in PCI procedures (RR: 0.58; 95%CI: 0.43–0.80; $P = 0.0007$; **Figure 1**). Conservative treatment for ACS management was largely stable across 2018, 2019, and 2020 time-intervals (RR: 0.83, 95%CI: 0.58–1.18, $P = 0.299$). Although there was a decline in the rates of STEMI across the examined time periods, this did not reach statistical significance probably due the small number of events. In the STEMI subgroup analyses, a numerical trend toward later (>24 h) admissions ($P = 0.014$) and increased patient mortality were noted ($P = 0.03$), whereas the rates of thrombolysis did not differ across the 3 time periods examined (**Table 2**). A numerical trend toward lower incident coronary angiograms without significant findings or ensuing treatment (thrombolysis, PCI or CABG) was also noted (RR: 0.73; 95%CI: 0.53–1.01; $P = 0.055$) in line with the rest of the aforementioned findings on the incidence of overall ACS events (**Table 2**). Stratified analyses of individual risk factors identified a significant reduction in ACS incidence in most cases in the absence of the known CAD risk factors (i.e., family history of coronary artery disease, smoking, diabetes, dyslipidemia, renal dysfunction, peripheral arterial disease, renal dysfunction, atrial fibrillation; $P < 0.001$ in all cases; **Figure 2**). There was an inverse association between the reduction in the incidence of ACS during the Covid-19 lockdown period and the burden of registered cardiovascular risk factors (**Figure 3**).

The number of all-cause deaths registered across the municipal regions (population, $n = 411{,}576$) was largely stable across the examined years (2018, $n = 604$; 2019, $n = 672$, and 2020, $n = 604$). All-cause mortality per 100,000 population during the examined time period, in the years 2018, 2019, and 2020 was 146.7, 163.7, and 146.7, in the years 2018, 2019, and 2020, respectively, with no differences among the examined 3 municipalities.

For the telephone survey, a total of 10,917 contacts were made, but 7,777 refused to answer. In 2,126 cases the interview was not conducted because the specifications were not met (e.g., age <35 years, etc.). Hence, the sample size for the telephone survey was 1,014, of whom 48.7% were women. These included 509, 302, and 203, inhabitants of the 3 municipalities. The sample was weighted by using external data on age and sex from the 2011 census in order to prevent the bias due to sample design and distribution, as well as non-response variance while making estimations. The most important results regarding lifestyle habit changes during quarantine are presented in **Figure 4**.

There were significant changes in most of the lifestyle variables reported with the exception of active smoking. Briefly, passive smoking (13.5% of the sample), was reduced in 44.0% of non-smokers, mainly men, and the younger ($P < 0.001$). Regular smoking (31% of the sample), was not affected during the lockdown. However, a proportion of 34.5% of occasional smokers (9% of smokers) reported a reduction in smoking during quarantine. Amongst people who reported alcohol consumption, the latter was reduced in 34.3%. This was more evident in men, younger people, the unemployed, and more highly educated individuals ($P < 0.001$). Most participants (61.1%), reported reduced working hours during the lockdown period compared to the pre-lockdown time. This was more evident in women, those aged <75 years, those with higher education status, and people with a lower family income ($P < 0.001$). The proportion of people sleeping >7 h during the lockdown was significantly greater compared to previous habits ($P < 0.001$), mainly in the younger people and those with higher income ($P < 0.001$). The increase in sleeping time and the reduction in working hours, smoking, and junk food consumption were more pronounced in participants with fewer risk factors ($P < 0.01$ for all).

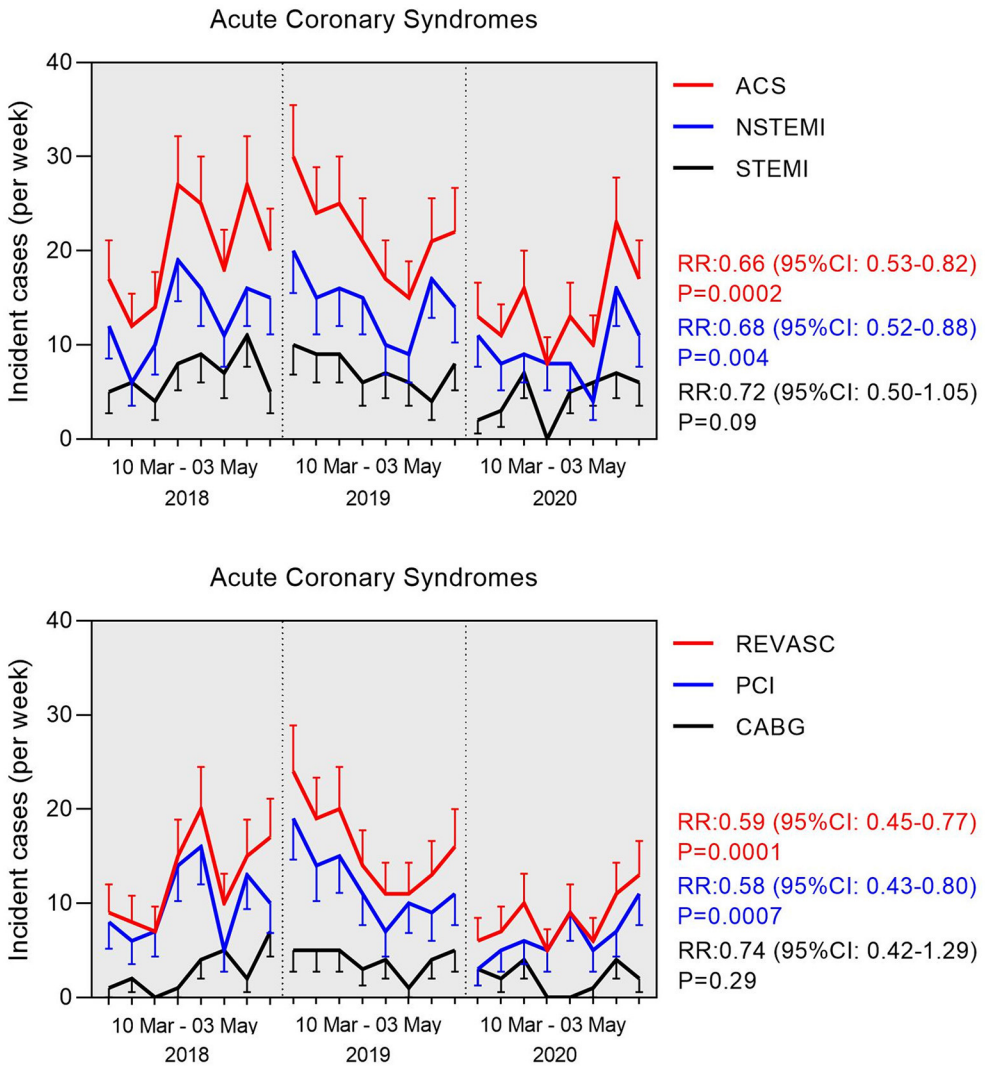

FIGURE 1 | Weekly counts of incident cases of acute coronary syndrome (ACS) between 10 March and 04 May (period of 8 weeks) during 2018, 2019, and 2020. **(Top)** Overall ACS incidence stratified by type of ACS (STEMI vs. NSTEMI). **(Bottom)** Total counts of ACS cases that underwent revascularization (REVASC) stratified by mode (PCI vs. CABG). Relative risks (RR) were calculated by Poisson regression adjusting for Covid-19 epidemic.

There was no difference in self-reported compliance with medications for chronic diseases. People who did not exercise regularly (<3–4 times per week), reported an increase in exercise time. Among people who did not previously exercise at all, 15% reported exercising during the lockdown. Junk food, snack, and salt consumption were reduced in 25.5, 18.8, and 10.3%, respectively, in this poll. Anxiety related to the pandemic was reported by 45.1% and lack of motivation and satisfaction by the 38.4% of the participants. A proportion of 76.9% of the participants reported that if they experienced chest pain they would seek the assistance of their personal doctor.

An inverse association (negative coefficients) between some of the observed lifestyle changes and the number of registered patient risk factors, was noted in the survey (**Figure 5**). In particular, reduced work hours, less smoking, less junk food intake, and more sleeping time were reported more frequently in patients with a lower number of modifiable cardiovascular risk factors. Finally, survey participants aged <65 years reported more exercise, reduced alcohol consumption, less junk food consumption ($P < 0.001$), and more sleeping hours compared to older people (>65 years) during the lockdown period.

DISCUSSION

This study shows that during the lockdown period imposed by the Hellenic Republic Greek government because of the COVID-19 pandemic, there was a significant reduction in the incidence of ACS admissions in 3 spoke, and 1 hub university hospital covering 3 neighboring municipalities in southwestern Greece. This observation is in accord with worldwide experience from the USA, Europe, and other continents (1–7). The prevailing hypothetical explanations for this phenomenon include fear of

TABLE 2 | Clinical events and outcomes in patients with ACS.

	2018	2019	2020	*p*-value
Late presentation (>24 h)	2/53 (3.6%)	3/59 (5.0%)	7/29 (19.4%)	0.014
Thrombolysis	8/53 (15.1%)	7/59 (11.9%)	7/29 (24.1%)	0.33
STEMI	55/160 (34.4%)	59/175 (33.7%)	36/111 (32.4%)	0.09
NSTEMI	105/160 (65.6%)	116/175 (66.3%)	75/111 (67.6%)	0.0037
Conservative treatment*	70/160 (43.8%)	65/175 (37.1%)	49/111 (44.1%)	0.055
Mortality	2/160 (1.3%)	6/175 (3.4%)	8/111 (7.2%)	0.03

P-values have been calculated with a Poisson regression model of weekly counts of events.

**Conservative treatment group includes all the patients who have received only medical treatment after coronary angiography, without further interventional (PCI or CABG) procedures.*

contagion at the hospital, reassignment of medical services to care of COVID-19 patients, use of thrombolysis for STEMI in district hospitals, and STEMI misdiagnosis (1–7). All the above imply a false decrease in the incidence of ACS (8–10), which could potentially lead to a corresponding increase in cardiovascular and possibly all-cause morbidity and mortality (6, 11). Indeed an alarming four times higher rate of out-of-hospital cardiac arrest was reported in New York City from 30 March to 5 April 2020, associated with an eight times higher mortality compared to the same period of the previous year (12). Very recently Nef et al. reported an 11.8% increase of cardiac mortality in 2020 compared to 2019 (IRR: 1.12, 95% CI: 1.05–1.19; $p < 0.001$), in central Germany (Hesse), suggesting that patients probably presented, or were referred too late to the hospitals (6).

In our study the observed incidence of STEMI and all-cause regional mortality was largely stable and the significant

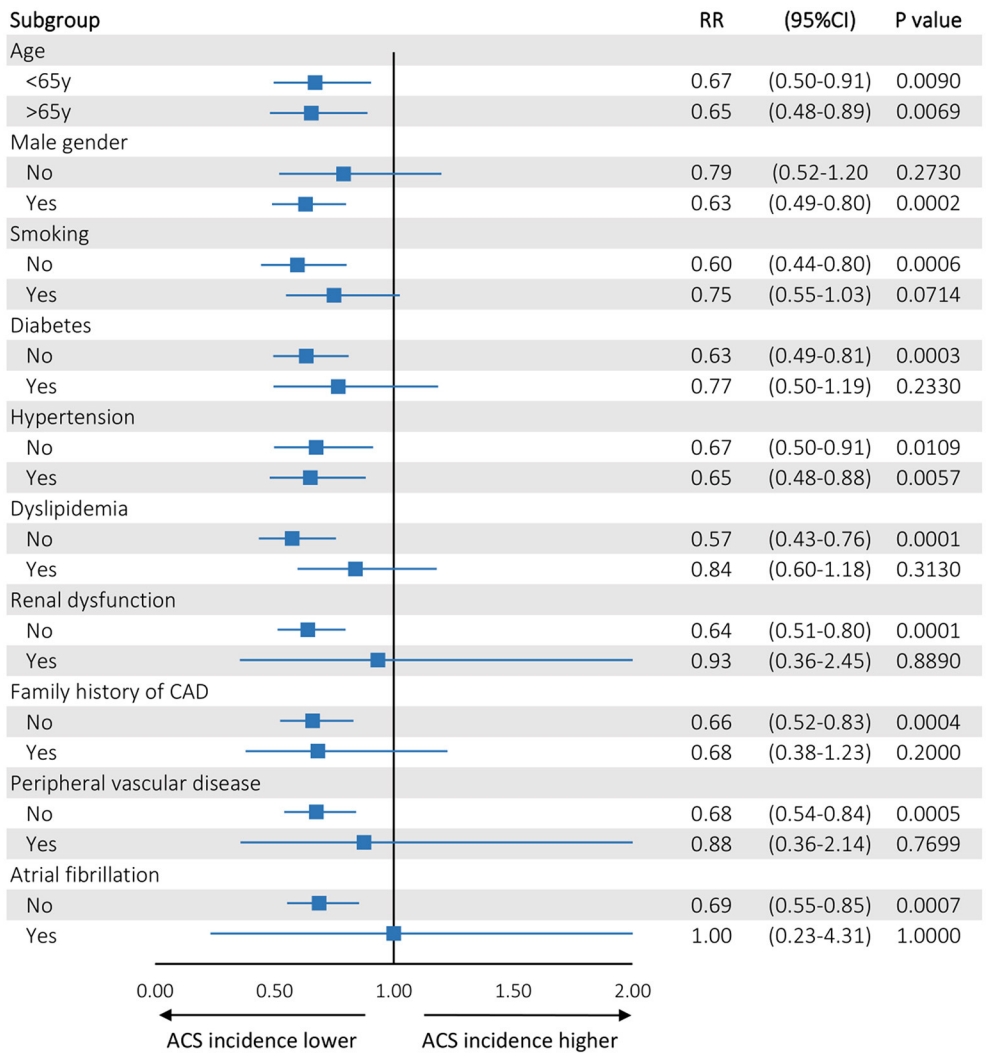

FIGURE 2 | Forest plot showing the results (Risk Ratios) of regression analysis of the weekly incidence of ACS across different strata of registered patient characteristics. Age was dichotomized at its median value (65 years)—rest of the remaining categorical explanatory variables were stratified by factor (GLM with a Poisson log-likelihood function). Statistical significance was assumed for $P < 0.001$ to account for multiple testing.

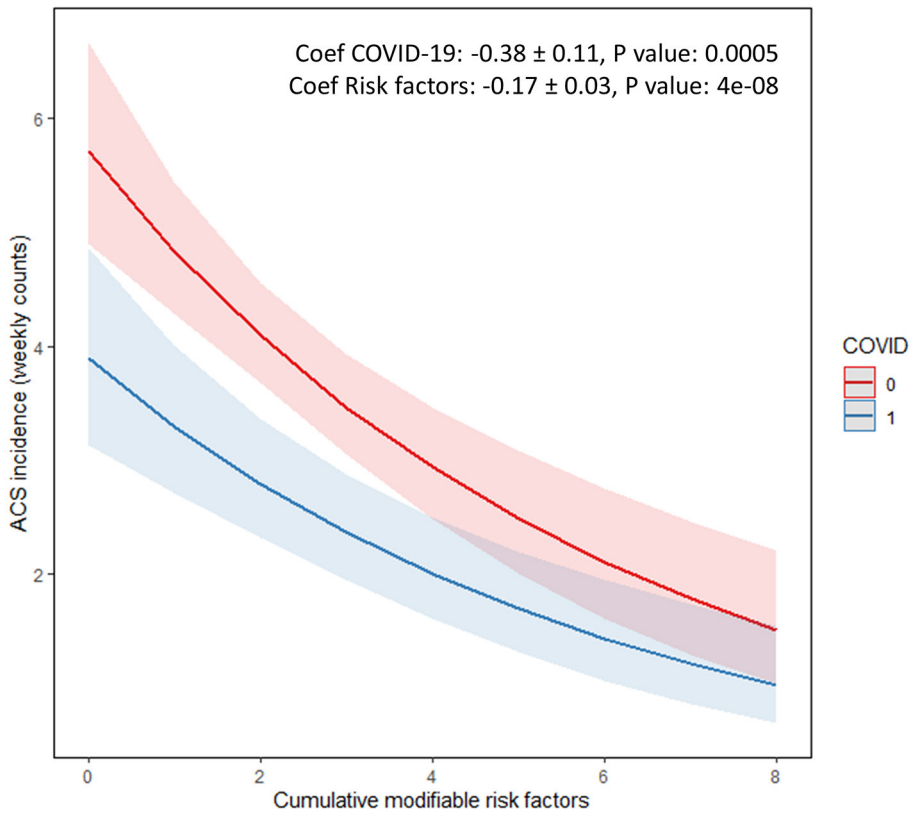

FIGURE 3 | Interaction plot of Poisson regression model showing an inverse association between the reduction of the incidence of Acute Coronary Syndrome during the Covid-19 lockdown period and the number of registered patient risk factors. Cumulative modifiable risk factors (*n*) included smoking, hypertension, diabetes, dyslipidemia, renal disease, peripheral arterial disease, atrial fibrillation, familial history, and previous history of coronary artery disease. The log coefficients for the Covid-19 disease and for each added individual risk factor are noted (R package sjPlot) (0, Non-covid-19 era 2019 and 2020; 1, Covid-19 era 2020).

cumulative decline in ACS admissions was mainly driven by a reduced incidence of NSTEMI observed primarily in patients with low cardiovascular risk. In the STEMI subgroup analyses, a numerical trend toward later (>24 h) admissions, and increased patient mortality were noted. We cannot exclude that the reduction in STEMI admissions and higher in-hospital mortality did not reach statistical significance due to the small number of events. This would be in accord with the observed higher in-hospital mortality in patients admitted for cardiac catheterization during the COVID-19 pandemic compared with 2019 (58/1,801 vs. 55/3,030, $p = 0.002$), reported by Nef et al. (6) This would support the theory of a phenomenal reduction in ACS incidence due to patients' denial to seek medical care under the fear of the pandemic. Nevertheless, an alternative scenario of "Life in a Standstill" where a real reduction of ACS incidence (mainly NSTEMI) could be related to lifestyle changes induced by lockdown measures also cannot be excluded. This hypothesis has also been proposed by others, however without any data regarding lifestyle changes during quarantine/ lockdown (13). Indeed, our survey of 1,014 citizens in our area during the lockdown revealed a significant reduction in the rate of occasional and passive smoking, working hours, and alcohol, salt and junk food consumption, along with a significant increase in sleeping hours and light to moderate exercise (in people who did not exercise before the lockdown). Many of these lifestyle habits that were changed favorably during quarantine are well-known risk factors for ACS (14–18). Hence, modification of such factors in the setting of quarantine could reduce the chance of stable coronary plaque destabilization and rupture. Most interestingly, these lifestyle changes were reported significantly more often by people with less risk factors for CAD and by relatively younger people. This parallels with the observation that the decline in ACS admissions was more pronounced in lower risk patients. It may be that the latter experienced a significant lifestyle change, thus reducing the chance for an acute plaque rupture and myocardial infarction.

It is important to stress that in contrast to other countries in Europe and the USA, Greece did not experience a severe outbreak of COVID-19 thanks to the very early institution of lockdown measures before the infection could spread in the community. Therefore, the dramatic scenes seen in hospitals of other countries such as Italy, Spain, or the USA were not observed in our country, potentially inducing less fear and hesitation in citizens to seek medical assistance if needed. Indeed, 54.9% of the participants in the survey did not report any anxiety related to the pandemic, while 61.6% did not report any depressive feelings. It

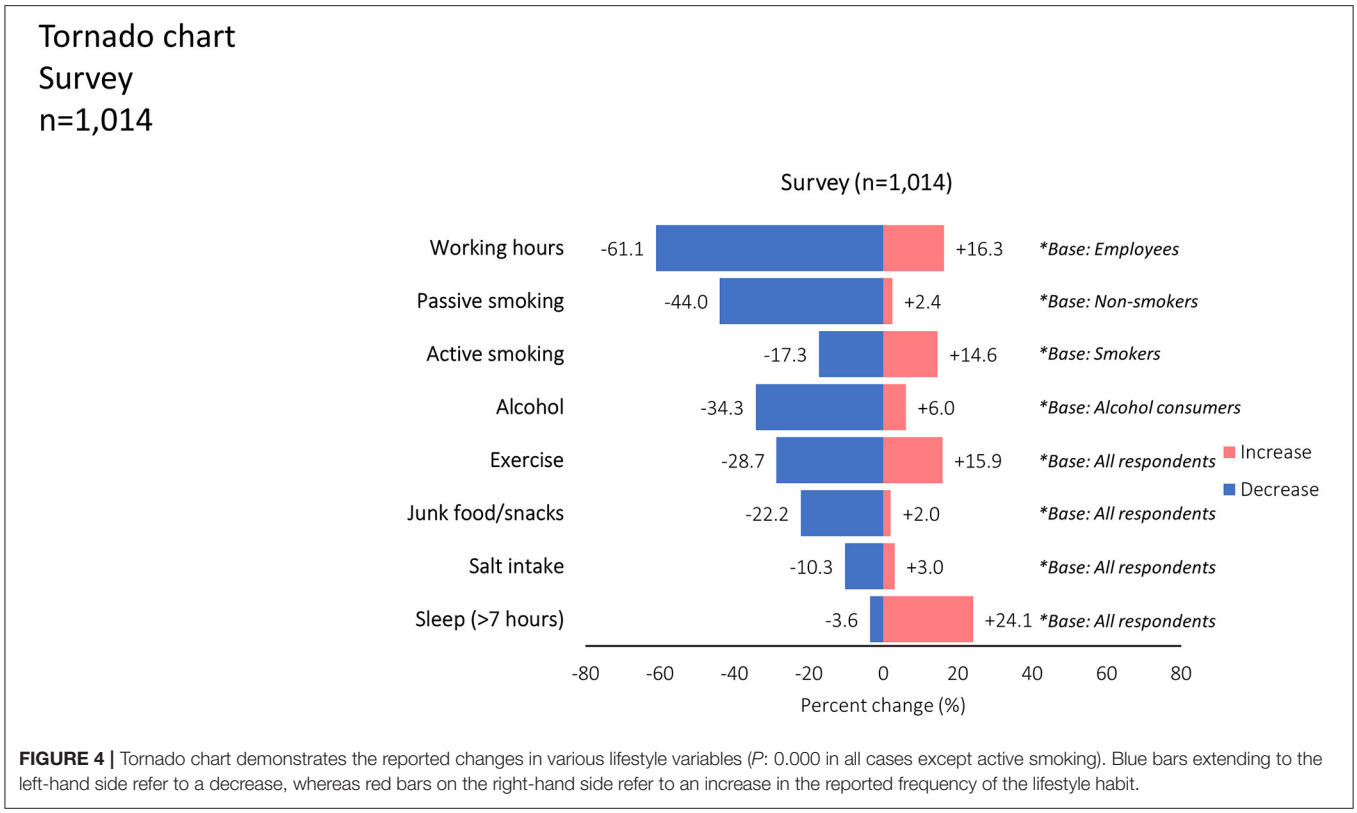

FIGURE 4 | Tornado chart demonstrates the reported changes in various lifestyle variables (*P*: 0.000 in all cases except active smoking). Blue bars extending to the left-hand side refer to a decrease, whereas red bars on the right-hand side refer to an increase in the reported frequency of the lifestyle habit.

should also be emphasized that the medical system in Greece is largely based on private general, and specialized medical services, largely affordable for the vast majority of citizens. Most private cardiology medical offices remained active during this period, and cardiologists were easily accessible to their private patients. Furthermore, at least 70% of the participants in our poll answered that they would seek medical care from their private physician without delay in case of chest pain, or dyspnoea. The decline in ACS admissions during the lockdown was not associated with any increase in total mortality per 100,000 population in the area covered in this study. Additionally, the rates of thrombolysis for STEMI did not increase in 2020 compared to 2018, and 2019, and our hub hospital did not discourage referral of ACS patients for catheterization. The lower proportional reduction of ACS during lockdown in patients with a higher burden of cardiovascular risk factors implies that high-risk patients with established atherosclerosis continued to suffer ACS and presented to the hospital during lockdown, whereas lower-risk patients may have actually experienced a real decrease in ACS incidence. The latter could be explained by lifestyle changes during the lockdown period, as it is an established knowledge that biological pathways, correlated with daily activities and the circadian rhythm could play an important role on the onset of ACS.

Conclusion

A significant decline in ACS admissions during the COVID-19 lockdown was noted, affecting mainly NSTEMI patients with a lower burden of cardiovascular risk factors. This was accompanied by significant lifestyle changes. Thus, it is tempting to speculate that to some extend the latter might be associated with the observed decline in ACS admissions.

Limitations

The exact number of the population in the area examined was based on the last nationwide census of 2011. Since then, according to the Greek Statistical Agency (ELSTAT), there is a stable decline of the country population of 25,000–30,000. Thus, we do not expect major population changes during the years 2018–2020 in the above area. The actual causes of death in the 3 municipalities during the period of lockdown and the corresponding period in 2018, and 2019 were not available. However, there was no difference in the number of total deaths over time. Because of the study design, no direct correlation can be demonstrated between the decrease in ACS admissions and lifestyle changes by the design of the study. Thus, our results are mainly hypothesis generating, and certainly apply to the very specific scenario of countries that did not experience the devastating effects from the pandemic thanks to the early institution of preventive measures. The number of observations for both STEMI and NSTEMI were small, as the study was not nationwide; nevertheless, they were statistically significant. Furthermore, we cannot also exclude that the observed reduced incidence of ACS admissions could be partially driven by reduced rates of type II NSTEMI events that presented with negative angiograms. However, our analysis is most likely underpowered to discern between STEMI and NSTEMI subtypes. Finally,

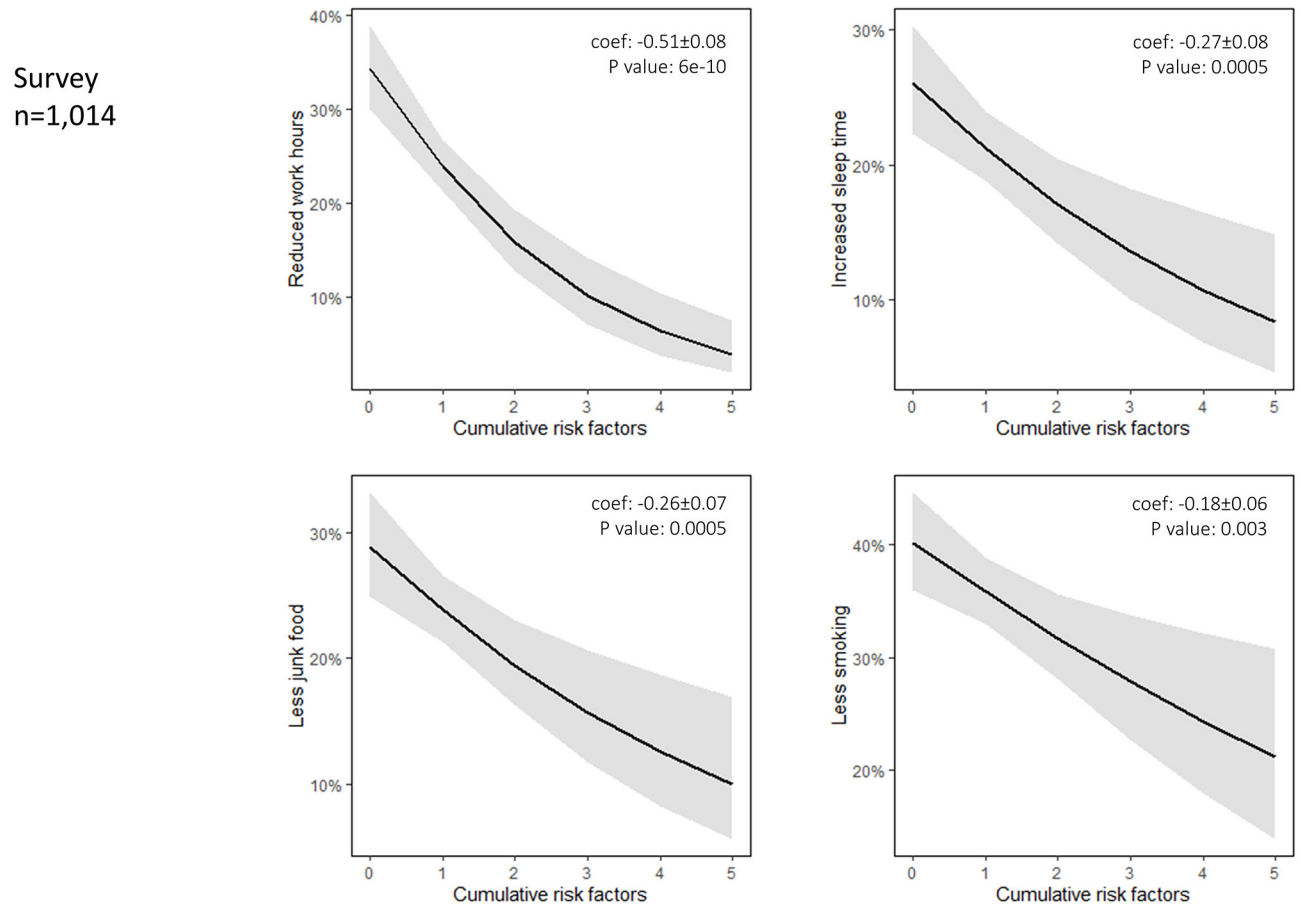

FIGURE 5 | Interaction plot of a logistic regression model denotes an inverse association (negative coefficients) between the observed change in the frequency of the lifestyle habit and the number of registered patient risk factors. Cumulative modifiable risk factors (*n*) registered in the survey and introduced in the logistic regression analysis included smoking, hypertension, diabetes, dyslipidemia, and previous history of coronary artery disease. Reduced working hours, less smoking, less junk food intake, and more sleeping time were reported more frequently in patients with a lower number of modifiable cardiovascular risk factors.

regardless of the aforementioned study limitations, in a purely observational cohort, the Covid-19 lockdown circumstances could hardly be reproduced under controlled experimental conditions (e.g., a randomized study), to confirm or refute our hypothesis and findings.

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 of Patras University Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

GT, E-EK, KK, PV, and PD contributed to conception design, analysis and interpretation, drafted, and critically revised the manuscript. PP, AM, IM, IC, FP, TD, and AK contributed to analysis and interpretation. GA contributed to analysis, drafted, and critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. 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
2. De Filippo O, D'Ascenzo F, Angelini F, Bocchino PP, Conrotto F, 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
3. 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
4. Metzler B, Siostrzonek P, Binder RK, Bauer A, Reinstadler SJ. Decline of acute coronary syndrome admissions in Austria since the outbreak of COVID-19: the pandemic response causes cardiac collateral damage. *Eur Heart J.* (2020) 41:1852–53. doi: 10.1093/eurheartj/ehaa314
5. Abdi S, Salarifar M, Mortazavi SH, Sadeghipour P, Geraiely B. COVID-19 sends STEMI to quarantine!? *Clin Res Cardiol.* (2020) 109:1567–8. doi: 10.1007/s00392-020-01664-3
6. Nef HM, Elsässer A, Möllmann H, Abdel-Hadi M, Bauer T, Brück M, et al. Impact of the COVID-19 pandemic on cardiovascular mortality and catheterization activity during the lockdown in central Germany: an observational study. *Clin Res Cardiol.* (2020) 1–10. doi: 10.1007/s00392-020-01780-0
7. Roffi M, Guagliumi G, Ibanez B. The obstacle course of reperfusion for STEMI in the COVID-19 pandemics. *Circulation.* (2020) 141:1951–3. doi: 10.1161/CIRCULATIONAHA.120.047523
8. Trabattoni D, Montorsi P, Merlino L. Late STEMI and NSTEMI patients' emergency calling in COVID-19 outbreak. *Can J Cardiol.* (2020) 1161.e7–e8. doi: 10.1016/j.cjca.2020.05.003
9. Tam CF, 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 Cardiovas Qual Outcomes.* (2020) 13:e006631. doi: 10.1161/CIRCOUTCOMES.120.006631
10. Toner L, Koshy AN, Hamilton GW, Clark D, Farouque O, Yudi MB. Acute coronary syndromes undergoing percutaneous coronary intervention in the COVID-19 era: comparable case volumes but delayed symptom onset to hospital presentation. *Eur Heart J Qual Care Clin Outcomes.* (2020) 6:225–6. doi: 10.1093/ehjqcco/qcaa038
11. Toniolo M, Negri F, Antonutti M, Masè M, Facchin D. Unpredictable fall of severe emergent cardiovascular diseases hospital admissions during the COVID-19 pandemic: experience of a single large center in Northern Italy. *J Am Heart Assoc.* (2020) 9:e017122. doi: 10.1161/JAHA.120.017122
12. 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.* (2020) 6:408–9. doi: 10.1093/ehjcvp/pvaa054
13. Piccolo R, Bruzzese D, Mauro C, Aloia A, Baldi C, Boccalatte M, et al. Population trends in rates of percutaneous coronary revascularization for acute coronary syndromes associated with the COVID-19 outbreak. *Circulation.* (2020) 141:2035–7. doi: 10.1161/CIRCULATIONAHA.120.047457
14. Whitman IR, Agarwal V, Nah G, Dukes JW, Vittinghoff E, Dewland TA, et al. Alcohol abuse and cardiac disease. *J Am Coll Cardiol.* (2017) 69:13–24. doi: 10.1016/j.jacc.2016.10.048
15. Kälsch T, Elmas E, Nguyen XD, Leweling H, Klüter H, Borggrefe M, et al. Alimentary lipemia enhances procoagulatory effects of inflammation in patients with a history of acute myocardial infarction complicated by ventricular fibrillation. *Int J Cardiol.* (2008) 123:131–7. doi: 10.1016/j.ijcard.2006.11.249
16. Daghlas I, Dashti HS, Lane J, Aragam KG, Rutter MK, Saxena R, et al. Sleep duration and myocardial infarction. *J Am Coll Cardiol.* (2019) 74:1304–14. doi: 10.1016/j.jacc.2019.07.022
17. Raupach T, Schäfer K, Konstantinides S, Andreas S. Secondhand smoke as an acute threat for the cardiovascular system: a change in paradigm. *Eur Heart J.* (2006) 27:386–92. doi: 10.1093/eurheartj/ehi601
18. Cheng Y, Du CL, Hwang JJ, Chen IS, Chen MF, Su TC. Working hours, sleep duration and the risk of acute coronary heart disease: a case-control study of middle-aged men in Taiwan. *Int J Cardiol.* (2014) 171:419–22. doi: 10.1016/j.ijcard.2013.12.035

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REVIEW
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Right Ventricular Damage in COVID-19: Association Between Myocardial Injury and COVID-19

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Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2, is a global pandemic. It has resulted in considerable morbidity and mortality around the world. The respiratory system is the main system invaded by the virus involved in COVID-19. In addition to typical respiratory manifestations, a certain proportion of severe COVID-19 cases present with evidence of myocardial injury, which is associated with excessive mortality. With availability of an increasing amount of imaging data, right ventricular (RV) damage is prevalent in patients with COVID-19 and myocardial injury, while left ventricular damage is relatively rare and lacks specificity. The mechanisms of RV damage may be due to increased RV afterload and decreased RV contractility caused by various factors, such as acute respiratory distress syndrome, pulmonary thrombosis, direct viral injury, hypoxia, inflammatory response and autoimmune injury. RV dysfunction usually indicates a poor clinical outcome in patients with COVID-19. Timely and effective treatment is of vital importance to save patients' lives as well as improve prognosis. By use of echocardiography or cardiovascular magnetic resonance, doctors can find RV dilatation and dysfunction early. By illustrating the phenomenon of RV damage and its potential pathophysiological mechanisms, we will guide doctors to give timely medical treatments (e.g., anticoagulants, diuretics, cardiotonic), and device-assisted therapy (e.g., mechanical ventilation, extracorporeal membrane oxygenation) when necessary for these patients. In the paper, we examined the latest relevant studies to investigate the imaging features, potential mechanisms, and treatments of myocardial damage caused by COVID-19. RV damage may be an association between myocardial damage and lung injury in COVID-19. Early assessment of RV geometry and function will be helpful in aetiological determination and adjustment of treatment options.

Keywords: COVID-19, right ventricular damage, myocardial injury, cardiovascular magnetic resonance, echocardiography, ARDS

INTRODUCTION

Coronavirus disease 2019 (COVID-19) has spread rapidly and triggered a terrible global pandemic that involves more than 200 countries/regions. On 6 December 2020, there were more than 66.9 million confirmed cases and 1,534,954 deaths internationally (1). Although respiratory symptoms are usually predominant in COVID-19, elevated troponin levels have been found at the early stage in some cases, indicating that COVID-19 also affects the heart. In particular, there is an increased prevalence of cardiovascular complications, including new or worsening heart failure, arrhythmia, acute myocarditis, and myocardial infarction, in severe and critically ill patients with COVID-19. Recent studies have shown that the incidence of acute myocardial injury in hospitalized patients with COVID-19 is ∼20–28% (2–4). With an increase in imaging evidence, such as echocardiography and magnetic resonance imaging (MRI), right ventricular (RV) involvement has been observed more commonly than left ventricular (LV) involvement in patients with COVID-19, with ∼40% of patients experiencing RV dilatation and RV dysfunction (5, 6). RV damage is associated with a higher incidence of myocardial damage in COVID-19 and generally predicts a worse prognosis (7). This review aims to describe involvement of RV damage in patients with COVID-19, to determine the association of RV damage with COVID-19 and its plausible mechanisms, and to summarize the existing appropriate treatment strategies to improve patients' prognosis.

MYOCARDIAL INJURY IN COVID-19 IS COMMON

Previous influenza-related studies have shown that elevated cardiac enzymes are relatively uncommon (8). Cardiac abnormalities associated with influenza are usually subclinical and/or transient (9). However, COVID-9-related cardiac injury is significantly different from influenza. In a review of 26 studies that included 11,685 patients, the overall prevalence of COVID-19-related acute myocardial injury ranged from 5 to 38% (10). N-terminal pro-brain natriuretic peptide and cardiac troponin-I levels were shown to be significantly higher in critically ill patients with COVID-19 than in non-critically ill patients (2). These findings suggest that the magnitude of elevated cardiac troponin levels may be related to the severity and prognosis of the disease (11). Monitoring cardiac troponin-I levels is important for judging the status of COVID-19, while understanding myocardial injury in patients with COVID-19. Chinese guidelines recommend myocardial enzyme monitoring in patients who are admitted for COVID-19 (12). Troponins are often associated with LV ischaemia and infarction. However, previous studies have shown that the most common mechanism of elevated troponin levels in patients with COVID-19 is acute RV damage rather than LV functional impairment (5). Specific manifestations of myocardial structural damage require assessment of cardiac imaging. Early retrospective analysis did not show any specificity between electrocardiography and echocardiography (13). However, with publication of more imaging study results, there are particularities in cardiac structural changes. Therefore, imaging assessment of cardiac injury in COVID-19 is important and helpful for differential diagnosis of cardiac events.

Abbreviations: ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; ECMO, extracorporeal membrane oxygenation; LV, left ventricular; LVEF, left ventricular ejection fraction; LGE, late gadolinium enhancement; MRI, magnetic resonance imaging; PEEP, positive end-expiratory pressure; RV, right ventricular; RVLS, right ventricular longitudinal strain.

RV INVOLVEMENT FROM CARDIAC IMAGES IN PATIENTS WITH COVID-19

With the discovery of COVID-19-related myocardial damage, cardiac imaging is becoming more common, and it can help to better understand the structural characteristics of COVID-19-related myocardial damage. Imaging studies can not only detect lesions, but also guide further treatment. We searched PubMed, EMBASE, and Web of Science until August 2020 for RV clinical research. "Snowball sampling" by searching reference lists and citation tracking was performed in each retrieved article. No language restrictions were applied. Following search terms were used: ("magnetic resonance imaging" OR "echocardiography" OR "myocardial injury" OR "cardiac manifestations" OR "cardiac function" OR "right ventricular damage/injury" OR "right ventricular dysfunction" OR "right ventricular dilatation") AND ("coronavirus" OR "SARS-COV-2" OR "COVID-19"). Recent findings on imaging assessment of cardiac injury in COVID-19 were summarized in **Tables 1**, **2**.

MRI Findings

MRI can be used to quantitatively assess myocardial fibrosis and oedema (28, 29). This technique is currently the gold standard for evaluating cardiac morphology and function (30). MRI analysis includes conventional sequences and quantitative mapping sequences. Conventional sequences include short-axis and long-axis cine, T2-weighted imaging (T2WI), and late gadolinium-enhanced scanning (LGE). Quantitative mapping sequences include native T1/T2 mapping and post-contrast T1 mapping. T1 mapping is mainly applied to quantitatively assess diffuse fibrosis, while T2 mapping enables the quantification of edema. Post-contrast T1 mapping can better obtain extracellular volume fraction, which can be used as the most sensitive biomarker of myocardial fibrosis and is highly consistent with histopathological findings (31). Myocardial oedema is assessed on T2WI images, and LV and RV functional parameters are calculated by changes in endocardial and epicardial contours (14). A study of competitive athletes recovered from COVID-19 found that cardiac MRI (CMR) was more sensitive to identify myocarditis, helping to identify the high-risk population. CMR has a negative predictive value for exclusion of myocarditis (16). Two other studies, analyzing of patients who had already recovered from COVID-19 when undergoing MRI, showed increased T1 and T2 signals, positive LGE and/or pericardial enhancement in 58–78% of the population (14, 15). In Huang's study, 26 patients without previous cardiac diseases were all

TABLE 1 | Studies of CMR imaging assessments in patients with COVID-19 and cardiac injury.

Study, publish date	Study type	Location	Study period	Patients	Image type	Mean age & gender	Main test items	Main findings
Huang et al., May 4, 2020 (14)	Retrospective study	Tongji Hospital, Tongji Medical College, Wuhan, China	Since March, 2020	26 hospitalized patients, recovered from COVID-19 with cardiac symptoms, no previous cardiac disease or COPD	CMR	32–45 26% male	◇ Conventional sequences (cine, T2WI, LGE) ◇ Quantitative mapping sequences (T1, T2, T1/T2, ECV mapping) ◇ Oedema ratio ◇ Cardiac function	◇ 15 (58%) T2 signal ↑ and/or positive LGE ◇ 14 (54%) myocardial oedema ◇ Global native T1, T2, ECV values ↑ in COVID-19 patients with positive cardiac MRI findings ◇ RVEF, CO, CI, SV, SV/BSA ↓ in COVID-19 patients with positive cardiac MRI findings ◇ No significant differences of LV function among controls and patients
Puntmann et al., July 27, 2020 (15)	Prospective observational cohort study	University Hospital Frankfurt COVID-19 Registry, Germany	April to June, 2020	100, recovered from COVID-19 including mostly home-based recovery and hospitalized patients, 13% prior CAD, 21% prior COPD or asthma	CMR	45–53 53% male	◇ LVEF ◇ LVEDV index ◇ LV mass index ◇ RVEF ◇ Native T1 and T2 ◇ LGE ◇ Pericardial effusion	◇ LVEF ↓ ◇ RVEF ↓ ◇ 78% abnormal CMR: 73% native T1 ↑, 60% native T2 ↑, 32% myocardial LGE, 22% pericardial LGE ◇ LV volume and mass ↑ ◇ High-sensitivity troponin T was significantly correlated with native T1, native T2 and LV mass ◇ Native T1 and T2 were the best measures to detect COVID-19-related myocardial pathology
Rajpal et al., Sep 11, 2020 (16)	Prospective study	Ohio State, USA	June 2020 to August 2020	26 competitive college athletes, recovered from COVID-19 without hospitalization, no previous cardiac disease or COPD	CMR	19.5 ± 1.5 57.7% male	◇ LGE ◇ LVEF and RVEF ◇ T1 and T2 mapping ◇ LVEDV and RVEDV	◇ 4 athletes had CMR findings consistent with myocarditis ◇ 12 (46%) had LGE, of whom 8 (30.8%) had LGE without concomitant T2 elevation ◇ Mean (SD) T2 in those with suspected myocarditis was 59 ms compared with 51 ms in those without myocarditis. ◇ CMR may provide an excellent riskstratification assessment for myocarditis in athletes who have recovered from COVID-19.

BSA, body surface area; CI, cardiac index; CMR, cardiovascular magnetic resonance; CO, cardiac output; COVID-19, coronavirus disease 2019; ECV, extracellular volume; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LGE, late gadolinium enhancement; RVEF, right ventricular ejection fraction; SV, stroke volume; T2WI, T2-weighted imaging.

TABLE 2 | Studies of echocardiography assessments in patients with COVID-19 and cardiac injury.

Study, publish date	Study type	Location	Study period	Patients	Image type	Mean age & gender	Main test items	Main findings
Argulian et al., May 7, 2020 (17)	Retrospective study	Mount Sinai Morningside Hospital, New York, USA	March 26, 2020 to April 22, 2020	105 hospitalized patients, 31 of whom were intubated and mechanically ventilated during examination	TTE	66 ± 14.6 64% male	◇ RV and LV sizes and function	◇ 32 (31%) RV dilatation ◇ Renal dysfunction is more common in patients with RVD than those without ◇ No differences in LV size and function ◇ 21 (20%) patients died: 13 (41%) deaths were observed in patients with RV dilatation and 8 (11%) in patients without RV dilatation ◇ RV enlargement was significantly associated with mortality
Li et al., April 24, 2020 (7)	Retrospective study	The west branch of Union Hospital, Tongji Medical College, Wuhan, China	February 12, 2020 to March 15, 2020	120 hospitalized patients (Survivors 102 and non-survivors 18), 9.2% prior CVD, 5% prior COPD	TTE, examed in 3–10 days	61 ± 14 58% male	◇ RVFAC ◇ TAPSE ◇ Tricuspid tissue Doppler annular velocities (S') ◇ RVLS ◇ LV volume and function	◇ Male, ARDS, RVLS, RVFAC and TAPSE were significant univariate predictors of higher risk for mortality ◇ RVLS was found to predict higher mortality more accurately ◇ The best cut-off value of RVLS for prediction of outcome was −23%
Szekely et al., May 29, 2020 (5)	Prospective study	Tel Aviv Medical Center, Israel	March 21, 2020 to April 16, 2020	100 hospitalized patients, 16% prior IHD	TTE, examed within 24 h	66.1 ± 17.3 63% male	◇ LV systolic and diastolic function ◇ Valve hemodynamics ◇ RV assessment (TAPSE, RV-S', RVFAC, Tei index, pulmonary acceleration time) ◇ Lung ultrasound	◇ 32% normal echocardiography ◇ 39% RV dilatation with or without dysfunction ◇ 16% LV diastolic dysfunction ◇ 10% LV systolic dysfunction ◇ Patients with elevated troponin (20%) or worse clinical condition had worse RV function
Mahmoud-Elsayed et al., May 24, 2020 (6)	Retrospective study	Queen Elizabeth Hospital Birmingham, United Kingdom	March 22, 2020 to April 17, 2020	74 hospitalized patients, referred for TTE with ≥ 1 clinical indication(s), 9% prior CAD	TTE, examed in 3–10 days	59 ± 13 78% male	◇ Chamber sizes and function ◇ Valvular disease ◇ Pulmonary hypertension	◇ 41% RV dilatation ◇ 27% RVD ◇ 89% LV function was hyper-dynamic or normal ◇ RV impairment was associated with increased D-dimer and CRP levels
Jain et al., June 9, 2020 (18)	Retrospective study	Columbia University Irving Medical Center and New York-Presbyterian Allen Hospital, New York, USA	March 1, 2020 to April 3, 2020	72 hospitalized patients, referred for TTE when having clinical indications, 18.1% prior CAD	TTE, median time was 3 days	50.8–70.3 72.2% male	◇ LV Function ◇ Segmental LV Wall Motion ◇ RV size and systolic function	◇ 34.7% LVEF ≤ 50% ◇ 40.3% RV systolic function ↓ ◇ RV systolic dysfunction was more common than LV systolic dysfunction ◇ patients with elevated hs-cTnT and elevated NT-proBNP were more likely to exhibit reduced LV function
Dweck et al., June 2, 2020 (19)	Prospective international survey	69 countries	April 3 to 20, 2020	1,216, of whom 813 had confirmed COVID-19, and 298 had a high probability when scanning, 26% prior cardiac disease	TTE	52–71 70% male	Ventricular sizes and function	◇ 55% abnormal echocardiogram ◇ 39% LV abnormalities ◇ 33% RV abnormalities ◇ 3% new myocardial infarction ◇ 3% myocarditis ◇ 2% takotsubo cardiomyopathy 15% severe cardiac disease (severe ventricular dysfunction or tamponade)
Rath et al., May 28, 2020 (20)	Prospective study	University Hospital of Tübingen, Germany	February to March, 2020	123 hospitalized patients (Non-survivors 16 and survivors 107), 22.8% prior CAD	TTE, examed in 24 h	68 ± 15 70% male	◇ LVEF ◇ RV function (TAPSE, RV-FAC) ◇ Aortic stenosis/regurgitation ◇ Mitral regurgitation ◇ Tricuspid regurgitation	◇ Mean LV function 57% ◇ 48.9% RV dilatation ◇ 30.6% tricuspid regurgitation > 1 ◇ RV-FAC ↓ in non-survivors ◇ Visually estimated impaired RV function ↑ in non-survivors ◇ Impaired LV and RV function, and tricuspid regurgitation > grade 1 were significantly associated with higher mortality

(Continued)

TABLE 2 | Continued

Pagnesi et al., July 1, 2020 (21)	Single-center, observational, cross-sectional study	San Raffaele Scientific Institute in Milan, Italy	March 24, 2020 to April 29, 2020	200 non-ICU inpatients, 7.5% prior CAD, 8.5% prior MI	TTE	55–74 65.5 male	◇ RVEDD ◇ RV length ◇ TAPSE ◇ S'TDI ◇ SPAP ◇ Tricuspid regurgitation	◇ 12% PH, 14.5% RVD ◇ PH (and not RVD) was associated with signs of more severe COVID-19 and with worse in-hospital clinical outcome
D' Andrea et al., June 17, 2020 (22)	Prospective study	4 centers in Italy: "Umberto I Hospital, Monaldi Hospital, M. Scarlato COVID Hospital, Cardarelli Hospital	February 20, 2020 to April 20, 2020	115, 26 of whom suffering cardiac injury	TTE	20–88 60% male	◇ RV tract diameter ◇ Tricuspid Peak E/A ratio ◇ TRV ◇ PASP ◇ MPAP ◇ TAPSE	◇ RV function and pulmonary pressures as independent predictors of COVID pneumonia mortality ◇ Patients with PH and RVD had more frequently a history of prior cardiac comorbidities ◇ Only patients with PH showed signs of more severe SARS- CoV-2 infection
Vasudev et al., July 26, 2020 (23)	Retrospective study	Three hospitals in Northern New Jersey, USA	March 15, 2020 to April 15, 2020	45 hospitalized patients, 20% prior ACS	TTE, during hospitalization	61.4 ± 12.2 51% male	◇ Ventricular size and function ◇ SPAP ◇ Pressure and volume overload	◇ 31.1% LVEF ↓ ◇ 11.1% RVEF ↓ ◇ 13.3% RV dilatation ◇ 22.2% PH ◇ Echocardiography is essential for assessment of COVID-19
Baycan et al., August 8, 2020 (24)	Prospective, single-center study	Goztepe Training and Research Hospital, Istanbul, Turkey	April 15, 2020 to April 30, 2020	100 hospitalized patients, all of whom having normal LVEF (≥50%)	TTE, examed on the first day	55.6 ± 14.4 50% male	◇ LV-GLS ◇ RV-FAC ◇ RV-LS ◇ TAPSE ◇ SPAP	◇ LV-GLS and RV-LS were lower in the severe group compared to the non-severe group ◇ LV-GLS and RV-LS are independent predictors of in-hospital mortality in patients with COVID-19 ◇ RVD is important in determining circulation and respiratory management strategies
Krishnamoorthy et al., August 4, 2020 (25)	Single-center study	The Zena & Michael A Wiener Cardiovascular Institute, New York, USA	–	12, 5 of whom required intubation and/or died, 16.7% prior CAD	TTE	29–60 41.7% male	◇ LVGLS ◇ RVGS ◇ RVFWS ◇ RVSP	◇ 41.7% RVD ◇ 58.3% LVD ◇ RVGS and RVFWS were significantly decreased in the patients who had poor outcomes compared with those who did not ◇ LVGLS was decreased regardless of outcome
Van den Heuvel et al., July 8, 2020 (26)	Single center, cross-sectional study	Radboud University Medical Center, Nijmegen, The Netherlands	April 1, 2020 to May 12, 2020	51 hospitalized patients (ICU 19 and non-ICU 32), 22% prior Cardiac history	TTE	51–68 80% male	◇ LV and RV dimensions ◇ LV function (LVEF, GLS) ◇ RV function (TAPSE, RV S') ◇ Atrial dimensions	◇ 27% LVD ◇ 10% RVD ◇ No relation between elevated Troponin T or NT-proBNP and ventricular dysfunction ◇ Ventricular dysfunction by means of L VEF, GLS, TAPSE and RV S' were not significantly different between ICU and non-ICU patients
Zeng et al., July 28, 2020 (27)	Single-center retrospective study	Shenzhen Third People's Hospital, China	January 11, 2020 to April 1, 2020	416 (ICU 35 and non-ICU 381), 3% prior CAD	TTE, only for severe patients (ICU 31 and non-ICU 26)	33–68 47.6% male	◇ LV and RV sizes ◇ LV and RV function ◇ PASP ◇ Ventricular wall thickness	◇ Ventricular wall thickening ◇ LVEF ↓ in 5 (16%) ICU patients ◇ PASP ↑ in 9 (29%) ICU patients ◇ RV dilatation and RVD in 3 (10%) ICU patients

ARDS, acute respiratory distress syndrome; CAD, coronary atherosclerotic heart disease; COVID-19, coronavirus disease 2019; CVD, cardiovascular disease; ICU, intensive care unit; IHD, ischemic heart disease; LV, left ventricular; LVD, left ventricular dysfunction; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; MPAP, mean pulmonary artery pressure; PASP, pulmonary artery systolic pressure; PH, pulmonary hypertension; RV, right ventricular; RVD, right ventricular dysfunction; RVEDD, right ventricular end-diastolic diameter; RVEF, right ventricular ejection fraction; RVFAC, right ventricular fractional area change; RVFWS, right ventricular free wall strain; RVGS, right ventricular global strain; RVLS, right ventricular longitudinal strain; RV S', right ventricular systolic excursion velocity; RVSP, right ventricular systolic pressure; SPAP, systolic pulmonary artery pressure; S' TDI, tissue Doppler imaging S wave; TAPSE, tricuspid annular plane systolic excursion; TRV, tricuspid regurgitation velocity; TTE, transthoracic echocardiography.

recovered and isolated for 14 days, and myocardial edema was found in 54% of patients (14). In Puntmann's study, mostly non-hospitalized patients recovered from COVID-19, 60% of them found myocardial inflammation (16). While COVID-19 patients had cardiac injury, regardless of preexisting disease, severity and overall course of COVID-19 manifestations, time since initial diagnosis, or presence of cardiac symptoms (16). Decreased RV functional parameters, including the RV ejection fraction, cardiac output, the cardiac index, and stroke volume, were found in patients with positive cardiac MRI findings compared with healthy controls ($P < 0.05$). These findings suggest that sustained cardiac involvement, including oedema, fibrosis, and impaired RV contractile function, may remain in patients who recover from COVID-19. Similarly, Puntmann et al. showed that the RV ejection fraction was decreased in patients with COVID-19 compared with healthy controls (15). They also found a reduction in the LV ejection fraction in the recovered COVID-19 cohort. However, Huang et al. showed that LV function was hyperdynamic or normal in the same subgroup (14). The outcomes were inconsistent between these two studies. Regardless of the discrepancy, Puntmann et al. considered that native T1 and T2 were the best indicators with the ability to detect COVID-19-related myocardial pathology (15). Further investigation on the long-term cardiovascular consequences of COVID-19 is required (16).

Echocardiographic Findings

Echocardiography is commonly used for assessing cardiac damage. This technique is easier to perform than cardiac MRI. Conventional echocardiographic evaluation includes cardiac structural assessment, myocardial systolic and diastolic function, and valvular hemodynamics. According to the American Society of Echocardiography, RV dysfunction is present when the following parameters used to quantify RV function are less than low values in the normal range: pulsed Doppler systolic myocardial velocity < 9.5 cm/s, tricuspid annular plane systolic excursion < 17 mm, RV ejection fraction < 45%, and RV fractional area change < 35% (32, 33). RV dilatation is usually observed early in the pressure-overloaded right ventricle. Typically, in the RV-focused view, a basal diameter > 41 mm and an intermediate horizontal diameter > 35 mm indicate RV dilatation (32).

Most inpatients with COVID-19 have RV dilatation or dysfunction. However, LV dysfunction is less common. In a study of 74 patients with COVID-19, 27% presented with RV dysfunction, but LV function was hyperdynamic or normal in 89% (6). Szekely et al. (5) showed that RV dysfunction was more common in patients with elevated troponin levels and a poor clinical grade, whereas the total number of patients with an impaired LV function was relatively smaller. Notably, in several other studies, LV dysfunction was not rare in patients with COVID-19 (18, 23, 25). This discrepancy among studies may be due to differences in the study populations, but RV damage is still universally found by echocardiography in patients with COVID-19. We summarized the results of recent cardiac imaging studies (**Table 2**). Among patients with COVID-19-related myocardial injury, the proportion of RV dilatation ranged from 13.3 to 48.9% (5, 6, 17, 20, 23). RV dilatation associated with elevated D-dimer levels and C-reactive protein levels was more common in patients with COVID-19 (6, 17, 18, 20). There was no significant difference in the incidence of major comorbidities (hypertension, diabetes and known coronary artery disease), laboratory markers of inflammation (white blood cell count, C-reactive protein) or myocardial injury (troponin) in patients with right ventricular dilatation (17).

Conventional echocardiographic parameters are not sensitive to early RV systolic dysfunction, and therefore, cannot be used for early diagnosis (34). Two-dimensional speckle tracking echocardiography can more accurately evaluate myocardial function and detect subclinical cardiac functional impairment earlier than conventional echocardiography (35, 36), which can measure LV global longitudinal strain (LVGLS), RV longitudinal strain (RVLS), RV free wall strain (RVFWS), and RV global strain (RVGS). In a retrospective study, RVLS was found to predict mortality in patients with COVID-19 more accurately. Therefore, there is potential value of RVLS for risk stratification in COVID-19. The optimal cut-off values for prediction of outcome were calculated to be −23% for RVLS, 43.5% for RV fractional area change, and 23 mm for tricuspid annular systolic displacement (7). Baycan et al. (24) and Krishnamoorthy et al. (25) also evaluated the prognostic value of strain indices. RVGS and RVFWS were significantly reduced in patients with poor clinical outcomes. RVLS is an independent predictor of in-hospital mortality in patients with COVID, while the predictive value of LVGLS for mortality varies in different studies. However, speckle-tracking echocardiography is demanding on image quality. The structure of the chest wall in different patients has a large effect on imaging, and critically ill patients are unable to cooperate in adjusting positions, both of which affect the results.

RV Dysfunction and Prognosis in COVID-19

Cardiac imaging findings have shown that RV damage is common in patients with COVID-19. Concomitant RV damage usually indicates a poor prognosis and affects the clinical outcome of patients. In a study of 120 COVID-19 cases, non-survivors showed elevated pulmonary artery systolic pressure, dilated right heart chambers, and diminished RV function compared with survivors (7). In another study where 28 patients died of COVID-19, 14 had a RV abnormality, but only 2 had LV impairment (6). Indeed, these outcomes all indicate a strong relation between RV dysfunction and poor prognosis. One multivariate analysis revealed that RV enlargement was the only factor significantly associated with mortality (17). Patients with COVID-19 and RV dysfunction often have more severe symptoms (19). Argulian et al. found that renal dysfunction was more common in patients with RV dilatation than those without (17). Therefore, RV dysfunction often predicts the presence of some severe complications, and they may partly account for the high mortality in this population. Additionally, Pagnesi et al. (21) showed that pulmonary hypertension, instead of RV dysfunction, was associated with worse in-hospital clinical outcomes in patients with COVID-19. However, because their

study population was non-intensive care unit patients without mechanical ventilation, this may have eliminated the association between COVID-19 and RV involvement.

Although CMR imaging is the gold standard for assessing RV function (30), the high infectivity of COVID-19 and the inability of patients to hold their breath for a long time limit its application. Patients without pre-existing cardiovascular diseases are more likely to have normal echocardiography than those with pre-existing cardiovascular diseases (21). RV dysfunction is more common than LV dysfunction in COVID-19 (23). Patients with RV dysfunction had a higher rate of cardiac comorbidities compared with patients without RV dysfunction (37). The main reasons for performing echocardiography in the previous study were suspected heart failure and elevated cardiac biomarker concentrations (5, 21, 23). Independent predictors of RV abnormalities are suspected RV failure and moderate or severe COVID-19 symptoms (21). To minimize the risk of the spread of infection, at least echocardiography should be performed in patients with suspected heart failure, more cardiac comorbidities, elevated cardiac biomarkers, and severe COVID-19 symptoms. Abnormal transthoracic echocardiography ultimately affects decision-making of clinicians in 16–33.3% of patients (18, 19). It also showed that clinical management was altered in 24.2% of patients because of acute cardiovascular events observed with transthoracic echocardiography (18).

Male was an independent predictor of prognosis (7), while age, weight, and ethnicity were not significantly different in COVID-19 patients with cardiac injury. Patients with a history of established cardiovascular disease or elevated cardiac biomarkers have an increased susceptibility to infection and an increased risk of severe disease progression and death (4, 37, 38). These patients are more likely to have RV dysfunction and pulmonary hypertension, which are independent risk factors for poor prognosis (21, 22). The proportions of echocardiographic abnormalities and serious heart disease are similar after excluding patients with pre-existing heart disease (heart failure, valvular disease, or ischemic heart disease), suggesting that cardiac abnormalities are associated with COVID-19 infection in this population (19).

AETIOLOGY OF COVID-19 WITH RV FUNCTIONAL CHANGES MAY INVOLVE MULTIPLE FACETS

The Right Ventricle Is More Susceptible to Lung Injury Than the Left Ventricle

The transverse section of the right ventricle is crescent-shaped compared with the thick wall of the left ventricle, and the relative surface area of the right ventricle is higher and the volume is lower. The thin RV free wall has greater compliance than the left ventricle. These anatomical features allow acute dilatation of the right ventricle when there is a sharp increase in afterload. RV systolic function is sensitive to increased pressure, and a slight rise in pulmonary circulation resistance causes RV overload and impaired systolic function. The primary target organ of severe acute respiratory syndrome coronavirus-2 is the lungs. The right ventricle is vulnerable to a slight increase in pulmonary vascular resistance (39), making it more vulnerable to injury than the left ventricle. As the right ventricle continues to expand, RV geometry changes, and the tricuspid annulus dilates insufficiently, resulting in tricuspid regurgitation. Tricuspid regurgitation leads to further RV dilatation and volume overload, which shifts the interventricular septum to the left and affects LV filling and contraction. RV pressure overload increases wall tension, increases myocardial oxygen consumption, and decreases RV oxygen supply during systole. This further leads to myocardial ischaemia and reduces RV contractility. RV dilatation may precede development of acute cor pulmonale (40).

Acute Respiratory Distress Syndrome and RV Dysfunction

COVID-19 mainly affects the respiratory system and the incidence of acute respiratory distress syndrome (ARDS) reported in COVID-19 ranges from 19.6 to 31% (37, 38, 41). ARDS is a severe form of COVID-19, which leads to a dramatic increase in RV afterload and delayed contraction owing to its own pathological effects and mechanical ventilation with a high positive end-expiratory pressure (PEEP). This then reverses the end-systolic transseptal pressure gradient. The incidence of RV dysfunction in ARDS has been reported to be 22–50% (33). There is no robust evidence to verify a definitive causal relationship between RV dysfunction and mortality in ARDS. However, RV dysfunction is undoubtedly associated with increased mortality and poorer prognosis in patients with COVID-19-related ARDS (42). In the setting of ARDS, numerous factors can destroy the pulmonary circulation, including mechanical compression by interstitial oedema, microvascular thrombosis, hypoxic or mediator-induced pulmonary vasoconstriction, and pulmonary vascular muscular remodeling. These factors raise pulmonary arterial pressure and further rapidly increase RV afterload. Pulmonary vascular resistance abates RV ejection and LV pulmonary venous return, while RV dilatation results in LV compression by a septal shift because of an inextensible pericardium. Both of these mechanisms account for the decrease in LV ejection and RV coronary blood flow. Therefore, ARDS-derived pulmonary circulation injury in COVID-19 has a deleterious effect on RV dysfunction (43, 44).

RV dilatation secondary to mechanical ventilation during hospitalization for ARDS requires attention. In the ARDS population, a lung protective ventilation strategy is recommended and mainly refers to PEEP. High PEEP levels cause overinflation of the normal alveoli and compression of intra-alveolar vessels, which lead to high pulmonary vascular resistance and increased RV afterload (43). Therefore, RV dysfunction can be a haemodynamically significant and deleterious consequence of COVID-19-related mechanical ventilation. Notably, Sud et al. showed that there was no meaningful correlation between PEEP and RV dilation on echocardiography in their COVID-19 infection cohort (17). However, they did not deny the possible contribution on RV dilatation from mechanical ventilation.

Pulmonary Embolism and RV Dysfunction

Owing to risk factors, such as virus-induced endothelial injury, vascular inflammation, and hospitalization-related prolonged immobilization, most patients with COVID-19 stay in a hypercoagulable state, and they are vulnerable to venous thrombosis. Poissy et al. studied 107 patients with COVID-19 who were admitted to the intensive care unit (45). They reported a high incidence of pulmonary embolism (20.4%), which was significantly higher than the contemporaneous average level in patients with influenza and in in-hospital patients. An autopsy of patients with COVID-19 showed a high incidence of deep venous thrombosis (58%) and death-causing pulmonary embolism (33%) (46). When thrombus enters pulmonary vessels, it produces mechanical obstruction and stimulates endothelial cells and platelets to release vasoactive mediators (e.g., thromboxane A2, serotonin). This triggers obstruction-related vasoconstriction and increases RV afterload and pulmonary arterial pressure in patients. Oxygen demand from the right ventricle increases, while embolism-associated hypoxemia and hypotension decrease myocardial oxygen supply. This imbalance finally leads to RV dysfunction (47).

Myocardial Injury and a Cytokine Storm

Myocardial injury was recognized early in patients with COVID-19 in China, and it also partly accounts for RV dysfunction. Myocarditis can occur before pulmonary symptoms of shock (48). The possible mechanisms for myocardial injury are as follows. Angiotensin-converting enzyme 2 (ACE2) is highly expressed not only in the lungs, but also in the cardiovascular system, thus possibly mediating viral entry into cardiomyocytes to cause direct damage. Cardiac elevation of troponin-I levels is accompanied by an increase in other inflammatory markers, such as lactate dehydrogenase, ferritin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8). This could represent a cytokine storm syndrome or secondary haemophagocytic lymphohistiocytosis, which may result in cardiac involvement (49). After viral invasion into the body, T cells become activated, and they produce and release amounts of antiviral cytokines. Because of an imbalanced response among subtypes of T helper cells, a cytokine storm release is induced, which attributes to hyperactivation of monocytes/macrophages. This then leads to tissue damage to multiple organs and causes complications, such as ARDS and cardiac insufficiency.

In ARDS, increased levels of cytokines, such as IL-6, IL-8, TNF-α, can be tested. In particular, IL-6 is an important marker. A previous study reported that elevated circulating IL-6 levels were associated with increased mortality in COVID-19 (50). Targeted therapy against the IL-6 receptor with tocilizumab can be effective in severe COVID-19 cases. A cytokine storm is essentially a protective response to limit spread of the virus, but its exact mechanism of myocardial injury remains unclear. However, cardiomyocyte and endothelial cell death triggered by inflammatory cytokines, such as TNF-α, has been well-documented (51). Ventricular dilatation with a reduction in the ejection fraction may be an adaptive response to myocardial dysfunction. Myocardial depression results from the direct or indirect action of one or more cardioinhibitory substances. Besides, TNF-α and IL-1, which act as potent inducible nitric oxide synthase inducers, are associated with inhibition of cardiomyocyte function. For one thing, nitric oxide interferes with calcium metabolism in cardiomyocytes, which in turn impairs contractile function. For another, peroxynitrite generated by interaction of nitric oxide with superoxide ions is directly toxic to cardiomyocytes (52). Additionally, Hypoxemia caused by COVID-19 can also induce intracellular calcium overload, leading to apoptosis of cardiomyocytes (53). So, an inflammatory storm, as well as autoimmune activation, can induce extensive vascular and myocardial inflammation, while predisposing to diffuse thrombosis (54).

In summary, the mechanism of myocardial injury varies at different stages of COVID-19. Isolated RV dysfunction can be found in the presence of severe ARDS or pulmonary embolism (55), while diffuse myocardial damage caused by viral toxicity and the host immune response also partly weaken RV function (**Figure 1**). Because of ACE2 expression in the endothelium, virus-induced endothelial shedding and microvascular damage may lead to thrombosis and myocardial infarction (55). ACE2-mediated direct injury may be a major mechanism in the early stages of COVID-19. With aggravation of COVID-19, pulmonary and cardiac injury caused by hypoxia is gradually aggravated. Inflammatory reactions and autoimmune damage leading to exacerbation of disease play a major role in the later stages of COVID-19.

TREATMENT OF RV DYSFUNCTION WITH COVID-19

Medical Treatment

Medical treatment of RV functional impairment includes reducing volume load, enhancing RV contractility, and reducing pulmonary arterial pressure. Diuretics can reduce intravascular volume. The RV Starling curve is flat, and improvement in RV function can only be observed with a large negative fluid balance. Normally, the RV filling pressure needs to be maintained at a slightly increased level at ∼8–12 mmHg. The volume status can be further adjusted on this basis to achieve optimal RV function and cardiac output (56). RV pressure monitoring is also important when circulating hypovolemia results in decreased blood pressure and the requirement for appropriate fluid replacement. Central venous pressure and mixed venous oxygen saturation help determine RV filling and oxygen supply. Echocardiography also helps determine the volume status. RV dilatation with restriction of LV filling indicate excessive preload.

Levosimendan is a novel calcium sensitizer that stabilizes the spatial configuration of myocardial fibrin and increases myocardial contractility. This calcium sensitizer has the advantages of no effect on diastolic function or arrhythmia, and does not increase myocardial oxygen consumption. Levosimendan improves RV myocardial contractility and reduces RV afterload. Morelli et al. showed that levosimendan was an effective treatment option for ARDS with acute right heart dilatation, and it was believed to dilate the pulmonary circulation and improve RV contractility (57).

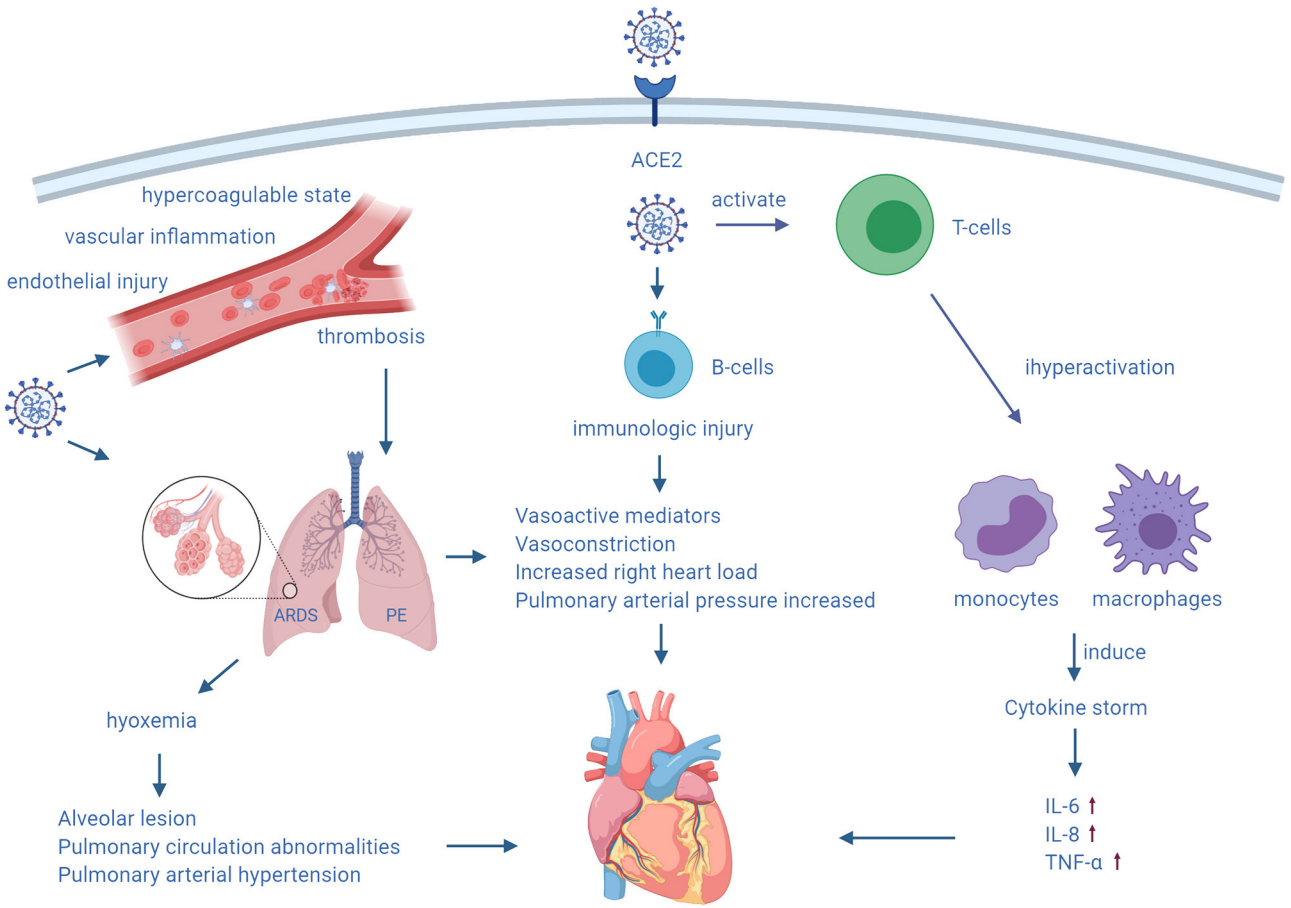

FIGURE 1 | Mechanism of RV damage caused by COVID-19.

Norepinephrine might improve RV function by restoring RV perfusion pressure as suggested in an experimental model of massive pulmonary embolism (58). Intravenous epoprostenol can improve symptoms, hemodynamics, and the survival rate, and enhance RV systolic function (59). Bosentan is a specific endothelin receptor antagonist, which reduces mean pulmonary arterial pressure and increases the cardiac index. Inhaled nitric oxide can selectively dilate pulmonary vessels, improve the ventilation-blood flow ratio, significantly reduce pulmonary vascular resistance, and increase cardiac output, while it has a slight effect on systemic vascular resistance. In patients with pulmonary heart disease caused by ARDS, inhaled nitric oxide reduces pulmonary arterial pressure and pulmonary inflammatory responses (60). In patients with pulmonary embolism and ARDS, prostacyclin is as effective as inhaled nitric oxide in reducing pulmonary arterial pressure, improving gas exchange and oxygenation, increasing cardiac output, and improving RV function (61, 62).

To alleviate inflammation and fibrosis, corticosteroids are considered as potential therapeutic agents for ARDS, which reduce morbidity and mortality, but remain controversial. High-dose corticosteroid therapy can accelerate improvement of ARDS, reduce mortality, and shorten the duration of invasive mechanical ventilation (63). However, the World Health Organization recommends that systemic corticosteroids should not be routinely used in patients with COVID-19 or COVID-19-associated ARDS (64).

Severe COVID-19 is often associated with thrombosis, and disseminated intravascular coagulation may be present in the majority of fatal cases (65). Prolonged immobilization and hormonal therapy increases the risk of venous thromboembolism. Patients with right heart enlargement are also prone to cardiac thrombosis. Coagulopathy due to COVID-19 may be associated with bacterially-induced infectious coagulopathy. Overproduction of inflammatory cytokines, vascular endothelial injury, and increased levels of damage-associated molecular patterns contribute to thrombosis. Patients who meet the sepsis-induced coagulopathy score criteria or have significantly elevated D-dimer levels may benefit from anticoagulant therapy by mainly using low-molecular-weight heparin (66). Among 449 patients with severe COVID-19, 99 received heparin (mainly low-molecular-weight heparin)

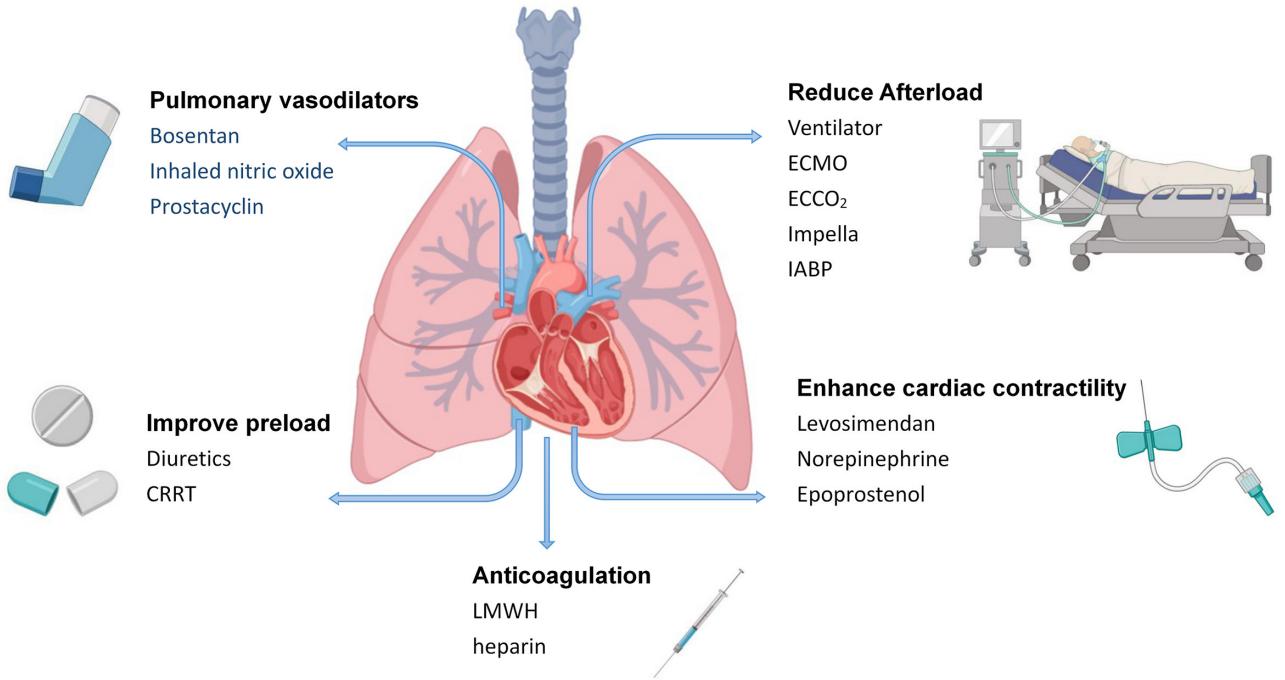

FIGURE 2 | Treatment of RV dysfunction with COVID-19. $ECCO_2$, extracorporeal carbon dioxide removal devices; IABP, intra-aortic balloon pump; LMWH, low molecular weight heparin.

for 7 days or longer, and 28-day mortality was significantly lower in patients with sepsis-induced coagulopathy scores $\geq$ 4 or D-dimer levels $>$ six times the upper limit of normal using heparin than in non-users ($P = 0.029$, $P = 0.017$). Lin et al. (67) also recommended the use of low-molecular-weight heparin in patients with D-dimer values $>$ four times the upper limit of normal. Thrombotic coagulopathy is common in severe patients with COVID-19, and D-dimer is more useful than other coagulation markers for prediction of this disease. However, bleeding complications are relatively uncommon in COVID-19. Therefore, anticoagulant therapy is necessary.

Device-Assisted Therapy

Mechanical ventilation, sedation, and analgesia may lead to increased afterload, increased transpulmonary pressure, and decreased cardiac output. Therefore, mechanical ventilation indications need to be strictly followed. For critically ill patients requiring mechanical ventilation, appropriate mechanical ventilation measures should be implemented to avoid hypoxemia, hypercapnia, a low or high lung volume, and high PEEP. Protective ventilation strategies should also be used when necessary. The principle of mechanical ventilation in patients with right heart failure is to limit plateau pressure and offer PEEP, avoiding hypercapnia, hypoxemia, and pulmonary vasoconstriction. Respiratory settings are adjusted according to the tolerance of the right ventricle, as assessed by ultrasound, to coordinate the balance between recruitment and hyperventilation resulting from ventilation according to RV function (68). PEEP can dilate the alveoli, compress extra-alveolar capillaries, and cause an increase in pulmonary vascular resistance. This increases afterload and RV volume, resulting in RV dilatation, which in turn affects LV filling. Appropriate PEEP is important for treatment.

When optimized ventilation measures still do not improve hypoxemia in mechanically ventilated patients, extracorporeal membrane oxygenation (ECMO) can be considered. ECMO is used as a rescue therapy for COVID-19 with refractory hypoxemia in accordance with provisional guidelines established by the World Health Organization (69) in 2020. However, because of a lack of relevant trials on the use of ECMO in patients with COVID-19, there is insufficient evidence that these patients can benefit from ECMO. For acute RV failure caused by severe ARDS, extracorporeal carbon dioxide removal devices can be considered for super-protective lung ventilation (tidal volume: 4 mL/kg) (70). Additionally, increased work of breathing, pulmonary oedema, and endogenous PEEP caused by weaning increase RV afterload. Worsening of RV function is an important cause of weaning failure in mechanically ventilated patients. When RV function is impaired in combination with severely impaired LV function, adjunctive therapy with Impella device or intra-aortic balloon counterpulsation can be used. Continuous renal replacement therapy may be considered when volume overload and drug therapy are not effective. COVID-19-related myocardial injury treatments are summarized in **Figure 2**.

FUTURE DIRECTIONS AND CONCLUSIONS

RV dysfunction usually indicates a poor prognosis in the wide array of cardiopulmonary diseases. Assessment of RV function is essential for managing ARDS, acute pulmonary embolism, and pulmonary hypertension. RV dilatation is common in patients with COVID-19. A full understanding of COVID-19-related RV dysfunction is conducive for early identification and precise treatment, and to help improve the prognosis of severe cases and reduce mortality. Early recognition of RV dysfunction allows appropriate treatment to be provided as soon as possible. How to identify RV dysfunction early is important for stratification of disease risk and prognostic evaluation. Echocardiography, cardiac MRI, right heart catheterization, and other examinations are helpful for early identification of RV dysfunction. At the same time, monitoring of biological indicators related to RV function, such as troponins and brain natriuretic peptide, should not be ignored for the suggestive role in RV function. It is recommended to assess RV function as soon as possible, for COVID-19 patients with suspected cardiac injury, elevated cardiac biomarkers, severe respiratory symptoms. RV function is often monitored to optimize haemodynamic and respiratory parameter settings. Timely medical treatment should be delivered. And device assistance should be implemented if necessary. RV damage reflects an association between myocardial injury and COVID-19. In future medical care, clinicians need to further focus on the morbidity of RV dysfunction in patients with COVID-19. Using cardiac imaging to detect RV dysfunction will provide early information concerning the severity of COVID-19 infection. Performing an appropriate strategy of the right ventricle will be helpful to reduce mortality and improve prognosis in this persistent epidemic.

AUTHOR CONTRIBUTIONS

WL contributed conception and constructing the overall structure and contents. YL wrote the draft and sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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REFERENCES

1. Medicine JHUa. *COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins.* (2020). Available online at: https://coronavirus.jhu.edu/map.html (accessed December 6, 2020).
2. Chen C, Chen C, Yan JT, Zhou N, Zhao JP, Wang DW. Analysis of myocardial injury in patients with COVID-19 and association between concomitant cardiovascular diseases and severity of COVID-19. *Zhonghua Xin Xue Guan Bing Za Zhi.* (2020) 48:567–71. doi: 10.3760/cma.j.cn112148-20200225-00123
3. 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
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. 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
6. 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
7. 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
8. Matsuura H, Ichida F, Saji T, Ogawa S, Waki K, Kaneko M, et al. Clinical features of acute and fulminant myocarditis in children-2nd nationwide survey by japanese society of pediatric cardiology and cardiac surgery. *Circ J.* (2016) 80:2362–8. doi: 10.1253/circj.CJ-16-0234
9. Ito T, Akamatsu K, Ukimura A, Fujisaka T, Ozeki M, Kanzaki Y, et al. The Prevalence and findings of subclinical influenza-associated cardiac abnormalities among Japanese patients. *Intern Med.* (2018) 57:1819–26. doi: 10.2169/internalmedicine.0316-17
10. Bavishi C, Bonow RO, Trivedi V, Abbott JD, Messerli FH, Bhatt DL. Acute myocardial injury in patients hospitalized with COVID-19 infection: a review. *Prog Cardiovasc Dis.* (2020) 6:682–9. doi: 10.1016/j.pcad.2020.05.013
11. 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
12. Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment (7th edition). Published by China National Health Commission on March 4, 2020. Available online at: http://kjfy.meeting.so/msite/news/show/cn/3337.html (accessed March 16, 2020).
13. 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
14. 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). doi: 10.1016/j.jcmg.2020.05.004
15. 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
16. 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) 13:e204916. doi: 10.1001/jamacardio.2020.4916
17. 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
18. Jain SS, Liu Q, Raikhelkar J, Fried J, Elias P, Poterucha TJ, et al. Indications and findings on transthoracic echocardiography in COVID-19. *J Am Soc Echocardiogr.* (2020) 33:1278–84. doi: 10.1016/j.echo.2020.06.009
19. 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) 2:949–58. doi: 10.1093/ehjci/jeaa178
20. 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. *Res Rep Clin Cardiol.* (2020) 109:1491–9. doi: 10.1007/s00392-020-01683-0

21. 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) 106:1324–31. doi: 10.1136/heartjnl-2020-317355
22. D'Andrea A, Scarafile R, Riegler L, Liccardo B, Crescibene F, Cocchia R, et al. Right ventricular function and pulmonary pressures as independent predictors of survival in patients with COVID-19 pneumonia. *JACC: Cardiovasc Imaging.* (2020) 13:2467–8. doi: 10.1016/j.jcmg.2020.06.004
23. 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
24. 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 Cardiovas Imag.* (2020) 15:1–10. doi: 10.1007/s10554-020-01968-5
25. Krishnamoorthy P, Croft LB, Ro R, Anastasius M, Zhao W, Giustino G, et al. Biventricular strain by speckle tracking echocardiography in COVID-19: findings and possible prognostic implications. *Future Cardiol.* (2020). doi: 10.2217/fca-2020-0100
26. van den Heuvel FMA, Vos JL, Koop Y, van Dijk APJ, Duijnhouwer AL, de Mast Q, et al. Cardiac function in relation to myocardial injury in hospitalised patients with COVID-19. *Neth Heart J.* (2020) 28:410–7. doi: 10.1007/s12471-020-01458-2
27. 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
28. Ferreira VM, Schulz-Menger J, Holmvang G, Kramer CM, Carbone I, Sechtem U, et al. Cardiovascular magnetic resonance in non-ischemic myocardial inflammation: expert recommendations. *J Am Coll Cardiol.* (2018) 72:3158–76. doi: 10.1016/j.jacc.2018.09.072
29. Kammerlander AA, Marzluf BA, Zotter-Tufaro C, Aschauer S, Duca F, Bachmann A, et al. T1 mapping by cmr imaging: from histological validation to clinical implication. *JACC Cardiovasc Imaging.* (2016) 9:14–23. doi: 10.1016/j.jcmg.2015.11.002
30. 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
31. Puntmann VO, Valbuena S, Hinojar R, Petersen SE, Greenwood JP, Kramer CM, et al. Society for Cardiovascular Magnetic Resonance (SCMR) expert consensus for CMR imaging endpoints in clinical research: part I—analytical validation and clinical qualification. *J Cardiovasc Magn Reson.* (2018) 20:67. doi: 10.1186/s12968-018-0484-5
32. 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
33. Zochios V, Parhar K, Tunnicliffe W, Roscoe A, Gao F. The Right Ventricle in ARDS. *Chest.* (2017) 152:181–93. doi: 10.1016/j.chest.2017.02.019
34. Carluccio E, Biagioli P, Alunni G, Murrone A, Zuchi C, Coiro S, et al. Prognostic value of right ventricular dysfunction in heart failure with reduced ejection fraction: superiority of longitudinal strain over tricuspid annular plane systolic excursion. *Circ Cardiovasc Imaging.* (2018) 11:e006894. doi: 10.1161/CIRCIMAGING.117.006894
35. Li Y, Xie M, Wang X, Lu Q, Zhang L, Ren P. Impaired right and left ventricular function in asymptomatic children with repaired tetralogy of Fallot by two-dimensional speckle tracking echocardiography study. *Echocardiography.* (2015) 32:135–43. doi: 10.1111/echo.12581
36. Park SJ, Park JH, Lee HS, Kim MS, Park YK, Park Y, et al. Impaired RV global longitudinal strain is associated with poor long-term clinical outcomes in patients with acute inferior STEMI. *Jacc-Cardiovasc Imag.* (2015) 8:161–9. doi: 10.1016/j.jcmg.2014.10.011
37. 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
38. 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
39. Repesse X, Charron C, Vieillard-Baron A. Right ventricular failure in acute lung injury and acute respiratory distress syndrome. *Minerva Anestesiol.* (2012) 78:941—8.
40. Boissier F, Katsahian S, Razazi K, Thille AW, Roche-Campo F, Leon R, et al. Prevalence and prognosis of cor pulmonale during protective ventilation for acute respiratory distress syndrome. *Intensive Care Med.* (2013) 39:1725–33. doi: 10.1007/s00134-013-2941-9
41. 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
42. Li X, Ma X. Acute respiratory failure in COVID-19: is it "typical" ARDS? *Crit Care.* (2020) 24:198. doi: 10.1186/s13054-020-02911-9
43. Nobre C, Thomas B. Right ventricle in ARDS. *Chest.* (2017) 152:215–6. doi: 10.1016/j.chest.2017.04.163
44. Repesse X, Charron C, Vieillard-Baron A. Acute respiratory distress syndrome: the heart side of the moon. *Curr Opin Crit Care.* (2016) 22:38–44. doi: 10.1097/MCC.0000000000000267
45. 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
46. 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
47. Huisman MV, Barco S, Cannegieter SC, Le Gal G, Konstantinides SV, Reitsma PH, et al. Pulmonary embolism. *Nat Rev Dis Primers.* (2018) 4:18028. doi: 10.1038/nrdp.2018.28
48. 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
49. Khan IH, Zahra SA, Zaim S, Harky A. At the heart of COVID-19. *J Cardiac Surg.* (2020) 35:1287–94. doi: 10.1111/jocs.14596
50. Ruan QR, Yang K, Wang WX, Jiang LY, Song JX. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Medicine.* (2020) 46:846–8. doi: 10.1007/s00134-020-05991-x
51. Zhu H, Rhee JW, Cheng P, Waliany S, Chang A, Witteles RM, et al. Cardiovascular complications in patients with COVID-19: consequences of viral toxicities and host immune response. *Curr Cardiol Rep.* (2020) 22:32. doi: 10.1007/s11886-020-01302-4
52. Nduka OO, Parrillo JE. The pathophysiology of septic shock. *Crit Care Clin.* (2009) 25:677–702. doi: 10.1016/j.ccc.2009.08.002
53. 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
54. 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
55. 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
56. Ventetuolo CE, Klinger JR. Management of acute right ventricular failure in the intensive care unit. *Ann Am Thorac Soc.* (2014) 11:811–22. d doi: 10.1513/AnnalsATS.201312-446FR
57. Morelli A, Teboul JL, Maggiore SM, Vieillard-Baron A, Rocco M, Conti G, et al. Effects of levosimendan on right ventricular afterload in patients with acute respiratory distress syndrome: a pilot study. *Crit Care Med.* (2006) 34:2287–93. doi: 10.1097/01.CCM.0000230244.17174.4F
58. Hirsch LJ, Rooney MW, Wat SS, Kleinmann B, Mathru M. Norepinephrine and phenylephrine effects on right ventricular function in experimental canine pulmonary embolism. *Chest.* (1991) 100:796–801. doi: 10.1378/chest.100.3.796
59. Kisch-Wedel H, Kemming G, Meisner F, Flondor M, Kuebler WM, Bruhn S, et al. The prostaglandins epoprostenol and iloprost increase

left ventricular contractility *in vivo*. *Intensive Care Med.* (2003) 29:1574–83. doi: 10.1007/s00134-003-1891-z
60. Hunt JL, Bronicki RA, Anas N. Role of inhaled nitric oxide in the management of severe acute respiratory distress syndrome. *Front Pediatr.* (2016) 4:74. doi: 10.3389/fped.2016.00074
61. Searcy RJ, Morales JR, Ferreira JA, Johnson DW. The role of inhaled prostacyclin in treating acute respiratory distress syndrome. *Ther Adv Respir Dis.* (2015) 9:302–12. doi: 10.1177/1753465815599345
62. Lang IM, Gaine SP. Recent advances in targeting the prostacyclin pathway in pulmonary arterial hypertension. *Eur Respir Rev.* (2015) 24:630–41. doi: 10.1183/16000617.0067-2015
63. Meduri GU, Siemieniuk RAC, Ness RA, Seyler SJ. Prolonged low-dose methylprednisolone treatment is highly effective in reducing duration of mechanical ventilation and mortality in patients with ARDS. *J Intensive Care Med.* (2018) 6:53. doi: 10.1186/s40560-018-0321-9
64. Organization WH. *Infection Prevention and Control During Health Care When Novel Coronavirus (ncov) Infection is Suspected.* (2020). Available online at: https://www.who.int/publications/i/item/10665-331495 (accessed March 19, 2020).
65. 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
66. Tang N, Bai H, Chen X, Gong JL, Li DJ, Sun ZY. 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
67. Lin L, Lu LF, Cao W, Li TS. Hypothesis for potential pathogenesis of SARS-CoV-2 infection-a review of immune changes in patients with viral pneumonia. *Emerg Microbes Infec.* (2020) 9:727–32. doi: 10.1080/22221751.2020.1746199
68. Repesse X, Charron C, Vieillard-Baron A. Acute cor pulmonale in ARDS: rationale for protecting the right ventricle. *Chest.* (2015) 14:259–65. doi: 10.1378/chest.14-0877
69. Organization WH. *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/i/item/10665-332299 (accessed January 12, 2020).
70. Tiruvoipati R, Botha JA, Pilcher D, Bailey M. Carbon dioxide clearance in critical care. *Anaesth Intensive Care.* (2013) 41:157–62. doi: 10.1177/0310057X1304100129

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Mitigating the Risk of COVID-19 Deaths in Cardiovascular Disease Patients in Africa Resource Poor Communities

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The novel coronavirus disease 2019 (Covid-19) pandemic has affected millions of patients in almost all countries with over one million cases recorded in Africa where it is a major health challenge. Covid-19 is known to have significant implications for those with pre-existing cardiovascular disease (CVD) and their cardiologists. Patients with pre-existing CVD are at increased risk of morbidity and mortality from Covid-19 due to associated direct and indirect life threatening cardiovascular (CV) complications. Mitigating the risk of such Covid-19 deaths in resource poor communities requires the institution of preventive measures at the primary, secondary and tertiary levels of preventive phenomenon with emphasis at the first two levels. General preventive measures, screening and monitoring of CVD patients for complications and modification of drug treatment and other treatment methods will need to be implemented. Health policy makers and manager should provide required training and retraining of CV health care workers managing Covid-19 patients with CVD, provision of health education, personal protective equipment (PPE), and diagnostic kits.

Keywords: COVID-19 deaths, cardiovascular disease, levels of prevention, Africa, resource poor communities

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INTRODUCTION

The novel corona virus disease referred to as Covid-19 was identified in December 2019 in Wuhan, China. It is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (1, 2) and the disease has since spread all over the world including resource poor communities in Africa (3).

Given the rapid spread of the virus in early 2020, the disease was declared a pandemic by the World Health Organization (WHO) on March 11, 2020 (2). Within a short time there was a litany of literature on the disease with physicians in all specialties expected to be aware of the impact of this disease in their respective clinical care areas and the medical community at large (4).

In Africa, the spread of Covid-19 was feared for so many reasons (5): Firstly, large and densely populated areas and townships with widespread poverty and high migration make such places vulnerable to airborne pandemics. Secondly, existing epidemics of human immunodeficiency virus (HIV), tuberculosis (TB), and malaria were thought to make Covid-19 more severe and thus lead to increased morbidity and mortality. Lastly, the high prevalence of non-communicable diseases in Africa such as hypertension, CVD and diabetes which are known risk factors for severe cases of Covid-19 portends a poor outcome (3), and this is of concern to the index authors.

The clinical presentation of this disease ranges from asymptomatic to mild, severe, and critical cases. Its symptoms which are similar to common viral and parasitic infections in sub-Saharan Africa include fever, cough, dyspnoea, myalgias, fatigue, and diarrhea. In severe and critical cases, it presents with pneumonia, acute respiratory distress syndrome (ARDS), cardiogenic, and septic shock. Over time, it was shown that elderly populations with pre-existing medical comorbidities are most vulnerable to severe disease (5–7).

In sub-Saharan Africa, the high prevalence of CVD and their relationship to the disease means cardiologists will be actively engaged in the management of Covid-19 patients. Aside Covid-19 infection being associated with CV complications, infected individuals with pre-existing CVD have elevated risk of severe disease and worse outcomes (8–10). Additionally, therapeutics for Covid-19 have potential adverse CV effects due to significant drug-drug interactions with regular CV medications. Finally, CVD drugs may interfere with the pathophysiology of Covid-19 especially with viral relationship to ACE2 receptors (11, 12).

The management of severe Covid-19 cases in patients with CVD and other high risk conditions is costly in resource poor countries of Africa, thus the need to activate primary and secondary levels of prevention. Presently, Covid-19 mortality in African countries are not as high as expected (5, 13). This is due to many factors including the implementation of primary and secondary preventive measures.

Hence, this review aim to discuss the need to mitigate Covid-19 deaths in CVD patients in resource poor countries of Africa, and the measures to be put in place toward realizing this goal.

VIROLOGY OF SARS-COV-2, EPIDEMIOLOGY AND PATHOPHYSIOLOGY OF COVID-19

The SARS-CoV-2 virus belongs to the family of Coronaviridae which are enveloped viruses with non-segmented, single stranded, positive-sense ribonucleic acid (RNA) genome (14). A number of the SARS—related coronaviruses have been found in bats, thus suggesting they may constitute the zoonotic host for SARS-CoV-2, especially given that the viral genome is 96.2% identical to a bat coronavirus (15). Typical of corona viruses such as SARS and the Middle East Respiratory syndrome virus (MERS-CoV), they commonly cause respiratory illnesses which are the predominant manifestation of Covid-19 disease. The infectivity of Covid-19 is greater than that of influenza with an estimated R_0 value of 2.28 (16). Similarly, death rate associated with Covid-19 is higher compared to <0.1% estimated recently for influenza by WHO, though it may be higher for the elderly, persons with comorbidities and persons in low resource settings (17). However, earlier coronaviruses infections such as SARS-CoV epidemic and MERS-CoV, had higher case fatality rates of 9.6 and 34.4%, respectively (18). Covid-19 disease however has spread more widely to affect larger populations and places than previous coronaviruses outbreaks (18, 19).

Since December 2019, Covid-19 disease has spread to all corners of the globe affecting over 37 million persons with more than 1 million deaths as of 11th October, 2020 (20) in over 100 countries across the world. Africa as of the same date has over 1 million cases with the highest number of 690,896 cases in South Africa and lowest of 414 in Eritrea. Nigeria with 60,103 cases of confirmed Covid-19 is the highest in West Africa sub-region (20). The crude case-fatality rate which was 3.8% in the USA in March 2020 (21, 22) fell to 2.8% in October, in same month it is 1.8% in Nigeria (20).

TABLE 1 | Reasons to mitigate the risk of Covid-19 deaths in CVD patients.

1.	Association between pre-existing CVD and severe Covid-19 disease.
2.	Life threatening CV complications are seen in Covid-19 patients with or without pre-existing CVD.
3.	Significant drug interactions exist between CVD medications and therapies under investigation for Covid-19.

The clinical cases can either be asymptomatic or mild in a large proportion of patients and severe in a smaller portion (18). In China it was found that 81.4% of cases were mild requiring only symptomatic treatment and isolation, with severe disease in 13.9% of cases that needed supplemental oxygen therapy, and critical in 4.7% requiring intensive care unit (ICU) treatment including mechanical ventilation (22).

Studies show that SARS-CoV-2 as well as other coronaviruses use angiotensin-converting enzyme 2 (ACE2) protein; a homolog of ACE1 (9) for cell entry. ACE2 which is a type 1 integral membrane protein is highly expressed in lung alveolar cells and may expose humans to increased viral entry (15). After ligand binding, SARS-CoV-2 enters cells via receptor-mediated endocytosis in a manner akin to entry of HIV viruses in to body cells (23). The viral take-over of ACE2 receptors in Covid-19 infection deregulates lung protective pathway occasioned by uninfected receptors, thus contributing to viral pathogenicity (24). ACE2 is found primarily in the lower respiratory tract, rather than the upper airways (10). This distribution can explain the few upper respiratory tract symptoms typical of flu and why Covid-19 is not just a common cold (10, 17).

Clinicians are concerned about a possible link between SARS-CoV-2 and angiotensin 2 receptor blockers (ARBs) which could increase chances of adverse effects of the disease in CVD patients on this class of antihypertensives (17), Hence it is important that doctors understand clinical and preventive measures to reduce morbidity and mortality from Covid-19 among CVD patients.

REASONS TO MITIGATE THE RISK OF COVID-19 DEATHS IN CVD PATIENTS

Several reasons portend the need to mitigate the risk of Covid-19 deaths in CVD patients (**Table 1**) details of which are given below.

Association Between Pre-existing CVD and Severe COVID-19 Disease

Different studies show the association between pre-existing CVD and severe Covid-19 disease. A meta-analysis of seriously ill Covid-19 patients found the prevalence of hypertension, cerebrovascular disease and diabetes to be 17.1, 16.4, and 9.7%, respectively, among them (8). The overall case fatality rate in Covid-19 patients is commonly <3% (18), but this increases to 10.5% in those with CVD, 7.3% in diabetes, and 6.3% in hypertensives (18).Similar findings showing more adverse events in CVD patients with Covid-19, have been reported in other investigations, whether in China, Europe, or sub-Saharan Africa (13, 25). In Ghana, the highest number of deaths occurred in Covid-19 patients with pre-existing hypertension and diabetes (13). This number will go up as more people become seriously ill with Covid-19 in the sub-region due to inadequate facilities and personnel.

Aside hypertension, other factors associated with increased deaths are age, diabetes, and hyperlipidemia. Age is a risk factor for hypertension, obesity, glucose intolerance, and reduced immunity (25–27); which are associated with increased risk of severe Covid-19 disease. Diabetes and hyperlipidemia causes dysregulation of the immune system in addition to deterioration of vascular integrity (27, 28). Thus, prevalent CVD may be a marker of accelerated immunologic aging/dysregulation and relate indirectly to Covid-19 prognosis. Other possible risk factors for severe disease in low income countries of sub-Saharan Africa include HIV, TB, Chronic Obstructive Pulmonary disease (COPD), Rheumatic Heart Disease (RHD), and cardiomyopathies (29).

Cardiovascular Complications of Covid-19 in Patients With or Without Pre-existing CVD

Several investigations suggest SARS-CoV-2 infection is associated with life threatening CV complications in those with or without pre-existing CVD (10, 18). The CV complications includes myocarditis, acute coronary syndromes, arrhythmias, heart failure, cardiogenic shock, and venous thromboembolism. The recognition of these complications must be possible in health facilities in Africa for improved survival of cardiac patients.

Myocarditis and Acute Coronary Syndromes

Myocardial injury is increased in patients with myocarditis and acute coronary syndrome as a results of ARDS and severe Covid-19 (10, 30, 31). Elevated serum troponin levels are seen in many Covid-19 patients, with significant differences noted in survivors and those who succumbed to the viral disease (32). Some authors found that mean cardiac troponin I levels was significantly higher in severe Covid-19-illness compared to non-severe disease (33).

Increased levels of troponin T (TnT) has been found to be associated with Covid-19 disease, especially in those with pre-existing CVD. Of note, the highest mortality rates were observed in those with elevated TnT levels whether due to Covid-19 or prior CVD.

Other markers of acute cardiac injury in Covid-19 patients are abnormal electrocardiographic and echocardiographic findings in patients.

Cardiac Arrhythmia and Cardiac Arrest

The arrhythmias observed in severe Covid-19 infections are cause for concern as it is a significant contributor to adverse outcomes (23). Arrhythmogenesis appears to be a feature of coronaviruses as these have been reported in SARS and MERS patients. The different forms of dysarrhythmias in coronaviruses infections include branch block, atrial fibrillation, premature beats, QT interval elongation, and even sudden cardiac death (34). In hospitalized Covid-19 patients, cardiac arrhythmias were noted in 16.7% of patients in a Chinese cohort especially in those in ICU (6). Up to 60% of fatal cases of Covid-19 have arrhythmias and in some patients the cardiac arrhythmias are independently associated with in-hospital mortality (35). This is more so as African Americans have been found to have genetic susceptibility for Covid-19 associated sudden cardiac death (36). It is advised that new onset of malignant tachyarrhythmias in the setting of troponin elevation should raise the suspicion of underlying myocarditis or acute coronary syndrome and potential arrhythmias (32, 37).

Arrhythmias should be considered a major complication of Covid-19 and be watched out for when medications are being considered in resource poor settings.

Cardiomyopathy and Heart Failure

Heart failure was reported in 23.0% of patients with Covid-19 presentations (10). Whether heart failure is most commonly due to exacerbations of pre-existing left ventricular (LV) dysfunction or new cardiomyopathy is unclear (38). Right heart failure and associated pulmonary hypertension should be considered, in particular in the context of severe parenchymal lung disease and ARDS, which are common findings in severe Covid-19 disease.

Cardiogenic and Mixed Shock

The appearance of ground glass opacities in severe Covid-19 patient similar to that in ARDS on chest imaging (39) should be distinguished from that of coexisting cardiogenic pulmonary oedema. A possibility of *in situ* cardiogenic or mixed cardiac plus primary pulmonary causes of respiratory manifestations in Covid-19 (mixed presentation), should be considered in clinical assessment of patients.

Venous Thromboembolic Disease

Patients with Covid-19 are at increased risk of venous thromboembolism and this is said to be as high as 31% in critically ill subjects (40). Studies suggest abnormal coagulation parameters like D-dimers are very useful in the diagnosis (41). In various places, elevated D-dimer levels (>1 g/l) are strongly associated with in-hospital death, even after multivariable adjustment (10, 41). The elevation of D-dimers and FDP (fibrin degradation products) are synonymous with poor survival in severely ill Covid-19 patients as this may indicate presence of disseminated intravascular coagulation (DIC) (41).

TABLE 2 | Drug therapy and Covid-19: interactions and cardiovascular complications.

1.	Therapies under investigation for Covid-19 may have significant drug-drug toxicity with CV medications.
2.	Therapies under investigation for Covid-19 have significant CV toxicities.
3.	Patient debilitation from severe Covid-19 may pose challenges in administering routine CV medications
4.	Drugs for patients with CVD could interfere with the pathophysiology of Covid-19

Drug Therapy and Covid-19: Interactions and Cardiovascular Complications

There are currently no specific effective therapies for Covid-19. However, it is worthy to note that significant drug interactions exist between CVD medications and therapies under investigation for Covid-19 (12, 42, 43) (**Table 2**).

MITIGATING THE RISK OF COVID-19 DEATHS IN CVD PATIENTS IN AFRICA RESOURCE POOR COMMUNITIES

Efforts to reduce Covid-19 deaths in CVD patients should involve three levels of prevention; primary, secondary, and tertiary levels. Primary prevention measures are those are put in place before the onset of illness. Secondary prevention refers to measures that ensure early diagnosis and prompt treatment, before development of CV complications. The tertiary prevention strategy is aimed at rehabilitation following significant illness.

In resource poor communities in sub-Saharan Africa, emphasis should be on primary and secondary preventive measures due to unsustainable financial requirements for tertiary measures of prevention.

Control measures will vary between:

1. Patients with CVD without Covid-19.
2. Patients with coexistence of CVD and Covid-19.
3. CV health workers.

Adequate consideration should be given to patients in resource poor communities where other immunosuppressive conditions such as HIV and TB could coexist with Covid-19 and CVD. Lifestyle measures, drug treatment and method of treatment modifications, as well as availability of necessary protective and medical equipment will all be required. Health care workers are also at risk of infection and should be protected.

Mitigating the Risk of Death in Patients With Pre-Existing CVD Without Covid-19

Measures to reduce the risk of death in resource poor settings should be emphasized at the primary and secondary levels of prevention for sustainability. Since disease transmission occurs most commonly via respiratory droplets and aerosols with the virus active on surfaces for several days (44), the recommendations are suggested for general prevention include:

a. All aged CVD patients should be taught to avoid close contact by practicing social distancing of at least 2 m away. They should be trained in community and personal hygiene and this should be more so with the uneducated subjects.
b. As much as possible, patients with known risk factors should avoid crowds, especially in door assembly. Possibly, very vulnerable subjects should practice voluntary isolation but be able to receive support from family members to prevent depressive events. This isolation is important for those in major congested cities in Africa (13).
c. Everyone must reduce or avoid touching their eyes, nose, and mouth, when up and about in their location where surfaces may be contaminated (44).
d. Subjects must wash their hands frequently under running water. The alternative is to use alcohol (65% w/v ethyl alcohol) based hand sanitizers for similar purpose.
e. The use of face masks should be mandatory for CVD subjects in resource poor settings.
f. Pseudo-telemedicine approaches such as use of internet based telephone consultations; these include WhatsApp and Facebook videos which are popular in Africa and can be used for patient consultations, during pandemics to reduce travels and social contacts at hospitals. This can help to promote viral containment (45).

Mitigating the Risk of Death in Covid-19 Patients With Pre-existing CVD in Sub-Saharan Africa

The majorly secondary and feasible tertiary levels of prevention features are as follows:

a. Screening for Covid-19 in all CVD patients for early diagnoses, especially when they are susceptible groups like health and other frontline workers, or those with immune compromise. This will enhance early diagnosis and closer monitoring for CV complications (10, 18) in resource poor communities.
b. Screening of Covid-19 patients for CVD and CV complications—The American College of Cardiology (ACC) has recommended the establishment of protocol for diagnosis, triage, and isolation of Covid-19 patients with CVD or CV complications (46).
c. Telemedicine and e-visits—as mentioned above, with the wide availability of cell phones in resource poor communities in sub-Saharan Africa consultations can be made by patients with specialty physicians without close contact.
d. Clear and prompt understanding of the effects of the virus and hypertension therapy in relation to ACE inhibitors and ARB therapy in Covid-19 patients, should be given early to reduce clinician and patient confusion (47). All CVD patients should be encouraged to continue their home blood pressure monitoring and medical regimen (48).
e. All drug—drug interactions with CV medications and direct CV toxicities should be avoided or reduced to the barest minimum, by retraining of clinicians and other healthcare workers.

f. As a tertiary measure, nationwide training of health workers on mechanical ventilatory support and Advanced Cardiac Life Support (ALCS) and all citizens on cardiac and vocational rehabilitation post Covid-19, should be commenced pending availability of resources for full implementation.

Recommendations for Healthcare Workers Managing CVD During Covid-19 Pandemic

a. Ensure the use of provided PPE and in the right manner as recommended by WHO, CDC and China's CDC, namely: facemask, goggles, disposable, or re-useable gowns and gloves (49–52).
b. Telemedicine and e-visits—this allows for triaging of patients and patient management while minimizing exposure of patients and health workers to potential infection.
c. Health care practitioners must be conversant with antiviral agents approved or under investigation for the treatment of Covid-19 and their CV toxicities (53).
d. Carefully managed rescheduling of elective procedures during the growth phase of the outbreak.
e. Ensure hospital equipment such as echocardiography, scanners et cetera are cleaned with antiseptic agents before and after each use.
f. When performing procedures that generates aerosol such as transesophageal echocardiography, endotracheal intubation, cardiopulmonary resuscitation, and bag mask ventilation, additional PPE may be required including controlled or powered air purifying respirators. Thorough infection control measures specific to the procedural cardiology specialty should be considered in light of Covid-19 outbreak.
g. In the event of cardiac arrest, use of external mechanical chest compression devices would help to minimize direct contact with infected patients.
h. The healthcare worker must self-report symptoms if present, and be excused from duty as health worker when symptomatic until tested and found negative.
i. Overall, as CV health workers are on the front lines treating Covid-19 patients, all possible measures should be implemented to reduce the risk of exposure (54).

Recommendations for Health Policy Officials and Manager

a. Provision of infrastructure for e-visits and telemedicine where possible.
b. Provision of sufficient PPEs for patient families and health care personnel.
c. Improving patient and public education concerning Covid-19 infection.
d. Provide adequate tests materials and personnel so that appropriate containment can be achieved.

CONCLUSION

The Covid-19 pandemic has affected thousands of patients globally, but its impact on resource poor communities in sub-Saharan Africa constitutes a major international health challenge. Where as many CVD patients have not died because of the virus, but a significant number had poor outcomes because of fear of going to the hospitals, or because hospitals have shut out routine care in most resource poor environment. Mitigating the risk of death from this disease will involve training and retraining of health care workers and ensuring provision of primary, secondary and tertiary levels preventions. Efficient resources channeled to combat this pandemic by health policy makers and managers will go a long way to mitigate risk of death, if actions are taken early and in right measures.

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

IO carried out data collection and manuscript writing. EO was involved in manuscript writing and editing of manuscript. All authors contributed to the article and approved the submitted version.

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*. (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
2. World Health Organization. *WHO Director-General's Opening Remarks at the Media Briefing on COVID-19- 11 March 2020*. (2020). Available online at: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19-$-$11-march-2020 (accessed March 12, 2020).
3. 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
4. Biondi-Zoccai G, Landoni G, Carnevale R, Cavarretta E, Sciarretta S, Frati G, et al. SARS-CoV-2 and COVID-19: facing the pandemic together as citizens and cardiovascular practitioners. *Minerva Cardioangiol*. (2020) 68:61–4. doi: 10.23736/S0026-4725.20.05250-0
5. Nkengasong JN, Mankoula W. Looming threat of COVID-19 infection in Africa: act collectively and fast. *Lancet*. (2020) 395:841–2. doi: 10.1016/S0140-6736(20)30464-5
6. 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
7. Murthy S, Gomersall CD, Fowler RA. Care for critically ill patients with COVID-19. *JAMA*. (2020) 323:1499–500. doi: 10.1001/jama.2020.3633
8. Li B, Yang J, Zhao F, Zhi L, Wang Y, Liu L, et al. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. *Clin Res Cardiol*. (2020) 109:531–8. doi: 10.1007/s00392-020-01626-9
9. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol*. (2020) 17:1–2. doi: 10.1038/s41569-020-0360-5
10. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult in-patients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3

11. Imai Y, Kuba K, Rao S, Huan Y, Gao F, Guan B, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*. (2005) 436:112–6. doi: 10.1038/nature03712
12. Ferrario CM, Jessup J, Chappell MC, Arerill DB, Brosnihan KB, Tallant EA, et al. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation*. (2005) 111:2605–10. doi: 10.1161/CIRCULATIONAHA.104.510461
13. Business Day. COVID-19: Sanofi to host 2-day virtual summit for healthcare practitioners. *Business Day* (2020, June 8).
14. Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, et al. Epidemiology, genetic recombination, and pathogenesis of coronavirus. *Trends Microbiol.* (2016) 24:490–502. doi: 10.1016/j.tim.2016.03.003
15. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A Pneumonia associated with a new coronavirus of probable bat origin. *Nature*. (2020) 579:270–3. doi: 10.1038/s41586-020-2012-7
16. Zhang S, Diao M, Yu W, Pei L, Lin Z, Chen D, et al. Estimation of the reproductive number of novel coronavirus (COVID-19) and the probable outbreak size on the Diamond Princess cruise ship: a data driven analysis. *Int J Infect Dis.* (2020) 93:201–4. doi: 10.1016/j.ijid.2020.02.033
17. Paules CI, Marston HD, Fauci AS. Coronavirus infections-more than just the common cold. *JAMA*. (2020) 323:707–8. doi: 10.1001/jama.2020.0757
18. 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 Centre for Disease Control and Prevention. *JAMA*. (2020) 323:1239–42. doi: 10.1001/jama.2020.2648
19. Mahase E. Coronavirus covid-19 has killed more people than SARS and MERS combined, despite lower case fatality rate. *BMJ*. (2020) 368:M641 doi: 10.1136/bmj.m641
20. Available online at: www.worldmeters.info
21. World Health Organization. *Coronavirus Disease 2019 (COVID-19) Situation Report – 46*. Available online at: https://20200306-sitrep-46-covid-19.pdf?sfvrsn=96b04adf_2 (accessed March 12, 2020).
22. CDC. *2019 Novel Coronavirus, Wuhan, China: 2019 Novel Coronavirus (2019 – nCoV) in the U.S Centres for Disease Control and Prevention (CDC)*. (2020). Available online at: https://www.cdc.gov/coronavirus/2019 -ncov/cases-in-us.html (accessed March 19, 2020).
23. Wang H, Yang P, Liu K, Guo F, Zhang Y, Zhang G, et al. SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. *Cell Res.* (2008) 18:290–301. doi: 10.1038/cr.2008.15
24. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med*. (2020) 46:586–90. doi: 10.1007/s00134-020-05985-9
25. Porcheddu R, Serra C, Kelvin D, Kelvin N, Rubino S. Similarity in case fatality rates (CFR) of COVID-19/SARS-COV-2 in Italy and China. *J Infect Dev Ctries*. (2020) 14:125–8. doi: 10.3855/jidc.12600
26. Liu WM, van der Zeijst BA, Boog CJ, Soethout EC. Aging and impaired immunity to influenza viruses: implications for vaccine development. *Hum Vacc*. (2011) 7(Suppl):94–8. doi: 10.4161/hv.7.0.14568
27. Tall AR, Yvan-Charvet L. Cholesterol, inflammation and innate immunity. *Nat Rev Immunol*. (2015) 15:104–16. doi: 10.1038/nri3793
28. Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. *J Clin Invest*. (2017) 127:1–4. doi: 10.1172/JCI92035
29. Thienemann F, Pinto F, Grobee DE, Boehm M, Bazargani N, Ge J, et al. World Heart Federation briefing on prevention: coronavirus disease 2019 (COVID-19) in low-income countries. *Global Heart*. (2020) 15:31. doi: 10.5334/gh.778
30. Sarkisian L, Saaby L, Poulsen TS, Gerke O, Jangaard N, Hosbond S, et al. Clinical characteristics and outcomes of patients with myocardial infarction, myocardial injury, and nonelevated troponins. *Am J Med*. (2016) 129:446. doi: 10.1016/j.amjmed.2015.11.006
31. 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
32. 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-centred, retrospective, observational study. *Lancet Respir Med*. (2020) 8:475–81. doi: 10.1016/S2213-2600(20)30079-5
33. 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
34. Wang Y, Wang Z, Tse G, Zhang L, Wan E, Guo Y et al. Cardiac arrhythmias in patients with COVID-19. *J Arrhythmia*. (2020) 36:1–10. doi: 10.1002/joa3.12405
35. Giudicessi JR, Roden DM, Wilde AMA, Ackerman MJ. Genetic susceptibility for COVID-19 associated sudden cardiac death in African Americans. *Heart Rhythm*. (2020) 17:1487–92. doi: 10.1016/j.hrthm.2020.04.045
36. Mehra MR, Desai SS, Kuy S, Henry TD. Cardiovascular Disease, drug therapy and mortality in Covid-19. *N Engl J Med*. (2020) 382:e102. doi: 10.1056/NEJMoa2007621
37. 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
38. Buzon J, Roignot O, Lemoine S, Perez P, Kimmoun A, Levy B, et al. Takotsubo cardiomyopathy triggered by influenza A virus. *Intern Med*. (2015) 54:2017–9. doi: 10.2169/internalmedicine.54.3606
39. Zompatori M, Ciccarese F, Fasano L. Overview of current lung imaging in acute respiratory distress syndrome. *Eur Respir Rev*. (2014) 23:519–30. doi: 10.1183/09059180.00001314
40. Klok FA, Kruip MJ, van der Meer NJ, Arbous MS, Gommers DA, Kaptein FH, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thrombosis Res.* (2020) 191:145–7. doi: 10.1016/j.thromres.2020.04.013
41. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thronb Haemost*. (2020) 18:844–7. doi: 10.1111/jth.14768
42. Itkonen MK, Tornio A, Lapatto-Reiniluoto O, Neuvonen M, Neuvonen PJ, Niemi M, et al. Clopidogrel increases dasabuvir exposure with or without ritonavir, and ritonavir inhibits the bioactivation of clopidogrel. *Clin Pharmacol Ther*. (2019) 105:219–28. doi: 10.1002/cpt.1099
43. Tonnesmann E, Kandolf R, Lewalter T. Chloroquine cardiomyopathy – a review of the literature. *Immunopharmacol Immunotoxicol*. (2013) 35:434–42. doi: 10.3109/08923973.2013.780078
44. van Doremalen N, Bushmaker T, Morris D, Holbook M, Gamble A, Williamson B, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS –COV-1. *N Engl J Med*. (2020) 382:1564–7. doi: 10.1056/NEJMc2004973
45. Hollander JE, Carr BG. Virtually imperfect? Telemedicine for Covid-19. *N Engl J Med*. (2020) 382:1679–81. doi: 10.1056/NEJMp2003539
46. American College of Cardiology. *COVID-19 Clinical Guidance for the Cardiovascular Care Team*. Available online at: https://www.acc.org/~/media/Non-Clinical/Files-PDFs-Excel-MS-Word-etc/2020/02/S20028-ACC-Clinical-Bulletin-Coronavirus.pdf (accessed March 10, 2020).
47. European Society of Cardiology. *Position Statement of the ESC Council on Hypertension on ACE-Inhibitors and Angiotensin Receptor Blockers*. (2020). Available online at: https://www.escardio.org/Councils/Council-on-Hypertension-(CHT)/News/position-statement-of-the-esc-council-on-hypertension-on-ace-inhibitors-and-ang (accessed March 27, 2020).
48. International Society of Hypertension. *A Statement From the International Society of Hypertension on COVID-19*. (2020). Available online at: https://ish-world.com/news/a/A-statement-from-the-International-Society-of-Hypertension-on-COVID-19/ (accessed March 27, 2020).
49. Welt FGP, Shah PB, Aronow HD, Bortnick AE, Henry TD, Sherwood MW, et al. Catheterization laboratory considerations during the coronavirus (COVID-19) pandemic: from tACC's Interventional Council and SCAI. *J Am Coll Cardiol*. (2020) 75:2372–5. doi: 10.1016/j.jacc.2020.03.021
50. *(CDC-1) Centres for Disease Control and Prevention. Coronavirus (COVID-19)*. Available online at: https://www.cdc.gov/coronavirus/2019-ncov/ (accessed May 1, 2020).

51. WHO/2019-nCoV/IPC_PPE_use/2020.4 Available online at: https://www.cdc.gov/coronavirus/2019-ncov/hcp
52. Livingstone E, Desai A, Berkwits M. Sourcing personal protective equipment during the COVID-19 pandemic. *JAMA*. (2020) 323:1912–4. doi: 10.1001/jama.2020.5317
53. 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
54. Adams JG, Wall RM. Supporting the health care workforce during the COVID-19 global epidemic. *JAMA*. (2020) 323:1439–40. doi: 10.1001/jama.2020.3972

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CASE REPORT
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Case Report: Hyperbilirubinemia in Gilbert Syndrome Attenuates Covid-19-Induced Metabolic Disturbances

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Gilbert syndrome (GS) is a liver disorder characterized by non-hemolytic unconjugated hyperbilirubinemia. On the other hand, Coronavirus disease 2019 (Covid-19) is a recent viral infectious disease presented as clusters of pneumonia, triggered by the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2). Little is known on the association between SARS-CoV-2 and GS, despite different studies have recently stated a link between hyperbilirubinemia and SARS-CoV-2 severity. In this case-report study we described a 47-year-old man, a known case of GS since the age of 4, presented to the emergency department with fever (39.8°C), dry cough, dyspnea, headache, myalgia, sweating and jaundice diagnosed with Covid-19-induced pneumonia. Interestingly, GS patient exhibited a rapid clinical recovery and short hospital stay compared to other SARS-CoV-2 positive patient, seeming that hyperbilirubinemia may exert a protective effect of against Covid-19 induced-cardiometabolic disturbances. Data obtained here underlines that the higher resistance against Covid-19 evidenced by the GS patient seems to be due to the antioxidant, anti-inflammatory, and antiviral effects of unconjugated bilirubin.

Keywords: gilbert syndrome, SARS-CoV-2, hyperbilirubinemia, COVID-19, metabolic disease

INTRODUCTION

Gilbert syndrome (GS) is a chronic liver disorder characterized by non-hemolytic unconjugated hyperbilirubinemia due to defect in the hepatic uptake of unconjugated bilirubin, which was first described by Augustin Gilbert in 1901 (1). GS is also called simple familial jaundice or icterus intermittent juvenilis, affects 5–10% of general population, being most common in male (2). Clinically, GS is presented with mild recurrent jaundice, fatigue and abdominal pain provoked by stress, infection, and menstruation. GS results from reduction in bilirubin uridine diphosphate glucuronyltransferase enzyme activity due to mutation in the UGT1A1 gene. There are more than 100 variants of UGT1A1 gene associated with GS phenotype, and generally, there is no effective treatment for GS, despite phenobarbital may be used in severe cases (3). Previously,

Maruhashi et al. (4) reported that hyperbilirubinemia in GS is associated with a cardioprotective effect attributed to the antioxidant and vasodilator effects of bilirubin.

On the other hand, coronavirus disease 2019 (Covid-19), a recent viral infectious disease presented as clusters of pneumonia and caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has triggered a huge attention among both medical and scientific communities with the intent of discovering an effective therapeutic agent (5). The clinical spectrum of Covid-19 is asymptomatic or mild flu-like illness in around 85%, mainly in young adults; however, 10% of cases develop a severe disease with risk of development of acute respiratory distress syndrome (ARDS) (6). However, in severe cases, Covid-19 may leads to extra-pulmonary manifestations, like acute cardiac injury, arrhythmias, acute kidney injury, acute brain injury, endocrine failure, multiple organ failure, metabolic disturbances, and even death (7). In this sense, as Covid-19 pandemic has full-grown public health issues, here we present a case-report study of a patient with GS who gets infected by the SARS-CoV-2. This case is particularly relevant regarding the ameliorative role of hyperbilirubinemia in GS patients during Covid-19 pneumonia.

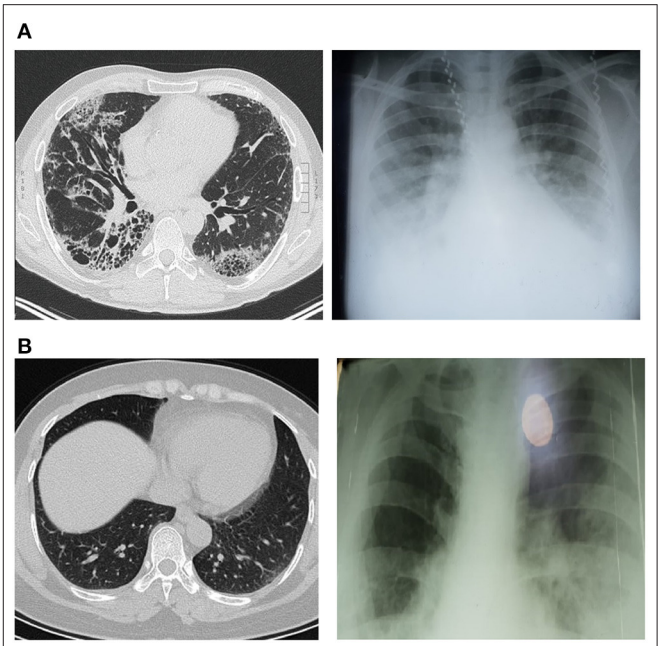

FIGURE 1 | Chest X-ray and CT scan imaging showed bilateral ground-glass appearance; **(A)** Covid-19 patients only, **(B)** Covid-19 patients with Gilbert syndrome.

CASE REPORT

Presenting Concerns

A 47-year-old man, a known case of GS since age of 4-year, presented to the emergency department with fever, dry cough, dyspnea, headache, myalgia, sweating, jaundice, and generalized poor health condition without response to the empiric antibiotics and analgesics for about 3 days. Besides, a 53-years-old man presented with fever (38.9°C), cough, headache, malaise and sweating diagnosed as Covid-19 pneumonia was regarded as a control. Informed verbal consent was attained from both patients, and this study was approved (MRT 7 August 2020) by the Scientific Editorial Board in College of Medicine, Al-Mustansiriyia University, Baghdad, Iraq.

Clinical and Laboratory Findings

General physical examination showed a conscious and febrile patient (39.8°C), with jaundice and poor health status. His blood pressure was 140/90 mmHg, heart rate was 110 beats/min and body mass index (BMI) of 33.73 kg/m^2 and hypoxemia (SaO_2 91%). Chest X-ray and chest computed tomography (CT) scan illustrated bilateral prominent bronchovascular marking and ground-glass opacities, respectively, suggestive of Covid-19-induced pneumonia (**Figure 1**). Radiological score was used to determine the radiological severity according to Wasilewski et al. (8).

Anti-SARS-CoV-2 antibody (IgM) was positive (2.9 U/mL) for Covid-19 patient with GS compared with (2.89 U/mL) for Covid-19 patient only, suggesting an acute SARS-CoV-2 infection in both. Complete blood count (CBC), fasting blood glucose (FBG), glycated hemoglobin (HbA1c), blood urea, serum creatinine, C-reactive protein (CRP), D-dimer, serum lactate dehydrogenase (LDH), and serum ferritin were done at the laboratory unit. Preliminary investigations showed high FBG (165 mg/dL), HbA1c (5.5%), total serum bilirubin (6.8 mg/dL), unconjugated bilirubin (6 mg/dL), conjugated bilirubin (0.8 mg mg/dL), and high white cell counts (16.073/μL) with lymphopenia (9.12 μ/L). Similarly, the inflammatory biomarkers were increased in regard to reference ranges. D-dimer (14.000 ng/mL), CRP (243 mg/L), ferritin (654 ng/mL), and LDH (674U/L).

Liver function test and ultrasonography imaging were done to exclude liver injury. Taken together, clinical findings, radiological examinations and laboratory findings of this GS patient with Covid-19 were compared with a matched COVID-19 patient without GS at time of hospitalization (**Table 1**).

Both patients were treated with the analgesic acetaminophen (500 mg/day), azithromycin (500 mg/day) for the first 5 days, ivermectin (12 mg/day), famotidine (40 mg/day), soluble insulin (10 units) 3 times/day, and montelukast (10 mg/day). Besides oxygen therapy by high flow nasal cannula for 10 days, patients also received subcutaneous enoxaparin (60 mg/day) during the hospitalization period as a prophylaxis against venous thromboembolism.

Follow-Up and Outcomes

Following 3 weeks of management, all laboratory investigations, radiological, and clinical findings return to normal except of unconjugated bilirubin (**Table 2**) and the patient was discharged to home. Particularly, the GS patient showed a rapid clinical improvement as compared to the Covid-19 patient without GS during the hospitalization period.

An outpatient follow-up through mobile dial-up within 2 weeks following discharge disclosed a complete recovery and the GS patient returned to his prior physical fitness and normal daily activities.

TABLE 1 | Cardiometabolic and inflammatory profiles of GS patient COVID-19 positive compared to a control patient at time of admission.

Variables	Reference range	COVID-19 patient with GS	COVID-19 patient	% Difference
BMI (kg/m^2)	20–25	33.73	34.71	2.86
SBP (mmHg)	110–120	140	153	8.87
DBP (mmHg)	70–90	90	92	2.19
Covid-19 IgM (U/mL)	0.9–1.1	2.9	2.89	0.34
Covid-19 IgG (U/mL)	0.9–1.1	0.00	0.00	0.00
SaO_2%	95–99	91	89	1.11
TSB (mg/dL)	0.2–1.0	6.8	0.8	157.89
Conjugated bilirubin (mg/dL)	0.1–0.3	0.8	0.7	13.33
Un-conjugated bilirubin (mg/dL)	0.1–0.7	6.0	0.1	193.44
FBG (mg/dL)	70–90	165	199	18.68
HbA1c (%)	4.5–5.5	5.5	5.9	7.01
Blood urea	20–40	41	39.7	3.22
Serum creatinine	0.5–1.5	1.2	1.1	8.69
CRP (mg/L)	0.5–200	243	422	53.83
D-dimer (ng/mL)	50–10.000	14.000	22.000	44.44
Ferritin (ng/mL)	20–250	654	907.84	32.50
LDH (U/L)	230–460	674	795.21	16.50
Hb (g/dL)	12–14	13.8	14.36	3.97
WBC (μ/L)	4,000–11,000	16.073	15.74	2.09
Lymphocytes %	20–40	9.12	7.53	19.09
Neutophils %	40–80	85.31	89.45	4.73
Radiological score	1–5	4	5	22.22

Data presented as number and %, BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TSB, total serum bilirubin; FBG, fasting blood glucose; HbA1c, glycated hemoglobin.

TABLE 2 | Cardiometabolic and inflammatory profiles of GS patient COVID-19 positive compared to a control patient at time of discharge.

Variables	Reference range	Covid-19 with GS	Covid-19 patient	% Difference
BMI (kg/m^2)	20–25	32.65	34.71	6.11
SBP (mmHg)	110–120	119	143	18.32
DBP (mmHg)	70–90	79	82	3.72
Covid-19 IgM (U/mL)	0.9–1.1	0.9	0.89	1.11
Covid-19 IgG (U/mL)	0.9–1.1	7.84	6.01	26.42
SaO_2%	95–99	98	95	3.10
TSB (mg/dL)	0.2–1.0	3.4	0.8	123.81
Conjugated bilirubin (mg/dL)	0.1–0.3	0.4	0.7	54.54
Un-conjugated bilirubin (mg/dL)	0.1–0.7	3.0	0.1	187.09
FBG (mg/dL)	70–90	95	179	61.31
HbA1c (%)	4.5–5.5	5.5	5.9	7.01
Blood urea	20–40	33	34.7	5.02
Serum creatinine	0.5–1.5	1.3	1.2	8
CRP (mg/L)	0.5–200	22	122	138.88
D-dimer (ng/mL)	50–10.000	452	631.71	33.16
Ferritin (ng/mL)	20–250	105	207.84	65.74
LDH (U/L)	230–460	321	395.21	20.72
Hb (g/dL)	12–14	13.8	14.36	3.97
WBC (μ/L)	4,000–11,000	8.832	9.44	6.65
Lymphocytes %	20–40	33.7	22.53	39.72
Neutophils %	40–80	72.88	80.45	9.87
Radiological score	1–5	1	2	66.66

Data presented as number and %, BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TSB, total serum bilirubin; FBG, fasting blood glucose; HbA1c, glycated hemoglobin.

CLINICAL COURSE SUMMARY

At time of hospitalization, both Covid-19 patients with or without GS presented comparable clinical presentations, like fever, headache, sweating, dry cough, fatigue, and generalized poor health status. However, these clinical features were less severe in Covid-19 patient with GS compared with Covid-19 patient only. In addition to high serum levels of unconjugated bilirubin in Covid-19 patient with GS, both laboratory and radiological findings were better as compared with Covid-19 patient only. In the management period, patients received the same course of supportive therapy, antibiotics, anticoagulants, and other drugs. During hospitalization period, the fasting blood glucose (FBG) was elevated in both Covid-19 patients (FBG = 165 mg/dL in GS, 199 mg/dL in Covid-19 control), managed through using soluble insulin subcutaneously 10 IU/day with frequent monitoring of FBG. In particular, Covid-19 patient with GS presented with a less needed for oxygen therapy compared with control Covid-19 patients who was more dependent on oxygen therapy. Near the end of hospitalization period, Covid-19 patient with GS showed a rapid clinical improvement as compared to the Covid-19 patient without GS. At the third week of disease management, clinical, radiological and laboratory findings were re-evaluated. All investigations and clinical findings return to normal with exception of unconjugated bilirubin, which remained higher in Covid-19 patient with GS (3 mg/dL) as compared with that in control Covid-19 patient (1 mg/dL). Both patients were discharged to home with complete recovery and returned to normal daily activities.

DISCUSSION

To our knowledge, this is the first reported case study of Covid-19 in a patient with GS. The GS patient with Covid-19 showed a rapid clinical improvement and short hospital stay as compared with a Covid-19 patient. Indeed, it has been proven that bilirubin exerts potent antioxidant effects which might alleviates Covid-19 induced-oxidative stress (9). Also, it has been reported that bilirubin has cardioprotective effects, improves endothelial function and provokes the nitric oxide (NO) release (10), thus, preventing from endothelial dysfunction and cardiovascular

complications in COVID-19 (11), as evident of hypertension in Covid-19 case compared to GS patient with Covid-19. Unfortunately, oxidative stress profile and endogenous antioxidant capacity were not measured in the present study to confirm the antioxidant potential of unconjugated bilirubin in Covid-19.

Liu et al. (12) found that SARS-CoV-2 infection leads to down-regulation of angiotensin converting enzyme 2 (ACE2) causing a reduction in the vasodilator angiotensins (Ang 1–7 and Ang 1–9) and augmenting of vasoconstrictor angiotensin II (AngII). These changes *per se* lead to acute lung injury (ALI), cardiovascular and metabolic disturbances in Covid-19 patients. Recently, Novák et al. (13) reported that high bilirubin levels attenuate the metabolic disorders through inhibition and attenuation of renin-angiotensin system (RAS). Besides, bilirubin has a protective effect against experimental ALI through inhibition of ischemic-reperfusion injury and exerting anti-proliferative effects (14). Therefore, high serum bilirubin level in patients with GS may lessen ALI and the development of ARDS through attenuation of AngII induced-pulmonary vasoconstriction and hyper-inflammation (15). These findings might explain a lower CT score 4 in Covid-19 with GS as compared with control Covid-19 score 5.

Lin et al. (16) also illustrated that bilirubin inhibits the nod-like receptor pyrin3 (NLRP3) inflammasomes over-activation through myeloperoxidase inhibition and subsequent reduction of inflammatory cytokines release. Thereby, high serum bilirubin levels in patients with GS may attenuate the development of cytokine storm during Covid-19 progression via inhibiting the release of interleukin (IL)-6, tumor necrosis factor (TNF)-α and IL-1β (17). These findings might explain the low rate of inflammatory biomarkers in GS patient with Covid-19 compared to the Covid-19 patient without GS. These protective effects of high bilirubin in GS are lacking in patients with Covid-19 pneumonia without GS. It has been shown that uncontrolled high pro-inflammatory cytokines, oxidative stress and unrestrained activation of NLRP3 inflammasomes contribute for development of ALI and progression of Covid-19 severity (18, 19).

Interestingly, fasting blood glucose (FBG) was increased at time of admission due to SARS-CoV-2 induced insulin resistance and transient pancreatic β-cells dysfunction. (20). However, FBG seem to be lower in Covid-19 patient with GS, since high unconjugated bilirubin in GS improves FBG and hyperinsulinemia through activation of peroxisome proliferative activated receptor alpha (PPAR-α) (21).

On the other hand, Santangelo et al. (22) disclosed that endogenous bilirubin has antiviral property against human herpes simplex virus type 1 (HSV-1), hepatitis C virus and enterovirus EV71 via up-regulation of mitogen activated protein kinase (MAPK) and c-Jun N-terminal (JNK). Both of MAPK and JNK are involved in the replication and pathogenesis of SARS-CoV-2 and other coronaviruses (23). Therefore, bilirubin may be the future endogenous agent against SARS-CoV-2. Nonetheless, Liu et al. (24) found that serum bilirubin levels are correlated with Covid-19 induced-liver injury and hemolysis, but the author ignored the antioxidant and anti-inflammatory properties of bilirubin.

The present case-report study had some limitations, including genetic sequence genotype and genetic information of family of patient with GS was not evaluated, relevant past interventions were not recorded, and antioxidant profile was not estimated. Even though this study is regarded as a baseline for future clinical trials and large-scale prospective to confirm the protective effect of unconjugated bilirubin against Covid-19.

CONCLUSION

Taken together, data obtained in this case report study shed light on the new modality for COVID-19 therapy through modulation of bilirubin metabolism. As well, high bilirubin levels in the GS patient with COVID-19 conferred a protective effect against COVID-19-derived cardiometabolic disturbances. In fact, the GS patient revealed higher resistance against COVID-19 associated cardiometabolic disturbances compared to the other COVID-19 patient without GS, directly linked to the antioxidant, anti-inflammatory and antiviral effects of unconjugated bilirubin. However, we cannot sketch any definitive conclusion from our observation; thus prospective, randomized, controlled studies are recommended in this regard.

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 Al-Mustansiriyia University. 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

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

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REFERENCES

1. Fretzayas A, Moustaki M, Liapi O, Karpathios T. Gilbert syndrome. *Eur J. Pediatrics*. (2012) 171:11. doi: 10.1007/s00431-011-1641-0
2. Aiso M, Yagi M, Tanaka A, Miura K, Miura R, Arizumi T, et al. Gilbert syndrome with concomitant hereditary spherocytosis presenting with moderate unconjugated hyperbilirubinemia. *Int Med*. (2017) 56:661–4. doi: 10.2169/internalmedicine.56.7362
3. Ha VH, Jupp J, Tsang RY. Oncology drug dosing in gilbert syndrome associated with UGT 1A1: a summary of the literature. *Pharmacotherapy*. (2017) 37:956–72. doi: 10.1002/phar.1946
4. Maruhashi T, Soga J, Fujimura N, Idei N, Mikami S, Iwamoto Y, et al. Hyperbilirubinemia, augmentation of endothelial function, and decrease in oxidative stress in Gilbert syndrome. *Circulation*. (2012) 126:598–603. doi: 10.1161/CIRCULATIONAHA.112.105775
5. Al-Kuraishy HM, Hussien NR, Al-Naimi MS, Al-Buhadily AK, Al-Gareeb AI, Lungnier C. Is ivermectin–azithromycin combination the next step for COVID-19? *Biomed Biotechnol Res J*. (2020) 4:101. doi: 10.4103/bbrj.bbrj_103_20
6. García LF. Immune response, inflammation, and the clinical spectrum of COVID-19. *Front Immunol*. (2020) 11:1441. doi: 10.3389/fimmu.2020.01441
7. Johnson KD, Harris C, Cain JK, Hummer C, Goyal H, Perisetti A. Pulmonary and extra-pulmonary clinical manifestations of COVID-19. *Front Med*. (2020) 7:526. doi: 10.3389/fmed.2020.00526
8. Wasilewski PG, Mruk B, Mazur S, Półtorak-Szymczak G, Sklinda K, Walecki J. COVID-19 severity scoring systems in radiological imaging–a review. *Polish J Radiol*. (2020) 85:e361. doi: 10.5114/pjr.2020.98009
9. Luckring EJ, Parker PD, Hani H, Grace MH, Lila MA, Pierce JG, et al. *In vitro* evaluation of a novel synthetic bilirubin analog as an antioxidant and cytoprotective agent for pancreatic islet transplantation. *Cell Transplant*. (2020) 29:0963689720906417. doi: 10.1177/0963689720906417
10. Bakrania B, Du Toit EF, Ashton KJ, Wagner KH, Headrick JP, Bulmer AC. Chronically elevated bilirubin protects from cardiac reperfusion injury in the male Gunn rat. *Acta Physiol*. (2017) 220:461–70. doi: 10.1111/apha.12858
11. 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
12. Liu N, Hong Y, Chen RG, Zhu HM. High rate of increased level of plasma Angiotensin II and its gender difference in COVID-19: an analysis of 55 hospitalized patients with COVID-19 in a single hospital, Wuhan, China. *medRxiv [Preprint]*. (2020) doi: 10.21203/rs.3.rs-51770/v1
13. Novák P, Jackson AO, Zhao GJ, Yin K. Bilirubin in metabolic syndrome and associated inflammatory diseases: new perspectives. *Life Sci*. (2020) 257:118032. doi: 10.1016/j.lfs.2020.118032
14. Leem AY, Kim YS, Lee JH, Kim TH, Kim HY, Oh YM, et al. Serum bilirubin level is associated with exercise capacity and quality of life in chronic obstructive pulmonary disease. *Respir Res*. (2019) 20:279. doi: 10.1186/s12931-019-1241-5
15. Karmouty-Quintana H, Thandavarayan RA, Keller SP, Sahay S, Pandit LM, Akkanti B. Emerging mechanisms of pulmonary vasoconstriction in SARS-CoV-2-induced Acute Respiratory Distress Syndrome (ARDS) and potential therapeutic targets. *Int J Mol Sci*. (2020) 21:8081. doi: 10.3390/ijms21218081
16. Lin Y, Wang S, Yang Z, Gao L, Zhou Z, Yu P, et al. Bilirubin alleviates alum–induced peritonitis through inactivation of NLRP3 inflammasome. *Biomed Pharmacother*. (2019) 116:108973. doi: 10.1016/j.biopha.2019.108973
17. Tran DT, Jeong YY, Kim JM, Bae HB, Son SK, Kwak SH. The anti-inflammatory role of bilirubin on "Two-Hit" sepsis animal model. *Int J Mol Sci*. (2020) 21:8650. doi: 10.3390/ijms21228650
18. 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
19. 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
20. 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
21. Hinds TD Jr, Stec DE. Bilirubin, a cardiometabolic signaling molecule. *Hypertension*. (2018) 72:788–95. doi: 10.1161/HYPERTENSIONAHA.118.11130
22. Santangelo R, Mancuso C, Marchetti S, Di Stasio E, Pani G, Fadda G. Bilirubin: an endogenous molecule with antiviral activity *in vitro*. *Front Pharmacol*. (2012) 3:36. doi: 10.3389/fphar.2012.00036
23. Wehbe Z, Hammoud S, Soudani N, Zaraket H, El-Yazbi A, Eid AH. Molecular insights into SARS COV-2 interaction with cardiovascular disease: role of RAAS and MAPK signaling. *Front Pharmacol*. (2020) 11:836. doi: 10.3389/fphar.2020.00836
24. Liu Z, Li J, Long W, Zeng W, Gao R, Zeng G, et al. Bilirubin levels as potential indicators of disease severity in coronavirus disease patients: a retrospective cohort study. *Front Med*. (2020) 7:598870. doi: 10.3389/fmed.2020.598870

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Commentary: Case Report: Hyperbilirubinemia in Gilbert Syndrome Attenuates Covid-19-Induced Metabolic Disturbances

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A Commentary on

Case Report: Hyperbilirubinemia in Gilbert Syndrome Attenuates Covid-19-Induced Metabolic Disturbances

by Al-Kuraishy, H. M., Al-Gareeb, A. I., Abdullah, S. M., Cruz-Martins, N., and Batiha, G. E. (2021). Front. Cardiovasc. Med. 8:642181. doi: 10.3389/fcvm.2021.642181

We have carefully read the recently published article by Al-Kuraishy et al. (1). In this case-report the authors described a patient with Gilbert Syndrome (GS), who presented to the emergency department with fever, dry cough, dyspnea, headache, myalgia, sweating and jaundice and was subsequently diagnosed with COVID-19-induced pneumonia. This patient exhibited a rapid clinical recovery and short hospitalization compared with another COVID-19 positive patient used as control. The authors speculated that hyperbilirubinemia exerted a protective effect in the GS patient due to the known antioxidant, anti-inflammatory and antiviral effects of unconjugated bilirubin. This is the first case in which bilirubin levels are correlated with the prognosis of a patient with COVID-19 infection.

However, based on the reported patient characteristics, we challenge the diagnosis of GS in this patient. This patient presented at time of admission a total serum bilirubin (TSB) level of 6.8 mg/dl with an unconjugated bilirubin level of 6.0, while at time of discharge his TSB had dropped to 3.4 mg/dl. Therefore, in our opinion, the reported TSB does not appear to be related to GS.

In GS patients, the TSB is usually below 3 mg/dl with <20% conjugated bilirubin. Only when associated with other pathological conditions, which increase hemolysis, TSB can be higher, but even then levels are usually below 6 mg/dl (2).

The most common genotype of GS is the homozygous polymorphism *A(TA)7TAA* in the promoter of the UDP-glucuronosyltransferase 1A1 *(UGT1A1)* gene (3). The extra bases reduce the affinity of the binding protein to the TATAA box causing reduced gene expression, which results in a 10% to 35% UGT1A1 enzyme activity reduction. However, more than 130 different pathogenic variants (PVs) in the *UGT1A1* gene are reported (4) and several PVs cause mild UGT activity reduction, which is consistent with GS. Conversely, intermediate TSB, consistent with other rarer forms of hereditary unconjugated hyperbilirubinemia, such as type II Crigler-Najjar syndrome

Abbreviations: GS, Gilbert Syndrome; TSB, total serum bilirubin; UGT1A1, UDP-glucuronosyltransferase 1A1; PVs, pathogenic variants; CNS-2, Crigler–Najjar syndrome type II.

(CNS-2: TSB: 5–20 mg/dl), is observed when the normal allele of a heterozygote CNS-2 PV carrier contains the Gilbert-polymorphism *A(TA)7TAA* (5).

We are aware that distinguishing GS from CNS-2 is often difficult, because many patients show intermediate TSB levels between the defined GS and CNS-2 cut-offs, which complicate a definitive diagnosis in these patients. In these cases, as in the Al-Kuraishy's patient, only genetic testing and clinical information of family members can help to discriminate the two syndromes from each other. In light of these evidences and considering TSB levels, we believe that this patient cannot be diagnosed as GS or CNS-2 according to the existing data, because the TSB level of the patient in the late stage dropped to 3.4 mg/dL.

In addition, the available evidence is insufficient to determine whether TSB level (6.8 mg/dL) is related or unrelated to COVID-19 infection, although a recent systematic review and meta-analysis highlighted that COVID-19 associated liver injury is generally mild and liver injuries are more common in patients with severe COVID-19.

Therefore, in the absence of other clinical evidences, the TSB level of 6.8 mg/dl in the patient described by Al-Kuraishy et al. could be related to genetic alterations of the *UGT1A1* gene and this value cannot be traced back to the presence of GS. However, we underline that the episodes of hyperbilirubinemia in GS patients can be triggered by several factors such as fasting, dehydration, inter-current illnesses, overexertion, and stress. Reducing the total calorie intake, these patients can have a rise up to three times their normal plasma bilirubin concentration within 48 h. The plasma bilirubin returns to normal levels within 24 h with a normal diet. We do not exclude that the TSB trend in this patient may be due to these factors. Unfortunately, in Al-Kuraishy's case report, no clinical information of the patient before admission to the emergency department was reported to justify the bilirubin value of 6.8 mg/dl.

In conclusion, we agree that the study of Al-Kuraishy et al. is particularly relevant regarding the antioxidant role of hyperbilirubinemia in coronavirus disease patients, as already reported by Liu et al. (6). Its beneficial role has been additionally highlighted by Khurana et al., who postulated the use of intravenous administration or inhalational delivery of bilirubin nanomedicine to combat systemic dysfunctions associated with COVID-19 (7). However, we believe that these evidences might involve CNS-2 patients, who are rarer than GS patients; in consequence, it is risky to consider this case as a GS patient based on bilirubin levels, which are apparently not compatible with a GS diagnosis. In our opinion it would have been more correct to describe the case report as *"COVID-19 patient with hereditary unconjugated hyperbilirubinemia."*

AUTHOR CONTRIBUTIONS

AM contributed to conception, design and draft the paper, and agreed to act as guarantor of this paper. AU contributed to draft the article and agreed to act as guarantor of this paper. MEO contributed to draft the paper and agreed to act as guarantor of this paper. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. 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) 8:642181. doi: 10.3389/fcvm.2021.642181
2. King D, Armstrong MJ. Overview of Gilbert's syndrome. *Drug Ther Bull.* (2019) 57:27–31. doi: 10.1136/dtb.2018.000028
3. Bosma P, Chowdhury JR, Jansen PH. Genetic inheritance of Gilbert's syndrome. *Lancet.* (1995) 346:314–5. doi: 10.1016/S0140-6736(95)92203-2
4. Canu G, Minucci A, Zuppi C, Capoluongo E. Gilbert and Crigler Najjar syndromes: an update of the UDP-glucuronosyltransferase 1A1 (UGT1A1) gene mutation database. *Blood Cells Mol Dis.* (2013) 50:273–80. doi: 10.1016/j.bcmd.2013.01.003
5. Minucci A, Ruggiero A, Canu G, Maurizi P, De Bonis M, Concolino P, et al. Co-inheritance of G6PD and PK deficiencies in a neonate carrying a Novel UGT1A1 genotype associated to Crigler-Najjar type II syndrome. *Pediatr Blood Cancer.* (2015) 62:1680–1. doi: 10.1002/pbc.25500
6. Liu Z, Li J, Long W, Zeng W, Gao R, Zeng G, et al. Bilirubin levels as potential indicators of disease severity in coronavirus disease patients: a retrospective cohort study. *Front Med.* (2020) 7:598870. doi: 10.3389/fmed.2020.598870
7. Khurana I, Allawadhi P, Khurana A, Srivastava AK, Navik U, Banothu AK, et al. Can bilirubin nanomedicine become a hope for the management of COVID-19? *Med Hypotheses.* (2021) 149:110534. doi: 10.1016/j.mehy.2021.110534

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Response: Commentary: Case Report: Hyperbilirubinemia in Gilbert Syndrome Attenuates Covid-19-Induced Metabolic Disturbances

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A Commentary on

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This is in response to the letter by Minucci et al. (1) addressing our recent article published in *Frontiers in Cardiovascular Medicine* (2). In the commentary, the authors suspected that the reported case was Crigler–Najjar syndrome type II (CNS-II) and not Gilbert syndrome (GS), based on the level of total serum bilirubin (TSB) and unconjugated fraction. CNS-II is a rare autosomal recessive disorder due to a mutation in the UGT1A1 gene, whose mutation can even cause other metabolic disorders, like CNS-I and GS, resulting in a reduction of the UDP-glucuronosyl transferase function, which is responsible for the conjugation of bilirubin (3). In addition, CNS-II is usually identified with persistent jaundice in the neonate and early childhood and very rarely in adults.

The TSB level in CNS-II patients commonly ranges from 10 to 20 mg/dL (mostly unconjugated), and increased up to 40 mg/dL during exacerbation and partly responds to the effect of phenobarbitone within 2–3 weeks (4). In a study, Kumar and colleagues (5) illustrated that CNS-II is an unwanted cause of jaundice in adults. In contrast, the prevalence of GS is between 4 and 16% for the general population compared to 1 per million for CNS-II. Moreover, hyperbilirubinemia in GS is completely normalized following phenobarbitone therapy and rarely exceeds 6 mg/dL (mostly unconjugated) (6). However, the serum TSB level in our reported case had a slightly higher serum TSB level (6.5 mg/dL), which might be due to the inflammatory burden caused by COVID-19. Skierka et al. (7) and Sood et al. (8) have shown that GS cases can have higher bilirubin levels than usually reported, despite that the TSB level varies continuously from GS to CNS-II, depending on

genotypes. In fact, because of the combination of polymorphisms and mutations, many patients experience intermediate TSB level between the two syndromes (9).

These findings rule out of CNS-II as a cause of inherited hyperbilirubinemia in the present study. Indeed, the case report presented is well-diagnosed since the age of 4 years by genetic analysis; however, this genetic analysis was not performed for other family members, as we mentioned in the limitations to the study. TSB alone is considered a hurdle in differentiating GS from CNS-II; nonetheless, the reduction in TSB level following phenobarbitone is regarded as a diagnostic clincher in differentiating GS (complete response) from CNS-II (partial response).

It is also worth noting that COVID-19 therapies used in our reported case, such as montelukast and prophylactic antibiotics (ceftriaxone), may increase the serum bilirubin level (5, 10). Besides, a pooled analysis study confirmed that the TSB level is associated and correlated with COVID-19 severity due to the alteration in bilirubin dynamics by an unknown mechanism (11).

Taken together, these points endorse the original diagnosis of GS, but not CNS-II, in contrast to the suggestion of Minucci et al. in their commentary.

AUTHOR CONTRIBUTIONS

All authors have significantly contributed to this work.

REFERENCES

1. Minucci A, Onori ME, Urbani A. Commentary: case report: hyperbilirubinemia in gilbert syndrome attenuates COVID-19-induced metabolic disturbances. *Front Cardiovasc Med.* 8:685835. doi: 10.3389/fcvm.2021.685835
2. Al-Kuraishy HM, Al-Gareeb AI, Cruz-Martins N, Batiha GE. Hyperbilirubinemia in Gilbert syndrome attenuates COVID-19 induced-metabolic disturbances: a case-report study. *Front Cardiovasc Med.* (2021) 8:71. doi: 10.3389/fcvm.2021.642181
3. Raffay EA, Liaqat A, Khan M, Awan AI, Mand B. A rare case report of crigler najjar syndrome type II. *Cureus.* (2021) 13:e12669. doi: 10.7759/cureus.12669
4. Liaqat A, Shahid A, Attiq H, Ameer A, Imran M. Crigler-Najjar Syndrome Type II diagnosed in a patient with jaundice since birth. *J Coll Physicians Surg Pak.* (2018) 28:806–8.
5. Kumar P, Sasmal G, Gupta S, Saxena R, Kohli S. Crigler Najjar Syndrome Type 2 (CNS Type 2): an unwonted cause of jaundice in adults. *J Clin Diagnost Res.* (2017) 11:OD05. doi: 10.7860/JCDR/2017/28195.10221
6. Fretzayas A, Moustaki M, Liapi O, Karpathios T. Gilbert syndrome. *Eur J Pediatr.* (2011) 171:11–5. doi: 10.1007/s00431-011-1641-0
7. Skierka JM, Kotzer KE, Lagerstedt SA, O'Kane DJ, Baudhuin LM. UGT1A1 genetic analysis as a diagnostic aid for individuals with unconjugated hyperbilirubinemia. *J Pediatr.* (2013) 162:1146–52. doi: 10.1016/j.jpeds.2012.11.042
8. Sood V, Lal BB, Sharma S, Khanna R, Siloliya MK, Alam S. Gilbert's syndrome in children with unconjugated hyperbilirubinemia–an analysis of 170 cases. *Indian J Pediatr.* (2021) 88:154–7. doi: 10.1007/s12098-020-03271-6
9. Maruo Y, Nakahara S, Yanagi T, Nomura A, Mimura Y, Matsui K, et al. Genotype of UGT1A1 and phenotype correlation between Crigler–Najjar syndrome type II and Gilbert syndrome. *J Gastroenterol Hepatol.* (2016) 31:403–8. doi: 10.1111/jgh.13071
10. Goldstein MF, Anoia J, Black M. Montelukast-induced hepatitis. *Ann Intern Med.* (2004) 140:586–7. doi: 10.7326/0003-4819-140-7-200404060-00042
11. Paliogiannis P, Zinellu A. Bilirubin levels in patients with mild and severe COVID-19: a pooled analysis. *Liver International.* (2020) 2020:14477. doi: 10.1111/liv.14477

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REVIEW
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Hypertension and COVID-19: Ongoing Controversies

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Coronavirus disease 2019 (COVID-19) has become a worldwide pandemic responsible for millions of deaths around the world. Hypertension has been identified as one of the most common comorbidities and risk factors for severity and adverse outcome in these patients. Recent investigations have raised the question whether hypertension represents a predictor of outcome in COVID-19 patients independently of other common comorbidities such as diabetes, obesity, other cardiovascular diseases, chronic kidney, liver, and pulmonary diseases. However, the impact of chronic and newly diagnosed hypertension in COVID-19 patients has been insufficiently investigated. The same is true for the relationship between blood pressure levels and outcomes in COVID-19 patients. It seems that the long discussion about the impact of angiotensin-converting enzyme inhibitors (ACEI) and blockers of angiotensin I receptors (ARB) on severity and outcome in COVID-19 is approaching an end because the large number of original studies and meta-analyses discarded the initial findings about higher prevalence of ACEI/ARB use in patients with unfavorable outcomes. Nevertheless, there are many controversies in the relationship between hypertension and COVID-19. The aim of this review article is to provide a clinical overview of the currently available evidence regarding the predictive value of hypertension, the effect of blood pressure levels, the impact of previously known and newly diagnosed hypertension, and the effect of antihypertensive therapy on the severity and outcomes in COVID-19 patients.

Keywords: hypertension, COVID - 19, blood pressure, antihypertensive therapy, comorbidities

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), induced by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has become a worldwide pandemic that is responsible for millions of deaths around the world. Hypertension, diabetes, and cardiovascular diseases were soon identified as common comorbidities in COVID-19 patients (1, 2). The following studies revealed that hypertension is an important risk factor for adverse outcomes in COVID-19 patients (3, 4). Initial studies reported hypertension as an independent predictor of hospitalization, an advanced stage of pneumonia, admission to the intensive care unit (ICU), and mortality in these patients (3–5). Later investigations raised the question whether hypertension would be a predictor of outcome in COVID-19 patients independently of diabetes, obesity, and other cardiovascular diseases (6, 7). Furthermore, the majority of studies did not make any distinction between patients with

chronic and new-onset of hypertension in COVID-19 patients, which could significantly impact final results. The relationship between blood pressure level and susceptibility to SARS-CoV-2 or outcome in COVID-19 patients has been insufficiently investigated, and potential blood pressure target value in these patients is still unknown.

There was a long discussion regarding the impact of antihypertensive therapy in COVID-19 patients and particularly of angiotensin-converting enzyme inhibitors (ACEI) and blockers of angiotensin I receptors (ARB). After initial reports that showed higher prevalence of use of these medications in COVID-19 patients with cardiac injury and more severe course of disease (8, 9), numerous original studies and meta-analysis reported no relationship with severity or mortality in COVID-19 patients (10, 11) or even benefit of taking renin-angiotensin-aldosterone inhibitors in COVID-19 patients (12, 13).

The aim of this review article is to provide an overview of the current evidence on controversies regarding hypertension in COVID-19 patients: predictive value of hypertension, effect of blood pressure (BP) level and control, influence of new-onset hypertension and impact of antihypertensive therapy.

IS HYPERTENSION AN INDEPENDENT PREDICTOR OF OUTCOME IN COVID-19 PATIENTS?

Initial studies were focused on prevalence of different comorbidities, including the impact of various risk factors on susceptibility, severity and mortality of COVID-19 (3–5). Later investigations revealed association between hypertension and more advanced stages of disease and mortality (14, 15). However, majority of them did not include diabetes and obesity in multivariable analysis.

A recent study demonstrated that hypertension alone was not an independent predictor of outcome, but only in combination with diabetes or some other risk factor (6). One should also notice that some researches did not show any impact of neither hypertension nor diabetes on outcome in COVID-19 patients (16), whereas other investigations reported that both hypertension and diabetes with or without obesity were independently associated with adverse outcome (3).

Bauer et al. suggested that hypertension was independent predictor of severe form of COVID-19 only in patients younger than 65 years, but not in the whole study population (17). On the other hand, diabetes and congestive heart failure were independent predictors both in patients younger than 65 years and in all participants. Barrera et al. included 15,794 participants and reported that hypertension and diabetes separately were significant predictors of ICU and mortality, but not with severe COVID-19 (18). Interestingly, concomitant presence of hypertension and diabetes was not predictor of severe COVID-19 (18).

An investigation that included almost 4,000 critically ill COVID-19 patients that were hospitalized in ICU showed that hypertension, diabetes, cardiovascular diseases, hypercholesterolemia, chronic kidney disease, and other comorbidities were predictors of mortality in these patients (19). However, among these comorbidities only diabetes and hypercholesterolemia were independent predictors (19). A study that involved only hypertensive patients reported that diabetes was not an independent prognostic factor, whereas age and chronic kidney disease were independent predictors. The same study demonstrated that hypertension, diabetes and obesity were independent predictors of severe COVID-19 in both sexes and obesity was stronger predictor in patients younger than 50 years, whereas the interaction between hypertension and diabetes with age was not noticed (20). However, the authors did not perform adjustment for all comorbidities like in the first mentioned from the same cohort of patients.

Furthermore, the National Cohort Study in England investigated 19,256 COVID-19–related ICU admissions and revealed that patients with type 2 diabetes were at increased risk of mortality independently of hypertension, chronic respiratory disease, chronic heart disease, chronic renal disease, chronic liver disease and other potential risk factors (21). Nevertheless, the recent investigation showed no association between hypertension and mortality or acute respiratory distress syndrome (ARDS) in COVID-19 patients (6). The authors reported that hypertension only together with diabetes was independent predictor of mortality and ARDS in COVID-19 patients (6). Moreover, diabetes alone was also independently related with adverse outcomes in these patients. On the other hand, Gupta et al. revealed that only body mass index $\geq$ 40 kg/m^2 and coronary artery disease were independent predictors of 28-day mortality in COVID-19 patients (16). Hypertension, diabetes, heart failure and chronic pulmonary obstructive disease were not independently associated with lethal outcome in these patients. **Table 1** summarizes findings from described studies.

There are several major limitations of mentioned studies: retrospective nature, confounding factors that were not measured, lack of information regarding duration of hypertension, diabetes and other comorbidities, as well as missing or incomplete data about antihypertensive and anti-diabetic therapy.

THE INFLUENCE OF BLOOD PRESSURE CONTROL IN COVID-19

Data regarding the impact of BP level on susceptibility, severity or outcome of COVID-19 patients are scarce. Majority of studies and particularly those published at the beginning of pandemic were based on anamnestic data and therefore were not fully reliable. Recently published studies investigated the impact of BP control on outcome in COVID-19 patients and provided more detailed insight (22, 23).

Ran et al. investigated 803 hypertensive patients with COVID-19 and found that average systolic BP was independent predictor of only heart failure development in these COVID-19 patients (22). After adjustment for confounding factors (systolic and diastolic BP on admission, age, sex, smoking, alcohol consumption, and comorbidities (cancer, diabetes, coronary

TABLE 1 | Hypertension as independent predictor of outcome in COVID-19.

Reference	Sample size	Age	Women (%)	Hypertension (%)	Other important findings
Sun et al. (6)	3,400	61 (50–68)	1,751 (51)	1,782 (52)	Hypertension only together with diabetes was independent predictor of mortality and ARDS.
Bauer et al. (17)	1,449	54.7 ± 22.5	920 (63)	525 (36)	Hypertension was independent predictor of severe form of COVID-19 only in patients younger than 65 years, but not in the whole study population.
Barrera et al. (18)	15,794	–	–	–	Hypertension and diabetes separately were significant predictors of ICU and mortality, but not with severe COVID-19.
Grasselli et al. (19)	3,988	63 (56–69)	800 (20)	1,643 (41)	Diabetes and hypercholesterolemia, but not hypertension, were independent predictors of mortality in COVID-19 patients hospitalized in ICU.
Dennis et al. (21)	19,256	66	7,683 (40)	5,657 (29)	Patients with type 2 diabetes were at increased risk of mortality independently of hypertension, chronic respiratory disease, chronic heart disease, chronic renal disease, chronic liver disease and other potential risk factors.
Gupta et al. (16)	2,215	60.5 ± 14.5	779 (35)	1,322 (60)	Body mass index $\geq$ 40 kg/m^2 and coronary artery disease, but not hypertension and diabetes, were independent predictors of 28-day mortality in COVID-19 patients.

ARDS, acute respiratory distress syndrome; ICU, intensive care unit.

heart disease, cerebrovascular disease, COPD, chronic liver disease, and chronic kidney disease), the remaining significant predictors for heart failure were average systolic BP and pulse pressure, and an increase in systolic BP variability was also marginally associated with an increased hazard of heart failure. Increased BP variability was significantly associated with higher risks of mortality and ICU admission, respectively. The authors showed that the risks of COVID-induced heart failure development significantly increased in patients with high systolic BP, but this trend was less evident for diastolic BP (22). This finding implies that high BP is an important predictor of adverse outcome and suggests that systolic BP should be the primary target of BP control in COVID-19 patients. However, high BP variability was related with high risks of mortality and ICU admissions, underlying the importance of maintenance of stable in-hospital BP in these patients. Increased BP variability could reflect increased arterial stiffness and endothelial dysfunction that are associated with cardiovascular events (24, 25). Additional explanation could be a sudden BP decline because of progressive deterioration of underlying conditions.

Chen et al. reported gradual increase in lethal outcome, septic shock, ARDS, respiratory failure, mechanical ventilation and ICU admission from normotensive patients with COVID-19, throughout patients with grade I hypertension, to those with grade II and III hypertension (23). Even though trend existed for all outcomes, one must admit that the significant difference was not noticed between normotensive patients and participants with grade I hypertension, as well as between grade II and III hypertension. Interestingly, the length of disease and symptoms gradually increased with grade of hypertension. In multivariable analysis hypertension grade $\geq$2 was independently associated with adverse events. However, diabetes was not included in multivariable analysis despite its significant proportion among COVID-19 patients in total study population and particularly in hypertensive participants (23).

Determination of the relationship between BP and COVID-19 outcome is not an easy task due to its high variability and dependency on comorbidities. Furthermore, both studies investigated hospitalized patients, which means that BP was measured after admission and not after symptoms onset and therefore COVID-19 could already influence BP.

CHRONIC VS. NEW-ONSET HYPERTENSION IN COVID-19

One of the main challenges in assessment of the relationship between hypertension and COVID-19 is the absence of data regarding the ratio of patients with hypertension before hospital admission. Namely, patients with chronic hypertension have significant endothelial dysfunction, which is crucial in the pathogenesis of cardiovascular complications in COVID-19 (25). Chronically hypertensive patients often have target organ damage that increases susceptibility for SARS-CoV-2 and elevates the risk of unfavorable outcomes in COVID-19 patients.

Data regarding the impact of known and newly diagnosed hypertension in COVID-19 patients are very limited. Ran et al. found that poor BP control was independently associated with adverse outcomes in COVID-19 patients with chronic hypertension (22). Chen et al. reported that stage I chronic hypertension was present in only 37% of hospitalized COVID-19 patients, whereas the prevalence of chronic hypertension stages II and III was significantly higher (61 and 70%, respectively) (23). This shows that newly diagnosed hypertension was present in a significant portion of COVID-19 patients. The investigators demonstrated that unfavorable outcomes (mortality, septic shock, respiratory failure, ARDS, ICU admission) gradually increased with BP elevation (23). However, the authors did not make separate analyses for patients with known and newly diagnosed hypertension.

Xiong et al. showed that almost 40% of patients with known hypertension did not receive any antihypertensive medication (26). Nevertheless, the occurrence of adverse events did not differ between patients who were previously treated with antihypertensive medications and those who did not receive therapy despite hypertension. A significant limitation is the small number of hypertensive patients in this study ($n = 71$), which is not enough to make a conclusion (26). One should also keep in mind that the majority of studies regarding COVID-19 have come from China, where traditional medicines are frequently used instead of formal medications, including antihypertensive drugs.

Data regarding the relationship between known and newly diagnosed diabetes among COVID-19 patients potentially might be used as the model for the association between chronic and newly diagnosed hypertension with outcome in these patients. It is already well-established that diabetes is associated with elevated risk of adverse outcomes in COVID-19 patients. Nevertheless, one should notice that COVID-19 could induce diabetes with its metabolic complications and insulin therapy requirement. Li et al. found that newly diagnosed diabetes was associated with higher mortality than known diabetes in hospitalized COVID-19 patients (27). Similar results were reported from the Italian group (28). Higher glucose levels at admission were related to COVID-19 severity, with a stronger association among patients without as compared to those with known diabetes.

It is evident that this topic deserves further investigation because whether newly diagnosed hypertension potentially has a more negative effect than chronic hypertension should be explained, in the same way as the comparison of newly diagnosed diabetes with known diabetes. This would have significant clinical and particularly therapeutic implications in COVID-19 patients.

ANTIHYPERTENSIVE THERAPY IN COVID-19

SARS-CoV-2 enters human host cells upon binding to angiotensin-converting enzyme 2 (ACE2)—a molecule functioning both as the main trans-membrane receptor for the virus and a component of the renin-angiotensin system —the key BP regulating cascade. Because renin-angiotensin-system inhibitors increase ACE2 levels, the potential negative effect of ACEI or ARB has been largely discussed since the beginning of COVID-19 pandemic. This hypothesis was supported by initial findings that these medications were more frequently used in COVID-19 patients with cardiac injury or in those with severe form of disease (8, 9). Nevertheless, later reports failed to show any negative relationship between adverse outcome and use of ACEI and ARB in COVID-19 patients (10–13).

A large study from the United Kingdom that included 16,866 patients with COVID-19 events and 70,137 matched controls showed that ACEIs and ARBs were associated with lower risk of COVID-19 diagnosis (28). In fully adjusted analyses, calcium channel blockers and thiazide diuretics were also associated with lower risk of COVID-19. Interestingly, beta-blockers were initially associated with increased risk, but this relationship disappeared in a multivariable-adjusted model (28). In adjusted analyses, patients treated with ACEIs or ARBs had similar mortality to patients treated with beta-blockers, calcium channel antagonists, and other antihypertensive medications or patients receiving no antihypertensive therapy (28).

A study that analyzed 880 COVID-19 patients from Germany and the Netherlands reported that use of ACEI/ARB and diuretics was not related to worse outcomes; instead, use of beta-blockers was associated with better outcomes, and use of calcium channel blockers with poorer outcomes (29). The model was adjusted only for age, sex, and diabetes and therefore not fully conclusive, if we consider the fact that many other confounding factors (comorbidities in the first place) were not included (29). There is a hypothesis that some beta-blockers, such as carvedilol, unlike ACEI and ARB, decrease the expression of ACE2 and suppress the properties of interleukin-6, which potentially could help in treatment of COVID-19 patients (30). However, this still remains in the domain of hypothesis.

Gao et al. reported no difference in mortality, time from onset of symptoms to discharge, COVID-19 severity, and percentage of ventilation between the cohort of patients who were treated with ACEI/ARB and those treated with beta-blockers, calcium channel blockers, and diuretics (31). Unfortunately, the authors did not investigate the influence of each antihypertensive class separately. Other Chinese study reported no association between any antihypertensive class (ACEI/ARB, beta-blockers, calcium channel blockers, and diuretics) and the composite endpoint, which was defined as admission to an ICU, need for mechanical ventilation, or a fatal outcome (26).

A Massachusetts community-based observational study showed that no antihypertensive medications were related to increased risk of severe COVID-19 (17). The authors investigated each of five antihypertensive classes separately. Similar results were reported in a large meta-analysis that included 2,100,587 participants (32). The investigators observed no association between prior usage of antihypertensive medications, including ACEIs/ARBs, calcium channel blockers, beta-blockers, or diuretics, and the risk or severity of COVID-19. Interestingly, when the analysis included only hypertensive patients, prior usage of ACEIs/ARBs was related to lower severity and mortality of COVID-19.

The large Italian population-based study that matched 6,272 COVID-19 patients and 30,759 subjects according to sex, age, and municipality of residence, showed that therapy with ACEIs and ARBs was more prevalent in COVID-19 patients than among their counterparts because of higher prevalence of CV disease in COVID-19 patients (33). Nevertheless, there was no association between use of ACEIs or ARBs and the risk of COVID-19.

Considering the confusion about the use of ACEI/ARBs in COVID-19 patients with hypertension that appeared at the beginning of the pandemic, hypertension societies around the globe were forced to publish statements that should encourage the maintenance of ongoing antihypertensive therapy and the following of current guidelines (34), which include the use of ACEI/ARB and the avoidance of replacing or switching ACEI/ARB to another antihypertensive medication (35–37).

Large recently published studies and meta-analysis have significantly reduced initial uncertainties regarding the use of ACEI and ARB in treatment of COVID-19 patients. Available data indicate that all antihypertensive classes are safe in this group of patients. However, prospective studies with a large number of patients with accurate data regarding antihypertensive therapy before and during COVID-19 would be very much appreciated.

DIFFERENCES AMONG COUNTRIES

Data regarding incidence and mortality of COVID-19 significantly changed during pandemic and particularly between different countries. Sorci et al. used data on the temporal trajectory of the case fatality rate provided by the European Center for Disease Prevention and Control, as well as country-specific data (38). The authors reported that temporal trajectories of case fatality rate vary significantly among countries. The main factors associated with temporal changes were comorbidities, demographic, economic, and political parameters. Countries with the highest prevalence of cardiovascular, cancer, and chronic respiratory diseases showed the highest levels of COVID-19 CFR (37). However, these are still preliminary data because information from all countries is updated on a daily basis and final conclusions will be published once the pandemic is over.

FUTURE DIRECTIONS

Many questions regarding the effects of hypertension, BP level, BP control, and antihypertensive therapy have been raised since the beginning of COVID-19 pandemic. A large number of studies have been published over a very short time period, which unfortunately does not guarantee their quality. Many questions remained without adequate answers. This is particularly true for the influence of BP levels and control on outcomes for COVID-19 patients. There is still not enough evidence about the effects of known and newly diagnosed hypertension on the severity and outcomes of COVID-19 for patients. A large number of studies considered the association of different antihypertensive classes of medications with the outcomes in these patients, but almost all of them are retrospective investigations or meta-analyses. It is evident that well-conducted research with a significant number of hypertensive patients is necessary to resolve current controversies in the relationship between hypertension and COVID-19.

AUTHOR CONTRIBUTIONS

MT: writing the article. SS: searching the literature and review. ST, GG, and GM: detailed review with constructive remarks that substantially changed the article. CC: conceptualization of the article and constructive review. All authors contributed to the article and approved the submitted version.

REFERENCES

1. 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
2. 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
3. Giannouchos TV, Sussman RA, Mier JM, Poulas K, Farsalinos K. Characteristics and risk factors for COVID-19 diagnosis and adverse outcomes in Mexico: an analysis of 89,756 laboratory-confirmed COVID-19 cases. *Eur Respir J.* (2020) 30:2002144. doi: 10.1183/13993003.02144-2020
4. Wang Z, Deng H, Ou C, Liang J, Wang Y, Jiang M, et al. Clinical symptoms, comorbidities and complications in severe and non-severe patients with COVID-19: A systematic review and meta-analysis without cases duplication. *Medicine.* (2020) 99:e23327. doi: 10.1097/MD.0000000000023327
5. de Almeida-Pititto B, Dualib PM, Zajdenverg L, Dantas JR, de Souza FD, Rodacki M, et al. Severity and mortality of COVID 19 in patients with diabetes, hypertension and cardiovascular disease: a meta-analysis. *Diabetol Metab Syndr.* (2020) 12:75. doi: 10.1186/s13098-020-00586-4
6. Sun Y, Guan X, Jia L, Xing N, Cheng L, Liu B, et al. Independent and combined effects of hypertension and diabetes on clinical outcomes in patients with COVID-19: a retrospective cohort study of Huoshen mountain hospital and Guanggu Fangcang Shelter Hospital. *J Clin Hypertens.* (2020). doi: 10.1111/jch.14146. [Epub ahead of print].
7. Mehraeen E, Karimi A, Barzegary A, Vahedi F, Afsahi AM, Dadras O, et al. Predictors of mortality in patients with COVID-19-a systematic review. *Eur J Integr Med.* (2020) 40:101226. doi: 10.1016/j.eujim.2020.101226
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. 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
10. Zhang G, Wu Y, Xu R, Du X. Effects of renin-angiotensin-aldosterone system (RAAS) inhibitors on disease severity and mortality in patients with COVID-19: a meta-analysis. *J Med Virol.* (2020). doi: 10.1002/jmv.26695. [Epub ahead of print].
11. Savarese G, Benson L, Sundström J, Lund LH. Association between renin-angiotensin-aldosterone system inhibitor use and COVID-19 hospitalization and death: A 1,4 million patient nation-wide registry analysis. *Eur J Heart Fail.* (2020). doi: 10.1002/ejhf.2060. [Epub ahead of print].
12. Ssentongo AE, Ssentongo P, Heilbrunn ES, Lekoubou A, Du P, Liao D, et al. Renin-angiotensin-aldosterone system inhibitors and the risk of mortality in patients with hypertension hospitalised for COVID-19: systematic review and meta-analysis. *Open Heart.* (2020) 7:e001353. doi: 10.1136/openhrt-2020-001353
13. Wang Y, Chen B, Li Y, Zhang L, Wang Y, Yang S, et al. The use of renin-angiotensin-aldosterone system (RAAS) inhibitors is associated with a lower risk of mortality in hypertensive COVID-19 patients: a systematic review and meta-analysis. *J Med Virol.* (2020). doi: 10.1002/jmv.26625. [Epub ahead of print].
14. Rodilla E, Saura A, Jiménez I, Mendizábal A, Pineda-Cantero A, Lorenzo-Hernández E, et al. Association of hypertension with all-cause mortality among hospitalized patients with COVID-19. *J Clin Med.* (2020) 9:3136. doi: 10.3390/jcm9103136
15. Zhang J, Wu J, Sun X, Xue H, Shao J, Cai W, et al. Association of hypertension with the severity and fatality of SARS-CoV-2 infection: a meta-analysis. *Epidemiol Infect.* (2020) 148:e106. doi: 10.1017/S095026882000117X
16. 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

17. Bauer AZ, Gore R, Sama SR, Rosiello R, Garber L, Sundaresan D, et al. Hypertension, medications, and risk of severe COVID-19: a Massachusetts community-based observational study. *J Clin Hypertens.* (2020). doi: 10.1111/jch.14101. [Epub ahead of print].
18. Barrera FJ, Shekhar S, Wurth R, Moreno-Pena PJ, Ponce OJ, Hajdenberg M, et al. Prevalence of diabetes and hypertension and their associated risks for poor outcomes in Covid-19 patients. *J Endocr Soc.* (2020) 4:bvaa102. doi: 10.1210/jendso/bvaa102
19. Grasselli G, Greco M, Zanella A, Albano G, Antonelli M, Bellani G, et al. Risk factors associated with mortality among patients with COVID-19 in intensive care units in Lombardy, Italy. *JAMA Intern Med.* (2020) 180:1345–55. doi: 10.1001/jamainternmed.2020.3539
20. Denova-Gutiérrez E, Lopez-Gatell H, Alomia-Zegarra JL, López-Ridaura R, Zaragoza-Jimenez CA, Dyer-Leal DD, et al. The association of obesity, type 2 diabetes, and hypertension with severe coronavirus disease 2019 on Admission among Mexican patients. *Obesity.* (2020) 28:1826–32. doi: 10.1002/oby.22946
21. Dennis JM, Mateen BA, Sonabend R, Thomas NJ, Patel KA, Hattersley AT, et al. Type 2 diabetes and COVID-19-related mortality in the critical care setting: a national cohort study in England, march-july (2020). *Diabetes Care.* (2020) 23:dc201444. doi: 10.2337/figshare.13034210
22. Ran J, Song Y, Zhuang Z, Han L, Zhao S, Cao P, et al. Blood pressure control and adverse outcomes of COVID-19 infection in patients with concomitant hypertension in Wuhan, China. *Hypertens Res.* (2020) 43:1267–76. doi: 10.1038/s41440-020-00541-w
23. Chen R, Yang J, Gao X, Ding X, Yang Y, Shen Y, et al. Influence of blood pressure control and application of renin-angiotensin-aldosterone system inhibitors on the outcomes in COVID-19 patients with hypertension. *J Clin Hypertens.* (2020). doi: 10.1111/jch.14038. [Epub ahead of print].
24. 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
25. Nägele MP, Haubner B, Tanner FC, Ruschitzka F, Flammer AJ. Endothelial dysfunction in COVID-19: Current findings and therapeutic implications. *Atherosclerosis.* (2020) 314:58–62. doi: 10.1016/j.atherosclerosis.2020.10.014
26. Xiong TY, Huang FY, Liu Q, Peng Y, Xu YN, Wei JF, et al. Hypertension is a risk factor for adverse outcomes in patients with coronavirus disease 2019: a cohort study. *Ann Med.* (2020) 52:361–66. doi: 10.1080/07853890.2020.1802059
27. Li H, Tian S, Chen T, Cui Z, Shi N, Zhong X, et al. Newly diagnosed diabetes is associated with a higher risk of mortality than known diabetes in hospitalized patients with COVID-19. *Diabetes Obes Metab.* (2020). doi: 10.1111/dom.14099. [Epub ahead of print].
28. Fadini GP, Morieri ML, Boscari F, Fioretto P, Maran A, Busetto L, et al. Newly-diagnosed diabetes and admission hyperglycemia predict COVID-19 severity by aggravating respiratory deterioration. *Diabetes Res Clin Pract.* (2020) 168:108374. doi: 10.1016/j.diabres.2020.108374
29. Rezel-Potts E, Douiri A, Chowienczyk PJ, Gulliford MC. Antihypertensive medications and COVID-19 diagnosis and mortality: population based case-control analysis in the United Kingdom. *medRxiv [Preprint].* (2020). doi: 10.1101/2020.09.25.20201731
30. Pinto-Sietsma SJ, Flossdorf M, Buchholz VR, Offerhaus J, Bleijendaal H, Beudel M, et al. Antihypertensive drugs in COVID-19 infection. *Eur Heart J Cardiovasc Pharmacother.* (2020) 6:415–6. doi: 10.1093/ehjcvp/pvaa058
31. 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
32. 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.* (2020). doi: 10.1016/j.jjcc.2020.10.015. [Epub ahead of print].
33. 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
34. 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. The task force for the management of arterial hypertension of the European society of cardiology and the European society of hypertension: the task force for the management of arterial hypertension of the European society of cardiology and the European society of hypertension. *J Hypertens.* (2018) 36:2284–309. doi: 10.1097/HJH.0000000000001961
35. 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
36. De Simone G. *Position Statement of the ESC Council on Hypertension on ACE-Inhibitors and Angiotensin Receptor Blockers.*
37. *BSH & BCS Joint Statement on ACEi or ARB in Relation to COVID-19.* Available online at: https://www.britishcardiovascularsociety.org/news/ACEi-or-ARB-and-COVID-19
38. Sorci G, Faivre B, Morand S. Explaining among-country variation in COVID-19 case fatality rate. *Sci Rep.* (2020) 10:18909. doi: 10.1038/s41598-020-75848-2

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CASE REPORT
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Case Report: Takotsubo Syndrome Associated With Novel Coronavirus Disease 2019

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Background: Takotsubo cardiomyopathy is triggered by emotional or physical stress. It is defined as a reversible myocardial dysfunction, usually with apical ballooning aspect due to apical akinesia associated with hyperkinetic basal left ventricular contraction. Described in cases of viral infections such as influenza, only few have been reported associated with novel coronavirus disease 2019 (COVID-19) in the recent pandemic.

Case summary: A 79-years-old man, with cardiovascular risk factors (type 2 diabetes and hypertension) and chronic kidney disease, presented to the emergency room for severe dyspnea after 8 days of presenting respiratory symptoms and fever. Baseline electrocardiogram (ECG) was normal, but he presented marked inflammatory syndrome. He was transferred to an intensive care unit to receive mechanical ventilation within 6 h, due to acute respiratory distress syndrome. He presented circulatory failure 2 days after, requiring norepinephrine support (up to up to 1.04 μg/kg/min). Troponin T was elevated (637 ng/l). ECG showed diffuse T wave inversion. Echocardiography showed reduced left ventricular ejection fraction (LVEF 40%), with visual signs of Takotsubo cardiomyopathy. Cardiac failure resolved after 24 h with troponin T decrease (433 ng/l) and restoration of cardiac function (LVEF 60% with regression of Takotsubo features). Patient died after 15 days of ICU admission, due to septic shock from ventilator-acquired pneumonia. Cardiac function was then normal.

Conclusion: Mechanisms of Takotsubo cardiomyopathy in viral infections include catecholamine-induced myocardial toxicity and inflammation related to sepsis. Differential diagnoses include myocarditis and myocardial infarction. Evidence of the benefit of immunomodulatory drugs and dexamethasone are growing to support this hypothesis in COVID-19.

Keywords: Tako-tsubo cardiomyopathy, COVID−19, heart failure, acute respiratory distress syndrome, sepsis

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INTRODUCTION

The outbreak of novel coronavirus disease 2019 (Covid-19) spread worldwide since the end of 2019. Takotsubo cardiomyopathy is a well-described reversible myocardial dysfunction, triggered by emotional or physical stress. Previously described in viral infections, causal mechanisms remain unclear between direct viral cardiac injury and secondary inflammation. Consequently, Takotsubo cardiomyopathies have been described in Covid-19 patients (1), and hereafter, we describe one such case, to discuss plausible mechanisms and management of these potentially severe occurrences.

CASE REPORT

A 79-years-old man presented to the emergency department for fever, cough, and increasing dyspnea. Previous medical history included hypertension, type 2 diabetes, and chronic kidney disease (estimated baseline glomerular filtration rate 59 ml/min), without any history of cardiac complication due to his cardiovascular risk factors. He had been symptomatic for a week and treated with cefpodoxime for 5 days.

At admission, he presented talking dyspnea, tachypnea (respiratory rate of 24 cycles/min), low pulse oxygen saturation (SpO_2 93%), and bilateral diffuse crackling. He required 3 l/min nasal O_2 support. He showed no fever (temperature 37.2°C). Electrocardiogram (ECG) showed sinus rhythm with neither conduction nor repolarization disorder (see **Figure 1**). Lung computed tomography scan showed typical bilateral opacity suggesting severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) infection (see **Figure 2**). Nasopharyngeal polymerase chain reaction confirmed diagnosis of SARS-Cov-2 infection. Present at baseline were moderate lymphopenia (1.33 g/l) and inflammatory syndrome (fibrinogen 7.88 g/l, ferritin 665 μg/l, interleukin-6 520 pg/ml, C-reactive protein 339.4 mg/l, procalcitonin 4.97 ng/ml, and neutrophil count 7.95 g/l). Creatinine was elevated (197 μmol/l) corresponding to an estimated glomerular filtration rate of 30 ml/min/1.73 m². Other lab results were normal (troponin was not assayed at admission).

The patient quickly deteriorated and required transfer to the intensive care medicine department for acute respiratory failure within the same day. He was supported by mechanical ventilation with sedation and neuromuscular blocking agents. Wide-spectrum antibiotic therapy with cefotaxime 6 g per day and rovamycin 9 million UI per day for 5 days was administered due to suspicion of bacterial coinfection, in association with lopinavir–ritonavir targeting COVID-19 (200 mg/50 mg per day). On day 2, he showed signs of circulatory failure (unassisted systolic arterial pressure 80 mmHg, metabolic acidosis with pH 7.28, and lactate 2.1 mmol/l) with acute kidney injury requiring catecholamine support by norepinephrine (up to 1.04 μg/kg/min). ECG showed non-elevated ST segment, prolonged QT interval, T wave inversion (see **Figure 3**), and increased highly sensitive troponin T (up to 637 ng/l). Transthoracic echography was inconclusive due to poor echogenicity and was completed by transesophageal echocardiography, which showed left ventricular failure with reduced ejection fraction (LVEF 40%) and typical apical ballooning suggesting Takotsubo cardiomyopathy (see **Supplementary Videos 1**, **2**). Coronary angiography was discussed; however, troponin spontaneously decreased within 24 h to 433 ng/l and follow-up echocardiography showed restoration of LVEF with decrease of apical ballooning aspect. ECG anomalies with T wave inversion persisted afterwards. Circulatory failure resolved within 2 days allowing catecholamine weaning. However, patient presented refractory acute respiratory distress syndrome and acute kidney injury requiring hemodialysis. Before cardiac magnetic resonance imagery (cMRI) could be performed, patient died 13 days later due to septic shock with multi-organ failure, secondary to ventilator-acquired pneumonia. Cardiac involvement was excluded as cardiac index was elevated (3 l/min/m^2) and Takotsubo cardiomyopathy was ruled out by transesophageal echocardiography. A timeline summarizing these events is presented in the **Supplementary Material**.

DISCUSSION

Takotsubo cardiomyopathy is an acute heart failure syndrome with specific dyskinetic abnormality, depicted after the traditional Japanese octopus-trap (2). Although described since 1990, various definitions still co-exist (see summary of diagnostic criteria in **Table 1**). The latest, described in the 2018 European Society of Cardiology Expert Consensus Document on Takotsubo cardiomyopathies listed criteria, required to assert this diagnosis (3). In the present case, the computation of the InterTAK diagnostic score yielded a total above 70 points, corresponding to a high probability of Takotsubo. Following the diagnostic algorithm, ECG was in favor with a lack of ST-segment elevation or depression, and QT interval was indeed prolonged. Echocardiography showed typical Takotsubo cardiomyopathy with circumferential wall motion abnormalities and apical ballooning, and left ventricular outflow tract obstruction, mitral regurgitation, and right ventricular failure were excluded. Patient being unstable, coronary computed tomography angiography was precluded. Coronary angiography was discussed; however, three elements prevented us to perform this exam: (i) patient was too unstable to be transported (requiring high doses of vasopressors and heavy oxygenation support due to severe acute respiratory distress syndrome), (ii) circumferential wall motion abnormalities could not be explained by a single coronary artery obstruction, and (iii) patient was already treated by adequate antithrombotic treatments. It must be further noted that, if performed, a coronary angiography may yield significant coronary artery disease; however, the presence of significant lesions do not exclude a diagnosis of Takotsubo cardiomyopathy; in this case, these lesions do not explain the observed regional wall motion abnormalities, which were circumferential (3). The resolution of these abnormalities with troponin and inflammatory biomarker decrease and restoration of LVEF and wall motion comforted this choice. Finally, at the time of caring for this patient, routinely performing coronary angiography in patients with COVID-19 was not easy due to safety risk for healthcare personnel not trained for viral outbreaks, a feature made easier since then (4).

Although traditionally associated with psychological or physical stress, cases have been reported during viral sepsis (5) and most recently in COVID-19 (6, 7). A case series reported by Giustino et al. described five cases of Takotsubo cardiomyopathy, out of 118 consecutive patients (4.2%) with COVID-19 who underwent transthoracic echocardiography exploration, with similar reported resolution of echocardiographic features (8).

Mechanisms are plural and include catecholamine-induced myocardial toxicity and inflammation related to sepsis, which may be intertwined.

Catecholamine-induced cardiotoxicity may be associated with the visual aspect of apical ballooning with relative hypokinesia,

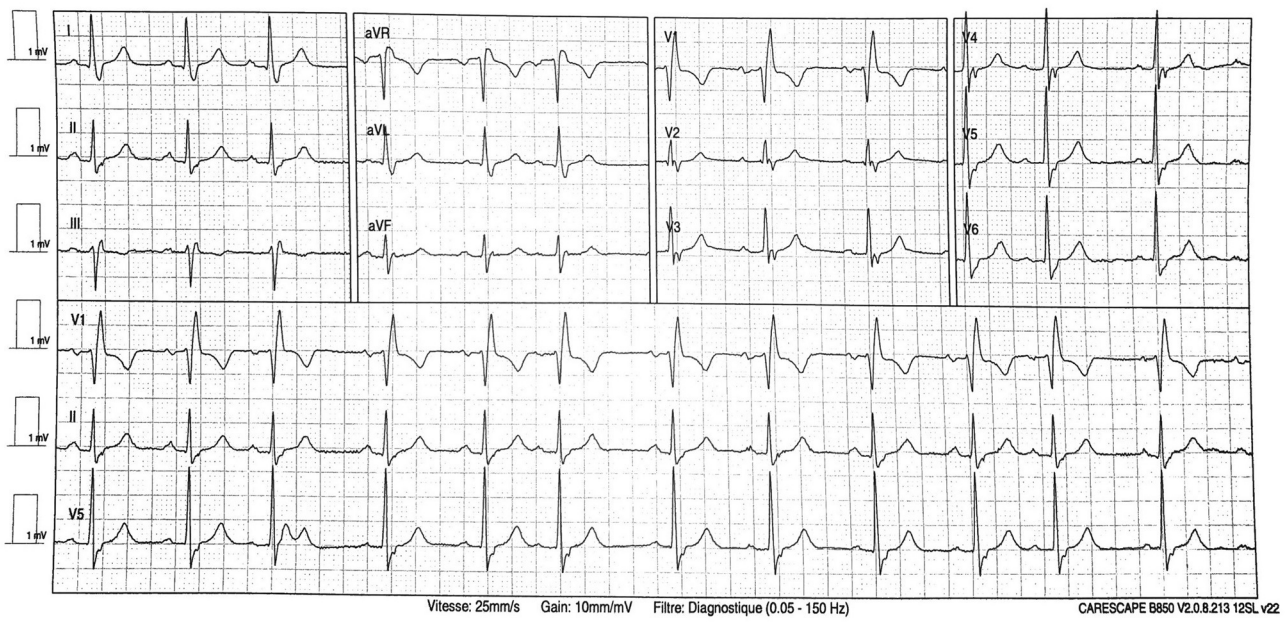

FIGURE 1 | Electrocardiogram at admission.

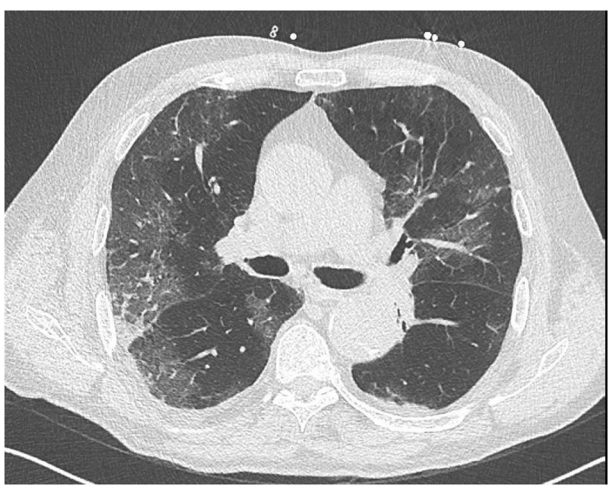

FIGURE 2 | Computed tomography lung scan at baseline.

due to the distribution of β2 adreno-receptors more prevalent in the apex (9). Indeed, myocardial beta-adrenergic toxicity is related to intra-cellular calcium dysregulation.

The sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCa) is key to calcium homeostasis in the myocardium, by regulating excitation/contraction coupling via calcium distribution around the sarcoplasmic reticulum. Its inhibition is associated with acute heart failure (10). This inhibition may be triggered by (i) sarcolipin protein, overexpressed during events such as inflammation, leading to a decrease in its calcium affinity (11) and (ii) phospholamban protein lack of phosphorylation that maintains SERCa inhibition. In the present case, the patient required high-dose norepinephrine during septic shock combined with acute heart failure. However, given the more pronounced beta-adrenergic effect of dobutamine, as compared to norepinephrine, dobutamine was not administrated to prevent further toxicity.

Added to the beta-adrenergic toxicity with SERCa inhibition, catecholamine storms have been associated with microcirculatory dysfunction due to diffuse vasoconstriction. A series of Takotsubo biopsies showed microvascular endothelial cells apoptosis. Reported histology described contraction band necrosis, hypercontracted sarcomeres, dense eosinophilic bands, and interstitial mononuclear infiltration (12). Furthering the microvascular injury hypothesis, stress microRNAs including endothelin-1 were associated with myocardial ischemia during Takotsubo cardiomyopathy (13). In COVID-19 infections, the prevalence of non-obstructive acute myocardial injury was reported elevated. Possible associated mechanisms include septic microvascular dysfunction with endothelial abnormalities, destabilization of atherosclerotic plaques, and hypoxic injury (14). In one case of Takotsubo cardiomyopathy related to COVID-19, endomyocardial biopsy showed diffuse T-lymphocytic inflammatory infiltrates with increased CD3 cell count (15). It must be noted, however, that endomyocardial biopsies are not required to confirm this diagnosis, all the more so in unstable patients (3).

While myocarditis and Takotsubo cardiomyopathy share common mechanisms, in the latter, beta-adrenergic cardiotoxicity seems prevalent, with a synergistic effect of inflammation. In SARS-Cov-2, a minor form of cytokine-release syndrome (CRS) has been related to the increased activation of

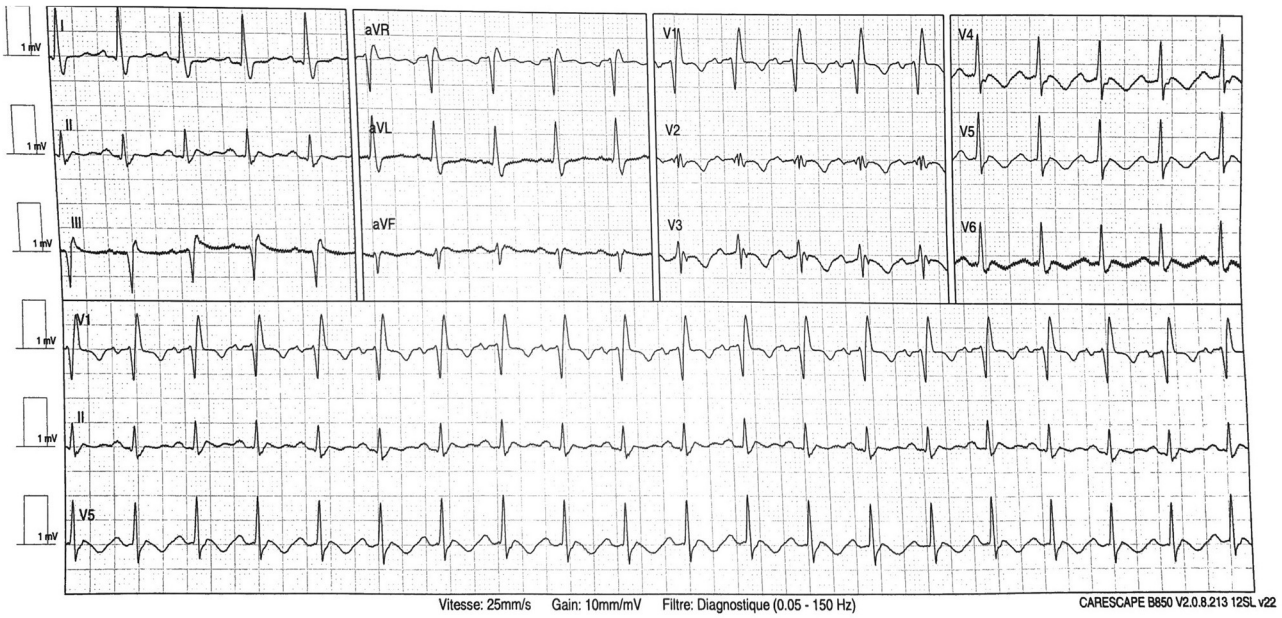

FIGURE 3 | Electrocardiogram on day 2, during circulatory failure.

TABLE 1 | Takotsubo diagnosis criteria, according to Mayo Clinic, European Society of Cardiology (ESC), and Heart Failure Association (HFA).

	Mayo Clinic	ESC, InterTAK criteria	HFA criteria
Echocardiography	Transient regional wall motion alteration	Apical ballooning Right ventricular involvement	Transient regional wall motion alteration
Coronary angiography	Absence of coronary artery disease which may explain the observed wall motion abnormalities	Possible coronary artery disease	Absence of coronary artery disease which may explain the observed wall motion abnormalities
Electrocardiogram (ECG)	New ECG repolarization abnormalities	New ECG repolarization abnormalities are present Possible no ECG changes	New and reversible repolarization ECG abnormalities
Cardiac Biomarkers	Modest elevation troponin	Modest troponin or brain natriuretic peptide elevation	Natriuretic peptide or troponin elevation
Differential diagnosis	Pheochromocytoma or Myocarditis	Infectious myocarditis	
Trigger	Possible stress trigger	Possible emotional, physical (neurologic disorders or pheochromocytoma) or combined Predominantly post-menopausal women	Possible stressful trigger

effector T cells and their production of high tumor necrosis factor (TNF) α, cytokine interleukin (IL)-6, IL-8, and chemokine ligand 1 (CXCL-1) level. These cytokines showed direct cardiac toxicity with negative inotropic effect and cell apoptosis associated with myocardial macrophage infiltration (16). In experimental models of CRS, catecholamines have been associated with immune dysregulation, through a self-amplifying loop in macrophages (17). In these models, atrial natriuretic peptides decreased catecholamine levels and, consequently, myeloid-derived cytokines including IL-1β, IL-6, and TNF. Because of this interplay between catecholamines and inflammation, both mechanisms may be involved in the genesis of Takotsubo cardiomyopathies in patients presenting with COVID-19 pneumonia. In our case, the patient presented elevated IL-6, which may give some substance to this hypothesis. As of yet, dexamethasone is one of the few molecules that showed unanimous efficacy in treating severe COVID-19 pneumonia, after the landmark Randomized Evaluation of COVID-19 Therapy (RECOVERY) trial (18). Likewise, other immunomodulatory molecules have been tested in these indications, however with less success, such as the Janus kinase inhibitor, baricitinib (19), and the IL-6 inhibitor, tocilizumab (20).

CONCLUSION

COVID-19 may be associated with Takotsubo cardiomyopathy in the context of marked inflammatory syndrome, and reasoned use

of catecholamines should be invoked whenever feasible, due to a plausible interplay between inflammation and catecholamines. Diagnostic algorithm may include coronary angiography; however, the presence of coronary lesions does not exclude a diagnosis of Takotsubo cardiomyopathy, if the observed regional motion wall abnormalities are not explained by the lesions.

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

SO wrote the initial draft. MJ and J-PM provided critical review to the manuscript. LN supervised this work and wrote the final manuscript. 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.2021.614562/full#supplementary-material

Supplementary Video 1 | Transesophageal echocardiography three-chamber view on day 2.

Supplementary Video 2 | Transesophageal echocardiography two-chamber view on day 2.

Supplementary Table 1 | Events timeline.

REFERENCES

1. Singh S, Desai R, Gandhi Z, Fong HK, Doreswamy S, Desai V, et al. Takotsubo syndrome in patients with COVID-19: a systematic review of published cases. *SN Compr Clin Med.* (2020) 1–7. doi: 10.1007/s42399-020-00557-w
2. Templin C, Ghadri JR, Diekmann J, Napp LC, Bataiosu DR, Jaguszewski M, et al. Clinical features and outcomes of takotsubo (stress) cardiomyopathy. *N Engl J Med.* (2015) 373:929–38. doi: 10.1056/NEJMoa1406761
3. Ghadri JR, Wittstein IS, Prasad A, Sharkey S, Dote K, Akashi YJ, et al. International expert consensus document on takotsubo syndrome (Part II): diagnostic workup, outcome, and management. *Eur Heart J.* (2018) 39:2047–62. doi: 10.1093/eurheartj/ehy077
4. Skulstad H, Cosyns B, Popescu BA, Galderisi M, Salvo GD, Donal EH. et al. COVID-19 pandemic and cardiac imaging: EACVI recommendations on precautions, indications, prioritization, and protection for patients and healthcare personnel. *Eur Heart J Cardiovasc Imaging.* 21 (2020) 592–8. doi: 10.1093/ehjci/jeaa072
5. Faircloth EL, Memon S. Stressing out from the flu: a case of influenza a-associated transient cardiomyopathy. *Cureus.* (2019) 11:e4918. doi: 10.7759/cureus.4918
6. 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 Reports.* (2020) 2:1321–5. doi: 10.1016/j.jaccas.2020.04.023
7. Roca E, Lombardi C, Campana M, Vivaldi O, Bigni B, Bertozzi B, et al. Takotsubo syndrome associated with COVID-19. *Eur J Case Rep Intern Med.* (2020) 7:001665. doi: 10.12890/2020_001665
8. Giustino G, Croft LB, Oates CP, Rahman K, Lerakis S, Reddy VY, et al. Takotsubo cardiomyopathy in COVID-19. *J Am Coll Cardiol.* (2020) 76:628. doi: 10.1016/j.jacc.2020.05.068
9. Paur H, Wright PT, Sikkel MB, Tranter MH, Mansfield C, O'gara P, et al. High levels of circulating epinephrine trigger apical cardiodepression in a β2-adrenergic receptor/Gi-dependent manner: a new model of Takotsubo cardiomyopathy. *Circulation.* (2012) 126:697–706. doi: 10.1161/CIRCULATIONAHA.112.111591
10. Nef HM, Möllmann H, Troidl C, Kostin S, Voss S, Hilpert P, et al. Abnormalities in intracellular Ca2+ regulation contribute to the pathomechanism of Tako-Tsubo cardiomyopathy. *Eur Heart J.* (2009) 30:2155–64. doi: 10.1093/eurheartj/ehp240
11. Martin L, Horst K, Chiazza F, Oggero S, Collino M, Brandenburg K, et al. The synthetic antimicrobial peptide 19-2.5 attenuates septic cardiomyopathy and prevents down-regulation of SERCA2 in polymicrobial sepsis. *Sci. Rep.* (2016) 6:37277. doi: 10.1038/srep37277
12. Wittstein IS, Thiemann DR, Lima JA, Baughman KL, Schulman SP, Gerstenblith G, et al. Neurohumoral features of myocardial stunning due to sudden emotional stress. *N Engl J Med.* (2005) 352:539–48. doi: 10.1056/NEJMoa043046
13. Jaguszewski M, Osipova J, Ghadri JR, Napp LC, Widera C, Franke J, et al. A signature of circulating microRNAs differentiates takotsubo cardiomyopathy from acute myocardial infarction. *Eur Heart J.* (2014) 35:999–1006. doi: 10.1093/eurheartj/eht392
14. 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
15. QSala 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) 41:1861–62. doi: 10.1093/eurheartj/ehaa286
16. Scally C, Abbas H, Ahearn T, Srinivasan J, Mezincescu A, Rudd A, et al. Myocardial and systemic inflammation in acute stress-induced (Takotsubo) cardiomyopathy. *Circulation.* (2019) 139:1581–92. doi: 10.1161/CIRCULATIONAHA.118.037975
17. Staedtke V, Bai RY, Kim K, Darvas M, Davila ML, Riggins GJ, et al. Disruption of a self-amplifying catecholamine loop reduces cytokine release syndrome. *Nature.* (2018) 564:273–7. doi: 10.1038/s41586-018-0774-y
18. Horby P, Mafham M, Linsell L, Bell JL, Staplin N, Emberson JR, et al. Effect of dexamethasone in hospitalized patients with COVID-19: preliminary report. *medRxiv.* (2020) 2020:20137273. doi: 10.1101/2020.06.22.20137273
19. Kalil AC, Patterson TF, Mehta AK, Tomashek KM, Wolfe CR, Ghazaryan V, et al. Baricitinib plus remdesivir for hospitalized adults with Covid-19. *N. Engl. J. Med.* (2020). doi: 10.1056/NEJMoa2031994. [Epub ahead of print].
20. Huang E, Jordan SC. Tocilizumab for Covid-19—the ongoing search for effective therapies. *N Engl J Med.* (2020) 383:2387–8. doi: 10.1056/NEJMe2032071

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frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
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Reduction of Emergency Calls and Hospitalizations for Cardiac Causes: Effects of Covid-19 Pandemic and Lockdown in Tuscany Region

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Introduction: Containment measures were established to flatten the curve of COVID-19 contagion in order to avoid a crash of the healthcare system. However, these measures influenced the rate of hospitalization of cardiac patients. In this study, we aimed to analyse the impact of COVID-19 and the effects of lockdown measures on hospital admissions and alerts of emergency medical system (EMS) for cardiac causes in the Tuscany region.

Methods: An observational, retrospective analysis from Italian Tuscany region was conducted. We evaluated consecutive patients contacting EMS or admitted to the 39 Emergency Departments (EDs) in Tuscany for cardiac causes in the first trimester of 2020. Data were compared with the same period in 2018/19.

Results: The alerts of EMS for cardiac causes significantly decrease in 2020 and the highest difference between 2018/19 and 2020 was found immediately after national lockdown ($\Delta = -47.4\%$, $p < 0.001$). The number of admissions for chest pain in the EDs also decreased, with a maximum difference of -67.6% ($p < 0.001$) vs. 2018/19. The number of hospital accesses for acute coronary syndromes, atrial fibrillation, and heart failure in the EDs significantly decreased in 2020 as compared to 2018/19 (maximum $\Delta = -58.9\%$, $p < 0.001$; maximum $\Delta = -63.0\%$, $p < 0.001$; maximum $\Delta = -72.7\%$, $p < 0.001$, respectively).

Conclusions: A significant decrease in the contacts to EMS for cardiac causes and in cardiac diagnoses was observed during the first trimester of 2020. Fear of contagion has likely played a relevant role. The lesson learnt from first wave of COVID-19 pandemic suggests that appropriate public information strategies and re-education of people are essential.

Keywords: lockdown, coronavirus, cardiovascular disorders, acute coronary syndrome, atrial fibrillation, heart failure

INTRODUCTION

The pandemic caused by COVID-19 has been associated with thousands of deaths worldwide and multiple cardiovascular risk factors and cardiac disorders have been recognized as high-risk conditions (1). The rapidly increasing number of patients affected by COVID-19 requiring hospitalization has imposed a relevant problem of sustainability for the healthcare system. Accordingly, during the first wave of COVID-19 pandemic, the Italian government has imposed measures promoting social distancing and a stepwise strategy starting from the quarantine for some Italy regions with subsequent lockdown measures adopted for the entire nation as of 11 March (https://www.gazzettaufficiale.it/eli/id/2020/03/08/20A01522/sg, https://www.gazzettaufficiale.it/eli/id/2020/03/11/20A01605/sg). Although these strategies were aimed to flatten the curve of the contagion in order to avoid a crash of the health care system, these measures have significantly influenced the rate of hospitalization of cardiac patients and changes in the pattern of hospital admissions have been noted, particularly in the Northern regions of Italy (2–4).

In this study, we aimed to analyse the epidemiologic impact of COVID-19 and the effects of lockdown measures on the contacts to emergency medical system (EMS) and hospital visits to the emergency department for cardiac causes for the entire Tuscany region. The number of final diagnoses of acute coronary syndrome (ACS), heart failure (HF), and atrial fibrillation (AF) was also considered. These data were compared with the trends observed in the same time frame of the previous 2 years.

METHODS

We conducted an observational, retrospective analysis from the Tuscany region aimed at evaluating the number of patients contacting the EMS for cardiac problems and symptoms, not occurring during COVID-19 infection (i.e., angina, arrhythmias, syncope, chest pain, etc.), with high dispatch priority, established by nurse triage, and the number of consecutive patients admitted to the Emergency Departments for cardiac causes, analyzing the final number of diagnoses of ACS, HF, and AF. In Tuscany there were 3.73 million inhabitants and 39 Emergency Departments that performed 1,537,031 visits (data for the year 2019). The period of observation lasted 3 months, i.e., the first trimester of 2020, from the 1st of January 2020 to the 31th of March 2020. This period was selected taking into account that the first cluster of cases of COVID-19 was identified in Italy the 20th of February and that lockdown measures were adopted for the entire nation as of 11th March. Weekly data observed during this period were compared to the trends observed in the same time frame of 2018 and 2019. Although the first cluster of cases of COVID-19 was identified in Italy the 20th of February 2020, the entire first trimester of 2020 was included in this analysis to show also pre-COVID 19 data and to demonstrate that differences in the rate of hospitalization in March were not due to physiologic fluctuations due to epidemiologic factors. A sub-analysis was also performed dividing the first trimester 2020 into three different periods, according to the events occurred during this trimester: 1st January-20th February; 21th February-10th March; 11th March-31th March. Number of accesses to Emergency Departments for stroke and sepsis were also analyzed.

The regional information systems of pre-hospital and hospital EMSs and hospital admission abstracts were used as data sources. These databases include calls to EMS, visits to emergency departments and hospital admissions in Tuscany region. In these data each individual has a unique and anonymous identifier that enables complete record linkage at individual level.

Although the comparison of the rate of mortality between the first trimester 2020 and 2018/2019 was beyond the primary scope of this study, the in-hospital mortality for patients admitted for ACS and HF was also analyzed. The rate of hospitalizations for patients admitted to the Emergency Departments and the number of patients with ACS and HF admitted to the intensive care units of the Tuscany Region during the hospitalization was also analyzed for the entire period. Data were analyzed and were checked for missing or contradictory entries and for values out of normal range by Regional Health Agency of Tuscany.

This study was conducted in accordance with the Helsinki Declaration. According to the Italian legislation (legislative decree 211/2003) and the regional procedures, the study does not need ethic approval as it is a purely observational study on routine collected anonymous data. Furthermore, because this was an observational retrospective study, patients had already been treated when the study protocol was written; therefore, it could not have modified their life-trajectories or care pathways in any way.

Statistical Analysis

Mean values of data obtained in the first trimester of 2018 and 2019 were calculated and compared with data collected in the same period of 2020. Ninety-five percentage confidence intervals of values observed in 2018-19 were calculated using Poisson model for each week and for the three periods considered in the study. Differences between periods of observation for 2018/2019 and 2020 were expressed as Δ and statistical significance was tested using Poisson models. The statistical significance was set for a two-tailed p-value < 0.05. Data were collected using Excel software (version 16.35 2019, Microsoft Corporation, Redmond, USA). The statistical software Stata 14 SE (StataCorp LP, College Station, Texas) was used for the data analyses.

RESULTS

A significant decrease in contacts of EMS by the patients for cardiac causes was found between 2019 and 2020, see **Figure 1**. The highest difference was found 1 week after the national lockdown was imposed ($\Delta = -47.4\%$ as compared to the same week of the previous years, $p < 0.001$).

The numbers of hospital visits for chest pain in the Emergency departments in Tuscany significantly decreased in 2020 as compared to 2018 and to 2019, reaching a Δ at the end of the week between 24 February-01 March of -24.0% ($p < 0.01$ vs. the same period of the previous years), see **Figure 2**. The week after the national lockdown, the number of visits for chest pain significantly dropped to -67.6% as compared to the same time

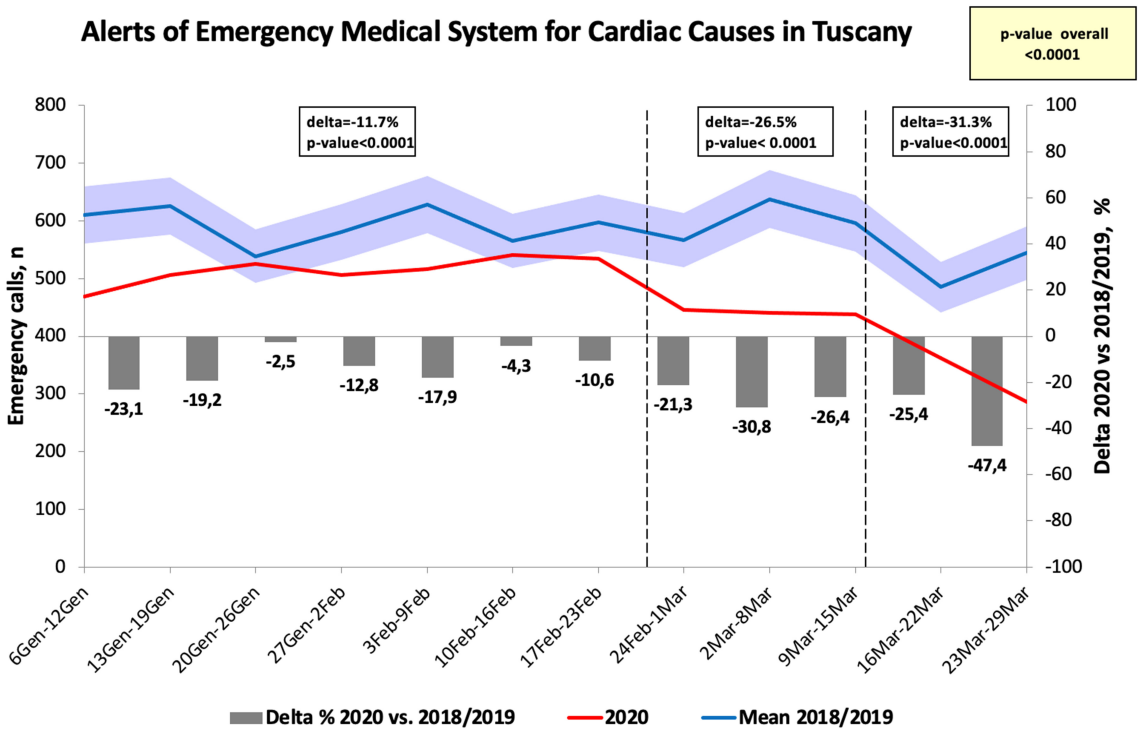

FIGURE 1 | Number of calls to the emergency medical system for cardiac causes in Tuscany. Data obtained in the first trimester were compared to data observed in 2018 and 2019 (an average value of this data was observed). For all the figures data were reported for each week separately (from the 6th of January to the 30th of March) and the delta between 2020 and 2018/2019 was reported and expressed by gray columns as percentage as well as delta and *p*-values for the three different periods.

frame of 2018 and 2019 ($p < 0.001$) and it represented the highest difference found between 2020 and the previous years. While no significant differences were found before the 24th of February for the visits to the Emergency departments for cardiac causes of chest pain ($p = 0.354$), they significantly decrease after this first period (see **Figure 3**).

The number of hospital visits for ACS in the Emergency departments significantly decreased at the end of February as compared to 2018 and 2019 ($\Delta = -18.3\%$, $p < 0.05$) and the greatest difference was identified at the end of March 2020 ($\Delta = -58.9\%$, $p < 0.001$) (**Figure 4**). Similarly, the diagnosis of AF in the Emergency departments significantly decreased at the end of February 2020 as compared to the same period in 2018 and 2019 ($p < 0.05$), reaching the greatest difference in the week after the national lockdown ($\Delta = -63\%$, $p < 0.001$) (**Figure 5**). The diagnosis of HF significantly decreased during COVID-19 pandemic, reaching the greatest difference in comparison with 2018/2019 data 1 week after the declaration of national lockdown ($\Delta = -72.7\%$, $p < 0.001$, **Figure 6**). The number of accesses to Emergency Departments due to stroke or sepsis were also decreased during the first wave of COVID-19 pandemic as compared to 2018 and 2019 (see **Supplementary Figures 1**, **2**).

The rate of hospitalizations for patients admitted to the Emergency Departments did not differ between 2020 and 2018/2019 (overall *p*-value = 0.68) for ACS and for HF (overall *p*-value = 0.49). The in-hospital mortality for patients suffering from an ACS did not differ between the first trimester 2020 and the first trimester of 2018 and 2019 (overall *p*-value = 0.166). During the three different periods no significant differences were observed ($p = 0.71$, $p = 0.0.92$, and $p = 0.364$, respectively). Among the patients admitted for ACS to the hospitals of the Tuscany Region, the number of patients requiring hospitalization in an intensive care unit did not differ between the first trimester 2020 and 2018/2019 (overall *p*-value = 0.11).

The in-hospital mortality for HF did not differ between 2020 and 2018/2019 (overall *p*-value = 0.102), with no differences among the three different periods ($p = 0.053$, $p = 0.269$, and $p = 0.208$, respectively). Among the patients admitted for HF to the hospitals of the Tuscany Region, the number of patients requiring hospitalization in an intensive care unit did not differ between the first trimester 2020 and 2018/2019 (overall *p*-value = 0.29).

DISCUSSION

The main finding of the present study is that a marked decrease in the number of patients alerting the EMS and visiting the Emergency departments for cardiac causes were observed in Tuscany after the diagnosis of the first cluster of COVID-19 cases in Italy and particularly after the national lockdown, as compared to the same time frame of the previous years (i.e., 2018 and 2019). As a consequence, the number of ACS and

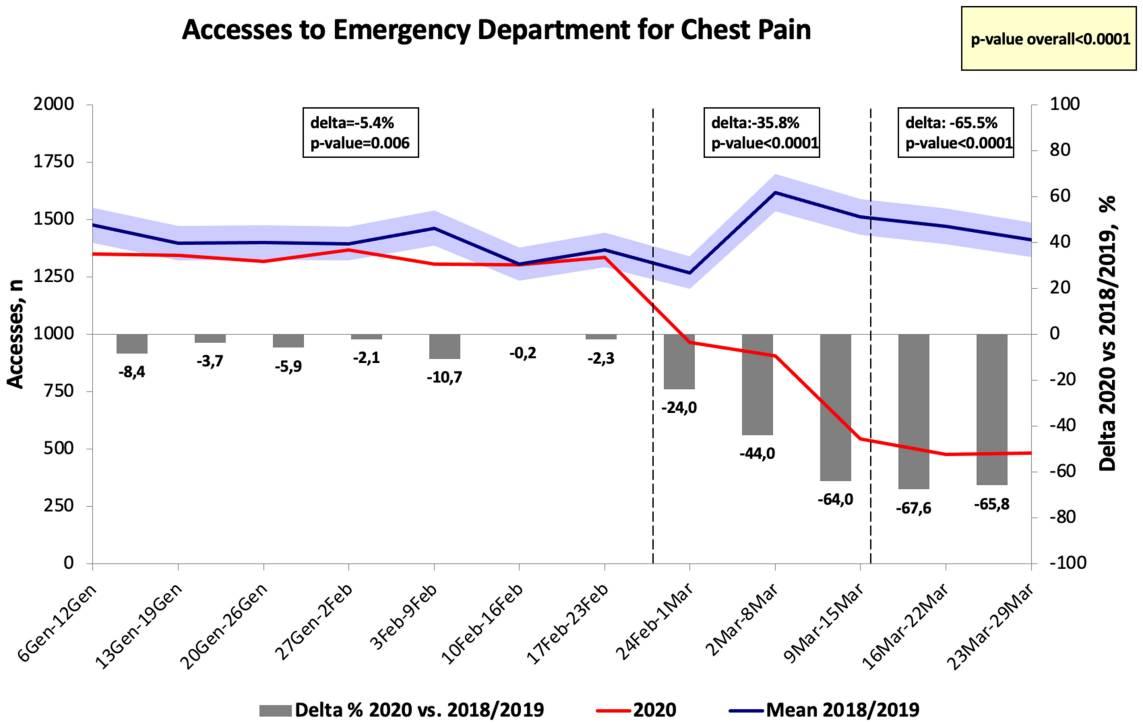

FIGURE 2 | Number of accesses to emergency department for chest pain in Tuscany.

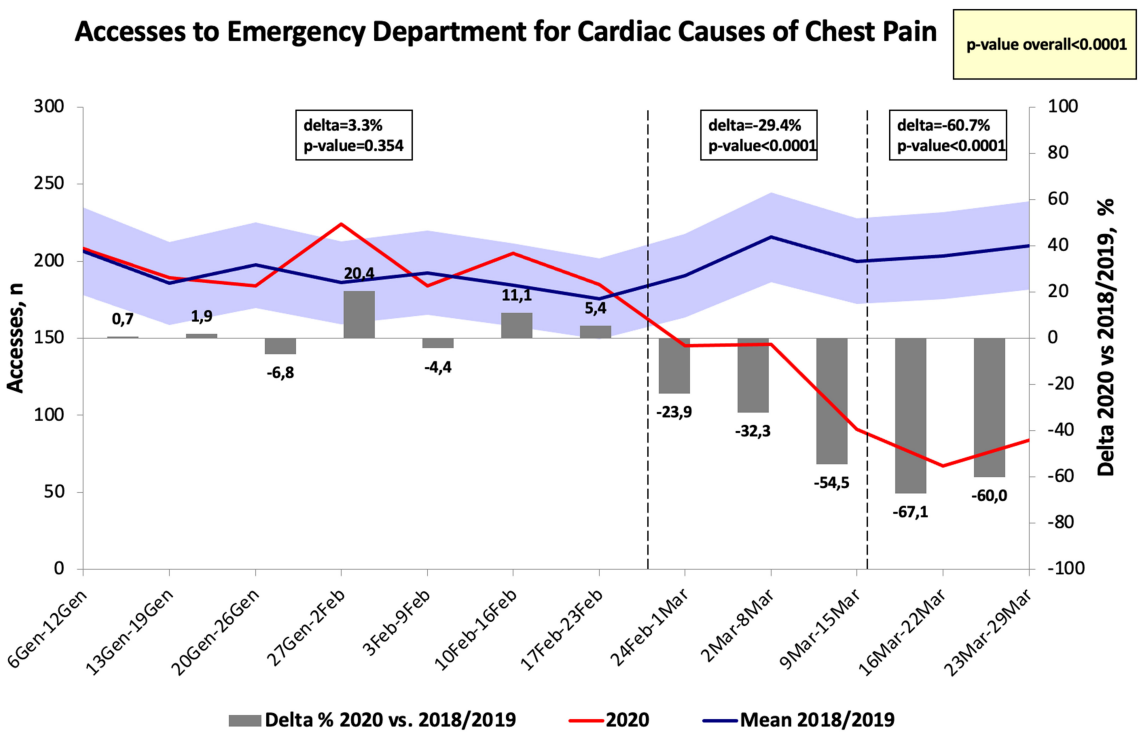

FIGURE 3 | Number of accesses to emergency department for cardiac causes of chest pain in Tuscany.

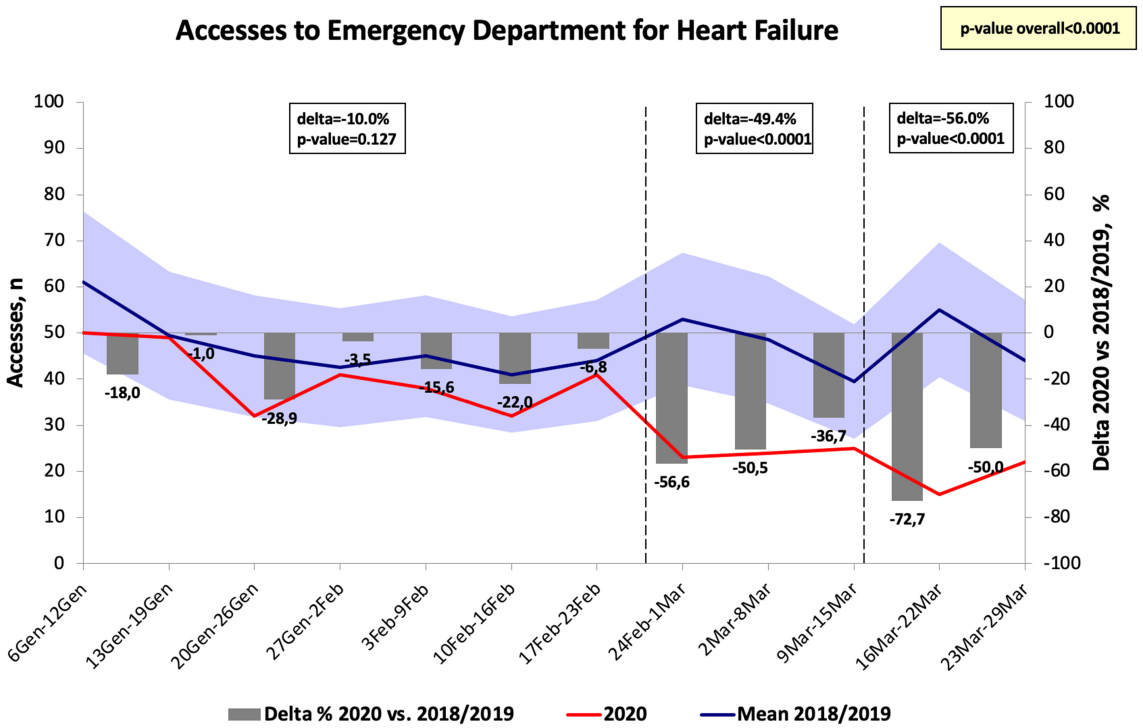

FIGURE 4 | Number of accesses to emergency department for acute coronary syndrome in Tuscany.

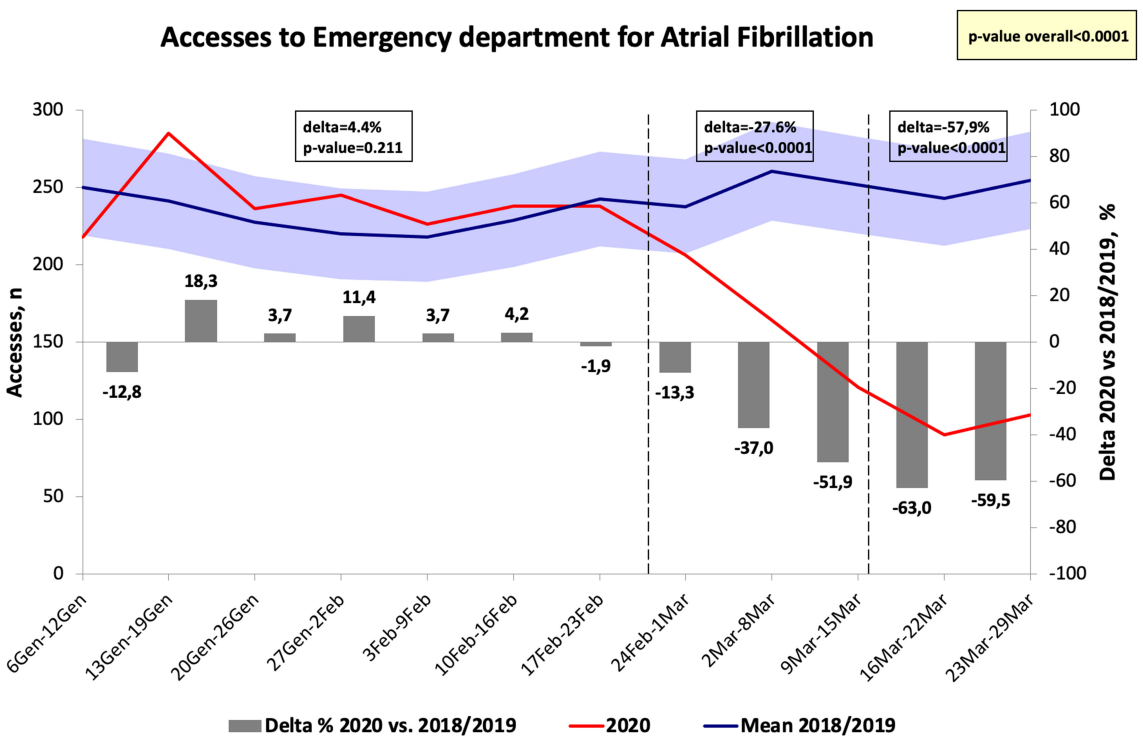

FIGURE 5 | Number of accesses to emergency department for atrial fibrillation in Tuscany.

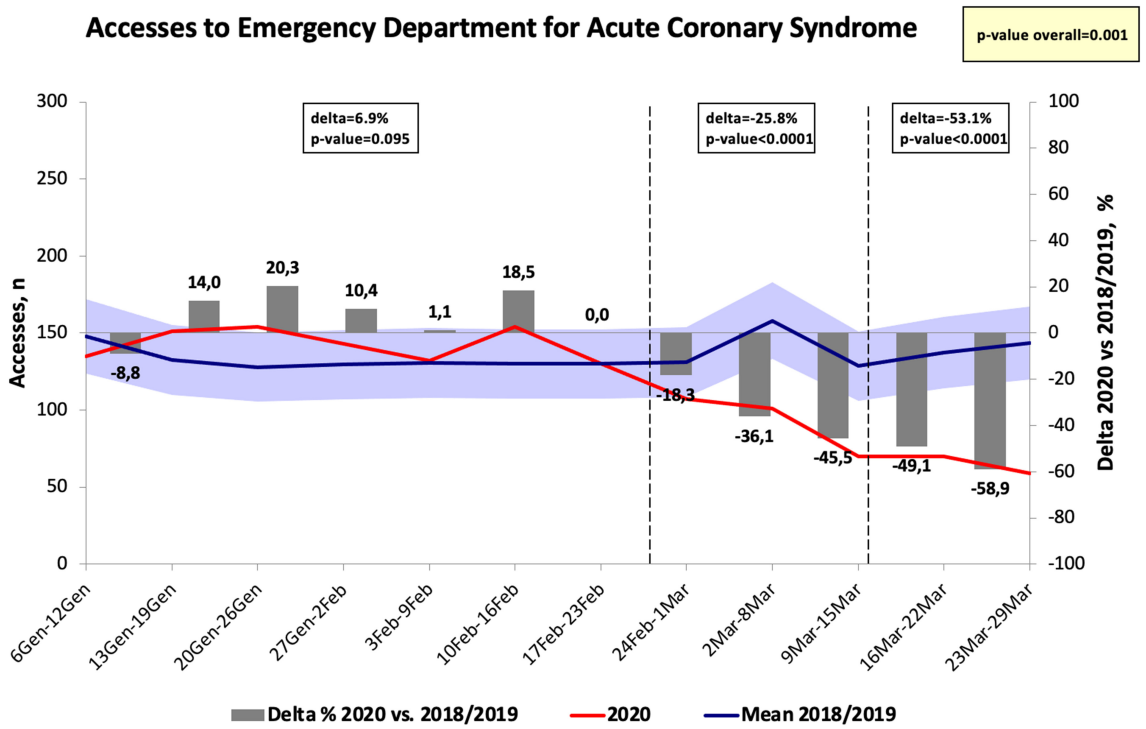

FIGURE 6 | Number of accesses to emergency department for heart failure in Tuscany.

AF diagnosed significantly decreased as compared to the same period of the previous years. Notably, the trend demonstrates a significant drop after the 20th of February and after the 11th of March 2020, i.e., after the first case diagnosed and after the national lockdown. Multiple factors may have affected the rate of visits and hospitalization for cardiac causes during the most dramatic periods of COVID-19 pandemic, as demonstrated also by the unpredictable reduction in hospitalizations for other causes, such stroke and sepsis. However, these findings indirectly suggest that the fear of contagion at the hospital probably have discouraged the patients to alert the EMS during the first wave of COVID-9 pandemic, particularly after the media diffused the news that infection was spread across hospitalized patients and healthcare personnel. The concerns raised by the mass media on the high mortality rate of COVID-19 pneumonia further discouraged patients with cardiac conditions to contact the EMS. As reported by De Rosa et al. (2), a second hypothesis can be that the emergency medical system was focused on COVID-19. However, our study demonstrates that the number of calls to the EMS significantly decrease during this dramatic period; while variations in the rate of ACS and cardiac disease have been demonstrated (5, 6) and cannot be definitely excluded, the marked difference between the same periods of 2018/2019 and 2020, reaching even more than −65% reduction in the visits, suggest that patients intentionally decided not to alert the EMS or to go to the hospital, irrespective of their cardiac conditions and their symptoms. Unfortunately, this phenomenon was not confined to Italy, but sharp drops in the numbers of persons seeking emergency medical care was observed also in United States, with the total number of US ED visits being 42% lower during the early pandemic period than during the same period a year earlier (7), and in Thailand (−36%) (8). Notably, also in US the decrease in ED visits for acute life-threatening health conditions was observed immediately before and after declaration of the COVID-19 pandemic as a national emergency (9). In agreement with our findings, also Wongtanasarasin et al. observed that the national lockdown in Thailand was associated with a significant reduction in average daily ED visits (8).

A reduction in ACS activations was reported also by US cardiac catheterization laboratories and was noticed also in Spain (10, 11) and in a recent survey conducted by the European Society of Cardiology the respondents declared a reduction in the admission of patients with ACS >40% (12). In Italy, a reduction in the rate of hospital admissions for ACS was reported by De Rosa et al. for the week 12–19 March (2), by Toniolo et al. (3), and by De Filippo et al. (4). Notably, the study by De Rosa was a national registry with analysis confined to 1 week while the other two articles included centers in the Northern part of Italy, i.e., the most affected by COVID-19 pandemic. Indeed, Lombardy and Piedmont regions had 89,526 and 30,758 confirmed cases of COVID-19, respectively, while in Tuscany 10,122 cases were diagnosed (http://opendatadpc.maps.arcgis.com/apps/opsdashboard/index.html#/b0c68bce2cce478eaac82fe38d4138b1 last access, 06/05/2020). In this study we extended the time frame

of observation reporting the data of the first trimester 2020 from a different region of Italy, i.e., the Tuscany, and we demonstrated that a low rate of contacts to EMS during this pandemic was observed also in regions of Italy less affected by the pandemic. We found that the reduction in admission was observed also for patients with heart failure, with a delta of −56% for the last period of observation in comparison with 2018 and 2019 ($p < 0.0001$), in agreement with data reported by Severino et al. and demonstrating a reduction of admission during the lockdown (13). These findings suggest that the ubiquitous presence of COVID-19 news on the mass media and social media and the lack of verified information have contributed to the perception of unsafe hospitals, even if hospital were not overwhelmed by the COVID-19 emergency, as in Tuscany, and an underestimation of mortality and morbidity risks due to cardiac conditions. Indeed, as demonstrated by Barbieri et al., the reduction in hospital admissions observed in 2020 ad compared to the same period of 2019 was associated with increased mortality (14).

Finally, we found in this study that, for the first trimester 2020, the in-hospital mortality did not differ for patients admitted for ACS and for HF, in comparison with the first trimester of 2018 and 2019. Furthermore, the number of patients with ACS and HF requiring hospitalization in an intensive care unit did not differ. Although the impact of the decrease in the number of hospitalizations and visits to the Emergency Departments on the cardiovascular mortality was not the primary scope of this study, these findings suggest that patients were treated with similar standards before and during the first wave of COVID-19 pandemic and with similar outcomes. However, the low rate of hospitalizations for ACS and AF may represent a warning alert for the future development of cardiac and cerebrovascular complications, such as end-stage heart failure, sudden death, or transient ischemic attack and stroke and the negative effects of this marked impact on the pattern of hospitalizations will likely be seen in the next future. Further studies extending the period of observation are needed to report a comprehensive analysis of this phenomenon. Furthermore, the negative impact of the reduction in hospitalization for cardiac causes may have cause an increase in out-of-hospital mortality. Unfortunately, these data were not available.

The present data further strengthen the need of adequate public information policies to reinforce the importance of timely care for medical emergencies. Furthermore, the lesson learnt from the first wave of COVID-19 pandemic suggests that the community of healthcare professionals should continue re-educating the general population to recognize early cardiac symptoms (2) and to be confident with the national healthcare system in case of hospitalization.

Limitations

In this study we observed a dramatic decrease of hospital admissions and emergency contacts, primarily due to the fear of contagion. Although the fear of contagion likely was the primary mechanisms leading to the reduction of hospital admissions, a multiplicity of factors, rather than a unique mechanism, contributed to this phenomenon. As reported by De Rosa et al. (2), we cannot completely exclude that a true reduction in the incidence of acute cardiovascular disease as the potential result of low physical stress and widespread prevalence of the resting state during the quarantine, especially in the initial phase of the social containment, might have partly contributed to the lower number of admissions.

Although patients affected by SARS-CoV-2 were excluded from the final analysis, we cannot definitively exclude that some cardiac symptoms suffered from patients contacting the EMS may be related to cardiac consequences of COVID-19 infection.

CONCLUSIONS

In Tuscany a significant decrease in the contacts to EMS for symptoms and disease related to cardiac causes and in the hospitalization rate for ACS, AF, and HF was observed during the COVID-19 pandemic. In the comparison with the same period of the previous years, the greatest difference was identified after the first case of COVID-19 in Italy and after the national lockdown. Fear of contagion among the patients has likely played the most relevant role. Therefore, the lesson learnt from the first wave of COVID-19 pandemic suggests that appropriate public information strategies are essential for a proper management of cardiac patients and a re-education of general population to recognize cardiac symptoms and life-threatening cardiovascular disorders and the consequent need of hospitalization should be guaranteed.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

FD'A, SM, and SV contributed to the conception of the study while FD'A, SF, FG, and MN contributed to the design of the study. FD'A wrote the manuscript. MC, SF, FG, CS, VD, MM, SM, and SV critically revised the manuscript. All the authors gave the final approval and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.625569/full#supplementary-material

Supplementary Figure 1 | Number of accesses to emergency department for stroke in Tuscany.

Supplementary Figure 2 | Number of accesses to emergency department for sepsis in Tuscany.

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. 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
3. Toniolo M, Negri F, Antonutti M, Mase M, Facchin D. Unpredictable fall of severe emergent cardiovascular diseases hospital admissions during the COVID-19 pandemic: experience of a single large center in Northern Italy. *J Am Heart Assoc*. (2020) 9:e017122. doi: 10.1161/JAHA.120.017122
4. 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
5. Nagarajan V, Fonarow GC, Ju C, Pencina M, Laskey WK, Maddox TM, et al. Seasonal and circadian variations of acute myocardial infarction: Findings from the Get With The Guidelines-Coronary Artery Disease (GWTG-CAD) program. *Am Heart J*. (2017) 189:85–93. doi: 10.1016/j.ahj.2017.04.002
6. Stewart S, Keates AK, Redfern A, McMurray JJV. Seasonal variations in cardiovascular disease. *Nat Rev Cardiol*. (2017) 14:654–64. doi: 10.1038/nrcardio.2017.76
7. Hartnett KP, Kite-Powell A, DeVies J, Coletta MA, Boehmer TK, Boehmer TK, et al. Impact of the COVID-19 pandemic on emergency department visits—United States, January 1, 2019-May 30, 2020. *MMWR Morb Mortal Wkly Rep*. (2020) 69:699–704. doi: 10.15585/mmwr.mm6923e1
8. Wongtanasarasin W, Srisawang T, Yothiya W, Phinyo P. Impact of national lockdown towards emergency department visits and admission rates during the COVID-19 pandemic in Thailand: a hospital-based study. *Emerg Med Australas*. (2020). doi: 10.1111/1742-6723.13666. [Epub ahead of print].
9. Lange SJ, Ritchey MD, Goodman AB, Dias T, Twentyman E, Fuld J, et al. Potential indirect effects of the COVID-19 pandemic on use of emergency departments for acute life-threatening conditions—United States, January-May 2020. *Am J Transplant*. (2020) 20:2612–7. doi: 10.1111/ajt.16239
10. 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
11. Aldama G, Rebollal F, Flores X, Pinon P, Rodriguez-Leor O, Vazquez JM. Decrease in the number of primary angioplasty procedures during the pandemic and its relationship with mortality from COVID-19. The role of competing risks. *Rev Esp Cardiol (Engl Ed)*. (2020). doi: 10.1016/j.rec.2020.11.008
12. 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 Heart J Qual Care Clin Outcomes*. (2020) 6:210–6. doi: 10.1093/ehjqcco/qcaa046
13. Severino P, D'Amato A, Saglietto A, D'Ascenzo F, Marini C, Schiavone M, et al. Reduction in heart failure hospitalization rate during coronavirus disease 19 pandemic outbreak. *ESC Heart Fail*. (2020) 7:4182–8. doi: 10.1002/ehf2.13043
14. Barbieri G, Spinelli S, Filippi M, Foltran F, Giraldi M, Martino MC, et al. COVID-19 pandemic management at the Emergency Department: the changing scenario at the University Hospital of Pisa. *Emerg Care J*. (2020) 16:9146.

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Echocardiographic Characteristics and Outcome in Patients With COVID-19 Infection and Underlying Cardiovascular Disease

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Background: The cardiac manifestations of coronavirus disease 2019 (COVID-19) patients with cardiovascular disease (CVD) remain unclear. We aimed to investigate the prognostic value of echocardiographic parameters in patients with COVID-19 infection and underlying CVD.

Methods: One hundred fifty-seven consecutive hospitalized COVID-19 patients were enrolled. The left ventricular (LV) and right ventricular (RV) structure and function were assessed using bedside echocardiography.

Results: Eighty-nine of the 157 patients (56.7%) had underlying CVD. Compared with patients without CVD, those with CVD had a higher mortality (22.5 vs. 4.4%, $p = 0.002$) and experienced more clinical events including acute respiratory distress syndrome, acute heart injury, or deep vein thrombosis. CVD patients presented with poorer LV diastolic and RV systolic function compared to those without CVD. RV dysfunction (30.3%) was the most frequent, followed by LV diastolic dysfunction (9.0%) and LV systolic dysfunction (5.6%) in CVD patients. CVD patients with high-sensitivity troponin I (hs-TNI) elevation or requiring mechanical ventilation therapy demonstrated worsening RV function compared with those with normal hs-TNI or non-intubated patients, whereas LV systolic or diastolic function was similar. Impaired RV function was associated with elevated hs-TNI level. RV function and elevated hs-TNI level were independent predictors of higher mortality in COVID-19 patients with CVD.

Conclusions: Patients with COVID-19 infection and underlying CVD displayed impaired LV diastolic and RV function, whereas LV systolic function was normal in most patients. Importantly, RV function parameters are predictive of higher mortality.

Keywords: COVID-19, cardiovascular disease, echocardiography, cardiac injury, cardiac function

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a global pandemic causing an escalating number of cases and fatalities worldwide. A large proportion of COVID-19 patients have comorbidities, with cardiovascular disease (CVD) being the most frequent. It was present in approximately 30–48% of patients (1–3). Patients with CVD are more likely to be infected with SARS-CoV-2 and to develop severe cases. In SARS, the presence of comorbidity increased the risk of death 12-fold (4). Therefore, COVID-19 patients with underlying CVD may suffer from a higher risk of mortality after SARS-CoV-2 infection (3, 5). A recent study revealed that hospitalized COVID-19 patients with concomitant cardiac disease have an exceptionally poor prognosis compared with those without cardiac disease (6). Nevertheless, the detailed features of cardiac function were not yet established in the aforementioned study. In clinical practice, echocardiography is the first-line imaging modality in cardiac assessment and is an indispensable bedside tool, allowing non-invasive quantification of heart performance in COVID-19 patients in isolated wards (7). Currently, there are limited data regarding the cardiac manifestations of COVID-19 patients with CVD. Therefore, we aimed to investigate the echocardiographic characteristics and explore the prognostic value of echocardiographic parameters in COVID-19 patients with CVD.

METHODS

Study Population

This observational study was performed at the west branch of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology of Wuhan, China, which was a designated hospital to treat patients with COVID-19. We enrolled a total of 157 consecutive adult patients who were confirmed to have COVID-19 infection according to the WHO interim guidance from February 12, 2020 to March 16, 2020 (8). Bedside echocardiography was performed in all patients from three wards managed by the investigators for evaluation of cardiac function. The study was approved by Union Hospital Tongji Medical College, Huazhong University of Science and Technology Ethics Committee (KY-2020-02.06). Written informed consent was waived for all participants with emerging infectious diseases as per the Ethics Committee.

Data Collection and Definitions

Epidemiological, medical history, comorbidities, laboratory, treatment, and outcomes data were collected from electronic medical records. The data were analyzed by a trained team of physicians. The timing of laboratory measurements was within 3 days of echocardiographic examination with a mean interval of 1 day [interquartile range (IQR), 1–2]. The median time from admission to echocardiographic examination was 7 days (IQR, 3–11). Clinical outcomes (death or discharge) were monitored through to April 7th, 2020.

Underlying CVD included a history of hypertension, coronary artery disease, heart failure, cardiomyopathy, and arrhythmia. Acute cardiac injury was defined as serum levels of cardiac high-sensitivity troponin I (hs-TNI) above the 99th percentile upper reference limit.

Echocardiography

Bedside echocardiography examinations were performed with an EPIQ 7C machine (Philips Medical Systems, Andover, MA, USA) at the designated COVID-19 isolation wards or intensive care units (ICU). Two-dimensional and Doppler echocardiography were performed in standard views according to the American Society of Echocardiography (ASE) guidelines (9). All scans were conducted by trained individuals in full personal protective equipment (PPE) (B.W., L.H., D.Z., Y.Z., H.Y., C.W., and H.L.). Personal protection at the time of echocardiographic assessment included wearing protective clothing, double gloving, shoe covers, head covers, N95 respirator masks, goggles, face shields. All images were stored in the ultrasound machine. At the end of the day, images were copied to hard disk and saved in Digital Imaging for subsequent offline analysis to reduce exposure contamination. Echocardiographic image readers (S.Z., W.S., Y.C., and L.C.) were blinded to epidemiological, clinical, laboratory, treatment, and outcomes findings.

Left Heart Assessment

Left ventricular (LV) ejection fraction (LVEF) and volumes were calculated using Simpson's biplane method. LV mass was calculated according to Devereux's formula. LV diastolic function was estimated using the ratio of early transmitral flow velocity (E) to the late transmitral flow velocity (A) and the ratio of transmitral E to the early diastolic LV septal tissue velocity (e′). LV systolic dysfunction was defined as a LVEF <50%, and LV diastolic dysfunction was determined according to the published guideline of the American Society of Echocardiography (ASE) and the European Association of Cardiovascular Imaging (EACVI) (10).

Right Heart Evaluation

RV function was assessed by tricuspid annular plane systolic excursion (TAPSE), fractional area change (FAC), peak systolic velocity (S′) of the tricuspid lateral annulus, and myocardial performance index (MPI) (9). RV dysfunction was defined as the aforementioned parameters measured to be lower than the published reference values (9). Representative examples of RVFAC and TAPSE measurements from COVID-19 patients without and with CVD are shown in **Figure 1**. The degree of tricuspid regurgitation (TR) was defined as moderate, moderate to severe, or severe TR. Pulmonary artery systolic pressure (PASP) was estimated according to published guidelines (9).

Statistical Analysis

Continuous numeric variables are expressed as mean ± SD or medians (interquartile range), and categorical variables are expressed as frequency (percentage). Continuous variables were compared using a two-sample *t*-test or Mann–Whitney test. Categorical variables were compared using the χ^2-test or Fisher's exact test. Correlations between echocardiographic and biomarker parameters were examined using Spearman's correlation coefficient. Receiver operator characteristic (ROC)

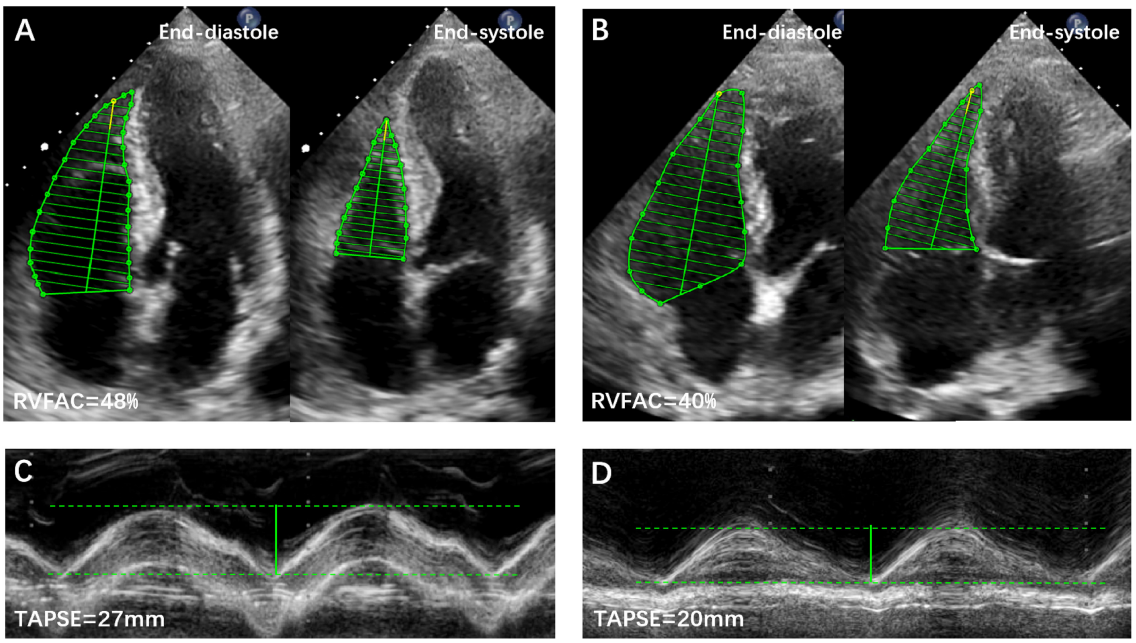


FIGURE 1 | Representative examples of RVFAC and TAPSE measurements from COVID-19 Patients without and with CVD. **(A)** RVFAC in COVID-19 patient without CVD. **(B)** RVFAC in COVID-19 patient with CVD. **(C)** TAPSE in COVID-19 patient without CVD. **(D)** TAPSE in COVID-19 patient with CVD. CVD, cardiovascular disease; RVFAC, right ventricular fractional area change; TAPSE, tricuspid annular plane systolic excursion.

curves were used to evaluate the optimal cutoff value (maximum Youden index) of LV and RV function parameters for detecting poor outcome. Survival curves were plotted using the Kaplan–Meier analysis and compared using the log-rank test. To investigate the risk factors associated with in-hospital death, univariate and multivariate Cox regression models were used. All potential explanatory variables entered into univariate analyses, including age, sex, laboratory findings, LV and RV echocardiographic parameters, and comorbidities. Variables with $p < 0.05$ in univariate Cox proportional hazard regression were included in the multivariate model. To assess the additional prognostic value of echocardiographic parameters over other clinical variables, likelihood ratio tests were performed, and Akaike information criterion (AIC) and Harrell's C statistic were calculated. All statistical analyses were performed using SPSS version 24.0 (SPSS Inc., Chicago, Illinois) and R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). Statistical charts were generated using Prism 7 (GraphPad) and Minitab (Version 18). A two-sided $p < 0.05$ was considered as statistically significant.

RESULTS

Clinical and Echocardiographic Characteristics in Patients With COVID-19 and CVD

Clinical characteristics of patients with COVID-19 with and without CVD are shown in **Table 1**. Among the 157 hospitalized patients with COVID-19, 134 (85.4%) patients were discharged and 23 (14.6%) patients died. The mean age was 62 ± 13 years, and 79 (50.3%) were men. Eighty-nine (56.7%) patients had underlying CVD. Among the CVD patients, hypertension, coronary artery disease, heart failure, and arrhythmia were present in 78.7, 29.2, 4.5, and 6.7% of the patients, respectively. Compared with patients without CVD, those with pre-existing CVD were older, and a higher proportion were men (42.7% female). Patients with underlying CVD were more likely to have a higher systolic arterial pressure, lower level of lymphocyte count and partial pressure of arterial oxygen to percentage of inspired oxygen ratio (PaO_2: FIO_2), higher levels of serum hs-TNI and B-type natriuretic peptide (BNP), more treatment with antibiotic, high-flow oxygen and mechanical ventilation, higher rate of ICU admissions, and higher incidence of acute respiratory distress syndrome (ARDS), acute heart injury, and deep vein thrombosis (DVT). Mortality was significantly higher in CVD compared with non-CVD patients (22.5 vs. 4.4%, $p = 0.002$).

Echocardiographic characteristics of COVID-19 patients with and without CVD are depicted in **Table 2**. Compared with patients without CVD, those with CVD had impaired LV diastolic and RV function and a higher PASP. No differences were identified in LV wall thickness and mass, LV volumes, LVEF, and mitral regurgitation (MR) or TR severity. The most frequent cardiac abnormality in CVD patients was RV dysfunction (27/89, 30.3%), followed by LV diastolic dysfunction (8/89, 9.0%) and LV systolic dysfunction (5/89, 5.6%).

At the time of echocardiographic examination, 27 (30%) COVID-19 patients with CVD were treated with

TABLE 1 | Clinical characteristics of patients with COVID-19 infection with and without cardiovascular disease.

Variables	All patients (*n* = 157)	With CVD (*n* = 89)	Without CVD (*n* = 68)	*P*-value
Clinical characteristic				
Age, years	62 ± 13	66 ± 11	58 ± 14	<0.001
Male, *n* (%)	79 (50.3%)	51 (57.3%)	28 (41.2%)	0.045
Body mass index, kg/m^2	24.1 ± 3.1	24.0 ± 3.0	24.3 ± 3.1	0.445
Heart rate, beats/min	90 ± 17	89 ± 16	92 ± 17	0.164
Respiratory rate, breaths/min	25 ± 6	25 ± 6	25 ± 6	0.780
Systolic arterial pressure, mm Hg	133 ± 81	138 ± 17	126 ± 17	<0.001
Diastolic arterial pressure, mm Hg	81 ± 12	82 ± 13	80 ± 10	0.096
Smoker, *n* (%)	17 (10.8%)	11 (12.4%)	6 (8.8%)	0.480
Comorbidities				
Hypertension, *n* (%)	70 (44.6%)	70 (78.7%)	0 (0%)	<0.001
Diabetes, *n* (%)	23 (14.6%)	17 (19.1%)	6 (8.8%)	0.071
Obesity, *n* (%)	24 (15.3%)	15 (16.9%)	9 (13.2%)	0.532
COPD, *n* (%)	9 (5.7%)	6 (6.7%)	3 (4.4%)	0.534
Coronary artery disease, *n* (%)	26 (16.6%)	26 (29.2%)	0 (0%)	<0.001
Heart failure, *n* (%)	4 (2.5%)	4 (4.5%)	0 (0%)	0.077
Arrhythmia, *n* (%)	6 (3.8%)	6 (6.7%)	0 (0%)	0.029
Chronic kidney disease, *n* (%)	3 (1.9%)	2 (2.2%)	1 (1.5%)	0.725
Chronic liver disease, *n* (%)	6 (3.8%)	2 (2.2%)	4 (5.8%)	0.234
Malignancy, *n* (%)	11 (7.0%)	3 (3.4%)	8 (11.8%)	0.041
Laboratory findings				
Lymphocyte count, $\times 10^9$/L	1.0 (0.6, 1.4)	0.9 (0.5, 1.2)	1.0 (0.7, 1.5)	0.012
D-dimer, mg/L	1.1 (0.4, 2.7)	1.5 (0.4, 2.4)	1.0 (0.5, 4.2)	0.295
PT, s	13.5 (12.5, 15.0)	13.4 (12.6, 15.2)	13.7 (12.5, 14.5)	0.99
APTT, s	37.4 (33.3, 44.6)	38.0 (33.1, 45.6)	37.0 (33.7, 42.2)	0.555
CK-MB, U/L	11 (8, 18)	12 (8, 25)	10 (8, 13)	0.05
hs-TNI, ng/L	4.8 (2.2, 31.2)	10.6 (3.3, 53.7)	2.7 (1.7, 7)	0.043
BNP, pg/ml	79.1 (35.7, 163.9)	85.3 (34.6, 162.5)	57.9 (38.7, 153.2)	0.049
CRP, mg/L	26.5 (3.7, 67.6)	27.5 (7.1, 75.4)	25.3 (2.8, 63.2)	0.44
PCT, ng/ml	0.08 (0.05, 0.20)	0.10 (0.05, 0.20)	0.07 (0.05, 0.21)	0.244
IL-6, pg/ml	5.2 (2.4, 20.7)	8.9 (3.5, 21.6)	4.6 (2.5, 21.7)	0.269
PaO_2:FIO_2, mmHg	232.0 (151.0, 268.97)	212.1 (140.6, 241.5)	254.0 (212.1, 330.5)	0.016
Treatments				
Antiviral therapy, *n* (%)	150 (95.5%)	86 (96.6%)	64 (94.1%)	0.45
Antibiotic therapy, *n* (%)	119 (75.8%)	73 (82.0%)	46 (67.6%)	0.037
Glucocorticoid therapy, *n* (%)	65 (41.4%)	36 (40.4%)	29 (42.6%)	0.782
Intravenous immune globulin, *n* (%)	56 (35.9%)	37 (41.6%)	19 (27.9%)	0.089
Anticoagulant therapy, *n* (%)	81 (51.6%)	52 (58.4%)	29 (42.6%)	0.05
Diuretics, *n* (%)	39 (24.8%)	32 (36.0%)	7 (10.3%)	<0.001
Beta-blockers, *n* (%)	33 (21.0%)	28 (31.5%)	5 (7.4%)	<0.001
Calcium channel blockers, *n* (%)	48 (30.6%)	43 (48.3%)	5 (7.4%)	<0.001
ACE-I/ARB, *n* (%)	17 (10.8%)	15 (16.9%)	2 (2.9%)	0.005
Oxygen therapy, *n* (%)	139 (88.5%)	83 (93.3%)	56 (82.3%)	0.034
High-flow oxygen, *n* (%)	90 (57.3%)	61 (68.5%)	29 (42.6%)	0.001
Mechanical ventilation, *n* (%)	37 (23.6%)	27 (30.3%)	10 (14.7%)	0.022
IMV, *n* (%)	26 (16.6%)	19 (21.3%)	7 (10.3%)	0.065
NIMV, *n* (%)	11 (7.0%)	8 (9.0%)	3 (4.4%)	0.266
ICU admission, *n* (%)	27 (17.2%)	20 (22.5%)	7 (10.3%)	0.045
Complications				
Acute kidney injury, *n* (%)	20 (12.8%)	12 (13.5%)	8 (11.8%)	0.775

(Continued)

TABLE 1 | Continued

Variables	All patients ($n = 157$)	With CVD ($n = 89$)	Without CVD ($n = 68$)	*P*-value
ARDS, *n* (%)	64 (40.8%)	47 (52.8%)	17 (25.0%)	<0.001
Acute heart injury, *n* (%)	48 (20.6%)	35 (39.3%)	13 (19.1%)	0.006
Coagulation dysfunction, *n* (%)	29 (18.5%)	19 (21.3%)	10 (14.7%)	0.288
DVT, *n* (%)	63 (40.1%)	42 (47.2%)	21 (30.9%)	0.039
Shock, *n* (%)	1 (0.6%)	1 (1.1%)	0 (0%)	0.567
Prognosis				
Discharge, *n* (%)	134 (85.4%)	69 (77.5%)	65 (95.6%)	0.002
Death, *n* (%)	23 (14.6%)	20 (22.5%)	3 (4.4%)	0.002

Values are mean ± SD, n (%), median (interquartile range).
ACE-I, angiotensin-converting enzyme inhibitors; APTT, activated partial thromboplastin time; ARB, angiotensin II receptor blockers; ARDS, acute respiratory distress syndrome; BNP, B-type natriuretic peptide; CK-MB, creatine kinase muscle–brain; COVID-19, coronavirus disease 2019; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CVD, cardiovascular disease; DVT, deep vein thrombosis; FIO_2, fraction of inspiration oxygen; HF, heart failure; hs-TNI, high-sensitivity troponin I; ICU, intensive care unit; IL-6, interleukin-6; IMV, invasive mechanical ventilation; NIMV, non-invasive mechanical ventilation; PCT, procalcitonin; PT, prothrombin time; PaO_2, partial pressure of oxygen.

TABLE 2 | Echocardiographic characteristics of patients with COVID-19 with and without cardiovascular disease.

Variables	All patients ($n = 157$)	With CVD ($n = 89$)	Without CVD ($n = 68$)	*P*-value
Left heart				
LA dimension, mm	35.4 ± 5.5	36.7 ± 5.9	33.3 ± 4.3	< 0.001
LV dimension, mm	45.7 ± 5.1	45.7 ± 5.0	45.7 ± 5.2	0.967
IVS, mm	9.6 ± 1.2	9.7 ± 1.3	9.5 ± 1.0	0.125
PW, mm	9.1 ± 1.3	9.2 ± 1.4	8.9 ± 1.2	0.291
LVMI, g/m^2	86.9 ± 21.0	88.4 ± 23.4	84.7 ± 16.9	0.331
Mitral DT, ms	203 ± 55	206 ± 53	200 ± 58	0.561
Mitral E/A	0.91 ± 0.36	0.88 ± 0.33	0.96 ± 0.39	0.473
Mitral E/e′	9.2 ± 3.2	9.7 ± 3.4	8.5 ± 2.8	0.043
LVEDVI, ml/m^2	51.3 (43.8, 62.5)	53.5 (43.0, 64.7)	50.7 (44.0, 58.0)	0.173
LVESVI, ml/m^2	19.3 (15.6, 25.7)	21.7 (15.6, 28.1)	18.6 (15.6, 23.8)	0.085
LVEF, %	63.4 ± 7.0	62.5 ± 8.3	64.7 ± 4.7	0.063
Moderate-severe MR, *n* (%)	6 (3.9%)	5 (5.6%)	1 (1.5%)	0.179
Right heart				
RA dimension, mm	35.8 ± 5.0	36.6 ± 5.3	34.9 ± 4.4	0.042
RV dimension, mm	34.6 ± 5.5	34.9 ± 5.6	34.2 ± 5.3	0.390
Tricuspid E/A	0.96 ± 0.29	0.92 ± 0.29	1.0 ± 0.29	0.134
Tricuspid E/e′	5.5 ± 1.8	5.7 ± 1.7	5.2 ± 2.0	0.577
TAPSE, mm	22.2 ± 3.8	21.5 ± 3.7	23.2 ± 3.9	0.007
RV FAC, %	47.5 ± 6.8	46.0 ± 5.3	49.3 ± 7.3	0.009
S′, cm/s	13.5 ± 3.2	13.4 ± 3.1	13.5 ± 3.4	0.946
RV MPI	0.46 ± 0.14	0.48 ± 0.16	0.43 ± 0.10	0.011
Moderate-severe TR, *n* (%)	6 (3.9%)	5 (5.6%)	1 (1.5%)	0.179
PASP, mmHg	32 (24, 47)	42 (27, 50)	28 (24, 39)	0.033

Values are mean ± SD, n (%), median (interquartile range). COVID-19, coronavirus disease 2019; CVD, cardiovascular disease; DT, deceleration time; IVS, interventricular septum; LA, left atrium; LV, left ventricular; LVEDVI, left ventricular end diastolic volume index; LVESVI, left ventricular end systolic volume index; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; MR, mitral regurgitation; RA, right atrium; RV, right ventricular; TAPSE, tricuspid annular plane systolic excursion; RV FAC, RV fractional area change; RV MPI, RV myocardial performance index; TR, tricuspid regurgitation; PASP, pulmonary artery systolic pressure; PW, posterior wall of left ventricle.

mechanical ventilation. These mechanically ventilated patients had decreased TAPSE and RVFAC and higher PASP, suggesting impaired RV function (**Supplementary Table 1**). In contrast, LV systolic or diastolic function was not different between patients with and without mechanical ventilation therapy.

Biomarker Levels and Echocardiography in COVID-19 Patients With CVD

Echocardiographic findings in COVID-19 patients with CVD stratified by hs-TNI level are shown in **Table 3**. Patients with high hs-TNI levels had worse RV function, as evidenced by lower TAPSE and RVFAC, and higher MPI, whereas LV diastolic or systolic function did not differ between patients with and without hs-TNI elevation. Correlations of hs-TNI level with LV and RV parameters are displayed in **Supplementary Table 2**. hs-TNI level negatively correlated with tricuspid E/A, TAPSE, and RVFAC and positively correlated with LA and right heart dimension, mitral E/e′, and RVMPI.

Clinical and Echocardiographic Characteristics of Survivors and Non-survivors Among CVD Patients

Clinical characteristics of survivors and non-survivors among CVD patients are presented in **Supplementary Table 3**. Compared with CVD patients who were alive, those who died were more likely to have been male and have a lower lymphocyte count, higher levels of biomarkers, more likely to be treated with

TABLE 3 | Clinical and echocardiographic characteristics of COVID-19 patients with CVD stratified by hs-TNI level.

Variables	Normal hs-TNI (*N* = 58)	Elevated hs-TNI (*N* = 31)	*P*-value
Age, years	65 ± 11	68 ± 10	0.185
Male, *n* (%)	27 (46.6%)	24 (77.4%)	0.003
Body mass index, kg/m2	23.8 ± 2.9	24.2 ± 3.3	0.629
Heart rate, beats/min	88 ± 17	91 ± 15	0.426
Respiratory rate, times/min	25 ± 6	25 ± 7	0.637
Systolic arterial pressure, mm Hg	139 ± 18	134 ± 16	0.216
Diastolic arterial pressure, mm Hg	83 ± 13	80 ± 13	0.236
CK-MB, U/L	10 (7, 14)	22 (13, 33)	0.072
BNP, pg/ml	53.2 (26.6, 111.8)	138.6 (86.9, 279)	0.062
CRP, mg/L	16.2 (4.2, 16.2)	62.9 (22.7, 124.5)	0.002
PCT, ng/ml	0.07 (0.05, 0.11)	0.21 (0.08, 0.40)	0.003
IL-6, pg/ml	4.5 (3.0, 14.8)	14 (10.5, 71)	0.126
D-dimer, mg/L	0.9 (0.3, 2.1)	1.7 (0.9, 3.0)	0.262
Left heart			
LA dimension, mm	35.7 ± 5.2	38.6 ± 6.5	0.029
LV dimension, mm	45.7 ± 4.9	45.8 ± 5.3	0.913
IVS, mm	9.8 ± 1.2	9.7 ± 1.5	0.653
PW, mm	9.0 ± 1.4	9.4 ± 1.3	0.206
LVMI, g/m^2	87.4 ± 20.5	90.2 ± 28.3	0.628
Mitral E/A	0.82 ± 0.29	0.97 ± 0.38	0.050
Mitral E/e′	9.1 ± 3.0	10.5 ± 3.9	0.084
LVEDVI, ml/m^2	53.0 (42.1, 68.8)	53.5 (45.5, 62.5)	0.079
LVESVI, ml/m^2	21.6 (16.0, 31.1)	23.4 (15.0, 25.3)	0.061
LVEF, %	61.6 ± 8.9	64.2 ± 6.8	0.203
Right heart			
RA dimension, mm	35.6 ± 4.6	38.1 ± 6.1	0.038
RV dimension, mm	34.2 ± 5.3	36.1 ± 6.0	0.134
Tricuspid E/A	0.92 ± 0.30	0.92 ± 0.30	0.985
Tricuspid E/e′	4.8 ± 2.2	5.5 ± 2.4	0.147
TAPSE, mm	22.2 ± 3.7	20.1 ± 3.3	0.013
RVFAC, %	47.2 ± 6.1	43.6 ± 5.0	0.020
S′, cm/s	13.5 ± 3.3	13.4 ± 2.8	0.855
RV MPI	0.45 ± 0.14	0.54 ± 0.17	0.018
PASP, mmHg	32 (26, 40)	47 (34, 56)	0.009

Data are mean ± SD, n (%), median (IQR). hs-TNI elevation was defined as higher than 26.5 ng/L.

SD, standard deviation; IQR, interquartile range. BNP, B-type natriuretic peptide; CK-MB, creatine kinase muscle-brain; CRP, C-reactive protein; hs-TNI, high-sensitivity troponin I; IL-6, interleukin-6; PCT, procalcitonin; COVID-19, coronavirus disease 2019; CVD, cardiovascular disease; IVS, interventricular septum; LA, left atrium; LV, left ventricular; LVEDVI, left ventricular end diastolic volume index; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end systolic volume index; LVM, left ventricular mass; MPI, myocardial performance index; PW, posterior wall of left ventricle; RA, right atrium; RV, right ventricular; RVFAC, right ventricular fractional area change; RV MPI, RV myocardial performance index; TAPSE, tricuspid annular plane systolic excursion; PASP, pulmonary artery systolic pressure.

glucocorticoids, intravenous immune globulins, anticoagulants, diuretics, high-flow oxygen, and mechanical ventilation, and had a higher rate of admission to the ICU. Among the complications, acute kidney injury, acute heart injury, ARDS, coagulation dysfunction, and DVT were more common in non-survivors than survivor.

Echocardiographic characteristics of survivors and non-survivors among CVD patients are depicted in **Table 4**. Compared with survivors, non-survivors had enlarged left atrial size, lower RV function, and higher PASP, while LV systolic or diastolic function was similar between survivors and non-survivors. Of these non-survivors, 12/20 (60%) patients had RV dysfunction, while only 1/20 (5%) had LV diastolic dysfunction.

Predictors of Mortality in COVID-19 Patients With CVD

LV and RV function parameters were studied by a receiver operating characteristic (ROC) analysis to evaluate the probability of mortality. RV functional indices were associated with a higher risk of mortality in COVID-19 patients with CVD (**Figure 2**). Area under the curve was 0.74 for RVFAC and 0.81 for TAPSE.

Kaplan–Meier survival curves for mortality are displayed **Figures 3A,B**. When stratified by cutoff values, RVFAC <44.3% or TAPSE <18.6 mm was associated with higher mortality ($p < 0.001$). To determine the relationship between levels of hs-TNI, RV function parameters, and mortality, a contour plot was performed. Our findings revealed that decreased RV function was associated with increased mortality, which was pronounced in patients with higher levels of hs-TNI (**Figures 3C,D**).

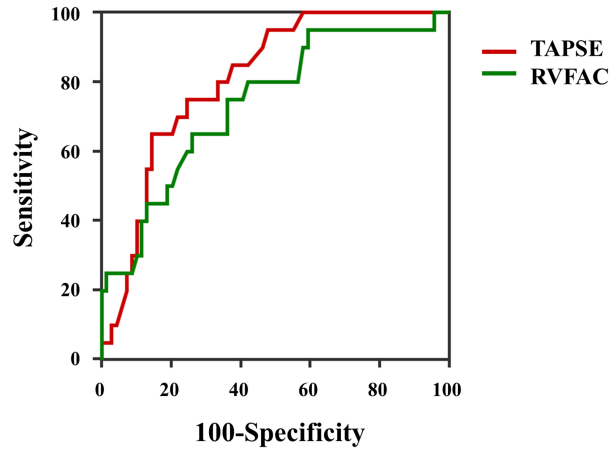
In univariate and multivariate Cox analysis, higher level of hs-TNI, TAPSE, and RVFAC were independent predictors of higher risk of mortality (**Figures 4**, **5**). To determine the incremental prognostic value of TAPSE over RVFAC and clinical variables in COVID-19 patients with CVD, a likelihood ratio test was performed. **Figure 6** compares the additional chi-square statistic value of TAPSE and RVFAC to increase predictive value for mortality. After the addition of RVFAC to the baseline model, an increase in the chi-square value was observed (chi-square difference = 4.9; $p = 0.027$). After the addition of TAPSE to the baseline model, an increased chi-square value was noted (chi-square difference = 10.4; $p = 0.001$). The incremental chi-square value of TAPSE was higher than that of RVFAC, demonstrating the additional prognostic value of TAPSE in COVID-19 patients

TABLE 4 | Echocardiographic characteristics of COVID-19 patients with CVD stratified by vital status.

	With CVD ($n = 89$)	Survivors ($n = 69$)	Non-survivors ($n = 20$)	*P*-value
Left heart				
LA dimension, mm	36.7 ± 5.9	36.2 ± 6.2	38.3 ± 4.3	0.035
LV dimension, mm	45.7 ± 5.0	46.0 ± 5.1	44.9 ± 4.6	0.460
IVS, mm	9.7 ± 1.3	9.9 ± 1.3	9.4 ± 1.3	0.230
PW, mm	9.2 ± 1.4	9.1 ± 1.4	9.3 ± 1.2	0.853
LVMI, g/m^2	88.4 ± 23.4	90.8 ± 24.6	80.4 ± 17.4	0.141
Mitral DT	206 ± 53	210 ± 54	187 ± 45	0.142
Mitral E/A	0.88 ± 0.33	0.80 (0.67, 1.00)	0.72 (0.67, 0.80)	0.110
Mitral E/e′	9.7 ± 3.4	9.7 ± 3.5	9.7 ± 3.0	0.713
LVEDVI, ml/m^2	53.5 (43.0, 64.7)	52.4 (40.3, 67.2)	53.6 (46.4, 59.4)	0.257
LVESVI, ml/m^2	21.7 (15.6, 28.1)	20.9 (15.8, 28.1)	23.4 (14.6, 29.8)	0.505
LVEF, %	62.5 ± 8.3	61.7 ± 8.6	65.4 ± 6.6	0.083
Moderate-severe MR, *n* (%)	5 (5.6%)	2 (2.8%)	3 (15%)	0.073
Right heart				
RA dimension, mm	36.6 ± 5.3	36.0 ± 5.1	38.1 ± 5.8	0.136
RV dimension, mm	34.9 ± 5.6	33.4 ± 5.1	36.7 ± 6.7	0.198
Tricuspid E/A	0.92 ± 0.29	1.0 ± 0.33	1.06 ± 0.24	0.502
Tricuspid E/e′	5.7 ± 1.7	5.9 ± 2.0	5.4 ± 1.3	0.618
TAPSE, mm	21.5 ± 3.7	22.2 ± 3.5	19.1 ± 3.1	0.002
RV FAC, %	46.0 ± 5.3	47.2 ± 5.6	41.6 ± 5.5	0.001
S′, cm/s	13.4 ± 3.1	13.6 ± 3.3	12.9 ± 2.7	0.340
RV MPI	0.48 ± 0.16	0.46 ± 0.15	0.54 ± 0.19	0.045
Moderate-severe TR, *n* (%)	5 (5.6%)	3 (4.3%)	2 (10%)	0.313
PASP, mmHg	42 (27, 50)	33 (27, 43)	48 (34, 59)	0.042

Values are mean ± SD, n (%), median (interquartile range).

COVID-19, coronavirus disease 2019; DT, deceleration time; IVS, interventricular septum; LA, left atrium; LV, left ventricular; LVEDVI, left ventricular end diastolic volume index; LVESVI, left ventricular end systolic volume index; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; MR, mitral regurgitation; RA, right atrium; RV, right ventricular; TAPSE, tricuspid annular plane systolic excursion; RV FAC, RV fractional area change; RV MPI, RV myocardial performance index; TR, tricuspid regurgitation; PASP, pulmonary artery systolic pressure; PW, posterior wall of left ventricle.



Variables	AUC	95%CI	Sensitivity	Specificity	P-value
RVFAC	0.74	0.64-0.83	65%	74%	<0.001
TAPSE	0.81	0.71-0.88	65%	86%	<0.001

FIGURE 2 | Receiver operating characteristic curves of RVFAC and TAPSE for adverse clinical outcome. RVFAC, right ventricular fractional area change; TAPSE, tricuspid annular plane systolic excursion.

with CVD. Moreover, the model with TAPSE (AIC = 129, C index = 0.86) was the best in predicting mortality compared with those with RVFAC (AIC = 137, C index = 0.84), and baseline model (AIC = 138, C index = 0.81).

DISCUSSION

To the best of our knowledge, this may be the first study describing the echocardiographic features and its prognostic value in patients with COVID-19 and CVD. COVID-19 patients with CVD displayed poorer LV diastolic and RV function than non-CVD patients. The most common cardiac abnormality in CVD patients was RV dysfunction, followed by LV diastolic dysfunction and LV systolic dysfunction. Furthermore, diminished RV function was associated with higher mortality in CVD patients, suggesting that RV measurements may be important for detecting COVID-19 patients with CVD who are at higher risk of mortality.

COVID-19 Patients With CVD and Cardiac Injury

Consistent with a previous study, we found that COVID-19 patients with CVD had a significantly higher mortality compared to those without (11). The mechanism of poor outcomes in patients of COVID-19 with CVD remains unknown. Previous reports suggest that coronavirus viral infections may trigger cardiovascular events and exacerbate heart failure (11–13). Direct viral damage, aggravation of a systemic inflammatory response, and hypoxemia may result in cardiac injury. Our study showed that COVID-19 patients with pre-existing CVD are more susceptible to cardiac injury. Furthermore, CVD patients with hs-TNI elevation are more likely to develop severe illness. Prior studies demonstrated that cardiac injury was associated with poor clinical outcome, irrespective of a history of CVD (3, 14, 15). In the present study, CVD patients who died had a significantly higher incidence of cardiac injury compared to those who were alive. Moreover, our results further revealed that the level of hs-TNI could help identify patients at higher risk and requiring earlier or more aggressive treatment strategies.

Cardiac Characteristics of COVID-19 Patients With CVD

Our study showed that patients with COVID-19 infection and underlying CVD had impaired LV diastolic function. This is in keeping with the study of Li et al., which demonstrated that only subclinical LV diastolic impairment was identified in patients with severe acute respiratory syndrome (16). In line with the results of Inciardi et al. (6), no difference was observed in LVEF between patients with or without CVD. Furthermore, LVEF was preserved in the majority of hospitalized CVD patients, in agreement with the results of Churchill et al., demonstrating that LVEF was normal/hyperdynamic in most patients with COVID-19 (17). Several case reports also demonstrate that the majority of patients with uncomplicated myocarditis displays normal cardiac function (17–19). In addition, diminished RV performance was the most common in patients with CVD, consistent with recent reports in unselected COVID-19 patients (20–22).

Generally, the etiology of RV dysfunction in COVID-19 infection has not been well-established. In addition to myocardial injury, it is though that the RV dysfunction may be reflective of conditions that can increase RV afterload during this viral infection, including hypoxic pulmonary vasoconstriction, hypercarbia, excessive positive end-expiratory pressure (PEEP), pneumonia, elevated left atrial pressure, or combination of all these factors (21). In a recent study of 26 symptomatic patients with COVID-19 infection (and without a history of coronary artery disease or myocarditis), Huang et al. investigated cardiac involvement using magnetic resonance imaging and found that 58% of patients displayed impaired RV function (23). Furthermore, myocardial edema and fibrosis were observed in these patients. Indeed, 30% of COVID-19 patients with CVD required mechanical ventilation at the time of echocardiogram. RV dysfunction has been demonstrated to be a complication of hypoxemic injury including ARDS and may deteriorate following mechanical ventilation due to the presence of higher PEEP causing higher RV afterload (24, 25). Importantly, we noticed that LV diastolic and RV function was further diminished in patients with CVD compared with those without. Recent evidence suggests that patients with CVD are more likely to develop severe and critical illness that may partially explain why these patients present with worsening cardiac function (3). Another possible explanation may be that SARS-CoV-2 infection might aggravate a pre-existing cardiovascular condition (26). The poorer cardiac function in COVID-19 patients with CVD may alert physicians to pay greater attention to the management of these patients.

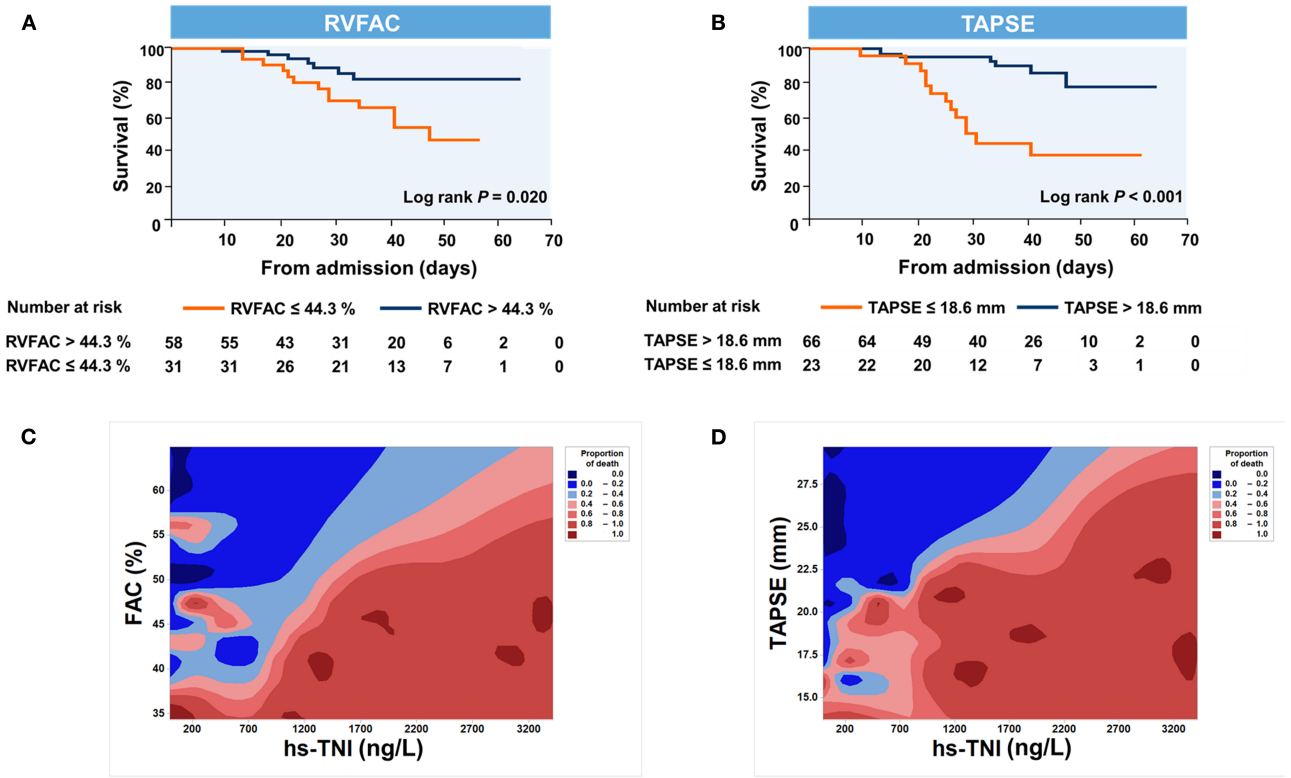


FIGURE 3 | Kaplan–Meier plots and contour plots of survival probability in hospitalized COVID-19 patients with CVD. **(A,B)** Survival significantly declined with diminished TAPSE and RVFAC. **(C,D)** Decreased TAPSE and RVFAC were associated with higher mortality, which were pronounced in patients with higher levels of hs-TNI. RVFAC, right ventricular fractional area change; TAPSE, tricuspid annular plane systolic excursion; hs-TNI, high-sensitivity troponin I.

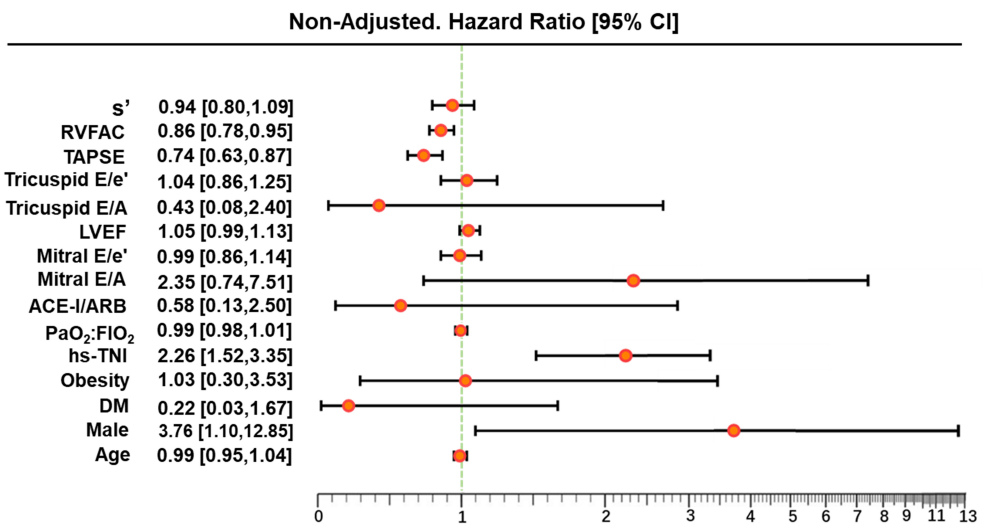


FIGURE 4 | Univariate Cox regression analysis of clinical and echocardiographic parameters. Forest plot for association of clinical and echocardiographic parameters with mortality. Impact of clinical and echocardiographic indicators on mortality in COVID-19 patients with CVD. ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; CI, confidence interval; COVID-19, coronavirus disease 2019; CVD, cardiovascular disease; DM, diabetes mellitus; FIO_2, fraction of inspiration oxygen; hs-TNI, hypersensitive troponin I; LVEF, left ventricular ejection fraction; RVFAC, right ventricular fractional area change; TAPSE, tricuspid annular plane systolic excursion; PaO_2, partial pressure of oxygen.

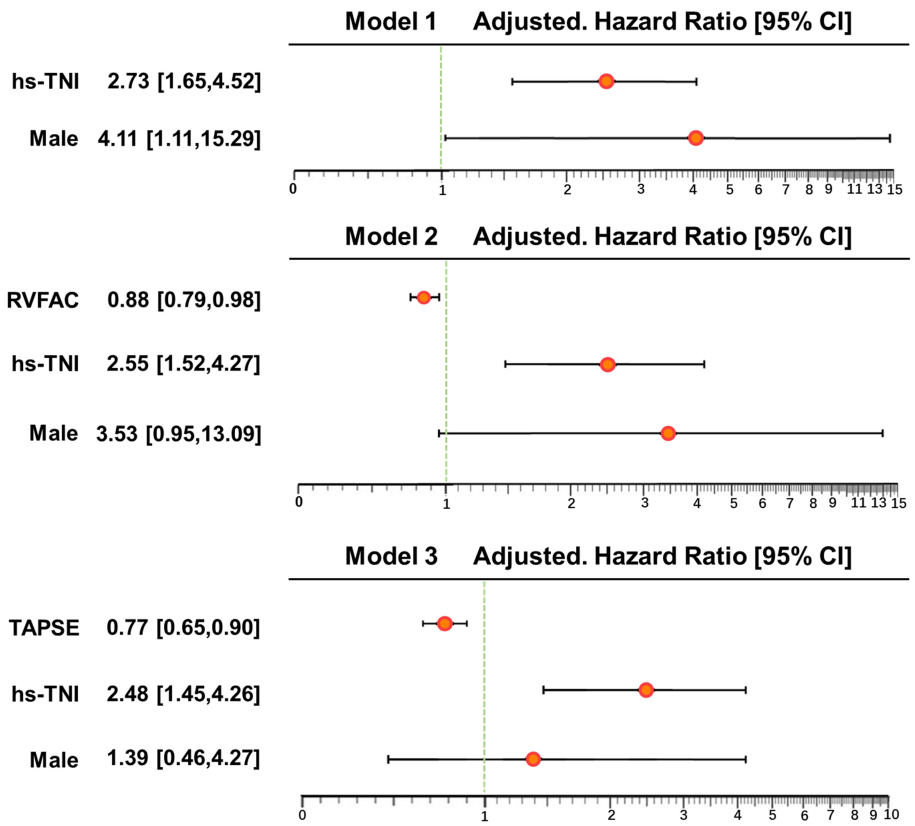


FIGURE 5 | Multivariate Cox regression analysis of clinical and echocardiographic parameters. Forest plot for association of clinical and echocardiographic parameters with mortality. Impact of clinical and echocardiographic indicators on mortality in COVID-19 patients with CVD. CI, confidence interval; COVID-19, coronavirus disease 2019; CVD, cardiovascular disease; hs-TNI, hypersensitive troponin I; RVFAC, right ventricular fractional area change; TAPSE, tricuspid annular plane systolic excursion.

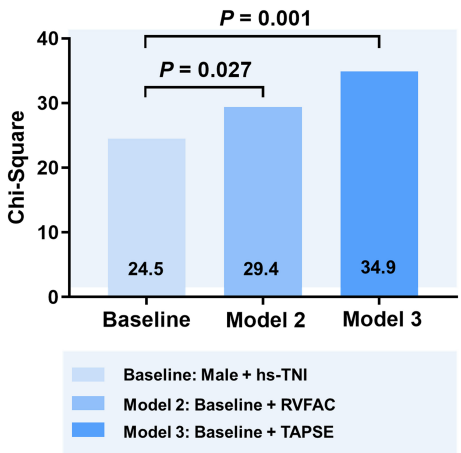


FIGURE 6 | Likelihood ratio test for the incremental prognostic value of TAPSE. The incremental value of TAPSE over clinical and RVFAC for the prediction of mortality. RVFAC, right ventricular fractional area change; TAPSE, tricuspid annular plane systolic excursion; hs-TNI, high-sensitivity troponin I.

Prognostic Value of Echocardiographic Parameters in COVID-19 Patients With CVD

Considering that patients with COVID-19 infection and underlying CVD are more likely to have a more severe course of their illness and a poorer clinical outcome, it is imperative to identify this high-risk group for consideration of earlier or more intensive therapy. Thus far, some prognostic indicators of poor outcome, in particular elevated level of hs-TNI, have been recognized (3, 27, 28). Our current study not only verified the role of these previously reported risk prognosticators but also reported the novel and additive prognostic value of RV measurements in patients with COVID-19 infection and underlying CVD.

In our study, patients found to have reduced RV function by echocardiography were at higher risk of deterioration and death. Our results demonstrate that RV function serves as a novel imaging biomarker that predicts higher mortality in patients with COVID-19 infection and underlying CVD. These findings were consistent with our previous work showing that RV dysfunction predicted poorer outcome in unselected patients

with COVID-19 (with or without CVD) (25). Similarly, in a recent study of 110 patients with COVID-19, Argulian et al. demonstrated that RV dilation was an independent predictor of in-hospital mortality (29). Importantly, our study reveals that TAPSE appears to be the best predictor of higher mortality compared with RVFAC and other clinical variables. RVFAC depends on imaging quality, resulting in relatively poor inter- and intraobserver reproducibility in subjects with suboptimal endocardial definition. In contrast, TAPSE is less dependent upon image quality, is simple to perform, and is reproducible. TAPSE is widely used on a daily basis in most echocardiographic laboratories. Considering the reduced time of exposure during echocardiographic examination in patients with COVID-19, the present study revealed the key clinical implication of TAPSE, as it can be easily obtained during bedside echocardiography. Our results highlights that the additional prognostic value of TAPSE over the other clinical parameters and RVFAC is important for risk stratification in COVID-19 patients with CVD.

Limitations

Although our results demonstrated the presence of cardiac impairment in COVID-19 patients with underlying CVD, the time course for the development of these cardiac abnormalities remained unknown, as we did not have serial echocardiography available for these patients. Another limitation to consider is that although RV functional parameters were revealed to be important predictors of risk in this study, we only carried out the basic, commonly used measures of RV function such as TAPSE and RVFAC (30), as opposed to more advanced measures such as RV myocardial strain and RV three-dimensional imaging, which are now recommended for consideration by the ASE (31) and EACVI (32).

Finally, the main limitation of our study was that it was a single-center study, with a relatively limited sample size and a homogenous population. As a center designated to treat patients with COVID-19 in our region, our study subjects may not be representative of populations elsewhere, limiting extrapolation of our results. Future studies, involving larger sample sizes, multiple centers, and international collaboration, are needed to determine the true prognostic value of echocardiographic parameters in patients with COVID-19 infection and allow for further refinement of stratification by determinants such as sex, age, and ethnicity.

CONCLUSIONS

Right ventricular dysfunction is more common than LV dysfunction among COVID-19 patients with underlying CVD. Importantly, RV function parameters are associated with higher mortality, suggesting that RV measurement may serve as a novel imaging biomarker for the risk stratification of patients with COVID-19 infection and underlying CVD. The study highlights the importance of bedside cardiovascular ultrasound in the assessment and prognostication of hospitalized patients with COVID-19 infection.

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 authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Tongji Medical College, Huazhong University of Science and Technology. 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

Conception and design of the study: YL, LF, SZ, YX, JW, YY, QL, AJ, MX, and LZ. Acquisition of data: BW, LH, DZ, YoZ, and HY. Analysis and interpretation of data: CW, HL, WS, YaZ, ML, YC, and LC. Drafting the article: YL, LF, SZ, and YX. Revising the article: YL, LF, SZ, YX, LZ, and MX. Final approval of the article: SZ, YX, LY, and LZ. All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.642973/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. 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

3. 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
4. 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
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. Inciardi RM, Adamo M, Lupi L, Cani DS, Pasquale MD, 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
7. Zhang L, Wang B, Zhou J, Kirkpatrick J, Xie MX, Johri AM. Bedside focused cardiac ultrasound in COVID-19 infection from the Wuhan epicenter: the role of cardiac point of care ultrasound (POCUS), limited transthoracic echocardiography and critical care echocardiography. *J Am Soc Echocardiogr*. (2020) 33:676–82. doi: 10.1016/j.echo.2020.04.004
8. Hendren NS, Drazner MH, Bozkurt B, Cooper LT. Description and proposed management of the acute COVID-19 cardiovascular syndrome. *Circulation*. (2020) 141:1903–14. doi: 10.1161/CIRCULATIONAHA.120.047349
9. 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
10. 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 Echocardiogr*. (2016) 29:277–314. doi: 10.1016/j.echo.2016.01.011
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. Udell JA, Rosamond W, Temte J, Udell JA, Rosamond W, Temte J, et al. Association of influenza-like illness activity with hospitalizations for heart failure: the atherosclerosis risk in communities study. *JAMA Cardiol*. (2019) 4:363–9. doi: 10.1001/jamacardio.2019.0549
13. 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
14. 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
15. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARSCoV-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
16. Li SS, Cheng CW, Fu CL, Chan Y, Lee M, Chan J, et al. Left ventricular performance in patients with severe acute respiratory syndrome: a 30-day echocardiographic follow-up study. *Circulation*. (2003) 108: 1798–803. doi: 10.1161/01.CIR.0000094737.21775.32
17. 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
18. Hu H, Ma F, Wei X, Fang Y. Coronavirus fulminant myocarditis saved with glucocorticoid and human immunoglobulin. *Eur Heart J*. (2021) 42:206. doi: 10.1093/eurheartj/ehaa190
19. Danzi GB, Loffi M, Galeazzi G, Gherbesi E. Acute pulmonary embolism and COVID-19 pneumonia: a random association. *Eur Heart J*. (2020) 41:1858. doi: 10.1093/eurheartj/ehaa254
20. Li Y, Li H, Zhu S, Wang B, He L, Zhang D, 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
21. Szekely Y, Lichter Y, Taieb P, Banai A, Hochstadt A, Merdler I, et al. The spectrum of cardiac manifestations in coronavirus disease 2019 (COVID-19) - a systematic echocardiographic study. *Circulation*. (2020) 142:342–53. doi: 10.1161/CIRCULATIONAHA.120.047971
22. Mahmoud-Elsayed HM, Moody WE, Bradlow WM, Khan-Kheil AM, Senior J, Hudsmith LE, et al. Echocardiographic findings in Covid-19 pneumonia. *Can J Cardiol*. (2020) 36:1203–7. doi: 10.1016/j.cjca.2020.05.030
23. Huang L, Zhao P, Tang D, Zhu T, Han R, Zhan C, et al. Cardiac involvement in patients recovered COVID-19 patients identified by magnetic resonance imaging. *JACC Cardiovasc Imaging*. (2020) 13:2330–9. doi: 10.1016/j.jcmg.2020.05.004
24. Zochios V, Parhar K, Tunnicliffe W, Roscoe A, Gao F. The right ventricle in ARDS. *Chest*. (2017) 152:181–93. doi: 10.1016/j.chest.2017.02.019
25. Vieillard-Baron A, Millington SJ, Sanfilippo F, Chew M, Diaz-Gomez J, McLean A, et al. A decade of progress in critical care echocardiography: a narrative review. *Intensive Care Med*. (2019) 45:770–88. doi: 10.1007/s00134-019-05604-2
26. Zheng Y, Ma Y, Zhang J, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol*. (2020) 17:259–60. doi: 10.1038/s41569-020-0360-5
27. 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
28. Capone V, Cuomo V, Esposito R, Canonico ME, Ilardi F, Prastaro M, et al. Epidemiology, prognosis, and clinical manifestation of cardiovascular disease in COVID-19. *Expert Rev Cardiovasc Ther*. (2020) 18:531–9. doi: 10.1080/14779072.2020.1797491
29. 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
30. Johri AM, Galen B, Kirkpatrick JN, Lanspa M, Mulvagh S, Thamman R, et al. 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. Kirkpatrick JN, Grimm R, Johri AM, Kimura BJ, Kort S, Labovitz AJ, et al. Recommendations for echocardiography laboratories participating in cardiac point of care cardiac ultrasound (POCUS) and critical care echocardiography training: report from the American Society of Echocardiography. *J Am Soc Echocardiogr*. (2020) 33:409–22 e4. doi: 10.1016/j.echo.2020.01.008
32. Skulstad H, Cosyns B, Popescu BA, Galderisi M, Salvo GD, Donal E, et al. COVID-19 pandemic and cardiac imaging: EACVI recommendations on precautions, indications, prioritization, and protection for patients and healthcare personnel. *Eur Heart J Cardiovasc Imaging*. (2020) 21:592–8. doi: 10.1093/ehjci/jeaa072

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OPINION
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Vascular Inflammation as a Therapeutic Target in COVID-19 "Long Haulers": HIITing the Spot?

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BACKGROUND

In the wake of the first wave of the ongoing global pandemic, it has become imminently clear that coronavirus disease 2019 (COVID-19) has brought with it a whole new clinical syndrome: "long COVID" (1, 2). Hence, after recovery from the acute viral infection, a remarkably large proportion of patients, who initially coined themselves "long haulers" in social media-based patient communities for COVID-19 survivors suffer from persistent and often invalidating symptoms, including dyspnoea, chest pain, tachycardia, post-viral brain fog, exercise intolerance, and extreme fatigue to mention a few (3, 4). According to recent studies ∼10% of all individuals infected with the causative acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), and as many as nine out of 10 patients that have required hospitalization because of COVID-19 develop long COVID that persists for at least 4 months, according to the currently available data (4). Time will tell whether the symptoms associated with long COVID are transient or ever-lasting phenomena.

Long COVID will expectedly have a huge impact on the morbidity burden and quality of life in many COVID-19 survivors in the future, and when considering the extent of the global pandemic with currently more than 40 million verified cases, it will expectedly have substantial consequences, both in terms of economic cost and health care capacity throughout the world. It is thus widely recognized that there is an impending need for implementing evidence-based patient-tailored safe and effective rehabilitation schemes, but due to the paucity of data on this, the structure and specificity of such schemes remain obscure. While it is widely recognized that some exercise is better than none and more intense exercise is superior to less intense exercise, opinion papers and guidelines published over the past year have consistently refuted high-intensity interval training (HIIT) as an option for rehabilitation after COVID-19 (5–10). On the basis of the known pathophysiology of COVID-19 and the physiological effects of HIIT, we will however argue in favor of the opposite stand, that is, that HIIT should be considered as one of the rehabilitation interventions of choice for alleviating or even reversing the symptoms of long COVID.

COVID-19 IS (ALSO) A VASCULAR DISEASE

Even though COVID-19 is primarily a viral pneumonia, its multiorgan involvement, both in the acute phase and when considering the persistent systems in long COVID, stresses that this is far from the whole story. Over the past months, several studies have highlighted the presence of a substantial vascular component in the pathophysiology of the disease (11–14). Indeed, COVID-19 is associated with severe vascular inflammation, both in the

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pulmonary and extrapulmonary vasculature, both on the macro- and microvascular level (11). This involves diffuse endothelial damage with pyroptosis and apoptosis as well as a procoagulant change of the vascular endothelium. Consequently, both pulmonary and extrapulmonary thromboembolism are common complications, that may both determine the initial clinical presentation and the long-term consequences of COVID-19 in many patients (15).

The main mechanisms of the universal vascular component of COVID-19 may both involve the mode of entry of the virus into host cells and the immune response to the virus. The causative severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) invades host endothelial cells through endocytosis which is facilitated by the angiotensin converting enzyme 2 receptor and the transmembrane protease serine 2 which are expressed in practically all organs throughout the body (16).

In terms of the immune response, a type 3 hypersensitivity reaction has been reported to contribute to vascular inflammation in COVID 19, at least in some cases (17). This type of immune reaction takes place when an excess or slight excess of soluble antigens lead to the accumulation of immune complexes, which then precipitate inside the tissues, in particular blood vessels, where they may cause so-called "leukocytoclastic vasculitis," which is a procoagulant condition that affects both the macro- and microvasculature.

Another immune mechanism, which is probably important regardless of whether a type 3 reaction takes place, is the highly proinflammatory cytokine response to SARS-CoV-2, which is prominent both in milder and very severe cases, and which some have designated a "cytokine storm" (18, 19). This involves vast elevations in the classical pro-inflammatory cytokines, TNF-α and IL-1β, which have prominent effects on the endothelium. Hence, TNF-α facilitates the development of a procoagulant endothelium by increasing the expression of endothelial cellular adhesion molecules and genes critical for coagulation, such as tissue factor and decreased thrombomodulin, resulting in a pro-thrombotic state (20, 21). Moreover, TNF-α suppresses endothelial nitric oxide synthase and cyclooxygenase 1, which further compounds endothelial dysfunction (22). Furthermore, IL-1β, which is a downstream cytokine of TNF-α in the initial cytokine cascade triggered by an invading pathogen, is a potent trigger of vascular inflammation, among other things by enhancing monocyte and leukocyte infiltration in the vascular wall. This has most convincingly been demonstrated in studies of infants with non-functional IL-1 receptor antagonist (IL-1ra) function and thus uninhibited IL-1β signal transduction, which leads to severe universal vasculitis (23, 24).

In the following sections we will argue that because the multiorgan involvement of COVID-19 may largely reflect universal vascular inflammation, HIIT is an alluring contender for alleviating and perhaps preventing long COVID.

THE ANTI-INFLAMMATORY EFFECT OF EXERCISE

Physical exercise is a fundamental physiological stressor that is capable of inducing ubiquitous adaptations in nearly all cells, in nearly all tissues and organs (25). This involves the skeletal muscle "secretome" of myokines that are released from contracting skeletal muscle, and which exerts various functions through autocrine, paracrine, and endocrine functions, including marked immunomodulatory effects (**Figure 1**) (26). To this end, the low-grade inflammation, which is a common manifestation of aging has been demonstrated to be reversed by exercise of both moderate to strenuous intensity in randomized controlled trials in the elderly (27). Of note, IL-6 is the first detectable myokine released into the bloodstream during exercise. This is triggered by contraction-induced glycogen depletion in skeletal muscle and its concentration in blood increases exponentially depending on the intensity and duration of exercise (25). Therefore, exercise modalities involving large muscle groups produce the greatest IL-6 response. HIIT regimens or marathons can result in IL-6 increase of 100-fold, although increases of 2–10-fold are more common in exercise regimes of more moderate intensity or duration (28).

Once released, recent studies indicate that IL-6 directly stimulates cardiac exercise adaptations (29) and also affects the vasculature by mobilizing natural killer and dendritic cells to the blood stream (30), which are critically involved in viral clearance. The principal immunomodulatory function of IL-6 released during exercise is however to stimulate the release of IL-10 and IL-1ra by monocytes (31), while also reducing the expression of genes encoding several pro-inflammatory cytokines, including TNF-α and IL-1β. IL-10 also directly inhibits the synthesis of TNF-α (32) while IL-1ra inhibits IL-1β signaling. Additionally, IL-10 negatively interferes with tissue factor expression, thus exerting an anti-coagulant effect in the vasculature (**Figure 1**) (33).

By increasing viral clearance, while also aberrating TNF-α and IL-1β signaling, and alleviating the associated procoagulant state, exercise may thus reduce vascular inflammation in COVID-19.

HIIT: IS IT EFFECTIVE AND/OR SAFE IN COVID-19?

Given that the anti-inflammatory effects of exercise depends critically on the intensity of exercise, intense modalities that involve large muscle groups, such as HIIT protocols, have the potential to produce marked anti-inflammatory effects in target tissues in a time-efficient fashion (28, 34, 35).

HIIT has become increasingly popular in various rehabilitation schemes in patients with lung diseases, mostly because patients with respiratory symptoms are often unable to engage in classical continuous exercise regimens at an intensity sufficient to induce a training adaptation, but during HIIT relatively high intensities are often tolerated (36). Another advantage of HIIT, which is also a benefit in the scientific study of exercise adaptations, is its highly standardized and reproducible nature and that it evokes measurable physiological adaptations much faster than continuous training, i.e., within 2 weeks in healthy volunteers (36). Hence, although an acute HIIT bout elicits apparently similar plasma IL-6 as an iso-energetic continuous exercise bout, the higher intensities and total workloads that may be tolerated during HIIT in

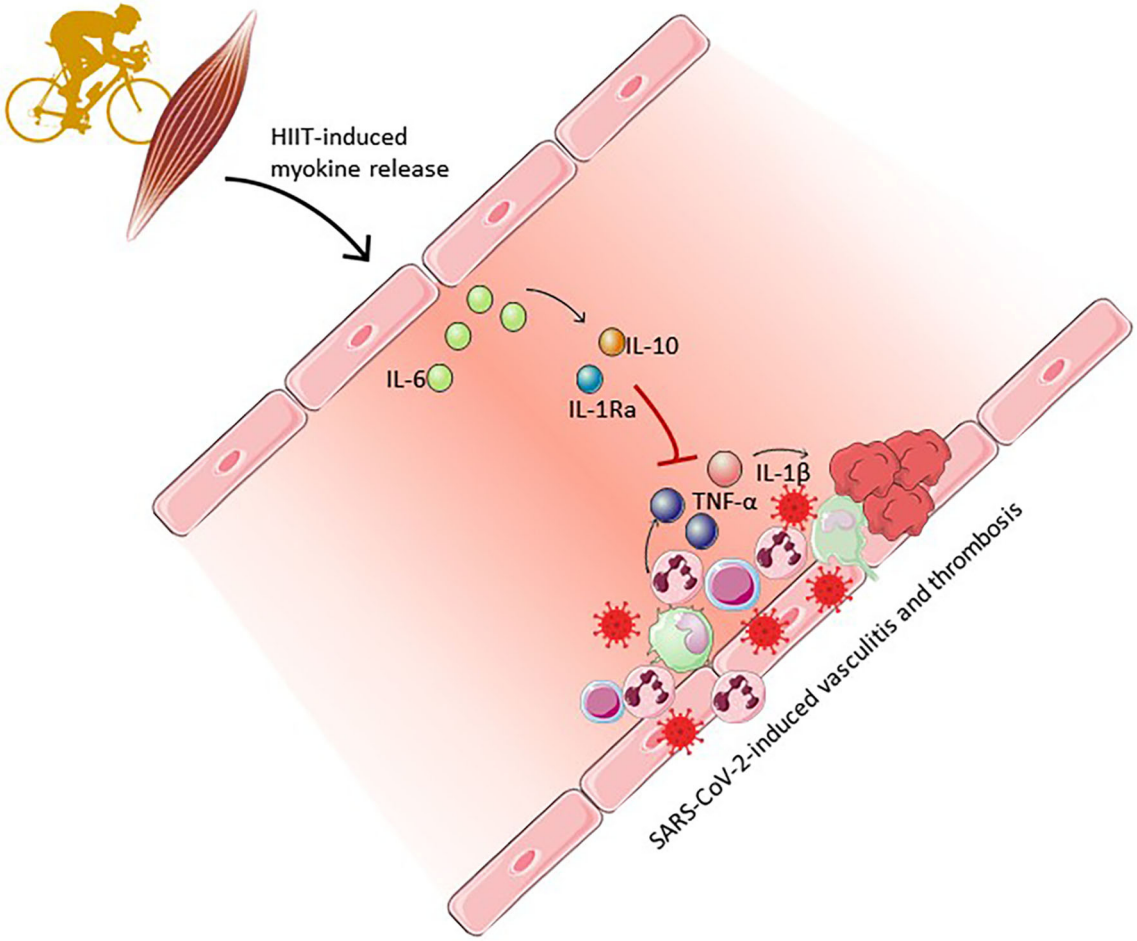

FIGURE 1 | High-intensity interval training (HIIT)-induced myokines (IL-6, IL-10 and IL-1Ra) may counter-act systemic vasculitis through an anti-inflammatory response, namely inhibition of TNFα and IL-1β-mediated activation of pro-coagulant and pro-inflammatory pathways. Clipart provided by Servier Medical Art (60).

various disease states compounds the exercise-induced anti-inflammatory effects (37, 38). Hence, HIIT has been shown to reduce disease-related TNF-α in an animal model of diabetes (39), and furthermore has specific suggested effects related to vascular inflammation, including reduced chemokine chemotaxis and enhanced endothelial repair reported in reviews and meta-analyses conducted on diverse populations of both normal overweight and obese individuals (40–42). This may both reflect the imminent effects of the high-intensity intervals on the IL-6 response as well as on the vasculature *per se*, i.e., due to the pronounced changes in vascular shear stress between intervals (43).

Of all the potential exercise interventions that may be prescribed in COVID-19, HIIT is nonetheless the most controversial. Several aspects of HIIT have been highlighted to disfavor it in this context, including presumed immunosuppressive effects that could increase viral susceptibility and decrease viral clearance (5, 44) and the potential risk of sudden cardiac arrest due to COVID-19-induced residual cardiovascular pathology (45). Due to the latter, the American College of Sports Medicine (ASCM) and experts endorsed by the section of Sports Cardiology & Exercise of the European Association of Preventive Cardiology (EAPC) have recommend that even athletes accustomed to high exercise intensities should resume to exercise only after a complete cardiovascular evaluation and in a gradual manner following a COVID-19 infection (6–10).

Concerns relating to viral susceptibility and clearance are directly contradicted by the known effects of exercise on immune function, including the effects on NK and dendritic cells described above (30). Accordingly, others have also stressed the potential of HIIT as a means to enhance immune surveillance and regulation while also exerting anti- rather than pro-inflammatory effects in COVID-19 survivors (46, 47).

In terms of the concerns of increasing the risk of adverse cardiovascular outcomes by HIIT in COVID-19 survivors, other reports suggest otherwise (48). Hence, a recent, admittedly small retrospective study of 28 discharged

COVID-19 survivors reported that rehabilitation triggered by HIIT, with endurance training at the maximum tolerated exercise load was both safe and feasible (49). To this end HIIT has successfully been implemented as a rehabilitation strategy in other "high risk" populations, as demonstrated in larger studies on patients at risk or with prevalent ischaemic heart disease, heart failure, chronic obstructive pulmonary disease, cystic fibrosis, and asthma with effects on parameters such as cardiorespiratory fitness (VO_2 peak) and exercise capacity with few reports of severe adverse events, even in patients with left ventricular assist devices (36, 41, 50–59). The rate of cardiovascular complications has been reported of 1 per 23,182 h of high-intensity exercise (51) and later studies have confirmed that HIIT is safe in patients with cardiovascular disease (53). As of now, no studies have thus provided any documentation to indicate that high intensity exercise regimes such as HIIT are not safe in COVID-19 survivors.

CONCLUSION

While the major focus in handling the burgeoning COVID-19 pandemic has hitherto been on reducing the spread of disease and mortality rates, the startlingly high prevalence and severity of long COVID in survivors heralds an aftermath of similar proportions. This may put health care systems throughout the world on the spot in the years to come, and clinical studies that seek to identify and implement effective rehabilitation strategies are thus of utmost importance. We thus believe that the following questions should be addressed by such studies in the very near near future: "When should HIIT be initiated in COVID-19 patients?," "Which specific HIIT protocol should be instigated in COVID-19 patients?" and "What are the effects on HIIT-based rehabilitation on cardio-pulmonary function, symptom burden, and quality of life in patients with long COVID?". HIIT may comprise a valuable component of the rehabilitation intervention in this context, given that its anti-inflammatory effects may target the prominent disease-specific vascular inflammation that is likely a substantial pathogenetic component of the "long haul" of COVID-19.

AUTHOR CONTRIBUTIONS

RC and RB conceived and wrote the initial draft of the manuscript. All authors provided critical input at all stages, and were involved in drafting and editing subsequent versions of the manuscript, read, and approved the final version of the manuscript.

REFERENCES

1. Alwan NA, Attree E, Blair JM, Bogaert D, Bowen MA, Boyle J, et al. From doctors as patients: a manifesto for tackling persisting symptoms of covid-19. *BMJ*. (2020) 370:m3565. doi: 10.1136/bmj.m3565
2. Bos LDJ, Brodie D, Calfee CS. Severe COVID-19 infections—knowledge gained and remaining questions. *JAMA Intern Med*. (2020) 181:9–11. doi: 10.1001/jamainternmed.2020.6047
3. Rubin R. As their numbers grow, COVID-19 "Long Haulers" stump experts. *JAMA*. (2020) 1:23–5. doi: 10.1001/jama.2020.17709
4. Carfì A, Bernabei R, Landi F. Persistent symptoms in patients after acute COVID-19. *JAMA*. (2020) 324:603–5. doi: 10.1001/jama.2020.12603
5. Rahmati-Ahmadabad S. Exercise against SARS-CoV-2 (COVID-19): does workout intensity matter? (A mini review of some indirect evidence related to obesity). *Obes Med*. (2020) 19:100245. doi: 10.1016/j.obmed.2020.100245
6. 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
7. Verwoert GC, de Vries ST, Bijsterveld N, Willems AR, vd Borgh R, Jongman JK, et al. Return to sports after COVID-19: a position paper from the dutch sports cardiology section of the Netherlands society of cardiology. *Netherlands Hear J*. (2020) 28:391–5. doi: 10.1007/s12471-020-01469-z
8. Dores H, Cardim N. Return to play after COVID-19: a sport cardiologist's view. *Br J Sports Med*. (2020) 54:8–9. doi: 10.1136/bjsports-2020-102482
9. Denay KL, Breslow RG, Turner MN, Nieman DC, Roberts WO, Best TM. ACSM call to action statement: COVID-19 considerations for sports and physical activity. *N Engl J Med*. (2020) 383:120–8. doi: 10.1249/JSR.0000000000000739
10. Kennedy FM, Sharma S. COVID-19, the heart and returning to physical exercise. *Occup Med*. (2020) 70:467–9. doi: 10.1093/occmed/kqaa154
11. 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
12. 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
13. Vacchi C, Meschiari M, Milic J, Marietta M, Tonelli R, Alfano G, et al. COVID-19-associated vasculitis and thrombotic complications: from pathological findings to multidisciplinary discussion. *Rheumatology*. (2020) 59:e147–50. doi: 10.1093/rheumatology/keaa581
14. 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
15. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of Coronaviruses on the cardiovascular system: a review. *JAMA Cardiol*. (2020) 10:1–10. doi: 10.1001/jamacardio.2020.1286
16. 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
17. Roncati L, Ligabue G, Fabbiani L, Malagoli C, Gallo G, Lusenti B, et al. Type 3 hypersensitivity in COVID-19 vasculitis. *Clin Immunol*. (2020) 217:108487. doi: 10.1016/j.clim.2020.108487
18. Ronit A, Berg RMG, Bay J, Haugaard AK, Ahlström MG, Burgdorf KS, et al. Compartmental immunophenotyping and cytomorphology in COVID-19 ARDS: a case series. *J Allergy Clin Immunol*. (2020) 147:81–91. doi: 10.1016/j.jaci.2020.09.009
19. Cao X. COVID-19: immunopathology and its implications for therapy. *Nat Rev Immunol*. (2020) 20:269–70. doi: 10.1038/s41577-020-0308-3
20. Tremoli E, Camera M, Toschi V, Colli S. Tissue factor in atherosclerosis. *Atherosclerosis*. (1999) 144:273–83. doi: 10.1016/S0021-9150(99)00063-5
21. Hot A, Lenief V, Miossec P. Combination of IL-17 and TNFα induces a pro-inflammatory, pro-coagulant and pro-thrombotic phenotype in human endothelial cells. *Ann Rheum Dis*. (2012) 71:768–76. doi: 10.1136/annrheumdis-2011-200468

22. Vallance P, Collier J, Bhagat K. Infection, inflammation, and infarction: does acute endothelial dysfunction provide a link? *Lancet.* (1997) 349:1391–2. doi: 10.1016/S0140-6736(96)09424-X
23. Dinarello CA, Simon A, van der Meer JWM. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov.* (2012) 11:633–52. doi: 10.1038/nrd3800
24. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood.* (2011) 117:3720–32. doi: 10.1182/blood-2010-07-273417
25. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev.* (2008) 88:1379–406. doi: 10.1152/physrev.90100.2007
26. Pedersen BK. Muscle as a secretory organ. *Compr Physiol.* (2013) 3:1337–62. doi: 10.1002/cphy.c120033
27. Woods JA, Wilund KR, Martin SA, Kistler BM. Exercise, inflammation and aging. *Aging Dis.* (2012) 3:130–40.
28. Fischer C. Interleukin-6 in acute exercise and training: what is the biological relevance. *Exerc Immunol Rev.* (2006) 12:6–33.
29. Christensen RH, Wedell-Neergaard AS, Lehrskov LL, Legaard GE, Dorph EB, Larsen MK, et al. Effect of aerobic and resistance exercise on cardiac adipose tissues: secondary analyses from a randomized controlled trial. *JAMA Cardiol.* (2019) 4:778–87. doi: 10.1001/jamacardio.2019.2074
30. Bay ML, Heywood S, Wedell-Neergaard A, Schauer T, Lehrskov LL, Christensen RH, et al. Human immune cell mobilization during exercise – effect of IL-6 receptor blockade. *Exp Physiol.* (2020) 105:2086–98. doi: 10.1113/EP088864
31. Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J Physiol.* (1999) 515(Pt 1):287–91. doi: 10.1111/j.1469-7793.1999.287ad.x
32. Starkie R, Ostrowski SR, Jauffred S, Febbraio M, Pedersen BK. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. *FASEB J.* (2003) 17:884–6. doi: 10.1096/fj.02-0670fje
33. Pedersen BK. Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease. *Eur J Clin Invest.* (2017) 47:600–11. doi: 10.1111/eci.12781
34. Helge JW, Stallknecht B, Pedersen BK, Galbo H, Kiens B, Richter EA. The effect of graded exercise on IL-6 release and glucose uptake in human skeletal muscle. *J Physiol.* (2003) 546:299–305. doi: 10.1113/jphysiol.2002.030437
35. Cullen T, Thomas AW, Webb R, Hughes MG. Interleukin-6 and associated cytokine responses to an acute bout of high-intensity interval exercise: the effect of exercise intensity and volume. *Appl Physiol Nutr Metab.* (2016) 41:803–8. doi: 10.1139/apnm-2015-0640
36. Sawyer A, Cavalheri V, Hill K. Effects of high intensity interval training on exercise capacity in people with chronic pulmonary conditions: a narrative review. *BMC Sports Sci Med.* (2020) 12:22. doi: 10.1186/s13102-020-00167-y
37. de Souza DC, Matos VAF, dos Santos VOA, Medeiros IF, Marinho CSR, Nascimento PRP, et al. Effects of high-intensity interval and moderate-intensity continuous exercise on inflammatory, leptin, IgA, and lipid peroxidation responses in obese males. *Front Physiol.* (2018) 9:1–9. doi: 10.3389/fphys.2018.00567
38. Peake JM, Tan SJ, Markworth JF, Broadbent JA, Skinner TL, Cameron-Smith D. Metabolic and hormonal responses to isoenergetic high-intensity interval exercise and continuous moderate-intensity exercise. *Am J Physiol Endocrinol Metab.* (2014) 307:E539–52. doi: 10.1152/ajpendo.00276.2014
39. Kim JS, Lee YH, Kim JC, Ko YH, Yoon CS, Yi HK. Effect of exercise training of different intensities on anti-inflammatory reaction in streptozotocin-induced diabetic rats. *Biol Sport.* (2014) 31:73–9. doi: 10.5604/20831862.1093775
40. Li Y, Liu D, Wu H. HIIT: a potential rehabilitation treatment in COVID-19 pneumonia with heart disease. *Int J Cardiol.* (2020) 320:186. doi: 10.1016/j.ijcard.2020.07.030
41. Batacan RB, Duncan MJ, Dalbo VJ, Tucker PS, Fenning AS. Effects of high-intensity interval training on cardiometabolic health: a systematic review and meta-analysis of intervention studies. *Br J Sports Med.* (2017) 51:494–503. doi: 10.1136/bjsports-2015-095841
42. Pal S, Radavelli-Bagatini S, Ho S. Potential benefits of exercise on blood pressure and vascular function. *J Am Soc Hypertens.* (2013) 7:494–506. doi: 10.1016/j.jash.2013.07.004
43. Williams JS, Del Giudice M, Gurd BJ, Pyke KE. Reproducible improvement in endothelial function following two separate periods of high-intensity interval training in young men. *J Appl Physiol.* (2020) 129:725–31. doi: 10.1152/japplphysiol.00054.2020
44. Leandro CG, Ferreira E Silva WT, Lima-Silva AE. Covid-19 and exercise-induced immunomodulation. *Neuroimmunomodulation.* (2020) 27:75–8. doi: 10.1159/000508951
45. Baggish AL, Levine BD. Icarus and sports after COVID 19: too close to the sun? *Circulation.* (2020) 142:615–7. doi: 10.1161/CIRCULATIONAHA.120.048335
46. Wang M, Baker JS, Quan W, Shen S, Fekete G, Gu Y. A preventive role of exercise across the Coronavirus 2 (SARS-CoV-2) pandemic. *Front Physiol.* (2020) 11:1–8. doi: 10.3389/fphys.2020.572718
47. da Silveira MP, da Silva Fagundes KK, Bizuti MR, Starck É, Rossi RC, de Resende e Silva DT. Physical exercise as a tool to help the immune system against COVID-19: an integrative review of the current literature. *Clin Exp Med.* (2020) 21:15–28. doi: 10.1007/s10238-020-00650-3
48. Batatinha HAP, Krüger K, Neto JCR. Thromboinflammation and COVID-19 : the role of exercise in the prevention and treatment. *Front Cardiovasc Med.* (2020) 7:8–11. doi: 10.3389/fcvm.2020.582824
49. Hermann M, Pekacka-Egli A-M, Witassek F, Baumgaertner R, Schoendorf S, Spielmanns M. Feasibility and efficacy of cardiopulmonary rehabilitation after COVID-19. *Am J Phys Med Rehabil.* (2020) 99:865–9. doi: 10.1097/PHM.0000000000001549
50. Ellingsen Ø, Halle M, Conraads V, Støylen A, Dalen H, Delagardelle C, et al. High-intensity interval training in patients with heart failure with reduced ejection fraction. *Circulation.* (2017) 135:839–49. doi: 10.1161/CIRCULATIONAHA.116.022924
51. Rognmo O, Moholdt T, Bakken H, Hole T, Mølstad P, Myhr NE, et al. Cardiovascular risk of high-versus moderate-intensity aerobic exercise in coronary heart disease patients. *Circulation.* (2012) 126:1436–40. doi: 10.1161/CIRCULATIONAHA.112.123117
52. Keech A, Way K, Holgate K, Fildes J, Indraratna P, Yu J. HIIT for post-COVID patients within cardiac rehabilitation: response to letter to the editor. *Int J Cardiol.* (2020) 322:291–2. doi: 10.1016/j.ijcard.2020.08.086
53. Wewege MA, Ahn D, Yu J, Liou K, Keech A. High-intensity interval training for patients with cardiovascular disease-is it safe? A systematic review. *J Am Heart Assoc.* (2018) 7:1–19. doi: 10.1161/JAHA.118.009305
54. Alvarez Villela M, Chinnadurai T, Salkey K, Furlani A, Yanamandala M, Vukelic S, et al. Feasibility of high-intensity interval training in patients with left ventricular assist devices: a pilot study. *ESC Hear Fail.* (2020) 8:498–507. doi: 10.1002/ehf2.13106
55. Angadi SS, Mookadam F, Lee CD, Tucker WJ, Haykowsky MJ, Gaesser GA. High-intensity interval training vs. moderate-intensity continuous exercise training in heart failure with preserved ejection fraction: a pilot study. *J Appl Physiol.* (2015) 119:753–8. doi: 10.1152/japplphysiol.00518.2014
56. Guadalupe-Grau A, Aznar-Laín S, Mañas A, Castellanos J, Alcázar J, Ara I, et al. Short- and long-term effects of concurrent strength and HIIT training in octogenarians with COPD. *J Aging Phys Act.* (2017) 25:105–15. doi: 10.1123/japa.2015-0307
57. Trachsel LD, David LP, Gayda M, Henri C, Hayami D, Thorin-Trescases N, et al. The impact of high-intensity interval training on ventricular remodeling in patients with a recent acute myocardial infarction—A randomized training intervention pilot study. *Clin Cardiol.* (2019) 42:1222–31. doi: 10.1002/clc.23277
58. Gomes Neto M, Durães AR, Conceição LSR, Saquetto MB, Ellingsen Ø, Carvalho VO. High intensity interval training versus moderate intensity continuous training on exercise capacity and quality of life in patients with heart failure with reduced ejection fraction: a systematic review and meta-analysis. *Int J Cardiol.* (2018) 261:134–41. doi: 10.1016/j.ijcard.2018.02.076
59. Villelabeitia-Jaureguizar K, Vicente-Campos D, Senen AB, Jiménez VH, Garrido-Lestache MEB, Chicharro JL. Effects of high-intensity interval versus continuous exercise training on post-exercise heart rate recovery in coronary heart-disease patients. *Int J Cardiol.* (2017) 244:17–23. doi: 10.1016/j.ijcard.2017.06.067

60. Les Laboratories Servier. *Servier Medical Art*. Available online at: http://servier.com/Powerpoint-image-bank (accessed October 21, 2020).

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Application of Machine Learning in Diagnosis of COVID-19 Through X-Ray and CT Images: A Scoping Review

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Coronavirus disease, first detected in late 2019 (COVID-19), has spread fast throughout the world, leading to high mortality. This condition can be diagnosed using RT-PCR technique on nasopharyngeal and throat swabs with sensitivity values ranging from 30 to 70%. However, chest CT scans and X-ray images have been reported to have sensitivity values of 98 and 69%, respectively. The application of machine learning methods on CT and X-ray images has facilitated the accurate diagnosis of COVID-19. In this study, we reviewed studies which used machine and deep learning methods on chest X-ray images and CT scans for COVID-19 diagnosis and compared their performance. The accuracy of these methods ranged from 76% to more than 99%, indicating the applicability of machine and deep learning methods in the clinical diagnosis of COVID-19.

Keywords: COVID-19, machine learning, detection, biomarker, X-ray image

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INTRODUCTION

First identified in Wuhan, China, severe pneumonia caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) quickly spread all over the world. The resultant disorder was named coronavirus disease (COVID-19) (1, 2). COVID-19 has various clinical symptoms, including fever, cough, dyspnea, fatigue, myalgia, headache, and gastrointestinal complications (3–5). Diagnosis of COVID-19 infection through RT-PCR on nasopharyngeal and throat swab samples has been reported to yield positive results in 30–70% of cases (6, 7). On the other hand, chest CT scans and X-ray images have been reported to have sensitivity values of 98 and 69%, respectively (7–9). The most typical radiological signs in these patients include multifocal and bilateral ground-glass opacities and consolidations, particularly in the peripheral and basal sites (10). However, interpretation of the results of these imaging techniques by expert radiologists might encounter some problems leading to reduced sensitivity (11). Artificial intelligence has recently gained the attention of both clinicians and researchers for the appropriate management of the COVID-19 pandemic (12). As an accurate method, artificial intelligence is able to identify abnormal patterns of CT and X-ray images. Using this method, it is possible to assess certain segment regions and take precise structures in chest CT images facilitating diagnostic purposes. Artificial intelligence methods have been shown to detect COVID-19 and distinguish this condition from other pulmonary disorders

and community-acquired pneumonia (13). Both deep learning and machine learning approaches have been used to predict different aspects of the COVID-19 outbreak. Support vector and random forest are among the most applied machine learning methods, while Convolutional Neural Network (CNN), Long Short-Term Memory (LSTM), Generative Adversarial Networks (GAN), and Residual Neural network are among the deep learning methods used in this regard (14). In this study, we reviewed studies which used machine and deep learning methods on chest X-ray images and CT scans for the purpose of COVID-19 diagnosis and compared their performance.

METHODS

Search Strategy

The research question was: "What are the applications of machine learning techniques and their performances in COVID-19 diagnosis using X-ray images?". The search of the present review was based on the PICO elements, which were as follows:

- **P (Problem/Patient/Population):** Patients' CT scans and Chest X-rays.
- **I (Intervention/Indicator):** Machine/deep learning models for diagnosis of Covid-19 patients
- **C (Comparison):** Ground truth or reference standards
- **O (Outcome):** Performance measurements including accuracy, AUC score, sensitivity, and specificity.

In other words, we were looking for publications that evaluated the performance of any machine learning or deep learning approaches based on inclusion and exclusion criteria. Studies that used other types of medical image modalities (e.g., ultrasound images) were excluded. An electronic search was conducted on PubMed, Google Scholar, Scopus, Embase, arXiv, and medRxiv for finding the relevant literature. Duplicate studies were removed. Studies that were cited within the retrieved papers were reviewed for finding missing studies. For identifying proper journal papers and conference proceedings, investigators screened the title and abstracts based on inclusion and exclusion criteria independently. Finally, considering the inclusion and exclusion criteria, investigators identified the eligible publications in this stage independently.

Inclusion Criteria

The following inclusion criteria were used in the selection of the articles: (1) Studies that applied machine learning or deep learning algorithms, (2) Studies that evaluated the measurement of model outcomes in comparison with ground truth or gold standards, and (3) Studies that used algorithms to analyze radiographic images (CT scan, Chest X-ray, etc.).

Exclusion Criteria

The following studies were excluded: (1) Studies that used any machine learning or deep learning approaches for problems not directly related to the COVID-19 imaging, (2) Studies that used other artificial intelligence techniques or classic computer vision approaches, (3) Studies that did not provide a clear explanation of the machine learning or deep learning model that was used to solve their problem, and (4) Review studies. The latter were excluded as we did not aim to review the data on an original level without any second-hand interpretations (summation, inferences, etc.).

Figure 1 shows the flowchart of the study design.

RESULTS

We obtained 105 studies that used machine or deep learning methods to assess chest images of COVID-19 patients. These studies have used different analytical methods. For instance, Ardakani et al. (15) have assessed radiological features of CT images obtained from patients with COVID-19 and non-COVID-19 pneumonia. They used decision tree, K-nearest neighbor, naïve Bayes, support vector machine, and ensemble classifiers to find the computer-aided diagnosis system with the best performance in distinguishing COVID-19 patients from non-COVID-19 pneumonia. They reported that site and distribution of pulmonary involvement, the quantity of the pulmonary lesions, ground-glass opacity, and crazy-paving as the most important characteristics for differentiation of these two sets of patients. Their computer-aided diagnosis method yielded the accuracy of 91.94%, using an ensemble (COVIDiag) classifier. Alazab et al. (16) have developed an artificial-intelligence method based on a deep CNN to evaluate chest X-ray images and detection of COVID-19 patients. Their method yielded an F-measure ranging from 95 to 99%. Notably, three predicting strategies could forecast the numbers of COVID-19 confirmations, recoveries, and mortalities over the upcoming week. The average accuracy of the prediction models were 94.80 and 88.43% in two different countries. Albahli has applied deep learning-based models on CT images of COVID-19 patients. He has demonstrated a high performance of a Deep Neural Network model in detecting COVID-19 patients and has offered an efficient assessment of chest-related disorders according to age and sex. His proposed model has yielded 89% accuracy in terms of GAN-based synthetic data (17). Automatic detection of COVID-19 based on X-ray images has been executed through the application of three deep learning models, including Inception ResNetV2, InceptionNetV3, and NASNetLarge. The best results have been obtained from InceptionNetV3, which yielded the accuracy levels of 98.63 and 99.02% with and without application of data augmentation in model training, respectively (18). Alsharman et al. (19) have used the CNN method to detect COVID-19 based on chest CT images in the early stages of disease course. Authors have reported high accuracy of GoogleNet CNN architecture for diagnosis of COVID-19. Altan et al. (20) have used a hybrid model comprising two-dimensional curvelet transformation, chaotic salp swarm algorithm, and deep learning methods for distinguishing COVID-19 from other pneumonia cases. Application of their proposed model on chest X-ray images has led to accurate diagnosis of COVID-19 patients (Accuracy = 99.69%, Sensitivity = 99.44% and Specificity = 99.81%). Apostolopoulos et al. (21) have used a certain CNN strategy, namely MobileNet on X-Ray images of COVID-19 patients. This method has yielded more than 99% accuracy

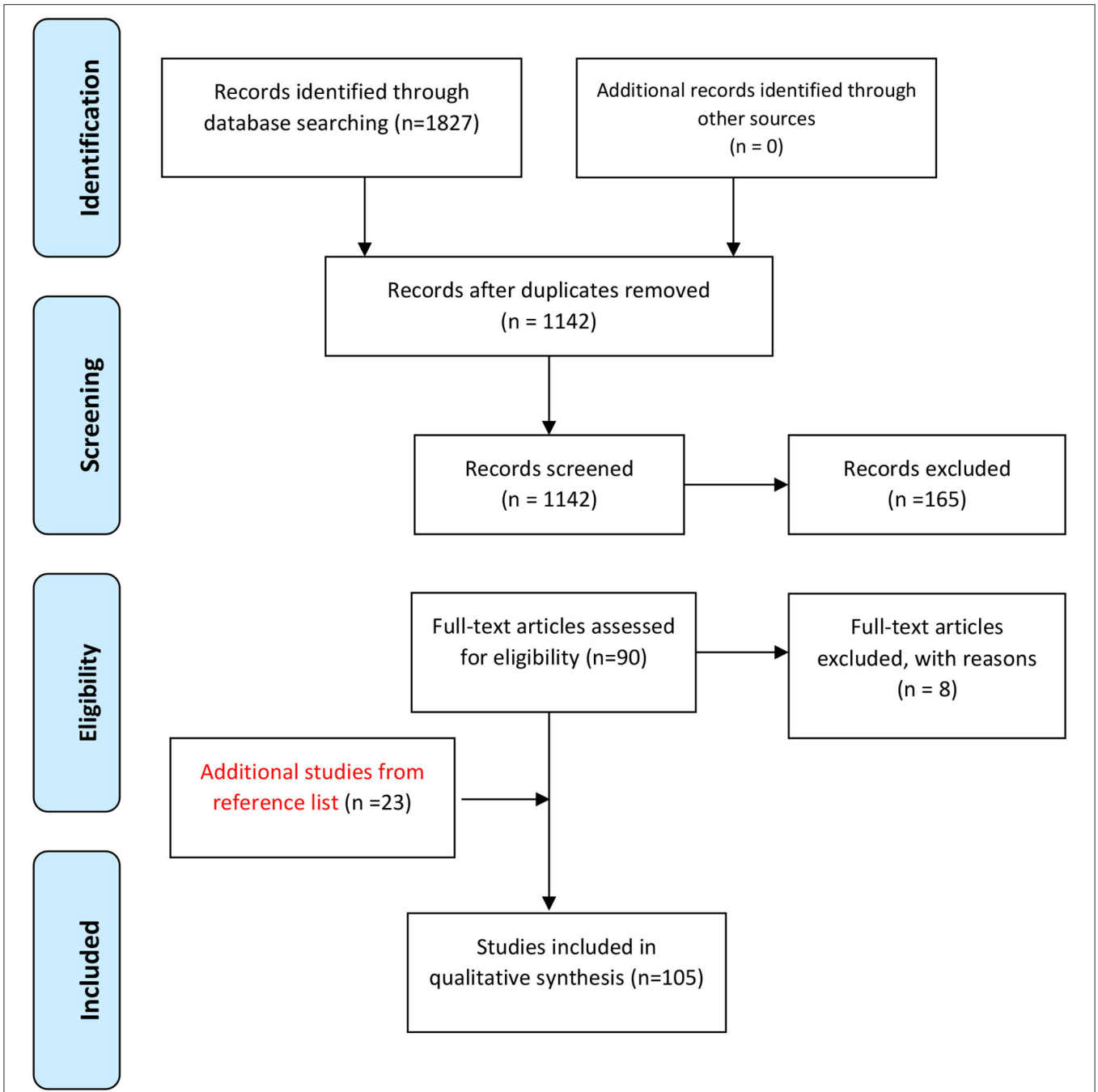

FIGURE 1 | PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) chart showing the process of systematic identification, screening, and selection of articles.

in the diagnosis of COVID-19. In another study, Ardakani et al. (22) used 10 CNN strategies, namely AlexNet, VGG-16, VGG-19, SqueezeNet, GoogleNet, MobileNet-V2, ResNet-18, ResNet-50, ResNet-101, and Xception, to differentiate COVID-19 cases from non-COVID-19 patients. They have demonstrated the best diagnostic values for ResNet-101 and Xception, both of them having area under curve (AUC) values higher than 0.99 which is superior to the performance of the radiologist. Das et al. (23) have used the CNN model Truncated InceptionNet to diagnose COVID-19 from other non-COVID and/or healthy cases based on chest X-ray. Their suggested model yielded AUC of 1.0 in distinguishing COVID-19 patients from combined Pneumonia and healthy subjects. **Tables 1**, **2** summarize the features of studies which adopted machine learning methods in CT images and chest X-ray of COVID-19 patients, respectively.

TABLE 1 | Characteristics of papers that used CT images or a combination of X-ray and CT images.

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Abbasian et al. (2020)	Iran University of Medical Sciences (IUMS)	306 COVID-19 patients; 306 COVID-19 pneumonia (CT images)	Extracting 20 features of CT images	Ensemble	91.94%	0.965	93.54%	90.32%	(15)
Alsharman et al. (2020)	"COVID-CT-dataset"	CT images	Binarization (the separation of the object and background is known as Binarization); Converting input image from 2D Grayscale to 3D Color	GoogleNet CNN	82.14%				(19)
Ardakani et al. (2020)	Private dataset	108 COVID-19 patients; 86 viral pneumonia diseases (CT images)	Converted to the gray-scale Cropped and resized to 60 * 60 pixels	ResNet-101 Xception	Resnet: 99.51% Xception: 99.02% (compared to 86.7% in human)	Resnet: 0.994 Xception: 0.994% (compared to 0.873 in human)	Resnet: 100% Xception: 98.04% (compared to 89.21% in human)	Resnet: 99.02% Xception: 100% (compared to 83.33% in human)	(22)
Aswathy et al. (2020)	"National Cancer Institute and the Cancer Image Archive"	1,763 normal patients; 63 pneumonia patients	Thresholding; Texture-based feature extractionwith a wrapper	CNN	99%	–	–	–	(24)
Bai et al. (2020)	Private dataset	521 COVID-19 patients; 665 other pulmonary diseases (CT images)	Lung segmentation; Generate an 8-bit image for each axial slice by applying Lung windowing to the Hounsfield units	EfficientNet B4	96% (compared to 85% in human)	0.95	95% (compared to 79% in human)	96% (compared to 88% in human)	(11)
Bridge et al. (2020)	"Toy dataset;" "Italian Society of Radiology;" "Shenzhen Hospital X-Ray dataset;" "ChestX-Ray8;" "COVID-CT-Dataset"	129 COVID-19 patients; 62,267 normal patients; 5,689 pneumonia patients (X-ray images) 30 COVID-19 patients; 1,919 normal patients (CT images)	Using the GEV activation function for unbalanced data	Inception V3	100%	–	100%	100%	(25)
Butt et al. (2020)	Not mentioned	219 images from 110 COVID-19 patients; 399 normal patients (CT images)	Image processing method base on HU values	3D CNN	–	0.996	98.2%	92.2%	(26)

(Continued)

TABLE 1 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Dey et al. (2020)	"COVID-19 CT segmentation dataset;" "Chest X-rays (Radiopaedia)"	200 COVID-19 patients; 200 normal patients (grayscale lung CTI images)	Segmenting lung area related to pneumonia infection; Extracting CWT, DWT, EWT features from original image and Haralick, Hu moments from binary segmented area Feature selection based on statistical tests	KNN	87.75%	–	89.00%	86.50%	(27)
El Asnaoui et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; Kermany et al. (28)	2,780 Bacterial pneumonia patients; 1,493 Coronavirus patients; 231 COVID-19 patients; 1,583 normal patients (X-ray and CT images)	Intensity Normalization; Contrast Limited Adaptive Histogram Equalization	Inception ResNetV2; Densnet201	Inception-ResNetV2: 92.18% Densnet201: 88.09%	Inception-ResNetV2: 0.920 Densnet201: 0.879	Inception-ResNetV2: 92.11% Densnet201: 87.99%	Inception-ResNetV2: 96.6% Densnet201: 94.00%	(29)
Han et al. (2020)	"COVID-19 hospitals in Shandong Province"	79 COVID-19 patients; 100 pneumonia patients; 130 normal patients (CT images)	Data augmentation	AD3D-MIL	97.9%	0.99	97.9%	97.9%	(30)
Harmon et al. (2020)	Private dataset	386 COVID-19 patients; 1,011 negative COVID-19 patients (CT images)	Lung segmentation; clipping images to HU range (−1,000, 500); Data augmentation (flipping, rotation, image intensity and contrast adjustment, adding random Gaussian noise);	Hybrid 3D based on Densnet-121	90.8%	–	84%	93%	(31)
Hasan et al. (2020)	"Radiopaedia and the cancer imaging archive websites"	118 COVID-19 patients; 96 pneumonia patients; 107 normal patients (CT images)	Histogram Thresholding; Dilation; Hole Filling	LSTM	99.68%	–	100%	–	(32)

(Continued)

TABLE 1 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Hu et al. (2020)	"Hospital of Wuhan Red Cross Society;" "Shenzhen Hospital;" "TCIA dataset;" "Cancer Centre Archive (TCIA) Public Access;" "MD Anderson Cancer Centre;" "Memorial Sloan-Kettering Cancer Center;" "MAASTRO clinic"	150 COVID-19 patients; 150 pneumonia patients; 150 normal patients (CT images)	Data augmentation	CNN	96.2%	0.970	94.5%	95.3%	(33)
Jaiswal et al. (2020)	"The SARS-CoV-2 CT scan dataset"	1,262 COVID-19 patients; 1,230 non-COVID-19 patients (CT images)	Data augmentation (rotation up to 15, slant-angle of 0.2, horizontal flipping, filling new pixels as "nearest" for better robustness)	DenseNet201	96.25%	0.97	96.29%	96.21%	(34)
Kang et al. (2020)	"Tongji Hospital of Huazhong University of Science and Technology;" "China-Japan Union Hospital of Jilin University;" "Ruijin Hospital ofShanghai Jiao Tong University"	1,495 COVID-19 patients; 1,027 community-acquired pneumonia (CAP) patients (CT images)	Normalization; Standardization	NN	93.90%	–	94.60%	91.70%	(35)
Lessmann et al. (2020)	"Emergency wards of an Academic center and teaching hospital in the Netherlands in March and April 2020"	237 COVID-19 patients; 606 normal patients (CT images)	Resampling; Normalization	CORADS-AI	–	0.95	85.7%	89.8%	(36)
Li et al. (2020)	Private	1,296 COVID-19 patients; 1,325—patients; 1,735 community-acquired (CT images)	Segmenting lung area with U-net	COVNet (ResNet-50)	–	0.96	90%	96%	(13)

(Continued)

TABLE 1 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Li et al. (2020)	More than 10 medical centers between Nov. 11th, 2010 and Feb. 9th, 2020	305 images from 251 COVID-19 patients; 872 images from 869 pneumonia patients; 1,498 images from 1,475 non-pneumonia patients (CT images)	DL-based algorithm Image processing method base on HU values; Data augmentation	3D ResNet-18	Recall = 88% Precision = 89.6% F1 score = 87.8%				(37)
Liu et al. (2020)	Private	73 COVID-19 patients; 27 general pneumonia patients (CT images)	ROI delineation based on ground-glass opacities (GGOs); 13 gray level co-occurrence matrix (GLCM) features, 15 gray level-gradient co-occurrence matrix (GLGCM) features, and six histogram features were extracted; Feature selection by ReliefF;	An ensemble of bagged tree (EBT)	94.16%	0.99	88.62%	100%	(38)
Mei et al. (2020)	Private	419 COVID-19 patients 486 non-COVID-19 patients (CT images)	Selecting pertinent slices by image segmentation to detect parenchymal tissue; Segmenting lung in CT images;	ResNet-18	79.6%	0.86	83.6%	75.9%	(39)
Panwar et al. (2020)	"COVID-chest X-ray;" "SARS-COV-2 CT-scan;" "Chest X-Ray Images (Pneumonia);"	206 COVID-19 patients; 364 Pneumonia patients (X-ray and CT images)	–	VGG-19	95.61% (COVID-19 vs. Pneumonia)	–	96.55% (COVID-19 vs. Pneumonia)	95.29% (COVID-19 vs. Pneumonia)	(40)
Pathak et al. (2020)	2 different COVID-19 datasets of chest-CT images	CT images	–	Deep bidirectional long short-term memory network with mixture density network (DBM)	96.19% (multi-class)	0.96 (multi-class)	96.22% (multi-class)	96.16% (multi-class)	(41)
Pathak et al. (2020)	"COVID-19 open datasets of chest CT images"	413 COVID-19 patients; 439 normal or pneumonia infected patients (CT images)	–	ResNet-50	93.01%	–	91.45%	94.77%	(41)
Peng et al. (2020)	Collected from PMC	606 COVID-19 patients; 222 Influenza; 397 Normal or other disease patients (CT images)	–	DenseNet121	–	0.87	72.3%	85.2%	(42)

(Continued)

TABLE 1 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Pu et al. (2020)	Private	498 COVID-19 patients; 497 community-acquired pneumonia (CAP) (CT images)	Data augmentation [rotation, translation, vertical/horizontal flips, Hounsfield Unit (HU) shift, smoothing (blurring) operation, Gaussian noise]	3D CNNs	99%	0.7	–	–	(43)
Raajan et al. (2020)	X-ray images on public medical Github repositories; Kaggle chest X-ray database	349 images from 216 COVID-19 patients; 1,341 Normal patients (CT images)	Normalization	ResNet-16	95.09%	–	100%	81.89%	(44)
Rajaraman et al. (2020)	"Pediatric CXR dataset;" "RSNA CXR dataset;" "Twitter COVID-19 CXR dataset;" "Montreal COVID-19 CXR dataset"	313 COVID-19 patients; 7,595 pneumonia of unknown type patients; 2,780 bacterial pneumonia; 7,595 Normal patients (X-ray images)	Median filtering; Normalization; Standardization	Inception-V3	99.01%	0.997	98.4%	–	(45)
Sakagianni et al. (2020)	COVID-19 articles on medRxiv and bioRxiv	349 COVID-19 patients; 397 non-COVID-19 patients (CT images)	–	AutoML Cloud Vision	–	0.94	88.31%	–	(46)
Sharma (2020)	Dataset from Italian Society of Medical and Interventional Radiology; COVID-CT available in GitHub; Dataset from hospitals in Moscow, Russia; Dataset from SAL Hospital, Ahmedabad, India;	800 COVID-19 patients; 600 Viral Pneumonia; 800 normal patients (CT images)	Ground-glass opacities (GGO), consolidation and pleural effusion are the features	ResNet	91%	–	92.1%	90.29%	(47)
Singh et al. (2020)	Not mentioned	CT images	–	Multi-objective differential evolution (MODE) based CNN	90.22%	–	91.17%	89.23%	(48)
Song et al. (2020)	Private (two hospitals in China);	98 COVID-19 patients; 103 non-COVID-19 pneumonia (CT images)	–	BigBiGAN	–	0.972	92%	91%	(49)

(Continued)

TABLE 1 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Wang et al. (2020)	Private	1,315 COVID-19 patients; 2,406 ILD patients; 936 Normal patients (CT images)	Lobe Segmentation by 3D-Unet; Converting CT numbers to grayscale	PA-66 model	93.3%	0.973	97.6%	–	(50)
Wang et al. (2020)	COVID-19 dataset (private); CT-epidermal growth factor receptor (CT-EGFR) dataset (private);	754 COVID-19 patients; 271 bacterial pneumonia 29 viral pneumonia; 42 Other pneumonia (CT images) *The CT-EGFR dataset was used for auxiliary training of the DL system	Lung segmentation; Using a fully automatic DL model (DenseNet121-FPN); suppress the intensities of non-lung areas inside the lung ROI;	COVID-19Net (DenseNet-like architecture)	Test-set1: 78.32% Test-set2: 80.12%	Test-set1: 0.87 Test-set2: 0.88	Test-set1: 80.39% Test-set2: 79.35%	Test-set1: 76.61% Test-set2: 81.16%	(51)
Warman et al. (2020)	"Public sources"	606 COVID-19 patients; 224 viral pneumonias patients; 74 Normal patients (CT images)	Data augmentation	YOLOv3 model	96.80%	0.966	98.33%	94.95%	(52)
Wu et al. (2020)	Private	368 COVID-19 patients; 127 other pneumonia (CT images)	Lung region in each axial, coronal and sagittal CT slices were segmented using threshold segmentation and morphological optimization algorithms; The slice with the most pixels in the segmented lung area from each of the axial, coronal and sagittal views was selected as the inputs of the deep learning network;	Multi-view fusion ResNet50 architecture	76%	0.819	81.1%	61.5%	(53)
Xu et al. (2020)	Private "Hospitals in Zhejiang Province, China."	219 images from 110 COVID-19 patients; 224 Influenza-A viral pneumonia patients; 175 Normal patients (CT images)	Image processing method base on HU values	3D CNN segmentation model	86.7%	–	86.7%	–	(54)

(Continued)

TABLE 1 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Xu et al. (2020)	Private	432 COVID-19 patients; 76 other viral pneumonia; 350 bacterial pneumonia; 418 normal patients (CT images)	Sampling 5 subsets of CT slices from all sequential images of one CT case to picture the infected lung regions.	3D-Densenet	–	0.98	97.5% (differentiating COVID-19 from three types of non-COVID-19 cases) (compared to 79% in human)	89.4% (differentiating COVID-19 from three types of non-COVID-19 cases) (compared to 90% in human)	(55)
Yan et al. (2020)	Private	416 images from 206 COVID-19 patients; 412 common pneumonia patients (CT images)	Transferring image slices to JPG; Normalization	MSCNN	97.7%	0.962	99.5%	95.6%	(56)
Yang et al. (2020)	Private	146 COVID-19 patients; 149 normal patients (CT images)	For patients, images containing round-glasses opacity (GGO), GGO with consolidation was selected; for healthy control, every 3 slices containing pulmonary parenchyma were selected; Lung windowing is performed over all image slices;	DenseNet	92% (compared to 95% in human)	0.98	97% (compared to 94% in human)	87% (compared to 96% in human)	(57)
Yu et al. (2020)	Private	202 COVID-19 patients (CT images)	–	DenseNet-201 with the cubic SVM model	95.2%	0.99	91.87%	96.87%	(58)
Al-Karawi et al. (2020)	"COVID-CT-Dataset"	275 COVID-19 patients; 195 normal patients (CT images)	Adaptive winner filter followed by inversion; Feature extraction by the FFT-spectrum	SVM	95.37%	–	95.99%	94.76%	(59)
Alom et al. (2020)	Publicly available datasets; "Kaggle repository"	3,875 pneumonia patients; 1,341 normal patients (X-Ray images) 178 COVID-19 patients; 247 normal patients (CT images)	Data augmentation; Adaptive Thresholding Approach	IRRCNN model; NABLA-3 network model	X-ray images: 84.67% CT images: 98.78%	0.93	–	–	(60)
Barstugan et al. (2020)	From the Italian Society of Medical and Interventional Radiology	150 COVID-19 patients (CT images)	13 features were extracted by Gray Level Size Zone Matrix (GLSZM)	SVM	98.77%	–	97.72%	99.67%	(61)

(Continued)

TABLE 1 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Chen et al. (2020)	Private dataset	25,989 images from 51 COVID-19 patients; 20,107 images from 55 normal patients (retrospective dataset); 13,911 images from 27 consecutive patients (prospective dataset) (CT images)	Filtering	Deep learning model	Retrospective dataset: 95.24%; Prospective dataset: 92.59% (per patient)	–	Retrospective dataset: 100%; Prospective dataset: 100% (per patient)	Retrospective dataset:93.55%; Prospective dataset: 81.82% (per patient)	(62)
Farid et al. (2020)	Kaggle database	51 COVID-19 patients (CT images)	Feature extraction (MPEG7 Histogram Filter, Gabor Image Filter, Pyramid of Rotation-Invariant Local Binary Pattern, Fuzzy 64-bin Histogram Image Filter); Feature selection by composite hybrid feature selection	CHFS-Stacked (jrip, RF) with Naïve Bayes classifier	96.07%	–	–	–	(63)
Gozes et al. (2020)	Dataset1:ChainZ; Dataset2: Private; Dataset3: ChainZ;	50 suspicious COVID-19 patients from dataset1 used for training; 56 COVID-19 patients; 51 normal patients (CT images) used for testing	Data augmentation (rotation, horizontal flips and cropping)	Resnet-50-2D	–	0.996	98.2%	92.2%	(64)
Jin et al. (2020)	Three centers in China; "LIDC-IDRI;" "Tianchi-Alibaba;" "CC-CCII"	2,529 images from 1,502 COVID-19 patients; 1,338 images from 1,334 CAP patients; 135 images from 83 influenza-A/B patients; 258 images from 258 normal patients (CT images)	–	CNN	–	0.977	90.19%	95.76%	(65)
Jin et al. (2020)	Data from three different centers in Wuhan; Data from three publicly available databases, LIDC-IDRI26, Tianchi-Alibaba27, and CC-CCII18;	1,502 COVID-19 patients; 83 influenza-A/B patients; 1,334 CAP patients except for influenza; 258 healthy subjects (CT images)	Segmenting lung area with U-net	ResNet152	–	0.971	90.19%	95.76%	(66)

(Continued)

TABLE 1 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Hosseinzadeh Kassani et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; "Kaggle chest X-ray database;" "Kaggle RSNA Pneumonia Detection dataset"	117 COVID-19 patients; 117 normal patients (X-Ray images); 20 COVID-19 patients; 20 normal patients (CT images)	Normalization	DenseNet121 with Bagging tree classifier	99%	–	96%	–	(67)
Ozkaya et al. (2020)	From the Italian Society of Medical and Interventional Radiology	53 COVID-19 patients (CT images)	Feature vectors obtained from Pre-trained VGG-16, GoogleNet and ResNet-50 networks and fusion method; Feature ranking by *t*-test method	SVM	98.27%	–	98.93%	97.60%	(68)
Shi et al. (2020)	From Tongji Hospital, Shanghai Public Health Clinical Center, and China-Japan Union Hospital (all in China)	183 COVID-19 patients; 5,521 Pneumonia patients (CT images)	Segmentation by a deep learning network (VB-Net)	Infection size-aware random forest	87.9%	0.942	90.7%	83.3%	(69)
Song et al. (2020)	From the Renmin Hospital of Wuhan University	88 COVID-19 patients (CT images)	We extracted the main regions of lungs and filled the blank of lung segmentation with the lung itself	Details Relation Extraction neural network	86%	0.96	96%	–	(3)
Wang et al. (2020)	Private dataset	44 COVID-19 patients; 55 Pneumonia patients (CT images)	Random selection of ROI; Feature extraction using Transfer Learning	Fully connected network and combination of Decision tree and Adaboost	82.9%	0.90	81%	84%	(6)
Zheng et al. (2020)	Private dataset	313 COVID-19 patients; 229 non-COVID-19 patients (CT images)	Data augmentation; Producing lung masks by a trained UNet	3D deep convolutional neural network	90.8%	0.959	–	–	(70)

Data Source: The source(s) that images were acquired from, Data Structure and Size: Number of images, image modalities, sample groups, Data Preprocessing: cleaning, Instance selection, normalization, transformation, feature extraction, selection, etc. The product of data preprocessing is the final training set, Best Model Structure(s): Best machine algorithm or deep learning model reported in the selected paper based on its performance, Performance Measurements (on the best model): The measurement of the model's output performance based on accuracy, sensitivity, specificity, and AUC score.

TABLE 2 | Characteristics of papers that used X-ray images.

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Alazab et al. (2020)	Kaggle database	70 COVID-19 patients 28 normal patients (X-ray images)	Augmented to 1,000 images	VGG-16	F1 Score: 0.99				(16)
Albahli et al. (2020)	"ChestX-ray8" combined with the few samples of rare classes from the Kaggle challenge	108,948 X-ray images of 32,717 unique patients. Including 15 kinds of chest disease	Data augmentation (rotation, height shift, zoom, horizontal flip)	ResNet	89%	–	–	–	(17)
Albahli et al. (2020)	Open source COVIDx dataset	850 COVID-19 patients; 500 non-COVID-19 pneumonia cases; 915 normal patients (X-ray images)	Data augmentation	InceptionNetV3	99.02%	–	–	–	(18)
Altan et al. (2020)	Not mentioned	7,980 chest X-ray image (2,905 real raw 5,075 synthetic chests X-ray images)	Data augmentation; The feature matrix is formed by 2D Curvelet transformation Coefficients; Optimizing the coefficients in the feature matrix with the CSSA	Hybrid model	99.69%	–	99.44%	99.81%	(20)
Apostolopoulos et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; Common Bacterial and Viral Pneumonia X-ray Images by Kermany et al.; Public datasets (Radiological Society of North America, Radiopaedia, and the Italian Society of Medical and Interventional Radiology); "NIH Chest X-ray Dataset"	455 COVID-19 patients; 910 viral pneumonia; 2,540 other pulmonary diseases (X-ray images)	Data augmentation (randomly rotated by a maximum of 10° and randomly shifted horizontally or vertically by a maximum of 20 pixels toward any direction)	MobileNet v2	99.18%	–	97.36%	99.42%	(21)
Apostolopoulos et al. (2020)	X-ray images on public medical Github repositories; "Radiological Society of North America;" "Radiopaedia, and Italian Society of Medicine and Interventional Radiology"	Dataset 1: 224 COVID-19 patients; 700 bacterial pneumonia patients; 504 normal patients (X-ray images) Dataset 2: 224 Covid-19 patients; 714 bacterial and viral pneumonia patients; 504 normal patients (X-ray images)	-	MobileNet v2	96.78%	–	98.66%	96.46%	(71)
Brunese et al. (2020)	COVID-19 image data collection; COVID-19 X-ray image database developed by Cohen JP; "ChestX-ray8;" "NIH Chest X-ray Dataset"	250 COVID-19 patients; 2,753 other pulmonary diseases; 3,520 normal patients (X-Ray images)	Data augmentation (15 degrees rotation clockwise or counterclockwise)	VGG-16	96% (comparison between COVID-19 and other pulmonary diseases)	–	87% 96%	94% 98%	(72)

(Continued)

TABLE 2 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Chowdhury et al. (2020)	Kaggle chest X-ray database; "Italian Society of Medical and Interventional Radiology COVID-19 database;" "Novel Corona Virus 2019 Dataset;" GitHub database; "COVID-19 Chest imaging at thread reader;" "RSNA-Pneumonia-Detection-Challenge"	423 COVID-19 patients; 1,485 viral pneumonia patients; 1,579 normal patients (X-ray images)	Data augmentation	CNN	99.7%	–	99.7%	99.55%	(73)
Civit-Masot et al. (2020)	COVID-19 and Pneumonia Scans Dataset	132 COVID-19 patients; 132 normal patients; 132 Pneumonia patients (X-ray images)	Histogram equalization	VGG16	85%	–	85%	92%	(74)
Das et al. (2020)	COVID-19 collection; "Kaggle CXR collection;" "Tuberculosis collections;" "U.S. National Library of Medicine;" "National Institutes of Health;" Pneumonia collections	162 COVID-19 patients; 1,583 normal patients	Histogram matching	Truncated Inception Net	100% (Pneumonia collections)	1.0	100%	100%	(23)
Elaziz et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; "Chest X-Ray Images Pneumonia;" Italian Society of Medical and Interventional Radiology COVID-19 DATABASE;	219 COVID-19 patients; 1,341 negative COVID-19 patients (X-ray images)	Feature extraction by Fractional Multichannel Exponent Moments (FrMEMs); Feature selection by modified Manta-Ray Foraging Optimization based on differential evolution	KNN	98.09	–	98.91	–	(75)
Hassantabar et al. (2020)	"COVID-CT-Dataset"	315 COVID-19 patients; 367 non-COVID-19 patients (X-ray images)	–	CNN	93.2%	–	96.1%	99.71%	(76)
Islam et al. (2020)	"GitHub;" "Radiopaedia;" "Cancer Imaging Archive;" "Italian Society of Radiology;" "Kaggle repository;" NIH dataset	1,525 COVID-19 patients; 1,525 pneumonia patients; 1,525 normal patients (X-ray images)	Normalization	CNN-LSTM	99.4%	0.999	99.3%	99.2%	(77)
Khan et al. (2020)	"Covid-chestxray-dataset" "Chest X-Ray Images (Pneumonia)"	284 COVID-19 patients; 330 Pneumonia Bacterial 327 Pneumonia Viral; 310 normal patients (X-ray images)	Random under-sampling (to overcome the unbalanced data problem)	CoroNet (based on Xception)	89.6%	–	89.92%	96.4%	(78)

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TABLE 2 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Khuzani et al. (2020)	"GitHub"	140 COVID-19 patients; 140 non-COVID-19 pneumonia patients; 140 normal patients (X-ray images)	PCA method; Min-Max Normalization; Adaptive Histogram Equalization	ML	94%	0.91	100%	–	(79)
Ko et al. (2020)	Private; Italian Society of Medical and Interventional Radiology COVID-19 DATABASE;	1,194 COVID-19 patients; 1,442 non-pneumonia patients; 1,357 Pneumonia patients (X-ray images)	Data augmentation (rotation, zoom)	FCONet (ResNet-50)	99.58%	–	99.58%	100%	(80)
Loey et al. (2020)	COVID-19 X-ray image database developed by Cohen JP	69 COVID-19 patients; 79 pneumonia bacterial patients; 79	Data augmentation	Googlenet	80.56% (Four classes)	–	80.56%	–	(81)
Mahmud et al. (2020)	Private	1,583 normal patients; 1,493 non-COVID viral pneumonia; 2,780 bacterial pneumonia; 305 COVID-19 patients (X-ray images)	–	CovXNet (CNN based architecture)	90.2% (multi-class)	0.911 (multi-class)	89.9% (multi-class)	89.1% (multi-class)	(82)
Martínez et al. (2020)	COVID-19 X-ray image database developed by Cohen JP	120 COVID-19 patients; 120 normal patients (X-ray images)	Data augmentation; Normalization	NASNet-type convolutional	97%	–	97%	97%	(83)
Minaee et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; "ChexPert dataset"	40 COVID-19 patients; 3,000 normal patients (X-ray images)	Regularization	SqueezeNet	97%	–	97.5%	97.8%	(84)
Narayan Das et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; "ChestX-ray8"	125 COVID-19 patients; 500 pneumonia patients; 500 normal patients (X-ray images)	–	Xception	97.4%	0.986	97.09%	97.29%	(85)
Nour et al. (2020)	"Public COVID-19 radiology database;" "Italian Society of Medical and Interventional Radiology;" "COVID-19 Database;" "Novel Corona Virus 2019 Dataset;" "COVID-19 positive chest X-ray images from different articles;"	219 COVID-19 patients; 1,345 Viral Pneumonia patients; 1,341 Normal patients (X-ray images)	Data augmentation	CNN	97.14%	0.995	94.61%	98.29%	(86)
Novitasari et al. (2020)	GitHub and Kaggle	102 COVID-19 patients; 204 Pneumonia and Normal patients (X-ray images)	Feature extraction by Googlenet, Resnet18, Resnet50, Resnet101; Feature selection by PCA, Relief;	SVM	97.33% (multi class)	–	96%	98%	(87)

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TABLE 2 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Oh et al. (2020)	"Japanese Society of Radiological Technology;" "SCR database;" "U.S. National Library of Medicine"	180 COVID-19 patients; 20 Viral Pneumonia patients; 54 pneumonia bacterial patients; 57 Tuberculosis patients; 191 Normal patients (X-ray images)	Data normalization; Data type casting; Histogram equalization; Gamma correction	(FC)-DenseNet103	88.9%	–	85.9%	96.4%	(88)
Ozturk et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; "ChestX-ray8;"	(X-ray images)		DarkCovidNet inspired by the DarkNet architecture	87.02%	–	85.35%	92.18%	(89)
Pandit et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; Kaggle chest X-ray database	224 COVID-19 patients; 700 pneumonia bacterial patients; 504 Normal patients (X-ray images)	Data augmentation	VGG-16	92.53% (Three class output)	–	86.7%	95.1%	(90)
Panwar et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; Radiopedia.org website; Kaggle chest X-ray database	142 COVID-19 patients; 142 other ("Normal" "Bacterial Pneumonia" and "Viral Pneumonia") (X-ray images)	Data augmentation	nCOVnet	88.10%	0.880	97.62%	78.57%	(40)
Pereira et al. (2020)	"RYDLS-20;" Radiopedia Encyclopedia "Chest X-ray14"	90 COVID-19 patients; 1,000 Normal patients; 10 MERS patients; 11 SARS patients; 10 Varicella patients; 12 Streptococcus patients; 11 Pneumocystis patients (X-ray images)	Resampling algorithms; Fusion techniques;	Pre-trained CNN	F1 score = 89%				(91)
Rahaman et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; "Chest X-Ray Images (pneumonia)"	260 COVID-19 patients; 300 Pneumonia; 300 Normal patients (X-ray images)	Data augmentation (rotate, shift, shear, zoom, horizontal and vertical flip)	VGG19	89.3%	–	89%	–	(92)
Rahimzadeh et al. (2020)	"Covid chestxray dataset;" "RSNA pneumonia detection challenge"	180 COVID-19 patients; 6,054 Pneumocystis patients; 8,851 Normal patients (X-ray images)	Data augmentation	Xception ResNet50V2 concatenated	91.4%	–	80.53%	99.56%	(93)
Rajaraman et al. (2020)	Pediatric CXR dataset; RSNA CXR dataset; CheXpert CXR dataset; NIH CXR-14 dataset; Twitter COVID-19 CXR dataset; Montreal COVID-19 CXR dataset;	4,683 Bacterial Pneumonia; 3,883 Viral Pneumonia (X-Ray images)	Segmenting lung area with dilated dropout U-Net; Image thresholding to remove very bright pixels; In-painting missing pixels using the surrounding pixel values; Using median-filter to remove noise and preserve edges;	VGG-16	94.05%	0.96	98.77%	86.24%	(45)

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TABLE 2 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Rajaraman et al. (2020)	"Pediatric CXR dataset;" "RSNA CXR dataset;" "Twitter COVID-19 CXR dataset;" "Montreal COVID-19 CXR dataset"	313 COVID-19 patients; 7,595 pneumonia of unknown type patients; 2,780 bacterial pneumonia; 7,595 Normal patients (X-ray images)	Median Filtering; Normalization; Standardization	Inception-V3	99.01%	0.997	98.4%	–	(45)
Sethy et al. (2020)	X-ray images on public medical Github repositories; Kaggle chest X-ray database	127 COVID-19 patients; 127 Pneumonia patients; 127 Normal patients (X-ray images)	–	ResNet50 plus SVM	98.66%	–	95.33%	–	(94)
Shibly et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; "RSNA pneumonia detection challenge dataset;" Kaggle chest X-ray database; "COVIDx"	183 COVID-19 patients; 5,551 Pneumonia patients; 8,066 Normal patients (X-ray images)	–	Faster R-CNN	97.36%	–	97.65%	–	(95)
Togaçar et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; Kaggle COVID-19 dataset created by a team of researchers from Qatar University, medical doctors from Bangladesh, and collaborators from Pakistan and Malaysia.	295 COVID-19 patients; 98 Pneumonia; 65 normal patients (X-ray images)	Restructuring images using the Fuzzy Color technique and stacking them with the original images; Feature extracting using deep learning models (MobileNetV2, SqueezeNet) using the Social Mimic optimization method;	SVM	100%	–	100%	100%	(96)
Toraman et al. (2020)	COVID-19 X-ray image database developed by Cohen JP	231 COVID-19 patients; 1,050 Pneumonia patients; 1,050 Normal patients (X-ray images)	Data augmentation;	Convolutional capsnet	97.24% (Binary class)	–	97.42%	97.04%	(97)

(Continued)

TABLE 2 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Tsiknakis et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; Dataset originated from the QUIBIM imagingcovid19 platform database and various public repositories, including RSNA, IEEE, RadioGyan and the British Society of Thoracic Imaging; Publicly available X-ray dataset of patients with pneumonia;	137 COVID-19 patients; 150 Virus Pneumonia; 150 Bacteria Pneumonia; 150 normal patients (X-ray images)	Data augmentation (rotation, shear, zoom)	Inception V3	76% (multi-class)	0.93 (multi-class)	93% (multi-class)	91.8% (multi-class)	(98)
Tuncer et al. (2020)	GitHub website; Kaggle chest X-ray database	87 COVID-19 patients; 234 Normal patients (X-ray images)	Converting X-ray image to grayscale; ResExLBP and IRF based method	SVM	100%	–	98.29%	100%	(99)
Ucar et al. (2020)	"COVID chest X-ray dataset;" "Kaggle chest X-ray pneumonia dataset;"	403 COVID-19 patients; 721 normal patients (X-ray images)	Data augmentation (noise, shear, brightness increase, brightness decrease)	Bayes-SqueezeNet	98.26% (multi-class)	–	–	99.13% (multi-class)	(100)
Vaid et al. (2020)	Set of lately published articles; NIH dataset	181 COVID-19 patients; 364 Normal patients (X-ray images)	Normalization	VGG-19	96.3%	–	97.1%	–	(101)
Waheed et al. (2020)	"IEEE Covid Chest X-ray dataset;" "COVID-19 Radiography Database" "COVID-19 Chest X-ray Dataset;"	403 COVID-19 patients; 721 normal patients (X-ray images)	Data augmentation using CovidGAN	VGG16	95%	–	90%	97%	(102)
Yildirim et al. (2020)	"COVID-19 Chest X-Ray dataset;" Kaggle chest X-ray database	136 COVID-19 patients; 162 Pneumonia patients; 245 Normal patients (X-ray images)	–	Hybrid model	96.30%	–	96.30%	98.73%	(103)
Yoo et al. (2020)	"COVID-Chest XrayDataset;" Eastern Asian Hospital; Shenzen data;	162 COVID-19 Patients; 162 TB patients; 162 Non-TB patients (X-ray images)	Data augmentation (rotated, translated, and horizontally flipped)	ResNet18	95% Average of (COVID-19/TB) and (COVID-19/non-TB)	0.95 Average of (COVID-19/TB) and (COVID-19/non-TB)	97% Average of (COVID-19/TB) and (COVID-19/non-TB)	93% Average of (COVID-19/TB) and (COVID-19/non-TB)	(104)
Ghoshal et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; "Kaggle chest X-ray database"	68 COVID-19 patients; 2,786 Bacterial Pneumonia patients; 1,504 Viral Pneumonia patients; 1,583 normal patients (X-Ray images)	Standardization; Data augmentation	Bayesian ResNet50V2 model	89.82%	–	–	–	(105)

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TABLE 2 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Hall et al. (2020)	"X-ray images on public medical Github repositories;" "Radiopaedia;" "Italian Society of Medical and Interventional Radiology (SIRM)"	135 COVID-19 patients; 320 Viral and Bacterial Pneumonia patients (X-Ray images)	Data augmentation	Resnet50 and VGG16 plus CNN	91.24%	0.94	–	–	(106)
Hammoudi et al. (2020)	"Chest XRay Images (Pneumonia) dataset;" COVID-19 X-ray image database developed by Cohen JP;	148 Bacterial pneumonia; 148 Viral pneumonia; 148 Normal patients (X-Ray Images)	–	DenseNet169	95.72%	–	–	–	(107)
El-Din Hemdan et al. (2020)	COVID-19 X-ray image database developed by Cohen JP; COVID-19 X-ray image database by Dr. Adrian Rosebrock	25 COVID-19 patients; 25 normal patients (X-Ray images)	Scaling to 224*224 pixels; One-hot encoding	COVIDX-Net (VGG19 and DenseNet201 models)	VGG19 = 90%; DenseNet201 = 90%	VGG19 = 0.90; DenseNet201 = 0.90	VGG19 = 100%; DenseNet201 = 100%	–	(108)
Jain et al. (2020)	"Chest XRay Images (Pneumonia) dataset;" COVID-19 X-ray image database developed by Cohen JP;	250 COVID-19 patients; 300 Bacterial pneumonia; 350 Viral pneumonia; 315 Normal patients (X-Ray Images)	Normalize images according to the images in the ImageNet database; Data augmentation (rotation and Gaussian blur);	ResNet50	97.77%	–	97.14%	–	(109)
Luz et al. (2020)	"COVIDx dataset;" "RSNA Pneumonia Detection Challenge dataset;" "COVID-19 image data collection"	183 COVID-19 patients; 5,521 Pneumonia patients; 8,066 normal patients (X-Ray images)	Intensity normalization; Data augmentation	EfficientNet B3	93.9%	–	96.8%	–	(110)
Ozkaya et al. (2020)	From the Italian Society of Medical and Interventional Radiology	53 COVID-19 patients (CT images)	Feature vectors obtained from Pre-trained VGG-16, GoogleNet and ResNet-50 networks and fusion method; Feature ranking by *t*-test method	SVM	98.27%	–	98.93%	97.60%	(68)

(Continued)

TABLE 2 | Continued

Author, year	Data source	Data structure and size	Data preprocessing	Best model structure(s)	Performance measurements (on the best model)				References
					Accuracy	AUC score	Sensitivity	Specificity	
Ozturk et al. (2020)	"covid-chestxray-dataset available at: https://github.com/ieee8023/covid-chestxray-dataset"	4 ARds images, 101 COVID images, 2 No finding images, 2 pneumocystis-pneumonia images, 11 Sars images, and 6 streptococcus (X-Ray images)	Data augmentation; SMOTE oversampling; creating feature vectors with sAE and PCA; feature extraction by feature vectors, Gray Level Co-occurrence Matrix, Local Binary Gray Level Co-occurrence Matrix, Gray Level Run Length Matrix, and Segmentation-based Fractal Texture Analysis	SVM	94.23%	0.99	91.88%	98.54%	(111)
Wang et al. (2020)	COVIDx dataset	266 COVID-19 patients; 5,536 Pneumonia patients; 8,066 normal patients (X-Ray images)	–	COVID-Net Network Architecture using a "lightweight residual projection-expansion-projection-extension design pattern" (Customized CNN)	93.3%		91.0%	–	(1)
Zhang et al. (2020)	X-COVID, OpenCOVID	599 COVID-19 patients; 2,107 non-COVID-19 patients (non-viral pneumonia and healthy) (X-Ray images)	Data augmentation; Feature extraction using EfficientNet	Confidence-aware anomaly detection	78.57%	0.844	77.13%	78.97%	(112)

Data Source: The source(s) that images were acquired from, Data Structure and Size: Number of images, image modalities, sample groups, Data Preprocessing: cleaning, Instance selection, normalization, transformation, feature extraction, selection, etc. The product of data preprocessing is the final training set, Best Model Structure(s): Best machine algorithm or deep learning model reported in the selected paper based on its performance, Performance Measurements (on the best model): The measurement of the model's output performance based on accuracy, sensitivity, specificity, and AUC score.

DISCUSSION

Machine and deep learning methods have been proven as valuable strategies to assess massive high-dimensional characteristics of medical images. CT or X-Ray findings of COVID-19 patients have similarities with other atypical and viral pneumonia diseases. Therefore, machine and deep learning methods might facilitate automatic discrimination of COVID-19 from other pneumonia conditions. The differential diagnosis of COVID also includes drug-induced diseases or immune pneumonitis. However, most of the studies reviewed here lack these kinds of samples. This point is the limitation of these studies. Different methods, such as Ensemble, VGG-16, ResNet, InceptionNetV3, MobileNet v2, Xception, CNN, VGG16, Truncated Inception Net, and KNN, have been used for the purpose of assessment of chest images of COVID-19 patients. Notably, the application of these methods on X-rays has offered promising results. Such a finding is particularly important since X-rays are easily accessible and low cost. These methods not only can diagnose COVID-19 patients from non-COVID pneumonia cases, but can also predict the severity of COVID-19 pneumonia and the risk of short-term mortality. In spite of the low expense of X-ray compared with CT images, the numbers of studies that assessed these two types of imaging using machine/deep learning methods are not meaningfully different. However, few studies have used these methods on both types of imaging (25, 29, 40). CNN-based methods have achieved accuracy values above 99% in classifying COVID-19 patients from other cases of pneumonia or related disorders, as reported by several independent studies, suggesting these strategies as screening methods for initial evaluation of COVID-19 cases.

Although both deep learning and machine learning strategies can be used for the mentioned purpose, they differ in some respects. For instance, deep learning methods usually need a large amount of labeled training data to make a concise conclusion. However, machine learning can apply a small amount of data delivered by users. Moreover, deep learning methods need high-performance hardware. Machine learning, on the other hand, needs features to be precisely branded by users, deep learning generates novel features by itself, thus requires more time to train. Machine learning classifies tasks into small fragments and subsequently combines obtained results into one conclusion, whereas deep learning resolves the problems using end-to-end principles.

Several studies have diagnosed COVID-19 patients through the application of machine learning methods rather than using deep learning methods by retrieving the features from the images. These studies have yielded high recognition outcomes and have the advantage of high learning speed (12). Pre-processing is an essential step for reducing the impacts of intensity variations in CT slices and getting rid of noise. Subsequent thresholding and morphological operations have also enhanced the analytical performance. Data augmentation and histogram equalization are among the most applied preprocessing methods.

One of the most promising approaches used in the included studies was transfer learning. Transfer learning is defined as using model knowledge on a huge dataset (which is referred to as the "pre-trained model") and transferring it to use on a new problem. This is very useful in settings like medical imaging, where there is a limited number of labeled data (113). Previous studies showed favorable outcomes of the transfer learning approaches in medical imaging tasks (114, 115). Among the included studies, Bridge et al. (25) even reached 100% classification accuracy on COVID-19 using the pre-trained InceptionV3.

The availability of public databases of CT and X-ray images of patients with COVID-19 has facilitated the application of machine learning methods on large quantities of clinical images and execution of training and verification steps. However, since these images have come from various institutes using different scanners, preprocessing of the obtained data is necessary to make them uniform and facilitate further analysis (12). Appraisal of demographic and clinical data of COVID-19 patients and their association with CT/ X-ray images features as well as the accuracy of machine learning prediction methods would provide more valuable information in the stratification of COVID-19 patients. Moreover, one of the major challenges of deep learning models in medical applications is its unexplainable features due to its black-box nature, which should be solved (116). Future studies can focus on approaches that provide interpretation besides black-box predictions.

CONCLUSION

Deep and machine learning methods have high accuracy in the differentiation of COVID-19 from non-COVID-19 pneumonia based on chest images. These techniques have facilitated the automatic evaluation of these images. However, deep learning methods suffer from the absence of transparency and interpretability, as it is not possible to identify the exact imaging feature that has been applied to define the output (13). As no single strategy has the capacity to distinguish all pulmonary disorders based merely on the imaging presentation on chest CT scans, the application of multidisciplinary approaches is suggested for overcoming diagnostic problems (13).

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

HM-R, MN, and AG-L collected the data and designed the tables. MT and SG-F designed the study, wrote the draft, and revised it. All the authors read the draft and approved the submitted version.

REFERENCES

1. Wang L, Wong A. COVID-Net: a tailored deep convolutional neural network design for detection of covid-19 cases from chest X-ray images. *arXiv*. (2020) Preprint arXiv:200309871. doi: 10.1038/s41598-020-76550-z
2. Ghafouri-Fard S, Noroozi R, Vafaee R, Branicki W, Pośpiech E, Pyrc K, et al. Effects of host genetic variations on response to, susceptibility and severity of respiratory infections. *Biomed Pharmacother*. (2020) 128:110296. doi: 10.1016/j.biopha.2020.110296
3. Song Y, Zheng S, Li L, Zhang X, Zhang X, Huang Z, et al. Deep learning enables accurate diagnosis of novel coronavirus (COVID-19) with CT images. *medRxiv*. (2020).
4. Samsami M, Mehravaran E, Tabarsi P, Javadi A, Arsang-Jang S, Komaki A, et al. Clinical and demographic characteristics of patients with COVID-19 infection: statistics from a single hospital in Iran. *Human Antibodies*. (2020) 1–6. doi: 10.3233/HAB-200428
5. Ghafouri-Fard S, Noroozi R, Omrani MD, Branicki W, Pośpiech E, Sayad A, et al. Angiotensin converting enzyme: a review on expression profile and its association with human disorders with special focus on SARS-CoV-2 infection. *Vascular Pharmacol.* (2020) 130:106680. doi: 10.1016/j.vph.2020.106680
6. Wang S, Kang B, Ma J, Zeng X, Xiao M, Guo J, et al. A deep learning algorithm using CT images to screen for Corona Virus Disease (COVID-19). *medRxiv*. (2020) 14:1–9. doi: 10.1101/2020.02.14.20023028
7. Fang Y, Zhang H, Xie J, Lin M, Ying L, Pang P, et al. Sensitivity of chest CT for COVID-19: comparison to RT-PCR. *Radiology*. (2020) 296:1–2. doi: 10.1148/radiol.2020200432
8. Zhang J, Tian S, Lou J, Chen Y. Familial cluster of COVID-19 infection from an asymptomatic. *Crit Care*. (2020) 24:1–3. doi: 10.1186/s13054-020-2817-7
9. Lei Y, Zhang H-W, Yu J, Patlas MN. *COVID-19 Infection: Early Lessons*. Los Angeles, CA: Sage (2020).
10. Rousan LA, Elobeid E, Karrar M, Khader Y. Chest x-ray findings and temporal lung changes in patients with COVID-19 pneumonia. *BMC Pulmonary Med*. (2020) 20:1–9. doi: 10.1186/s12890-020-01286-5
11. Bai HX, Hsieh B, Xiong Z, Halsey K, Choi JW, Tran TML, et al. Performance of radiologists in differentiating COVID-19 from viral pneumonia on chest CT. *Radiology*. (2020) 296:1–8. doi: 10.1148/radiol.2020200823
12. Ozsahin I, Sekeroglu B, Musa MS, Mustapha MT, Uzun Ozsahin D. Review on diagnosis of COVID-19 from chest CT images using artificial intelligence. *Comput Math Methods Med*. (2020) 2020:1–10. doi: 10.1155/2020/9756518
13. Li L, Qin L, Xu Z, Yin Y, Wang X, Kong B, et al. Using artificial intelligence to detect COVID-19 and community-acquired pneumonia based on pulmonary CT: evaluation of the diagnostic accuracy. *Radiology*. (2020) 296:E65–71. doi: 10.1148/radiol.2020200905
14. rekha Hanumanthu S. Role of intelligent computing in COVID-19 prognosis: a state-of-the-art review. *Chaos Solitons Fractals*. (2020) 138:109947. doi: 10.1016/j.chaos.2020.109947
15. Abbasian Ardakani A, Acharya UR, Habibollahi S, Mohammadi A. COVIDiag: a clinical CAD system to diagnose COVID-19 pneumonia based on CT findings. *Eur Radiol*. (2020) 31:1–10. doi: 10.1007/s00330-020-07087-y
16. Alazab M, Awajan A, Mesleh A, Abraham A, Jatana V, Alhyari S. COVID-19 prediction and detection using deep learning. *Int J Comput Information Syst Indus Manage Appl*. (2020) 12:168–81. doi: 10.1016/j.chaos.2020.110338
17. Albahli S. Efficient GAN-based Chest Radiographs (CXR) augmentation to diagnose coronavirus disease pneumonia. *Int J Med Sci*. (2020) 17:1439–48. doi: 10.7150/ijms.46684
18. Albahli S, Albattah W. Detection of coronavirus disease from X-ray images using deep learning and transfer learning algorithms. *J Xray Sci Technol*. (2020) 28:841–50. doi: 10.3233/XST-200720
19. Alsharman N, Jawarneh I. GoogleNet CNN neural network towards chest CT-coronavirus medical image classification. *J Comput Sci*. (2020) 16:620–5 doi: 10.3844/jcssp.2020.620.625
20. Altan A, Karasu S. Recognition of COVID-19 disease from X-ray images by hybrid model consisting of 2D curvelet transform, chaotic salp swarm algorithm and deep learning technique. *Chaos Solitons Fractals*. (2020) 140:110071. doi: 10.1016/j.chaos.2020.110071
21. Apostolopoulos ID, Aznaouridis SI, Tzani MA. Extracting possibly representative COVID-19 biomarkers from X-ray images with deep learning approach and image data related to pulmonary diseases. *J Med Biol Eng*. (2020) 40:1–8. doi: 10.1007/s40846-020-00529-4
22. Ardakani AA, Kanafi AR, Acharya UR, Khadem N, Mohammadi A. Application of deep learning technique to manage COVID-19 in routine clinical practice using CT images: results of 10 convolutional neural networks. *Comput Biol Med*. (2020) 121:103795. doi: 10.1016/j.compbiomed.2020.103795
23. Das D, Santosh KC, Pal U. Truncated inception net: COVID-19 outbreak screening using chest X-rays. *Phys Eng Sci Med*. (2020) 43:1—11. doi: 10.21203/rs.3.rs-20795/v1
24. Aswathy SU, Jarin T, Mathews R, Nair LM, Rroan M. CAD systems for automatic detection and classification of COVID-19 in nano CT lung image by using machine learning technique. *Int J Pharm Res*. (2020) 12:1865–70. doi: 10.31838/ijpr/2020.12.02.247
25. Bridge J, Meng Y, Zhao Y, Du Y, Zhao M, Sun R, et al. Introducing the GEV activation function for highly unbalanced data to develop COVID-19 diagnostic models. *IEEE J Biomed Health Inform*. (2020) 24:1–10. doi: 10.1109/JBHI.2020.3012383
26. Butt C, Gill J, Chun D, Babu BA. Deep learning system to screen coronavirus disease 2019 pneumonia. *Appl Intell*. (2020) 6:1–7. doi: 10.1007/s10489-020-01714-3
27. Dey N, Rajinikanth V, Fong SJ, Kaiser MS, Mahmud M. Social group optimization-assisted Kapur's entropy and morphological segmentation for automated detection of COVID-19 infection from computed tomography images. *Cognit Comput*. (2020) 12:1–13. doi: 10.20944/preprints202005.0052.v1
28. Kermany D, Zhang K, Goldbaum M. Labeled optical coherence tomography (OCT) and Chest X-Ray images for classification. *Mendeley Data*. (2018) 2. doi: 10.17632/RSCBJBR9SJ.2
29. El Asnaoui K, Chawki Y. Using X-ray images and deep learning for automated detection of coronavirus disease. *J Biomol Struct Dyn*. (2020) 1—12. doi: 10.1080/07391102.2020.1767212
30. Han Z, Wei B, Hong Y, Li T, Cong J, Zhu X, et al. Accurate screening of COVID-19 using attention-based deep 3D multiple instance learning. *IEEE Trans Med Imaging*. (2020) 39:2584–94. doi: 10.1109/TMI.2020.2996256
31. Harmon SA, Sanford TH, Xu S, Turkbey EB, Roth H, Xu Z, et al. Artificial intelligence for the detection of COVID-19 pneumonia on chest CT using multinational datasets. *Nat Commun*. (2020) 11:4080. doi: 10.1038/s41467-020-17971-2
32. Hasan AM, Al-Jawad MM, Jalab HA, Shaiba H, Ibrahim RW, Al-Shamasneh AR. Classification of Covid-19 coronavirus, pneumonia and healthy lungs in CT scans using Q-deformed entropy and deep learning features. *Entropy*. (2020) 22:517. doi: 10.3390/e22050517
33. Hu S, Gao Y, Niu Z, Jiang Y, Li L, Xiao X, et al. Weakly supervised deep learning for COVID-19 infection detection and classification from CT images. *IEEE Access*. (2020) 8:118869–83. doi: 10.1109/ACCESS.2020.3005510
34. Jaiswal A, Gianchandani N, Singh D, Kumar V, Kaur M. Classification of the COVID-19 infected patients using DenseNet201 based deep transfer learning. *J Biomol Struct Dyn*. (2020) 1—8. doi: 10.1080/07391102.2020.1788642
35. Kang H, Xia L, Yan F, Wan Z, Shi F, Yuan H, et al. Diagnosis of coronavirus disease 2019 (COVID-19) with structured latent multi-view representation learning. *IEEE Trans Med Imaging*. (2020) 39:2606–14. doi: 10.1109/TMI.2020.2992546
36. Lessmann N, Sánchez CI, Beenen L, Boulogne LH, Brink M, Calli E, et al. Automated assessment of CO-RADS and chest CT severity scores in patients with suspected COVID-19 using artificial intelligence. *Radiology*. (2020) 202439.
37. Li Y, Dong W, Chen J, Cao S, Zhou H, Zhu Y, et al. Efficient and effective training of COVID-19 classification networks with self-supervised dual-track learning to rank. *IEEE J Biomed Health Inform*. (2020) 24:1–10. doi: 10.1109/JBHI.2020.3018181
38. Liu C, Wang X, Liu C, Sun Q, Peng W. Differentiating novel coronavirus pneumonia from general pneumonia based

on machine learning. *Biomed Eng Online*. (2020) 19:66. doi: 10.1186/s12938-020-00809-9
39. Mei X, Lee HC, Diao KY, Huang M, Lin B, Liu C, et al. Artificial intelligence-enabled rapid diagnosis of patients with COVID-19. *Nat Med.* (2020) 26:1224–8. doi: 10.1038/s41591-020-0931-3
40. Panwar H, Gupta PK, Siddiqui MK, Morales-Menendez R, Singh V. Application of deep learning for fast detection of COVID-19 in X-Rays using nCOVnet. *Chaos Solitons Fractals*. (2020) 138:109944. doi: 10.1016/j.chaos.2020.109944
41. Pathak Y, Shukla PK, Tiwari A, Stalin S, Singh S, Shukla PK. Deep transfer learning based classification model for COVID-19 disease. *Ing Rech Biomed*. (2020) 1–6. doi: 10.1016/j.irbm.2020.05.003
42. Peng Y, Tang YX, Lee S, Zhu Y, Summers RM, Lu Z. COVID-19-CT-CXR: a freely accessible and weakly labeled chest X-ray and CT image collection on COVID-19 from biomedical literature. *ArXiv*. (2020). doi: 10.1109/TBDATA.2020.3035935
43. Pu J, Leader J, Bandos A, Shi J, Du P, Yu J, et al. Any unique image biomarkers associated with COVID-19? *Eur Radiol*. (2020) 30:1–7. doi: 10.1007/s00330-020-06956-w
44. Raajan NR, Lakshmi VSR, Prabaharan N. Non-invasive technique-based novel corona (COVID-19) virus detection using CNN. *Natl Acad Sci Lett*. (2020) 1–4. doi: 10.1007/s40009-020-01009-8
45. Rajaraman S, Siegelman J, Alderson PO, Folio LS, Folio LR, Antani SK. Iteratively pruned deep learning ensembles for COVID-19 detection in chest X-rays. *IEEE Access*. (2020) 8:115041–50. doi: 10.1109/ACCESS.2020.3003810
46. Sakagianni A, Feretzakis G, Kalles D, Koufopoulou C, Kaldis V. Setting up an easy-to-use machine learning pipeline for medical decision support: a case study for COVID-19 diagnosis based on deep learning with CT scans. *Stud Health Technol Inform*. (2020) 272:13–6. doi: 10.3233/SHTI200481
47. Sharma S. Drawing insights from COVID-19-infected patients using CT scan images and machine learning techniques: a study on 200 patients. *Environ Sci Pollut Res Int.* (2020) 27:1–9. doi: 10.21203/rs.3.rs-23863/v1
48. Singh D, Kumar V, Vaishali, Kaur M. Classification of COVID-19 patients from chest CT images using multi-objective differential evolution-based convolutional neural networks. *Eur J Clin Microbiol Infect Dis*. (2020) 39:1379–89. doi: 10.1007/s10096-020-03901-z
49. Song J, Wang H, Liu Y, Wu W, Dai G, Wu Z, et al. End-to-end automatic differentiation of the coronavirus disease 2019 (COVID-19) from viral pneumonia based on chest CT. *Eur J Nucl Med Mol Imaging.* (2020) 47:1–9. doi: 10.1007/s00259-020-04929-1
50. Wang J, Bao Y, Wen Y, Lu H, Luo H, Xiang Y, et al. Prior-attention residual learning for more discriminative COVID-19 screening in CT images. *IEEE Trans Med Imaging*. (2020) 39:2572–83. doi: 10.1109/TMI.2020.2994908
51. Wang S, Zha Y, Li W, Wu Q, Li X, Niu M, et al. A fully automatic deep learning system for COVID-19 diagnostic and prognostic analysis. *Eur Respir J*. (2020) 56:2000775. doi: 10.1183/13993003.00775-2020
52. Warman A, Warman P, Sharma A, Parikh P, Warman R, Viswanadhan N, et al. Interpretable artificial intelligence for COVID-19 diagnosis from chest CT reveals specificity of ground-glass opacities. *medRxiv*. (2020) 1–13. doi: 10.1101/2020.05.16.20103408
53. Wu X, Hui H, Niu M, Li L, Wang L, He B, et al. Deep learning-based multi-view fusion model for screening 2019 novel coronavirus pneumonia: a multicentre study. *Eur J Radiol*. (2020) 128:109041. doi: 10.1016/j.ejrad.2020.109041
54. Xu X, Jiang X, Ma C, Du P, Li X, Lv S, et al. A deep learning system to screen novel coronavirus disease 2019 pneumonia. *Engineering.* (2020) 6:1–7. doi: 10.1016/j.eng.2020.04.010
55. Xu Y, Ma L, Yang F, Chen Y, Ma K, Yang J, et al. A collaborative online AI engine for CT-based COVID-19 diagnosis. *medRxiv*. (2020). doi: 10.1101/2020.05.10.20096073
56. Yan T, Wong PK, Ren H, Wang H, Wang J, Li Y. Automatic distinction between COVID-19 and common pneumonia using multi-scale convolutional neural network on chest CT scans. *Chaos Solitons Fractals*. (2020) 140:110153. doi: 10.1016/j.chaos.2020.110153
57. Yang S, Jiang L, Cao Z, Wang L, Cao J, Feng R, et al. Deep learning for detecting corona virus disease 2019 (COVID-19) on high-resolution computed tomography: a pilot study. *Ann Transl Med*. (2020) 8:450. doi: 10.21037/atm.2020.03.132
58. Yu Z, Li X, Sun H, Wang J, Zhao T, Chen H, et al. Rapid identification of COVID-19 severity in CT scans through classification of deep features. *Biomed Eng Online*. (2020) 19:63. doi: 10.1186/s12938-020-00807-x
59. Al-Karawi D, Al-Zaidi S, Polus N, Jassim S. Machine learning analysis of chest CT scan images as a complementary digital test of coronavirus (COVID-19) patients. *medRxiv*. (2020) 1–8. doi: 10.1101/2020.04.13.20063479
60. Alom MZ, Rahman M, Nasrin MS, Taha TM, Asari VK. COVID_MTNet: COVID-19 detection with multi-task deep learning approaches. *arXiv*. (2020) Preprint arXiv:200403747.
61. Barstugan M, Ozkaya U, Ozturk S. Coronavirus (covid-19) classification using ct images by machine learning methods. *arXiv*. (2020) Preprint arXiv:200309424.
62. Chen J, Wu L, Zhang J, Zhang L, Gong D, Zhao Y, et al. Deep learning-based model for detecting 2019 novel coronavirus pneumonia on high-resolution computed tomography. *Sci Rep*. (2020) 10:1–11. doi: 10.1101/2020.02.25.20021568
63. Farid AA, Selim GI, Awad H, Khater A. A novel approach of CT images feature analysis and prediction to screen for corona virus disease (COVID-19). *Int J Sci Eng Res*. (2020) 11:1–9. doi: 10.14299/ijser.2020.03.02
64. Gozes O, Frid-Adar M, Greenspan H, Browning PD, Zhang H, Ji W, et al. Rapid ai development cycle for the coronavirus (covid-19) pandemic: initial results for automated detection & patient monitoring using deep learning CT image analysis. *arXiv*. (2020) Preprint arXiv:200305037.
65. Jin C, Chen W, Cao Y, Xu Z, Zhang X, Deng L, et al. Development and evaluation of an AI system for COVID-19 diagnosis. *medRxiv*. (2020) 11:1–14. doi: 10.1101/2020.03.20.20039834
66. Jin S, Wang B, Xu H, Luo C, Wei L, Zhao W, et al. AI-assisted CT imaging analysis for COVID-19 screening: building and deploying a medical AI system in four weeks. *medRxiv*. (2020). doi: 10.1101/2020.03.19.20039354
67. Kassani SH, Kassasni PH, Wesolowski MJ, Schneider KA, Deters R. Automatic detection of coronavirus disease (COVID-19) in X-ray and CT images: a machine learning-based approach. *arXiv*. (2020) Preprint arXiv:200410641.
68. Ozkaya U, Ozturk S, Barstugan M. Coronavirus (COVID-19) classification using deep features fusion and ranking technique. *arXiv*. (2020) Preprint arXiv:200403698. doi: 10.1007/978-3-030-55258-9_17
69. Shi F, Xia L, Shan F, Wu D, Wei Y, Yuan H, et al. Large-scale screening of covid-19 from community acquired pneumonia using infection size-aware classification. *arXiv*. (2020) Preprint arXiv:200309860. doi: 10.1088/1361-6560/abe838
70. Zheng C, Deng X, Fu Q, Zhou Q, Feng J, Ma H, et al. Deep learning-based detection for COVID-19 from chest CT using weak label. *medRxiv*. (2020) 1–13. doi: 10.1101/2020.03.12.20027185
71. Apostolopoulos ID, Mpesiana TA. Covid-19: automatic detection from X-ray images utilizing transfer learning with convolutional neural networks. *Phys Eng Sci Med*. (2020) 43:635–40. doi: 10.1007/s13246-020-00865-4
72. Brunese L, Mercaldo F, Reginelli A, Santone A. Explainable deep learning for pulmonary disease and coronavirus COVID-19 detection from X-rays. *Comput Methods Programs Biomed*. (2020) 196:105608. doi: 10.1016/j.cmpb.2020.105608
73. Chowdhury MEH, Rahman T, Khandakar A, Mazhar R, Kadir MA, Mahbub ZB, et al. Can AI help in screening viral and COVID-19 pneumonia? *IEEE Access*. (2020) 8:132665–76. doi: 10.1109/ACCESS.2020.3010287
74. Civit-Masot J, Luna-Perejón F, Morales MD, Civit A. Deep learning system for COVID-19 diagnosis aid using X-ray pulmonary images. *Appl Sci*. (2020) 10:4640. doi: 10.3390/app10134640
75. Elaziz MA, Hosny KM, Salah A, Darwish MM, Lu S, Sahlol AT. New machine learning method for image-based diagnosis of COVID-19. *PLoS ONE*. (2020) 15:e0235187. doi: 10.1371/journal.pone.0235187
76. Hassantabar S, Ahmadi M, Sharifi A. Diagnosis and detection of infected tissue of COVID-19 patients based on lung x-ray image using

convolutional neural network approaches. *Chaos Solitons Fractals*. (2020) 140:110170. doi: 10.1016/j.chaos.2020.110170
77. Islam MZ, Islam MM, Asraf A. A combined deep CNN-LSTM network for the detection of novel coronavirus (COVID-19) using X-ray images. *Inform Med Unlocked*. (2020) 20:100412. doi: 10.1016/j.imu.2020.100412
78. Khan AI, Shah JL, Bhat MM. CoroNet: a deep neural network for detection and diagnosis of COVID-19 from chest x-ray images. *Comput Methods Programs Biomed*. (2020) 196:105581. doi: 10.1016/j.cmpb.2020.105581
79. Khuzani AZ, Heidari M, Shariati SA. COVID-Classifier: an automated machine learning model to assist in the diagnosis of COVID-19 infection in chest x-ray images. *medRxiv*. (2020).
80. Ko H, Chung H, Kang WS, Kim KW, Shin Y, Kang SJ, et al. COVID-19 pneumonia diagnosis using a simple 2D deep learning framework with a single chest CT image: model development and validation. *J Med Internet Res*. (2020) 22:e19569. doi: 10.2196/19569
81. Loey M, Smarandache F, Khalifa NEM. Within the lack of chest COVID-19 X-ray dataset: a novel detection model based on GAN and deep transfer learning. *Symmetry*. (2020) 12:651. doi: 10.3390/sym12040651
82. Mahmud T, Rahman MA, Fattah SA. CovXNet: a multi-dilation convolutional neural network for automatic COVID-19 and other pneumonia detection from chest X-ray images with transferable multi-receptive feature optimization. *Comput Biol Med*. (2020) 122:103869. doi: 10.1016/j.compbiomed.2020.103869
83. Martínez F, Martínez F, Jacinto E. Performance evaluation of the NASnet convolutional network in the automatic identification of COVID-19. *Int J Adv Sci Engin Information Technol*. (2020) 10:662–7. doi: 10.18517/ijaseit.10.2.11446
84. Minaee S, Kafieh R, Sonka M, Yazdani S, Jamalipour Soufi G. Deep-COVID: predicting COVID-19 from chest X-ray images using deep transfer learning. *Med Image Anal*. (2020) 65:101794. doi: 10.1016/j.media.2020.101794
85. Narayan Das N, Kumar N, Kaur M, Kumar V, Singh D. Automated deep transfer learning-based approach for detection of COVID-19 infection in chest X-rays. *Ing Rech Biomed*. (2020) 1–7. doi: 10.1016/j.irbm.2020.07.001
86. Nour M, Cömert Z, Polat K. A novel medical diagnosis model for COVID-19 infection detection based on deep features and Bayesian optimization. *Appl Soft Comput*. (2020) 97:1–14. doi: 10.1016/j.asoc.2020.106580
87. Novitasari DCR, Hendradi R, Caraka RE, Rachmawati Y, Fanani NZ, Syarifudin A, et al. Detection of COVID-19 chest x-ray using support vector machine and convolutional neural network. *Commun Math Biol Neurosci*. (2020) 2020:1–19. doi: 10.28919/cmbn/4765
88. Oh Y, Park S, Ye JC. Deep Learning COVID-19 Features on CXR using limited training data sets. *IEEE Trans Med Imaging*. (2020) 39:2688–700. doi: 10.1109/TMI.2020.2993291
89. Ozturk T, Talo M, Yildirim EA, Baloglu UB, Yildirim O, Rajendra Acharya U. Automated detection of COVID-19 cases using deep neural networks with X-ray images. *Comput Biol Med*. (2020) 121:103792. doi: 10.1016/j.compbiomed.2020.103792
90. Pandit MK, Banday SA. SARS n-CoV2-19 detection from chest x-ray images using deep neural networks. *Int J Pervasive Comput Commun*. (2020) 16:1–9. doi: 10.1108/IJPCC-06-2020-0060
91. Pereira RM, Bertolini D, Teixeira LO, Silla CN, Jr., Costa YMG. COVID-19 identification in chest X-ray images on flat and hierarchical classification scenarios. *Comput Methods Programs Biomed*. (2020) 194:105532. doi: 10.1016/j.cmpb.2020.105532
92. Rahaman MM, Li C, Yao Y, Kulwa F, Rahman MA, Wang Q, et al. Identification of COVID-19 samples from chest X-Ray images using deep learning: a comparison of transfer learning approaches. *J Xray Sci Technol*. (2020) 28:1–19. doi: 10.3233/XST-200715
93. Rahimzadeh M, Attar A. A modified deep convolutional neural network for detecting COVID-19 and pneumonia from chest X-ray images based on the concatenation of Xception and ResNet50V2. *Inform Med Unlocked*. (2020) 19:100360. doi: 10.1016/j.imu.2020.100360
94. Sethy PK, Behera SK, Ratha PK, Biswas P. Detection of coronavirus disease (COVID-19) based on deep features and support vector machine. *Int J Math Eng Manage Sci*. (2020) 5:643–51. doi: 10.33889/IJMEMS.2020.5.4.052
95. Shibly KH, Dey SK, Islam MT, Rahman MM. COVID faster R-CNN: a novel framework to diagnose novel coronavirus disease (COVID-19) in X-ray images. *Inform Med Unlocked*. (2020) 20:100405. doi: 10.1016/j.imu.2020.100405
96. Togaçar M, Ergen B, Cömert Z. COVID-19 detection using deep learning models to exploit social mimic optimization and structured chest X-ray images using fuzzy color and stacking approaches. *Comput Biol Med*. (2020) 121:103805. doi: 10.1016/j.compbiomed.2020.103805
97. Toraman S, Alakus TB, Turkoglu I. Convolutional capsnet: a novel artificial neural network approach to detect COVID-19 disease from X-ray images using capsule networks. *Chaos Solitons Fractals*. (2020) 140:110122. doi: 10.1016/j.chaos.2020.110122
98. Tsiknakis N, Trivizakis E, Vassalou EE, Papadakis GZ, Spandidos DA, Tsatsakis A, et al. Interpretable artificial intelligence framework for COVID-19 screening on chest X-rays. *Exp Ther Med*. (2020) 20:727–35. doi: 10.3892/etm.2020.8797
99. Tuncer T, Dogan S, Ozyurt F. An automated residual exemplar local binary pattern and iterative ReliefF based COVID-19 detection method using chest X-ray image. *Chemometr Intell Lab Syst*. (2020) 203:104054. doi: 10.1016/j.chemolab.2020.104054
100. Ucar F, Korkmaz D. COVIDiagnosis-Net: deep Bayes-SqueezeNet based diagnosis of the coronavirus disease 2019 (COVID-19) from X-ray images. *Med Hypotheses*. (2020) 140:109761. doi: 10.1016/j.mehy.2020.109761
101. Vaid S, Kalantar R, Bhandari M. Deep learning COVID-19 detection bias: accuracy through artificial intelligence. *Int Orthop*. (2020) 44:1539–42. doi: 10.1007/s00264-020-04609-7
102. Waheed A, Goyal M, Gupta D, Khanna A, Al-Turjman F, Pinheiro PR. CovidGAN: data augmentation using auxiliary classifier GAN for improved Covid-19 detection. *IEEE Access*. (2020) 8:91916–23. doi: 10.1109/ACCESS.2020.2994762
103. Yildirim M, Cinar A. A deep learning based hybrid approach for covid-19 disease detections. *Traitement Signal*. (2020) 37:461–8. doi: 10.18280/ts.370313
104. Yoo SH, Geng H, Chiu TL, Yu SK, Cho DC, Heo J, et al. Deep learning-based decision-tree classifier for COVID-19 diagnosis from chest X-ray imaging. *Front Med*. (2020) 7:427. doi: 10.3389/fmed.2020.00427
105. Ghoshal B, Tucker A. Estimating uncertainty and interpretability in deep learning for coronavirus (COVID-19) detection. *arXiv*. (2020) Preprint arXiv:200310769.
106. Hall LO, Paul R, Goldgof DB, Goldgof GM. Finding covid-19 from chest x-rays using deep learning on a small dataset. *arXiv*. (2020) 40:1–14. doi: 10.36227/techrxiv.12083964
107. Hammoudi K, Benhabiles H, Melkemi M, Dornaika F, Arganda-Carreras I, Collard D, et al. Deep learning on chest X-ray images to detect and evaluate pneumonia cases at the Era of COVID-19. *arXiv*. (2020) Preprint arXiv:200403399.
108. Hemdan EE-D, Shouman MA, Karar ME. Covidx-net: a framework of deep learning classifiers to diagnose covid-19 in x-ray images. *arXiv*. (2020) Preprint arXiv:200311055.
109. Jain G, Mittal D, Thakur D, Mittal MK. A deep learning approach to detect Covid-19 coronavirus with X-Ray images. *Biocybernet Biomed Eng*. (2020). doi: 10.1016/j.bbe.2020.08.008
110. Luz E, Silva PL, Silva R, Moreira G. Towards an efficient deep learning model for covid-19 patterns detection in x-ray images. *arXiv*. (2020) 31:1–10.
111. Ozturk S, Ozkaya U, Barstugan M. Classification of coronavirus images using shrunken features. *medRxiv*. (2020). doi: 10.1101/2020.04.03.20048868
112. Zhang J, Xie Y, Li Y, Shen C, Xia Y. Covid-19 screening on chest x-ray images using deep learning based anomaly detection. *arXiv*. (2020) Preprint arXiv:200312338.
113. Ravishankar H, Sudhakar P, Venkataramani R, Thiruvenkadam S, Annangi P, Babu N, et al. Understanding the mechanisms of deep transfer learning for medical images. In: *Deep Learning and Data Labeling for Medical Applications*: Springer (2016). p. 188–96.

114. Hosny KM, Kassem MA, Foaud MM. Classification of skin lesions using transfer learning and augmentation with Alex-net. *PLoS ONE*. (2019) 14:e0217293. doi: 10.1371/journal.pone.0217293
115. Khan S, Islam N, Jan Z, Din IU, Rodrigues JJC. A novel deep learning based framework for the detection and classification of breast cancer using transfer learning. *Pattern Recogn Lett*. (2019) 125:1–6. doi: 10.1016/j.patrec.2019.03.022
116. Singh A, Sengupta S, Lakshminarayanan V. Explainable deep learning models in medical image analysis. *J Imaging*. (2020) 6:52. doi: 10.3390/jimaging6060052

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Implementation and Evaluation of Virtual Anticoagulation Clinic Care to Provide Incessant Care During COVID-19 Times in an Indian Tertiary Care Teaching Hospital

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Background: COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-II) has become a global pandemic disrupting public health services. Telemedicine has emerged as an important tool to deliver care during these situations. Patients receiving Vitamin K antagonists (VKA) require structured monitoring which has posed a challenge during this pandemic. We aimed to evaluate the impact of Virtual anticoagulation clinic (VAC), a Telehealth model on the quality of anticoagulation, adverse events, and patient satisfaction vis-a-vis standard Anticoagulation clinic (ACC) care.

Materials and methods: A bidirectional cohort study was conducted in the Department of Cardiology, JSS Hospital, Mysore. Two hundred and twenty-eight patients in the VAC and 274 patients in the ACC fulfilling inclusion criteria were the subjects of the study. Telehealth tools like WhatsApp and telephone were used. Time in therapeutic range (TTR), Percentage of International normalized ratio in range (PINRR), and adverse events were analyzed and compared between the VAC group and the ACC group, between pre-COVID and COVID ACC groups, and between the VAC group and the same pre-COVID cohort. Patient satisfaction was assessed by a questionnaire at the end of 8 months. Descriptive statistics were used for the patient characteristics and inferential statistics for the comparisons between pre-VAC and VAC care.

Results: The mean TTR was 75.4 ± 8.9% and 71.2 ± 13.4% in the VAC group and ACC group, respectively ($p < 0.001$). The mean PINRR was 66.7 ± 9.4% and 62.4 ± 10.9% in the VAC group and ACC group respectively, ($p < 0.001$). There was no significant difference in TTR between the VAC group and the same pre-COVID cohort. The TTR differential between the pre-COVID and COVID ACC groups was significant. In either group, no major adverse events were seen. The most common tools used for data exchange were WhatsApp (83%) and SMS (17%). Seventy-four percent of patients were extremely satisfied with the overall VAC care.

Conclusions: Virtual anticoagulation clinic, a telehealth model can be used as an alternative option to deliver uninterrupted anticoagulation care during pandemic times.

Keywords: anticoagulation clinic, vitamin K antagonist, time in therapeutic range, percentage of international normalized ratio in range, telehealth

INTRODUCTION

COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-II) has become a global pandemic disrupting public health services (1). In these time frames, effective clinical care for patients with various chronic cardiovascular and other disorders has gained considerable attention from various stakeholders (2). In this predicament, Telehealth a virtual platform for the care provider and seeker has great potential in providing cardiovascular care which is evidently quite ideal (3). Its utility for patients on oral anticoagulants is one domain that needs to be addressed. Of the anticoagulants, vitamin K antagonists (VKAs) have a narrow therapeutic index with variable dose-response and diet/drug interactions (4). Patients taking VKAs require International normalized ratio (INR) monitoring and dose titration to achieve therapeutic INR for optimal outcomes (5). Patients taking VKAs may have multiple comorbidities like advanced age, hypertension, diabetes mellitus, and others. Studies have shown that patients with these risk factors are susceptible to severe COVID-19 infection necessitating a strategy to mitigate exposure of such patients (6, 7).

Telehealth services help to provide patients with the necessary care while minimizing the risk of transmitting SARS-CoV-II to healthcare workers and patients (8). The notion of telemedicine was incorporated in the Anticoagulation clinic to provide uninterrupted virtual care to patients taking VKAs. This study was conducted to evaluate the impact of Virtual anticoagulation clinic care (VAC) on the quality of anticoagulation, adverse events, and patient satisfaction vis-a-vis standard ACC care.

MATERIALS AND METHODS

Study Design and Participants

A bidirectional observational cohort study was conducted on patients enrolled in the VAC and ACC at the Department of Cardiology, JSS Hospital, Mysore from March to November 2020. Institutional ethical committee approval was taken. A total of 521 patients were registered in ACC till March 2020. Among these, 234 patients opted for VAC care and 287 patients opted for ACC care. For calculation of TTR, patients who had more than 3 months of ACC care before March 2020 with at least 3 INR values in both groups were included in the study. Newly enrolled patients in the ACC and those patients who had less than 3 months of ACC care before March 2020 were excluded from the study. A total of 228 patients in the VAC care group and 274 patients in the standard ACC care group were eligible for analysis. The patient enrolment process is depicted in **Figure 1**.

Anticoagulation Quality Assessment Tools

The anticoagulation related quality measures like Percentage Time in Therapeutic Range (%TTR) (9), Percentage of INR within Range (PINRR) (10), extreme INRs, and adverse events were analyzed. Patient satisfaction toward VAC care was assessed by administering five items self-developed questionnaire with scores 0 to 4 from extremely satisfied to not at all satisfied at the end of 8 months.

Anticoagulation Clinic (ACC)

JSS Hospital, Mysore has an established ACC since February 2017 comprising a multidisciplinary team comprising a Senior cardiologist, Junior cardiologist, Clinical Pharmacist, Clinical Pharmacy interns, and trained nursing staff. Key issues such as patient education (VKA risks/benefits, potential diet/drug interactions), ordering relevant laboratory tests (once a month INR testing), titrating the dose of VKAs to meet the INR target, facilitating procedures requiring interruption of VKAs, and adverse effects associated with VKAs were addressed.

Virtual Anticoagulation Clinic (VAC)

VAC was initiated in March 2020 to provide sustained care to patients taking VKAs registered in ACC during the COVID-19 pandemic. Telehealth tools like WhatsApp and telephone were used as per Telemedicine practice guidelines (11). WhatsApp and SMS were used for the asynchronous exchange of the data. Patients were supposed to undergo INR testing once a month and communicate the INR report and if any symptoms related to bleeding, Transient Ischemic Attack (TIA), or stroke by any of the tools quoting their ACC identification number. Based on the INR value, dose titration was done and advice regarding the next INR testing was given. Patients with INR <1.5 and INR >5.0, major bleeding, and systemic embolic events were advised for the hospital visit. TTR and PINRR were calculated by Rosendaal linear interpolation technique for each patient. Calculations were performed with the assistance of a template made available by INR Pro (12). Major bleeding was defined by the International Society on Thrombosis and Haemostasis criteria (13). Stroke/Systemic embolic events were defined as the combined endpoints of ischaemic stroke, TIA, and systemic embolic events.

Statistical Analysis

Data was entered in MS Office Excel 2019 and analyzed by using IBM SPSS Statistics Version 25. Continuous variables were expressed as mean $\pm$ standard deviation (SD). Categorical variables were expressed as absolute numbers and percentages. Descriptive statistics were

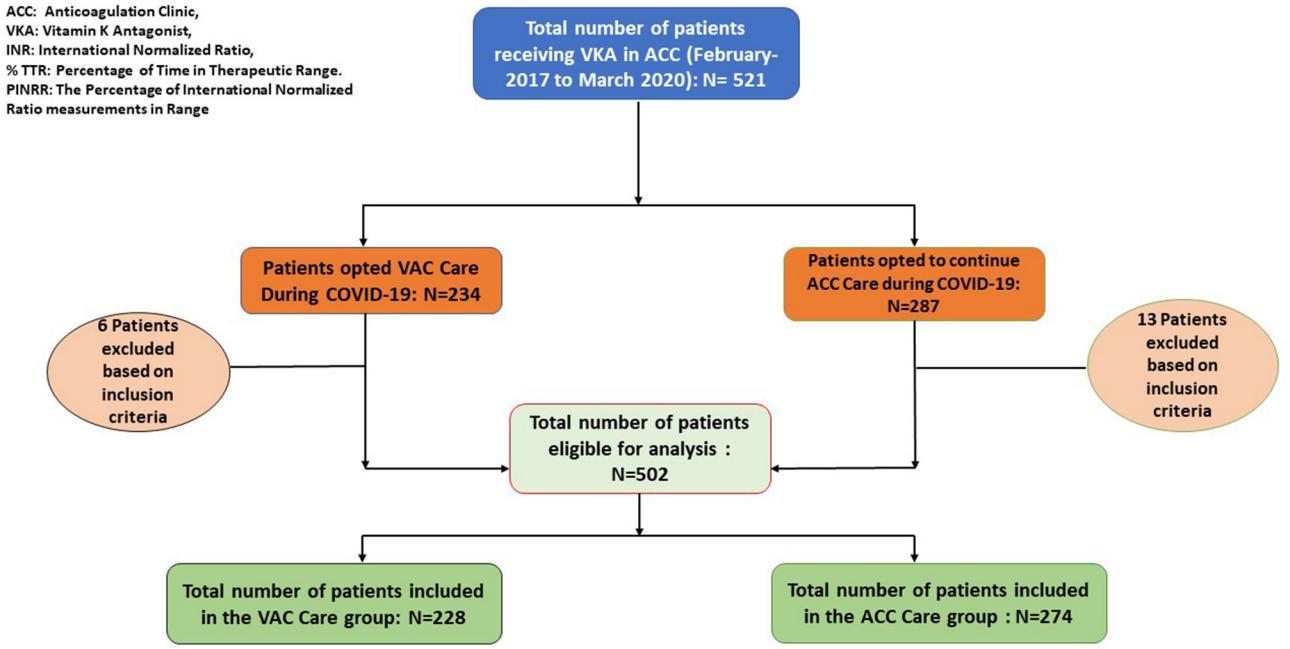

FIGURE 1 | Participant enrolment process. ACC, Anticoagulation clinic; VKA, Vitamin K Antagonist; INR, International Normalized Ratio; % TTR, Percentage of Time in Therapeutic Range; PINRR, The percentage of International Normalized Ratio measurements in Range. * EXCLUSION CRITERIA: Patients having less than 3 months of ACC exposure pre COVID-19 phase and less than 3 INR values were excluded from the study.

used for patient characteristics. *T*-test and chi-square tests (χ^2) were used for comparisons between groups. All tests were two-tailed, $p < 0.05$ was considered to be statistically significant.

RESULTS

The mean age of the patients in the VAC group and ACC group was 55.62 ± 13.77 years and 53.72 ± 11.8 years, respectively. The majority of the patients in the VAC group were from rural areas (57%). On the contrary, only 30% of the patients were from rural areas in the ACC group. Patients characteristics are depicted in **Table 1**. Atrial fibrillation was the most common indication for VKA therapy in both groups. Acenocoumarol was the most common VKA prescribed. Mean TTR in VAC group and ACC group was 75.4 ± 8.9% and 71.2 ± 13.4%, respectively (p-value = 0.001). Mean PINRR in the VAC group and ACC group was 66.7 ± 9.4% and 62.4 ± 10.9%, respectively (p-value = 0.0002). Patients in the VAC group underwent more frequent INR testing when compared to those in the ACC group. Two patients had a minor lower gastrointestinal bleed in the VAC group. None of the patients had major adverse events in either group. Three patients were scheduled for an in-person visit in the VAC group. Anticoagulation related parameters in the VAC group and ACC group are depicted in **Table 2**. There was no significant difference in TTR between the VAC group and the same group during pre-COVID ACC care. There was a significant difference in TTR and PINRR between the pre-COVID and COVID-ACC groups (p <0.0001). The number of INR tests performed per patient was less in the ACC group during the COVID pandemic. Anticoagulation related parameters between the groups are depicted in **Table 3**.

WhatsApp 189 (83%), followed by SMS 39 (17%) were the most common tools used for the exchange of data. One hundred and sixty-nine (74%) of patients were extremely satisfied with overall VAC care and 187 (82%) of patients were extremely satisfied to continue virtual care as assessed by a 5-item questionnaire. The patient satisfaction score and questionnaire are depicted in **Figure 2** and **Table 4**.

DISCUSSION

In our study, the principal findings were (1) Patients in the VAC group had greater control of anticoagulation in the form of more time spent in the therapeutic range compared to ACC during the COVID pandemic (75.4 and 71.2%, respectively). (2) There was no significant difference in TTR between the VAC group and the same patients in the Pre-COVID ACC care 3). There was a significant difference in TTR between the pre-COVID and COVID ACC groups.

TABLE 1 | Patient characteristics and anticoagulation related parameters.

Variables	VAC ($N = 228$)	ACC ($N = 274$)	p-value
Age (years)			
<60	118 (51.6)	156 (57.07)	0.9077
>61	110 (48.4)	118 (42.93)	0.2208
Gender			
Men	129 (57)	167 (60.84)	0.3841
Women	99 (43)	107 (39.16)	
Comorbidities			
Type 2 Diabetes Mellitus	53 (23.4)	89 (32.54)	0.0239
Hypertension	73 (32)	90 (33.01)	0.8102
Congestive heart failure	18 (7.8)	36.1 (13.20)	0.0520
Vascular disease[#]	29 (12.5)	31 (11.32)	0.6842
Educational status			
Literate	162 (71.1)	247 (90.09)	<0.0001[††]
Illiterate	66 (28.9)	27 (9.9)	<0.0001[††]
Location of residence			
Urban	98 (43)	194 (70.82)	<0.0001[††]
Rural	130 (57)	80 (29.18)	<0.0001[††]
HASBLED score			
≥3	69 (30.4)	57 (20.75)	0.0132[††]
<3	158 (69.5)	217 (79.25)	0.0123[††]
Vitamin K Antagonist			
Warfarin	16 (7)	6 (2.36)	0.0121[††]
Acenocoumarol	212 (93)	268 (97.64)	0.0123[††]
Indications for VKA*			
Atrial fibrillation	137 (60)	192 (70.28)	0.0159[††]
Mechanical Valve replacement	8 (3.4)	44 (16.03)	<0.0001[††]
Deep vein thrombosis / Pulmonary embolism	80 (35.1)	38 (13.69)	<0.0001[††]
Cortical venous thrombosis	3 (1.5)	0	–

*[#]Vascular disease: Coronary artery disease, Peripheral arterial disease; *VKA: Vitamin K antagonist.*
[††] statistically significant p-value has been obtained by performing chi-squared test.

TABLE 2 | Anticoagulation related Quality Parameters.

Variables	VAC ($N = 228$)	ACC ($N = 274$)	p-value
Number of INR[†] draws (1,544)	1,324	1,019	-
Average number of INR[†] draws/ Patient	5.8	3.72	-
Mean TTR%[††]	75.4 ± 8.91	71.2 ± 13.4	0.0018[†]
Mean PINRR %**	66.7 ± 9.4 %	62.4 ± 10.9%	0.0002[†]
Tests Over Range	129 (9.7%)	113 (11.11%)	0.2660
Tests Below range	151 (11.7%)	142 (13.9%)	0.1124
Extreme INRs			
INR >5.0	14 (1.06)	15 (1.51)	0.3323
INR < 1.5	30 (2.26)	75 (7.32)	<0.0001
Adverse events			
Major	0 (0%)	0	-
Minor bleeding	2 (0.8%)	0	

*[†]INR: International Normalized Ratio; **PINRR: Percentage of International Normalized Ratio in the Therapeutic Range; [††]TTR: Time in Therapeutic Range.*
[††] statistically significant p-value has been obtained by performing chi-squared test.
[†] statistically significant p-value has been obtained by performing t-test.

Due to the COVID pandemic, healthcare was inaccessible to the majority of the patients. Telehealth-based VAC initiated during that period could deliver uninterrupted care to the patients on chronic VKA therapy. Patients in the virtual care group could maintain their mean TTR similar to that of ACC care during the pre-COVID state. Wherein patients in the ACC care group were unable to maintain the mean TTR because of less frequent INR testing and in-person visits. Similar telehealth-based studies conducted on patients with chronic warfarin therapy have reported mean TTRs ranging from 66 to 74% (14–16).

Several meta-analyses of randomized and real-world trials have found that TTRs and PINRRs are generally equal to or below 60% (10, 17, 18). The European consensus document recommends a TTR of >70% for optimal outcomes (19). NICE guidelines recommend a TTR of > 65% for patients with AF on VKA therapy (20). In our study, achieved TTRs in both groups were above the proposed benchmark of >65–70%. One of the main reasons to achieve mean TTR > 70% in our study was because our cohort of patients were those registered in the ACC managed by a multidisciplinary team. Even randomized controlled trials and studies related to Anticoagulation clinics have documented better control of INR compared to community settings that were possible due to frequent monitoring, organized care, and improvement in adherence to VKAs (10, 17).

Other important and desirable points to note were that these patients had multiple comorbidities and could be treated with the reduced risk of exposure to COVID-19 infection during transit to the hospital, cost savings for travel, and no major adverse events. The majority of the patients were satisfied with overall virtual care and opted for virtual care even in the post-COVID state.

The tenable reasons for the patients to continue to benefit from following up in VAC are several. Patients were educated during their initial visits to the regular anticoagulation clinic about the importance of regular follow-up with PT/INR testing, risks of discontinuation, clinical benefits of continuous and uninterrupted use of VKAs. Also, the ease of contacting the care provider through dedicated service like a 24/7 contactable phone number could have helped the patients. Prior consultation on a one-to-one basis with the care provider may also have increased the confidence as it is reflected in the data on the satisfactory questionnaire. In our study, the majority of the patients (74%) were satisfied with overall virtual care. Eighty-two percent of the patients were extremely satisfied in continuing virtual care even in the post-COVID scenario.

In our study, 57% of the patients who availed virtual care were from rural areas. WhatsApp was the most common chat platform used. A recent study by the Internet & Mobile Association of India (IAMAI) and

research by Neilsen, reported that there are 227 million active internet users in rural areas in India as of November 2019 (21). This digital penetration can transform the delivery of virtual care to patients with chronic diseases in remote locations.

Preferably, patients who require VKAs, must visit in person initially and ideally should achieve at least two consecutive INRs in the therapeutic range before they could be transitioned to virtual anticoagulation clinic care for optimal patient-centered outcomes.

This pilot study has paved a path of utilizing telehealth to manage patients on chronic VKA therapy during the COVID pandemic. Though short-term results are promising, more extensive and larger multi-centric studies with a longer duration of follow-up are required to assess the feasibility and efficacy of the virtual anticoagulation clinic.

TABLE 3 | Assessment of Anticoagulation parameters among Pre-VAC (pre COVID) and VAC care.

Anticoagulation parameters	VAC care ($n = 228$)	Pre-COVID care ($n = 228$)	*p*-value	ACC COVID-19 care ($n = 274$)	ACC pre-COVID care ($n = 274$)	*p*-value
Number of INR† draws	1,324	1,467		1,019	1,551	
Average number of INR† draws/ Patient	5.8	6.43	-	3.72	5.66	-
Mean TTR%*	75.4 ± 8.91	77.58 ± 8.85	0.0506#	71.2 ± 13.4	79.12 ± 9.3	<0.0001#
Mean PINRR %**	66.7 ± 9.4 %	69.68 ± 11.50	0.0241#	62.4 ± 10.9%	67.8 ± 10.4	<0.0001#
Tests Over Range	129 (9.7)	118 (8.1)	0.1375	113 (11.11)	96 (6.2)	<0.0001††
Tests Below range	151 (11.7)	106 (7.26)	0.0001††	142 (13.9)	129 (8.3)	<0.0001††
Extreme INRs						
INR >5.0	14 (1.06)	0	-	15 (1.51)	10 (0.66)	0.0339††
INR < 1.5	30 (2.26)	11 (0.75)	0.0009††	75 (7.32)	8 (0.5)	< 0.0001††
Adverse events						
Major	0 (0%)	0	-	0	0	-
Minor bleeding	2 (0.8%)	0		0	0	

† *INR: International Normalized Ratio; **PINRR: Percentage of International Normalized Ratio in the Therapeutic Range *TTR: Time in Therapeutic Range.*
†† *statistically significant p-value has been obtained by performing chi-squared test.*
# *statistically significant p-value has been obtained by performing t-test.*

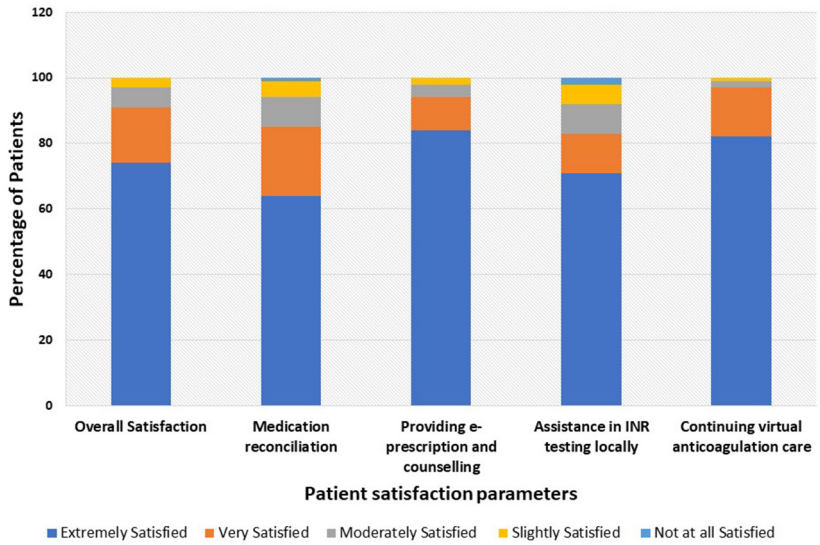

FIGURE 2 | Patient satisfaction toward VAC care during COVID-19.

TABLE 4 | Patient satisfaction toward virtual anticoagulation care (VAC) during COVID-19 pandemic ($N = 228$).

S. No	Parameter*		Response** *n* (%)
1.	Overall satisfaction of patients on VAC care during COVID 19	Extremely satisfied (4)	168 (74)
		Very satisfied (3)	39 (17)
		Moderately satisfied (2)	14 (6)
		Slightly satisfied (1)	7 (3)
		Not at all satisfied (0)	(0)
2.	Medication reconciliation	Extremely satisfied (4)	146 (64)
		Very satisfied (3)	48 (21)
		Moderately satisfied (2)	20 (9)
		Slightly satisfied (1)	12 (5)
		Not at all satisfied (0)	2 (1)
3.	Providing e-prescription and education reinforcement (counseling)	Extremely satisfied (4)	192 (84)
		Very satisfied (3)	23 (10)
		Moderately satisfied (2)	8 (4)
		Slightly satisfied (1)	5 (2)
		Not at all satisfied (0)	(0)
4.	Assistance in INR monitoring locally despite lockdown during COVID 19	Extremely satisfied (4)	162 (71)
		Very satisfied (3)	27 (12)
		Moderately satisfied (2)	20 (9)
		Slightly satisfied (1)	14 (6)
		Not at all satisfied (0)	5 (2)
5.	Continuing virtual anticoagulation care	Extremely satisfied (4)	187 (82)
		Very satisfied (3)	34 (15)
		Moderately satisfied (2)	5 (2)
		Slightly satisfied (1)	2 (1)
		Not at all satisfied (0)	0

**Feedbacks for Q1 – Q7 were obtained through a 5-point Likert scale with scoring 0 – 4, 0 = Not at all Satisfied, 1 = Slightly Satisfied, 2 = Moderately Satisfied, 3 = Very Satisfied, 4 = Extremely Satisfied. **Data represented as frequency and proportion.*

STRENGTHS AND LIMITATIONS

The virtual anticoagulation clinic, a telehealth model that was developed during the onset of the COVID-19 pandemic to facilitate uninterrupted anticoagulation care, which could help maintain the quality of anticoagulation and minimize the risk of exposure to COVID-19. Our study has limitations such as single-center, lack of randomization, small patient population, and shorter duration of follow-up.

CONCLUSIONS

This preliminary study showed that a virtual anticoagulation clinic can serve as a feasible alternate care model to provide uninterrupted anticoagulation care for patients on chronic Vitamin K antagonist therapy during 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/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by JSS Medical College and Hospital, JSS AHER. 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. SKS, SPSB, and OJG designed and formulated the hypothesis. RV and OJG performed data collection. SKS and OJG prepared manuscript. ND and RM reviewed the manuscript. MB, OJG, and SKS performed statistical planning and analysis. All the authors approved the manuscript for publication.

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REFERENCES

1. Castagnoli R, Votto M, Licari A, Brambilla I, Bruno R, Perlini S, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-II) infection in children and adolescents: a systematic review. *JAMA Pediatr*. (2020) 174:882–9. doi: 10.1001/jamapediatrics.2020.1467
2. 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
3. Cutler DM, Nikpay S, Huckman RS. The business of medicine in the era of COVID-19. *JAMA*. (2020) 323:2003–4. doi: 10.1001/jama.2020.7242
4. Vranckx P, Valgimigli M, Heidbuchel H. The Significance of drug—Drug and drug—Food interactions of oral anticoagulation. *Arrhythmia Electrophysiol Rev*. (2018) 7:55. doi: 10.15420/aer.2017.50.1
5. Witt DM, Clark NP, Kaatz S, Schnurr T, Ansell JE. Guidance for the practical management of warfarin therapy in the treatment of venous thromboembolism. *J Thrombosis Thrombolysis*. (2016) 41:187–205. doi: 10.1007/s11239-015-1319-y
6. Shoeb M, Fang MC. Assessing bleeding risk in patients taking anticoagulants. *J Thrombosis Thrombolysis*. (2013) 35:312–9. doi: 10.1007/s11239-013-0899-7
7. 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 Respiratory J*. (2020) 55:2000547. doi: 10.1183/13993003.00547-2020
8. Bhaskar S, Bradley S, Chattu VK, Adisesh A, Nurtazina A, Kyrykbayeva S, et al. Telemedicine as the new outpatient clinic gone digital: position paper from the pandemic health system REsilience PROGRAM (REPROGRAM) international consortium (Part 2). *Front Public Health*. (2020) 8:410. doi: 10.3389/fpubh.2020.00410

9. Schmitt L, Speckman J, Ansell J. Quality assessment of anticoagulation dose management: comparative evaluation of measures of time-in-therapeutic range. *J Thrombosis Thrombolysis*. (2003) 15:213–6. doi: 10.1023/B:THRO.0000011377.78585.63
10. Mearns ES, White CM, Kohn CG, Hawthorne J, Song JS, Meng J, et al. Quality of vitamin K antagonist control and outcomes in atrial fibrillation patients: a meta-analysis and meta-regression. *Thrombosis J*. (2014) 12:1–20. doi: 10.1186/1477-9560-12-14
11. Telemedicine Practice Guidelines. *Enabling Registered Medical Practitioners to Provide Healthcare Using Telemedicine*. Available online at: https://www.mohfw.gov.in/pdf/Telemedicine.pdf (accessed July 1, 2020).
12. INR Pro. *Rosendaal method for % INR in range*. INR Pro. Available online at: www.inrpro.com/rosendaal.asp (accessed July 8, 2020).
13. Schulman S, Kearon C, Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. *J Thrombosis Haemostasis*. (2005) 3:692–4. doi: 10.1111/j.1538-7836.2005.01204.x
14. Kelly JJ, Sweigard KW, Shields K, Schneider D. Safety, effectiveness, and efficiency: a Web-based virtual anticoagulation clinic. *Joint Commission J Quality Safety*. (2003) 29:646–51. doi: 10.1016/S1549-3741(03)29076-6
15. Chan FW, Wong RS, Lau WH, Chan TY, Cheng G, You JH. Management of Chinese patients on warfarin therapy in two models of anticoagulation service–a prospective randomized trial. *Br J Clin Pharmacol*. (2006) 62:601–9. doi: 10.1111/j.1365-2125.2006.02693.x
16. Ryan F, Byrne S, O'shea S. Randomized controlled trial of supervised patient self-testing of warfarin therapy using an internet-based expert system. *J Thrombosis Haemostasis*. (2009) 7:1284–90. doi: 10.1111/j.1538-7836.2009.03497.x
17. Haas S, Ten Cate H, Accetta G, Angchaisuksiri P, Bassand JP, Camm AJ, et al. Quality of vitamin K antagonist control and 1-year outcomes in patients with atrial fibrillation: a global perspective from the GARFIELD-AF registry. *PLoS ONE*. (2016) 11:e0164076. doi: 10.1371/journal.pone.0164076
18. Erkens PM, ten Cate H, Büller HR, Prins MH. Benchmark for time in therapeutic range in venous thromboembolism: a systematic review and meta-analysis. *PLoS ONE*. (2012) 7:e42269. doi: 10.1371/journal.pone.0042269
19. Camm AJ, Lip GY, De Caterina R, Savelieva I, Atar D, Hohnloser SH, et al. 2012 focused update of the ESC Guidelines for the management of atrial fibrillation: an update of the 2010 ESC Guidelines for the management of atrial fibrillation Developed with the special contribution of the European Heart Rhythm Association. *Eur Heart J*. (2012) 33:2719–47. doi: 10.1093/eurheartj/ehs253
20. National Clinical Guideline Centre (UK). *Atrial Fibrillation: The Management of Atrial Fibrillation*. London: National Institute for Health and Care Excellence (UK) (2014).
21. The Logical Indian. *Internet Usage In Rural India Surpasses Urban Areas For The First Time: Report*. Avaialble online at: https://thelogicalindian.com/news/internet-usage-rural-urban-india-20946 (accessed July 17, 2020).

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ORIGINAL RESEARCH
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Outcomes of Patients With ST-Segment Elevation Myocardial Infarction Admitted During COVID-19 Pandemic Lockdown in Germany – Results of a Single Center Prospective Cohort Study

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Objective: Since the outbreak of the COVID-19 pandemic, healthcare professionals reported declining numbers of patients admitted with ST-segment myocardial infarction (STEMI) associated with increased in-hospital morbidity and mortality. However, the effect of lockdown on outcomes of STEMI patients admitted during the COVID-19 crisis has not been prospectively evaluated.

Methods: A prospective, observational study on STEMI patients admitted to our tertiary care center during the COVID-19 pandemic was conducted. Outcomes of patients admitted during lockdown were compared to those patients admitted before and after pandemic-related lockdown.

Results: A total of 147 patients were enrolled in our study, including 57 patients in the pre-lockdown group (November 1, 2019 to March 20, 2020), 16 patients in the lockdown group (March 21 to April 19, 2020), and 74 patients in the post-lockdown group (April 20 to September 30, 2020). Patients admitted during lockdown had significantly longer time to first medical contact, longer door-to-needle-time, higher serum troponin T levels, worse left ventricular end-diastolic pressure, and higher need for circulatory support. After a median follow-up of 142 days, survival was significantly worse in STEMI patients of the lockdown group (log-rank: $p = 0.0035$).

Conclusions: This is the first prospective study on outcomes of STEMI patients admitted during public lockdown amid the COVID-19 pandemic. Our results suggest that lockdown might deteriorate outcomes of STEMI patients. Public health strategies to constrain spread of COVID-19, such as lockdown, have to be accompanied by distinct public instructions to ensure timely medical care in acute diseases such as STEMI.

Keywords: COVID-19, STEMI, myocardial infarction, lockdown, outcome, epidemiology, Germany

INTRODUCTION

Soon after the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread globally, physicians warned about potential side effects of the COVID-19 pandemic compromising medical care (1, 2). It has been suggested that the pandemic keeps patients from seeking and receiving needed medical attention despite suffering from physical symptoms. Social containment measures (i.e., lockdown and stay-at-home orders), stress, and fear of COVID-19 may influence an individual's health behavior (3–5). Patients with ST-segment elevation myocardial infarction (STEMI) are an especially vulnerable population, as total ischemic time severely influences their outcome (6). There have been several reports on diminishing numbers of STEMI admissions during the outbreak in both epicenters and non-epicenters of the COVID-19 pandemic. This has been associated with significantly prolonged times from symptom onset to first medical contact (FMC) and increased in-hospital morbidity and mortality (7–11). However, the reasons underlying this phenomenon have rarely been assessed. The influence of factors such as lockdown, stress, and fear of COVID-19 on the patient's long-term outcome, has not yet been evaluated.

METHODS

Study Design and Study Population

In this prospective, observational cohort study, we aimed for inclusion of all patients with STEMI admitted between March 21, 2020 and September 30, 2020. STEMI patients admitted between November 1, 2019 and March 20, 2020 were enrolled retrospectively.

Patients had to be ≥18 years old and give written informed consent to be eligible for inclusion. Diagnosis of STEMI was made according to contemporary guidelines and all STEMI patients underwent cardiac catheterization and subsequent percutaneous coronary intervention (PCI) immediately after admission as indicated by current recommendations (6). During the COVID-19 pandemic, all patients were treated with personal protection gear in the case of an unknown COVID-19 status. The study complies with the Declaration of Helsinki and was approved by the local ethics committee (number of application and positive vote 250/20). This study adheres to the STROBE statement (12).

Data Collection

Demographic, clinical, laboratory, interventional, and in-hospital outcome data were extracted from our patient management system by two medical practitioners (CW and LS) and adjudicated by a third one (MR) in case of any kind of difference. Left ventricular systolic function at admissions was measured by cardiac ventriculography during cardiac catheterization and categorized as normal, mildly impaired, moderately impaired, or severely impaired, according to the expertise of the attending physician. Left ventricular systolic function at follow-up was assessed by automated echocardiographic quantification (outpatient visit: EPIQ 7, Koninklijke Philips N.V., Eindhoven, Netherlands; home visit: Butterfly IQ, Butterfly Network. Inc., Guilford, CT, USA).

Clinical Follow-Up

Patients were scheduled for outpatient clinic visits (clinical assessment, 12-lead ECG, and echocardiography) after 1 month, 3 months and, then, at least every 3 months after discharge. If, for any reason, an outpatient clinic visit could not be realized, a home visit was offered to the patient.

Laboratory Measurements

Blood samples were drawn at the time of hospital admission or at the outpatient clinic visits for measurements of high sensitivity cardiac troponin T (hsTnT), NT-pro BNP, and creatinine (ElectroChemiLumineszenz ImmunoAssay "ECLIA" Roche, Cobas 8000, Modul e801 and e601) as part of the clinical routine. In addition, every patient was tested for SARS- CoV-2 by throat swab test at admissions (Sigma-Virocult® with 2 ml Virocult® medium, Check Diagnostics GmbH, Germany) and analyzed by RT-PCR at the local Institute for Virology.

Assessment of the Effect of Lockdown on STEMI Patients

Measures of social restriction in Germany came into effect on March 21, 2020 and public reopening was partly initiated on April 20, 2020. Consequently, patients admitted between November 1, 2019 and March 20, 2020 were classified as the "pre-lockdown" (pre-COVID-19) group, patients admitted between March 21 and April 19, 2020 were assigned to the "lockdown" group, and patients admitted between April 20 and September 30, 2020 to the "post-lockdown" group. Comparisons were made on patient characteristics, clinical data, and outcomes of patients of the lockdown group and the combined pre-/post-lockdown group. Outcomes were heart failure symptoms as measured by NYHA class, serum levels of cardiac biomarkers, left ventricular ejection fraction, and survival. Additionally, baseline characteristics, laboratory parameters, in-hospital clinical characteristics and time to FMC were assessed and compared between the groups.

Assessment of the Effect of Stress and Fear of COVID-19 on STEMI Patients Admitted During the COVID-19 Pandemic

To assess the level of stress and fear of COVID-19 at baseline in STEMI patients admitted during the pandemic, we utilized well-established questionnaires. The COVID Stress Scales (CSS) were used to assess COVID-19 related distress and the Fear of COVID-19 Scale (FCV-19S) was implemented to measure COVID-19 related fear (13, 14).

Statistical Analysis

Continuous variables were described as mean ± standard deviation or median together with interquartile range (IQR), as appropriate. Categorical variables were described as absolute and relative frequencies, respectively. Group comparison (lockdown vs. pre-/post-lockdown combined) of continuous variables was performed by two-sample *t*-test or Wilcoxon rank sum test

TABLE 1 | Patient characteristics at baseline.

	Total $n = 147$	Lockdown $n = 16$	Pre-/Post-Lockdown $n = 131$	p-value
Age	64 ± 13	69 ± 12	64 ± 14	0.1519*
Sex (male)	112 (76)	12 (75)	100 (76)	1.0000§
Arterial hypertension	89 (61)	11 (69)	78 (60)	0.4768§§
Diabetes	39 (27)	3 (19)	36 (27)	0.5608§
Family history	35 (24)	3 (19)	32 (24)	0.7627§
Smoking	71 (48)	8 (50)	63 (48)	0.8853§§
Obesity	21 (14)	2 (13)	19 (15)	1.0000§
TIA/stroke	8 (5)	2 (13)	6 (5)	0.2109§
OSAS	7 (5)	1 (6)	6 (5)	0.5616§
COPD	2 (3)	1 (6)	4 (3)	0.4427§
CKD	35 (24)	5 (31)	30 (23)	0.5345§
FCV-19S questionnaire (score)	14 (9, 17)	12 (9, 17)	14 (9, 17)	0.8976**
CSS questionnaire (score)	38 (25, 70)	31 (13, 50]	39 (27, 71)	0.2018**

TIA, transient ischemic attack; OSAS, obstructive sleep apnea syndrome; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; FCV-19S, Fear of COVID-19 Scale; CSS, COVID-19 Stress Scales.
**two-sample t-test.*
***Wilcoxon rank sum test.*
§Fisher's exact test.
§§ chi^2 test.

as appropriate. Group comparison (pre-lockdown vs. lockdown vs. post-lockdown) of continuous variables was performed by one-way ANOVA or Kruskal-Wallis test as appropriate. The chi² test or Fisher's exact test was used for group comparison of categorical variables. The Fisher's exact test was used if >20% of cells of the table contain expected values of <5, as appropriate. Otherwise the chi-squared test was used. The Kaplan-Meier estimator was used to assess the time to event and groups were compared using the log-rank test. Logistic regression analysis was done to investigate potential predictors on delayed presentation. Association of outcomes and total sums of both CSS and FCV-19S were assessed by scatter plots and either point-biserial correlation coefficient (in the case of dichotomous variables) or Spearman rank correlation coefficient (in the case of continuous variables).

Statistical analysis was performed by SAS version 9.4 under Windows. A two-sided p-value of <0.05 was considered statistically significant. Due to the explorative nature of this study, all results from statistical tests have to be interpreted as hypothesis generating. An adjustment for multiple testing was not done.

RESULTS

Patient Characteristics

From March 21, 2020, when measures of social restrictions were implemented for the first time during the COVID-19 pandemic in Germany, until the end of our inclusion period on September 31, 2020, 90 patients with STEMI had been admitted to our tertiary care center. Amongst those, 16 patients had been admitted during the lockdown period (March 21, 2020 to April 19, 2020; "lockdown group") and 74 patients in the post-lockdown period (April 20, 2020 to September 30, 2020; "post-lockdown group"). Furthermore, characteristics of 57 STEMI patients admitted before the COVID-19 pandemic ("pre-lockdown group") were assessed. For main analyses, the "pre-lockdown group" and the "post-lockdown group" were combined ("pre-/post-lockdown group"). In total, the mean age was 64 ± 13 years with 76% (112 out of 147 patients) being male. There were no significant differences in baseline characteristics between groups. No patients tested positive for SARS-CoV-2 virus during hospitalization. No patient was lost to follow-up. Detailed baseline characteristics are shown in **Table 1** and **Supplementary Table 1**.

Clinical Characteristics at Admission

To assess the effect of lockdown on STEMI patients admitted to hospital during the COVID-19 outbreak, clinical characteristics were assessed and compared to patients admitted before the outbreak and those admitted after measures of social restrictions had been lifted. Remarkably, a significantly higher rate of patients in the lockdown period reported that they intentionally did not go to the hospital or inform the emergency medical services immediately after the onset of symptoms (pre-/post-lockdown: 39 out of 120 patients (33%), lockdown: 11 out of 13 patients (85%); $p = 0.0004$). Likewise, 46% of patients in the lockdown group acknowledged that the time from symptom onset to FMC was longer than 24 h compared to 11% of patients in the pre-/post-lockdown group. Overall, time to FMC (in hours) was significantly prolonged in the lockdown group [pre-/post-lockdown: 2.0 (0.3, 16.0), lockdown: 11.0 (2.0, 144.0); $p = 0.0193$]. Additionally, door-to-needle time (in minutes) was significantly prolonged in patients admitted during lockdown [pre-/post-lockdown: 46 (28, 74); lockdown: 83 (59, 117); $p = 0.0277$]. Interestingly, patients in the lockdown group were more symptomatic at admission, as measured by NYHA class. However, there was no significant difference for measurements of vital signs at admission. Evaluation of laboratory parameters at admissions revealed that patients admitted due to STEMI during lockdown had significantly higher serum troponin T levels compared to those admitted before and after the pandemic lockdown [pre-/post-lockdown: 244 (53, 1124) ng/L, lockdown: 746 (292, 3899) ng/L; $p = 0.0105$]. Additionally, measures for NT-pro BNP and creatinine showed no significant difference. However, mean left ventricular end diastolic pressure (LVEDP) was significantly higher in the lockdown group compared to the pre-/post-lockdown group [pre-/post-lockdown: 24 (17, 29) mmHg, lockdown: 34 (27, 36) mmHg; $p = 0.0116$]. Lastly, STEMI patients admitted during lockdown had significantly higher need for circulatory support than those admitted before after the lockdown period [pre-/post lockdown: 18 out of 122 patients (15%), lockdown: nine out of 16 patients (56%), $p = 0.0005$]. Clinical characteristics at admission are summarized in **Table 2** and **Supplementary Table 2**.

TABLE 2 | Clinical characteristics at baseline.

	Total	Lockdown	Pre-/Post-Lockdown	*p*-value
	n = 147	*n* = 16	*n* = 131	
NYHA class				
I	34 (27)	1 (8)	33 (29)	**0.0087**§
II	25 (20)	0 (0)	25 (22)	
III	10 (8)	3 (23)	7 (6)	
IV	57 (45)	9 (69)	48 (42)	
Delayed presentation				
Yes	50 (38)	11 (85)	39 (33)	**0.0004**§
No	83 (62)	2 (15)	81 (68)	
Time to FMC				
Immediately	60 (45)	2 (15)	58 (49)	**0.0032**§
≤ 3 h	27 (20)	2 (15)	25 (21)	
≤ 12 h	14 (11)	3 (23)	11 (9)	
≤ 24 h	12 (9)	0 (0)	12 (10)	
> 24 h	19 (14)	6 (46)	13 (11)	
Time to FMC (hours)	2.0 (0.3, 24.0)	11.0 (2.0, 144.0)	2.0 (0.3, 16.0)	**0.0193****
Systolic bp (mmHg)	117 ± 28	116 ± 29	117 ± 29	0.9009*
Diastolic bp (mmHg)	67 ± 20	76 ± 19	65 ± 19	0.0579*
Troponin T (ng/L)	318 (63, 1,301)	746 (292, 3,899)	244 (53, 1,124)	**0.0105****
NT-pro BNP (pg/ml)	354 (91, 1,879)	1,120 (237, 6,459)	331 (83, 1,712)	0.0717**
Creatinine (μmol/L)	84 (71, 110)	86 (74, 115)	84 (71, 109)	0.6503**
Laevocardiography				
Normal	4 (3)	0 (0)	4 (3)	0.2620§
Mildly reduced	31 (23)	4 (27)	27 (22)	
Moderately reduced	55 (40)	3 (20)	52 (43)	
Severely reduced	46 (34)	8 (53)	38 (31)	
LVEDP (mmHg)	26 (17, 32)	34 (27, 36)	24 (17, 29)	**0.0116****
Door-to-needle-time (min)	54 (28, 80)	83 (59, 117)	46 (28, 74)	**0.0277****
Culprit lesion				
LAD	67 (49)	11 (79)	56 (46)	0.0815§
LCX	19 (14)	1 (7)	18 (15)	
RCA	51 (37)	2 (14)	49 (40)	
Circulatory support				
Yes	27 (20)	9 (56)	18 (15)	**0.0005**§
No	111 (80)	7 (44)	104 (85)	
Time at hospital (days)	4 (3, 6)	5 (2, 6)	4 (3, 6)	0.9445**

FMC, first medical contact; BNP, brain natriuretic peptide; bp, blood pressure; LVED, left ventricular end diastolic pressure; LAD, left anterior descending; LCX, left circumflex artery; RCA, right coronary artery.

**two-sample t-test.*

***Wilcoxon rank sum test.*

§Fisher's exact test.

§§chi^2 test.

Boldface denotes significance of p-values.

Clinical Outcomes

Patients included in our analysis had a median follow-up time of 142 days. Intriguingly, a comparison of survival time of patients of the lockdown group and patients of the pre-/post-lockdown group showed that STEMI patients admitted during lockdown had a significantly lower survival (confirmed deaths; pre-/post-lockdown: 21 out of 131 patients; lockdown: 7 out of 16 patients; log-rank test: $p = 0.0035$; **Figure 1**). This was associated with a higher rate of patients in the lockdown group (30%) reporting the presence of heart failure symptoms at rest compared to the pre-/post-lockdown period (11%). However, the overall difference in NYHA-class between both groups was only a non-significant result ($p = 0.1367$; **Table 3**). Analysis of laboratory measures at follow-up showed no significant difference. Clinical outcomes are shown in **Table 3** and **Supplementary Table 3**.

Effect of Stress of COVID-19 and Fear of COVID-19 on Outcomes

Since the association of fear of COVID-19 and outcomes of STEMI patients has not been comprehensively evaluated so far, we assessed the level of stress and fear of COVID-19 by two well-established questionnaires (FCV-19S and CSS). Patients in the lockdown period had a median FCV-19S Score of 12 (9, 17) and CSS score of 31 (13, 50), compared to a FCV-19S score of 14 (9, 17) ($p = 0.8976$), and CSS Score of 39 (27, 71) ($p = 0.2018$) in the pre-/post-lockdown group (**Table 1**). Association analysis of total test scores with baseline and follow-up parameters showed a significant relationship between the total CCS score and left ventricular contractile function as assessed by laevocardiography. However, no association between the total FCS-19V score and laevocardiography at admission could be demonstrated. There was no relationship between anxiety of COVID-19 and other parameters (**Supplementary Table 4**).

Predictors of Intentionally Delayed Presentation

To identify predictors of delayed presentation, we performed both univariate as well as multiple logistic regression analysis of the parameters that potentially keep STEMI patients from seeking timely medical care amid the COVID-19 pandemic. After multiple analysis, only "admission during lockdown" remained significantly associated with intentionally delayed presentation (**Table 4**).

DISCUSSION

To the best of our knowledge, this is the first study prospectively evaluating the outcome of STEMI patients admitted during the COVID-19 pandemic caused lockdown, which also analyzes the effects of stress and fear of COVID-19 on patient outcomes. We found that patients with STEMI admitted during the lockdown period to our tertiary center showed lower survival compared to both those admitted before the COVID-19 pandemic and after measures of social restriction have been partly lifted. This was associated with a longer time from symptom onset to FMC and

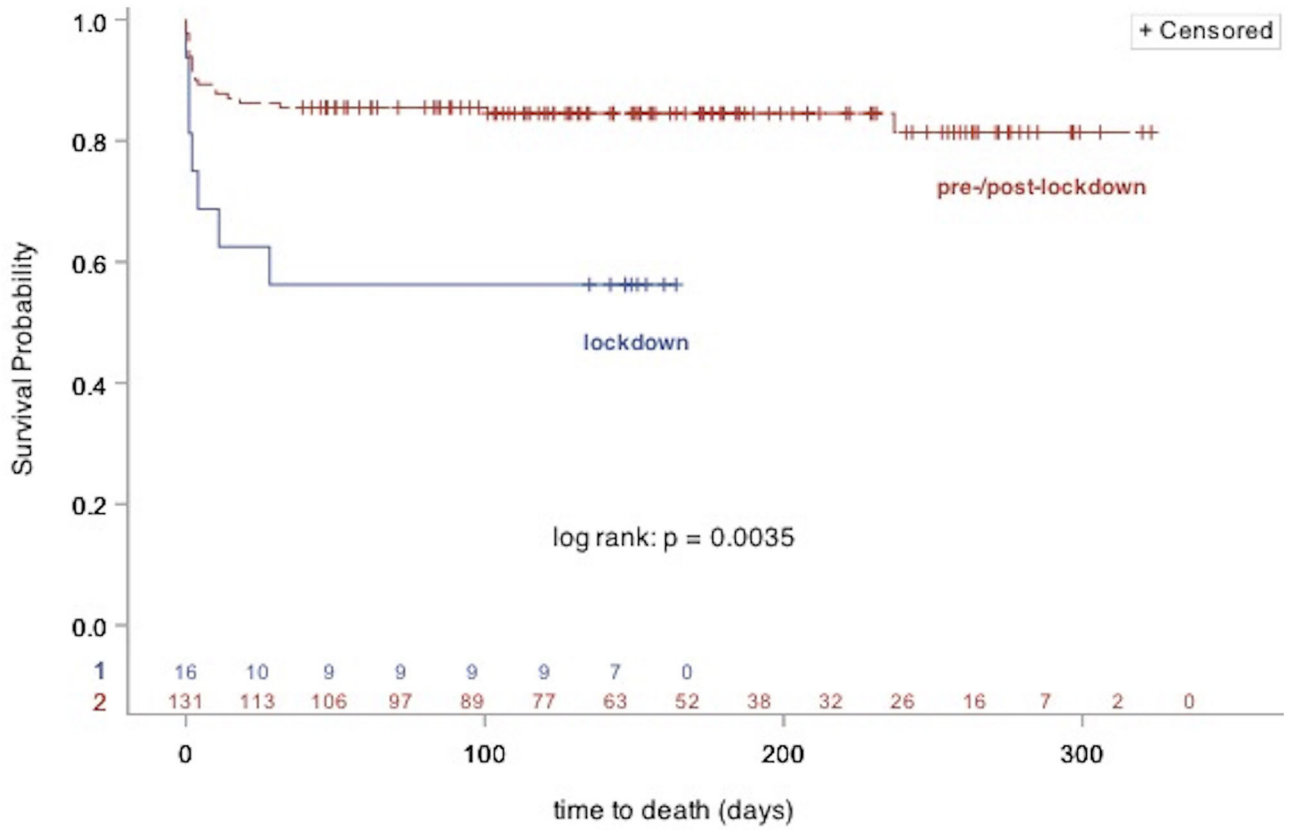

FIGURE 1 | Kaplan Meier plot showing survival of STEMI patients admitted during (lockdown group), and outside of COVID-19 associated lockdown (pre-/post-lockdown group).

a prolonged door-to-needle time. Additionally, patients in the lockdown group had significantly higher serum troponin T levels, a worse left ventricular end-diastolic pressure, and a higher need of circulatory support at admission.

Effect of Fear of COVID-19 and Stress of COVID-19 on STEMI Patients

Observations since the beginning of the COVID-19 crisis have suggested that various factors, such as altruistic behavior, information by the media, and especially fear of contagion with SARS-CoV-2 in hospital, contributed to reduced admissions of patients with acute myocardial infarction and prolonged times from symptom onset to FMC (4, 15). It has been reported, that patients who avoided an emergency room visit timely because they feared getting infected with SARS-CoV-2 in hospital, suffered catastrophic complications such as ventricular septal defect, which has become rare due to steadily improving medical care (16). As fear displays one characteristic of infectious disease and is associated with its transmission rate, morbidity, and mortality, Ahorsu et al. developed and validated a 7-item scale (Fear of COVID-19 Scale, FCV-19S) assessing the level of fear of SARS-CoV-2 (14). As there is currently no systematic study on the effect of fear on patients with myocardial infarction, we employed the FCV-19S to estimate if the extent of fear of COVID-19 is associated with worsened outcomes in STEMI patients. To do so, we conducted association analysis of the scores of the FCV-19S, and relevant clinical patient characteristics. Our results indicate that overall fear of COVID-19 is not related to adverse outcomes in STEMI patients. To substantiate this finding, we applied the COVID Stress Scales, a 36-item scale developed by Taylor et al. to better understand and assess COVID-19-related stress and anxiety (13). Besides a single relationship between the total CSS score and left ventricular systolic contractility as assessed by laevocardiography, we could not demonstrate an association between the totaled item scores and outcomes of STEMI patients. Therefore, fear of COVID-19 was not associated with lockdown, higher measures of cardiac biomarkers, outcomes at follow-up, and prolonged times from symptom onset to FMC. Since our study population represents a region which has been rather spared from overwhelming infection rates in the early phase of the pandemic, these results might deviate if STEMI patients in epicenters of the pandemic are interviewed.

Effect of Lockdown on STEMI Patients

Soon after SARS-CoV-2 surfaced around the world, several countermeasures were initiated to contain further spread as

TABLE 3 | Patient characteristics at follow up.

	Total	Lockdown	Pre-/Post-Lockdown	*p*-value
	***n* = 147**	***n* = 16**	***n* = 131**	
NYHA class				
I	46 (45)	5 (50)	41 (44)	0.1367[§]
II	35 (34)	1 (10)	34 (37)	
III	9 (9)	1 (10)	8 (9)	
IV	13 (13)	3 (30)	10 (11)	
LVEF	53 (45, 60)	47 (35, 63)	53 (45, 60)	0.4327**
Troponin T (ng/L)	19 (10, 39)	26 (19, 81)	17 (10, 33)	0.1300**
NT-pro BNP (pg/ml)	483 (187, 1,092)	1,014 (187, 3,559)	483 (195, 964)	0.4976**
Creatinine (μmol/L)	81 (74, 93)	83 (74, 131)	80 (74, 93)	0.5377**

NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; BNP, brain natriuretic peptide.
***Wilcoxon rank sum test.*
[§]*Fisher's exact test.*

TABLE 4 | Identification of predictors of intentionally delayed presentation.

	Multiple analysis		
Variables	**Odds Ratio (OR)**	**95% CI of OR**	***p*-value**
Age	0.965	0.924–1.008	0.1133
Sex (female)	2.187	0.567–8.437	0.2558
Admission during lockdown	16.393	1.692–166.67	**0.0159**
FCV-19S questionnaire (score)	1.083	0.939–1.249	0.2760
CSS questionnaire (score)	0.986	0.952–1.021	0.4280

FCV-19S, Fear of COVID-19 Scale; CSS, COVID-19 Stress Scales. Boldface denotes significance of p-values.

much as possible. Drastic measures of social distancing and public lockdown were implemented, among other policies. In Germany, public facilities were closed, sporting events were canceled, and the physical contact of more than two persons outside of families was prohibited (9). Depending on the region, even curfews were enforced to minimize the inter-personal contact. For patients suffering from acute myocardial infarction, it has been suggested that measures of public lockdown might interfere with timely and adequate medical care (1, 2, 4, 10). It has been observed that the implementation of regional lockdown was associated with a significant decline in admission numbers of STEMI patients compared to times before the pandemic (10, 17). Furthermore, a concomitant increase of patient-related as well as system-related delay times, as measured by the time from symptom onset to FMC and door-to-balloon time, has been registered (18). However, it is difficult to distinguish if these findings were related specifically to the lockdown or to the COVID-19 crisis as a whole. To date, there are only a few retrospective cohort and register studies available with data on STEMI patients admitted during and after regional lockdown (19, 20). While these studies confirm the decrease in incidence during lockdown period, there is a lack of information regarding delay times, mortality and survival (19, 20). To compensate for this issue, we prospectively assessed and compared survival of STEMI patients admitted during the COVID-19 pandemic and outside of lockdown. Intriguingly, we found that the lockdown group had a significantly lower survival. This might be attributed to our finding that during lockdown, patients were admitted in worse condition. This is substantiated by (1) worse symptoms as measured by NYHA-class, (2) significantly increased serum troponin T levels, (3) a significantly higher LVEDP, and (4) significantly higher need for circulatory support in the lockdown-group. This could be related to a significantly prolonged time from symptom onset to FMC during lockdown, which is known to be associated with larger infarct size and infarct transmurality (21). Additionally, we observed that the door-to-needle time was significantly prolonged in the lockdown-group, too. Evidently, this is related to the indispensable adaptation of emergency processes, such as employment of personal protective gear, to mitigate the risk of getting infected with SARS-CoV-2 (22). Nevertheless, it remains possible that the increase in system-related delay time, as measured by door-to-needle time, contributed to the worse outcome of STEMI patients admitted during lockdown. Assessment of other outcomes did not show differences, which might be related to the higher number of deceased patients in the lockdown group, who, therefore, did not receive a follow-up visit.

Moreover, by multiple logistic regression analysis, we show that amongst several aforementioned factors that potentially keep STEMI patients from seeking timely medical attention despite experiencing ischemic symptoms, that lockdown (not stress or fear of COVID-19) was significantly associated with an intentionally delayed presentation. These findings substantiate our hypothesis that measures of social distancing such as lockdown adversely affect the health behavior and outcomes of STEMI patients.

As a consequence, public lockdown appears to considerably deteriorate the prognosis of patients suffering from myocardial ischemia. In the presence of the currently rising incidence of SARS-CoV-2 virus infections worldwide and imminent recurrence of lockdown measures, public health policy has to carefully decide on the extent of social policies to avoid potential excess morbidity and mortality. Implementation of lockdown measures have to be accompanied by distinct public instructions on how to act in health emergencies such as STEMI and others.

Limitations

As this is a prospective, observational explorative study on the outcomes of STEMI patients admitted during and outside of social lockdown related to the COVID-19 pandemic, it inherently has limitations. Since this is a study from a single center, only a limited number of patients could be included. Due to the explorative character of this study, our results have to be interpreted as hypothesis generating. Studies reporting the outcomes a larger number of participants, which might be achievable by a multi-center design or a prolonged time to select

cases, are of the essence to verify our results and to further investigate the drivers of increased mortality (e.g., by pathway analysis). Nonetheless, these results are the first prospective data on the outcomes of STEMI patients admitted during, before and after lockdown, which reveal a significant decrease in survival during lockdown. For further analysis, the raw data underlying our analyses are available upon publication.

CONCLUSION

This is the first prospective study comparing the outcomes of STEMI patients admitted during lockdown, to outcomes of patients admitted before and after public lockdown in a non-COVID-19 epicenter. Our results suggest that enforced lockdown is associated with reduced survival of STEMI patients, which supposedly is related to prolonged patients delay times. Patient related factors such as the fear of getting infected in the hospital or stress factors related to COVID-19 seem to have less impact on outcomes among these patients. Public health care strategies to constrain SARS-CoV-2 or other pandemics at present and in future including public lockdown measures have to assure timely medical treatment beyond COVID-19. Implementation of lockdown measures should be accompanied by distinct public instructions on how to act in acute life-threatening diseases such as STEMI and others.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. Mendeley Data can be found with 10.17632/8pzrkkjrz3.1.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ulm University Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MR and AI had the idea for and designed the study and had full access to all data and take responsibility for the integrity of the data and the accuracy of the data analysis. MR, CW, and LS collected the data. JD performed the statistical analysis. MR and KT mainly wrote the manuscript with support from AI, CW, LS, SM, and MB. MR, AI, and WR were mainly responsible for the interpretation of the data. AI and WR supervised the project. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.638954/full#supplementary-material

REFERENCES

1. Rosenbaum L. The untold toll — the pandemic's effects on patients without Covid-19. *N Engl J Med.* (2020) 382:2368–71. doi: 10.1056/NEJMms2009984
2. Kittleson MM. The invisible hand — medical care during the pandemic. *N Engl J Med.* (2020) 382:1586–7. doi: 10.1056/NEJMp2006607
3. Dubey S, Biswas P, Ghosh R, Chatterjee S, Dubey MJ, Chatterjee S, et al. Psychosocial impact of COVID-19. *Diabetes Metab Syndr Clin Res Rev.* (2020) 14:779–88. doi: 10.1016/j.dsx.2020.05.035
4. 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
5. Ahmed T, Lodhi SH, Kapadia S, Shah G V. Community and healthcare system-related factors feeding the phenomenon of evading medical attention for time-dependent emergencies during COVID-19 crisis. *BMJ Case Rep.* (2020) 13:e237817. doi: 10.1136/bcr-2020-237817
6. 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
7. 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
8. 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
9. Rattka M, Baumhardt M, Dreyhaupt J, Rothenbacher D, Thiessen K, Markovic S, et al. 31 days of COVID-19-cardiac events during restriction of public life-a comparative study. *Clin Res Cardiol.* (2020) 109:1476–82. doi: 10.2139/ssrn.3594561
10. Claeys MJ, Argacha J-F, Collart P, Carlier M, Van Caenegem O, Sinnaeve PR, et al. Impact of COVID-19-related public containment measures on the ST elevation myocardial infarction epidemic in Belgium: a nationwide, serial, cross-sectional study. *Acta Cardiol.* (2020). doi: 10.1080/00015385.2020.1796035. [Epub ahead of print].
11. Gramegna M, Baldetti L, Beneduce A, Pannone L, Falasconi G, Calvo F, et al. ST-segment–elevation myocardial infarction during COVID-19 pandemic. *Circ Cardiovasc Interv.* (2020) 13:e009413. doi: 10.1161/CIRCINTERVENTIONS.120.009413
12. Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. STROBE initiative. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet.* (2007) 370:1453–7. doi: 10.1016/S0140-6736(07)61602-X
13. Taylor S, Landry CA, Paluszek MM, Fergus TA, McKay D, Asmundson GJG. Development and initial validation of the COVID stress scales. *J Anxiety Disord.* (2020) 72:102232. doi: 10.1016/j.janxdis.2020.102232
14. Ahorsu DK, Lin C-Y, Imani V, Saffari M, Griffiths MD, Pakpour AH. The fear of COVID-19 scale: development and initial validation. *Int J Ment Health Addict.* (2020). doi: 10.1007/s11469-020-00270-8. [Epub ahead of print].

15. Hammad TA, 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 Card Interv*. (2021) 97:208–14. doi: 10.1002/ccd.28997
16. Masroor S. Collateral damage of COVID-19 pandemic: delayed medical care. *J Card Surg*. (2020) 35:1345–7. doi: 10.1111/jocs.14638
17. Rebollal-Leal F, Aldama-López G, Flores-Ríos X, Piñón-Esteban P, Salgado-Fernández J, Calviño-Santos R, et al. Impact of COVID-19 outbreak and public lockdown on ST-segment elevation myocardial infarction care in Spain. *Cardiol J*. (2020) 27:425–6. doi: 10.5603/CJ.a2020.0098
18. Kwok CS, Gale CP, Kinnaird T, Curzen N, Ludman P, Kontopantelis E, et al. Impact of COVID-19 on percutaneous coronary intervention for ST-elevation myocardial infarction. *Heart*. (2020) 106:1805–11. doi: 10.1136/heartjnl-2020-317650
19. 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
20. Wu J, Mamas M, Rashid M, Weston C, Hains J, Luescher T, et al. Patient response, treatments and mortality for acute myocardial infarction during the COVID-19 pandemic. *Eur Hear J Qual Care Clin Outcomes*. (2020) 53:1689–99. doi: 10.1093/ehjqcco/qcaa062
21. Thiele H, Kappl MJ, Linke A, Erbs S, Boudriot E, Lembcke A, et al. Influence of time-to-treatment, TIMI-flow grades, and ST-segment resolution on infarct size and infarct transmurality as assessed by delayed enhancement magnetic resonance imaging. *Eur Heart J*. (2006) 28:1433–9. doi: 10.1093/eurheartj/ehm173
22. Han Y. A treatment strategy for acute myocardial infarction and personal protection for medical staff during the COVID-19 epidemic: the Chinese experience. *Eur Heart J*. (2020) 41:2148–9. doi: 10.1093/eurheartj/ehaa358

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.

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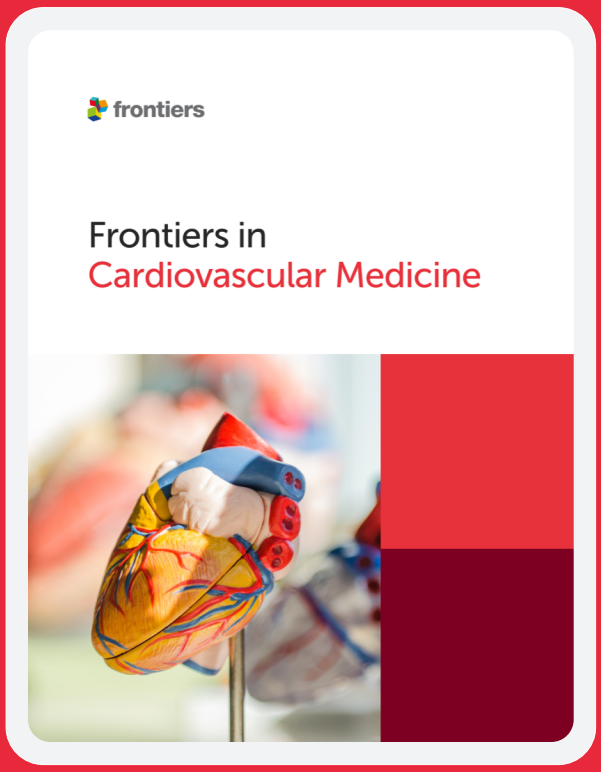

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