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Post-COVID-19 cardiovascular sequelae

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Post-COVID-19 cardiovascular sequelae

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Editorial: Post-COVID-19 cardiovascular sequelae

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Editorial on the Research Topic
Post-COVID-19 cardiovascular sequelae

1. Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) commonly presents as a pulmonary illness but may also affect extrapulmonary organs including the heart (1). Cardiovascular complications have been documented at all stages of the disease, from direct acute myocardial injury during severe acute illness (2) to prolonged cardiovascular involvement in the post-acute phase (3), and more indolent symptoms beyond the acute infection commonly referred to as Long COVID (4). The COVID-19 pandemic, particularly in its early stages, also led to major disruptions in healthcare delivery, which indirectly affected all aspects of cardiovascular care from preventive assessments to acute cardiovascular treatments. Although vaccinations against SARS-CoV-2 attenuated disease severity and mortality after COVID-19, vaccine-induced adverse effects involving the heart such as myopericarditis started emerging (5). The aim of this Research Topic in Frontiers Cardiovascular Medicine was to present research examining these direct and indirect cardiovascular consequences of the COVID-19 pandemic.

2. Cardiovascular complications of COVID-19

Growing reports highlight the multisystem manifestations of COVID-19. In particular, existing literature highlights cardiovascular complications of COVID-19 in the acute and early post-acute phase of the illness. In a retrospective cohort study, Lu et al. studied the prognostic impact of multi-organ injury in hospitalised patients with acute COVID-19. They analysed the demographics, clinical variables, and the likelihood of inpatient mortality of COVID-19 patients with combined injury Acute Kidney Injury (AKI) and Acute myoCardial Injury (ACI) and compared them with those with AKI only, acute cardiac injury only, and no injury (NI). Of the 5,896 hospitalized COVID-19 patients,

44% had NI, whilst 19%, 9%, and 28% had AKI, ACI, and AKI-ACI, respectively. They observed that COVID-19 patients with both AKI and ACI had markedly worse outcomes compared to those with NI or each organ injury alone (AKI, ACI). These patients had worse clinical and laboratory variables, markedly worse disease courses, and increased in-hospital mortality. The adjusted odds ratios for in hospital-mortality were 17.1, 7.2 and 4.7 for AKI-ACI, ACI, and AKI, respectively, relative to NI.

Some studies have suggested that there may be persistent cardiovascular involvement in the context of COVID-19, even after apparent recovery from the acute illness. These assertions were mainly based on abnormalities in imaging metrics. Shanmuganathan et al. present a prospective study of middle-aged (median age 56 years) unvaccinated patients ($n = 23$) hospitalised with COVID-19. They observed evidence of myocardial oedema, as demonstrated by significantly elevated myocardial T1 and T2 signals on cardiovascular magnetic resonance (CMR) performed during the acute hospitalisation, in some cases compared to non-COVID-19 and asymptomatic controls matched for cardiovascular risk factors ($n = 19$). Follow up CMR scans performed 6 months after discharge from hospital suggested that acute myocardial oedema tends to normalize over time during the convalescent phase. Ventricular function was preserved throughout. Consistent with these observations, Gao et al. reported no elevation of high-sensitivity troponin I (cTnI) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) or echocardiographic structural and functional abnormalities in survivors of COVID-19 compared with healthy control and risk factor-matched control, almost one year (327 days) after recovery from the acute illness. The authors suggested that any myocardial injury and echocardiographic structural and functional abnormalities observed in the acute phase of COVID-19 infection might be reversible.

Many studies have reported elevated risk of incident cardiovascular events in individuals recovering from COVID-19. In particular, the elevated risk of venous thromboembolism (VTE) after COVID-19 has been consistently demonstrated in different studies and settings (6). These observations have raised the question of potential benefit of prophylactic antithrombin therapy after COVID-19, with current uncertainty about the duration and type of treatment. A study performed in Russia by Motloch et al. investigated the benefits of prophylactic anticoagulation or antiplatelet therapy in Covid-19 patients on long-term survival and cardiovascular outcomes (hospitalization due to pulmonary embolism (PE), myocardial infarction (MI) and stroke). In mid-2020, dipyridamole or prophylactic direct anticoagulation (DOAC) were routinely prescribed in the early post-discharge period (30-days post-discharge) in several medical centres in Russia based on Russian Ministry of Healthcare recommendations. This single centre, retrospective study showed that post-discharge thromboprophylaxis with DOAC/Dipyridamole for 30 days reduced the risk of cardiovascular events and all-cause mortality compared to no anticoagulation, emphasizing the ongoing thromboembolic and inflammatory burden in COVID-19 in the early post-discharge period following the acute phase of the disease.

A growing body of research highlights persistent cardiovascular symptoms in patients apparently recovered from COVID-19, raising questions about the longer-term consequences of infection (7). An observational study conducted by Marques et al. analysed the presence of alterations in cardiac autonomic functioning in 155 patients with long-COVID-19 (asymptomatic or mildly to moderately symptomatic) and compared them with a Covid-19 negative control group ($n = 94$). The study concluded that long COVID clinical group showed reduced heart rate variability (HRV). These findings suggest potential alteration of sympathetic tone following COVID-19 which may help explain some of the symptoms of long COVID-19 as also proposed by Sze et al. in a mini-review in this Topic.

3. Post-COVID-19 vaccine cardiovascular complications

Whilst the availability of vaccinations heralded a new phase of the pandemic with a reduction in frequency of severe infections and mortality (8), it was also accompanied by concerns around their safety. In particular a number of case reports and registry data (Wu et al. Cui et al. Sciaccaluga et al.) suggest that myocarditis is a side effect of COVID-19 vaccination. In this series, two case reports have linked both viral-vector based and inactivated SARS-CoV-2 vaccines to incidences of fulminant myocarditis (Wu et al. Cui et al.). Another case report linked mRNA-based COVID-19 vaccine to myocarditis and pericarditis in two young male patients (Sciaccaluga et al.). Shiyovich et al. suggest that the CMR imaging findings of myocarditis following the administration of the BNT162b2 mRNA COVID-19 booster vaccine were relatively mild.

Given concerns raised in such early case reports, a number of researchers undertook a more systematic assessments of this research question. Researchers from the CMR-Center of University Hospital Muenster, Germany (Meier et al.) enrolled 41 healthy volunteers for a CMR-based screening study before and after the third booster vaccination to assess the effect of the booster on the myocardium. 30% of the subjects received mRNA- 1273% and 70% received BNT162b2 for booster. The study showed no significant changes in the myocardial tissue characteristics or function following the third booster vaccination; however, they did report one case of subclinical pericarditis in a female patient. The third booster vaccination significantly raised the SARS CoV-2-IgG antibody titre, and curiously, it did so more in females than in males, according to the results. While reassuring, the size of this study limits its generalisability with regards to real-world prevalence of myopericardial injury following mRNA vaccination.

4. Impact of pandemic on cardiovascular healthcare

The COVID-19 pandemic has significantly impacted cardiovascular care across key areas of health care delivery including preventive interventions as well as the management of acute and chronic disease. A study performed in Lombardi, Italy by Ferlini et al. explored the impact of the COVID-19 pandemic

on the presentation, time of care, and mortality data of patients with diagnoses of ACS, including ST-elevation myocardial infarction (STEMI) during the second SARS-CoV-2 pandemic spread (November 2020-January 2021) within the "macro-hubs" network implemented by the Lombardy region, to keep the regional healthcare system from being overwhelmed, and to guarantee timely optimal care to patients with acute coronary syndromes (ACS). The observational study included 941 ACS patients and a total of 59 patients (6.3%) presented a concomitant confirmed SARS-CoV-2 infection out of which 42. 4% of patients had pneumonia. STEMI was the clinical presentation in 56% of SARS-CoV-2 infected patients.

The study revealed that patients with ACS (STEMI) and positive SARS-Cov-2 (based on the positive nasopharyngeal swab, pulmonary TAC diagnostic for interstitial pneumonia, as a single test or in combination) had a higher GRACE (Global Registry of Acute Coronary Events) score of 139 (IQR 105–158) and a considerably greater rate of in-hospital death than those without infection (16.9 vs. 3.6%), whereas post-discharge mortality was not affected (4.2 vs. 4.1%). This excess mortality risk appeared to be attributable to the presence of concomitant pneumonia (Ferlini et al.). Ferlini et al. reported the centralized model used in Lombardy did not show a negative impact on time to treatment of STEMI patients. Furthermore, almost all patients with ACS received coronary angiography (97%) for STEMI, corroborating the beneficial effect of the organizational strategy adopted.

Contrasting this approach in Italy, Tang et al. observed a decline in percutaneous coronary intervention (PCI) activity during COVID19 pandemic outbreak (January 23, 2020 to April 8, 2020) in Hunan province, China. When compared with prepandemic levels there was a 12.7% reduction in PCI procedures in COVID-19 negative patients. The authors also reported a 10.5% drop of STEMI admissions. Overall, in this study, restructuring health care services during the COVID-19 pandemic outbreak did not appear to significantly adversely influence in-hospital mortality and major cardiac events. The authors suggested that multiple factors might contribute to this decline in admissions of patients with STEMI including misdiagnosis because of complex cardiovascular manifestations under the circumstance of COVID-19 as well as reluctance of symptomatic patients to seek acute medical care due to a fear of catching COVID-19.

There were also changes in uptake of cardiovascular assessments in other areas. Li et al. performed a study using an online ECG platform in China to investigate how COVID19 affected the health-seeking behaviour of patients with various arrhythmias with non-COVID-19 diseases, during and after COVID-19 epidemic. Compared with the same period during pre-COVID years, the number of medical visits decreased during the lockdown (a 38% reduction), followed by a rebound post-lockdown (a 17% increase) and a fall to the baseline level in post-SARS-CoV-2 period. The ECG utilization patterns of patients with arrhythmias exhibited a decrease-rebound-fallback pattern following the COVID-19 lockdowns. Lockdowns had less of an impact on medical visits for illnesses with more severe symptoms, demonstrating a persistent need for healthcare.

5. Conclusion

In summary, this Research Topic has covered a range of articles from around the world, covering matters relevant to our understanding of the direct and indirect cardiovascular consequences of COVID19 (**Figure 1**). While acute cardiac

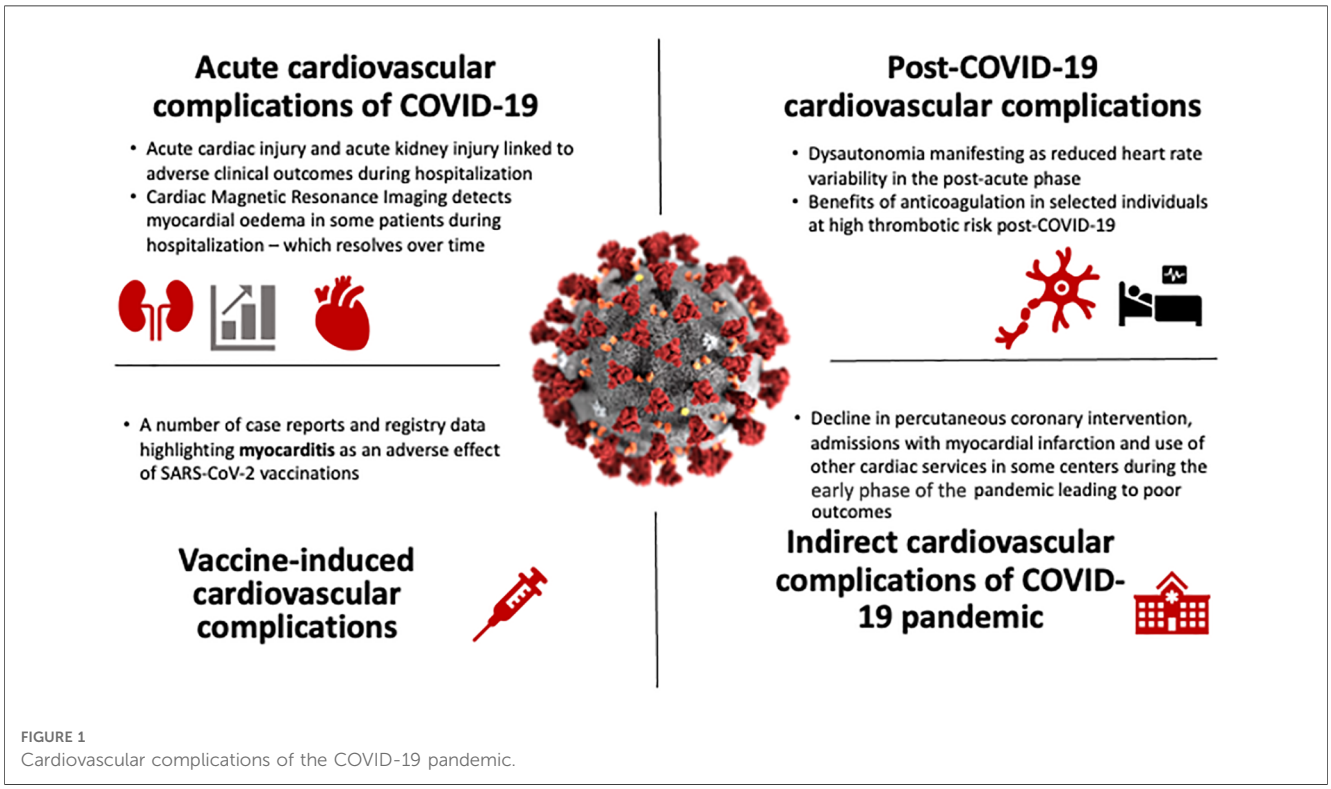

FIGURE 1
Cardiovascular complications of the COVID-19 pandemic.

manifestations were reported in the early phase of the pandemic, the decreased virulence of evolving SARS-CoV-2 variants and the protective effects of vaccines and acquired immunity from natural infections have reduced the rates of severe complications and mortality from COVID-19. However, one must be vigilant of the potential of even mild COVID-19 to cause ongoing symptoms (e.g., dysautonomia) and of the infrequent presentation of myopericarditis after COVID-19 vaccinations. The high cardiovascular mortality in the early pandemic period could have also resulted from a failure of health care systems to rapidly adapt to health care needs during a global health crisis and reinforces the need for greater investment into agile services in preparation for future pandemics.

Author contributions

D-GC wrote the initial draft. ZR-E, MS and BR critically reviewed and provided feedback on the final version of the manuscript. BR supervised the work and is the guarantor of integrity of the work. All authors contributed to the article and approved the submitted version.

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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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OPINION
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Hospitalized Children With Familial Hypercholesterolemia and COVID-19: A Case for Preventive Anticoagulation

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INTRODUCTION

Heterozygous familial hypercholesterolemia (HeFH) affects about one in 200 to 250 persons or over 30 million people worldwide, of whom about 20–25% are children and adolescents (1, 2). In those with HeFH, the level of serum low-density lipoprotein cholesterol (LDL-C) is elevated about two-fold from birth (3). If left untreated, the severe hypercholesterolemia causes pre-mature atherosclerosis. The standard treatment in HeFH children is statin therapy, which should start when the child is between 8 and 12 years of age (4). Homozygous familial hypercholesterolemia (HoFH) is the severe form of familial hypercholesterolemia (FH) affecting approximately 1 in 300,000 persons worldwide and causing four- to five-fold elevated levels of serum LDL-C (5). Despite the availability of multiple lipid-lowering therapies most HoFH patients do not achieve sufficiently low LDL-C levels, and accordingly are at high risk of symptomatic atherosclerotic cardiovascular disease already in childhood (6). In fact, there have been several case reports of sudden cardiac death due to fatal myocardial infarction in children with HoFH before the age of 10 years (7). Of note, the majority of the clinical studies performed on FH patients have included only the much more common form of FH, i.e., HeFH. Accordingly, when we refer to mere "FH," we refer to studies with HeFH patients, unless specified otherwise.

ENDOTHELIAL DYSFUNCTION IN FAMILIAL HYPERCHOLESTEROLEMIA

The significantly elevated serum LDL-C causes endothelial dysfunction already in young children with FH (8, 9). Additionally, many FH patients have raised serum levels of lipoprotein(a) [Lp(a)] (10). Thus, endothelial function in FH children can be severely compromised when both LDL-C and Lp(a) levels are increased (8). Moreover, compared with unaffected controls, FH children display a proinflammatory and prothrombotic phenotype which is associated with vascular dysfunction (11). Because Lp(a) is circulating in the blood, both proinflammatory and antifibrinolytic (i.e., prothrombotic) effects may extend from the macrovascular to the microvascular level, so affecting the entire circulatory system. Furthermore, because Lp(a) inhibits fibrinolysis, the risk of forming non-occluding or occluding thrombi is increased in FH children, in contrast to non-FH children with a primarily healthy endothelium (12).

COVID-19–AN ENDOTHELIAL DISEASE

COVID-19 is considered to be an endothelial disease (13). Thus, the effect of this disease on vessel wall endothelial linings should particularly affect FH patients with COVID-19, in whom the already dysfunctional endothelium is acutely exposed to additional damaging insults caused by the excessive immunoinflammatory response of the host (i.e., the cytokine storm) and because the coronavirus can damage the endothelial cells also directly thereby leading to "endotheliitis" (13, 14). When exposed to inflammatory and infectious signals, the normally anticoagulant, antithrombotic, and profibrinolytic endothelial cells become activated and locally promote the activation of the coagulation cascade and thrombus formation. The pro-coagulant/pro-aggregatory, pro-inflammatory, vasoconstrictor, pro-oxidant, and barrier function-impairing properties of such damaged endothelium then critically contribute to the multiorgan failure characteristic of advanced stages of COVID-19.

COVID-19 IS A PROTHROMBOTIC STATE

A recent autopsy study revealed that adult COVID-19 patients frequently have fibrin microthrombi in the heart without acute ischemic injury (15). The risk of developing such non-occluding or even occluding cardiac microthrombi is likely to be higher in children with FH. According to the results of a recent meta-analysis, among hospitalized adult patients with COVID-19, the prevalence of acute myocardial infarction was 3.3% (95% CI 0.3–8.5) (16). Therefore, the possibility that children with FH, particularly those with HoFH and COVID-19, are at increased risk of coronary thrombus formation and, despite their young age, may be at risk for an ischemic cardiac event (6).

Current data have demonstrated a COVID-19-induced prothrombotic state in children, as reflected by elevated D-dimer levels (17). This prothrombotic state can be further followed in the clinical setting by using a diagnostic disseminated intravascular coagulation (DIC) score, which has been established by The International Society on Thrombosis and Haemostasis (ISTH) (18, 19). The ISTH DIC score is taking into account several mechanisms related to the DIC syndrome which is characterized by widespread intravascular activation of coagulation. The pathophysiological mechanisms include, among others, cytokine-initiated inflammatory activation of coagulation and insufficient control of anticoagulant pathways, which together lead to endothelial dysfunction and microvascular thrombosis (19). The usefulness of the ISTH DIC score was shown in a retrospective large cohort study of 1,127 adult COVID-19 patients in Spain (20). In this study, the initial ISTH DIC score was significantly higher among the ultimately non-surviving patients.

Al-Ghafry et al. (21) recently published a case series of eight hospitalized COVID-19 pediatric patients, in which the coagulation profiles were determined. Six children had elevated D-dimer levels and required oxygen supplementation, and five children also required intensive care unit treatment. The authors carried out rotational thromboelastometry and found an increased blood clot firmness with a contribution from fibrinogen. Based on these laboratory findings, the children of whom the youngest were 8 years old received anticoagulation according to institutional adult anticoagulation guidelines, and no thromboembolic complications were observed in the treated children. Based on the above findings there is a potential need to expand and study the indication for prophylactic anticoagulation in hospitalized children with COVID-19 (22) to children with FH, provided there are no contraindications to anticoagulant therapy. Furthermore, in FH children, it is essential to continue effective statin therapy because statins not only improve endothelial function but also decrease serum D-dimer levels by about 15%, thus providing additional mild anticoagulation (23, 24). Moreover, because proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors effectively lower serum LDL-C concentration, reduce the Lp(a) level by about 30%, and may also enhance the antiviral action of interferon in patients with hypercholesterolemia, the use of these inhibitors could be considered in hospitalized pediatric FH patients with COVID-19, particularly those with HoFH, if not already in use (25–27).

DISCUSSION

Results from controlled studies investigating the clinical effects of anticoagulation in hospitalized children with COVID-19 are lacking. Meanwhile, Loi et al. (22) have recommended that children with COVID-19 are eligible for anticoagulation. Based on the considerations presented here and on a recent expert consensus-based pediatric opinion (28), anticoagulant prophylaxis in children should be carried out (in the absence of any contraindications) by using low-dose low-molecular-weight heparin. Loi et al. also recommend that, in hospitalized children with COVID-19, it is important to trend the disseminated intravascular coagulation score with attention to the D-dimer level. Additionally, a pediatric risk assessment and consideration of prophylactic anticoagulation to prevent thrombosis should be performed at baseline and daily thereafter. When considering that FH is a prothrombotic condition by itself, the above recommendations would particularly apply to hospitalized FH children with COVID-19 (10, 29). This idea is supported by the above consensus-based clinical recommendation for anticoagulation in children hospitalized for COVID-19-related illnesses (28).

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AV: writing the first draft. AV, FR, and PK: reviewing and editing to produce the final draft. All authors contributed to the article and approved the submitted version.

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Clinical Implications of IL-32, IL-34 and IL-37 in Atherosclerosis: Speculative Role in Cardiovascular Manifestations of COVID-19

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Atherosclerosis, which is a primary cause of cardiovascular disease (CVD) deaths around the world, is a chronic inflammatory disease that is characterised by the accumulation of lipid plaques in the arterial wall, triggering inflammation that is regulated by cytokines/chemokines that mediate innate and adaptive immunity. This review focuses on IL-32, -34 and -37 in the stable vs. unstable plaques from atherosclerotic patients. Dysregulation of the novel cytokines IL-32, -34 and -37 has been discovered in atherosclerotic plaques. IL-32 and -34 are pro-atherogenic and associated with an unstable plaque phenotype; whereas IL-37 is anti-atherogenic and maintains plaque stability. It is speculated that these cytokines may contribute to the explanation for the increased occurrence of atherosclerotic plaque rupture seen in patients with COVID-19 infection. Understanding the roles of these cytokines in atherogenesis may provide future therapeutic perspectives, both in the management of unstable plaque and acute coronary syndrome, and may contribute to our understanding of the COVID-19 cytokine storm.

Keywords: IL-32, IL-34, IL-37, implication, COVID-19

ATHEROSCLEROSIS

Cardiovascular disease (CVD) is the leading cause of death in the world (1). Cerebrovascular disease and coronary artery disease (CAD) are the most prevalent subtypes of cardiovascular disease that result in a high morbidity as well as large economic burden in developing countries (1). Atherogenesis, referring to the development of atherosclerotic plaques, progresses through endothelial dysfunction; leukocytes recruitment; differentiation of monocytes; formation of foam cells; and proliferation of vascular smooth muscle cells (VSMC) (2). The abnormal steps of atherogenesis are regulated by both innate and adaptive immunity *via* cytokines/chemokines modulating the cross-talk between inflammatory and vascular cells (2, 3). Despite the aggressive management of modifiable risks factors for atherosclerosis, for example, lipid-lowering treatments and anti-hypertensives, which promise effective management for atherosclerosis, the mortality and morbidity of CVD are still rather unacceptably high (4). The *Canakinumab Anti-Inflammatory Thrombosis Outcomes Study* is a large-scaled clinical trial

which demonstrates a decrease in major adverse cardiovascular events following anti-IL-1β, antibody treatment, supporting the critical role of inflammation during atherogenesis (5).

ATHEROGENESIS

Circulating low-density lipoproteins (LDL) are deposited in the intima at lesion-prone sites and undergo oxidative modification to generate oxidised LDL (OxLDL), which is a potent inflammatory mediator that triggers endothelial dysfunction (6, 7). Endothelial cells respond to OxLDL by expressing adhesion molecules such as ICAM-1 and chemokines including monocyte chemotactic protein-1 (MCP-1/CCL2) for recruitment of leukocytes (7, 8). Macrophages perform a protective role to metabolise lipids *via* scavenger receptors that internalise OxLDL and ATP-binding cassette (ABC) transporters A-1 and G-1 that mediate the efflux of OxLDL (9). However, imbalance of cholesterol influx and efflux results in the accumulation of lipids within macrophages, which contributes to foam cells formation (3, 9). Continuous low grade inflammation within the vessel wall subsequently progressively transforms a fatty streak into a fibro-fatty plaque, which is characterised by a fibrous cap covered by a necrotic core within the grossly thickened arterial intima (3, 10). The fibrous cap is formed by proliferating VSMC that migrate from the media, synthesising and releasing extracellular matrix to stabilise the plaque; whereas the necrotic core is formed by apoptotic macrophages/foam cells that have become exhausted by excessive lipid metabolism (3). Thinning of the fibrous cap is induced by inflammatory mediators triggering apoptosis of VSMC and the production of collagenolytic enzymes that degrade the collagen within the cap (11). Ineffective clearance of apoptotic cells contributes to secondary necrosis, releasing damage-associated molecular patterns (DAMP) to sustain the inflammation, thus enlarging the necrotic core (11). These features characterise the unstable symptomatic plaque that is susceptible to rupture, which results in the release of pro-thrombotic materials to cause intra-vascular thrombosis (10), which in medium sized vessels, such as the major coronary or cerebral vessels, becomes an obstructive atherothrombosis, causing ischaemia and eventual infarction of the tissue perfused by that vessel.

Plaque Phenotypes

Atherosclerotic plaque is classified into stable and unstable phenotypes (3). The stable atherosclerotic plaque is characterised by a thick fibrous cap covering a small necrotic core, which can withstand haemodynamic changes and stresses and is therefore less susceptible to rupture (3, 12). In contrast, the unstable atherosclerotic plaque that is prone to rupture is associated with a thin fibrous cap covering a large necrotic core (10).

IL-32

IL-32, formerly named natural killer cell transcript 4 (NK4), is constitutively produced by peripheral blood mononuclear (PBMC), epithelial and endothelial cells (13, 14). IL-32 consists of eight splice variants, however, only the IL-32α, IL-32β and IL-32γ isoforms have been extensively studied (15). An abundance of IL-32α is found in haematopoietic cells; whereas IL-32β and IL-32γ are the major isoform in endothelial cells and are the most active isoforms, respectively (13, 14, 16) (**Figure 1**).

Overexpression of IL-32 has been reported in rheumatoid arthritis (RA) (17) and Crohn's disease (18), as well as, in human symptomatic atherosclerotic plaques (19), compared to asymptomatic individuals (20). Interestingly, anti-inflammatory activity has been demonstrated in a murine model of asthma with allergic airways inflammation (21). Although the precise explanation for this apparent discrepancy in the activity of IL-32 remains unknown, it may be due to differences in inflammatory regulators between species and/or diseases.

IL-32 and Atherogenesis

IL-32 has been detected in human endothelial cells of atherosclerotic plaques (22) and different isoforms have been demonstrated to exhibit distinct functional roles (23). IL-32α is associated with the suppression of ICAM-1 and VCAM-1 expression on endothelial cells, resulting in attenuation of atherosclerotic lesions, with decreased leukocyte infiltration being observed following overexpression of IL-32α in the IL-32α tg *Apoe*$^{-/-}$ mouse model of atherosclerosis, suggesting that IL-32α is anti-inflammatory during atherogenesis (24). This is consistent with the finding that IL-32α enhances lipid accumulation and inhibits cholesterol efflux from ox-LDL-exposed THP-1 macrophages *via* the PPARγ-LXRα-ABCA1 pathway (25).

On the other hand, IL-32β promotes vascular inflammation, based on the observation of increased leukocyte adhesion on endothelial cells following overexpression of IL-32β in a transgenic mouse model of atherosclerosis (26), perhaps *via* upregulation of ICAM-1/VCAM-1 expression by IL-32β, as observed on human umbilical vein endothelial cells (HUVECs) following IL-32β stimulation (27). In addition, IL-32 regulates the function of endothelial cells within the aortic, coronary and pulmonary circulations, via IL-1β and other pro-inflammatory cytokines, particularly regulating I-CAM (27).

Thus, taken together, these data support the hypothesis that atherosclerotic development is accelerated by unbalanced expression of IL-32α and IL-32β facilitating vascular inflammation.

Furthermore, IL-32β and IL-32γ have been detected in macrophages of human atherosclerotic plaques, while IL-32γ is associated with greater MCP-1/CCL2 production from monocytic THP-1 cells, suggesting that IL-32γ amplifies local inflammation *via* recruitment of monocytes/macrophages (20). These data are consistent with the finding that IL-32γ enhances monocytes differentiation into macrophage-like cells (28), suggesting that IL-32γ is important for the regulation of the host response against antigens that the immune system detects within atherosclerotic plaques.

It is well known that macrophage heterogeneity is involved in atherogenesis, which consists of pro-inflammatory M1 and anti-inflammatory M2 macrophages (29). Interestingly, M2 macrophages shift towards a pro-atherogenic profile when in a

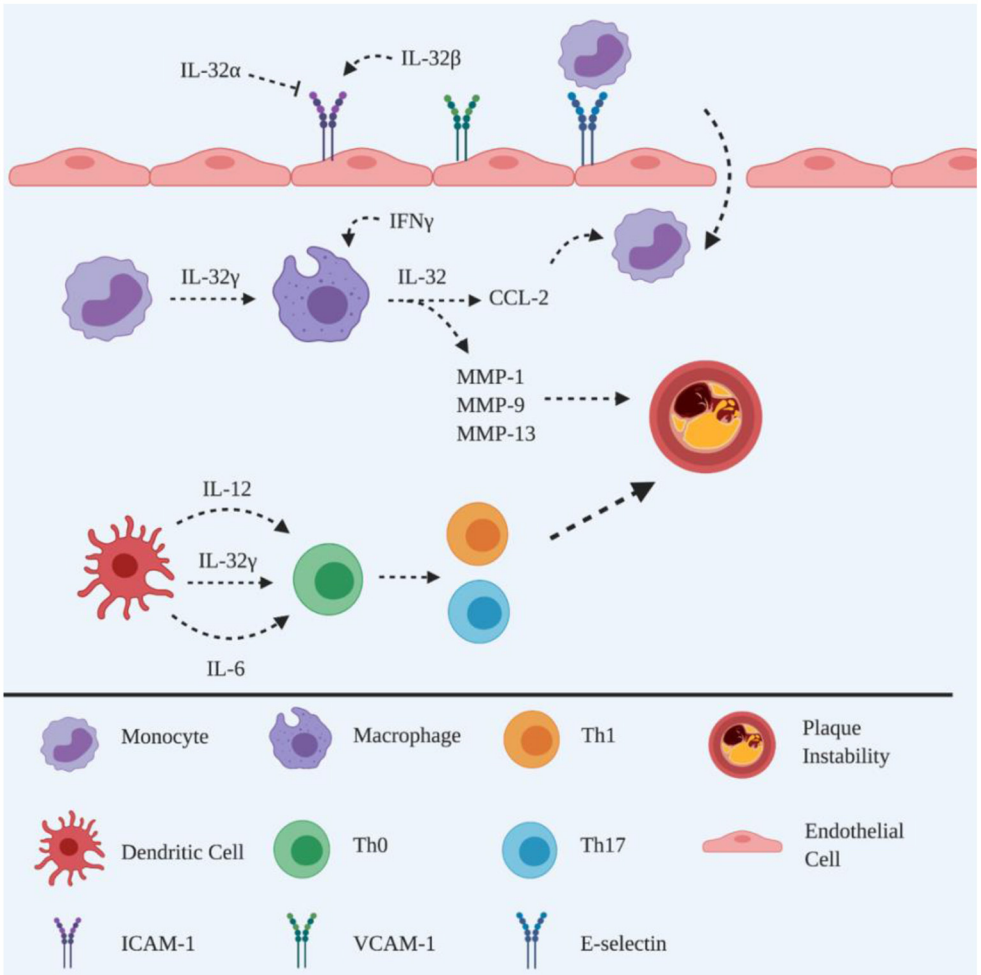

FIGURE 1 | Schematic representation of the roles of IL-32 in atherogenesis. Adhesion molecules are promoted by IL-32β to facilitate monocyte recruitment, whereas recruitment can also be inhibited by IL-32α. The differentiation of monocytes into phagocytic macrophages is induced by IL-32γ, which in turn triggers the release of CCL-2 to recruit circulating monocytes. IL-32γ induces the maturation of DCs, releasing IL-12 and IL-6 to polarise naïve $CD4^+$ T cells into Th1 and Th17 subsets. IL-32γ induces macrophages to produce MMPs leading to atherosclerotic plaque instability. Created with BioRender.com.

pro-inflammatory micro-environment, as reported by the finding that M2 macrophages transform into foam cells *via* upregulation of scavenger receptor CD36 to internalise OxLDL at a higher capacity than M1 macrophages, following their exposure to OxLDL (30). In relation to the IL-32s, M2 rather than M1 macrophages demonstrate a significant upregulation of IL-32 expression in the presence of IFNγ, suggesting that IL-32 is an effector molecule mediating pro-atherogenic responses in the presence of pro-inflammatory stimuli (20). Since IL-32β is a less bioactive form, the upregulation of IL-32β in macrophages may be a form of reverse regulation that is generated by the alternative splicing of the IL-32γ transcript to reduce the overall pro-atherogenic effect (20).

The maturation of murine dendritic cells (DC) is promoted in the presence of rhIL-32γ (31). Specifically, rhIL-32γ increases the production of IL-12 and IL-6 in murine DCs, promoting the polarisation of $CD4^+$ T cells into Th1 and Th17 subsets, accompanied by increased production of IFNγ and IL-17, respectively (31). This is an important mechanism in atherogenesis, in which IFNγ destabilises atherosclerotic plaques *via* the inhibition of VSMC proliferation leading to a thin fibrous cap (10). It is the degradation of the extracellular matrix, i.e., collagen, by matrix metalloproteinases (MMP) that causes thinning of the fibrous cap (3), which can be promoted by IL-32γ *via* increasing the secretion of MMP-1, MMP-9 and MMP-13 from macrophages (20). These data suggest that IL-32 contributes to plaque instability, which supports the finding of a strong correlation between IL-32 and symptomatic plaque phenotype in human atherosclerosis (19).

However, the more controversial role of IL-32, i.e., its anti-inflammatory role, has also been reported. It is well accepted that disruption of the removal of excessive cholesterol in the arterial wall is important in atherogenesis (2), which is regulated by the reverse cholesterol transport (RCT) mechanism *via*

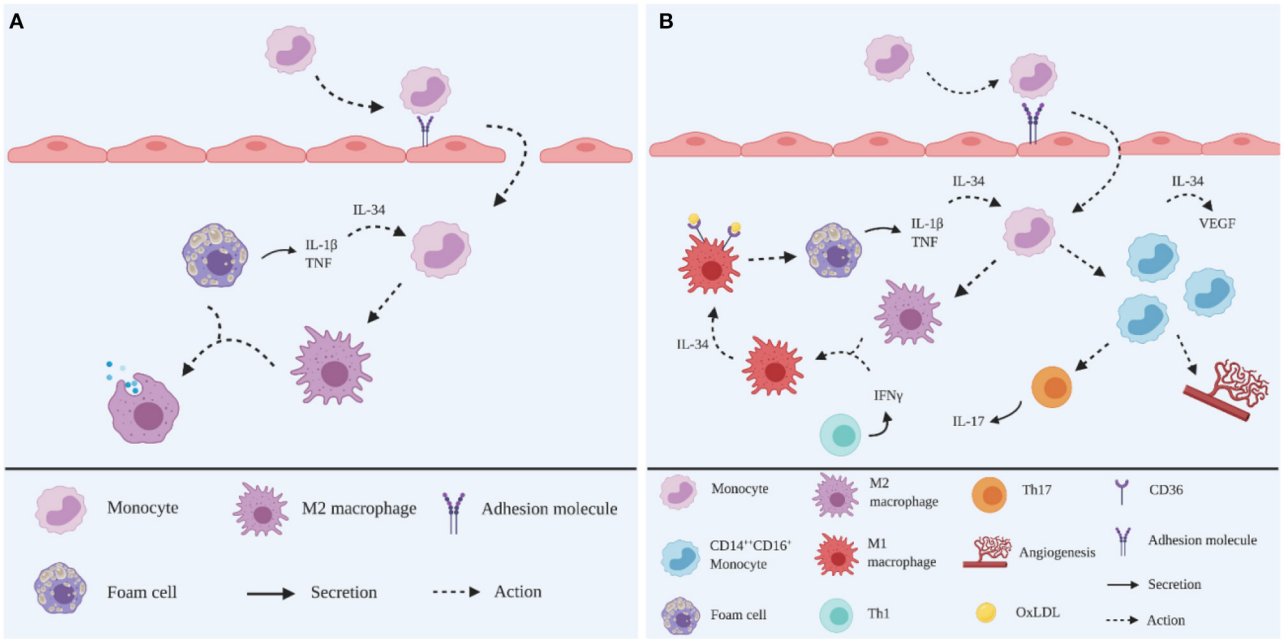

FIGURE 2 | Schematic representation of the roles of IL-34 in atherogenesis. **(A)** In the early stage, TNF and IL-1β produced in the plaque microenvironment stimulate IL-34 production. Infiltrated monocytes are induced by IL-34 to differentiate into M2 macrophages to dampen the inflammatory responses by digesting OxLDL. **(B)** In the advanced stage, IFNγ produced from overwhelming inflammation skews M2 macrophages into an M1 phenotype. These M1 macrophages are induced by IL-34 to upregulate scavenger receptor CD36 to ingest OxLDL, leading to foam cell formation. IL-34 induces the expansion of $CD14^{bright}CD16^{+}$ monocytes subpopulations, increasing Th17 polarisation and angiogenesis, together with increased VEGF production. Created with BioRender.com.

high density lipoproteins (HDL) transporting cholesterol to the liver for excretion (32). Increased HDL is associated with ameliorated human coronary atherosclerosis (32). Interestingly, increased HDL has been associated with an IL-32 promoter single nucleotide polymorphism (SNP) in rheumatoid arthritis patients (33), implying an anti-inflammatory role of IL-32 in CVD (33). This is supported by the findings that cholesterol is eliminated *via* ABCA-1, which can be induced by intracellular IL-32γ in hepatocytes (34). In the same study, both IL-32γ and ABCA-1 mRNA have been found in human carotid artery plaques (34). However, this relationship remains to be clarified, since this study did not show that IL-32γ and ABCA-1 can be colocalised *in vivo* in macrophages.

Taken together, the role of IL-32 during the development of atherosclerosis remains to be elucidated. However, we speculate that IL-32 acts differently in different stages of atherogenesis, perhaps depending on the different stimuli occurring within the plaque at various stages of development, based on the data described above. The precise underlying mechanism of IL-32 in atherogenesis, particularly in the presence of M1 vs M2 macrophages warrants further study.

IL-34

IL-34 is a haematopoietic cytokine that shares similar functions with CSF-1/M-CSF, to maintain the viability of the myeloid cells lineage (35). Overexpression of IL-34 is associated with autoimmune diseases, such as RA (36), inflammatory bowel disease (IBD) (37) and Sjogren's syndrome (38). Upregulated IL-34 is also detected in human atherosclerotic plaques, particularly correlating with unstable plaques (19), suggesting that the pro-inflammatory activities of IL-34 in the advanced stages of plaque development may contribute to acute coronary syndrome and premature death (39). In addition, a substantial circulating IL-34 level has been detected in CAD patients and is associated with the severity of comorbid CAD in heart failure (40, 41) (**Figure 2**).

Roles in Atherogenesis

IL-34 upregulates the scavenger receptor CD36 on murine bone-marrow derived macrophages to promote foam cell formation *via* the internalisation of OxLDL *in vitro* (42). In addition, IL-34 increases the mRNA expression of IL-1β, IL-6 and TNF in murine bone-marrow derived macrophages *in vitro* in the presence of OxLDL (42). These observations are consistent with the finding that IL-34 can elevate the production of chemokines and cytokines, including IL-6, in human PBMC (43). Moreover, IL-34 is upregulated in the presence of TNF and IL-1β (36, 38), suggesting IL-34 may act as a pro-atherogenic factor in both a paracrine and autocrine fashion to enhance foam cell formation in the plaque microenvironment.

Angiogenesis, which is known to promote plaque growth, is promoted in the presence of IL-34 *in vitro* (44, 45). Human PBMCs produce a significant level of VEGF in response to recombinant human (rh) IL-34 (45). Additionally, it is increasingly recognised that monocytes are classified

into different subsets based on phenotypic characteristics and have distinct roles during the inflammatory response of atherosclerosis (46), including in relation to angiogenesis. Briefly, these subsets are: classical $CD14^{bright}CD16^{-}$, intermediate $CD14^{bright}CD16^{+}$ and non-classical $CD14^{dim}CD16^{+}$ monocytes, of which the intermediate $CD14^{bright}CD16^{+}$ monocytes are pro-atherogenic (46). It has also been shown that $CD14^{bright}CD16^{+}$ monocytes express vascular growth factor receptor-2 (VEGFR2) and respond to VEGF, suggesting a pro-angiogenic property (47). Since $CD14^{bright}CD16^{+}$ monocytes are abundantly detected in CAD patients (48), it is reasonable to speculate that IL-34 may promote angiogenesis *via* $CD14^{bright}CD16^{+}$ monocytes stimulation.

In addition, IL-34 induces Th17 polarisation, as evidenced by an increased Th17 cell population following the coculture of IL-34 treated macrophages and naïve $CD4^{+}$ T cells (49). In the presence of IL-34, Th17 polarisation is promoted *via* upregulating IL-6 from human fibroblast-like synoviocytes (50). IL-23 has been shown to be produced by $CD14^{bright}CD16^{+}$ monocytes to induce Th17 polarisation *in vitro* (51). These observations correlate with the high expression of IL-34 in Sjogren's syndrome, in conjunction with an increased expression of IL-17 and IL-23 *in vivo*, suggesting that IL-34 may be linked to the IL-23/Th17 axis (38). Thus, it is reasonable to speculate that IL-34 induces Th17 polarisation during atherogenesis.

In contrast, IL-34 also exhibits an anti-inflammatory capacity. Human monocytes have been shown to differentiate into M2 macrophages in response to IL-34 *in vitro* (44, 52). Interestingly, M2 macrophages that are differentiated in the presence of IL-34, skew towards a pro-inflammatory M1 phenotype in response to IFNγ (52). This finding suggests that IL-34 plays an immunoregulatory role in the early stage of atherogenesis by inducing M2 macrophages to dampen the inflammatory responses and tissue remodelling. This is supported by the report from Boulakirba et al., showing IL-34 promotes M2 polarisation (53).

However, subsequently these M2 macrophages skew towards an M1 phenotype in response to increased IFNγ, which results from overwhelming inflammation in the plaque microenvironment.

Taken together, the role of IL-34 in atherogenesis remains ambiguous due to the complexity of the immune system. However, it is reasonable to suggest that the differential role of IL-34 in different stages of atherogenesis may depend on the specific anti-inflammatory or pro-inflammatory microenvironment in the early or advanced stages of atherogenesis.

IL-37

IL-37 is an anti-inflammatory cytokine member of the IL-1 family (54, 55). IL-37 is constitutively expressed by immune cells including macrophages and DCs, as well as epithelial cells, and is upregulated in response to pro-inflammatory stimuli such as cytokines and TLR ligation (55). IL-37 functions through a heterodimeric receptor, which is composed of IL-18Rα and IL-1R8 (55). Elevated IL-37 expression is detected in autoimmune diseases such as RA (56) and IBD (57). Elevated IL-37 expression has also been observed in a murine model of atherosclerosis (58) as well as in plasma from acute coronary syndrome patients (59).

IL-37 in Atherogenesis

IL-37 Host Immunity Mediated Atherogenesis

The activity of IL-37 was initially suggested to be pro-atherogenic because high levels of IL-37 are detected in foam cells within atherosclerotic plaques (59). However, interestingly, treatment with recombinant IL-37 has been shown to ameliorate the size of atherosclerotic plaque in diabetic $Apoe^{-/-}$ mice, and is associated with increased anti-inflammatory IL-10, but not pro-inflammatory TNF or IL-18 (60). This striking finding is further supported by another study, showing that plaque size is reduced in IL-37 tg $Apoe^{-/-}$ mice (61) and bone marrow transplanted $Ldlr^{-/-}$ mice with increased endogenous IL-37 expression (62). Moreover, IL-37 reduces atherogenesis *via* decreasing circulating pro-inflammatory and increasing anti-inflammatory cytokines in IL-37 tg $Apoe^{-/-}$ mice (63) and IL-37 treated $Apoe^{-/-}$ mice (58).

Human coronary artery endothelial cells that have been transfected with IL-37 demonstrate downregulation of ICAM-1 in the presence of TLR2 ligand stimuli *in vitro* (64). IL-1β, which is known to upregulate adhesion molecules, is reduced in the presence of IL-37 in OxLDL-treated macrophages *in vitro* (62). These findings, in conjunction with evidence of reduced production of TNF and IL-1β, as well as reduced leukocytes infiltration, in the inflamed colon of IL-37 tg mice with colitis (65), suggest that IL-37 reduces leukocytes recruitment *via* downregulation of TNF and IL-1β during atherogenesis. Furthermore, IL-37-expressing mouse bone marrow-derived macrophages not only reduce uptake of OxLDL, but also decrease macrophage transmigration towards MCP-1 (62). These findings suggest that IL-37 plays an anti-atherogenic role *via* a negative regulatory mechanism to dampen the inflammation in atherosclerosis, perhaps by reducing foam cell formation, pro-inflammatory cytokines, as well as macrophage infiltration. The anti-inflammatory function of IL-37 during atherosclerosis is supported by data from others showing an inverse correlation between IL-37 and M1 macrophage polarisation in human calcified aortic valves (66), as well as in an animal atherosclerotic model (67), perhaps *via* suppressing M1 polarisation. However, while IL-37 reduces systemic inflammation, it does not influence atherosclerosis development in hyperlipidemic LDLr-deficient mice, which might be due to LDLr depletion (68). These mechanisms require future elucidation due to the potential for a major discrepancy between the human and murine context.

IL-37 functions in a dual fashion in DCs to maintain an anti-inflammatory environment by implementing its anti-inflammatory actions intracellularly or by being released as a regulatory cytokine (69). Isolated bone marrow-derived DCs from IL-37 tg mice generate a tolerogenic phenotype in the presence of LPS by downregulating MHC-II and the costimulatory molecule CD40 (70). The findings which show the downregulation of MHC-II and CD86 in DCs from rhIL-37 treated $Apoe^{-/-}$ mice (58) and IL-37 tg $Apoe^{-/-}$ mice (63) suggest that atherogenesis is attenuated *via* reduced antigen presentation (**Figure 3**).

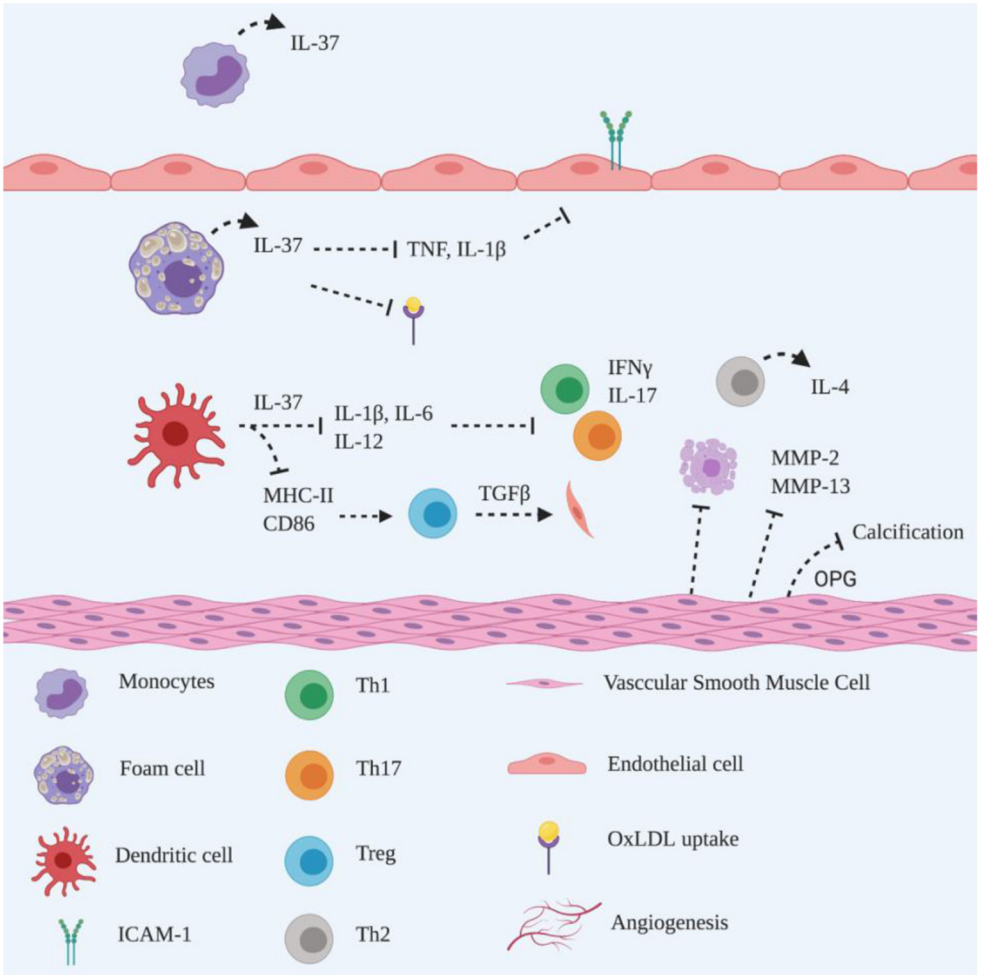

FIGURE 3 | Schematic representation of the roles of IL-37 in atherogenesis. IL-37 is constitutively expressed by monocytes in the unstimulated state. In pathological conditions, IL-37 is upregulated by foam cells to suppress pro-inflammatory cytokines secretion and reduce OxLDL uptake and adhesion molecules expression on endothelial cells. IL-37 downregulates MHC-II and CD86 on dendritic cells to induce Treg activation, promoting collagen deposition via TGFβ production. Additionally, IL-37 reduces IL-1β, IL-6 and IL-12 production, to suppress Th1 and Th17 polarisation accompanied by reduced IFNγ and IL-17 secretion. It remains unclear whether the Th2 population is induced by dendritic cells or IL-37 producing T lymphocytes. IL-37 triggers VSMC to reduce MMP-2 and−13 production, attenuating collagen degradation and inhibiting apoptosis. IL-37 functions closely with VSMC-derived OPG, inhibiting vascular calcification. Created with BioRender.com.

A reduction of Th1 cells is detected in rhIL-37 treated *Apoe*$^{-/-}$ mice (58) and IL-37 tg *Apoe*$^{-/-}$ mice (61), which is consistent with the observed reduction in Th1 cells in IL-37 treated splenic lymphocytes, which is accompanied by decreased IFNγ secretion (58, 61). However, there was no significant reduction of Th17 cells observed in the latter study (61), which suggests that IL-37 promotes Th polarisation during atherogenesis. T regulatory (Treg) cells play an athero-protective role in atherosclerosis *via* IL-10 inhibition of disease progression and TGFβ stimulation of collagen deposition to maintain plaque stability (10). The development of Treg cells is promoted in the presence of isolated bone marrow-derived DCs from IL-37 tg mice *in vitro* (70). This finding is supported by others, showing that Treg cells are increased in rhIL-37 treated *Apoe*$^{-/-}$ mice *in vivo* and increased production of TGFβ and IL-10 is induced during the coculture of CD4^{+} T cells with OxLDL plus IL-37-treated bone marrow-derived DCs (58). Interestingly, Th2 cells, but not Treg cells, together with IL-4, are abundant in IL-37 tg *Apoe*$^{-/-}$ mice (61), suggesting that different signalling mechanisms may be exerted by exogenous and/or endogenous IL-37. CD4^{+} T cells have been shown to be the major source of IL-37 in human atherosclerotic plaques (58, 61). Since Th1 cells shift towards Th2 cells in the presence of IL-37 *in vitro* (61), the hypothesis emerges that Th2 polarisation may be spontaneously induced by CD4^{+} T cell-derived IL-37 in the plaque microenvironment. These data are in line with others who have shown that IL-37 contributes to the anti-inflammatory response in the development of atherosclerosis, perhaps *via* enhancing Treg cells (71). Interestingly, elevated circulating and local IL-37 in atherosclerotic rabbits is suppressed

by atorvastatin (72), suggesting that atorvastatin dampens systemic and local inflammation, resulting in a reduction of IL-37.

IL-37 and Plaque Stability

It is recognised that plaque vulnerability is also promoted by VSMC apoptosis (73). IL-37 inhibits VSMC apoptosis, as evidenced by the reduced apoptotic VSMC area in atherosclerotic plaques of IL-37 tg *Apoe*$^{-/-}$ mice (61). Such findings are supported by attenuated atherosclerotic plaque in rhIL-37 treated *Apoe*$^{-/-}$ mice, showing a larger VSMC- and collagen-positive staining area than a mock treated group (58). An increased amount of collagen content, with reduced mRNA expression of MMP-2/-13 within atherosclerotic plaque has been observed in IL-37 tg *Apoe*$^{-/-}$ mice, compared to *Apoe*$^{-/-}$ mice only (61), suggesting that IL-37 plays an important role in maintaining plaque stability. VSMC proliferation is reparative and advantageous for atherogenesis in both early and advanced stages, to maintain plaque stability (74). As IL-37 is expressed by VSMC to maintain plaque stability in human atherosclerotic plaques (58, 61), it is reasonable to speculate that IL-37 also induces VSMC proliferation *via* an autocrine mechanism.

Vascular calcification is also one of the key features of atherosclerosis and serves as an independent predictor for acute coronary events (75). Spotty microcalcifications that are dispersed within the necrotic core and fibrous cap drive plaque instability (75). It is well recognised that calcification is driven by VSMC plasticity *via* trans-differentiation into osteoblast, chondrocyte and macrophage-like phenotypes in response to pro-inflammatory cytokines in atherosclerotic plaques, which release pro-calcific factors accompanied by a loss of calcification inhibitors (76). Reduced calcification in the aortic root has been observed in rhIL-37 treated *Apoe*$^{-/-}$ mice (60), which is consistent with findings in humans, where IL-37 is highly detected in calcified human aortic valve interstitial cells *in vivo*, as well as reduced calcification in calcified human aortic valve interstitial cells in the presence of rIL-37 *in vitro* (77). Osteoprotegrin (OPG), which is a calcification inhibitor, is highly detected in VSMCs of atherosclerotic plaques in rhIL-37 treated *Apoe*$^{-/-}$ mice (60). However, in the presence of anti-OPG antibody, increased calcified areas are observed, implicating a close relationship between IL-37 and OPG for calcification regulation (60). These finding are indirectly supported by the observation that IL-37 is abundantly detected in human calcified coronary arteries, particularly in VSMCs, compared to normal arteries, suggesting that the purpose of upregulation of IL-37 is to alleviate arterial calcification (78). In addition, a positive correlation between plasma IL-37 and OPG has been detected in patients with severe coronary artery calcification, suggesting that IL-37 is a potential biomarker of arterial calcification (79).

Since an effective treatment to mitigate vascular calcification remains undetermined (75, 76), investigation of the underlying mechanisms of IL-37 in VSMC may provide future therapeutic opportunities.

In addition elevated plasma IL-37 has been detected in acute ischemic stroke patients, and IL-37 is an independent association with poorer prognoses (80), which is consistent with others, showing elevated circulating IL-37 is associated with a poor outcome in ST-segment elevation acute myocardial infarction in acute coronary syndrome patients (81, 82), although this finding remains controversial (83).

Taken together, IL-37 plays an anti-atherogenic role in atherogenesis. Although the exact mechanism is not well understood, data support speculation that elevation of IL-37 expression is a compensatory mechanism to suppress plaque inflammation, however, inflammatory cells may fail to respond effectively to IL-37 due to exhaustion or the complex nature of the plaque microenvironment, resulting in a continuous release of ineffective IL-37. In relation to COVID-19, IL-37 has been suggested to be a potential treatment based on its anti-inflammatory profile to inhibit IL-1β, IL-6 and TNF, which are the main players of the cytokine storm (84).

CLINICAL IMPLICATIONS OF IL-32, IL-34 AND IL37 IN ATHEROSCLEROSIS

The role of IL-32 during the development of atherosclerosis has been illustrated, showing that IL-32 promotes angiogenesis on endothelial cells, suggesting IL-32 boosts the development of atherosclerosis (85). This is in line with others, showing that the protective role of IL-32 during the development of atherosclerosis is related to a single promoter single-nucleotide polymorphism (SNP) in IL-32, contributing to modified lipid profiles, especially in rheumatoid arthritis patients (33). Furthermore, the benefit of the SNP in IL-32 is related to reduce pro-inflammatory cytokines and increases HDLc concentration (15), further supporting the role of IL-32 during atherogenesis. This may also in line with the findings following influenza viral challenge, showing that increased IL-32 is beneficial against the viral infection (86).

The role of IL-34 during the development of atherosclerosis has been demonstrated, since there is an association between the level of IL-34 and severity of coronary artery disease in patients with heart failure, and IL-34 is an independent risk factor for CAD among heart failure patients, regardless of the systolic function (41). In addition, there is evidence from others, showing that IL-34 is significantly induced in influenza infected patients in an autocrine and paracrine fashion (87), supporting a role for IL-34 in the course of SARS-COV-2 viral infection. Furthermore, the possible mechanisms utilised by IL-34 in atherogenesis have been demonstrated via a linkage among IL-34, obesity, chronic inflammation, and insulin resistance, suggesting that IL-34 enhances atheroma *via* insulin resistance in obese patients (88).

Finally, increased circulating IL-37 levels have been correlated with high coronary calcium score levels, suggesting that IL-37 may contribute to the activation of inflammation. Furthermore, IL-37 has been proposed as a predictor of severe coronary artery disease (79). In addition, the importance of elevated serum and urine IL-37 has been demonstrated in post-ischemic stroke patients (89). However, it is unclear whether the increased IL-37 results from or results in such clinical manifestations. The possible mechanism of the anti-inflammatory role of IL-37

may be by antagonising inflammatory responses while retaining type I interferon, subsequently maintaining the functionalities of vital organs (90). The role of IL-37 in COVID-19 is supported by the findings in influenza viral infection, showing that IL-37 ameliorates influenza pneumonia *in vivo* (91). However, we have reviewed the mechanisms of action of IL-32, -34 and -37 in atherosclerosis, allowing us to speculate on the possible pathogenesis of SARS-CoV-2 involvement in CVD.

SPECULATIVE ROLE OF IL-32, IL-34 AND IL-37 IN ATHEROSCLEROSIS AND COVID-19

COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (92), which is similar to severe acute respiratory syndrome coronavirus (SARS-CoV) (92) and Middle East respiratory syndrome coronavirus (MERS-CoV) (93). SARS-CoV-2 infects host cells by binding to the cell surface receptor angiotensin converting enzyme 2 (ACE2) receptor *via* the viral spike (S) protein (92). The original COVID-19 was first reported in Wuhan (94), then other regions of China (95, 95) and the became a pandemic (96).

Based on the current information available, during the course of COVID-19, particularly in moderate to severe COVID-19 patients, there is likely to be a contribution of COVID-19 in atherosclerosis, perhaps due to the cytokine storm causing vascular dysfunction via the ACE2 pathway, which likely further enhances local inflammation (97) and subsequently results in further activation of endothelial cells in large vessels (98), in addition to the microvascular system. Such insults from the cytokine storm also contribute to hyper-coagulation (99), but this will not be discussed further in the current review.

The role of IL-32 may be induced in local macro-vessels and micro-vessels, which may be due to SARS-COV-2 viral challenge via the ACE2-spike protein pathway. IL-32 may contribute to quench both systemic and local inflammation, which may be effective in moderate COVID-19 patients, but likely fails in severe patients. Subsequently, major organ failure would be induced due to infarction, e.g., heart, lung and kidney (100), particularly in the more susceptible COVID-19 patients. This speculation is supported by others, who have shown that steroids may help to reduce clinical symptoms and shorten the course of COVID-19 (101).

In contrast, IL-34 may contribute to atherosclerosis, but its role in COVID-19 remains unclear. We believe that IL-34 would be secreted by infiltrating inflammatory leucocytes, particularly macrophages and lymphocytes following the cytokine storm in COVID-19 patients (102). More obvious vascular manifestations would then result.

It has been reported that circulating IL-37 is elevated in COVID-19 infected patients. Interestingly, the patients with higher IL-37 had a shorter hospitalisation period than the lower group, suggesting that IL-37 may provide protection during the course of COVID-19 infection (90).

However, there is not yet any solid evidence to clearly state the direct involvement among IL-32, 34 and 37 in the atherogenesis in COVID-19 patients.

In addition there is a strong association between cardiovascular disease (CVD) and the susceptibility to, and the outcomes of, COVID-19 (103), including coronary artery disease (CAD), particularly among those patients with co-existing diabetes mellitus (104). Patients with pre-existing CVD, including hypertension, coronary artery disease (CAD) and diabetes mellitus are more susceptible to SARS-CoV-2 infection and are more likely to develop exaggerated cardiovascular sequelae (105), hence there is a higher prevalence of severe disease in the elderly population (106). A major contributing factor to the higher susceptibility among patients with pre-existing CVD is the higher levels of cell surface expression of ACE2, which makes the patients more vulnerable to SARS-CoV-2 viral infection (106, 107). Additionally, a small proportion of young adults without pre-existing CVD also develop cardiovascular complications following SARS-CoV-2 infection (108), which may be related to their exaggerated host immunity (cytokine storm) (109). One of the key contributing factors for the higher mortality and morbidity in COVID-19 patients is excess local production of pro-inflammatory cytokines, such as IL-1β, IL-6, IL-8 and TNF in key organs (heart, lungs and liver) (110–112), which is termed a cytokine storm (113). Consequently, substantial damage occurs in the heart, lungs, liver and kidneys, which contributes to the disease severity in COVID-19 patients (110). Although the underlying mechanism of SARS-CoV-2 viral attack is not well understood, these findings above suggest that a relationship exists between COVID-19 and CVD outcomes that is both bidirectional and multifactorial (106, 114). Thus, it is reasonable to speculate that many COVID-19-related heart problems are due to a cytokine storm, either in the heart or major arteries (115).

Interestingly, there is some limited data emerging in the literature supporting the view that COVID-19 may increase the rate of acute plaque rupture (116, 117). Respiratory infections such as influenza are known to be capable of triggering acute coronary syndrome (118), so it is likely that COVID-19 will act in a similar manner. A recent case report of an ACS event during COVID-19 infection supports this likelihood (116). Similarly, the likely mechanisms underpinning increased plaque instability during COVID-19 infection have been explored (107, 117).

CONCLUSION

We conclude that IL-32 provides athero-protection *via* differential regulation of polarisation of macrophages in different stages of atherogenesis, perhaps depending on the different stimuli occurring within the plaque at various stages of development. Subsequently IL-32 down-regulates the activities of CCL-2 and MMPs, and finally ABCA1 pathway

IL-34 is pro-atherogenic and its role is stage dependent. In the early stage, recruited monocytes are induced by IL-34 to differentiate into M2 macrophages to dampen the inflammation in the presence of stimuli, e.g., OxLDL, in

an autocrine and paracrine fashion. In the advanced stage, particularly in some SNP populations, macrophages are skewed towards the M1 phenotype, especially in the presence of a large amount of IFNγ. IL-34 induced M1 macrophages upregulate scavenger receptor CD36 to ingest OxLDL, leading to foam cell formation. Subsequently, IL-34 induces the expansion of $CD14^{bright}CD16^{+}$ monocytes subpopulations, further boosting the pro-inflammatory responses, including increasing Th17.

IL-37 is also athero-protective. Constitutively expressed IL-37 can be upregulated by foam cells to dampen proinflammatory cytokines secretion, reduce OxLDL uptake and adhesion molecules expression on endothelial cells, as well as downregulate MHC-II and CD86 on dendritic cells to induce Treg activation *via* TGFβ production. In addition, IL-37 reduces IL-1β, IL-6 and IL-12 to suppress Th1/Th17 polarisation, and subsequently down-regulates IFNγ and IL-17 secretion. IL-37 also reduces MMPs on VSMC and attenuates collagen degradation and inhibits apoptosis. Finally, IL-37 inhibits vascular calcification via VSMC-derived OPG.

Finally IL-32 and IL-37 may be protective while IL-34 may contribute to the development of atherosclerosis. In addition, we speculate that the role of IL-32 and 37 may also be beneficial, but IL-34 may be harmful, during the course of COVID-19. Such information highlights gaps in our current understanding for future studies to investigate. Our figures offer a very dynamic summary of these cytokines during the development of atherosclerosis. We believe that our review provides more in-depth information for both basic scientists and clinicians.

AUTHOR CONTRIBUTIONS

CL: conceptualised, drafted, and wrote the manuscript. RP and JFa: conceptualised. JFe: revised the manuscript. BH: revised and edited the manuscript. SB: conceptualised, drafted, and edited the manuscript. All authors contributed to the article and approved the submitted version.

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SYSTEMATIC REVIEW
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The Predictive Value of Myoglobin for COVID-19-Related Adverse Outcomes: A Systematic Review and Meta-Analysis

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Objective: Cardiac injury is detected in numerous patients with coronavirus disease 2019 (COVID-19) and has been demonstrated to be closely related to poor outcomes. However, an optimal cardiac biomarker for predicting COVID-19 prognosis has not been identified.

Methods: The PubMed, Web of Science, and Embase databases were searched for published articles between December 1, 2019 and September 8, 2021. Eligible studies that examined the anomalies of different cardiac biomarkers in patients with COVID-19 were included. The prevalence and odds ratios (ORs) were extracted. Summary estimates and the corresponding 95% confidence intervals (95% CIs) were obtained through meta-analyses.

Results: A total of 63 studies, with 64,319 patients with COVID-19, were enrolled in this meta-analysis. The prevalence of elevated cardiac troponin I (cTnI) and myoglobin (Mb) in the general population with COVID-19 was 22.9 (19–27%) and 13.5% (10.6–16.4%), respectively. However, the presence of elevated Mb was more common than elevated cTnI in patients with severe COVID-19 [37.7 (23.3–52.1%) vs.30.7% (24.7–37.1%)]. Moreover, compared with cTnI, the elevation of Mb also demonstrated tendency of higher correlation with case-severity rate (Mb, $r = 13.9$ vs. cTnI, $r = 3.93$) and case-fatality rate (Mb, $r = 15.42$ vs. cTnI, $r = 3.04$). Notably, elevated Mb level was also associated with higher odds of severe illness [Mb, OR = 13.75 (10.2–18.54) vs. cTnI, OR = 7.06 (3.94–12.65)] and mortality [Mb, OR = 13.49 (9.3–19.58) vs. cTnI, OR = 7.75 (4.4–13.66)] than cTnI.

Conclusions: Patients with COVID-19 and elevated Mb levels are at significantly higher risk of severe disease and mortality. Elevation of Mb may serve as a marker for predicting COVID-19-related adverse outcomes.

Prospero Registration Number: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020175133, CRD42020175133.

Keywords: COVID-19, myoglobin, cardiac troponin I, predictive value, severe illness, mortality

INTRODUCTION

Coronavirus disease 2019, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in Wuhan City, Hubei province of China in December 2019 (1). The pandemic spread rapidly worldwide from China, resulting in 230 million confirmed cases and more than 4 million deaths by September 22, 2021. Clinical manifestations differ greatly among patients with coronavirus disease 2019 (COVID-19), ranging from asymptomatic infections to severe or critical disease and even death (2). Although SARS-CoV-2 was initially thought to be a respiratory tract virus, it has been widely reported that the adverse prognosis of patients with COVID-19 relates largely to the involvement of multisystem organs such as the heart, liver, kidney, brain, and the nervous system (3–5).

Cardiac injury, manifested as the elevation of cardiac biomarkers, namely, cardiac troponin I (cTnI), lactate dehydrogenase (LDH), creatine kinase (CK), CK isomer-MB (CK-MB), myoglobin (Mb), and B-type natriuretic peptide (BNP) or N-terminal pro-B type natriuretic peptide (NT-proBNP), has been detected in numerous patients with COVID-19, and is closely related to the clinical prognosis (6–9). In particular, elevation of cTnI, which was widely reported in several studies, has been identified as an independent variable associated with in-hospital mortality (10).

Nevertheless, elevation of Mb in patients with COVID-19 has been widely mentioned in several studies (11–15). More importantly, Mb presents a potential predictive value in COVID-19-related adverse outcomes. In a study reported by Qin et al., elevated Mb presented with higher frequency on admission and showed the highest overall performance for predicting the risk of COVID-19 mortality among the various cardiac biomarkers (16). However, to the best of our knowledge, a pooled analysis regarding the advantage of Mb in predicting the prognosis of COVID-19 is lacking. Therefore, we conducted a systematic review and meta-analysis to explore the predictive value of elevated Mb for adverse outcomes of patients with COVID-19.

METHODS

Study Protocol

This study was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and Meta-analysis of Observational Studies in Epidemiology (MOOSE) reporting guidelines (17, 18). The protocol was preregistered in the International prospective register of systematic reviews (PROSPERO, CRD42020175133). The detailed definitions of laboratory-confirmed COVID-19 cases and severe illness are described in **Supplementary Method S1**.

Search Strategy and Study Selection

Two investigators (DT and JG) independently searched the PubMed, Embase, and Web of Science Core Collection (Clarivate Analytics) databases for relevant articles published between December 2019 and September 8, 2021 using the following keywords: "coronavirus," "nCoV," "HCoV," "SARS-CoV-2," "COVID*," "NCP*," "cardiac injury," "cardiac," "biomarker*," "myocardial," "heart," "troponin," and "myoglobin" alone and in combination. The detailed search strategies are presented in **Supplementary Methods S2**. After removing duplicate studies, three reviewers (CM, DT, and JG) were assigned to independently screen the titles and abstracts, and then examine the full texts. Any disagreement was resolved by the senior authors (YB and XZ). The inclusion criteria were as follows: (1) diagnosis of COVID-19 according to the World Health Organization interim guidance (19), (2) gives the specific number of COVID-19 patients with the elevation of cTnI and/or Mb, (3) studies in English only, and (4) sample size of $\geq$10 individuals. The exclusion criteria were as follows: (1) studies with data that could not be reliably extracted, and (2) editorials, comments, expert opinions, case reports.

Data Extraction and Quality Assessment

Using a predesigned spreadsheet, three authors (DT, CM, and JG) independently extracted the relevant data from the included studies. Corresponding authors were asked via email to clarify or provide additional information. Study quality assessments were performed using the Quality Assessment Forms recommended by the Agency for Healthcare Research and Quality (AHRQ) for cross-sectional studies (**Supplementary Methods S3**). Studies were defined as high quality if a score of $\geq$7 was attained. Any conflicts with the assessments were resolved either by consensus or by the adjudicators (XZ and PL).

Statistical Analysis

Effect estimates were presented as pooled prevalence or odds ratio (OR) with 95% confidence interval (CI) and visualized with forest plots. A fixed or random-effects model was used according to heterogeneity across studies (if I^2 $\leq$50%, fixed-effects model; if I^2 >50%, random-effects model) (20). We performed Egger's test and the test performed by Peters et al., and visually inspected the funnel plots to investigate publication bias (21). Sensitivity analyses were performed by systematically removing each study in turn to explore its effect on the outcome. All the analyses were performed using R (version 3.5.3), RStudio (version 1.2.1335), and Comprehensive Meta-Analysis.

Patient and Public Involvement

Patients or the public were not involved in the design, conduct, reporting, and dissemination plans of our research.

RESULTS

Literature Search and Study Characteristics

A total of 106,925 articles were initially retrieved, of which the full texts of 6,542 articles were reviewed (**Figure 1**). Finally, 63 studies were eligible for our analysis (**Table 1** and **Supplementary Tables S1**, **S2**), and included 64,319 confirmed patients with COVID-19 who presented to a hospital. All these studies were retrospective observational ones. Of the 63 studies, 31 were conducted in China, 18 in the United States, 5 in Italy, 4 in Spain, 2 in Turkey, and 3 in other countries (Libya, Finland,

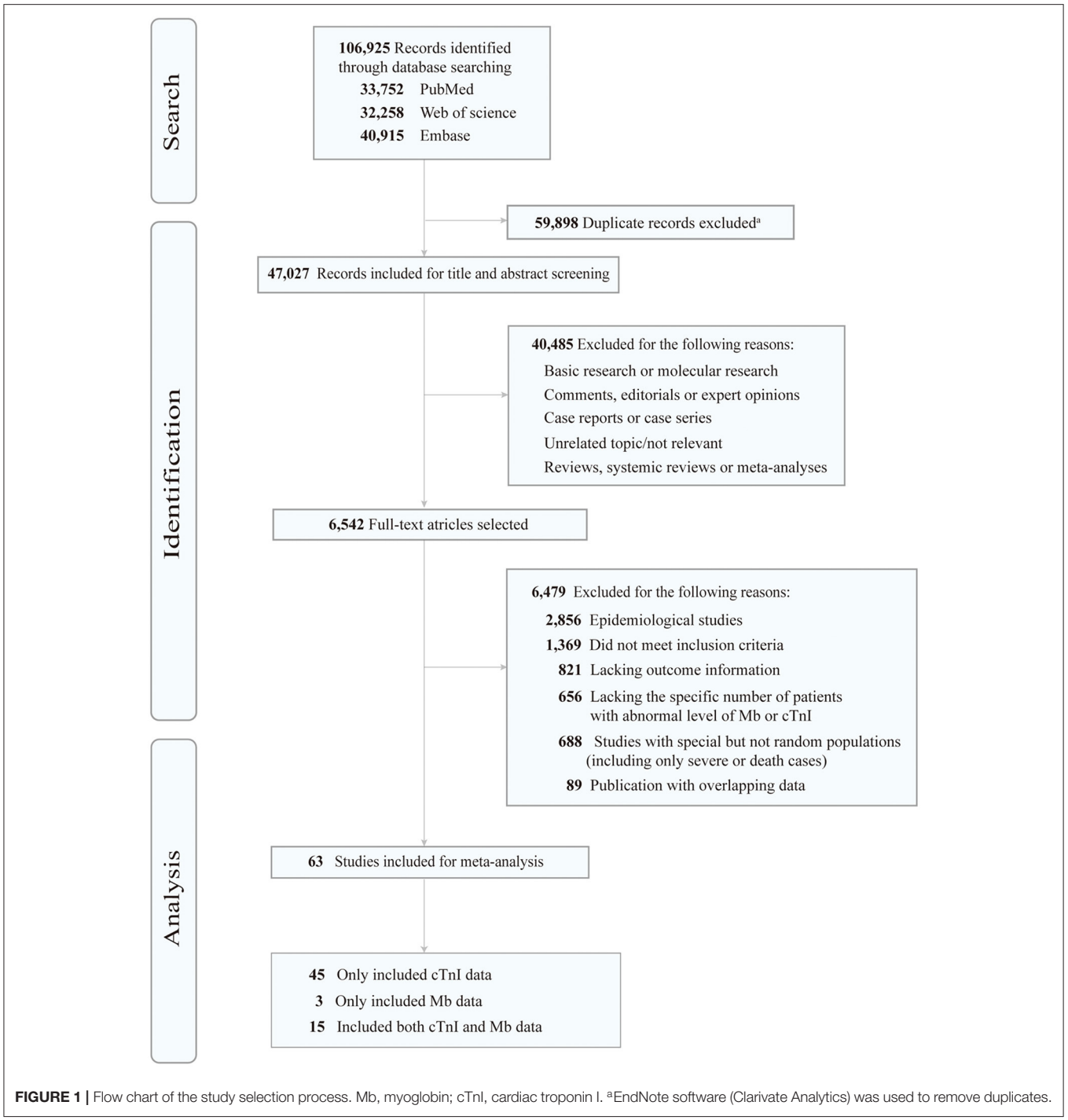

FIGURE 1 | Flow chart of the study selection process. Mb, myoglobin; cTnI, cardiac troponin I. [a]EndNote software (Clarivate Analytics) was used to remove duplicates.

and Iran) (**Supplementary Table S2**). Among them, 45 studies only mentioned data of cTnI, 3 studies only mentioned Mb, and 15 studies included both Mb and cTnI. Regarding the differences in Mb or cTnI detection methods and criteria among different hospitals, we listed in **Table 1** the average level of Mb or cTnI, cut-off value of abnormal Mb or cTnI, and number of patients with elevated Mb or cTnI in each study. In addition, preexisting cardiovascular conditions, such as the prevalence of coronary artery disease (CAD) and heart failure (HF), and the average level of BNP or NT-proBNP were also summarized (**Table 1**).

Incidence of cTnI/Mb Elevation

Among the 63 included studies, the pooled case-severity rate (CSR), case-fatality rate (CFR), and intensive-care unit (ICU)-admission rate were 31.3 (95% CI 23.2–39.4%, $I^2 = 99\%$), 12.5

TABLE 1 | Characteristics of the included studies.

Authors	No.	Cardiovascular condition	Mb	cTnI	Outcome
Arcari L et al.	111	CAD, 12 (11.0); HF, 8 (7.0)	NA	Average level of cTnI, 17 (5–47) pg/mL; cut-off value, 14 pg/ml; elevated patients, 39/103 (37.9%)	Death
Bardaji' A et al.	186	CAD, 20 (10.8); HF, 14 (7.5)	NA	Elevated patients, 41 (22.0%)	Death, admission to ICU
Bhatla A et al.	700	CAD, 76 (11.0); HF, 88 (13.0); BNP, 2,940 (7,962) pg/mL	NA	Cut-off value, 0.01 ng/mL; elevated patients, 82/373 (22.0%)	NA
Cai Q et al.	298	CAD, 25 (8.4); HF, 7 (2.3)	Average level of Mb, 37.1 (29.2–51.5) μg/L; elevated patients, 10/260 (3.8%)	NA	Death, discharge
Calvo-Fernández A et al.	872	CAD, 59 (6.83); HF, 41 (4.73)	NA	Cut-off value, 14.0 ng/L; elevated patients, 225/651 (34.6%)	Death, admission to ICU, mechanical ventilation
Cao J et al.	102	CAD, 5 (4.9); BNP, 12.2 (0–63.1) pg/mL; NT-pro BNP, 417 (132–1,800) pg/mL	NA	Average level of cTnI, 8.0 (3.0–35.7) pg/mL; cut-off value, 0.026 ng/mL; elevated patients, 15/55 (27.3%)	Discharge, death
Cao J et al.	244	NA	Average level of Mb in severe patients, 39.35 (29.21–74.19) μg/L; Cut-off value, 110 μg/L	Cut-off value, 0.04 ng/mL; elevated patients, 27/244 (11.1%)	Severe COVID-19, death, mechanic ventilation
Cao M et al.	198	CAD, 12 (6.0)	Average level of Mb, 5.9 (2.8–15.7) μg/L; cut-off value, 48.8 μg/L; elevated patients, 33/194 (17.0%)	Average level of cTnI, 0.02 (0.01–0.04) ng/ml; cut-off value, 0.04 ng/mL; elevated patients, 22/194 (11.3%)	Severe COVID-19
Chen N et al.	99	CAD, 40 (40.0)	Average level of Mb, 49.5 (32.2–99.8) μg/L; cut-off value, 146.9 μg/L; elevated patients, 15 (15.2%)	NA	Discharge, death
Chorin E et al.	204	CAD, 25 (12.0); HF, 7 (3.0)	NA	Average level of cTnI, 0.02 (0.01–0.04) ng/Ml; cut-off value, 0.05 ng/mL; elevated patients, 84 (41.2%)	Death
Cipriani A et al.	109	CAD, 18 (17.0); HF, 16 (15.0%); BNP, 90 (22–262) pg/ml	NA	Average level of cTnI, 18.0 (7.0–96.0) ng/L; cut-off value, 32 ng/L for males, 16 ng/L for females; elevated patients, 46 (42.2%)	Death, admission to ICU, discharge
Deng Q et al.	112	CAD, 15 (13.4); HF, 6 (5.4); NT-pro BNP, 430.1 (100.6–2859.3) ng/L	NA	Average level of cTnI, 0.01 (0.00–0.14) ng/ml; cut-off value, 0.04 ng/mL; elevated patients, 42 (37.5%)	Severe COVID-19, death
Elhadi M et al.	1,207	CAD, 25 (2.1)	NA	Cut-off value, 26 pg/mL; elevated patients, 90/292 (30.8%)	Death, admission to ICU
Feng Y et al.	476	CAD, 38 (8.0); BNP, 40.85 (21.64–79.37) pg/ml	Average level of Mb, 18.85 (4.8–51.48) μg/L	Elevated patients, 86/384 (22.4%)	Death, discharge, severe COVID-19
Ferguson J et al.	72	NA	NA	Cut-off value, 0.055 ng/mL; elevated patients, 2/45 (4.4%)	Death, mechanical ventilation, admission to ICU
Ferrante G et al.	332	CAD, 49 (14.5); BNP, 72.5 (34.5–198.0) pg/mL	NA	Average level of cTnI, 11.4 (4.7–37.3) mg/L; cut-off value, 0.02 ng/L; elevated patients, 123 (37.0%)	Death, admission to ICU

(Continued)

TABLE 1 | Continued

Authors	No.	Cardiovascular condition	Mb	cTnI	Outcome
Franks C et al.	182	NA	NA	Cut-off value, 0.03 ng/mL; elevated patients, 80/143 (55.9%)	Death
García de Guadiana-Romualdo L et al.	1,280	CAD, 328 (25.6)	NA	Elevated patients, 344 (26.9%)	Death, admission to ICU
Garibaldi BT et al.	832	CAD, 266 (32.0); HF, 127 (15.0); NT-pro BNP 214 (45–960) pg/mL	NA	Elevated patients, 194/682 (28.4%)	Death, severe COVID-19
Guo T et al.	187	CAD, 21 (11.2); NT-pro BNP, 268.4 (75.3–689.1) pg/mL	Average level of Mb, 38.5 (21.0–78.0) μg/L	Elevated patients, 52 (27.8%)	Death
Han H et al.	273	NA	Cut-off value, 110 μg/L; elevated patients, 29/273 (10.6%)	Cut-off value, 0.04 ng/mL; elevated patients, 27/273 (9.9%)	Death, severe COVID-19
Harmouch F et al.	560	Vascular disease, 36 (6.4); HF, 54 (9.6)	NA	Cut-off value, 0.05 ng/mL; elevated patients, 97/482 (20.1%)	Death, mechanical ventilation, admission to ICU
He F et al.	288	CAD, 85 (29.5); BNP, 35 (13–117.5) pg/mL	Elevated patients, 8/276 (2.9%)	Cut-off value, 0.03 ng/mL; elevated patients, 22/190 (11.6%);	Death, admission to ICU
He X et al.	1,031	CAD, 83 (8.1); NT-pro BNP 124 (43–374) pg/mL	NA	Average level of cTnI, 5.3 (2.5–14.0) pg/Ml; elevated patients, 215 (20.9%)	Death
Hu L et al.	323	CAD, 41 (12.7)	NA	Cut-off value, 0.04 pg/mL; elevated patients, 68 (21.1%)	Death, severe COVID-19, mechanical ventilation
Huang C et al.	41	CAD, 6 (15.0)	NA	Average level of cTnI, 3.4 (1.1–9.1) pg/mL; cut-off value, 0.028 ng/mL; elevated patients, 5/41 (12.2%)	Death, severe COVID-19, discharge
Huang J et al.	98	CAD, 6 (6.0); BNP 119 (54–392) pg/mL	NA	Cut-off value, 0.0229 ng/Ml; elevated patients, 7 (7.1%)	Death, discharge, severe COVID-19
Huang R et al.	202	CAD, 5 (2.5)	NA	Elevated patients, 2/103 (1.9%)	Admission to ICU, mechanical ventilation, severe COVID-19
Karbalai Saleh S et al.	386	CAD, 97 (25.1)	NA	Cut-off value, 26 ng/L for males, 11 ng/L for females; elevated patients, 115 (29.8%)	Death, admission to ICU
Lala A et al.	2,736	CAD, 453 (16.6); HF, 276 (10.1)	NA	Cut-off value, 0.03 ng/mL; OR for in-hospital mortality, 1.75 (1.37–2.24); elevated patients, 985 (36.0%)	Death
Li C et al.	2,068	CAD, 182 (8.8); HF, 14 (0.7); NT-pro BNP 108 (36–370) pg/mL	Average level of Mb, 40.7 (28.4–73.8) μg/L; elevated patients, 174/1,554 (11.2%)	Average level of cTnI, 4.2 (1.9–11.0) pg/mL; elevated patients, 181 (8.8%)	Death, severe COVID-19
Li X et al.	548	CAD, 34 (6.2)	NA	Cut-off value, 15.6 pg/mL; elevated patients, 119 (21.7%)	Discharge, death, severe COVID-19
Maeda T et al.	181	CAD, 36 (19.9); HF, 24/180 (13.3)	NA	Elevated patients, 54 (29.8%)	Death
Majure D et al.	6,247	CAD, 833 (13.0); HF, 529 (9.0)	NA	Cut-off value, 0.045 ng/mL; elevated patients, 1,821 (29.1%)	Death, admission to ICU, mechanical ventilation

(Continued)

TABLE 1 | Continued

Authors	No.	Cardiovascular condition	Mb	cTnI	Outcome
Manocha KK et al.	446	CAD, 94 (21.1); HF, 38 (8.5); BNP 84 (25–300) pg/mL	NA	Average level of cTnI, 0.05 (0–0.34) ng/Ml; cut-off value, 0.34 ng/mL; elevated patients, 112 (25.1%)	Death, admission to ICU
Merugu GP et al.	217	NA	NA	Elevated patients, 34/201 (16.9%)	Death
Mikami T et al.	6,493	NA	NA	Average level of cTnI, 0.03 (0.02–0.10) ng/dl; cut-off value, 0.03 ng/dL; elevated patients, 1,312/2,526 (51.9%)	Death
Özyilmaz S et al.	105	CAD, 14 (21.1)	NA	Average level of cTnI, 2.6 (0–1774.5) pg/mL[a]; cut-off value, 7.8 ng/mL; elevated patients, 21 (20.0%)	Death
Palaiodimos L et al.	200	CAD, 33 (16.5); HF, 34 (17.0)	NA	Cut-off value, 0.01 ng/mL; elevated patients, 56 (28.0%)	Mortality, intubation, O_2 requirement, ARDS, ICU, AKI, RRT, length of stay
Peiró ÓM et al.	196	CAD, 19 (9.7); HF, 14 (7.1)	NA	Average level of cTnI, 14 (4–37) ng/L; cut-off value, 21 ng/L; elevated patients, 77 (39.3%)	Death, admission to ICU, mechanical ventilation
Price-Haywood E et al.	3,481	CAD, 139 (4.0); HF, 128 (3.7)	NA	Cut-off value, 0.06 ng/mL; elevated patients, 270/1,084 (24.9%)	Death, admission to ICU
Qin J et al.	3,219	CAD, 206 (6.4)	Elevated patients, 228/1,895 (12.0%); HR for in-hospital mortality, 6.84 (4.95–9.45) AUC for mortality, 0.83 (0.80–0.86)	Elevated patients, 95/1,462 (6.5%); HR for in-hospital mortality, 9.59 (6.36–14.47); AUC for in-hospital mortality, 0.78 (0.73–0.84)	Death
Richardson S et al.	5,700	CAD, 595 (11.1); HF, 371 (6.9); BNP, 385.5 (160–1996.8), $n = 1,818$	NA	Elevated patients, 801/3,533 (22.7%)	Admission to ICU, mechanical ventilation, kidney replacement therapy, Death
Schiavone M et al.	674	HF, 111 (16.5)	NA	Average level of cTnI, 18 (8–40) ng/L; elevated patients, 130 (19.3%)	Death, admission to ICU, mechanical ventilation
Shah P et al.	309	CAD, 28 (9.1); HF, 65 (21.0)	NA	Elevated patients, 116 (37.5%)	Death, admission to ICU, mechanical ventilation
Shen Y et al.	325	NA	Cut-off value, 48.8 μg/L; elevated patients, 28/325 (8.6%)	Cut-off value, 0.04 ng/mL; elevated patients, 80/325 (24.6%)	Death, discharge
Singh N et al.	276	Vascular disease, 49 (17.8); HF, 56 (20.3)	NA	Cut-off value, 0.017 ng/mL; elevated patients, 132/276 (47.8%) OR for in-hospital mortality, 4.43 (1.61–12.19)	Death
Stefanini G et al.	397	Prior MI, 33/395 (8.4); HF, 18/395 (4.6); BNP, 67 (30–191) pg/mL	NA	Average level of cTnI, max 10.8 (4.3–39.5) ng/L, baseline 7.8 (4.5–25.6) ng/L; elevated patients, 130 (32.7%)	Death, admission to ICU, discharge
Suleyman G et al.	463	CAD, 59 (12.7); HF, 49 (10.6)	NA	Elevated patients, 107 (23.1%)	Death, admission to ICU

(Continued)

TABLE 1 | Continued

Authors	No.	Cardiovascular condition	Mb	cTnI	Outcome
Tanboga IH et al.	14,855	CAD, 2,341 (15.3); HF, 776 (5.1)	NA	Average level of cTnI, 0.08 (0.00–0.28) ng/mL; elevated patients, 1,027 (6.9%)	Death, admission to ICU, mechanical ventilation
Tomasoni D et al.	692	CAD, 148 (21.4); HF, 90 (13.0); NT-pro BNP 303 (96–1,201) pg/mL	NA	Elevated patients, 272/605 (45.0%)	Death
Wang D et al.	138	CAD, 20 (14.5)	NA	Average level of cTnI, 6.4 (2.8–18.5) pg/mL; cut-off value, 0.0262 ng/mL; Elevated patients, 10 (7.2%)	Admission to ICU
Wang Z et al.	293	CAD, 21 (7.2)	Average level of Mb, 57.6 (30.8–116.4) μg/L; cut-off value, 110 μg/L; elevated patients, 58/213 (27.2%)	Average level of cTnI, 0.007 (0.006–0.046) ng/mL; cut-off value, 0.0796 ng/mL; elevated patients, 36/216 (16.7%)	Death
Wei J et al.	101	CAD, 5 (5.0); NT-pro BNP, 71.2 (31.6–237.5) pg/mL	NA	Average level of cTnI, 6.8 (4.3–10.1) pg/mL; cut-off value, 0.014 ng/mL; elevated patients, 16 (15.8%)	Death, severe case, admission to ICU, mechanical ventilation
Wu Y et al.	125	CAD, 11 (8.8); BNP, 65.0 (23.0–178.0) pg/mL	Average level of Mb, 35.0 (27.7–75.65) μg/L; cut-off value, 154.9 μg/L; elevated patients, 14 (11.2%)	Average level of cTnI, 3.9 (1.9–10.3) pg/ml; cut-off value, 0.0342 ng/mL; elevated patients, 10 (8.0%)	Long-term hospitalization
Xu P et al.	703	CAD, 35 (5.0)	Elevated patients, 33/181 (18.2%)	NA	Death, admission to ICU, mechanical ventilation
Zeng J et al.	416	CAD, 13 (3.1); HF, 5/57 (8.8)	Cut-off value, 100 μg/L; elevated patients, 30/174 (17.2%)	Cut-off value, 0.026 ng/mL; elevated patients, 29/345 (8.4%)	Death, discharge
Zhang G et al.	221	CAD, 22 (10.0)	NA	Average level of cTnI, 7.6 (3.6–21.5) pg/mL; cut-off value, 0.0262 ng/mL; elevated patients, 17 (7.7%)	Discharge, death, severe COVID-19
Zhang Q et al.	41	CAD, 1 (2.4)	Average level of Mb, 26.0 (19.7–118.6) μg/L; elevated patients, 11 (26.8%)	Average level of cTnI; 1.5 (0.8–5.0) ng/mL; elevated patients, 41 (100%)	Severe COVID-19
Zhang Y et al.	166	CAD, 30 (18.1); NT-proBNP, 179.0 (67.0–457.0) pg/mL	Average level of Mb, 54.8 (33.8–127.2) μg/L; cut-off value, 106 μg/L; elevated patients, 28/166 (16.9%)	Average level of cTnI, 5.0 (2.2–10.7) pg/mL; cut-off value, 0.0156 ng/mL; elevated patients, 17 /166 (10.2%)	Discharge, death
Zhao M et al.	1,000	CAD, 60 (6.0)	Average level of Mb, 44.54 (28.5–85.05) μg/L; cut-off value, 110 μg/L; elevated patients, 132/754 (17.5%)	Average level of cTnI, 0.006 (0.006–0.018) ng/mL; cut-off value, 0.0796 ng/mL; elevated patients, 66/758 (8.7%)	Death, discharge
Zhao X et al.	91	HF, 14 (15.4)	NA	Cut-off value, 0.01 ng/mL; elevated patients, 3/88 (3.4%)	Death, discharge
Zhou F et al.	191	CAD, 15 (8.0); HF, 44 (23.0)	NA	Average level of cTnI, 4.1 (2.0–14.1) ng/mL; cut-off value, 28 ng/mL; elevated patients, 24/145 (16.6%)	Death, admission to ICU

No., confirmed number of patients with coronavirus disease 2019(COVID-19); Mb, myoglobin; cTnI, cardiac troponin I; CAD, coronary artery disease; HF, heart failure; BNP, B-type natriuretic peptide; NT-proBNP, N-terminal pro-B type natriuretic peptide; ICU, intensive-care unit; NA, data not available. [a]Median (range).

(95% CI 10.7–14.6%, $I^2 = 98\%$), and 20.1% (95% CI 15.3–24.9%, $I^2 = 99\%$) (**Supplementary Figure S1**). The prevalence of elevated cTnI and Mb in the general population with COVID-19 was 22.9 (95% CI 19–27%, $I^2 = 99\%$) and 13.5% (95% CI 10.6–16.4%, $I^2 = 92\%$), respectively (**Figure 2**). Furthermore, the meta-analysis showed that elevated cTnI occurred in 30.7% (24.7–37.1%, $I^2 = 86\%$) of the patients in the severe disease group, while the estimated rate of elevated Mb was 37.7% (23.3–52.1%, $I^2 = 90\%$) in patients with severe COVID-19. For the non-survivor group, the elevation rate of Mb and cTnI was 53.4 (95% CI 46.9–59.9%, $I^2 = 0\%$) and 55.5% (95% CI 47.1–64%, $I^2 = 94\%$), respectively (**Figure 3**).

Meta-regression demonstrated that both CSR and CFR were positively associated with the proportion of patients with elevated cTnI or Mb. Regarding logit CSR, the prevalence of elevated Mb showed tendency of higher regression coefficient compared with cTnI (Mb: $r = 13.9$, [95% CI 3.51–24.29, $p < 0.01$] vs. cTnI: $r = 3.93$, [95% CI 0–8.52, $p < 0.05$]). A similar trend was observed in logit CFR (Mb: $r = 15.42$, [95% CI 11.2–19.65, $p < 0.0001$] vs. cTnI: $r = 3.04$, [95% CI 1.84–4.25, $p < 0.0001$]) (**Figure 4**).

Risk of Elevated cTnI/Mb for Adverse Outcomes

The ORs of elevation of Mb/cTnI for the development of severe illness and death were further estimated. In the overall analysis, patients COVID-19 and elevated cTnI were at higher risk of severe illness (OR = 7.06, 95% CI 3.94–12.65, n = 15, $I^2 = 88\%$). Nevertheless, elevated Mb showed tendency of better predictive value for severe illness (OR = 13.75, 95% CI 10.2–18.54, $n = 6$, $I^2 = 39\%$) compared with cTnI. Regarding in-hospital mortality, elevated cTnI (OR = 7.75, 95% CI 4.4–13.66, $n = 13$, $I^2 = 95\%$) and Mb (OR = 13.49, 95% CI 9.3–19.58, $n = 3$, $I^2 = 0\%$) were associated with COVID-19-related deaths (**Figure 5**).

Sensitivity Analysis and Publication Bias

Sequential removal of each trial from the analysis revealed no meaningful differences (**Supplementary Figure S2**). We observed no evidence of publication bias by inspecting the funnel plot or with Egger's test, Begger's test or the test used by Peters et al. ($p > 0.05$; **Supplementary Figure S3**).

DISCUSSION

This systematic review and meta-analysis of 63 high-quality retrospective studies systematically investigated the predictive value of Mb for COVID-19-related severe disease or death compared with cTnI. The main findings of the study are as follows: (1) more patients with COVID-19-related severe disease showed elevated Mb compared with elevated cTnI; (2) elevated Mb presented obvious superiority over cTnI for predicting severe illness, showing 3-fold higher meta-regression coefficient and 2-fold higher OR; (3) furthermore, Mb elevation was more strongly associated with high risk of COVID-19-related death compared with cTnI.

Severe acute respiratory syndrome coronavirus 2 has been reported to be more contagious than previously discovered human coronaviruses (22), with the progression of the COVID-19 pandemic worldwide, there has been increasing concern regarding the "destructive power" of SARS-CoV-2 for multiple system organ damage, such as in the heart, liver, kidney, brain, and the nervous system (5, 23). Among them, myocardial injury is an important manifestation (6). Madjid et al. reported that up to 15% of hospitalized patients with COVID-19 exhibit myocardial injury, with some developing significant cardiac complications, such as biventricular heart failure, arrhythmias, and cardiogenic shock (9, 24). Liu et al. demonstrated that the mortality rate of patients with COVID-19 and cardiovascular disease was as high as 10.5%, which was 11.67 times higher than that of patients with COVID-19 with no preexisting conditions (25). Consistently, our analysis showed that the pooled incidence rate of cardiac injury was 22.9% in the general population, while the rate increased to 55.5% in the non-survivor group, indicating that cardiac injury was common in patients with COVID-19, especially those with poor prognosis.

Abnormal levels of cardiac biomarkers, including cTnI, CK-MB, Mb, and NT-proBNP, have been identified as indicators for COVID-19-related poor prognosis, such as severe illness (26), ICU admission and in-hospital mortality (27, 28). However, there is no consensus on the optimal biomarker for predicting COVID-19-related outcomes. cTnI elevation has been widely studied for its high prevalence in patients with COVID-19. However, in a study by Qin et al., elevated Mb presented with obviously higher frequency on admission compared with cTnI (12 vs. 6.5%) (16). Similarly, our subgroup analysis revealed that elevated Mb was more common in patients with severe COVID-19 than cTnI. Several recent studies have highlighted elevated cTnI as an important risk factor for adverse outcomes, such severe illness (29, 30), ICU admission (31, 32), and death (10, 26, 33). However, our meta-regression analysis suggested that the elevation rate of Mb presented 3-fold stronger association with CSR and 5-fold stronger association with CFR than cTnI. Notably, elevated Mb level showed higher risk of severe illness and mortality compared with cTnI. The results suggested that Mb may serve as a better biomarker for the severity of COVID-19. Accordingly, the dynamic monitoring of Mb might facilitate timely initiation of intensive care, thereby reducing the risk of other adverse events, such as COVID-19-related death.

Myoglobin is an iron and oxygen-binding protein that plays an important role in the storage of oxygen in skeletal and cardiac muscles (34). Previously, it was generally believed that Mb, while sensitive, was not specific for cardiac injury *per se*. Therefore, the prognostic value of Mb as a marker of myocardial injury in patients with COVID-19 has not been taken seriously (35). However, our meta-analysis suggested that Mb has a potential advantage over cTnI in predicting COVID-19-related adverse outcomes, such as the occurrence of severe illness and death. The mechanistic link between Mb and COVID-19 prognosis is unclear, but it may be the distribution of Mb in skeletal muscle besides myocardium, making it more sensitive to the dynamics of systemic states (36). de Andrade-Junior et al. reported that patients with severe COVID-19 are prone to develop muscle wasting and impaired muscle function (37). Moreover, Mb can be rapidly released into the blood in response

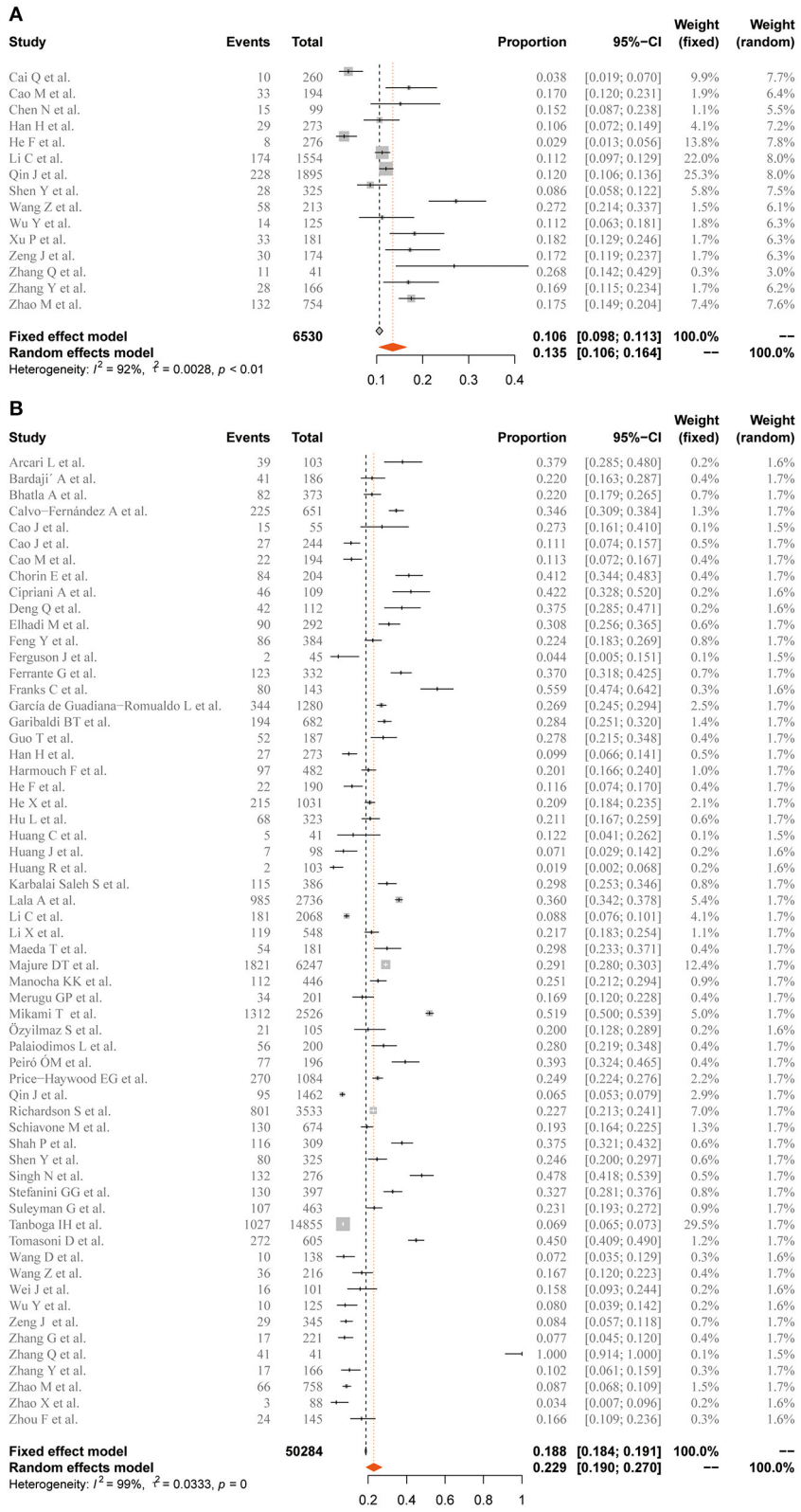

FIGURE 2 | Forest plot for the pooled prevalence of elevated **(A)** Mb and **(B)** cTnI in general population. Mb, myoglobin; cTnI, cardiac troponin I. Proportions are presented with fixed-effects when $I^2 \leq 50\%$ and random-effects otherwise.

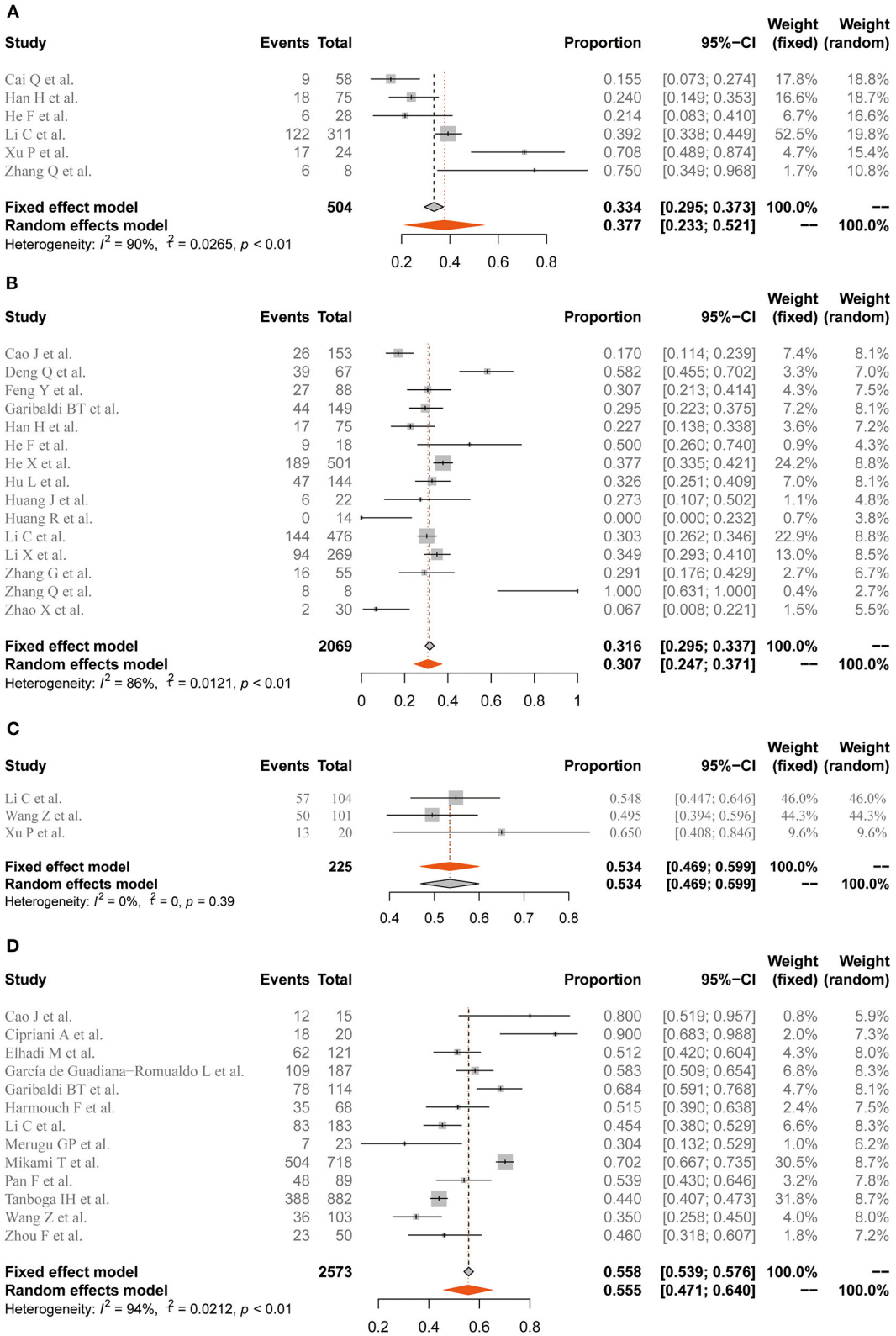

FIGURE 3 | Forest plot for the pooled prevalence of elevated Mb and cTnI in the severe disease and non-survivor groups. **(A)** Prevalence of elevated Mb in the severe disease group. **(B)** Prevalence of elevated cTnI in the severe disease group. **(C)** Prevalence of elevated Mb in the non-survivor group. **(D)** Prevalence of elevated cTnI in the non-survivor group. Mb, myoglobin; cTnI, cardiac troponin I. Proportions are presented with fixed-effects when I^2 ≤50% and random-effects otherwise.

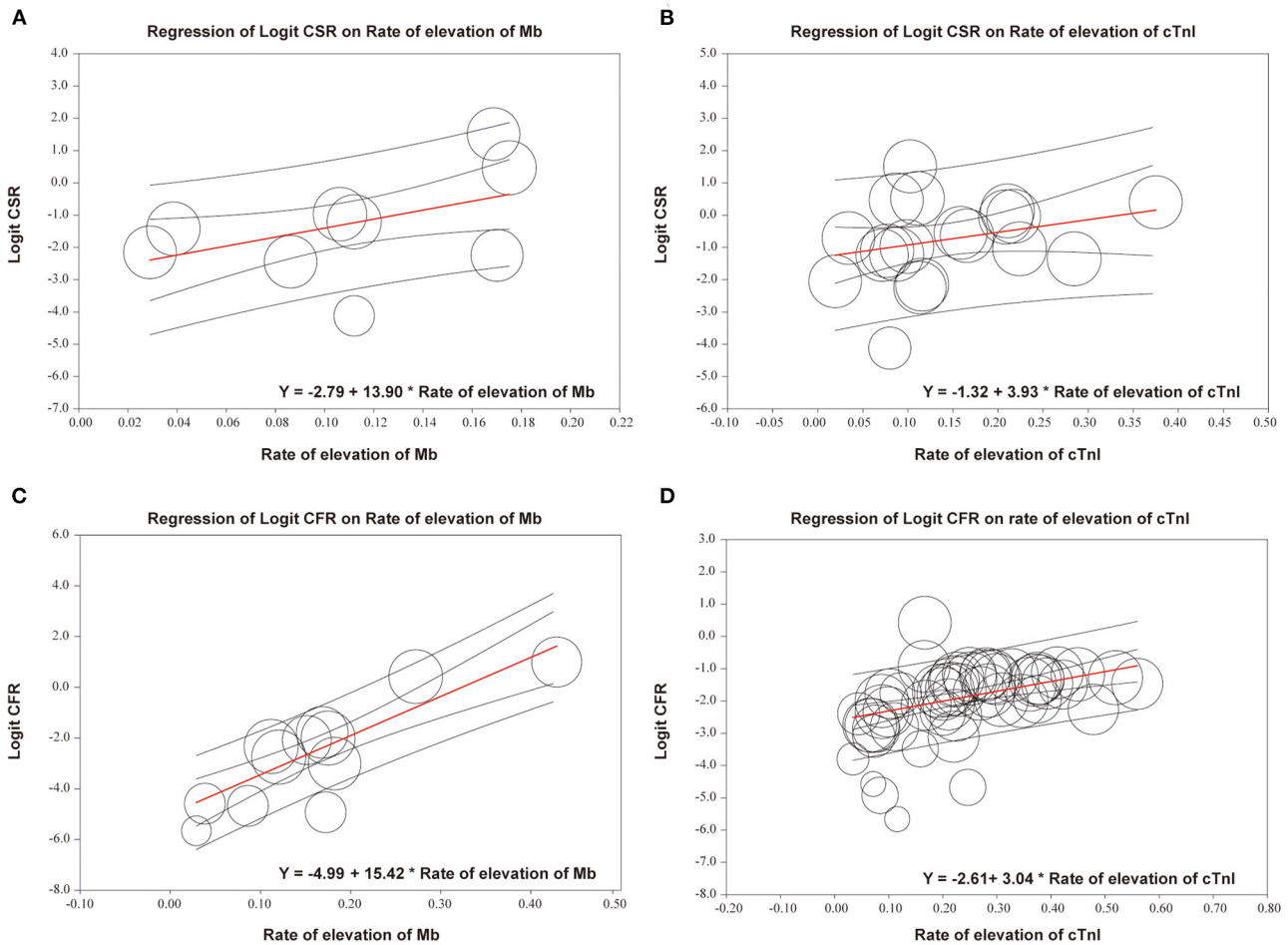

FIGURE 4 | Meta-regression of logit CSR or CFR on the rate of elevation of Mb or cTnI. **(A)** Regression of logit CSR on rate of elevation of Mb; $R = 13.9$, 95% CI 3.51–24.29, $p < 0.01$. **(B)** Regression of logit CSR on rate of elevation of cTnI; $r = 3.93$, 95% CI 0–8.52, $p < 0.05$. **(C)** Regression of logit CFR on rate of elevation of Mb; $r = 15.42$, 95% CI 11.2–19.65, $p < 0.0001$. **(D)** Regression of logit CFR on rate of elevation of cTnI; $r = 3.04$, 95% CI 1.84–4.25, $p < 0.0001$. CSR, case-severity rate; CFR, case-fatality rate; Mb, myoglobin; cTnI, cardiac troponin I. Each circle represents one study; size of the circle is proportional to the population size of each study.

to inflammatory stimuli (38). Wang et al. reported that oxidized Mb can act as a useful marker of myocardial inflammation (39). Furthermore, emerging evidence suggests that inflammatory responses, such as lymphopenia and cytokine storm, are closely associated with severe COVID-19 and high mortality (40, 41). Therefore, besides myocardial injury, the link between elevated Mb and COVID-19 prognosis may also be explained by inflammation and muscle injury. In addition to SARS-CoV-2 infection, increased Mb may also be caused by other preexisting comorbidities, such as chronic obstructive pulmonary disease (COPD), liver diseases, kidney diseases, and cardiovascular diseases, which have also been identified as risk factors for COVID-19 severity and mortality (42–45). Taken together, elevated Mb may be involved in damage directly caused by SARS-COV-2 infection and subsequent multiple organ failure, which partly explains the predictive value of Mb for adverse prognosis of COVID-19.

In the past year, the development and application of vaccines against SARS-CoV-2 brought hope to people worldwide. Notably, for the prevention of adverse outcomes of COVID-19, Chung et al. reported that two doses of mRNA COVID-19 vaccines were highly effective against symptomatic infection and severe consequences (46). Cornberg et al. demonstrated that priority vaccination for COVID-19 in patients with chronic liver diseases may be an important measure to intervene in the course of severe COVID-19 (47). However, the exact efficacy of COVID-19 vaccines against various comorbidities associated with myoglobin elevation is unknown and remains to be elucidated.

This meta-analysis had several potential limitations. First, all the studies included in this meta-analysis were retrospective, and there were relatively few studies involving both Mb and cTnI. Hence, the superiority of Mb over cTnI in predicting value should be interpreted as an observational conclusion.

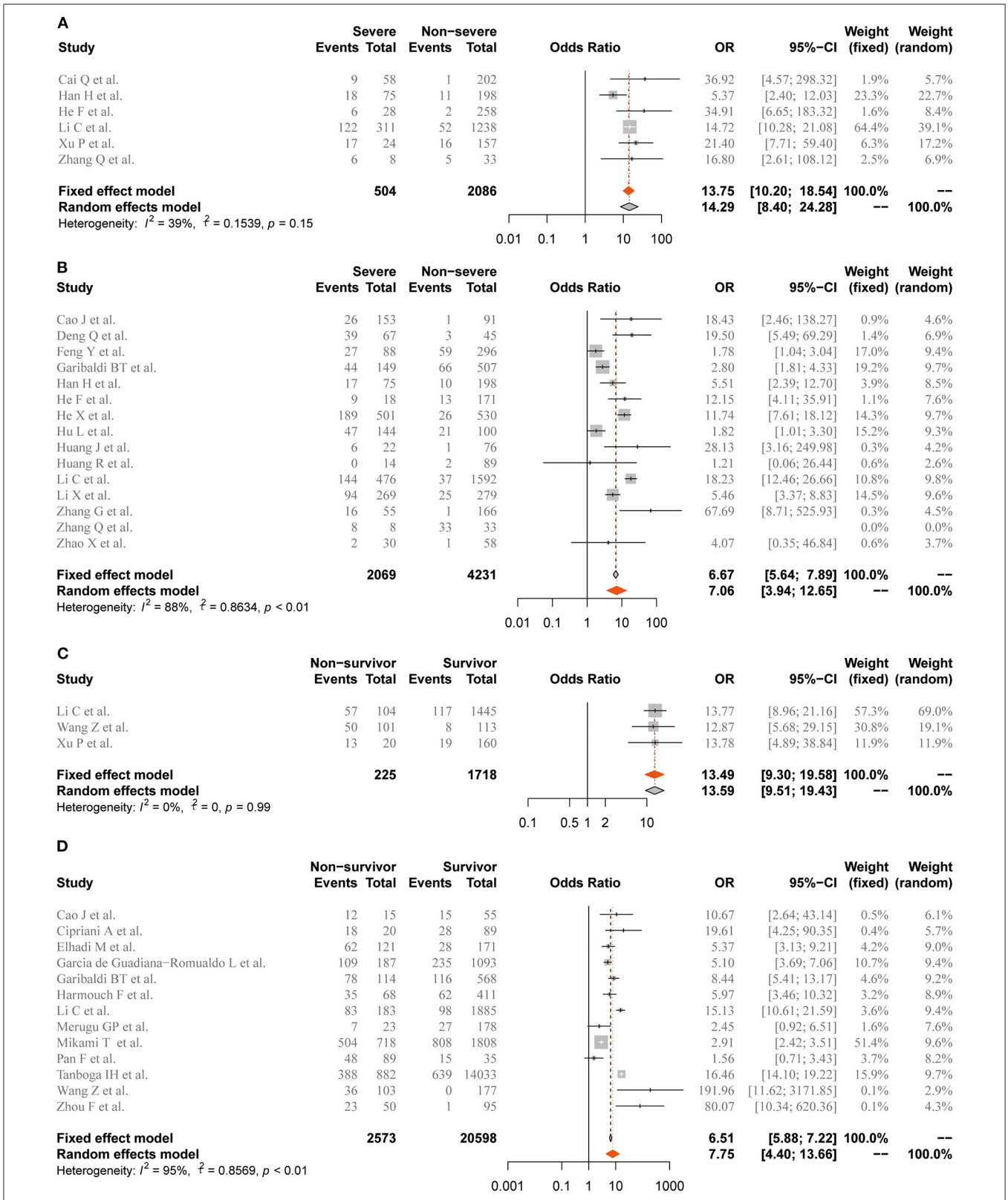

FIGURE 5 | Forest plot for the association of coronavirus disease 2019 (COVID-19)-related adverse outcomes with abnormal level of Mb or cTnI. **(A)** Severe illness and elevation of Mb. **(B)** Severe illness and elevation of cTnI. **(C)** In-hospital mortality and elevation of Mb. **(D)** In-hospital mortality and elevation of cTnI. Mb, myoglobin; cTnI, cardiac troponin I. Odds ratios (ORs) are presented with fixed-effects when $I^2 \leq 50\%$ and random-effects otherwise.

Further high-quality comparative studies are needed to confirm the difference between Mb and cTnI in predicting prognosis of COVID-19. Second, because of the nature of meta-regression and high heterogeneity across the analyses, we were unable to obtain a definite causal relationship between elevated Mb and poor prognosis of COVID-19. The potential sources of heterogeneity include different cutoffs of elevated cTnI or Mb, mean ages (48, 49), and sex ratios (50) in different studies. Therefore, considering the confounding factors, our results need to be further confirmed by rigorous prospective studies and randomized controlled trials. Third, because of the limited number of included studies, this meta-analysis did not analyze the predictive value of CK-MB, NT-proBNP, LDH, and other cardiac markers except Mb and cTnI. Fourth, studies enrolled in this meta-analysis had a relatively short follow-up period. Therefore, the predictive value of Mb for long-term prognosis of COVID-19 needs to be further explored.

In summary, this meta-analysis showed that patients with COVID-19 and elevated Mb levels are at higher risk of severe disease and mortality. Hence, elevated Mb could be used as a predictor of adverse outcomes in COVID-19. However, high-quality studies are required to confirm these findings and establish the link between elevated Mb and prognosis 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.

AUTHOR CONTRIBUTIONS

PL, XZ, and YB were the judicators and contributed to the conception of the study. CM, DT, JG, YB, ZG, and HW designed the protocol. CM, DT, and JG searched the databases and finished data extraction, quality assessment, and statistical analysis. CM and DT wrote the first draft of the manuscript. All authors reviewed the manuscript, provided critical revision, and have approved the final version 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.2021.757799/full#supplementary-material

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Normalized Cardiac Structure and Function in COVID-19 Survivors Late After Recovery

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Background: Coronavirus disease 2019 can result in myocardial injury in the acute phase. However, information on the late cardiac consequences of coronavirus disease 2019 (COVID-19) is limited.

Methods: We conducted a prospective observational cohort study to investigate the late cardiac consequences of COVID-19. Standard echocardiography and myocardial strain assessment were performed, and cardiac blood biomarkers were tested in 86 COVID-19 survivors 327 days (IQR 318–337 days) after recovery. Comparisons were made with 28 age-matched and sex-matched healthy controls and 30 risk factor-matched patients.

Results: There were no significant differences in all echocardiographic structural and functional parameters, including left ventricular (LV) global longitudinal strain, right ventricular (RV) longitudinal strain, LV end-diastolic volume, RV dimension, and the ratio of peak early velocity in mitral inflow to peak early diastolic velocity in the septal mitral annulus (E/e') among COVID-19 survivors, healthy controls and risk factor-matched controls. Even 26 patients with myocardial injury at admission did not have any echocardiographic structural and functional abnormalities. There were no significant differences among the three groups with respect to serum concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-sensitivity cardiac troponin I (cTnI).

Conclusion: This study showed that COVID-19 survivors, including those with myocardial injury at admission and those with severe and critical types of illness, do not have any echocardiographic evidence of cardiac structural and functional abnormalities 327 days after diagnosis.

Keywords: COVID-19, speckle tracking echocardiography, myocardial strain, NT-proBNP, troponin

INTRODUCTION

Coronavirus disease-2019 (COVID-19) is now the deadliest pandemics caused by the novel severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) (1). Though it primarily affects the respiratory system, cardiovascular complications are common in COVID-19 (2, 3). Myocardial injury reflected through elevated troponin concentration was reported in the acute stage of COVID-19 (4, 5). Left ventricular (LV) and right ventricular (RV) enlargements and dysfunctions were found with conventional and speckle tracking echocardiography in patients with COVID-19 (6–8). Since most COVID-19 patients recover from the illness, the understanding of the late cardiovascular consequences of infection was important. Until now, there are only a few studies on the cardiac outcome of COVID-19 survivors (9–13). These studies have reported residual cardiac structural and functional abnormalities even after recovery from COVID-19 using cardiac magnetic resonance (CMR) imaging (11–13) and echocardiography (9–11). However, these studies have been limited by their short time interval between COVID-19 diagnosis and follow-up study from 26 to 140 days which may not be long enough for cardiac abnormalities to resolve. Therefore, we performed the present study to examine the myocardial mechanical function with speckle tracking echocardiography as well as cardiac blood biomarkers in COVID-19 survivors 327 days after diagnosis.

METHODS

Study Design and Participants

This is a single-center, prospective observational cohort study undertaken in Tongji Hospital of Huazhong University of Science and Technology, a designated medical unit for treating patients with COVID-19. COVID-19 survivors were identified from the hospital medical record system and recruited through posting recruitment notices. Exclusion criteria were unwillingness to participate, incapability of communication, acute conditions such as infection, organ dysfunction and active autoimmune disease, and other illness requiring hospitalization. Patients with unsatisfactory recordings of echocardiograms were also excluded. Finally, a total of 86 consecutive patients with a history of confirmed SARS-CoV-2 infection using reverse transcription-polymerase chain reaction swab test of the upper respiratory tract were recruited between December 2020 and January 2021. In total, 28 healthy subjects matched for age and sex were recruited as the healthy controls. While the other 30 matched for age, sex, hypertension, diabetes mellitus, smoking, hypercholesterolemia, and coronary artery disease were also recruited as the risk factor-matched controls. All control group subjects were recruited from communities with consent of each participant. Our research was in concordance with the Declaration of Helsinki and the International Conference on Harmonization of Good Clinical Practice. The Tongji Hospital Ethics Committee approved the study (TJ-C20200156) and informed consent was obtained from each participant before their enrollment in the study.

Clinical characteristics, laboratory test results, and treatment for the acute phase of illness were collected from electronic medical records or patient discharge summaries. After recording the present clinical characteristics, all subjects underwent blood sampling, standard echocardiography, and myocardial strain assessment.

Standard Echocardiography and Myocardial Strain Assessment

All participants underwent echocardiographic examinations according to the recommendation of the American Society of Echocardiography using a Vivid E95 digital ultrasound system (GE Medical System, Horten, Norway) equipped with a 1.7–3.4 MHz M5Sc phased array transducer (14). All images were analyzed offline using commercially available software (EchoPac version 203, GE Vingmed, Horten, Norway). LV dimension, wall thickness, and LV mass were obtained from M-mode echocardiography. The biplane Simpson's method was used to calculate LV volume and ejection fraction. Left atrial (LA) volume was measured with the modified Simpson's method. LA volume and LV volume, and mass were indexed to the body surface area. Peak early (E) and late diastolic (A) velocities in mitral inflow, and peak early diastolic velocity (e') in septal mitral annulus were measured, and the E/A and E/e' ratios were calculated. Each parameter was averaged in three cardiac cycles.

Right atrial and RV dimensions and RV area were measured in the apical four-chamber view. RV fractional area change was calculated by dividing the difference between RV end-diastolic and end-systolic areas by the end-diastolic area. The tricuspid annular plane systolic excursion was obtained from the M-mode recording as the systolic displacement of the tricuspid lateral annulus. Tricuspid lateral annular systolic tissue velocity was measured in apical four-chamber view. The presence and severity of tricuspid regurgitation and pulmonary artery systolic pressure were assessed on color Doppler and continuous wave Doppler spectrum according to current guidelines.

Myocardial strain off-line analysis was performed using software (EchoPac version 203, GE Vingmed, Horten, Norway) on the two-dimensional gray-scale image with a frame rate of 70–90 frames/s according to the recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging (15). LV myocardial strain was obtained from the apical four-chamber, apical two-chamber, apical long-axis using a 17-segmental model with speckle tracking echocardiographic method. The LV global longitudinal strain was calculated by averaging peak strain values in 17 LV segments. RV free wall longitudinal strain for basal, mid, and apical segments was obtained in the apical four-chamber view. RV longitudinal strain was calculated by averaging the peak strain values in the three segments of the RV free wall.

Laboratory Examination

Peripheral venous blood samples were drawn at least 30 min before echocardiographic examination. Blood samples were processed using standardized commercially available test kits for analysis of high-sensitivity troponin I [(cTnI), Roche

Diagnostics, Rotkreuz, Switzerland] and N-terminal pro-B-type natriuretic peptide [(NT-proBNP), Abbott, Illinois, USA]. Myocardial injury was defined as a serum cTnI above the upper 99th percentile value. Serum NT-proBNP level was considered elevated according to the age-specific diagnostic threshold for heart failure. The local laboratory cTnI values above the upper 99th percentile counted as a significant increase were 15.6 pg/ml for women and 34.2 pg/ml for men. The age-specific diagnostic thresholds of serum NT-proBNP for heart failure were as follows: <62.9 pg/ml for men and <116 pg/ml for women (18–44 years old); <89.3 pg/ml for men and <169 pg/ml for women (45–54 years old); <161 pg/ml for men and <247 pg/ml for women (55–64 years old); <241 pg/ml for men and <285 pg/ml for women (65–74 years old); <486 pg/ml for men and <738 pg/ml for women (above 75 years old).

Statistical Analysis

Statistical analysis was carried out using SPSS version 21 software (IBM, Armonk, NY, USA). Normality was evaluated using the Shapiro-Wilk test. Categorical variables were expressed as counts and percentage, and continuous variables as mean ± *SD* or median [interquartile range (IQR)]. Wilcoxon test was utilized for comparisons of the data obtained at the acute phase and recovery of the illness. Unpaired Student's *t*-test was used to compare clinical data between two groups if normally distributed, and Mann-Whitney *U*-test if not normally distributed. Comparisons among three groups were performed using one-way ANOVA with Bonferroni corrected *post-hoc* comparisons for normal distribution or Kruskal-Wallis tests for non-normal distribution, as appropriate. Differences in proportions were analyzed with the Chi-square test or the Fisher exact test. A p-value < 0.05 was considered to indicate statistical significance.

RESULTS

Patient Characteristics

A total of 86 patients were enrolled in this study (**Table 1**). Median (IQR) age was 58 (39–70) years and 32 (37%) were men. Among the 86 patients, 45 (52%) were diagnosed as having moderate-type COVID-19 illness, 27 (31%) as having severe-type, and 14 (17%) as having critical-type from January to February 2020 according to the Diagnosis and Treatment Protocol of Novel Coronavirus issued by the National Health Commission of the People's Republic of China.[1] Furthermore, 78 (91%) patients required hospitalization. Among these 78 hospitalized patients, 1 patient (1%) underwent extracorporeal membrane oxygenation, 6 (8%) underwent mechanical ventilation, and 10 (13%) underwent non-invasive ventilation with positive airway pressure. Nasal cannula oxygen support was needed in 68 (87%) patients. All patients received antiviral and antibiotics therapy. Corticosteroid was used in 41 of 78 hospitalized patients (53%). Histories of cardiovascular conditions included hypertension in 32 (37%) patients, diabetes mellitus in 14 (16%), hypercholesterolemia in 16 (19%), and coronary heart disease in 13 (15%). During hospitalization, serum cTnI and NT-proBNP levels were available in 64 and 45 patients, respectively. Among them, a significant rise in cTnI was detected in 26 patients (26/64, 41%) while an elevated NT-proBNP level was found in 25 patients (25/45, 56%)

Patient characteristics, echocardiographic findings, and cardiac biomarkers on the day of echocardiographic strain are shown in **Table 1**. The median (IQR) interval between the COVID-19 diagnosis and echocardiographic examination was 327 (318–337) days. Exertional shortness of breath and chest discomfort was reported in 25 (29%) and 33 (38%), respectively, on the day of echocardiographic examination.

Echocardiographic Findings

No difference was found among COVID-19 survivors, healthy controls, and risk factor-matched patients with respect to age, percentage of male subjects, body mass index, body surface area, heart rate, and blood pressure. Hypertension, diabetes mellitus, coronary artery disease, and hypercholesterolemia were more common in COVID-19 survivors than those in healthy controls, but there were no differences between COVID-19 survivors and risk factor-matched patients (**Table 1**).

There were no significant differences in all echocardiographic structural and functional parameters, including LV global longitudinal strain, RV longitudinal strain among COVID-19 survivors, healthy controls, and risk factor-matched controls (**Figures 1A,B**, **Table 1**). There were even no significant differences in echocardiographic structural and functional parameters among groups classified according to disease severity and the presence of myocardial injury at admission, healthy control, and risk-matched control (**Figures 1C–G**).

Blood Biomarkers

There were no significant differences among the three groups with respect to serum concentrations of NT-proBNP and cTnI (**Table 1**). In a proportion of survivors with obtainable data in the acute phase, NT-proBNP and cTnI concentrations were both significantly decreased 327 days after diagnosis when compared with those in the acute phase (**Figure 2**).

DISCUSSION

Our study showed that there were no significant differences in echocardiographic structural and functional parameters among COVID-19 survivors, healthy control, and risk factor-matched control 327 days after diagnosis regardless of the presence of myocardial injury in the acute phase and severity of the illness at admission. In addition, blood biomarkers of myocardial injury and function revealed no significant differences among COVID-19 survivors, healthy and risk-factor matched controls.

Coronavirus disease 2019 is a global pandemic leading to high morbidity and mortality (1). A significant proportion of patients with COVID-19 were reported to suffer from a myocardial injury in the acute phase. Echocardiographic abnormalities, including

[1]National Health Commission of the People's Republic of China. Diagnosis and treatment protocol of novel coronavirus (trial version 7th). National Health Commission of the People's Republic of China Website. http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml. Accessed March 4, 2020.

TABLE 1 | Clinical characteristics, echocardiographic findings, and laboratory results of coronavirus disease 2019 (COVID-19) survivors 327 days after diagnosis.

	Healthy control ($n = 28$)	Risk factor-matched control ($n = 30$)	COVID-19 ($n = 86$)	*p*-value
Patient characteristics				
Age, years	56 (37–65)	62 (39–67)	58 (39–70)	0.392
Male, *n*%	10 (36%)	11 (37%)	32 (37%)	0.990
Body mass index, kg/m^2	23 ± 3	24 ± 3	24 ±3	0.304
Body surface area, m^2	1.7 ± 0.2	1.7 ± 0.2	1.7 ± 0.2	0.561
Heart rate, beats/min	67 (61–81)	69 (63–73)	73 (65–79)	0.119
Systolic blood pressure, mm Hg	125 ± 12	126 ± 16	131 ± 18	0.132
Diastolic blood pressure, mm Hg	73 (67–82)	72 (67–79)	77 (70–82)	0.228
Oxygen saturation, %	NA	NA	98 (97–99)	NA
Hypertension, *n*%	0 (0%)	10 (33%)*	32 (37%)*	0.001
Diabetes mellitus, *n*%	0 (0%)	2 (7%)	14 (16%)*	0.032
Coronary heart disease, *n*%	0 (0%)	3 (10%)	13 (15%)	0.076
Hypercholesterolemia, *n*%	0 (0%)	9 (30%)*	16 (19%)*	0.003
Echocardiographic findings				
LA dimension, mm	31 (28–33)	31 (28–33)	32 (29–34)	0.388
LV dimension, mm	45 (43–50)	45 (43–49)	46 (44–49)	0.780
IVS thickness, mm	8 (7–8)	8 (7–9)	8 (7–9)	0.180
LV posterior wall thickness, mm	8 (7–8)	8 (7–8)	8 (7–9)	0.094
LV mass, g/m^2	73 (63–87)	78 (64–86)	80 (67–96)	0.346
LV end-diastolic volume, ml/m^2	47 (43–51)	48 (44–52)	45 (40–54)	0.866
LV end-systolic volume, ml/m^2	18 (15–19)	17 (15–19)	17 (14–21)	0.889
LV ejection fraction, %	63 (61–67)	63 (61–67)	63 (61–68)	0.870
LA volume, ml/m^2	22 (18–26)	22 (18–27)	21 (18–25)	0.750
E/A ratio	1.1 (0.8–1.4)	1.1 (0.8–1.2)	0.9 (0.7–1.3)	0.190
E/e' ratio	8 ± 3	9 ± 4	9 ± 2	0.426
LV GLS, %	21 ± 2	21 ± 2	20 ± 2	0.381
LV GLS < 16%, *n*%	0 (0%)	0 (0%)	4 (5%)	0.476
RA dimension, mm	34 (30–36)	34 (30–35)	33 (30–38)	0.554
RV dimension, mm	31 (27–33)	30 (27–34)	32 (28–36)	0.217
TAPSE, mm	27 (24–29)	26 (23–28)	26 (24–28)	0.346
RV fractional area change, %	47 ± 9	49 ± 8	51 ± 9	0.158
S', cm/s	14 (13–17)	14 (13–17)	14 (13–16)	0.936
PASP, mm Hg	23 (19–28)	24 (19–28)	25 (21–30)	0.707
RV longitudinal strain, %	30 ± 5	30 ± 6	29 ± 6	0.722
RV longitudinal strain < 20%, *n*%	1 (4%)	1 (3%)	2 (2%)	1.000
Pericardial effusion, *n*%	0 (0%)	0 (0%)	1 (1%)	1.000
Laboratory results				
NT-proBNP, pg/mL	36 (15–65)	41 (19–72)	51 (24-104)	0.113
cTnI, pg/mL	1.9 (1.9–2.5)	1.9 (1.9–2.8)	1.9 (1.9–4.9)	0.159

Numbers are given as median (interquartile range) or mean ± standard deviation or as case numbers with percentages in parentheses.
NA, not applicable; LA, left atrium; LV, left ventricle; IVS, interventricular septum; E, peak early diastolic velocity in mitral inflow; A, late diastolic velocity in mitral inflow; e', peak early diastolic velocity in septal mitral annulus; GLS, global longitudinal strain; RA, right atrium; RV, right ventricle; TAPSE, tricuspid annular plane systolic excursion; S', tricuspid lateral annular systolic tissue velocity; PASP, pulmonary artery systolic pressure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; cTnI, high-sensitivity cardiac troponin I.
$^*p < 0.01$, vs. healthy control.

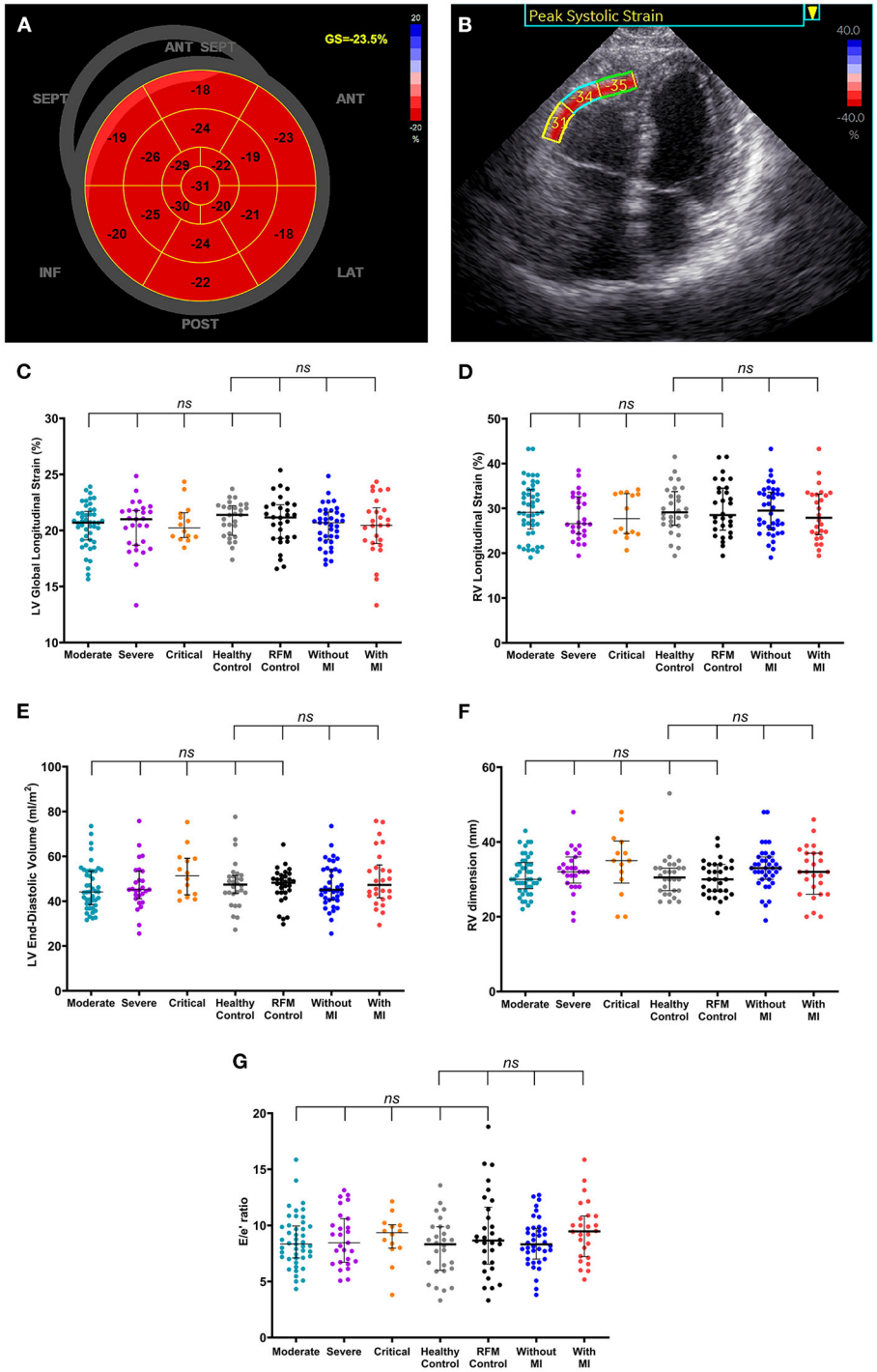

FIGURE 1 | Normalized cardiac structure and function in coronavirus disease 2019 (COVID-19) survivors late after the recovery. **(A,B)** A patient (75–80 years old) with no history of hypertension, diabetes, and/or coronary heart disease was diagnosed with severe-type COVID-19 illness. High-sensitivity troponin I level was 1,137 pg/ml at admission and 4.3 pg/ml on the day of echocardiographic examination (316 days after COVID-19 diagnosis). **(A)** Shows normal left ventricular (LV) global longitudinal strain (GS) and panel B shows normal right ventricular (RV) free wall longitudinal strain for basal, mid, and apical segments. **(C–G)** There were no significant differences in LV global longitudinal strain **(C)**, RV longitudinal strain **(D)**, LV end-diastolic volume **(E)**, RV dimension **(F)**, and the ratio of peak early velocity in mitral inflow to peak early diastolic velocity in the septal mitral annulus [E/e', **(G)**] among groups classified according to disease severity and the presence of myocardial injury at admission, healthy control, and risk-matched control. Longer black lines indicate the medians and shorter black lines indicate the interquartile ranges. Each dot represents a value. ANT, anterior; LAT, lateral; POST, posterior; INF, inferior; SEPT, septum; ANT SEPT, anterior septum; RFM, risk-factor matched; MI, myocardial injury; LV, left ventricular; RV, right ventricular.

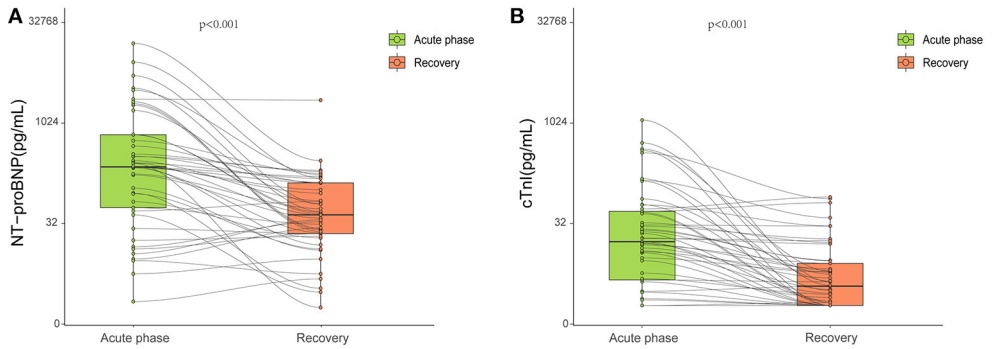

FIGURE 2 | Blood biomarkers obtained at the acute phase and late after the recovery. During hospitalization, serum NT-proBNP **(A)** and cTnI **(B)** levels were available in 45 and 64 patients, respectively. Both were significantly decreased 327 days after diagnosis compared with those in the acute phase ($p < 0.001$). Each small circle represents a value. The top and bottom of the rectangle represent the interquartile range. Bold black lines in the rectangle indicate medians.

global LV dysfunction, regional wall motion abnormalities, diastolic dysfunction, RV dysfunction, and pericardial effusion were detected in patients with COVID-19 in the acute phase and a higher prevalence of echocardiographic abnormalities was found in patients with biomarker evidence of myocardial injury (4). CMR also revealed myocarditis, LV dysfunction, pericarditis, and Takotsubo cardiomyopathy in the acute phase of COVID-19 illness, indicated by abnormalities in T1 and T2 mapping and late gadolinium enhancement images (16–18). Nevertheless, it is still unclear whether the myocardial injury at the acute phase of illness leaves persistent lesions and how significant these abnormalities are in the long run. A few studies on the cardiovascular consequences of COVID-19 with limited follow-up intervals have been published (9–13, 19–24). In a study of cohort patients 71 days after recovery of COVID-19, magnetic resonance revealed cardiac involvement, including myocardial late gadolinium enhancement, raised myocardial native T1 and T2 in 78% of patients independent of preexisting conditions, severity, and overall course of the acute illness (12). Echocardiographic studies showed similar findings. The study of Zhou et al. reported LV dysfunction with decreased LV ejection fraction after a short period of 1–4 weeks following discharge (20). Another study showed that despite normalized blood concentrations of troponin and NT-proBNP, 29% of survivors had an abnormality in echocardiography after 3 months of admission, with reverse RV remodeling in the majority reflected by dilated RV dimension and decreased RV fractional area change (9). To notice, 80% of patients in this study had undergone mechanical ventilation, indicating severely impaired pulmonary structure and function. Thus, the above observed persistent RV dysfunction could not simply be attributed to direct myocardial injury. Preservation in cardiac consequence has been reported (10, 21–23). The study of Catena et al. reported no structural and functional sequelae in the heart of survivors of COVID-19 more than 1 month after recovery from illness (10). Daher et al. also demonstrated no echocardiographic impairments in 33 patients with severe illness after 6 weeks following discharge (23). However, these studies were limited by their short time periods at follow-up, leaving long-term cardiovascular consequences of COVID-19 poorly understood. In the present prospective study, COVID-19 survivors were evaluated after a relatively long time period with a median interval of 327 days after diagnosis, and no elevation of cTnI and NT-proBNP were detected nor echocardiographic structural and functional abnormalities were found when compared with healthy control and risk factor-matched control, including those with myocardial injury in the acute phase. Our finding was consistent with previously published longer period follow-up studies. After a median interval of 6 months, echocardiographic measurements in COVID-19 survivors were not different between patients with and without myocardial injury during the acute COVID-19 phase (24). Combining our findings and previous follow-up results, it is suggested that myocardial injury and echocardiographic structural and functional abnormalities observed in the acute phase of COVID-19 infection might be reversible. The resolution of CMR abnormalities in COVID-19 athletes seems to be an example of this reversibility. In a consecutive follow-up study on athletes, CMR imaging revealed elevated T1, elevated T2, and late gadolinium enhancement in 2.3% of patients after a short interval (10–77 days) from diagnosis. However, a repeated CMR 4–14 weeks later from the first follow-up demonstrated resolution of T2 elevation in 100% and late gadolinium enhancement in 41% of patients (13). Thus, the cardiac abnormalities observed in COVID-19 survivors in previous studies (9, 12, 13) might be due to the short follow-up period and they might resolve in the long run. Another possible explanation for those observed persistent cardiac abnormalities in survivors could be the effect of pre-existing conditions in COVID-19 patients, such as hypertension, coronary artery disease, diabetes which are usually seen in the seniors. These patients tend to suffer more severe pneumonia (3), which further heavies the burden of the heart with mechanical ventilation. To avoid such confounders, COVID-19 survivors in our study were compared with a group of risk-factor matched control, with no significant cardiac abnormalities being found in the COVID-19 survivor group. Taken together, COVID-19 *per se* does not appear to cause long-term cardiac sequelae after recovery from acute illness.

The proposed mechanism of myocardial injury and dysfunction in patients with COVID-19 infection include cytokine-mediated damage, oxygen supply-demand imbalance, ischemic injury from microvascular thrombi formation, a direct viral infection of the myocardium, and pulmonary hypertension-induced RV dysfunction (4, 25, 26). The oxygen saturation was quite normal in COVID-19 survivors on the day of echocardiographic examination, lowering the possibility of oxygen supply-demand imbalance. Pulmonary artery systolic pressure in the COVID-19 survivors was also not different from that in healthy control. Previous studies have demonstrated that the cardiac structural and functional abnormalities caused by ischemic injury resolved after successful revascularization (27, 28). Longitudinal studies have demonstrated gradual declines of serum concentration of inflammatory biomarkers including IL-6, IL-8, tumor necrosis factor-α, and high-sensitivity C-reactive protein (hs-CRP) at the late stage of illness in COVID-19 survivors (29). Another study reported slight increased CRP levels in 16% of COVID-19 patients 2 months after symptom onset (30). Fulminant myocarditis is an inflammatory disease of the myocardium most often caused by a viral infection with severe impairment of LV systolic function in the acute phase. Previous reports showed that LV ejection fraction recovered at follow-up in survivors with fulminant myocarditis (31, 32). It is speculated that when the underlying pathogenic conditions were eliminated, the myocardial dysfunction would be reversed. Those findings in inflammatory biomarkers, oxygen saturation, and pulmonary artery systolic pressure in our study and previous studies (27, 28, 31, 32) support the observations in the present study that no significant differences exist in cTnI concentration, and echocardiographic structural and functional parameters among COVID-19 survivors, healthy control, and risk factor-matched control 327 days after diagnosis of COVID-19 infection.

Some limitations existed in our study. First, the quantitative echocardiographic data were unavailable at the onset of COVID-19 in isolation wards, which makes the longitudinal comparison of echocardiographic parameters impossible. Second, we did not perform segmental strain comparisons among groups. A previous study (33) has shown basal longitudinal strain dysfunction in COVID-19 patients in the acute phase of illness. Nevertheless, this study also showed decreased global longitudinal strain. Global longitudinal strain was calculated by averaging peak strains in 17 segments in our study. If one or several segment(s) has or have significantly decreased strain, the global longitudinal strain would be decreased concomitantly. Since no significant differences in global longitudinal strain were found in our study, we did not do further analysis in the segmental strain. Third, our study was based on a small sampling of survivors, thus, multicenter study with a larger population and longer follow-up period would be needed to provide more valuable information on the long-term cardiac consequences of COVID-19 infection.

CONCLUSIONS

This study showed that COVID-19 survivors, including those with significantly elevated cTnI at admission and those with the severe and critical types of illness, did not have evident echocardiographic proof of cardiac structural and functional abnormalities 327 days after diagnosis.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Tongji Hospital Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Y-BD, Y-NL, X-JB, H-YL, and YZ conceived and designed the study. Y-PG, WZ, P-NH, X-QC, R-YS, Y-NL, and Y-BD collected clinical and ultrasound data. Y-PG, WZ, LL, Q-YT, JZ, and JS analyzed data and performed the statistical analysis. Y-BD, Y-NL, Y-PG, and WZ drafted the manuscript. All authors approved the manuscript.

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frontiers in Cardiovascular Medicine

CASE REPORT
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Case Report: COVID-19 Vaccination Associated Fulminant Myocarditis

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Herein, we describe a novel finding of fulminant myocarditis (FM) in two subjects the day after administration of the first dose of the currently available inactivated SARS-CoV-2 vaccine (Vero cell). Cardiac magnetic resonance imaging revealed extensive myocardial edema and necrosis. A pathologic evaluation of the endocardial biopsy tissues revealed inflammatory cell (lymphocytes) infiltration and interstitial edema, myocyte necrosis, and focal areas of fibrosis. A life-support-based comprehensive treatment regimen comprising mechanical circulatory support using intra-aortic balloon pulsation and immunomodulatory therapy—glucocorticoids and intravenous immunoglobulin—was used to treat the patients with FM; eventually, the patients recovered and were discharged. To our knowledge, these are the first two reported cases of FM, with no other identified cause or associated illness, after receiving the inactivated SARS-CoV-2 vaccine (Vero cell). These findings suggest a novel pathogenesis of myocarditis which mentions to pay more attention to this rare, but lethal complication of COVID-19 vaccination.

Keywords: myocarditis, COVID-19 vaccine, immunomodulatory therapy, myocyte necrosis, pathogenesis

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BACKGROUND

Myocarditis refers to the inflammation of the heart muscle due to microbial infections, toxic substances, or autoimmune processes. Fulminant myocarditis, which is characterized by severe and sudden cardiac inflammation with cardiogenic shock and arrhythmias and a high mortality rate of approximately 40–70% (1, 2), is a less common, but not rare, clinical emergency; however, it is not specifically mentioned in the Dallas Criteria or in the report of the World Health Organization/International Society and Federation of Cardiology classification of cardiomyopathies (3). Since coronavirus disease (COVID-19) was first described in December 2019, COVID-19-related fulminant myocarditis has been reported several times (4–7). Current knowledge suggests that it is a combination of systemic inflammation due to cytokine storm, severe myocardial injury caused by the patient's immune response, and direct viral injury of the myocardium [since the identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) particles in the reverse transcription polymerase chain reaction (RT-PCR) myocardial biopsy of rare patients], which suggests the eventual cardiotropism of the virus (8, 9). Currently, vaccines represent the most powerful approach in controlling the COVID-19 pandemic. However, several adverse events, especially vaccination-associated deaths, have been reported in the news and on social platforms (10, 11), and these adverse events often occur within 5–24 days of the vaccination. In this report, we describe a novel finding in two cases of fulminant myocarditis following the administration of the first dose of the currently available inactivated SARS-CoV-2 vaccine (Vero cell).

CASE PRESENTATION

Case 1

A 57-year-old woman presenting with chest distress, fatigue, fever, and chills for 4 days was hospitalized. Her highest recorded body temperature was 38.5°C. Her symptoms of chest tightness were aggravated, accompanied by palpitations. No discomfort, such as chest pain, nausea and vomiting, amaurosis and syncope, or acid regurgitation were observed. The woman had received the COVID-19 vaccine 4 days before. She had good health, apart from a history of hypertension.

Physical examination revealed a body temperature of 37.2°C, blood pressure of 102/58 mmHg, pulse of 99 bpm, and respiratory rate of 16 breaths/minute, and oxygen saturation of 99% while the patient was breathing ambient air. Physical examination of the heart revealed low and dull heart sounds.

Investigations

Biochemical analysis was performed when the patient was admitted (**Table 1**). Laboratory results reflected severe myocardial damage [troponin I > 50,000 pg/ml, creatine kinase (CK) 1,186 U/L, lactate dehydrogenase (LDH) 764 U/L], and elevated levels of white blood cell (WBC) (8.83×10^9/L) and neutrophils (92.6%) while decreased lymphocyte (0.44×10^9/L) levels. Additionally, high-sensitivity C-reactive protein, erythrocyte sedimentation rate, and inflammatory cytokines (ILβ, 8.9 pg/ml; TNFα, 11 pg/ml) were all elevated on the day of administration.

Given the patient's symptoms, two nucleic acid amplification tests for COVID-19 were performed, and the result was negative. Tests for influenza A and B, parainfluenza, respiratory syncytial virus, rhinovirus, adenovirus, and four common coronavirus strains known to cause illness in humans (HKU1, NL63, 229E, and OC43) as well as COVID-19 antibodies (IgG and IgM) were also negative.

The admission electrocardiogram showed a right bundle branch block (**Figure 1A**). Urgent coronary angiography excluded coronary artery disease (**Figures 2A–C**); therefore, transthoracic echocardiography (TTE) with strain analysis revealed diffuse left ventricular hypokinesia and increased thickness of the mid-ventricular septal wall (septal wall 13 mm; inferior wall 11 mm), and markedly reduced LV ejection fraction (LVEF 30%) (**Figures 3B,D**). Based on all these clinical and laboratory data, fulminant myocarditis was diagnosed, and treatments were immediately initiated with an intra-aortic balloon pump, which elevated the systolic blood pressure from 95 to 110 mm Hg and heart rate reduced from 100 to 85 bpm; intravenous drip of methylprednisolone (400 mg intravenous drip on the first day and then 200 mg per day for 4 more days) and intravenous immunoglobulin 20 g per day for 5 days. After these treatments, the patient's circulation stabilized and gradually recovered. On day 5, cardiac magnetic resonance (CMR) was performed, and the results revealed a corresponding extensive myocardial edema and necrosis with predominant subepicardial/mid-ventricular septal distribution highly suggestive of a myocarditis pattern (**Figures 3G,H**). Additionally, late gadolinium enhancement imaging in different positions detected massive myocardial necrosis in the medial septum, thinning of the lateral wall of the myocardium, and fibrosis. Ventricular septal myoedema was observed on T1 mapping, and the value of myocardial T1 was significantly increased (1,364 ms, **Figure 3J**). Furthermore, endocardial biopsy was performed, and histological analysis showed mildly increased cardiomyocyte diameter with some perinuclear halos and dysmetric and dysmorphic nuclei, interstitial edema with lymphocytic aggregates, myocyte necrosis, and focal areas of fibrosis were observed (**Figure 3L**). All these results helped in establishing the final diagnosis of fulminant myocarditis, which is associated with the inactivated SARS-CoV-2 vaccination.

Case 2

A 63-year-old man had received a COVID-19 vaccine injection 4 days prior and was admitted for fever, fatigue of 3 days, and chest tightness for 1 day. The patient developed fever and fatigue 1 day after the vaccination, with the highest body temperature of 39°C, no palpitation, chest tightness, cough, dizziness, headache, abdominal pain, diarrhea, nausea, or vomiting. The patient visited the local clinic and took Tylenol orally, and his body temperature was within the normal range. One day before, the patient had sudden chest tightness, palpitation, dizziness, and loss of consciousness lasting for several seconds. An emergency electrocardiogram revealed a third-degree atrioventricular block (AVB) (**Figure 1B**). The patient was immediately transferred to Tongji Hospital directly through the chest pain center. His blood pressure was 90/60 mmHg, and his heart rate was 30 beats per minute with third AVB. Emergency coronary angiography revealed no obvious coronary stenosis. At this time, a diagnosis of fulminant myocarditis was suspected. After 20 mg intravenous dexamethasone, the patient was urgently implanted with a temporary pacemaker to maintain heart rate and was also implanted with an intra-aortic balloon pump to support his circulation; his blood pressure increased to 105/60 mm Hg. The patient was transferred to the cardiac intensive care unit.

Investigations

Physical examination revealed a body temperature of 36.2°C, blood pressure of 101/60 mmHg, pulse of 77 bpm (pacemaker heart rate, **Figure 1C**), respiratory rate of 18 bpm, and blood oxygen saturation of 99% while the patient was breathing ambient air. His heart sounds were low and dull. A biochemical analysis was also performed when the patient was admitted (**Table 1**). The results reflected severe myocardial damage (cTnI was 17,961.8 pg/ml, CK 586 U/L, LDH 401 U/L). The WBC was in a normal range (5.16×10^9/L) while elevated levels of neutrophils (90.1%) and decreased levels of lymphocyte (0.47×10^9/L). Immediate transthoracic echocardiography with strain analysis documented diffuse left ventricular hypokinesia and increased thickness in the mid-ventricular septal wall (13 mm), and LVEF severely reduced to 26% (**Figures 3A,C**). The patient was immediately treated as case one, including IVIG and methylprednisolone. After these treatments, including IABP

TABLE 1 | Clinical laboratory results.

Patient	Measure	Reference range	Illness Day 4, Hospital Day 1	Illness Day 6, Hospital Day 3	Illness Day 8, Hospital Day 5	Illness Day 10, Hospital Day 7
Case 1	White-cell count (*10^9/L)	3.5–9.5	8.83	14.66	11.76	7.94
	Red-cell count (10^{12}/L)	3.8–5.1	4.49	3.74	4.1	3.98
	Absolute neutrophil count (*10^9/L)	1.8–6.3	8.18	12.98	9.92	6.2
	Absolute lymphocyte count (*10^9/L)	1.1–3.2	0.44	1.02	1.22	1.23
	Platelet count (*10^9/L)	125.0–350.0	156	171	234	254
	Hemoglobin (g/L)	115.0–150.0	132	111	124	135
	Hematocrit (%)	35.0–45.0	39.3	34.3	36.9	40.2
	Sodium (mmol/L)	136.0–145.0	133.3	136.8	/	/
	Potassium (mmol/L)	3.5–5.1	3.47	4.41	3.69	4.17
	Chloride (mmol/L)	99.0–110.0	95.4	103.4	/	/
	Calcium (mmol/L)	2.20–2.55	2.19	2.14	/	/
	Bicarbonate radical (mmol/L)	22.0–29.0	20.7	22.7	26.6	22.2
	Glucose (mmol/L)	3.9–6.1	14.18	5.6	6.1	/
	Blood urea nitrogen (mmol/L)	2.6–7.5	6.26	10.10	7.93	11.75
	Creatinine (μmol/L)	45–84	79	63	59	69
	Total protein (g/L)	64.0–83.0	73.3	65.9	72	67.2
	Albumin (g/L)	35.0–52.0	36.3	30.4	28.9	31.5
	Total bilirubin (μmol/L)	≤21.0	18.6	4.4	3.4	5.7
	Procalcitonin (ng/ml)	0.02–0.05	0.6	/	/	0.33
	Alanine aminotransferase (U/L)	≤33	43	32	80	36
	Aspartate aminotransferase (U/L)	≤32	231	78	79	130
	Alkaline phosphatase (U/L)	35–105	106	85	98	86
	Fibrinogen (g/L)	2.0–4.0	5.93	3.22	/	/
	Lactate dehydrogenase (g/L)	135.0–214.0	764	688	629	550
	Prothrombin time (s)	11.5–14.5	13.4	13.4	/	/
	International normalized ratio	0.8–1.2	1.01	1.03	/	/
	Creatine kinase (U/L)	≤190	1186	846	647	274
	Venous lactate (mmol/L)	0.5–2.2	/	1.77	/	1.82
Case 2	White-cell count (*10^9/L)	3.5–9.5	5.16	9.36	7.81	4.87
	Red-cell count (10^{12}/L)	3.8–5.1	3.25	3.79	4.3	4.1
	Absolute neutrophil count (*10^9/L)	1.8–6.3	4.65	8.08	7.16	5.3
	Absolute lymphocyte count (*10^9/L)	1.1–3.2	0.47	0.64	0.38	0.98
	Platelet count (*10^9/L)	125.0–350.0	91	83	61	110
	Hemoglobin (g/L)	130.0–175.0	98	111	125	134
	Hematocrit (%)	40.0–50.0	29.7	34.2	38.6	42
	Sodium (mmol/L)	136.0–145.0	138.8	137.8	/	139.3
	Potassium (mmol/L)	3.5–5.1	3.86	3.97	4.08	4.18
	Chloride (mmol/L)	99.0–110.0	108.9	107.0	/	/
	Calcium (mmol/L)	2.20–2.55	2.08	1.95	/	/
	Bicarbonate radical (mmol/L)	22.0–29.0	17.9	22.8	26.6	25.7
	Glucose (mmol/L)	3.9–6.1	8.49	/	5.5	/
	Blood urea nitrogen (mmol/L)	3.6–9.5	8.14	7.7	5.68	5.9
	Creatinine (μmol/L)	59–104	83	76	72	77
	Total protein (g/L)	64.0–83.0	63.4	61.7	69.5	68.4
	Albumin (g/L)	35.0–52.0	34.4	30.1	31.2	33.8
	Total bilirubin (μmol/L)	≤26.0	5.8	4.9	4.3	6.9
	Procalcitonin (ng/ml)	0.02–0.05	0.03	/	/	/
	Alanine aminotransferase (U/L)	≤33	28	41	80	85
	Aspartate aminotransferase (U/L)	≤32	93	34	44	36
	Alkaline phosphatase (U/L)	40–130	45	42	52	51

(Continued)

TABLE 1 | Continued

Patient	Measure	Reference range	Illness Day 4, Hospital Day 1	Illness Day 6, Hospital Day 3	Illness Day 8, Hospital Day 5	Illness Day 10, Hospital Day 7
	Fibrinogen (g/L)	2.0–4.0	3.94	/	3.87	/
	Lactate dehydrogenase (g/L)	135.0–214.0	586	459	501	334
	Prothrombin time (s)	11.5–14.5	14.2	15.5	14.6	12
	International normalized ratio	0.8–1.2	1.09	/	/	/
	Creatine kinase (U/L)	≤190	586	687	423	211
	Venous lactate (mmol/L)	0.5–2.2	1.41	/	1.23	/

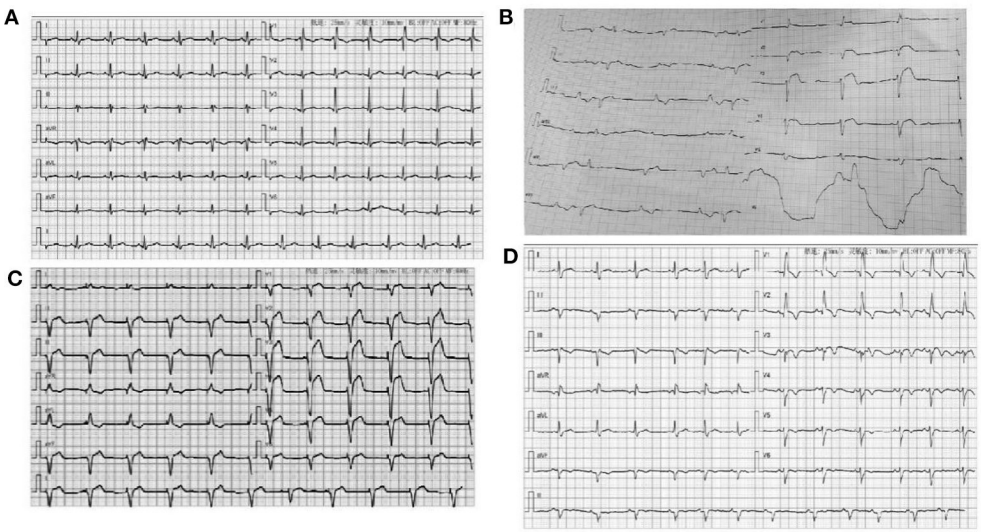

FIGURE 1 | (A) ECG for the woman patient when admitted to hospital: Sinus rhythm Right bundle branch block; **(B)** ECG of the man patient when admitted to hospital; V1–V3 lead ST-elevation and third-degree atrioventricular block; **(C)** ECG of the man patient when implanted with temporary pacing; **(D)** ECG of the man patient at discharge: Sinus rhythm, Right bundle branch block.

for circulatory support and immunomodulation therapy using sufficient doses of methylprednisolone and IVIG, the patients became stable soon. Five days later, his temporary pacemaker was withdrawn with regular sinus rhythm, and at this time, the CMR test revealed a corresponding extensive myocardial edema and necrosis with predominant subepicardial/mid-ventricular septal distribution highly suggestive of a myocarditis pattern (**Figures 3E,F**). Ventricular septal myoedema was observed on T1 mapping, and the value of myocardial T1 was significantly increased (1,380 ms in the male patient, **Figures 3I,J**).

Histological analysis of the endocardial biopsy confirmed the diagnosis of fulminant myocarditis with interstitial edema and lymphocytic lymphocyte infiltration (**Figure 3K**).

OUTCOME AND FOLLOW-UP

Case 1

After treatment for 10 days, her LVEF recovered to 52%, and she was discharged from the hospital with oral beta-blockers (47.5 mg/day), perindopril (4 mg/day), and prednisone 20 mg/day. At the first follow-up after 1 month, her LVEF was 60%, cTnI level reduced from 12,000 pg/ml at discharge to 4,700 pg/ml and NT-proBNP reduced to close to normal levels (108 ng/L).

Case 2

After 9 days, the LVEF recovered to 59% with a normal sinus rhythm (**Figure 1D**) when he was discharged with oral beta-blockers (47.5 mg/day), perindopril (4 mg/day), and prednisone 20 mg/day. At the first follow-up after 1 month, her LVEF was 62%, cTnI level reduced from 17,961.8 pg/ml at discharge to 45 pg/ml and NT-proBNP reduced to normal levels (76 ng/L).

DISCUSSION

Previous studies reported only mild or moderate adverse events following the COVID-19 vaccine, including thrombosis and even pulmonary thrombosis (12). To the best of our knowledge, this is the first report of inactivated COVID-19 vaccine-associated fulminant myocarditis cases.

The two patients reported in this study had no previous history of myocarditis. They were in good health and had no

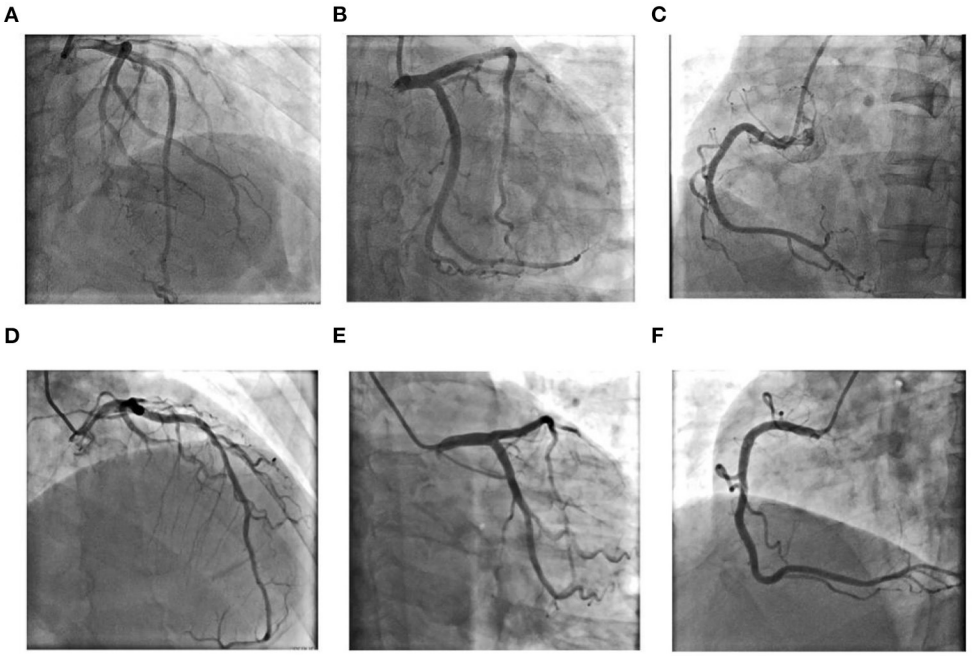

FIGURE 2 | Coronary angiography results for Case 1 **(A–C)**: **(A)** Left coronary artery: Cranial 30°; **(B)** Left coronary artery: Caudal 30°; **(C)** Right coronary artery: Left anterior oblique 45°; Coronary angiography results for Case 2 **(D–F)**: **(D)** Left anterior oblique 30°+ Cranial 30°; **(E)** Left coronary artery: Caudal 30°; **(F)** Right coronary artery: Left anterior oblique 45°.

recent travel history. They were all at home in a community free of COVID-19 case. However, we found that they both had clinical symptoms that appeared the day after the COVID-19 vaccination. At present, we do not know whether the inactivated COVID-19 vaccine can directly cause myocarditis. However, based on the epidemiological analysis, these two cases of fulminant myocarditis may be possibly related to COVID-19 vaccination.

The term myocarditis refers to the inflammation of the heart muscle, which can be caused by infections, toxic substances, or autoimmune processes. A diagnosis of active myocarditis requires the presence of inflammatory infiltrates of non-ischemic origin in myocardial tissue associated with necrosis and/or degeneration of the adjacent cardiomyocytes. The diagnosis of myocarditis is a challenging diagnosis because of the heterogeneity of clinical presentations. Endomyocardial biopsy (EMB) is considered the reference standard for the diagnosis of myocarditis. In our report, both of our cases detected myocardial fibers, cardiomyocytes and myocardial interstitium all demonstrated edema of varying degrees with infiltration of chronic inflammatory (lymphocytic cells) cells, according to the Marburg criteria and Quantitative criteria (13, 14). Furthermore, myocyte necrosis and focal areas of fibrosis had also occurred in case 1. This phenomenon indicates the possibility of chronic transformation of acute myocarditis into inflammatory cardiomyopathy. The results of CMR imaging were also consistent with the typical findings of myocarditis. It is worth noting that the extent of LGE is a dynamic process in acute myocarditis, mainly related to tissue edema in the acute phase that progressively disappears over time, whereas in the late phase, LGE mainly reflects postinflammatory replacement fibrosis. Moreover, immunohistochemistry is the current standard method used to evaluate infiltrating immune cells in tissues. However, the quantification and comparison of the different cell subsets are sometimes difficult. Immunohistochemistry-specific antibodies for leukocytes (CD45), macrophages (CD68), T cells (CD3) and their main subtypes, helper (CD4) and cytotoxic (CD8) cells, and B cells (CD19/CD20) can also increase the sensitivity of EMB. These measures will be helpful in the diagnosis and differential diagnosis of myocarditis.

At present, the underlying pathogenetic mechanisms of fulminant myocarditis are not known clearly, but may involve virus or other pathogen-induced initial myocardial injury and, more importantly, subsequent severe injury by aggravation of inflammatory cells and cytokine storm through pattern recognition receptors via both pathogen-associated molecular patterns and damage-associated molecular patterns (15–17). In these two patients, no evidence of other infections was detected. The possibility of COVID-19 vaccine-induced immune-related myocarditis was considered based on epidemiological history.

We do not know exactly how vaccine injection induces fulminant myocarditis. Under desired conditions, antigen vaccination is initially recognized by innate immune cells, such as dendritic cells and macrophages, engulfed by phagocytosis, and present pathogen-derived peptide antigens to naïve T cells, which then activate and instruct the development of antigen-specific adaptive immunity. However, inactivated COVID-19 virus contains RNAs and proteins and induces

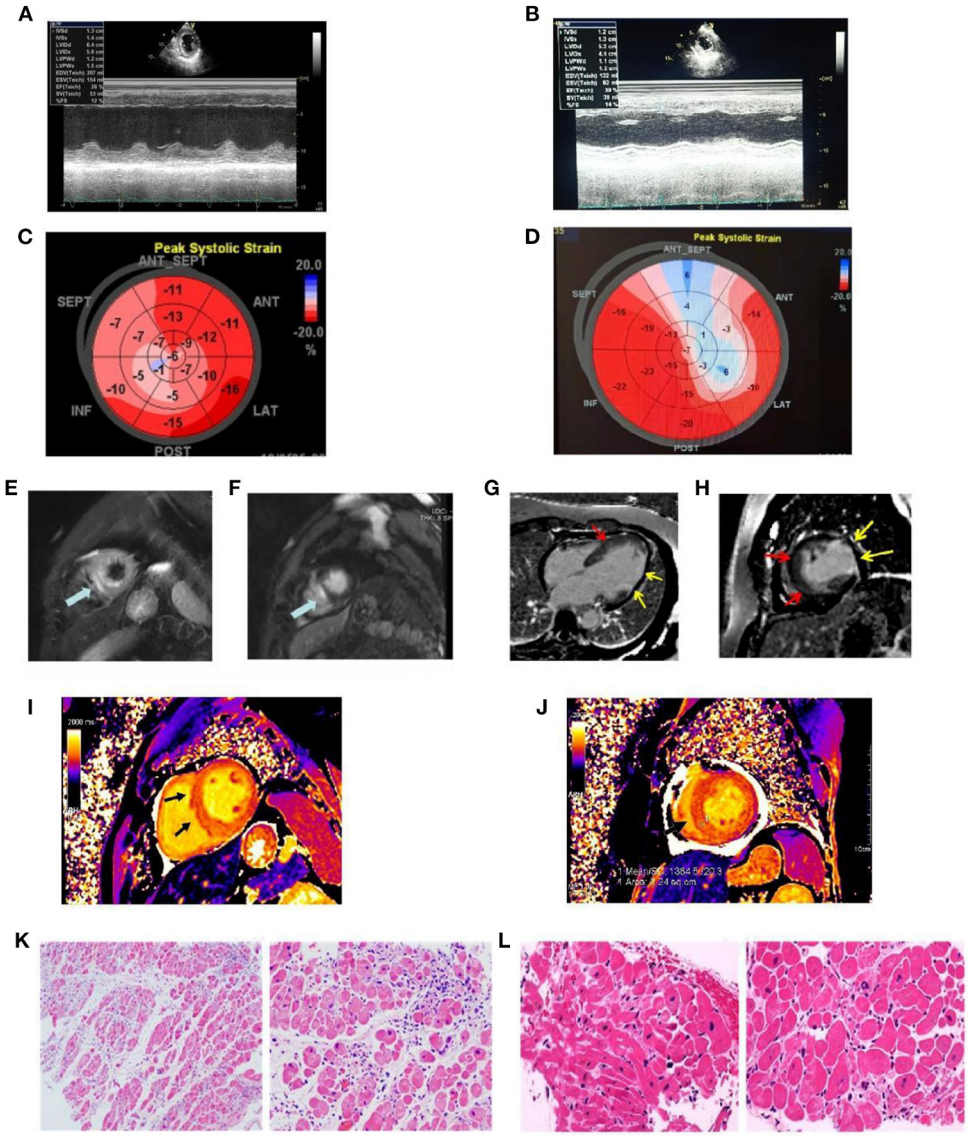

FIGURE 3 | The echocardiographic and cardiac magnetic resonance images recorded at admission and the findings of pathological specimens compatible with fulminant myocarditis. **(A)** LV ejection fraction was mildly reduced (EF value, 28%); **(B)** LV ejection fraction was mildly reduced (EF value = 30%); **(C)** Representative images of global longitudinal strains (GLS) presented as "bullseye" displays in case 1 (GLS = −12.1%); **(D)** Representative images of global longitudinal strains (GLS) presented as "bullseye" displays in case 2 (GLS = −9.8%); **(E)** Increased myocardial signal in the outer layer of the apical ventricular septum (edema) (arrow); **(F)** Late gadolinium enhancement imaging suggests myocardial enhancement in the outer layer of the apical ventricular septum (myocardial necrosis) (arrow); **(G)** Long-axis late gadolinium enhancement imaging suggests myocardial necrosis in the middle ventricular septum (red arrow), thinning, and enhancement of the lateral wall (yellow arrow); **(H)** Short axial late gadolinium enhancement imaging demonstrates myocardial necrosis in the middle ventricular septum (red arrow) with thinning of the lateral wall and formation of fibrosis (yellow arrow); **(I)** in T1 mapping, ventricular septal myocardial edema was observed, and the value of myocardial T1 was significantly increased, T1=1380 ms (normal value T1 = 1,180 ± 20 ms); **(J)** Myocardial edema in the lower interventricular septum was observed in T1 mapping, and the value of myocardial T1 was significantly increased, T1 = 1,364 ms (normal value T1 = 1,180 ± 20 ms); **(K)** Biopsy from myocardium showing myocardial fibers were slightly edematous and interstitial edema was accompanied by infiltration of inflammatory cells; **(L)** Biopsy from myocardium showing myocardial atrophy, hypertrophy of some cardiomyocytes, myocardial interstitial edema, local fibrosis, scattered focal necrosis of cardiomyocytes accompanied by infiltration of inflammatory cells.

a non-adaptive response, resulting in an overactivated inflammatory response, such as myocarditis or lethal fulminant myocarditis (15–18).

The mainstay of treatment for fulminant myocarditis is immunoregulatory therapy and an optimal heart failure medical regimen. Moreover, EMB is the basis for safe (infection-negative) immunosuppression or antiviral treatment. Our center has accumulated a lot of practical experience in the treatment of fulminant myocarditis, including COVID-19 related myocarditis (2, 6, 19, 20). In our report, a life-support-based comprehensive

treatment regimen was preferentially used to treat the patients according to expert consensus recommendations (21). In this treatment regimen, mechanical circulatory support is based and simultaneously, immunomodulatory therapy using sufficient doses of glucocorticoids and intravenous immunoglobulin plays an important role in the treatment of myocardial injury and the regulation of inflammatory response. In a previous study, we demonstrated that early application of IABP is sufficient to stabilize circulation in most patients with fulminant myocarditis (2). A combination of glucocorticoid and IVIG can modulate the overactivated immune response and inhibit severe cardiac inflammation (22–25); therefore, it was successfully used in these two cases.

SUMMARY

Both the clinical and endomyocardial biopsy analyses of these two cases related to the SARS-CoV-2 vaccines confirmed the diagnosis of fulminant myocarditis; hence, based on the frequency and social importance of vaccination, vaccine-related adverse reactions should be further investigated and pay close attention in a larger population and in different ethnic groups because fulminant myocarditis is lethal.

Limitations and Strengths

Strengths: This case serves as a reminder of the importance of the possibility of COVID-19 vaccine-induced immune-related myocarditis and its management. Weaknesses: Several mRNA COVID-19 vaccine-related myocarditis have been reported before. In this case, thorough etiologic tests for myocarditis did not reveal any specific cause for viral myocarditis. The mechanism is uncertain and there is no specific diagnostic method for this etiology. It is also unclear why patients do not have COVID abs after receiving vaccinations. The etiologic diagnosis of the inactivated COVID-19 vaccine-related myocarditis would be dependent on the manner of exclusion in a case with a temporal relationship. We need to wait for further cases to confirm this epidemiological relationship.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Health Commission of China and the Institutional Review Board at Tongji Hospital. 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

GC participated in the research design, carried out the epidemiological investigation, performed statistical analyses, and drafted the manuscript. RL and CZ collected samples, participated in the epidemiological investigation, and collected samples for this study. DW participated in the research design, carried out the epidemiological investigation. All authors have read and approved the final manuscript.

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frontiers in Cardiovascular Medicine

SYSTEMATIC REVIEW
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Cumulative Evidence for the Association of Thrombosis and the Prognosis of COVID-19: Systematic Review and Meta-Analysis

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Background: Although thrombosis events have been reported in patients with coronavirus disease 2019 (COVID-19), the association between thrombosis and COVID-19-related critical status or risk of mortality in COVID-19 has been inconsistent.

Objective: We conducted a meta-analysis of reports assessing the association between thrombosis and the prognosis of COVID-19.

Methods: The EMBASE, Ovid-MEDLINE, and Web of Science databases were searched up to December 9, 2021, and additional studies were retrieved *via* manual searching. Studies were included if they reported the risk of COVID-19-related critical status or COVID-19-related mortality in relation to thrombosis. The related data were extracted by two authors independently, and a random effects model was conducted to pool the odds ratios (ORs). In addition, stratified analyses were conducted to evaluate the association.

Results: Among 6,686 initially identified studies, we included 25 studies published in 2020 and 2021, with a total of 332,915 patients according to predefined inclusion criteria. The associations between thrombosis and COVID-19-related mortality and COVID-19-related critical status were significant, with ORs of 2.61 (95% CI, 1.91–3.55, $p < 0.05$) and 2.9 (95% CI, 1.6–5.24, $p < 0.05$), respectively. The results were statistically significant and consistent in stratified analyses.

Conclusions: Thrombosis is associated with an increased risk of mortality and critical status induced by COVID-19. Further prospective studies with large sample sizes are required to establish whether these associations are causal by considering more confounders and to clarify their mechanisms.

Observational studies cannot prove causality. However, autopsy studies show thrombosis events preceding COVID-19-related deaths. The results of this meta-analysis reported that thrombosis was associated with a 161% increased risk of mortality from COVID-19 and a 190% increased risk of COVID-19-related critical status. The type of thrombosis included in the original studies also seemed to be related to the results.

Keywords: thrombosis and COVID-19 thrombosis, SARS-CoV-2, COVID-19, 2019-nCoV, mortality

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), a novel infectious disease, is highly prevalent globally and has infected over 271 million patients to date (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and progressive respiratory failure is the primary cause of death (1) during the COVID-19 pandemic. Over 5 million individuals globally have succumbed to COVID-19 (https://covid19.who.int/). However, little is known about the causes of death. Histologic autopsy of pulmonary vessels in patients with COVID-19 showed widespread thrombosis with microangiopathy (1–3). Luca Spiezia et al. (4) reported that severe hypercoagulability rather than consumptive coagulopathy station was observed in patients with COVID-19 with acute respiratory failure. Fibrin formation and polymerization may contribute to thrombosis and correlate with critical status and a worse outcome in patients with COVID-19 (4, 5). An increased risk of thrombosis, such as venous thromboembolism (VTE), brain stroke, cardiac ischemia, and pulmonary embolism (PE), in patients with COVID-19 admitted to the intensive care unit (ICU) has been reported (6–9). The magnitude of this public health challenge is increasing, a concerning trend given that COVID-19 imposes a significant public health burden and large demand on health care systems. The association between thrombosis and COVID-19 prognosis should be recognized by clinical doctors globally.

There were four types of thrombosis found in patients with COVID-19: pale thrombus, mixed thrombus (arterial and venous thrombosis), red thrombus, and hyaline thrombus (microvascular thrombosis). A hypercoagulable state in the critically ill patients with COVID-19 was found due to the following mechanisms: severe hypofibrinolysis (10), endothelial dysfunction (11, 12), platelet activation (12, 13), endothelial-derived von Willebrand factor (vWF) activation (14), elevated soluble (s) P-selectin (13, 15), gene expression (13, 16), inflammatory cytokine activation (17, 18), and mannose-binding lectin (MBL)-related complement activation (19, 20). Serious adverse events, such as thrombosis and thrombocytopenia syndrome, after COVID-19 vaccination are rare (21) and are associated with a high mortality rate (22). Campello et al. found that no hypercoagulable condition was found after COVID-19 (ChAdOx1 or BNT162b2) vaccination (23).

A number of primary studies (24–28) have evaluated the association between thrombosis and the risk of adverse outcomes of COVID-19, including mortality and severity of COVID-19, with inconsistent results. We, therefore, conducted a meta-analysis to evaluate the association between thrombosis and the prognosis of COVID-19.

Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019; DVT, deep venous thrombosis; ICU, intensive care unit; PE, pulmonary embolism; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VTE, venous thromboembolism.

METHODS

Retrieval of Studies

The reporting of this meta-analysis of observational studies was in accordance with the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The Embase, Ovid-MEDLINE, and Web of Science databases were searched up to 9 December 2021. The search consisted of three terms: thrombosis, COVID-19, and study design. We used the following key words to search for the first term: "thrombosis" OR "embolism" OR "thrombotic" OR "thrombus" OR "thrombi" OR "thromboembol*" OR "emboli*" OR "embolus" OR "clot?" OR "DVT" OR "VTE" OR "PE." We used the following key words to search for the second term: "SARS-CoV-2" OR "COVID-19." The third term was associated with "risk," "mortality," and "cohort." Finally, we used "AND" to connect the three terms. For the search strategy, see **Supplementary Material**. The retrieved studies were first screened by reading the titles and abstracts. Two authors (Dongqiong Xiao and Hu Gao) independently read the full texts of the remaining studies. Fajuan Tang resolved any disagreements.

Definition

The critical status among patients with COVID-19 is with any of the following conditions—shock, respiratory failure requiring mechanical ventilation, and/or other organ dysfunction requiring admission to the intensive care unit (ICU) (24).

Study Selection

The inclusion criteria were as follows: (1) studies with participants who were investigated for the following outcomes: the incidence, prevalence, or risk or odds ratio (OR) of mortality and critical status in patients with COVID-19 with thrombosis relative to those without thrombosis; (2) studies that evaluated the association between thrombosis and prognosis of COVID-19 and reported unadjusted or adjusted ORs and their corresponding 95% confidence intervals (CIs) or the number of patients with COVID-19 with thrombosis relative to those without thrombosis; and (3) studies with case-control, cohort, or cross-sectional designs published in English.

The exclusion criteria were as follows: (1) studies that reported the results of few autopsy cases of COVID-19; (2) unrelated studies or studies in which the data overlapped with those of another study or studies that reported the association between the D-dimer level and COVID-19 without evidence of definite thrombosis; or (3) reviews, case reports, and meta-analyses.

Data Extraction

The data were independently extracted from the studies by Dongqiong Xiao and Hu Gao, and they were aggregated in a standardized form; the collected data included study author and year, study location and design, sample size, type of thrombosis, primary outcomes (presence or absence of critical status, COVID-19-related mortality), adjusted for confounding factors, and Newcastle-Ottawa Scale (NOS) scores for the included studies.

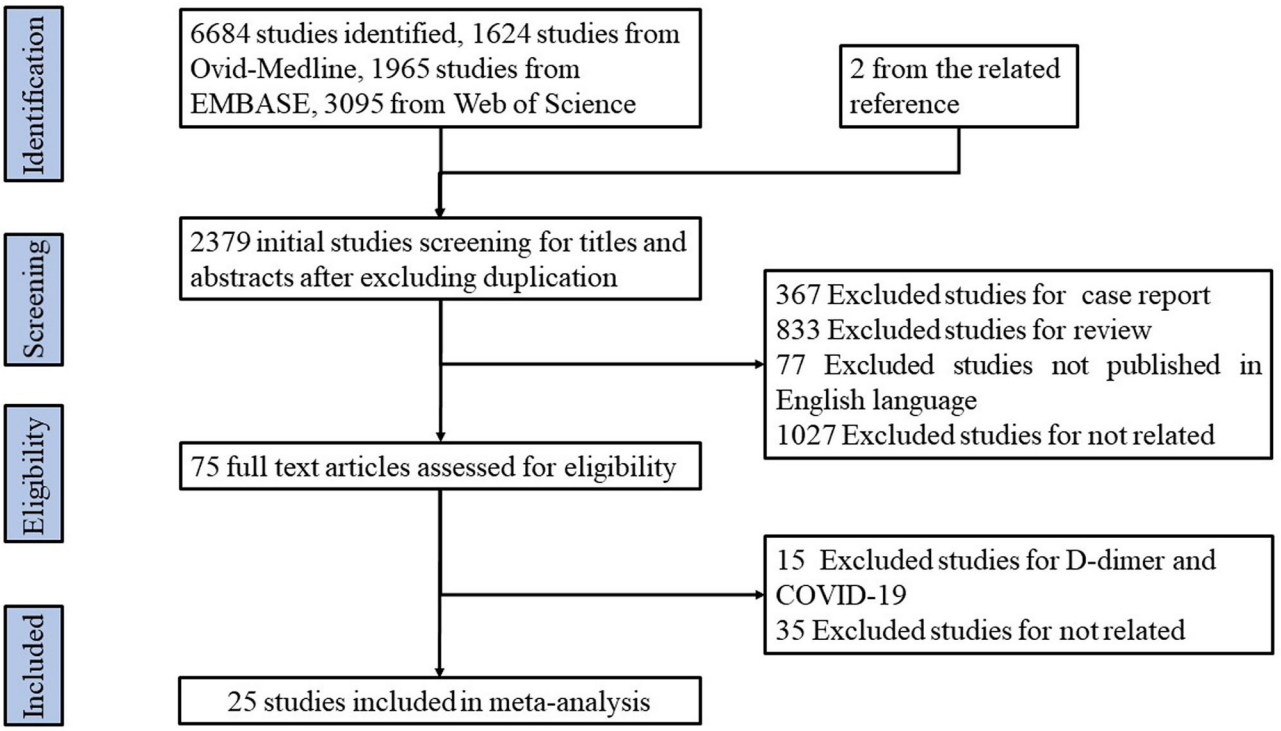

FIGURE 1 | A flow chart describing study selection.

Quality Evaluation

The methodological quality of all the included studies (**Supplementary Table 2**) was examined by Dongqiong Xiao and Hu Gao independently using the NOS (29), and Fajuan Tang resolved any disagreements. The reviewers assessed the quality scores (varying from 0 to 9) in three domains: selection of the study population, evaluation of exposure and outcomes, and comparability.

Statistical Analysis

The odds ratios (ORs) and 95% CIs were used as measures of the association between thrombosis and the prognosis of COVID-19 across studies. For original studies that compared the number of participants who developed critical status and death exposure to thrombosis compared with control groups, we calculated ORs and 95% CIs for each study (30). All data from the included studies were converted into log (ORs) and standard errors (SEs) (31). We pooled the log (ORs) and SEs of each study separately using the DerSimonian-Laird formula (random effects model) (32). We used the I^2 statistic to assess the statistical heterogeneity among the studies (33). High heterogeneity was indicated with values of $I^2 > 50\%$ and $p < 0.05$ (34).

We conducted stratified analyses based on the study location (Europe, the United States, and Asia), study design (cohort, cross-sectional), sample size ($\geq$ 1,000 $<$ 1,000), type of thrombosis (VTE, PE, DVT, and others), adjusted for confounding factors [not available (NA), adjusted $\leq$ 7 factors, adjusted $\geq$ 8 factors, $\leq$ 7 factors], adjusted for age (yes, no), adjusted for sex (yes, no), adjusted for body mass index (BMI) (yes, no), adjusted for diabetes (yes, no), and adjusted for comorbidities (yes, no).

We used Egger's tests, Begg's tests, and funnel plots in the meta-analysis to assess publication bias (33–36). We used Stata software, version 12.0 (StataCorp, College Station, TX) and Review Manager, version 5.3 to perform the statistical tests.

RESULTS

Literature Search

We identified 6,686 potential studies, including 1,624 from Ovid-MEDLINE, 1,965 from Embase, 3,095 from Web of Science, and 2 from the related references (**Supplementary Table 3**). After careful screening, 6,661 studies were excluded for the reasons listed in **Figure 1**, and 25 studies reporting the association between thrombosis and prognosis of COVID-19 met the inclusion criteria (see **Figure 1**). These 25 included studies are summarized in **Table 1**.

Characteristics and Quality of the Included Studies

Table 1 shows the characteristics of the 25 included studies. Among the included studies, 6 studies (24, 26, 37–40) were cross-sectional studies, and 19 studies (7, 25, 27, 28, 41–55) were cohort studies. The association between thrombosis and COVID-19-related mortality was the primary outcome of interest in 19

TABLE 1 | Characteristics of the included studies.

Study	Year	Study location	Sample size	Study design	Type of thrombosis	Outcomes	Adjusted for
Zhang	2020	China	143	CSS	VTE	Mortality and critical care status	NA
Yaghi, Shadi	2020	United States	3,556	Retrospective cohort	Brain stroke	Mortality	Age and NIHSS score
Stoneham, Simon M.	2020	UK	230	CSS	VTE	ICU hospitalization	NA
Middeldorp, S.	2020	Netherlands	198	Retrospective cohort	VTE	Mortality and critical care status	Age, sex, and ICU stay
Leonard-Lorant, Ian	2020	France	106	Retrospective cohort	PE	ICU hospitalization	NA
Klok, F. A.	2020	Netherlands	184	Retrospective cohort	Thrombotic complications	Mortality	NA
Jain, R.	2020	United States	3,218	Retrospective cohort	Brain stroke	Mortality	Age, BMI, and hypertension
Bhayana, R.	2020	United States	412	CSS	Abdominal ischaemia	ICU hospitalization	NA
Ren, B.	2020	China	48	CSS	VTE	Mortality	NA
Galloway, James B	2020	UK	1,157	Retrospective cohort	Cardiac ischaemia	Mortality and critical care status	>8 factors, age, sex, and with comorbidities (such as hypertension and diabetes mellitus)
Corrado Lodigiani	2020	Italy	338	Retrospective cohort	VTE	ICU hospitalization	NA
Avruscio	2020	Italy	85	Observational cohort	VTE	ICU hospitalization	NA
Contou	2020	France	92	CSS	PE	Mortality	NA
Abizaid	2021	Brazil	152	Prospective study	MI	Mortality	Age, prior coronary disease, and myocardial blush
Alharthy	2021	Saudi Arabia	352	Retrospective study	PE	Mortality	Age, ICU length of stay, SpO_2/FiO_2 ratio, WBCs, lymphocytes, D-dimer, lactate, and active smoking
Alwafi	2021	Saudi Arabia	706	CSS	VTE	Mortality	Age, sex, and comorbidities (diabetes mellitus, hypertension, coronary artery disease, end-stage renal disease, asthma, congestive heart failure, cerebrovascular accident, chronic obstructive pulmonary disease, chronic liver disease, and cancer)
Anderson	2021	UK	312,378	Cohort	VTE	Mortality Critical status	Comorbid cardiovascular disease (myocardial infarction, heart failure, angina, stroke, transient ischaemic attack, atrial fibrillation/flutter, and valve disease) and prevalent diabetes mellitus; use of exogenous oestrogens in women only
Arribalzaga	2021	Spain	5,966	Cohort	VTE	Mortality	Age, sex, follow-up (days), and time from admission to VTE diagnosis
Fournier	2021	France	531	Cohort	Arterial thrombotic events	Mortality	Age, sex, and comorbidities (cancer, HIV infection, inflammatory disorders, high blood pressure, smoking, and diabetes)
Purroy	2021	Spain	1,737	Cohort	Thromboem-bolism	Mortality	Age, diabetes, chronic obstructive pulmonary disease, ICU care, systolic blood pressure, and oxygen saturation
Riyahi	2021	USA	413	Retrospective cohort	PE	Mortality	NA

(Continued)

TABLE 1 | Continued

Study	Year	Study location	Sample size	Study design	Type of thrombosis	Outcomes	Adjusted for
Scudiero	2021	Italy	224	Retrospective cohort	PE	Mortality	Age, sex, and comorbidities
Violi	2021	Italy	373	Prospective multicentre study	Thrombotic events	Mortality	Age, sex, COPD, diabetes, and D-dimer
Wang	2021	China	88	Retrospective	DVT	Critical status	NA
Paz Rios	2021	USA	184	Retrospective observational study	VTE	Mortality	Age, sex, race, comorbidities (diabetes, hypertension, COPD, CKD, heart failure, cancer, and atrial fibrillation)

CSS, cross-sectional study; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; DVT, deep venous thrombosis; HIV, human immunodeficiency virus; ICU, intensive care unit; MI, myocardial infarction; NA, not available; PE, pulmonary embolism; USA, United States of America; VTE, venous thromboembolism; WBC, white blood cell.

studies, and the association between thrombosis and COVID-19-related critical status was the primary outcome in 10 studies.

The related studies were published in 2020 and 2021, and the sample size ranged from 48 to 312,378, for a total of 332,915 participants across studies.

Five studies (25, 38, 42, 51, 55) were conducted in the United States, 5 studies (24, 26, 39, 46, 54) were conducted in Asia, 14 studies (7, 27, 28, 37, 40, 41, 43, 44, 47–50, 52, 53) were conducted in Europe, and one study (45) was conducted in Brazil. All the included studies included both adult men and women.

Among the included studies, 13 studies (25–27, 39, 42, 45, 46, 48–50, 52, 53, 55) adjusted for age, 7 studies (27, 39, 48, 49, 52, 53, 55) adjusted for sex, one study (42) adjusted for BMI, 8 studies (26, 39, 47, 49, 50, 52, 53, 55) adjusted for diabetes mellitus, and 7 studies (39, 43, 46, 47, 49, 52, 55) adjusted for 8 or more confounding factors.

The quality scores of the included studies ranged from 6 to 8 (**Supplementary Table 1**), and they were considered high.

Quantitative Results (Meta-Analysis)

Among the 25 selected studies, 19 studies revealed the association between thrombosis and COVID-19-related mortality, and 10 studies investigated the association between thrombosis and COVID-19-related critical status. Among the included studies, 5 studies (26, 43, 47, 48, 51) found a non-significant association between thrombosis and COVID-19-related mortality, while the other 14 studies (24, 25, 27, 28, 39, 40, 42, 45, 46, 49, 50, 52, 53, 55) revealed that thrombosis would increase the risk of mortality from COVID-19. All 19 studies reported risks as ORs, ranging from 0.79 to 40.27. Any type of thrombosis was associated with an increased risk of mortality from COVID-19 compared with the control, with a pooled OR of 2.61 (95% CI, 1.91, 3.55). High heterogeneity was found in these studies ($I^2 = 84\%$, $p < 0.05$) (**Figure 2**).

Additionally, among the included studies, 4 studies (7, 37, 38, 43) found a non-significant association between thrombosis and COVID-19-related critical status, while the other 6 studies (24, 27, 41, 44, 47, 54) revealed that thrombosis would increase the risk of COVID-19-related critical status. All seven studies reported risks as ORs, ranging from 0.8 to 9.3. Any type of thrombosis was associated with an increased risk of COVID-19-related critical status compared with the control, with a pooled OR of 2.9 (95% CI, 1.6, 5.24). High heterogeneity was reported in the studies ($I^2 = 80\%$, $p < 0.05$) (**Figure 2**).

Stratified Analyses

Thrombosis and COVID-19-Related Mortality

Among the 25 selected studies, 19 studies revealed the association between thrombosis and COVID-19-related mortality. Stratified analyses of clinical factors and study characteristics were conducted to evaluate possible sources of heterogeneity in the included studies (**Table 2**). The association between thrombosis and COVID-19-related mortality was significant at 2.61 (95% CI, 1.91, 3.55), and this association was consistent in all of the stratified analyses (**Table 2**). Stronger associations between thrombosis and the COVID-19-related mortality were found in cross-sectional studies (OR: 4.86, 95% CI, 1.99, 11.83) when compared to that in cohort studies (OR: 2.39, 95% CI, 1.72, 3.33) in studies with small sample sizes (< 1,000) (OR: 2.95, 95% CI, 2.28, 3.82) when compared to studies with large sample sizes (≥ 1,000) (OR: 1.99, 95% CI, 1.1, 3.58), and in studies that were conducted in the United States compared with studies conducted in Europe and Asia (**Table 2**).

The type of thrombosis included in the original reports also seemed to be related to the results. For example, studies demonstrated a weaker association between thrombosis and the COVID-19-related mortality if the thrombosis was VTE (OR: 2.48, 95% CI, 1.17, 5.25) when compared to other types of thrombosis (OR: 3.17, 95% CI, 1.95, 5.16).

The association between thrombosis and the COVID-19-related mortality was strong when the studies were not adjusted for sex, diabetes, comorbidities, or <8 confounding factors (**Table 2**).

Thrombosis and COVID-19-Related Critical Status

Among the 25 selected studies, 10 studies investigated the association between thrombosis and COVID-19-related critical

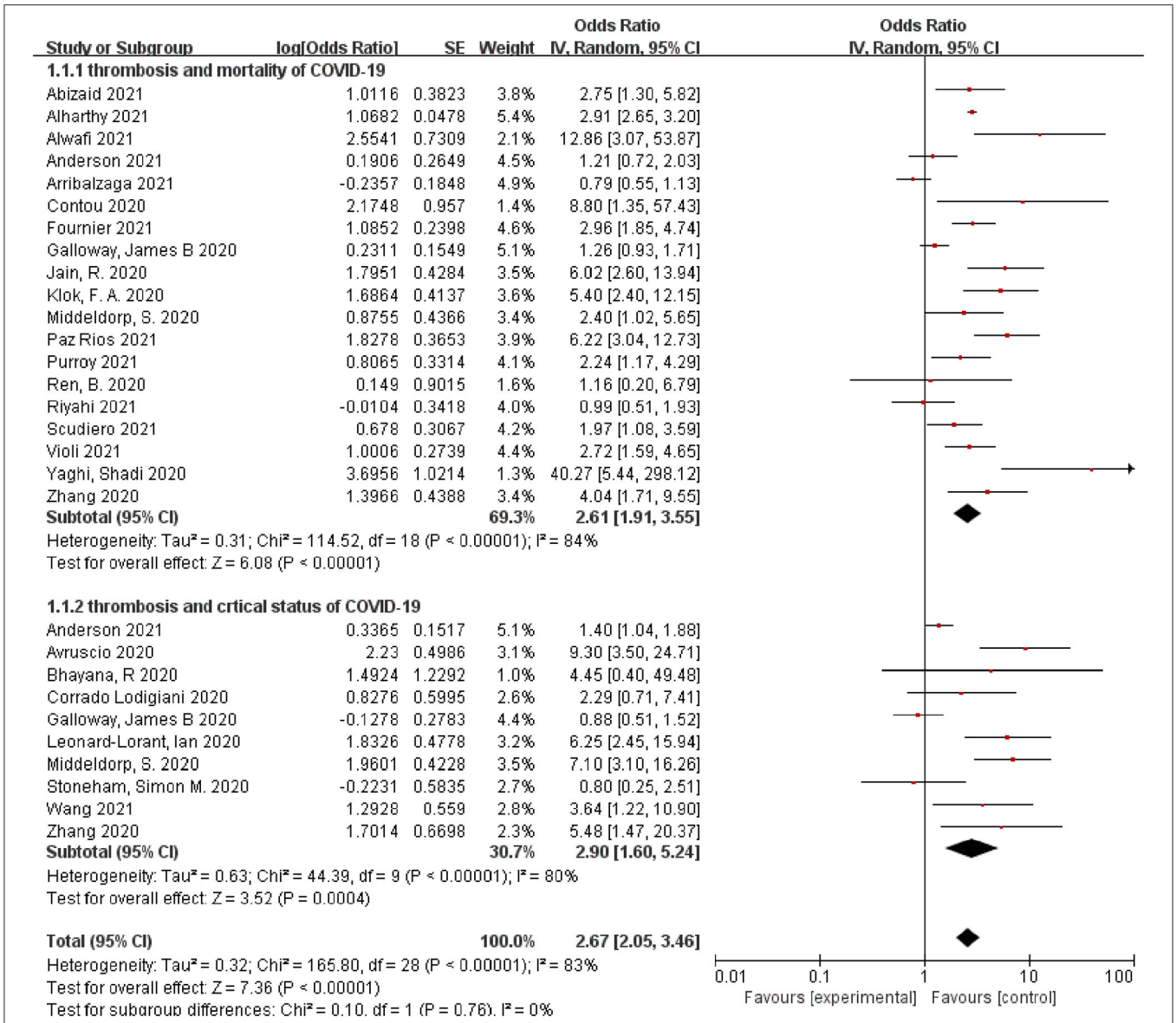

FIGURE 2 | A forest plot of the pooled odds ratio of the association between thrombosis and prognosis of COVID-19, including mortality and critical status.

status. The same stratified analyses were conducted (**Table 2**). The association between thrombosis and COVID-19-related critical status was significant (OR: 2.9, 95% CI, 1.6, 5.24), and it was consistent in all of the stratified analyses (**Table 2**). Sample size, study location, type of thrombosis, adjusted for more than 8 confounding factors, diabetes, and comorbidities seemed to be correlated with the results. For example, stronger associations between thrombosis and COVID-19-related critical status were found in studies that were conducted in Asia (OR: 4.31, 95% CI, 1.86, 9.99) when compared to those in studies that were conducted in Europe (OR: 2.58, 95% CI, 1.28, 5.19) and in studies with a small sample size (< 1,000) (OR: 4.17, 95% CI, 2.37, 7.35) when compared to those in studies with a large sample size (≥ 1,000) (OR: 1.18, 95% CI, 0.76, 1.83) (**Table 2**).

The association between thrombosis and COVID-19-related critical status was strong when the studies were not adjusted for diabetes, comorbidities, or <8 confounding factors (**Table 2**).

Publication Bias

Potential publication bias was revealed by asymmetrical funnel plots (**Figure 3**). The publication bias test for the association between thrombosis and COVID-19-related mortality was not significant (Begg's test with $p = 0.069$, $z = 1.82$), and publication bias was also not statistically significant for the association between thrombosis and COVID-19-related critical status with Begg's test ($p = 0.858$, $z = 0.18$) (**Supplementary Table 4**).

TABLE 2 | Stratified analysis of the associations between thrombosis and mortality and COVID-19-related critical status.

	Thrombosis and mortality				Thrombosis and critical status			
Variables	**Studies**	**OR (95% CI)**	**I^2 (*P-value*)**	***P***	**Studies**	**OR (95% CI)**	**I^2 (*P-value*)**	***P***
Total	19	2.61 (1.91, 3.55)	84% (<0.05)		10	2.9 (1.6, 5.24)	83% (<0.05)	
Study location								
Europe	10	2.01 (1.37, 2.95)	79% (<0.05)	**<0.05**	7	2.58 (1.28, 5.19)	85% (<0.05)	**<0.05**
Unites States-Brazil	5	4.24 (1.67, 10.76)	83% (<0.05)		1	4.45 (0.4, 49.48)	NA	
Asia	4	3.51 (1.95, 6.3)	47% (0.13)		2	4.31 (1.86, 9.99)	0 (0.64)	
Study design								
Cohort	15	2.39 (1.72, 3.33)	87% (<0.05)	**<0.05**	7	3.11 (0.55, 6.2)	85% (<0.05)	*>0.05*
Cross-sectional	4	4.86 (1.99, 11.83)	35% (0.18)		3	2.38 (0.58, 9.76)	61% (0.08)	
Sample size								
≥1,000	6	1.99 (1.1, 3.58)	85% (<0.05)	*>0.05*	2	1.18 (0.76, 1.83)	53% (0.14)	**<0.05**
<1,000	13	2.95 (2.28, 3.82)	53% (0.01)		8	4.17 (2.37, 7.35)	50% (0.05)	
Type of thrombosis								
VTE	7	2.48 (1.17, 5.25)	86% (<0.05)	**<0.05**	6	2.67 (1.28, 5.59)	75% (<0.05)	**<0.05**
PE	4	2.16 (1.18, 3.93)	76% (<0.05)		1	6.25 (2.45, 15.94)	NA	
DVT	0	NA	NA		1	3.64 (1.22, 10.90)	NA	
Other	8	3.17 (1.95, 5.16)	79% (<0.05)		2	1.27 (0.34, 4.38)	39%(0.2)	
Adjusted for confounding factors								
NA	5	2.81 (1.16, 6.78)	72% (<0.05)	**<0.05**	7	3.74 (1.95, 7.16)	52% (0.05)	**<0.05**
Adjusted (≤7 factors)	6	3.06 (1.35, 6.95)	88% (<0.05)		1	7.1 (3.1, 16.26)	NA	
Adjusted (≥8 factors)	8	2.25 (1.54, 3.31)	86% (<0.05)		2	1.18 (0.76, 1.83)	53% (0.14)	
Adjusted for age								
Yes	12	2.8 (1.91, 4.1)	88% (<0.05)	*>0.05*	2	2.44 (0.32, 18.87)	94% (<0.05)	*>0.05*
No	7	2.29 (1.26, 4.17)	68% (<0.05)		8	3.1 (1.59, 6.06)	74% (<0.05)	
Adjusted for sex								
Yes	8	2.39 (1.43, 3.97)	87% (<0.05)	*>0.05*	2	2.44 (0.32, 18.87)	94% (<0.05)	*>0.05*
No	11	2.84 (1.92, 4.18)	72% (<0.05)		8	3.1 (1.59, 6.06)	74% (<0.05)	
Adjusted for BMI								
Yes	1	6.02 (2.6, 13.64)	NA	**<0.05**	0	NA	NA	NA
No	18	2.49 (1.82, 3.42)	85% (<0.05)		10	2.9 (1.6, 5.24)	83% (<0.05)	
Adjusted for diabetes								
Yes	7	2.59 (1.56, 4.31)	81% (<0.05)	*>0.05*	2	1.18 (0.76, 1.83)	53% (0.14)	**<0.05**
No	12	2.69 (1.74, 4.16)	81% (<0.05)		8	4.17 (2.37, 7.35)	78% (<0.05)	
Adjusted for comorbidities								
yes	6	2.53 (1.44, 4.44)	84% (<0.05)	*>0.05*	2	1.18 (0.76, 1.83)	53% (0.14)	**<0.05**
no	13	2.71 (1.81,4.07)	83% (<0.05)		8	4.17 (2.37, 7.35)	78% (<0.05)	

BMI, body mass index; DVT, deep venous thrombosis; NA, not available; PE, pulmonary embolism; VTE, venous thromboembolism. Significantly different ($p < 0.05$).

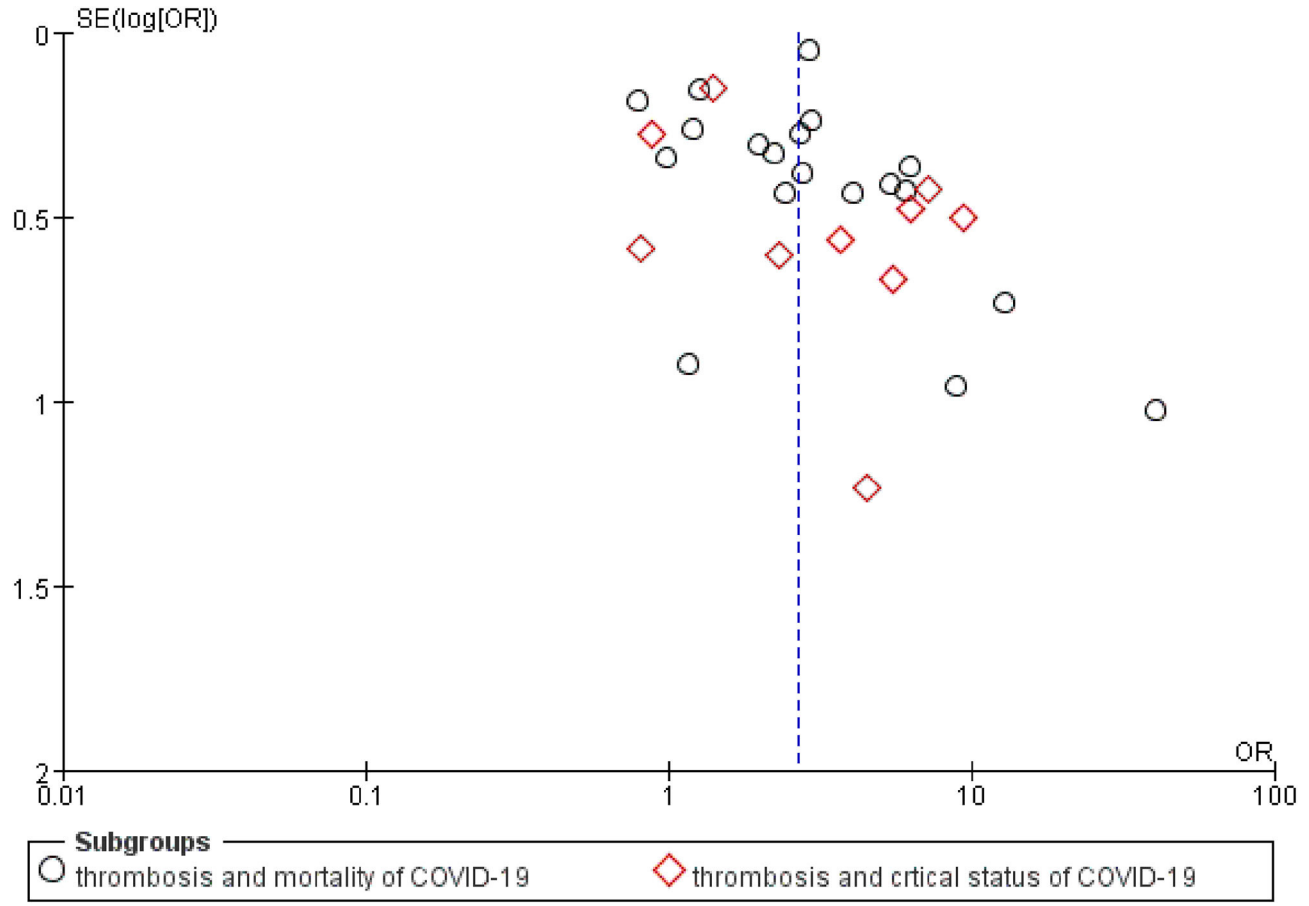

FIGURE 3 | A funnel plot of public bias of the association between thrombosis and prognosis of COVID-19.

DISCUSSION

To the best of our knowledge, this study tried to evaluate the association between thrombosis and the prognosis of COVID-19, which is often neglected by clinical physicians. The results of this meta-analysis, which included 25 studies, revealed that thrombosis was associated with a 161 and 190% increased risk of COVID-19-related mortality and COVID-19-related critical status, respectively. The association persisted and remained statistically significant in all of the stratified analyses.

Observational studies cannot prove causality. However, the following issues may explain the causation. First, there was an appropriate temporal relationship: thrombosis preceded COVID-19-related mortality in all studies. Second, there is theoretical biological plausibility for causality in that thrombosis may lead to organ dysfunction or prolong hypoxia, critical status, and death. The high rate of death-causing pulmonary embolism at autopsy is one of the strongest prognostic markers of a poor outcome (2). Additionally, the lungs of patients with COVID-19 displayed severe endothelial injury and diffuse thrombosis with microangiopathy (1, 56, 57). The association between deep venous thrombosis (DVT) and COVID-19 is uncertain, and the mechanisms may be related to the following factors: the coagulation system may be activated by SARS-CoV-2, viral infection-induced release of cytokine, which is also thrombogenic, the plausible role of angiotensin-converting enzyme receptors induced severe endothelial injury, a pro-coagulatory state by tissue factor pathway activation (2, 4, 8, 58). Third, the findings revealed stronger associations for other thromboses, such as brain stroke and PE, relative to VTE. Hypoxia of important organs may lead to critical status and death (59). Fourth, there was consistency of this association across the included studies, as shown by the forest plot (**Figure 2**).

Conversely, there are also possible non-causal explanations for this association. Thrombosis is often associated with other confounding factors, including lack of physical activity, obesity, diabetes, hypertension, older age, sex, and chronic organ diseases (60, 61). Some of these factors were adjusted for the studies included in our meta-analysis, but the extent to which these potential intervening factors were controlled for in the individual studies was generally limited. The lack of adjustment for age (only 13 studies adjusted for age), sex (only 9 studies), BMI, diabetes, and comorbidities (only 7 studies) could contribute to a non-causal association between thrombosis and the COVID-19-related critical status and COVID-19-related mortality.

Our meta-analysis reports a stronger association between thrombosis and mortality without adjusting for sex relative to adjusting for sex. In our meta-analysis, two studies reported an association adjusted for sex. Xie et al. (62) may explain that age and sex are related to the COVID-19-related mortality. The authors reported that ACE2 concentration decreased almost 67% in older female rats and 78% in older male rats relative to younger groups. Additionally, evidence shows that sex hormones may modulate the expression of ACE2 (63). Kuba et al. (64) identified that ACE2 protects against acute lung injury, and decreased ACE2 may be related to the adverse outcome of COVID-19. The risk of severe infection and mortality increase with male sex (65). Sex was a strong factor in the COVID-19-related mortality, and several studies support this result (66, 67).

Our meta-analysis has many limitations. First, the sample size of the included studies was small, and the results of this meta-analysis should be interpreted with caution. Second, some of the included studies reported the association among thrombosis and mortality and critical status without adjustment for confounding factors, such as crude ORs or number of participants, which may have led to high heterogeneity and an overestimation of the results of the meta-analysis. Third, some related studies may be omitted by the study selection. Fourth, potential publication bias existed because studies published in English and articles were included. Fifth, there was no analysis of the association between different types of thrombosis and different statuses of COVID-19 based on the original studies. Furthermore, quantitative synthesis could not eliminate the bias inherent to observational studies.

There are a few merits of this meta-analysis. First, this study evaluated the association among thrombosis and mortality and the COVID-19-related critical status globally. Considering the consistent finding of increased mortality and critical status associated with thrombosis, we recommend that further prospective cohort studies considering additional adjusted confounding factors should be performed to test this hypothesis. Second, this study demonstrated that study location, study design, sample size, type of thrombosis, and adjusted confounding factors were all sources of heterogeneity.

CONCLUSIONS

In conclusion, our pooled analyses provide evidence that participants with thrombosis were associated with an increased risk of COVID-19-related mortality and COVID-19-related critical status. Further prospective studies with large sample sizes are required to establish whether this association is causal by considering more confounders and to clarify its mechanisms.

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

DX, HG, and FT: conceptualization. HG, FT, LC, and DX: methodology. DX, HG, FT, LC, and XL: software, validation, formal analysis, investigation, resources, data curation, and visualization. DX and FT: writing—original draft preparation. DX and XL: writing—review and editing and supervision. 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.2021.819318/full#supplementary-material

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Outcomes of Hospitalized Patients With COVID-19 With Acute Kidney Injury and Acute Cardiac Injury

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Purpose: This study investigated the incidence, disease course, risk factors, and mortality in COVID-19 patients who developed both acute kidney injury (AKI) and acute cardiac injury (ACI), and compared to those with AKI only, ACI only, and no injury (NI).

Methods: This retrospective study consisted of hospitalized COVID-19 patients at Montefiore Health System in Bronx, New York between March 11, 2020 and January 29, 2021. Demographics, comorbidities, vitals, and laboratory tests were collected during hospitalization. Predictive models were used to predict AKI, ACI, and AKI-ACI onset. Longitudinal laboratory tests were analyzed with time-lock to discharge alive or death.

Results: Of the 5,896 hospitalized COVID-19 patients, 44, 19, 9, and 28% had NI, AKI, ACI, and AKI-ACI, respectively. Most ACI presented very early (within a day or two) during hospitalization in contrast to AKI ($p < 0.05$). Patients with combined AKI-ACI were significantly older, more often men and had more comorbidities, and higher levels of cardiac, kidney, liver, inflammatory, and immunological markers compared to those of the AKI, ACI, and NI groups. The adjusted hospital-mortality odds ratios were 17.1 [95% CI = 13.6–21.7, $p < 0.001$], 7.2 [95% CI = 5.4–9.6, $p < 0.001$], and 4.7 [95% CI = 3.7–6.1, $p < 0.001$] for AKI-ACI, ACI, and AKI, respectively, relative to NI. A predictive model of AKI-ACI onset using top predictors yielded 97% accuracy. Longitudinal laboratory data predicted mortality of AKI-ACI patients up to 5 days prior to outcome, with an area-under-the-curve, ranging from 0.68 to 0.89.

Conclusions: COVID-19 patients with AKI-ACI had markedly worse outcomes compared to those only AKI, ACI and NI. Common laboratory variables accurately predicted AKI-ACI. The ability to identify patients at risk for AKI-ACI could lead to earlier intervention and improvement in clinical outcomes.

Keywords: SARS-CoV-2, cardiovascular sequelae, cardiac injury, predictive model, AKI

INTRODUCTION

Acute kidney injury (AKI) and acute cardiac injury (ACI) are well-recognized complications of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1–3). AKI and ACI separately have been associated with increased risk of critical illness and mortality in COVID-19 patients (1–3). The mechanisms underlying the high incidence of AKI and ACI and their association with poor outcomes in COVID-19 are not well-understood and are likely multifactorial. SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) as docking and entry receptor on host cells, and the transmembrane serine protease 2 (TMPRSS2) is also involved in its cellular entry (4, 5). Though unproven, it has been hypothesized that SARS-CoV2 may directly induce AKI and ACI as the kidney and heart have a high density of ACE2 receptors. Indirect effects of COVID-19 that contribute to AKI and ACI include hypoxia, hypotension, inflammation, thromboembolism, cytokine storm, and sepsis (1–3, 6, 7). Endothelial dysfunction has been reported in patients with severe COVID-19 (8) and also likely plays a role in AKI and ACI.

In addition to age, pre-existing hypertension, diabetes, and obesity are major risk factors for severe COVID-19 and increased mortality (9–11). Black and Hispanic patients have been disproportionately affected by COVID-19 and have increased mortality. This may be due to a higher prevalence of cardiovascular risk factors in this population or socioeconomic factors such as crowding, food insecurity and poverty (12–15).

Observational studies have characterized the risk factors and outcomes of AKI (16–21) and ACI (22–25) separately among hospitalized patients with COVID-19. However, there have been no systematic studies comparing outcomes of COVID-19 patients with AKI to COVID-19 patients with ACI or evaluating the incidence, risk factors and clinical outcomes of COVID-19 patients who develop both AKI and ACI during hospitalization. Understanding the clinical characteristics and risk factors that make COVID-19 patients susceptible to in-hospital AKI and ACI could lead to better patient management and clinical outcomes.

The purpose of this study was to investigate the demographics and the clinical variables of COVID-19 patients with combined injury (AKI-ACI), and to compare them with those with AKI only, ACI only, and no injury (NI). Our study population came from Montefiore Health System in the Bronx, New York, which serves a large, low-income, and diverse population and which was hit hard by the COVID-19 pandemic. Mathematical models were developed to predict AKI-ACI onset. In addition, we analyzed the temporal progression of different clinical variables with time-lock to outcome (discharged alive or in-hospital death) and use them to predict likelihood of mortality. To our knowledge this is the first systematic documentation of the longitudinal clinical variables associated with AKI-ACI, with comparison with AKI, ACI, and NI in COVID-19.

METHODS

Study Design, Population, and Data Source

This retrospective study was approved by the Einstein-Montefiore Institutional Review Board (#2020-11389). All patients in this study were seen in The Montefiore Health System (MHS) and tested for SARS-CoV-2 infection using real-time polymerase chain reaction test (RT-PCR) on a nasopharyngeal swab between January 1, 2020, and January 29, 2021. The Montefiore Health System is one of the largest healthcare systems in New York City with 15 hospitals located in the Bronx, the lower Hudson Valley, and Westchester County serving a large, low-income, and racially and ethnically diverse population that was hit hard by COVID-19 early in the pandemic (13, 26).

Health data were searched and extracted as described previously (13, 27). In short, de-identified data were made available for research by the Montefiore Einstein Center for Health Data Innovations after standardization to the Observational Medical Outcomes Partnership (OMOP) Common Data Model (CDM) version 6. OMOP CDM represents healthcare data from diverse sources, which are stored in standard vocabulary concepts (28), allowing for the systematic analysis of disparate observational databases, including data from the electronic medical record system, administrative claims, and disease classifications systems (e.g., ICD-10, SNOWMED, LOINC, etc.). ATLAS, a web-based tool developed by the Observational Health Data Sciences and Informatics (OHDSI) community that enables navigation of patient-level, observational data in the CDM format, was used to search vocabulary concepts and facilitate cohort building. Data were subsequently exported and queried as SQLite database files using the DB Browser for SQLite (version 3.12.0).

The primary study outcome was in-hospital mortality as extracted from electronic medical record. Demographic data included age, sex, ethnicity, and race. Chronic comorbidities included obesity, diabetes, congestive heart failure (CHF), chronic kidney disease (CKD), coronary artery disease (CAD), chronic obstructive pulmonary disease (COPD), and asthma. Longitudinal laboratory tests and vitals included creatinine (Cr), estimated glomerular filtration rate (eGFR), albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), brain natriuretic peptide (BNP), C-reactive protein (CRP), D-dimer (DDIM), ferritin (FERR), lactate dehydrogenase (LDH), lymphocytes (LYMPH), troponin-T (TNT), white blood cells (WBC), fibrinogen, eosinophils, basophils, neutrophils, prothrombin time (PT), systolic blood pressure (SBP), body temperature, heart rate (HR), and pulse oximetry.

AKI and ACI Definitions

AKI was defined using the Kidney Disease Improving Global Outcomes criteria as either a 0.3 mg/dl increase in serum creatinine within 48 h or a 1.5x increase in serum creatinine within a 7-day iterative window. The baseline creatinine was determined as the mean of all serum creatinine values 8–365 days preceding hospitalization (20, 21, 29). For patients who did not have creatinine baseline values, the lowest creatinine value during hospitalization was used as the baseline creatinine (19, 30). Urine

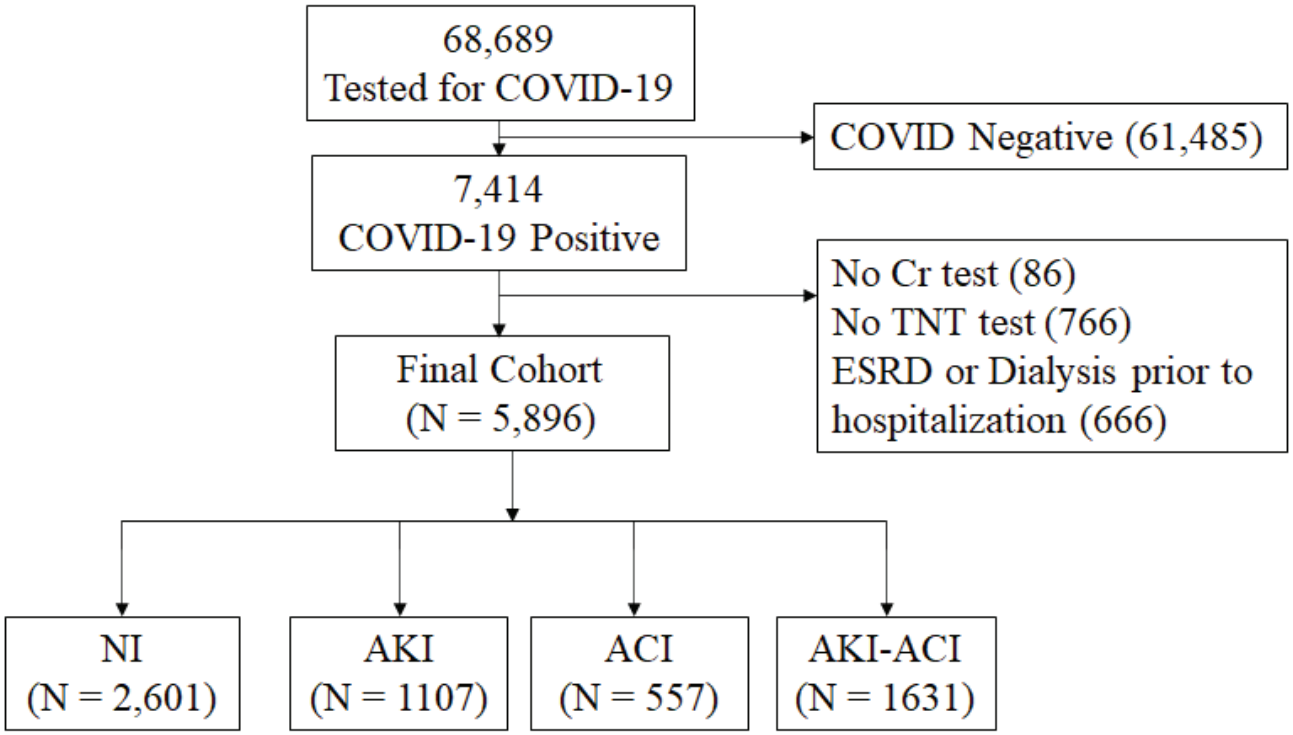

FIGURE 1 | Flowchart of hospitalized patient selection. From March 11, 2020 to January 29, 2021, there were a total of 68,689 hospitalized patients had tests for COVID-19 and 7,414 had a positive COVID-19 test. Cr, creatinine; TNT, troponin-T; NI, no injury; AKI, acute kidney injury; ACI, acute myocardial injury; ESRD, end-stage renal disease.

output was not used to define AKI due to significant missing data. ACI was defined using the 4th Universal Definition of Myocardial Infarction, with a high-sensitivity troponin T level above the 99th-percentile upper reference limit (0.0141 ng/mL) (31–33).

Patients without AKI or ACI were assigned to the no injury group. Note that we also evaluated isolated liver injury and found 713 patients had elevated liver enzymes [AST > 1ULN (>40U/L) and ALT > 1ULN (>35U/L)] (34).

From March 11, 2020 to January 29, 2021 (**Figure 1**), there were a total of 68,689 hospitalized patients were tested for COVID-19 and 7,414 had a positive COVID-19 test. Patients who were not hospitalized were excluded. Patients missing Cr or TNT data, and patients with ESKD on dialysis were excluded. This left 5,896 hospitalized COVID-19 patients for the final analysis. Of these, 2,601 had NI, 1,107 had AKI only, 557 had ACI only and 1,631 had AKI-ACI. There were no statistically significant differences in major baseline characteristics (i.e., age, gender, race, ethnicity, and comorbidities) between the included and excluded patients ($p > 0.05$).

Prediction of AKI, ACI, and AKI-ACI

Logistic regression models were used to rank the importance of clinical variables (demographics, comorbidities, vitals, and blood tests) and predict AKI, ACI, and AKI-ACI onsets using data at admission. Prediction of mortality was also performed using logistic regression. Performance was evaluated using the area under the curve (AUC) of the receiver operating characteristic (ROC) curve with 5-fold cross validation. Note that Cr and TNT, which were used to define AKI and ACI onset respectively, were included in the predictive models because their quantitative values at different days could be predictive of outcomes.

Temporal Profiles of Clinical Variables

Clinical variables were collected 5 days prior to outcome (death or hospital discharge). Temporal progression of clinical data was time-locked to outcome and compared between groups stratified by survivors and non-survivors. Logistic regression models were used to rank the importance of clinical variables. Prediction performance was evaluated using ROC analysis for individual top variables for different days prior to outcome.

Statistical Analysis

Statistical analyses were performed using Python and Statistical Analysis System (SAS) software (Cary, NC, USA). Group differences in frequencies and percentages for categorical variables were tested using χ^2 or Fisher's exact tests. Group comparison of continuous used the non-parametric Kruskal Wallis/ Mann-Whitney *U*-test. Mortality odds ratios (aOR) were adjusted for age, gender, ethnicity, and comorbidities and provided. Differences among AKI-ACI, AKI, ACI, and NI groups for clinical variables in time-series graphs were analyzed *via* linear mixed models and least-squares means. $P < 0.05$ was

TABLE 1 | Demographics, comorbidities, and laboratory variables at admission of NI, AKI, ACI, and AKI-ACI groups.

	NI	AKI	ACI	AKI-ACI	ACI vs. AKI	AKI-ACI vs. AKI	AKI-ACI vs. ACI
***N* (%)**	2,601 (44.11%)	1,107 (18.78%)	557 (9.45%)	1,631 (27.66%)			
Demographics							
Age in years, mean (SEM)	57.4 (0.4)	63.6 (0.5)	72.7 (0.7)	72.1 (0.4)	*	#	
Female sex, *n* (%)	1,394 (53.6%)	529 (47.7%)	231 (41.4%)	674 (41.3%)		#	
Race, *n* (%)							
White	210 (15.9%)	86 (12.7%)	66 (18.9)	157 (15.0%)			
Black/African American	719 (54.3%)	404 (59.8%)	193 (55.1%)	642 (61.5%)			
Asian	63 (4.8%)	26 (3.8%)	19 (5.4%)	45 (4.3%)			
Other	209 (15.8%)	87 (12.9%)	44 (12.6%)	115 (11.0%)			
Unknown	122 (9.2%)	73 (10.8%)	28 (8.0%)	85 (8.2%)			
Ethnicity, *n* (%)							
Hispanic	1,278 (49.1%)	431 (38.9%)	207 (37.2%)	587 (36.0%)			
Non-Hispanic	1,323 (50.9%)	676 (61.1%)	350 (62.8%)	1,044 (64.0%)			
Comorbidities, *n* (%)							
Hypertension	669 (21.5%)	343 (31.0%)	176 (31.5%)	643 (39.4%)		#	$
COPD and asthma	259 (10.0%)	91 (8.2%)	52 (9.3%)	136 (8.3%)			
Stroke	44 (1.7%)	28 (2.5%)	16 (2.9%)	79 (4.8%)		#	
Diabetes	587 (22.6%)	334 (30.1%)	136 (24.4%)	562 (34.4%)			$
Chronic kidney disease	189 (7.3%)	180 (16.3%)	134 (24.1%)	577 (35.4%)	*	#	$
Coronary artery disease	123 (4.7%)	59 (5.3%)	77 (13.8%)	192 (11.8%)	*	#	
Heart failure	50 (1.9%)	32 (2.9%)	44 (7.9%)	140 (8.6%)	*	#	
Liver disease	34 (1.3%)	21 (1.9%)	6 (1.0%)	36 (2.7%)			
Presenting laboratory values, mean, SEM							
Troponin, ng/mL	0.01 (0.00)	0.01 (0.00)	0.20 (0.04)	0.17 (0.03)	*	#	
Brain Natriuretic Peptide (pg/mL)	265 (28)	594 (71)	3,343 (288)	3,199 (171)	*	#	
Creatinine, mg/dL	0.9 (0.01)	1.4 (0.06)	2.3 (0.16)	3.7 (0.23)	*	#	$
eGFR, mg/mL	85 (0.8)	64 (2.3)	43 (2.3)	32 (1.6)	*	#	$
Alanine aminotransferase, U/L	35 (1.0)	35 (2.3)	48 (7.9)	74 (13.9)		#	$
Aspartate aminotransferase, U/L	39 (1.0)	50 (2.7)	73 (10.8)	102 (16.9)		#	$
C-reactive protein, mg/dL	6 (0.32)	11 (0.87)	13 (1.11)	16 (0.81)		#	
D-dimer, ug/mL	1.4 (0.10)	3.6 (0.44)	5.6 (0.60)	5.7 (0.43)	*	#	
Ferritin, ng/mL	554 (35)	1,062 (146)	1,446 (236)	2,137 (505)		#	
Lactate dehydrogenase, U/L	327 (6)	454 (17)	492 (36)	543 (25)		#	
White blood cell count, $x10^9$/L	6.9 (0.14)	8.8 (0.33)	9.5 (0.77)	10.4 (0.36)	*	#	
Lymphocytes, $x10^9$/L	1.5 (0.02)	1.2 (0.06)	1.5 (0.20)	1.3 (0.06)			
Basophil $x10^9$/L	0.02 (0.000)	0.02 (0.002)	0.03 (0.002)	0.03 (0.001)			
Neutrophils, $x10^9$/L	4.7 (0.08)	6.6 (0.28)	6.6 (0.26)	8.2 (0.27)		#	$
Eosinophil $x10^9$/L	0.07 (0.004)	0.04 (0.009)	0.05 (0.006)	0.03 (0.005)		#	
Prothrombin time, s	14 (0.11)	15 (0.26)	16 (0.32)	17 (0.23)	*	#	
Systolic Blood Pressure, mmHg	132 (0.5)	128 (1.5)	131 (2.1)	122 (1.6)			
Pulse Oximetry (%)	97 (0.08)	96 (0.24)	94 (0.66)	94 (0.46)	*	#	
Temperature, °F	99 (0.03)	99 (0.09)	99 (0.08)	99 (0.08)			
Heart Rate, bpm	90 (0.5)	91 (1.5)	90 (1.6)	97 (1.4)			
In-hospital mortality, *n* (%)	80 (3.1%)	190 (17.2%)	165 (29.6%)	710 (43.5%)	*	#	$

Group comparison of categorical variables in frequencies and percentages used chi-squared test or Fisher exact tests. Group comparison of continuous variables in means and SEMs (standard error of means) used the Kruskal Wallis/Mann-Whitney U-tests.

COPD, Chronic obstructive pulmonary disease.

All values are in n (%) unless otherwise specified. Note that all variables shown of all injury groups were significant compared to those of the NI group.

*, #, $ *Denote significance in pairwise comparisons.*

considered statistically significant and corrected for multiple comparison using the Bonferroni method.

RESULTS

Demographics and Comorbidities

The final hospitalized COVID-19 cohort (5,896) consisted of 2,602 (44%) NI patients, 1,107 (19%) AKI-only patients, 557 (9%) ACI-only patients, and 1,631 (28%) combined injury (AKI-ACI) patients. The AKI and ACI incidences were 46.4 and 37.1%, respectively. **Table 1** summarizes patient demographics, comorbidities, and laboratory values at admission for each group. The mean ages were 57, 64, 73, and 72 years old in the NI, AKI, ACI, and AKI-ACI groups, respectively ($p < 0.05$ across groups), with ACI or AKI-ACI patients being ∼15 years older compared to NI patients ($p < 0.05$). Percentages of female were 54, 48, 41, and 41% in the NI, AKI, ACI, and AKI-ACI groups, respectively ($p < 0.05$ across groups), with ∼13% more males in the ACI or AKI-ACI group compared to NI group ($p < 0.05$). There were no group differences across race ($p > 0.05$) and ethnicity ($p > 0.05$).

Patients with ACI-only had more comorbidities including CKD, CAD, and CHF compared to those with AKI-only ($p < 0.05$). Patients with AKI-ACI had a higher prevalence of hypertension, stroke, CKD, CAD, and CHF than those with AKI-only ($p < 0.05$) and had significantly more hypertension, diabetes, CKD than those with ACI-only ($p < 0.05$).

To assess the relative contribution of covariates on prediction of mortality, we performed a relative weight analysis (36) for the logistic regression. The relative weights of these organ injuries, age, CKD, and heart failure were 74.33, 19.24, 1.58, and 1.24, respectively. The relative weights of other comorbidities and demographics were all <1.

Markers of Organ Injury

At hospital admission, those with combined AKI-ACI had significantly worse levels of cardiac (TNT, BNP), kidney (Cr, eGFR), liver (ALT, AST), inflammatory/immunological (LDH, neutrophils and others) markers ($p < 0.05$) followed by those with ACI or AKI ($p < 0.05$) compared to those with NI. All laboratory values of the three injury groups were significantly different from NI group ($p < 0.05$), except pulse oximetry.

For between group comparisons, those with ACI had significantly higher levels of TNT, BNP, Cr, D-dimer, WBC, prothrombin time, and lower eGFR and pulse oximetry compared to those with AKI. Patients with AKI-ACI had significantly higher levels of TNT, BNP, Cr, eGFR, ALT, AST, CRP, D-dimer, ferritin, LDH, WBC, neutrophils, eosinophil, prothrombin time, and lower pulse oximetry than those with AKI alone and significantly higher levels of Cr, eGFR, ALT, AST, and neutrophil than those with ACI alone.

In-hospital Mortality

The unadjusted mortality rates of NI, AKI, ACI, and AKI-ACI were 3.1, 17.2, 29.6, and 43.5%, respectively. Odds ratios for in-hospital mortality with adjustment for sex, age and significantly different comorbidities are summarized in **Table 2**. AKI-ACI patients had 17-fold higher odds of in-hospital mortality [adjusted OR (aOR) = 17.11, 95% CI = 13.63–21.66, $p < 0.001$], ACI patients had 7-fold higher odds of in-hospital mortality (aOR = 7.17, 95% CI = 5.35–9.64, $p < 0.001$), and AKI patients had 4.7-fold higher odds of in-hospital mortality (aOR = 4.74, 95% CI = 3.66–6.13, $p < 0.001$) compared to the NI cohort.

TABLE 2 | Adjusted odds ratios and 95% confidence intervals for in-hospital mortality by group.

	OR	95% CI	*P*
AKI-ACI (ref = NI)	17.1	13.6–21.7	<0.001
ACI (ref = NI)	7.17	5.35–9.64	<0.001
AKI (ref = NI)	4.74	3.66–6.13	<0.001
AKI-ACI (ref = ACI)	1.98	1.61–2.44	<0.001
AKI-ACI (ref = AKI)	3.68	3.05–4.44	<0.001
ACI (ref = AKI)	1.78	1.39–2.29	<0.001

Covariates used in logistic regression were age, gender, ethnicity and comorbidities that showed statistically significant differences between groups.
AKI, acute kidney injury; ACI, acute cardiac injury; ref, reference; NI, no injury.

Those with combined AKI-ACI had higher risk of death than ACI alone (aOR = 1.98, 95% CI = 1.61–2.44, $p < 0.001$) and AKI alone (aOR = 3.68, 95% CI = 3.05–4.44, $p < 0.001$). The ACI group had a higher mortality rate than the AKI group (aOR = 1.78, 95% CI = 1.39–2.29, $p < 0.001$).

AKI and ACI Onset

In the AKI-only group, AKI onset peaked 1 day after hospital admission, but a significant proportion of patients developed AKI throughout the hospitalization (**Figure 2**). In contrast, in the ACI only group, ACI onset peaked and was predominantly localized to 1 day after admission. In the AKI-ACI group, the onsets of AKI and ACI were similar to those in the AKI-only and ACI-only groups.

Prediction of AKI, ACI, and AKI-ACI

The top predictors of AKI were Cr, WBC, age, diabetes, and AST, and the predictive model yielded 73 ± 5% accuracy, 93 ± 3% sensitivity, and 27 ± 10% specificity **Table 3**. The top predictors of ACI were TNT, BNP, Cr, Age, PT, and the predictive model yielded 93 ± 1% accuracy, 96 ± 1% sensitivity, and 82 ± 4% specificity. The top predictors of AKI-ACI were TNT, Cr, DDIM, BNP, PT, and the predictive model yielded 89 ± 2% accuracy, 93 ± 2% sensitivity, and 83 ± 2% specificity.

Temporal Profiles of Clinical Variables

Figure 3 depicts the time series of clinical variables relative to death or discharge for NI, AKI, ACI, and AKI-ACI groups. Overall, laboratory tests at admission were more abnormal, progressively worsened among non-survivors compared to survivors.

For non-survivors, AKI-ACI cardiac (TNT, BNP) and kidney markers (Cr, eGFR) markers were markedly worse days prior compared to the other groups, and liver markers (ALT, AST) markers were markedly elevated and early on only in the AKI-ACI, but not in AKI and ACI group. Furthermore, cell death (LDH), and immunological markers (lymphocyte, WBC,

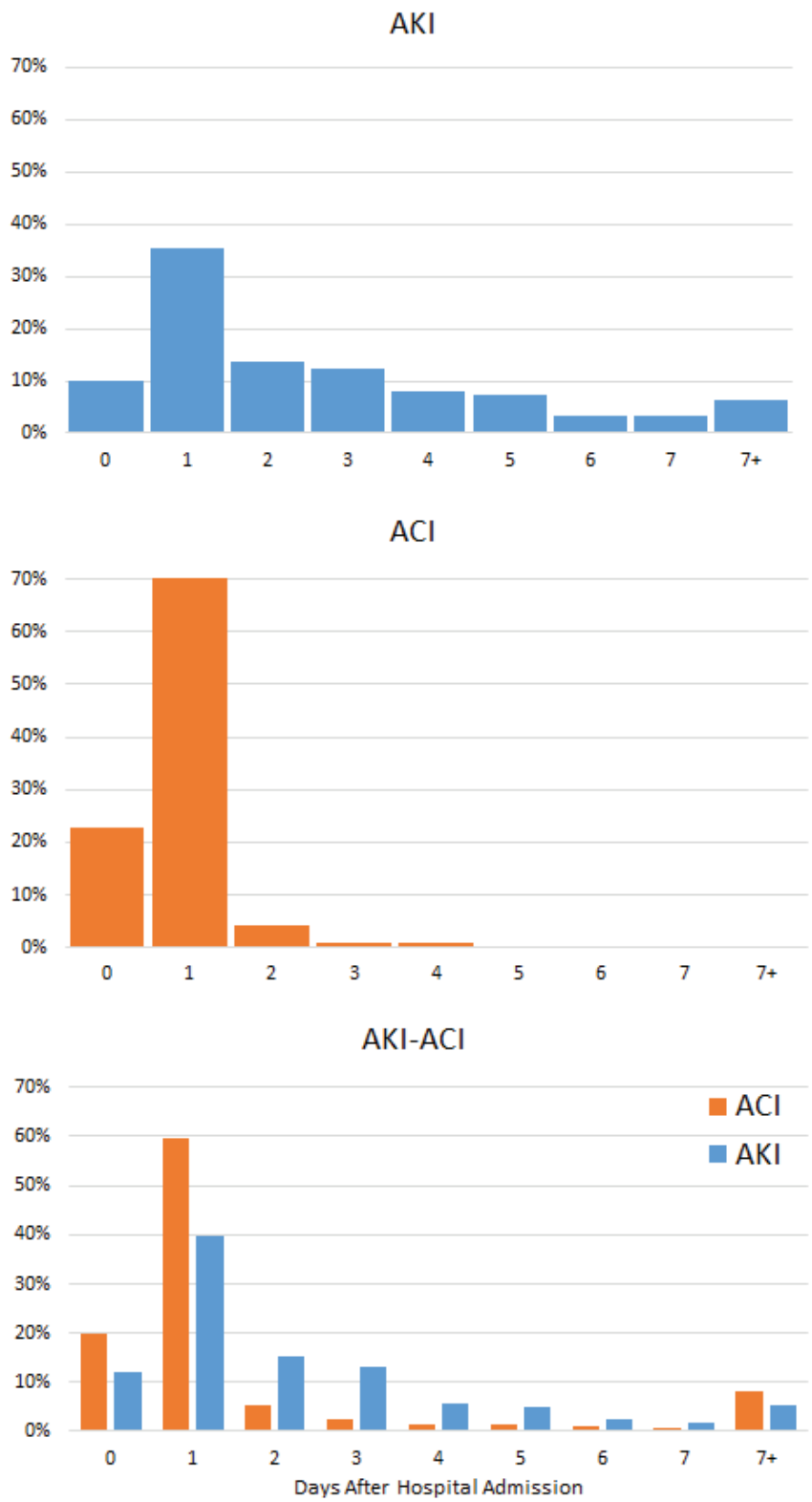

FIGURE 2 | Onsets of AKI and ACI from hospital admission. Percentage of patients who developed AKI, ACI, and AKI-ACI as a function of days after hospital admission.

TABLE 3 | Top predictors of AKI, ACI, and AKI+ACI and their performance metrics.

Cohorts	Top predictors	Accuracy	Sensitivity	Specificity
AKI	Cr, DDIM, LDH, CRP, Neutrophil	0.73 ± 0.05	0.93 ± 0.03	0.27 ± 0.10
ACI	TNT, BNP, Cr, Age, PT	0.93 ± 0.01	0.96 ± 0.01	0.82 ± 0.04
AKI-ACI	TNT, Cr, DDIM, BNP, PT	0.89 ± 0.02	0.93 ± 0.02	0.83 ± 0.02

Cr, creatine; DDIM, D-dimer; LDH, lactate dehydrogenase; CRP, C-reactive protein; TNT, troponin T; BNP, brain natriuretic peptide; PT, prothrombin time.

neutrophil, basophil, and eosinophil) were also worse days prior compared to the other groups, whereas inflammatory (CRP, D-dimer, and ferritin) and most vitals were similarly elevated in all groups.

Moreover, the temporal fluctuations of the most of these variables were markedly higher in the AKI-ACI compared to the AKI, ACI, and NI groups. These temporal fluctuations were most noticeable in the non-survivor group.

Predictors of Mortality

The top predictors of mortality in the AKI-ACI cohort were CRP, D-dimer, LDH, neutrophils, and WBC in AKI-ACI cohort. Prediction AUCs were high at days 0 and progressively decreased away from day of outcome, ranging from 0.68 to 0.89 (**Figure 4**).

DISCUSSION

This study investigated the clinical characteristics of COVID-19 patients who developed AKI and ACI during hospitalization. ACI onset occurred within a day of hospitalization in contrast to AKI onset which was more distributed across the hospitalization. Patients with AKI-ACI were significantly older, more often men and had significantly more comorbidities compared to those with AKI and NI. COVID-19 patients with AKI-ACI had more elevated levels of cardiac, kidney, liver, inflammatory and immunological markers, followed by those with ACI or AKI compared to those with NI. Patients with AKI-ACI, ACI, and AKI were, respectively, 17.1, 7.2, and 4.7 times more likely to die in the hospital compared to patients with NI. The top clinical predictors of AKI-ACI were TNT, age, Cr, WBC, BNP, and the predictive model yielded 97% accuracy, 94% sensitivity, and 72% specificity. Although physicians already know anecdotally that patients with AKI-ACI have worse outcomes, this study documented the incidence, likelihood of in-hospital mortality using odds ratio and the early clinical laboratory markers that predict which patient will develop AKI-ACI and die in the hospital.

Incidence of AKI and ACI

We observed a higher incidence of ACI (37.1%) among hospitalized COVID-19 patients compared to previously reported studies with incidences ranging from 16.1 to 23.8% (24). These differences may be explained due to differences in patient populations. Our cohort was minority-predominant and had a relatively high prevalence of cardiovascular comorbidities and lower socioeconomic status that may have been contributing factors to increased adverse cardiovascular outcomes in the setting of COVID-19. We also observed a high incidence of AKI-ACI (28%), suggesting a strong association between AKI and ACI. This is consistent with a previous study that reported an association between AKI and cardiovascular events among COVID-19 patients in the American Heart Association COVID-19 Cardiovascular Disease Registry (37).

Risk Factors Contributing to AKI and ACI

Compared to those with NI, ACI, and AKI-ACI patients were ∼15 years older and had 13% more men. Compared to AKI, ACI, and AKI-ACI patients were ∼10 years older and had 6% more men, suggesting that older age and male sex are risk factors for AKI-ACI and ACI. Moreover, preexisting CKD, CAD, CHF, and stroke carried additional risks of developing ACI relative to AKI. The contributions of these additional preexisting cardiovascular comorbidities are not surprising (35, 38). Similarly, preexisting hypertension and diabetes carried additional risk of developing AKI-ACI. Notably, CKD prevalence was remarkably high (35.4%) in the AKI-ACI group compared to only 7.3% in NI, 16.3% in AKI, and 24.1% in ACI group, suggesting that having preexisting CKD markedly increases susceptibility to developing both AKI and ACI.

Having both AKI-ACI signaled a patient is 17.11 times more likely to die in the hospital compared to those without injury, whereas ACI COVID-19 patients were 7.2 times and AKI COVID-19 patients were 4.74 times more likely to die. These observations reflect the multiplicative nature of cardiac and kidney injury on risk of death and that the COVID-19 related cardiovascular event may be the driver of markedly higher mortality.

ACI develops early compared to other organ injuries. The heart may be more susceptible to early damage than other organs as heart muscle has a high density of ACE2 receptors (4, 39). The early ACI onset suggests that ACI is a primary effect of COVID-19, whereas AKI (20, 21) and acute liver injury (40) occur later in the COVID-19 clinical disease course and with more distributed onsets, suggesting that AKI and acute liver injury may arise from secondary effects of COVID-19 (i.e., systemic hypoxia, hypotension, shock, sepsis, and cytokine storm) and/or COVID-19 treatments (6, 7). These secondary effects could also contribute to sustained ACI (41–43). Cardiac injury could lead to AKI or liver injury. Our findings support consideration of pre-emptive and prophylactic treatment early in the disease course and careful monitoring of clinical variables for AKI development.

Longitudinal Characterization of Clinical Variables Associated With AKI and ACI

Patients with AKI-ACI had markedly worse cardiac, kidney and liver markers days prior to death compared to other groups, suggesting higher incidence of and more severe multi-organ injury. Furthermore, immunological markers were also worse days prior compared to the other groups, whereas inflammatory markers and most vitals were similarly elevated in all groups. These observations indicate AKI-ACI patients had differentially high levels of clinical markers that included more severe multiorgan injuries and overwhelming inflammation and immunological responses.

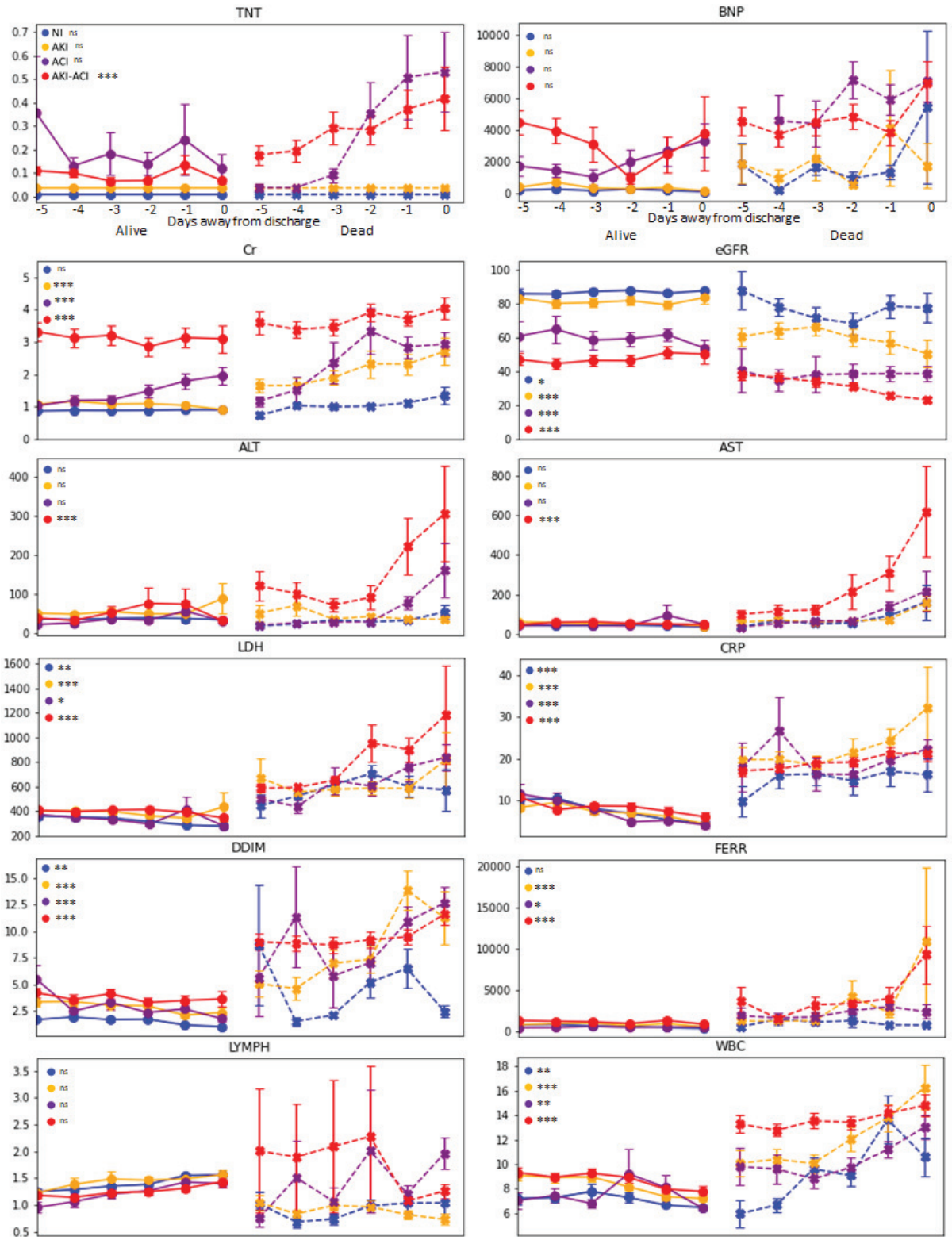

FIGURE 3 | Continued

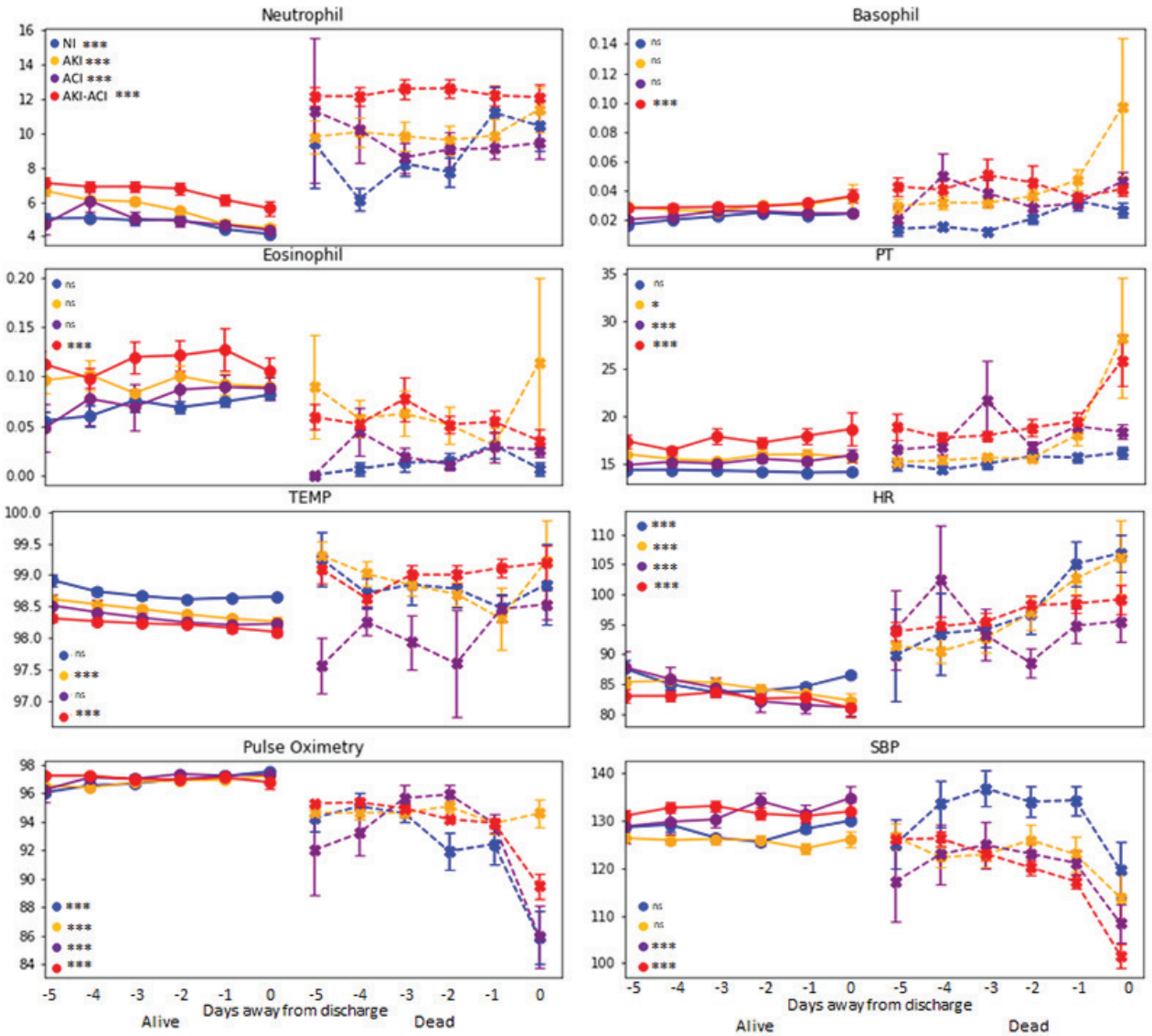

FIGURE 3 | Temporal progression of clinical variables days from outcome. Temporal progression of laboratory tests and vital signs with $t = 0$ representing day of death (for non-survivors) or day of discharge (for survivors). Error bars are SEM. *Indicates $p < 0.05$ between survivors and non-survivors. **Indicates $p < 0.01$ between survivors and non-survivors. ***Indicates $p < 0.001$ between survivors and non-survivors. ns, indicates no significant difference between survivors and non-survivors.

Significantly elevated TNT and BNP in both ACI and AKI-ACI non-survivor groups 2–3 days prior to death supports a hypothesis of heart attack or heart failure being a possible cause of death in these two groups. In contrast, TNT and BNP were not as elevated in the AKI and NI non-survivor groups. Elevated LDH and CRP seen in non-survivors in all groups are evidence of increased inflammation and immune response to infection. Similarly, elevated WBC in all non-survivors supports sepsis as a possible cause of death in all groups. The steep increase in both ALT and AST in the AKI-ACI non-survivor group points to liver damage close to death and lend evidence to multi-organ failure being a third possible cause of death.

Laboratory variables of the AKI-ACI group were temporally more unstable compared to those with AKI, ACI or NI, especially among non-survivors, suggesting these temporal profiles of clinical variables can also be used to predict mortality (44, 45).

Predicting of Mortality Associated With AKI and ACI

Understanding the temporal progression of these clinical markers allowed us to construct a prediction model. Longitudinal data accurately predicted mortality likelihood up to 5 days prior. These top predictors of mortality are consistent with a previous

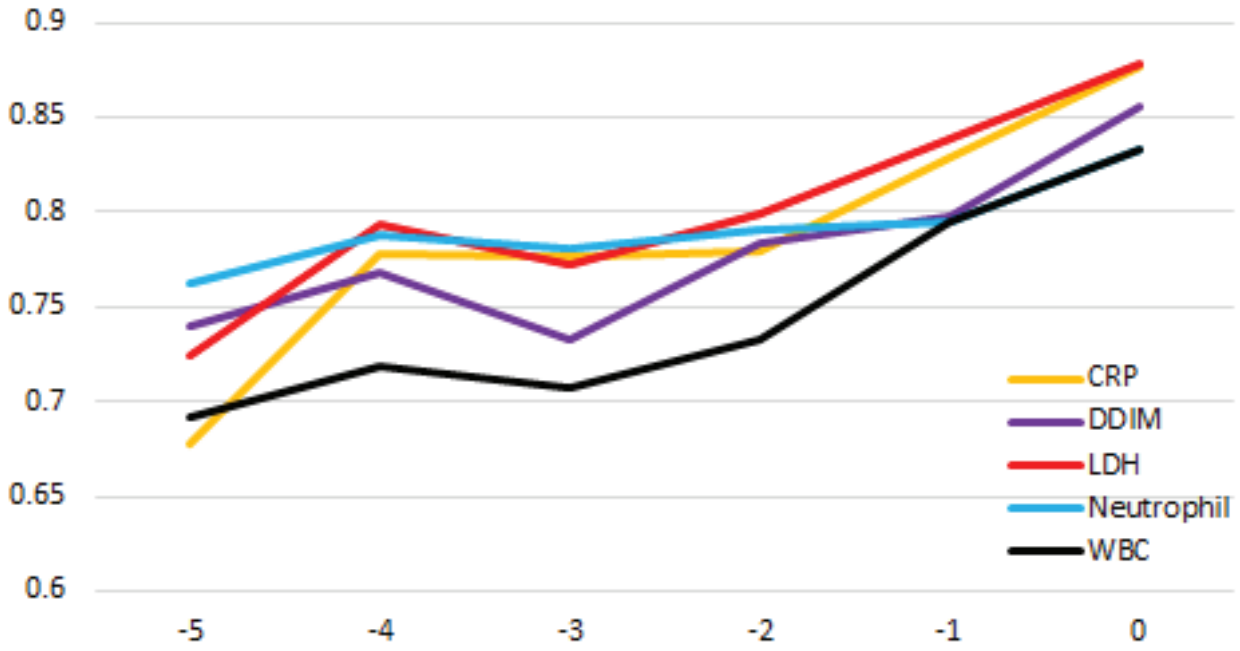

FIGURE 4 | Prediction of mortality likelihood of the AKI-ACI cohort. AUC at different days prior to outcome for individual top predictors and combined top predictors.

report (44) from a different hospital. Most published models used clinical data at admission, not longitudinal variables prior to outcomes (46–49). Prediction using the admission timepoint has relatively poor accuracy compared to a few days prior to outcome. While this finding is intuitively logical, this study provides evidence that our current model can yield a highly accurate prediction a few days prior to the outcome which may lead to earlier recognition, intervention and improvement in clinical outcomes.

Limitations

A strength of our study is that it addressed multiorgan injury with detailed clinical characteristics in a large diverse population. Our study has several limitations. This is a descriptive retrospective study that could not address the underlying cause of AKI and ACI among hospitalized patients with COVID-19. Missing certain laboratory variables could alter ranking of top predictors. We were unable to analyze how treatment of COVID-19 could have affected AKI and ACI. This study used TNT as indicator of ACI. We were unable to analyze other cardiovascular variables (such as EKGs and echocardiograms) because they would have required manual chart reviews of a large cohort of patients. We also did not study cardiac complications of atrial arrhythmias, ventricular arrhythmias, pericarditis, myocarditis, and heart failure, although this was found to be rare. Although ACI incidence and mortality among COVID-19 patients were generally higher than non-COVID-19 patients, comparison studies controlling for age, race, and ethnicity are needed. This study came from a large population of Black and Hispanic patients and these findings may not be generalizable to other populations. Additional and prospective studies are needed. We did not investigate the effects of anticoagulants on organ injuries (50, 51), the status of the COVID-19 survivors at discharge (52, 53) and the longer-term outcomes (54). As with any retrospective study, there could be unintended patient selection bias and unaccounted confounders.

CONCLUSION

A significant number of patients hospitalized with COVID-19 developed combined AKI and ACI. These patients had additional pre-existing risk factors, worse clinical and laboratory variables, markedly worse disease courses, and increased in-hospital mortality. Predictive models using readily available laboratory variables accurately predict which patients are at risk of AKI-ACI and death. Our study has potential clinical implications for hospitalized patients with COVID-19. First, the high incidence of AKI-ACI suggest that AKI-ACI is an important marker of future adverse outcomes in COVID-19. Second, health providers should increase awareness for kidney-cardiovascular complications when AKI-ACI is detected as these complications may assume a lower priority in individuals admitted with COVID-19 given the high respiratory morbidity and mortality of this illness. Third, initiation of kidney and cardiovascular

preventive therapies to mitigate kidney and cardiac damages in patients with COVID-19 may be warranted. The ability to identify patients at-risk of developing AKI-ACI early on could enable timely care.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Albert Einstein-Montefiore Institutional Review Board (#2020-11389). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JL: concept, design, collected data, analyzed data, created tables and figures, and drafted paper. AB: concept, design, collected data, analyzed data, and drafted paper. RF and WSH: concept, design, collected data, and edited paper. WH: analyzed data and drafted paper. MF and CR: concept, design, and edited paper. TD: concept, design, supervised, and edited paper. All authors contributed to the article and approved the submitted version.

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BRIEF RESEARCH REPORT
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A Case Series of Myocarditis Following Third (Booster) Dose of COVID-19 Vaccination: Magnetic Resonance Imaging Study

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Background: Myocarditis has been reported following the first two doses of Pfizer-BNT162b2 messenger RNA (mRNA) COVID-19 vaccination. Administration of a third dose (booster) of the vaccine was initiated recently in Israel.

Objective: The aim of this study was to describe the characteristics of patients referred for cardiac magnetic resonance (CMR) imaging with myocarditis following the booster.

Methods: Patients referred for CMR imaging with a clinical diagnosis of myocarditis within 21 days following the booster, between July 13 and November 11, 2021, were analyzed.

Results: Overall, 4 patients were included, 3/4 (75%) were men, and the mean age was 27 ± 10 years. The time from booster administration to the onset of symptoms was 5.75 ± 4.8 days (range 2–14). Obstructive coronary artery disease was excluded in 3 of the patients (75%). CMR was performed 34 ± 15 days (range 8-47 days) following the 3rd vaccination. The mean left ventricular ejection fraction was 61 ± 7% (range 53–71%), and regional wall motion abnormalities were present in one of the patients. Global T1 was increased in one of the patients, while focal T1 values were increased in 3 of the patients. Global T2 was increased in one of the patients, while focal T2 values were increased in all the patients. Global ECV was increased in 3 of the patients, while focal ECV was increased in all the patients. Median late gadolinium enhancement (LGE) was 4 ± 3% (range 1–9%), with the inferolateral segment as the most common location (3 of the 4 patients). All the patients met the Updated Lake Louise Criteria.

Conclusions: Patient characteristics and CMR imaging findings of myocarditis following the administration of the booster vaccine are relatively mild and consistent with those observed with the first two doses. Although larger-scale prospective studies are necessary, these initial findings are somewhat reassuring.

Keywords: myocarditis, BNT162b2 messenger RNA (mRNA) COVID-19 vaccination, third dose (booster), cardiac magnetic resonance imaging (CMR), COVID-19

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INTRODUCTION

Myocarditis has been reported to be a possible rare adverse event following the first or second dose of Pfizer-BNT162b2 messenger RNA (mRNA) COVID-19 vaccination (1–4). Incidence of such post-vaccine myocarditis was reported to be highest among younger males, with most cases being mild or moderate with favorable clinical outcomes (1, 4). As reported by us (5) and

others (6–8), cardiovascular magnetic resonance (CMR) imaging findings of these patients were consistently mild and in line with "classical myocarditis." Following the resurgence of COVID-19 morbidity, the Israeli Ministry of Health announced a campaign to administer the third dose (i.e., booster) of the BNT162b2 mRNA COVID-19 vaccine (Pfizer–BioNTech) to individuals who received the second dose > 5 months earlier, starting on July 13 (9). This third vaccine dose was reported to be effectively protected against severe COVID-19-related outcomes (9). Our aim in the current report was to describe the characteristics of patients referred for CMR with myocarditis following the administration of the BNT162b2 mRNA COVID-19 vaccine.

METHODS

Study Population

This study comprised consecutive patients who are members of Clalit Health Services (CHS), and who were referred for CMR at Mor Inside Ltd. (Kfar Saba, Israel), with a clinically suspected diagnosis of myocarditis within 21 days after receiving the third dose of the Pfizer-BNT162b2 mRNA COVID-19 vaccine between July 13, 2021, and November 11, 2021. Patient-specific data were available from referral letters and electronic medical records. Patients with prior history of myocarditis, with missing data of the third dose of the vaccine, or with an alternative competing diagnosis (i.e., COVID-19 infection) were excluded.

This study was approved by the CHS institutional review board and performed consistently with the Helsinki declaration. Exemption from informed consent was granted.

CMR Imaging

The patients underwent CMR imaging using a 1.5 T scanner (Ingenia; Philips Medical Systems). The CMR protocol included multiplanar cine imaging and late gadolinium enhancement (LGE) imaging. T1 mapping was performed using a balanced steady state free precession, single breath-hold modified inversion recovery Look-Locker (MOLLI). T2 mapping was performed using a navigator-gated black blood-prepared gradient spin-echo sequence. Native T1 and T2 mapping, and postcontrast T1 mapping were acquired in apical, mid-ventricular, and basal short-axis slices.

Data analysis was performed using a dedicated CMR workstation (Philips Intellispace Portal, version 11.0). Cardiac volume, function, and mass were measured using a semiautomated contour detection system, and extracellular volume (ECV) was calculated based on pre and postcontrast T1 images. Myocardial ROIs was placed accurately to minimize partial volume effects from adjacent blood pool or extra-myocardial tissues. Global T1 and T2 relaxation times and ECV were evaluated for the complete mid-ventricular slice using motion-corrected images as previously described (10). Consistent with Puntmann et al. (10), to avoid overestimation of T1 value due to partial volume effect, the apical slices were not analyzed. In addition, there are no differences in T1 value between basal and mid-ventricular slices (11), and in some cases, the basal slice may contain a part of the left ventricular outflow tract (11).

Regional T1, T2, and ECV were measured in LGE positive myocardium by manually drawing a region-of-interest (ROI) on the LGE image around the lesions and copying these ROIs to the corresponding T1 and T2 maps; respectively.

LGE was defined as an image intensity level ≥ 2 SDs above the mean of the remote myocardium. Abnormal native T1, T2, and ECV values were defined as >1,060 ms, >57 ms, and higher than 28%, respectively (12). The diameter of pericardial effusion was measured at the end-systolic frame, and pericardial LGE was considered present when enhancement involved parietal and visceral pericardial layers.

We evaluated the diagnosis of myocarditis by CMR using the Updated Lake Louise Criteria (13).

Statistical Analysis

A descriptive statistical methodology was used. Patient characteristics are presented as counts (%) for categorical variables and mean (±SD) or median (range) for continuous variables.

RESULTS

Overall, 4 patients met the inclusion criteria. A total of ¾ th (75%) were male, and the mean age was 27 ± 10 years (range: 18–44 years). Baseline characteristics are presented in **Table 1**. One of the patients had asthma, but the rest were otherwise healthy. The mean time from the third vaccine administration to the onset of symptoms was 5.7 5 ± 4.8 days (range 2–14). Of all the patients who experienced chest pain, ¾th (75%) had abnormal ECG mostly accounting for ST-segment elevations, and troponin levels were increased in all the patients, with peak values between 79 and 4,967 ng/L. Obstructive coronary artery disease was excluded in 3 (75%) of the patients, one had coronary angiography, and the other two had coronary computed tomography angiography.

The CMR imaging was performed after a median of 34 ± 15 days (range 8-47 days) following the 3rd vaccination. One of the patients underwent CMR during the acute phase, while the rest over a month following the acute episode. The CMR findings are presented in **Table 1**. CMR images of all the patients are presented in **Figure 1**.

The mean left ventricular ejection fraction was 61 ± 7% (range 53-71%), regional wall motion abnormalities were present in one of the patients only. Global T1 values were increased in one (25%) of the patients, while focal values were increased in 3 (75%) of the patients. Global T2 values were increased in one (25%) of the patients, while focal values were increased in all of the patients (100%). Global ECV was increased in 3 (75%) of the patients, while focal ECV was increased in all the patients (100%). LGE was present in all the patients; thus, all of the patients met the Updated Lake Louise Criteria. Mean LGE% was 4 ± 3% (range 1-9%), and the inferolateral segment was the most common location (3/4 patients). LGE patterns were as follows: epicardial 2 patients, mid-wall 1 patient, mid-wall and epicardial 1 patient. LGE in the pericardium was present in 2 of the 4 patients, and pericardial effusion was present in 2 of the 4 patients, circular in both. The diameter of pericardial effusion was 4 and 5 mm in the two latter patients.

TABLE 1 | Clinical characteristics and CMR findings of the study patients.

Age (years)	Sex	Past medical history	Symptoms	ECG	Peak Troponin (ng/L)	CAD ruled out	Time from 3rd vaccine and symptoms (days)	Time between 3rd vaccine and MRI (days)	LVEF %	Wall motion abnormality	LVEDV/BSA	LVESV/BSA	LV-mass/BSA	T1 global (ms)	T1 focal (ms)	T2 global (ms)	T2 focal (ms)	Global ECV (%)	Focal ECV (%)	LGE (%)	LGE localization	LGE pattern	LGE in pericard	Pericardial effusion	Diameter of effusion (mm)
21	M	None	Chest pain	Inferior STE	240	CA	4	8	53	Lateral wall	73.5	33.9	49.6	1,078 ± 107	1,135 ± 118	62 ± 8	69.2	30.1	36	9	Anterolateral, inferolateral (basal, mid) Lateral (apical)	Epicardial and mid-wall	Y	N	
44	F	None	Chest pain	Normal	80	CCT	2	40	63	N	70.6	25.8	31.7	1,039 ± 70	1,077 ± 66	52.4 ± 6	57.5	30.5	31.9	1	Apex, inferoseptal (basal)	Mid-wall	Y	N	
26	M	Asthma	Chest pain	Diffuse STE	4,967	N	14	47	71	N	76.7	22.2	46.9	1,045 ± 93	1,155 ± 89	50 ± 6.7	58.1	34.2	44.9	3	Inferior and inferolateral (basal)	Epicardial	N	Circular	5
18	M	None	Chest pain	Diffuse STE	79	CCT	3	42	59	N	74	31.4	45.8	1,008 ± 70	1,041 ± 80	49 ± 4.4	57.4	27.3	29.3	1	Inferior (basal)	Epicardial	N	Circular	4

M, male; Y, yes; N, no; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; ECG, electrocardiogram; LVESV, left ventricular end-systolic volume; BSA, body surface are; LGE, late gadolinium enhancement; CAD, coronary artery disease; CA, coronary angiography; CCT, cardiac computed tomography; STE, ST-segment elevation.

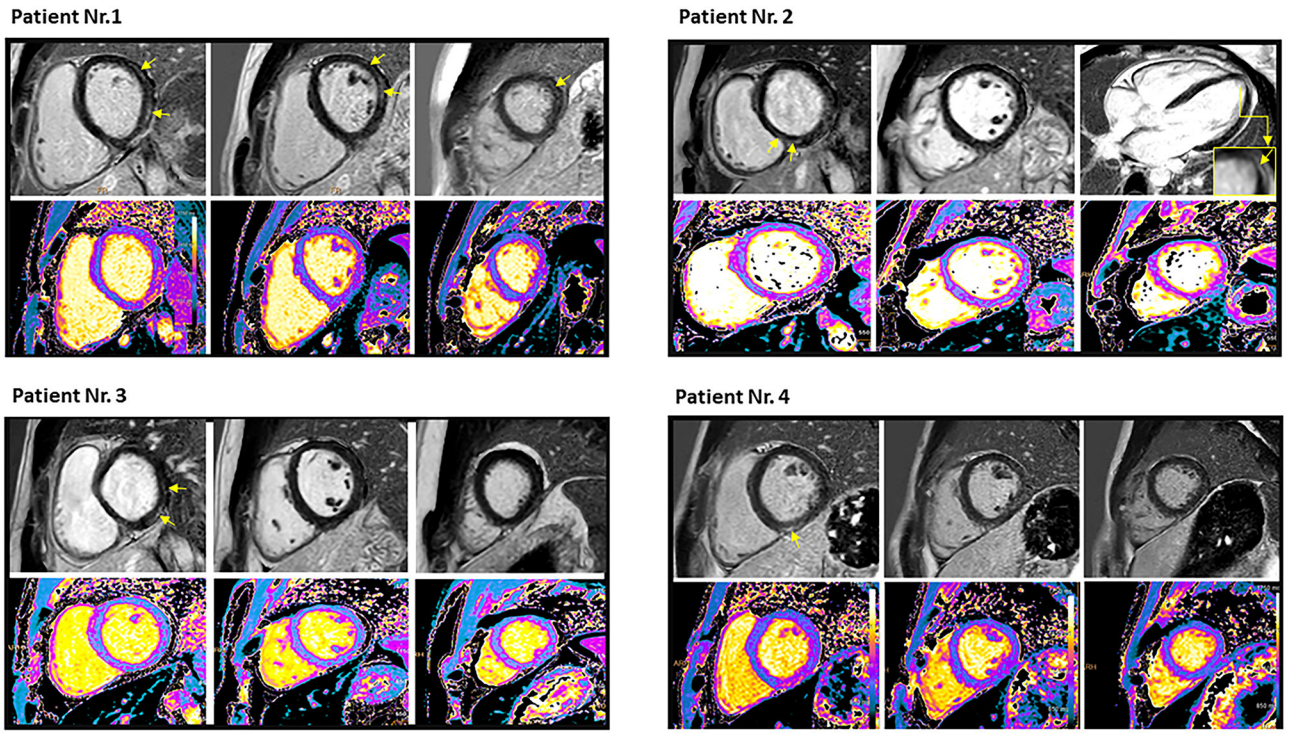

FIGURE 1 | Cardiac magnetic resonance imaging of the four patients who had myocarditis following the third dose of mRNA COVID-19 vaccination demonstrated late gadolinium enhancement (yellow arrows) and T1 mapping (lower row). Patient no. 1: Mid wall late gadolinium enhancement involving 9% of the myocardium with corresponding myocardial injury in native T1 mapping imaging in antero- and infero-lateral segments of basal and mid ventricular short-axis view, as well as in the lateral segment of apical short-axis view. Native T1 value was 1,135 ms, and T2 value was 69.2 ms. Peak troponin was 240 ng/L, and scan delay (from COVID-19 vaccine) was 8 days. Patient no. 2: Mid wall late gadolinium enhancement involving 1% of the myocardium with corresponding myocardial injury in native T1 mapping imaging in the lateral segment of apical and in the septal segment of the basal short-axis view. Native T1 value was 1,077 ms, and T2 value was 57.5 ms. Peak troponin was 80 ng/L, and scan delay (from COVID-19 vaccine) was 40 days. Patient no. 3: Epicardial late gadolinium enhancement involving 3% of the myocardium with corresponding myocardial injury in native T1 mapping imaging in the inferior and inferolateral segments of the basal short-axis view. Native T1 value was 1,155 ms, and T2 value was 58.1 ms. Peak troponin T was 4,967 ng/L, and scan delay (from COVID-19 vaccine) was 47 days. Patient no. 4: Mid wall late gadolinium enhancement involving 1% of the myocardium with corresponding myocardial injury in native T1 mapping imaging in inferior segments of the basal and mid-ventricular short axis view, as well as in the lateral segment of the apical short axis view. Native T1 value was 1,041 ms, and T2 value was 57.4 ms. Peak troponin T was79 ng/L, and scan delay (from COVID-19 vaccine) was 42 days. Reference (normal) values: T1: 950–1,060 ms, T2: < 57 ms, and troponin T < 13 ng/L.

DISCUSSION

The present study consists, to our knowledge, of the first report describing CMR as well as clinical findings of patients with myocarditis following the administration of BNT162b2 mRNA COVID-19 booster (i.e., 3rd dose) vaccine. The baseline characteristics of the patients in this report are consistent with those of people who developed myocarditis following the first two doses, as previously reported (5–8); most were young men without a significant past medical history. However, it should be mentioned that one of the patients (25%) was a 44-year-old woman, which could imply less dominance of men with myocarditis following the 3rd vaccine, yet this is a small cohort thus such inferences are significantly limited. The CMR findings are overall mild, with two patients having ∼1% LGE, and consistent with those previously reported following the first two doses of the vaccine (5–8). Although this could partially result from the delayed scan, it is probably consistent with the favorable outcome of these patients. Findings are also similar to those reported on patients who recently recovered from COVID-19, suggesting potential etiological common pathways for myocardial involvement (10). The severity of the CMR findings (e.g., LGE percentage, T1 values, etc.) was greater in one patient, in whom CMR was performed during the acute phase compared with over a month delay in the other patients. Although this may imply the natural course of the inflammation, a selection bias with a more severe case scanned earlier cannot be ruled out. Nevertheless, and despite the delay in CMR in 3 of the patients, all the patients met the Updated Lake Louise Criteria (13).

LIMITATIONS

The causality between myocarditis and the vaccine cannot be unequivocally determined. However, temporal proximity between the two events and the very similar characteristics of

the patients and previously reported CMR findings to support a probable causal association. An additional limitation is that CMR was performed over a month after the acute phase in 3 of the 4 patients, which might have attenuated some of the findings. We should also acknowledge the relatively small cohort, which limits the generalizability of the findings.

CONCLUSIONS

Patient characteristics and CMR findings of clinically suspected myocarditis following the administration of the booster vaccine are relatively mild and consistent with previous observations following the first two doses. Although more data are required to better characterize this clinical entity, these initial findings are somewhat reassuring with regard to the risk/benefit profile of the third dose of the vaccine.

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 Helsiniki Committee Clalit Healthcare Services. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AS, AH, and RK conceived and planned the study. AS and AH reviewed the CMR tests, contributed to the interpretation of the results, and drafted the manuscript. AS, AH, YA, and GW obtained patient related clinical data and contributed to sample preparation. All authors provided critical feedback and helped shape the research, analysis, and manuscript. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Case Report: Two Case Reports of Acute Myopericarditis After mRNA COVID-19 Vaccine

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Background: Cases of myocarditis and myopericarditis after mRNA COVID-19 vaccines have been reported, especially after the second dose and in young males. Their course is generally benign, with symptoms onset after 24–72 h from the dose.

Case Summary: We report two cases of myopericarditis after the second dose of the mRNA-1273 COVID-19 vaccine in two young males. Both the patients were administered the mRNA-1273 COVID-19 vaccine from the same batch on the same day and experienced fever on the same day of the vaccine, and symptoms consisted of myopericarditis 3 days after the dose.

Discussion: Myopericarditis is usually considered an uncommon adverse reaction after various vaccinations, reported also after the mRNA COVID-19 vaccine. Several explanations have been proposed, including an abnormal activation of the immune system leading to a pro-inflammatory cascade responsible for myocarditis development. Both patients experienced the same temporal onset as well as the same symptoms, it is also useful to underscore that both vaccines belonged to the same batch of vaccines. However, despite these cases, vaccination against COVID-19 far outweighs the risk linked to COVID-19 infection and remains the best option to overcome this disease.

Keywords: case report, myocarditis, myopericarditis, mRNA vaccine, COVID-19

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INTRODUCTION

The safety profile of mRNA vaccines for the prevention of COVID-19 disease has been demonstrated in several trials (1–3). Systemic reactions, generally mild and transient, are described mainly after the second dose of vaccine and especially in young people. Few cases of myocarditis post-mRNA vaccine (both BNT162b2 and mRNA-1273 vaccines) have also been reported (4, 5), typically with a benign course. In most case series, symptoms arose 24–72 h after the second dose, whereas only rare cases occurred after 1-week post-vaccine (4).

CASE DESCRIPTION

We report two cases of myopericarditis after the second dose of the mRNA-1273 COVID-19 vaccine, from the same batch of vaccines, administered on the same day. Two young males, 20-years old and 21-years old, with no past medical history, experienced fever (38 and 40°C, respectively) on the same day of the second dose of mRNA-1273 COVID-19 vaccine and chest pain, exacerbated with breathing, 3 days later, for which they were admitted to the emergency department. On admission, both patients had normal vital signs with no fever. Nasopharyngeal SARS-CoV2 polymerase chain reaction was negative in both patients. The 12-lead resting electrocardiograms on arrival showed sinus rhythm, normal atrioventricular conduction, incomplete right bundle branch block and no ventricular repolarization abnormalities (**Figure 1**). Both chest x-rays revealed no significant findings. Blood tests revealed a C-reactive protein = 1.9 mg/dL in both cases, with white blood count within normal limits and increased levels of high-sensitivity troponin (211 and 366 ng/L, respectively). Due to the clinical presentation and the elevation of high-sensitivity troponin, a complete transthoracic echocardiographic exam was performed. In the first case, transthoracic echocardiography showed no pericardial effusion, a mild inferolateral wall thickness (12 mm) with normal biventricular function, absence of wall motion abnormalities and no significant heart valve disease. The echocardiographic examination of the 21-year-old patient showed a minimal pericardial effusion (2 mm) with hyperreflective pericardial

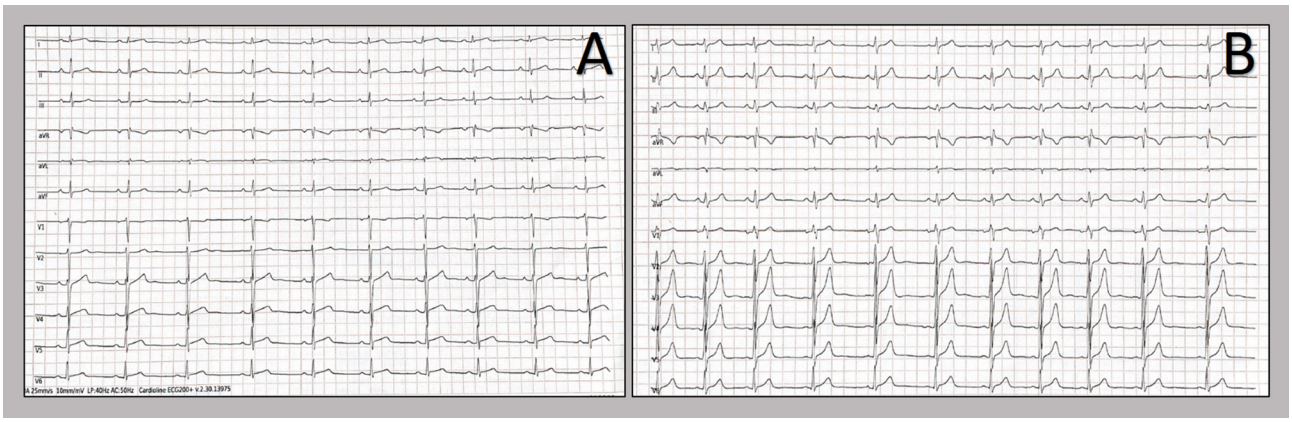

FIGURE 1 | Twelve-lead resting electrocardiograms collected at the hospital admission. Patients' electrocardiograms on admission: 20-year-old patient **(A)** and 21-year-old patient **(B)**. The resting ECGs showed sinus rhythm, normal atrioventricular conduction, incomplete right bundle branch block and no ventricular repolarization abnormalities.

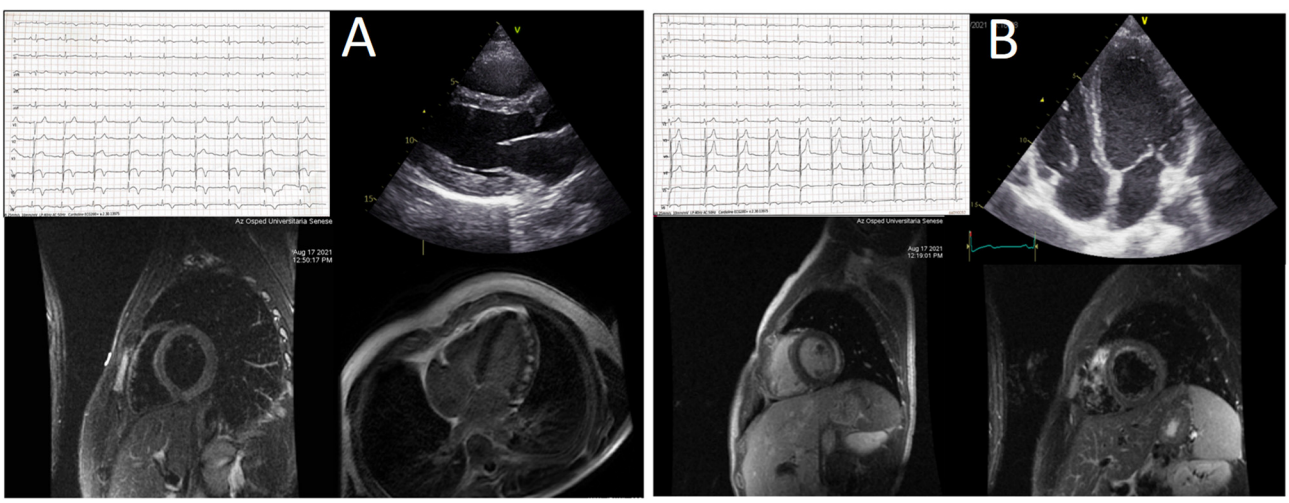

FIGURE 2 | (A,B) Central illustration. The picture summarizes the main non-invasive findings in the two patients experiencing acute myopericarditis after mRNA-1273 COVID-19 vaccine. See text for details.

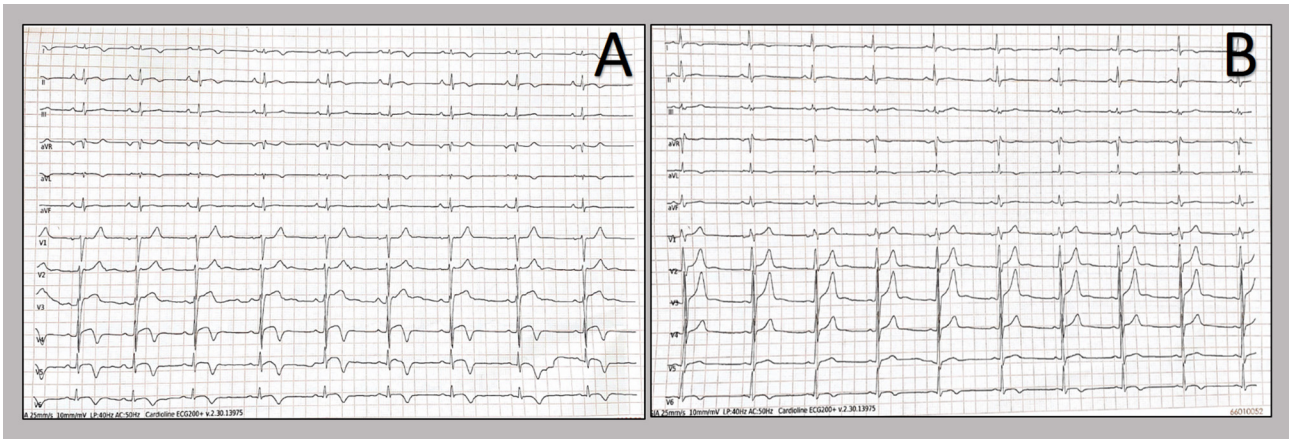

FIGURE 3 | T-wave inversion showed by twelve-lead resting electrocardiograms. Patients' electrocardiograms on day 2 from admission show T-wave inversion in the anterolateral leads in the 20-years old patient **(A)**, whereas in the other patient, the T-wave inversion occurred in the lateral leads **(B)**.

layers, normal biventricular function and no significant heart valve disease. Due to the temporal correlation between the symptom onset and the second dose vaccine, the hypothesized diagnosis was acute myopericarditis as an adverse reaction to the mRNA-1273 COVID-19 vaccine. Therefore, on the day after hospital admission, both patients underwent cardiac magnetic resonance (CMR), which confirmed the diagnosis of acute myopericarditis, with evidence of myocardial oedema and late gadolinium enhancement (LGE) with subepicardial pattern (**Figure 2A**). In particular, in the 20-year old patient, myocardial oedema was found in the middle inferolateral wall, whereas LGE involved the subepicardial region of the lateral wall, inferior basal wall, and anterior apical septum, with left ventricular ejection fraction (LVEF) = 55%. The CMR of the 21-year-old patient revealed myocardial oedema in the mid-basal lateral wall and LGE in the subepicardial region of the basal inferolateral wall and mid-basal lateral wall with LVEF 52% (**Figure 2B**). The disease course was benign in both patients, and only one patient presented rare ventricular arrhythmias on the admission day (isolated ventricular ectopic beats, 3 couplets and 1 triplet). Due to the patients' low-risk profile and the clear etiology of the myocarditis, we decided not to perform an endomyocardial biopsy. Both patients were treated with low doses of beta-blockers, angiotensin-converting enzyme, and antagonists of mineralocorticoid receptors. Non-steroidal anti-inflammatory drugs were introduced to control chest pain, whereas colchicine was not introduced due to the prevalent myocardial involvement. Serial electrocardiograms showed T-wave inversion in the anterolateral leads in the 20-years old patient, whereas in the other patient, the T-wave inversion occurred in the lateral leads, both occurred 2 days later from admission (**Figures 3A,B**). Blood tests revealed an initial increase in markers of myocardial injury (peak high-sensitivity troponin 2,474 and 1,414 ng/L and isozyme creatin-kinase MB 80.4 and 50 ng/ml respectively) and C-reactive protein levels (peak 1.9 and 2.16 mg/dl respectively), with a decreasing trend until complete normalization before the hospital discharge. They were both discharged on the 9th day of the in-hospital stay. **Figure 4** shows the timeline of these two patients from the day of the vaccine and symptoms onset to discharge. One month after hospital discharge, both patients were asymptomatic and were evaluated by clinical examination, resting ECG and echocardiograms which were all within normal limits. In particular, resting ECG showed almost complete resolutions of repolarization abnormalities (**Figure 5**).

Their 48-h Holter ECG did not show any brady- or tachyarritmias as well as ST-T dynamic changes. CMR was scheduled at 3 months from the acute event.

DISCUSSION

Myopericarditis is usually considered an uncommon adverse reaction after various vaccinations (5–7), and few cases have also been described after the mRNA COVID-19 vaccine, both after BNT162b2 and mRNA-1273 COVID-19 vaccines (4, 8). Both vaccines encode the stabilized prefusion spike glycoprotein of SARS-CoV2, and they were recommended as a 2-dose schedule. In certain individuals with genetic predisposition, nucleoside modifications of mRNA might trigger the immune system and the abnormal activation of both innate and acquired immune response (9), leading to a pro-inflammatory cascade responsible for myocarditis development. Besides mRNA immune reactivity, antibodies cross-reaction between SARS-CoV2 spike glycoproteins and myocardial proteins might play a role in post-vaccine myocarditis (10). Furthermore, both age and sex could be factors involved in the development of this adverse reaction (10). In fact, according to several case reports (11) and the large retrospective analysis conducted in Israel (12), the incidence of myocarditis after BNT162b2 mRNA vaccine is significantly higher in young male subjects, hinting that hormonal differences might play a central role in modulating

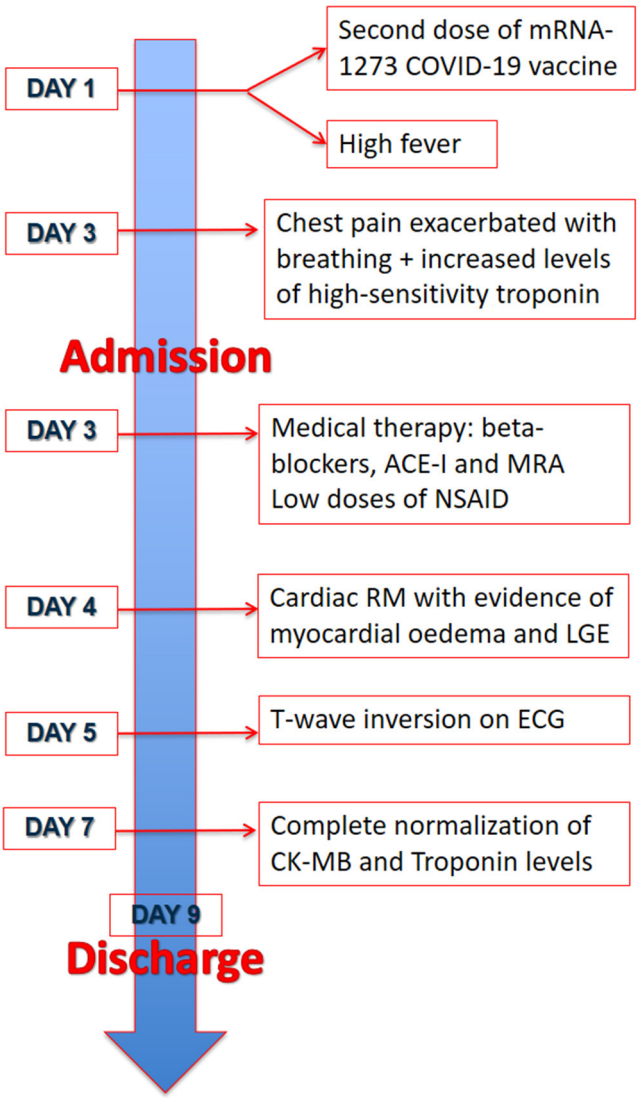

FIGURE 4 | Timeline. Timeline of the two patients from the day of the vaccine and symptoms onset to discharge.

the immune response. Within the Vaccine Adverse Event Reporting System (VAERS), 1,226 reports of myocarditis after mRNA vaccination during the first 6 months of 2021 (13). Furthermore, it has been widely demonstrated that the rate of adverse reactions to the vaccine is significantly lower compared to the rate of complications related to SARS-CoV2 infection (14), also in young individuals (15). In particular, Barda et al. showed that the risk of developing myocarditis after mRNA vaccine is much lower than after SARS-CoV2 infection (2.7 events vs. 11.0 events per 100,000 persons respectively) (16). In line with these results, the Italian Society of Cardiology still recommends vaccination against COVID-19 even in patients that developed myopericarditis after mRNA vaccine (17). However, it recognizes that these patients represent a vulnerable population and therefore some precautions might be taken such as prolonging the interval between the two doses and perhaps choosing a different vaccine for the second dose (17). The two cases we presented showed clinical characteristics in line with the other documented cases and the latest report by Rosner et al. (18): prevalence of male sex, symptoms onset 48–72 h after the second dose of vaccine, and uncomplicated course with mild symptoms. Indeed, both patients, of approximately the same age, were administered mRNA-1273 COVID-19 vaccine on the same day and in the same hospital, and both experienced fever on the same day and symptoms consisted of myopericarditis 3 days after the dose. Furthermore, it is useful to underscore that both vaccines belonged to the same batch of vaccines, questioning whether problems in vaccines storage may be at least in part responsible for these adverse reactions. To the best of our knowledge there are no clear reports linking a storage problem with the onset of systemic adverse reactions, either for COVID-19 vaccine or for other anti-viral vaccines. However, it is well-known that mRNA vaccines require specific handling which might be particularly challenging such as the need to guarantee a correct temperature (19). We actually cannot know whether there had been any problems with the transportation, storage or administration of this batch of vaccine and whether this might have had affected the onset of myocarditis, but it is important to stress this aspect for raising awareness of a possible correlation

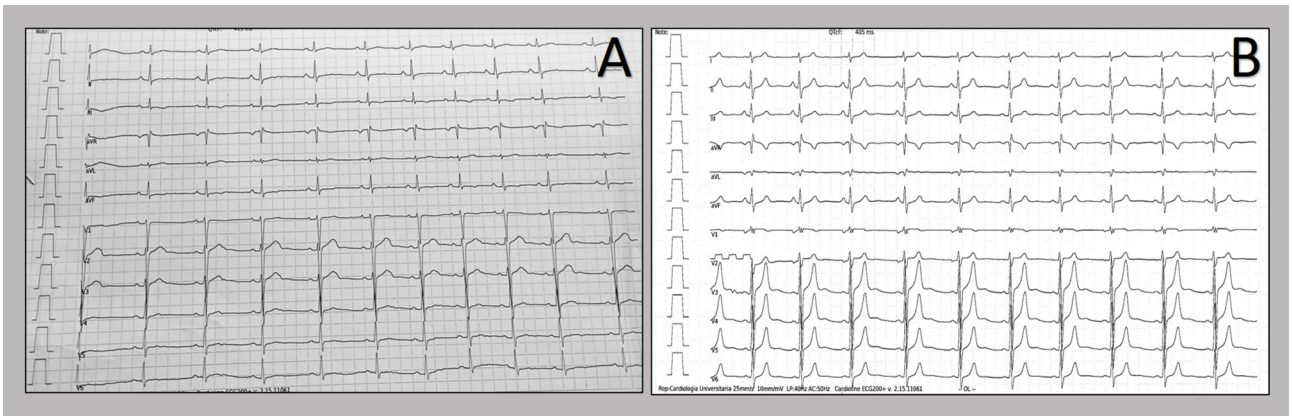

FIGURE 5 | Resting electrocardiograms after hospital discharge. Patients' electrocardiograms after one month from hospital discharge show almost complete resolution of repolarization abnormalities in both patients [in the 20-years old patient **(A)** and in the 21-year old patient **(B)**].

between these problems and the onset of side effects, which might therefore be explained with a possible toxic effect rather than an immunological pathophysiology.

CONCLUSIONS

We reported two cases of acute myopericarditis in two young males who developed chest pain three days after the second dose of the mRNA-1273 COVID-19 vaccine. Due to several cases of myocarditis after mRNA COVID-19 vaccination, clinical suspicion should be high, especially in young males. However, despite these cases, vaccination against COVID-19 far outweighs the risk linked to COVID-19 infection and remains the best option to overcome this disease.

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

CS, DM, VM, and BB collected the data upon which the manuscript was based. CS, FD'A, GA, and MG wrote the manuscript, while MC, SV, and MF critically revised it. All authors contributed to the article and approved the submitted version.

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MINI REVIEW
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Cardiovascular Complications of COVID-19 Vaccines

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Coronavirus disease 2019 (COVID-19) has become a global public health catastrophe. Vaccination against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is proven to be the most effective measure to suppress the pandemic. With the widespread application of the four vaccines, namely, ChAdOx1, Ad26.COV2.S, BNT162b2, and mRNA-1273.2, several adverse effects have been reported. The most serious type of complication is cardiovascularly related, including myocarditis, immune thrombocytopenia (ITP), cerebral sinus venous thrombosis, among others. All these adverse events undermine the health of the vaccinees and affect the administration of the vaccines. As the distribution of COVID-19 vaccines is surrounded by suspicion and rumors, it is essential to provide the public with accurate reports from trusted experts and journals. Monitoring the safety of COVID-19 vaccines is an important and ongoing process that is also urgent. Thus, we summarized the cardiovascular complications of the major types of COVID-19 vaccines, including mRNA vaccines, which are now generally considered to be innovative vaccines, and the future for vaccination against COVID-19, in addition to the underlying pathogenesis and potential therapeutics.

Keywords: COVID-19, vaccine, cardiovascular, complication, mRNA

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INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), also known as coronavirus disease 2019 (COVID-19), has spread rapidly throughout the world, leading to acute respiratory distress syndrome (ARDS). COVID-19 has become a global public health catastrophe. Vaccination against SARS-CoV-2 is now proven to be the most effective means of suppressing the pandemic (1). COVID-19 vaccines showed high efficacy against SARS-CoV-2 during the different phases of clinical trials (1). The first four vaccine preparations (i.e., ChAdOx1 and AD26.COV2·S, BNT162b2, and mRNA-1273) have received marketing authorization from the European Medicines Agency (EMA) (2). With the widespread application of these four vaccines, several adverse effects, such as pain at the site of inoculation, fever, and allergic reactions, have been reported (3). The most serious complications are cardiovascularly related, and these complications include myocarditis, immune thrombocytopenia (ITP), cerebral sinus venous thrombosis, and visceral

thrombosis. All of these adverse events impair the health of those receiving vaccinations and affect the administration of the vaccines (4, 5). Thus, it is necessary to summarize the cardiovascular complications of the major types of COVID-19 vaccines (**Figure 1**) and review the underlying pathogenesis and potential therapies.

mRNA Vaccines

At present, there are two types of mRNA vaccines used to prevent SARS-CoV-2, namely, Pfizer's BNT162b2 mRNA vaccine and Moderna's mRNA-1273 vaccine. mRNA vaccines use the mRNA that encodes the spike protein of SARS-CoV-2, surrounded by lipid nanoparticles (LNPs). The spike protein induces the body to produce the corresponding antibodies, which causes recipients to develop immunity to SARS-CoV-2. The BNT162b2 mRNA vaccine has been reported to be effective in a variety of COVID-19 clinical trials (6). However, the development of cardiovascular adverse events after the administration of these mRNA vaccines should be seriously considered.

Myocarditis After COVID-19 mRNA Vaccination

A total of 561,197 people in North Carolina were vaccinated from February 1 to April 30, 2021. Later, the Duke University Medical Center in Durham reported 4 cases of myocarditis; in all cases, the patients developed severe chest pain with biomarker evidence of myocardial damage, and all 4 patients were later hospitalized (4). In another case, one Filipino patient was diagnosed with myocarditis 3 days after receiving the second dose of the BNT162b2 mRNA vaccine (7). From January 30 to February 20, 2021, six patients with chest pain were treated in the Hillel Yaffe Medical Center, Israel, soon after vaccination with the BNT162b2 mRNA vaccine. All six of these patients were diagnosed with myocarditis, and one of these patients had received only the first dose of the vaccination (8). As mRNA vaccination becomes more widespread, an increasing number of myocarditis cases have been diagnosed. All patients with mild symptoms can be discharged within 4–8 days after treatment with non-steroidal anti-inflammatory drugs or colchicine (8). As on June 11, 2021, more than 296 million doses of COVID-19 mRNA vaccine had been administered in the United States, of which 52 million were administered to people aged 12–29 years. From December 29, 2020, to June 11, 2021, the Vaccine Adverse Event Reporting System (VAERS) received 1,226 reports of myocarditis after mRNA vaccination. The risk of myocarditis increases within 7 days after the first or second dose of an mRNA vaccine (9). The Centers for Disease Control and Prevention reported two cases of histologically confirmed myocarditis after COVID-19 mRNA vaccination on August 18, 2021. A 42-year-old man developed breathing difficulties and chest pain 2 weeks after receiving the second dose of the mRNA-1273 vaccine. It was also reported that he had no viral prodrome, and his PCR tests were negative for SARS-CoV-2. The patient developed tachycardia and fever. An electrocardiogram (ECG) showed diffuse ST-segment elevation, and Doppler echocardiography showed biventricular dysfunction (ejection fraction, 15%). The patient died of cardiogenic shock 3 days after the visit, and an autopsy revealed biventricular myocarditis (10). The potential mechanisms of mRNA vaccine-induced myocarditis are still unclear. It has been reported that the mRNA-1273 vaccine can induce a strong CD4 cytokine response involving type 1 helper T (Th1) cells, and CD4 cells are an important factor in myocarditis (11, 12). The detailed mechanisms warrant further clinical and basic research.

Thrombosis With COVID-19 mRNA Vaccination

The Medicines and Healthcare Products Regulatory Agency in the United Kingdom reported that, with the administration of 10.6 million doses of the BNT162b2 mRNA vaccine, there were 24 cases of cerebral venous sinus thrombosis (CVST), 3 cases of cerebral vascular thrombosis, 3 cases of superior sagittal sinus thrombosis, and 1 case of transverse sinus thrombosis (13). Among 4 million doses of mRNA-1273 that have been administered, 5 cases of suspected CVST have been reported (14). The mechanism underlying the association between mRNA vaccination and thrombosis is unelucidated. It is speculated that it is related to the encoding of the SARS-CoV-2 spike protein by mRNA vaccines. The spike protein, which is necessary to allow SARS-CoV-2 to invade human cells, enhances platelet aggregation and promotes the secretion of dense granules from platelets (15). Moreover, as a binding ligand of the ACE2 receptor, the SARS-CoV-2 spike protein induces an inflammatory response in brain endothelial cells and impairs the functional integrity of the blood-brain barrier, which promotes the activation of endothelial cells and the upregulation of leucocyte chemokines, pro-inflammatory cytokines (interleukin (IL)-1β and IL-6), and cell adhesion molecules [intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1)] (16). All of these mRNA vaccine-related pathophysiological activities might initiate the development of thrombosis. Thus, anti-spike protein monoclonal antibodies or recombinant human ACE2 proteins might assist in the treatment of patients with COVID-19 mRNA vaccine-induced thrombosis (15). However, at present, the current clinical treatment of choice is anticoagulation therapy with unfractionated heparin, followed by low-molecular-weight heparin and then warfarin (13).

Vaccine-Induced Thrombocytopenia

One day after receiving the first dose of the mRNA-1273 vaccine, a 60-year-old African-American man developed the symptoms of low-grade fever and chills, followed by the appearance of a severe generalized rash on his skin that quickly spread throughout his body. In addition, he was diagnosed with ITP (17). From mid-February to mid-March, nearly 5 million people in Israel were vaccinated with the BNT162b2 mRNA vaccine, and four patients were diagnosed with acquired thrombotic thrombocytopenic purpura (ATTP) (18). In the United States, more than 20 million people (as on February 2, 2021) have received at least one dose of either of the two available mRNA vaccines, and 20 of these patients developed thrombocytopenia after vaccination. Most of the 20 patients had symptoms, such as bruises or mucosal bleeding. Nine of these patients were vaccinated with the BNT162b2 mRNA vaccine, and 11 of these patients were vaccinated with the mRNA-1273 vaccine (19). Interestingly, in the United States, approximately 50,000 adults are diagnosed

with ITP each year. Thus, the incidence rate of mRNA vaccine-related ITP is almost the same as that of the baseline incidence rate for the population (19). However, most of these patients developed ITP symptoms after their first dose of COVID-19 mRNA vaccination; therefore, there seems to be a link between ITP and mRNA vaccine administration. However, the potential mechanism underlying the relationship between mRNA vaccines and ITP is unknown. Vaccines can activate autoimmunity through molecular mimicry, which induces the production of antiplatelet autoantibodies and causes thrombocytopenia (20).

ADENOVIRAL VECTOR VACCINES

At present, the cardiovascular complications that have been observed in association with adenoviral vector vaccines, i.e., the ChAdOx1 nCoV-19 (Oxford-AstraZeneca [AZ]; also known as Vaxzevria) vaccine and AD26.COV2·S (Johnson & Johnson [JJ]) vaccine, are primarily associated with thrombosis with thrombocytopenia syndrome (TTS). The ChAdOx1 nCoV-19 and AD26.COV2·S vaccines are composed of recombinant adenovirus vectors from chimpanzee adenovirus or human adenovirus, which encode the spike protein of SARS-CoV-2 (21). As on April 7, 2021, 34 million people had been vaccinated with ChAdOx1 in the European Economic Area and the United Kingdom, and the EMA reported 169 cases of cerebral venous thrombosis and 53 cases of splanchnic vein thrombosis (SVT) after vaccination (2). Six cases of suspected CVST have been reported among more than 7 million recipients of the AD26.COV2·S adenovirus vector vaccine (14). In the context of a worldwide vaccination campaign, these safety issues should not be ignored. Most patients with thrombosis are positive for anti-PF4 antibodies, which have effects similar to heparin-induced thrombocytopenia (HIT); therefore, this syndrome was named vaccine-induced immune thrombotic thrombocytopenia (VITT) (2, 14). VITT mainly occurs in women under the age of 55 years, often occurs 4–16 days after patients receive an adenovirus-based vaccine, and is associated with a high mortality rate (14, 22). A 35-year-old pregnant woman developed intracerebral hemorrhage in the left temporal lobe associated with VITT 12 days after off-label ChAdOx1 nCoV-19 vaccination. This pregnant woman, who was at 23 weeks of gestation, died on day 17 (after vaccination) of refractory intracranial hypertension despite the use of all available pressure control measures (23).

The activation and depletion of platelets in VITT do not rely on heparin (2, 21, 24). Why, then, do anti-PF4 antibodies appear? It has been reported that the presence of PF4-immunoglobulin G (IgG) antibodies increases with the severity of trauma (25); therefore, the production of PF4 antibodies may be associated with an inflammatory response following adenovirus vector vaccination. As PF4 is released by platelets and forms a complex with heparin in the pathogenesis of HIT, the human body forms an IgG against the PF4-heparin complex. Another hypothesis is that after vaccination, viral proteins and free DNA bind to PF4 and form a new antigen (26). These antibodies can bind to FcγRIIa on platelets, promoting platelet activation and aggregation and thus leading to thrombosis (27). In addition, the activation of von Willebrand factor (vWF) and P-selectin after the administration of adenoviral vector vaccines plays a key role in complexes of platelets, white blood cells, and endothelial cells and accelerates the activation and clearance of platelets (28, 29). The other underlying mechanism is that the ethylenediaminetetraacetic acid (EDTA) in vaccine preparations may increase vascular permeability at the injection site and may cause the vaccine components to spread through the bloodstream, which may produce a signal that leads to the production of anti-PF4 antibodies in B cells (2).

However, not all adenoviral vector vaccines induce similar symptoms. No VITT-related adverse events have been reported for the AD5 adenovirus vector vaccine produced by CanSino Biologics (2). The principles for the treatment of VITT are the administration of intravenous immunoglobulin, anticoagulation, the avoidance of heparin, and the transfusion of platelets (30). If the platelet levels are $>30 \times 10^9$/L and if fibrinogen is >1.5 g/L, non-heparin anticoagulation, including argatroban, bivalirudin, apixaban, or rivaroxaban, is suggested (20).

INACTIVATED VACCINES

Inactivated vaccines have been extensively studied and have the advantage of being easy to store and transport, making them suitable for many low-income countries (31). The BBIP-CorV inactivated vaccine was produced by Sinopharm in China and developed from the HB02 strain isolated from patients at the Jinyintan Hospital in Wuhan, China. The ZhongkangKewei (WIBP-CorV) inactivated vaccine, developed from the WIVO4 strain, was also isolated from patients at the Jinyintan Hospital (31). The CoronaVac vaccine is produced by Sinovac Life Sciences in China. In the 40,382 recipients of these vaccines, all three inactivated vaccines had a high level of safety and efficacy (>70%), and none of the patients developed serious cardiovascular adverse events related to vaccination (31). However, in Turkey, among more than 7 million vaccinated people, a 41-year-old woman without any cardiovascular risk factors developed symptoms that included facial flushing, chest palpitations, and chest pain 15 min after receiving the first vaccine dose. She was diagnosed with type one Kounis syndrome, which is a combination of acute coronary disease and hypersensitivity. After treatment with oral antihistamine and aspirin, the patient improved and was discharged from the hospital (32). Although this is the first reported case of allergic myocardial infarction secondary to the administration of an inactivated vaccine, more clinical data and research on the underlying mechanism are needed.

The cardiovascular complications associated with COVID-19 vaccines are presented in detail in **Table 1**.

DISCUSSION

The emergence of the delta variant (Pango lineage B.1.617.2) of SARS-CoV-2 has caused a global resurgence of the pandemic. However, fortunately, the latest real-world data have revealed that COVID-19 vaccines, (33) especially the BNT162b2 mRNA

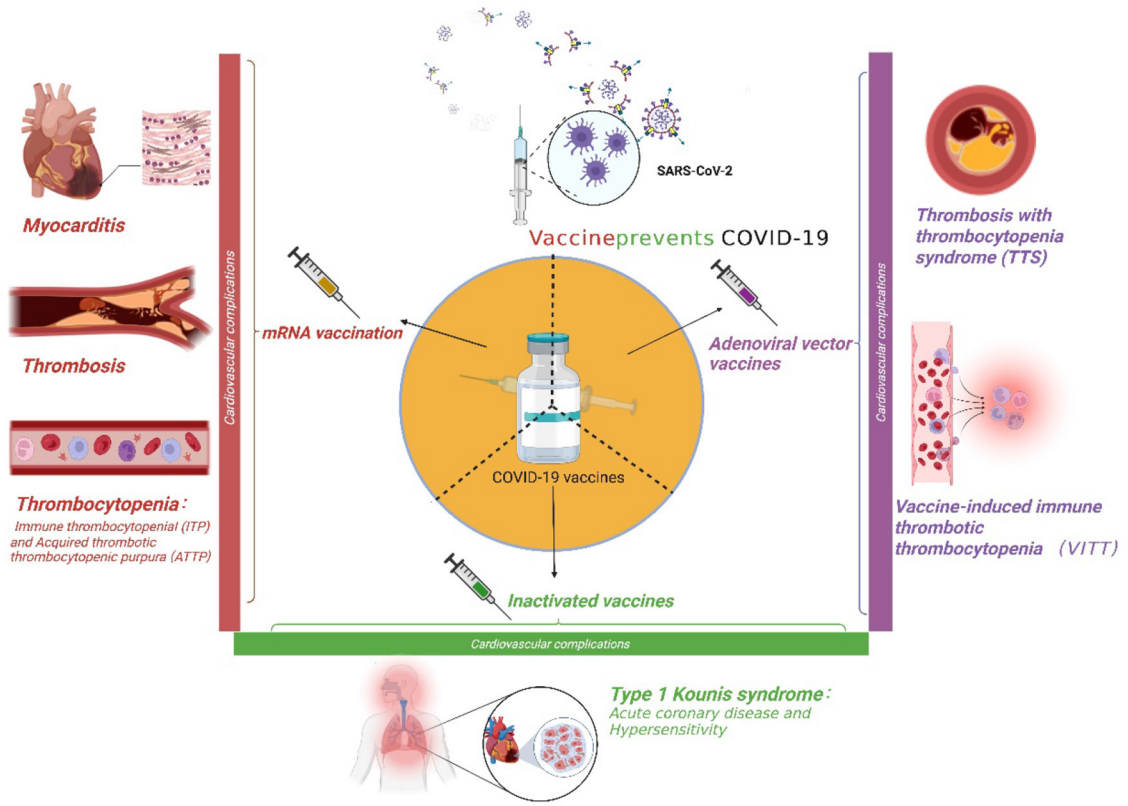

FIGURE 1 | The cardiovascular complications of three major coronavirus disease 2019 (COVID-19) vaccines.

vaccine, still have 88% efficacy for preventing the symptomatic morbidity of the delta variant of SARS-CoV-2 (34). With the increasingly widespread use of COVID-19 vaccines, safety issues associated with the vaccines are gradually becoming the focus of public concern.

Inactivated vaccines have been used for many years to prevent a variety of infectious diseases, and consequently, their safety is generally considered good. However, cardiovascular-related allergic events can occur during vaccination. According to the available literature, the frequency of severe allergic reactions after inactivated vaccine administration appears to be low. However, type one Kounis syndrome is one such rare serious adverse event. Physicians should be aware that Kounis syndrome is a rare but dangerous complication of inactivated coronavirus vaccines. Patients who develop chest pain or severe allergic reactions after vaccination should undergo ECG, echocardiography, and troponin measurement, and these patients should undergo adequate observation or hospitalization if necessary (32).

The VITT, a particularly rare cardiovascular complication, has been observed in adenoviral vector vaccines but not in the other types of vaccines; however, cases of thrombocytopenia after mRNA vaccination have been reported and may be due to an autoimmune mechanism. The key point is whether the VITT/TTS was observed with ChAdOx1 nCoV-19 and AD26.COV2·S vaccinations to represent side effects specific to adenovirus vector vaccines and the extent to which this may affect the administration of the adenoviral vector vaccines. There have been reports suggesting that the incidence of VITT/TTS may be much higher than previously assumed, and this incidence may further increase as physicians become increasingly aware of the syndrome. The estimated incidence of VITT varies among different reports, from ∼1 in 25,000 vaccinated with ChAdOx1 and 1 in more than 500,000 vaccinated with AD26.COV2·S (2). However, these findings should not be used as a reason to discontinue the use of ChAdOx1 nCoV-19 or AD26.COV2·S vaccines. The incidence of VITT complications following adenovirus vector vaccination remains low, while the COVID-19 infection rate and mortality rate are much higher (25).

The mRNA vaccines are innovative vaccines that represent the future of vaccination against COVID-19, and they have the advantages of low production costs and short production cycles. Although the BNT162b2 and mRNA-1273 vaccines were 95% effective after two doses in a phase III clinical trial, (35) the long-term efficacy of these mRNA vaccines is poorly understood. Although mRNA vaccines have been proven to be highly preventive, their cardiovascular side effects should also be seriously considered. Acute myocarditis is a critical adverse event after mRNA vaccination, especially in young males, and these adverse events should be considered in patients who

TABLE 1 | Cardiovascular complications of coronavirus disease 2019 (COVID-19) vaccines.

Vaccine	Data source or region	Time	Complication	Total number administered	Number of events	Complication rate
BNT162B2 (8)	Israel	To 2021.3.24	Myocarditis	More than 4 million	6	1.5/1 million
BNT162B2 (13)	MHRA	2020.12.9–2021.5.26	Thrombosis	10.6 million	33	3.11/1 million
BNT162B2 (13)	Singapore	To 2021.5.31	Thrombosis	1,766,493	3	1.70/1 million
BNT162B2 (14)	EMA	Unknown	Thrombosis	54 million	35	0.65/1 million
mRNA vaccine (19)	VAERS	To 2021.2.2	ITP	20 million	20	1.00/1 million
BNT162B2 (18)	Israel	2021.2–2021.3	ATTP	5 million	4	0.80/1 million
mRNA vaccine (4)	DUMC	2021.2.1–2021.4.30	Myocarditis	561,197	4	7.14/1 million
mRNA vaccine (9)	USA	2020.12.29–2021.6.1	Myocarditis	52 million	1,226	23.58/1 million
mRNA-1273 (14)	EMA	Unknown	Thrombosis	4 million	5	1.25/1 million
mRNA vaccine and ChAdOx1 (13)	VigiBase	2020.12.12–2021.3.16	Thrombosis	Unknown	2,169	Unknown
ChAdOx1 (1)	PRAC	To 2021.4.7	Thrombotic thrombocytopenia	34 million	222	6.53/1 million
ChAdOx1 (21)	UK	To 2021.4.14	Thrombotic thrombocytopenia	21.2 million	168	7.92/1 million
AD26.COV2·S (21)	USA	To 2021.4.13	Thrombosis	6.8 million	15	2.21/1 million
AD26.COV2·S (14)	EMA	Unknown	Thrombosis	More than 7 million	6	0.86/1 million

MHRA, Medicines and Healthcare Products Regulatory Agency of the United Kingdom; EMA, European Medicines Agency; VAERS, Vaccine Adverse Events Reporting System; DUMC, Duke University Medical Center; VigiBase, WHO Global Database for Individual Case Safety Reports; PRAC, the EMA's Pharmacovigilance Risk Assessment Committee.

develop cardiac symptoms after receiving an mRNA vaccine (7). However, the specific mechanism needs to be further explored in larger studies. The cases of mRNA vaccine-secondary ITP following the administration of the BNT162b2 mRNA vaccine or the mRNA-1273 vaccine have been reported and have raised public concern (17, 18). Public panic intensified after the first confirmation of a patient who died of an intracranial hemorrhage (19). The incidence of ITP after mRNA vaccination was actually not far from the estimated baseline annual incidence in the general population; however, post-vaccination ITP remains a possibility, especially in patients with an onset of 1–2 weeks after exposure (19). For patients with cardiovascular complications after mRNA vaccination, such as myocarditis, thrombosis, and ITP, further study is needed to determine whether a second dose of vaccine is needed, whether a different type of vaccine should be used, or whether ITP following the initial dose will exacerbate all of these problems. British researchers launched a phase I clinical trial of a second-generation COVID-19 vaccine on September 20, 2021. The new vaccine, named GRT-R910, (36) is a self-amplified mRNA vaccine. With the global trend of the development of new mRNA vaccines, it is important for researchers to pay increased attention to these possible fatal cardiovascular complications.

The attitudes of people in the community toward vaccination against COVID-19 have been volatile since the end of 2019. People were reluctant to be vaccinated at the initial stage of the application of the new COVID-19 vaccines, as the mid- and long-term data were lacking. As the pandemic worsened in 2020, many people were in favor of being vaccinated. However, as the number of adverse events, especially cardiovascular complications, of these COVID-19 vaccines merged, people became conflicted about whether to be vaccinated, even when the delta variant and the subsequent omicron variant merged. In contrast, due to a lack of scientific and prompt information and data, people are concerned about the possible complications of these vaccines and refuse to allow themselves or their children to be vaccinated. This contradiction also impacts their normal life, resulting in different degrees of anxiety (37). For countries around the world, spending on nationwide COVID-19 vaccination under the suspicion of these adverse events might have unprecedented budget implications for governments and commercial payers. Governments should focus on expanding health system infrastructure and subsidize payer coverage to deliver these vaccines effectively (38). Since the outbreak of the pandemic in 2019, people have been concerned about the complications of vaccines, which have sparked anti-vaccine movements. It is now most important to raise public awareness of COVID-19 vaccine complications through urgent education to reduce the negative impact of a lack of knowledge of COVID-19 vaccination decisions (39).

Although multiple COVID-19 vaccine-related cardiovascular adverse events have been reported, vaccines are still widely used because they are effective against the virus. Compared with the low incidence of complications, the high efficacy of the vaccines against COVID-19 suggests that COVID-19 vaccines should be widely administered. In fact, the cardiovascular complications caused by vaccines can be effectively treated, and most patients improve quickly. Furthermore, a recent study indicated that SARS-CoV-2 infection is itself a very strong risk factor for myocarditis, and the virus also substantially increases the risk of many other serious adverse events (40). A syndrome called Long-COVID-19 has recently emerged among COVID-19 survivors, which is characterized by persistent, typical acute symptoms accompanied by changes in inflammatory and coagulation parameters caused by endothelial damage. SARS-CoV-2 causes the activation of local and

circulating coagulation factors, inducing the production of diffuse coagulation. The similarities and differences between the cardiovascular complications caused by COVID-19 and those caused by mRNA vaccines *via* the spike protein need to be further studied (41). Thus, people should not refuse vaccinations or promote conspiracy theories out of fear of vaccine-related complications.

As the distribution of COVID-19 vaccines is surrounded by suspicion and rumors, it is essential to provide the public with accurate reports from trusted experts, such as medical professionals. Monitoring the safety of COVID-19 vaccines is an important and ongoing process that warrants urgent attention. We propose the establishment of a global database on COVID-19 vaccine adverse events to collect precise and continuous data. In addition, regional regulatory systems should regulate vaccine administration and monitor the occurrence of adverse events and their follow-up in vaccinated people.

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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Impact of the COVID-19 Pandemic on ST-Elevation Myocardial Infarction Management in Hunan Province, China: A Multi-Center Observational Study

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Background: This study aimed to investigate the impact of the COVID-19 pandemic on ST-segment elevation myocardial infarction (STEMI) care in China.

Methods: We conducted a multicenter, retrospective cohort study in Hunan province (adjacent to the epidemic center), China. Consecutive patients presenting with STEMI within 12 h of symptom onset and receiving primary percutaneous coronary intervention, pharmaco-invasive strategy and only thrombolytic treatment, were enrolled from January 23, 2020 to April 8, 2020 (COVID-19 era group). The same data were also collected for the equivalent period of 2019 (pre-COVID-19 era group).

Results: A total of 610 patients with STEMI (COVID-19 era group n = 286, pre-COVID-19 era group n = 324) were included. There was a decline in the number of STEMI admissions by 10.5% and STEMI-related PCI procedures by 12.7% in 2020 compared with the equivalent period of 2019. The key time intervals including time from symptom onset to first medical contact, symptom onset to door, door-to-balloon, symptom onset to balloon and symptom onset to thrombolysis showed no significant difference between these two groups. There were no significant differences for in-hospital death and major adverse cardiovascular events between these two groups.

Conclusion: During the COVID-19 pandemic outbreak in China, we observed a decline in the number of STEMI admissions and STEMI-related PCI procedures. However, the key quality indicators of STEMI care were not significantly affected. Restructuring health services during the COVID-19 pandemic has not significantly adversely influenced the in-hospital outcomes.

Keywords: COVID-19, ST-segment elevation myocardial infarction, primary percutaneous coronary intervention, thrombolysis, outcomes

INTRODUCTION

In late December 2019, an outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) occurred in Wuhan, China (1, 2). Within 3 months since the outbreak, COVID-19 has emerged as a pandemic and an international public health crisis (3). According to the dynamic real-time information provided by Johns Hopkins University Coronavirus Resource Center, as of January 7, 2022, the pandemic has infected over 303,204,268 people and caused 5,479,893 deaths globally (4). The ongoing pandemic of COVID-19 has imposed a serious threat on public health and the economy worldwide.

ST-segment elevation myocardial infarction (STEMI) remains a leading cause of death worldwide (5). Improvement in clinical outcomes after STEMI depends greatly on the timely effective reperfusion therapy. Primary percutaneous coronary intervention (PPCI) is the preferred reperfusion strategy and is the current standard of care for STEMI (6). However, the COVID-19 pandemic inevitably poses a severe challenge to the emergent care of STEMI patients, as the regional STEMI-network was reorganized to assist COVID-19 patients, and the screening and infectious control of COVID-19 procedures required to prevent nosocomial infection may substantially defer PPCI (7–9). Recently, the American College of Cardiology's Interventional Council and Society of Cardiovascular Angiography and Intervention have issued a statement on the management of STEMI in the context of the COVID-19 pandemic and it continues to recommend PPCI as the standard treatment of STEMI patients with unconfirmed COVID-19 status (10). In contrast, the Chinese Society of Cardiology has issued a consensus on the management of STEMI during the COVID-19 pandemic and recommended a strategy of thrombolytic therapy over PPCI due to concerns of resource allocation, as well as challenges in transfer of patients to facilities that perform PPCI (11).

To date, while there are isolated local and regional level reports that the COVID-19 pandemic is associated with a reduction in both presentations with STEMI and PPCI procedures (7, 8), there have been limited data regarding its impact on real-world reperfusion strategies decision making, key indicators of STEMI care, and clinical outcomes. Therefore, the present investigation was undertaken to investigate the real-world impact of the COVID-19 pandemic on time-sensitive STEMI care delivery in Hunan province, China, a so-called "hot-spot" province (adjacent to the epidemic center Wuhan) where the impact would be expected to be most pronounced and lab results.

MATERIALS AND METHODS

Study Design and Population

We conducted a multicenter, retrospective study involving 13 tertiary care cardiac catheterization centers in Hunan province, China. Consecutive patients, presenting with STEMI within 12 h of symptom onset and receiving reperfusion therapy with PPCI, pharmaco-invasive strategy, and only thrombolytic treatment, were enrolled from January 23, 2020 to April 8, 2020, when the city of Wuhan was on lockdown to constrain the spread of the virus. A group of STEMI patients from the equivalent period of last year (i.e., January 23, 2019 to April 8, 2019; pre-COVID-19 era group) was used as control.

During the COVID-19 pandemic, all STEMI patients were screened for COVID-19 first. All admitted patients were required to undergo temperature checks and complete an epidemiological survey at prescreening triage station, which was set up at the entrance of the emergency department. For patients with suspected COVID-19 infection, rapid chest scans and routine blood tests were performed. A nasopharyngeal swab was performed if the condition of the patient allows it. Patients were transferred to a COVID-19-designated hospital if COVID-19 is confirmed. In this study, patients with confirmed or suspected COVID-19 were excluded. Besides, Patients were excluded from the analysis if they presented with ischemic time > 12 h or unknown time, combined with neoplastic disease, discharged to other medical facilities within 48 h, received no acute reperfusion, or had records with missing or incomplete data. STEMI patients were classified into two groups: COVID-19 era group and pre-COVID-19 era group according to the time admitted in hospitals. Patients who underwent PCI were further categorized according to whether they received PPCI or pharmaco-invasive strategy to analyze the procedural characteristics and key time indicators. The diagnosis of STEMI was made based on the fourth universal definition (12). The pharmaco-invasive strategy was defined as fibrinolysis combined with routine early PCI strategy (in case of successful fibrinolysis) or rescue PCI (in case of failed fibrinolysis) (6). The study was conducted in accordance with the Declaration of Helsinki and approved by the local hospital Institutional Review Board, and the need for informed consent for using the medical records was waived owing to the retrospective nature of the study.

Data Collection

The clinical data were collected by trained staff reviewing the medical records of all patients. Data were collected retrospectively, in an anonymized fashion without any sensitive data. We collected detailed baseline variables including demographics, cardiovascular risk factors, medical history, physical findings, and Killip classification on admission, early medical treatments (within 24 h after hospital arrival), and laboratory tests. Treatment timelines delay including symptom onset to first medical contact (FMC), symptom onset to door, door to balloon, symptom onset to balloon, and symptom onset to thrombolysis time. In addition, angiographic and procedural characteristics were assessed.

Clinical Outcomes

All adverse clinical events were adjudicated through the use of original source documentation by an independent committee that was unaware of the treatment allocation. The primary outcome of interest was the number of STEMI admissions and STEMI-related PCI (including PPCI, rescue PCI, and routine early PCI) procedures during Wuhan lockdown and the

equivalent period in 2019. The secondary outcomes were in-hospital all-cause mortality and major adverse cardiovascular events (MACEs), which were defined as a composite of death, non-fatal reinfarction, target vessel revascularization, new-onset congestive heart failure, and stroke during hospitalization (13, 14).

Statistical Analysis

Continuous data were reported as median with 25th and 75th percentiles (interquartile range, IQR) and compared by the Mann–Whitney *U* test. Categorical data were expressed as numbers and percentages and compared by the chi-square test or Fisher's exact test. Multivariate logistic regression analysis was used to identify independent predictors of in-hospital mortality and MACEs. All statistical tests were performed using SPSS software, version 24.0 (SPSS Inc., Chicago, IL, United States). A *P* value of <0.05 was regarded as statistically significant.

RESULTS

Baseline Characteristics

A total of 953 consecutive patients (COVID-19 era group $n = 450$, pre-COVID-19 era group $n = 503$) were admitted for STEMI during the described time frames. Of these, no patient was confirmed or suspected COVID-19 and 610 patients (COVID-19 era group $n = 286$, pre-COVID-19 era group $n = 324$) fit the inclusion criteria for this study (**Figure 1**). Among this study population, 567 (93.0%) patients received PPCI. Forty (6.6%) patients received pharmaco-invasive treatment including routine early PCI ($n = 18$) and rescue PCI ($n = 22$). When comparing the study period of 2020 to the equivalent period of 2019, a reduction of 10.5% in STEMI admissions was observed (**Figure 2**). Also, there was a 12.7% decline in the number of all STEMI-related PCI procedures (including PPCI, rescue PCI and routine early PCI) when compared to the same time interval in 2019 (**Figure 3**). The remaining 3 (0.5%) patients received only thrombolysis treatment.

Demographic, baseline clinical characteristics and laboratory variables of the enrolled patients are listed in **Table 1**. Compared with the pre-COVID-19 era group, the COVID-19 era group was more likely to have a history of previous PCI. Otherwise, there were no significant differences between the two groups in terms of patient demographics, medical history, the prevalence of coronary risk factors, physical findings on admission and concomitant medications. Patients were presented with a slightly higher cardiac troponin I (cTnI) level on admission during the COVID-19 pandemic era compared with the pre-COVID-19 era. Patients in the COVID-19 era group tended to have a lower blood urea nitrogen, low-density lipoprotein cholesterol and C-reactive protein level on admission (**Table 1**).

The baseline angiographic features and procedural data are summarized in **Table 2**. For patients who received PPCI, the time from symptom onset to FMC, symptom onset to door and symptom onset to balloon were not substantially longer during the COVID-19 pandemic era. The door-to-balloon time and the total procedure time were similar between two groups. For pharmaco-invasive patients, the key time interval, including the time from symptom onset to FMC, symptom onset to door and symptom onset to thrombolysis, showed no significant increase during the COVID-19 pandemic era. Patients who received PPCI were less likely to have a right coronary artery occlusion, multivessel disease, and radial access during the COVID-19 pandemic era compared with the pre-COVID-19 era. Moreover, patients who received PPCI during the COVID-19 pandemic had a greater proportion of direct stenting and thrombus aspiration. Among patients admitted with PPCI, the intra-aortic balloon pump (IABP) use and extracorporeal membrane oxygenation use were no difference during the COVID-19 pandemic and pre-COVID-19 era. A high procedural success rate (97.7 vs. 99.0%) and low complications rate (1.5 vs. 1.3%) were similarly observed between two groups. Among patients who received pharmaco-invasive strategy, no significant difference was observed between the two groups with regard to the location of culprit artery, initial and final TIMI flow grade, and prevalence of multivessel diseases. IABP was less used in these patients during the COVID-19 pandemic era.

Clinical Outcomes

The in-hospital outcomes are shown in **Table 3**. No significant difference was observed in the median hospital length of stay between these two groups. There was no significant difference in in-hospital mortality between these two groups (2.4 vs. 3.4%, $P = 0.490$). One non-fatal myocardial infarction occurred in pre-COVID-19 era group. Two patients in the COVID-19 era group and one in the pre-COVID-19 era group experienced non-fatal stroke in hospital in COVID-19 era group. The rate of in-hospital heart failure decreased from 8.0 to 4.9% during the outbreak period. The rate of target vessel revascularization increased slightly from 0.9 to 2.4% during the outbreak period. Finally, the cumulative MACEs were similar between two groups (9.8 vs. 10.8%, $p = 0.682$). The adjusted odds of in-hospital death and MACEs are shown in **Table 4**. Following adjustment for covariates, no significant differences were found for in-hospital death (odds ratio [OR] 1.180, 95% confidence interval [CI] 0.181–7.679, $P = 0.862$) or MACEs (OR 1.390, 95%CI 0.612–3.161, $P = 0.431$).

DISCUSSION

In response to the COVID-19 pandemic, many countries have implemented strict infection containment measures such as "lockdown" and encouraged a "stay-at-home" lifestyle, to reduce the spread of the pandemic (8, 9, 15). Moreover, the routine hospital services including cardiac catheterization have been restructured in order to increase hospital capacity for COVID-19 patients and prevent cross-infection. These strict restriction measures would inevitably have a profound impact on routine medical care, in particular, acute cardiovascular disease management.

In the present study, we conducted a retrospective analysis in 610 STEMI patients receiving acute reperfusion treatment including PPCI, pharmaco-invasive strategy and systematic

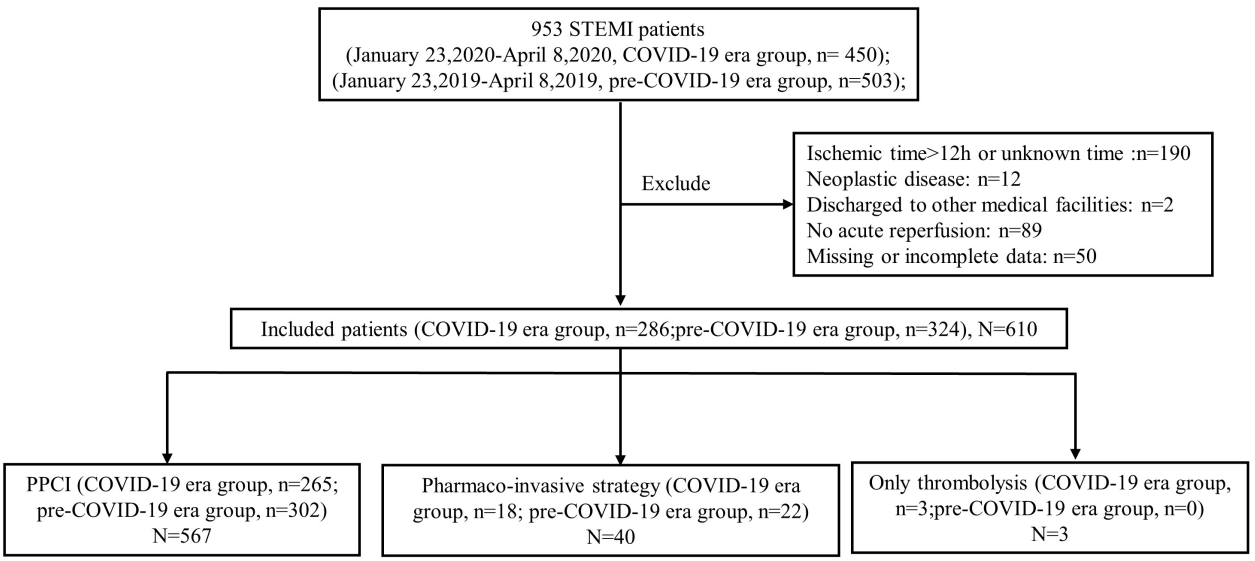

FIGURE 1 | Study flow chart. STEMI, ST-segment elevation myocardial infarction; COVID-19, coronavirus disease 2019; PPCI, primary percutaneous coronary intervention.

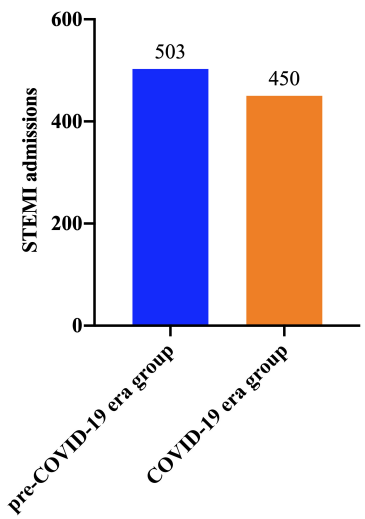

FIGURE 2 | Admissions for STEMI during Wuhan lockdown and equivalent time period in 2019. STEMI, ST-segment elevation myocardial infarction; COVID-19, coronavirus disease 2019.

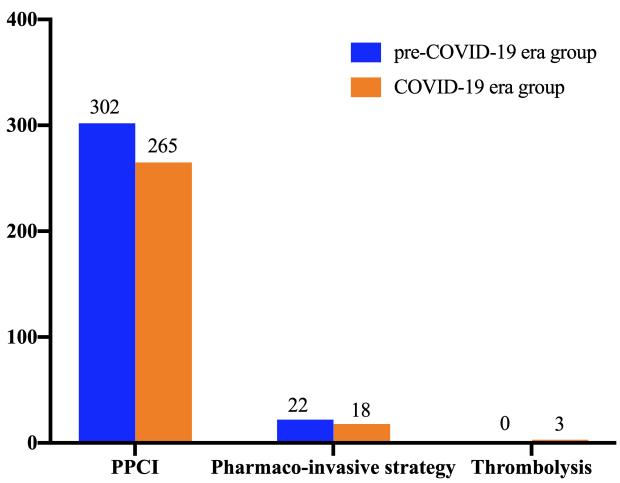

FIGURE 3 | Reperfusion strategy for STEMI patients during Wuhan lockdown and equivalent time period in 2019. STEMI, ST-segment elevation myocardial infarction; COVID-19, coronavirus disease 2019; PPCI, primary percutaneous coronary intervention.

thrombolysis and compared the in-hospital clinical outcomes of patients presenting during the COVID-19 pandemic vs. pre-COVID-19 era. First, we demonstrated a 10.5% drop in STEMI volumes, a 12.7% decline in STEMI-related PCI procedures and a significantly higher cTnI level on admission during the COVID-19 outbreak. Second, in terms of time delay, the pandemic of COVID-19 incurred no additional time delay whether in the PPCI subgroup or pharmaco-invasive strategy subgroup. Finally, there were no differences in clinical outcomes including in-hospital mortality and MACEs before and after lockdown.

Previous studies have reported a common decrease in STEMI admissions while the degree of decline varied considerably among countries affected by the COVID-19 pandemic. During the early phase of the COVID-19 pandemic, Xiang et al. (15) reported a 26.3% reduction in STEMI patients' access to care in non-Hubei provinces in China based on the Chest Pain Center database. A similar reduction of 23% in admissions for STEMI was reported in England (9). Scholz et al. (16) reported a mild decrease in the absolute number of STEM patients treated in systems of STEMI care in Germany (12.6%). Our results supported this finding but presented a relatively

milder decrease in STEMI volumes (10.5%). In contrast, reports from other countries (e.g., Singapore, France, and Denmark) report no appreciable decrease and even a modest increase in STEMI volumes (17–19). The largely discrepant reports of STEMI hospitalization across countries could be partly explained by disparities in healthcare organizations.

Multiple factors might contribute to this decline in admissions of patients with STEMI during the COVID-19 pandemic. One

TABLE 1 | Baseline clinical characteristics.

	COVID-19 group (*n* = 286)	Pre-COVID-19 group (*n* = 324)	Statistic	*P* value
Demographics				
Age (years)	63 (53.5–70)	63 (53–75)	−0.727‡	0.467
Male sex, *n* (%)	229 (80.1)	250 (77.2)	0.763†	0.383
Cardiovascular risk factors, *n* (%)				
Diabetes mellitus	51 (17.8)	77 (23.8)	3.225†	0.073
Hypertension (> 140/90 mmHg)	143 (50.0)	148 (45.7)	1.137†	0.286
Hyperlipidemia	90 (31.5)	113 (34.9)	0.795†	0.373
Current smoker	129 (45.1)	164 (50.6)	1.849†	0.174
Number of cardiovascular risk factors			0.658†	0.883
≥3	48 (16.8)	60 (18.5)		
2	99 (34.6)	106 (32.7)		
1	90 (31.5)	98 (30.2)		
0	49 (17.1)	60 (18.5)		
Medical history, *n* (%)				
History of PCI	16 (5.6)	6 (1.9)	6.120†	0.013
History of CABG	0 (0.0)	0 (0.0)	—	—
Previous MI	8 (2.8)	10 (3.1)	0.440†	0.833
Physical findings on admission				
Systolic blood pressure (mm Hg)	126 (110–145.25)	127 (110–141.5)	−0.126‡	0.900
Heart rate (beats/min)	77.96 ± 18.083	78.43 ± 17.135	−0.329*	0.742
Killip classification on admission, *n* (%)			2.836†	0.242
Class I	191 (66.8)	225 (69.4)		
Class II	71 (24.8)	64 (19.8)		
Class III–IV	24 (8.4)	35 (10.8)		
Medication within 24 h of hospital arrival, *n* (%)				
Aspirin	285 (99.7)	322 (99.4)	-	1.000
P2Y12 receptor inhibitor	286 (100.0)	323 (99.7)	-	1.000
GP IIb/IIIa receptor inhibitor	149 (52.1)	151 (46.6)	1.834†	0.176
β-blockers	230 (80.4)	279 (86.1)	3.562†	0.059
Statins	271 (94.8)	314 (96.9)	1.800†	0.180
Angiotensin-converting enzyme inhibitors/Angiotensin receptor blockers	211 (73.8)	240 (74.1)	0.007†	0.933
Laboratory tests				
White blood cell × 10^9/L	9.8 (8.0–12.9)	10.6 (8.0–13.3)	−0.560‡	0.575
Neutrophil × 10^9/L	7.9 (5.7–10.9)	8.4 (5.8–10.7)	−0.402‡	0.608
Lymphocyte × 10^9/L	1.3 (1.0–1.8)	1.3 (0.9–2.0)	−0.553‡	0.580
Platelets × 10^9/L	204.0 (169.0–250.5)	209.5 (177.0–243.3)	−0.097‡	0.923
Hs-cTnI (pg/ml)	5.3 (0.8–23.8)	3.0 (0.3–18.2)	−2.707‡	0.007
CK (U/L)	877.0 (225.5–2590.7)	939.1 (236.0–2199.0)	−0.243‡	0.808
CK-MB (U/L)	92.7 (27.2–232.0)	99.0 (34.6–235.7)	−0.704‡	0.482
BNP (pg/ml)	100.0 (31.3–225.1)	105.0 (67.3–419.3)	−1.687‡	0.092
NT-proBNP (pg/ml)	560.4 (160.3–1727.5)	392.0 (91.4–1541.0)	−1.534‡	0.125
ALT (U/L)	35.2 (24.1–60.0)	39.8 (26.0–61.4)	−1.144‡	0.253
AST (U/L)	91.5 (37.9–208.6)	114.5 (38.5–247.9)	−1.378‡	0.168
BUN (mmol/L)	5.7 (4.6–7.2)	6.4 (5.0–8.0)	−3.181‡	0.001
TC (mmol/L)	4.7 (3.9–5.5)	4.7 (4.0–5.4)	−0.647‡	0.517
TG (mmol/L)	1.5 (1.0–2.3)	1.7 (1.1–2.4)	−1.224‡	0.221
HDL-C (mmol/L)	1.0 (0.9–1.2)	1.1 (0.9–1.3)	−1.735‡	0.083

(Continued)

TABLE 1 | (Continued)

	COVID-19 group (*n* = 286)	Pre-COVID-19 group (*n* = 324)	Statistic	*P* value
LDL-C (mmol/L)	2.8 (2.2–3.5)	3.1 (2.5–3.7)	−2.557‡	0.011
Hs-CRP (mg/L)	4.2 (1.7–11.7)	7.6 (2.9–21.4)	−2.737‡	0.006
PT (s)	12.5 (11.1–14.6)	12.0 (10.3–15.3)	−2.539‡	0.011
APTT (s)	34.1 (27.5–55.5)	32.4 (26.8–41.1)	−2.292‡	0.022
D-dimer (mg/L)	0.4 (0.2–1.9)	0.4 (0.2–0.8)	−1.830‡	0.067

*Data are expressed as mean ± SD, as percentages, or as median (Q_1, Q_3). *t value; † χ^2 value; ‡Z value. —: Data not available (Fisher exact test). COVID-19, coronavirus disease 2019; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; MI, myocardial infarction; Hs-cTnI, high sensitivity cardiac troponin I; CK, creatine phosphokinase; CK-MB, creatine phosphokinase-MB; BNP, B-type natriuretic peptide; NT-proBNP, N terminal pro-hormone BNP; ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Hs-CRP, high-sensitivity C-reactive protein; PT, prothrombin time; APTT, activated partial thromboplastin time.*

possibility is that the case of misdiagnosis increased because of complex cardiovascular manifestations under the circumstance of COVID-19. It is challenging to differentiate STEMI patients from COVID-19 patients, who might simulate a STEMI manifestation and present with cardiac troponin elevation and/or ST changes (20). Therefore, a proportion of critical STEMI with dyspnea and pulmonary edema could be mistaken with the coronavirus features and managed as a COVID-19 case from the outset. The fear of medical system might be another important factor. The soaring confirmed infections, no effective therapeutic drugs, no vaccines and lack of personal protective equipment may have created an atmosphere of fear. The symptomatic patients might avoid seeking acute medical care for fear of getting in contact with COVID-19 patients (21).

Additionally, our finding showed a decline of 12.7% in STEMI-related PCI procedures, which supports the decline in PCI procedures for STEMI reported in other studies, but we add some additional value to such observations by describing clinical and procedural characteristics and outcomes after the COVID-19 lockdown using last year as a reference. A preliminary analysis from multiple United States centers showed during the early phase of the COVID-19 pandemic, an estimated 38% reduction in cardiac catheterization laboratories activations for STEMI care (22). Another survey of 73 centers in Spain reported a 40% reduction in procedures performed in the STEMI settings (23). Using the British Cardiovascular Intervention Society database, Kwok et al. (24) reported a 43% reduction in all STEMI-related PCI procedures in England in the month after the lockdown. In the present study, we observed a slight decline in STEMI-related PCI procedures. The different degrees of decline in nations and regions indicated the huge differences in terms of local healthcare resources, the pandemic density of the COVID-19 outbreak and changes of the pandemic over time. In China, since Hubei province started lockdown on January 23, 2020, Hunan province had activated Level one major public health emergency response on the same day. With the joint efforts of the government and people, the epidemic was quickly controlled. Subsequently, the government degraded the major public health emergency response to Level two on March 10 due to a sustained decrease in the number of new cases. The regional medical system had the capacity to continue to provide emergency STEMI care according to current clinical practice guidelines.

Undoubtedly, the COVID-19 pandemic is a major burden on the time-dependent emergency healthcare networks and is imposing a change on STEMI care especially in region heavy involvement in the epidemic. An important issue merit consideration is how changes in patients' health-seeking behavior, health service delivery and government strategies to restrict virus spread impact clinical characteristics and outcomes of the patients (24). Our study showed patients admitted during the COVID-19 pandemic were more likely to have a history of previous PCI with a significant increase in the baseline cTnI level compared to a similar time frame last year. Similar observations have also been reported from England and Germany (21, 25). The fear of getting infected within the hospitals and government calls to stay at home and seek medical care only in case of an emergency may lead to patients' delay seeking a doctor, and aggravation of their symptoms (21, 24). As a consequence, a substantial reduction in admissions for STEMI and an increase in the number of out-of-hospital cardiac arrests were observed (26). In the present study, we found relatively fewer patients receiving PCI during the COVID-19 pandemic and no overall increase in in-hospital mortality and MACEs among patients admitted for STEMI. Despite this fact, caution must be exercised in interpreting the results. On the one hand, many patients had STEMI but receive no reperfusion therapy in hospital because of deaths out of hospital. On the other hand, it warrants much investigation to assess whether the long-term clinical outcome was not different before and after the COVID-19 pandemic outbreak.

In present study, there was no difference between two groups in terms of key time interval and short term in-hospital outcomes for STEM patients. This result was in line with studies in other regions of China. A single center report from Beijing by Guan et al. (27) showed door to balloon time, operation time and the incidence of MACEs were similar pre and during COVID-19 pandemic. Similar results were found in Shenzhen, a metropolitan city in southern of China (28). Therefore, above results indicated that safety measures to prevent nosocomial COVID-19 infection did not compromise the in-hospital outcomes as compared with PCI under normal condition. The regional collaborative STEMI treatment network established in China worked well and ensured timely acute cardiac care even in the context of the COVID-19 pandemic.

TABLE 2 | Angiographic characteristics and procedural data.

	PPCI (*n* = 567)				Pharmaco-invasive (*n* = 40)			
	COVID-19 era group (*n* = 265)	**Pre-COVID-19 era group (*n* = 302)**	**Statistic**	***P* value**	**COVID-19 era group (*n* = 18)**	**Pre-COVID-19 era group (*n* = 22)**	**Statistic**	***P* value**
Time delays, min								
Symptom onset to FMC	141 (67–282)	147 (70.25–270)	−0.292‡	0.77	88 (30–121.75)	72 (30–193.75)	−0.015‡	0.988
Symptom onset to door	185 (105.75–301)	210 (103–327.5)	−0.668‡	0.504	349 (220–592.5)	382.5 (186.25–579)	−0.184‡	0.854
Door-to-balloon	79 (61–102.5)	77 (55.5–99.5)	−0.855‡	0.393	–	–	–	–
Symptom onset to balloon	274 (177.75–372.75)	278.5 (182.75–425.75)	−0.957‡	0.339	–	–	–	–
Total procedure time	55 (43–72)	53 (42–67)	−1.083‡	0.279	–	–	–	–
symptom onset to thrombolysis	–	–	–	–	139 (70.25–185.5)	120 (70–225.5)	−0.155‡	0.877
Infarct-related artery, *n* (%)								
LM	18 (6.8)	14 (4.6)	1.233†	0.267	1 (5.6)	0 (0.0)	–	0.45
LAD	150 (56.6)	170 (56.3)	0.006†	0.94	9 (50.0)	14 (63.6)	0.753†	0.385
LCX	69 (26.0)	96 (31.8)	2.262†	0.133	3 (16.7)	5 (22.7)	–	0.632
RCA	124 (46.8)	169 (56.0)	4.751†	0.029	11 (61.1)	11 (50.0)	0.494†	0.482
Multivessel disease Procedural issues, *n* (%)	77 (29.1)	124 (41.1)	8.887†	0.003	8 (44.4)	12 (54.5)	0.404†	0.525
Procedural issues, *n* (%)								
Radial access	254 (95.8)	276 (91.4)	4.599†	0.032	17 (94.4)	22 (100.0)	–	0.45
Stent use	236 (89.1)	281 (93.0)	2.794†	0.095	17 (94.4)	22 (100.0)	–	0.45
Direct stenting	100 (37.7)	86 (28.5)	5.489†	0.019	11 (61.1)	16 (72.7)	0.609†	0.435
Thrombus aspiration	34 (12.8)	22 (7.3)	4.876†	0.027	2 (11.1)	0 (0.0)	–	0.196
IABP use	16 (6.0)	22 (7.3)	0.351†	0.554	2 (11.1)	10 (45.5)	5.560†	0.018
ECMO use	2 (0.8)	1 (0.3)	–	0.602	0 (0.0)	0 (0.0)	–	–
Procedural success	259 (97.7)	299 (99.0)	–	0.225	18 (100.0)	22 (100.0)	–	–
Complications	4 (1.5)	4 (1.3)	–	1	1 (5.6)	0 (0.0)	–	0.45
Initial TIMI flow grade (pre-PCI), *n* (%)			0.774†	0.379			0.609†	0.435
TIMI flow grade 0–1	223 (84.2)	262 (86.8)			11 (61.1)	16 (72.7)		
TIMI flow grade 2–3	42 (15.8)	40 (13.2)			7 (38.9)	6 (27.3)		
Final TIMI flow grade (post-PCI), n (%)			–	1.000			–	–
TIMI flow grade 0–1	2 (0.8)	2 (0.7)			0 (0.0)	0 (0.0)		
TIMI flow grade 2–3	263 (99.2)	300 (99.3)			18 (100.0)	22 (100.0)		

Data are expressed as mean ± SD, as percentages, or as median (Q1, Q3). † *χ2 value;* ‡ *Z value. —: Data not available (Fisher exact test). COVID-19, coronavirus disease 2019; PCI, percutaneous coronary intervention; PPCI, primary PCI; LM, left main; LAD, left anterior descending; LCX, left circumflex; RCA, right coronary artery; IABP, intra-aortic balloon pump; ECMO, extracorporeal membrane oxygenation; TIMI, thrombolysis in myocardial infarction.*

TABLE 3 | Clinical outcomes data.

	COVID-19 era group (*n* = 286)	Pre-COVID-19 era (*n* = 324)	Statistic	*P* Value
Length of stay, *d*	8 (6–10)	8 (6–11)	−0.988‡	0.323
In-hospital death, *n* (%)	7 (2.4)	11 (3.4)	0.476†	0.490
Non-fatal MI, *n* (%)	0 (0.0)	1 (0.3)	–	1.000
Non-fatal stroke, *n* (%)	2 (0.7)	1 (0.3)	–	0.602
Congestive heart failure, *n* (%)	13 (4.5)	24 (7.4)	2.184†	0.139
Target vessel revascularization, *n* (%)	7 (2.4)	3 (0.9)	–	0.202
Cumulative MACEs, *n* (%)	29 (10.1)	40 (12.3)	0.737†	0.391

Data are expressed as mean ± SD, as percentages, or as median (Q_1, Q_3). †χ^2 value; ‡Z value. —: Data not available (Fisher exact test). COVID-19, coronavirus disease 2019; MI, myocardial infarction; MACEs, major adverse cardiovascular events.

TABLE 4 | Multivariate logistic regression analysis.

	Comparison of COVID-19 era group versus Pre-COVID-19 era group	
	Adjusted OR (95% CI)*	*P* value
In-hospital death	3.935 (0.511, 30.310)	0.188
MACEs	1.074 (0.416, 2.770)	0.883

*COVID-19, coronavirus disease 2019; MACEs, major adverse cardiovascular events. *Adjusted for age, sex, hypertension, hypercholesterolemia, diabetes mellitus, smokers, previous myocardial infarction, previous percutaneous coronary intervention, previous coronary artery bypass graft, aspirin, P2Y12 receptor antagonist, glycoprotein IIb/IIIa inhibitor use, β-blockers, statins, angiotensin converting enzyme inhibitors/Angiotensin II receptor blockers, symptom-to-hospital time, door-to-balloon time, radial access, multivessel disease, vessel of intervention, flow, intra-aortic balloon pump, extracorporeal membrane oxygenation.*

Our data demonstrated that a better public communication approach should be adopted to reassure patients in critical conditions to obtain timely medical contact. Public health, political, and physician leaders in China have taken aggressive measures to encourage patients with heart attack symptoms to seek medical care. Social media including WeChat, Weibo, Tik Tok, and so on was applied as a tool for grassroots health promotion initiatives during the COVID-19 pandemic. Based on social media platforms, healthcare professionals reeducated the general population to recognize and act on heart attack signs and symptoms and call an ambulance immediately. Furthermore, it's necessary to stress that the national healthcare system still had the capacity to provide prompt and effective care in a manner that was safe for both patients and healthcare workers. Meanwhile, hospitals had to take appropriate precautions to protect patients and healthcare workers from COVID-19 infection.

STUDY LIMITATIONS

Our study has several limitations. First, although patients affected by COVID-19 were excluded from the final analysis, we cannot definitively exclude the possibility that patients in the COVID-19 era may have COVID-19 infection because it's hard to make an absolutely accurate diagnosis in the early phase of the pandemic. However, we believe this possibility was very small because all enrolled patients were lack of the epidemiological history and clinical manifestations. Second, we assessed only in-hospital outcomes, as data on post-discharge follow-up are currently not available. Third, the onset of symptoms was a subjective parameter and might not be precisely recorded. Finally, self-report of in-hospital outcomes generally along with early discharge may have resulted in under-reporting of adverse outcomes.

FUTURE DIRECTIONS

Every effort should be made to educate the public to recognize symptoms of life-threatening cardiac conditions and seek appropriate care in a timely fashion. Health authorities should implement strategies to further optimize the STEMI care system in response to emerging infectious diseases like COVID-19.

CONCLUSION

The COVID-19 pandemic outbreak led to a decline in the number of admitted STEMI cases as well as STEMI-related PCI procedures in Hunan province, China. The key quality indicators of reperfusion treatment including median time from symptom onset to FMC, symptom onset to door, door-to-balloon, symptom onset to balloon, and symptom onset to thrombolysis, were not significantly affected during the pandemic outbreak. Restructuring health services during the COVID-19 pandemic has not significantly adversely influenced the in-hospital outcomes.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of The Second Xiangya Hospital,

Central South University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LT and Z-JW participated in the design of the study, collected clinical data, performed statistical analysis, and drafted the manuscript. X-QH, Z-FF, Z-FZ, J-PZ, L-PJ, FO, C-HL, and G-FZ participated in the treatment for the patients and collected clinical data. Y-HG and S-HZ participated in the design of the study, revised the final version of the manuscript, and supervised the study. All authors participated in the research and reviewed the final version of the manuscript.

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CASE REPORT
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Acute Fulminant Myocarditis After ChAdOx1 nCoV-19 Vaccine: A Case Report and Literature Review

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According to recent literatures, myocarditis is an uncommon side effect of mRNA vaccines against COVID-19. On the other hand, myocarditis after adenovirus based vaccine is rarely reported. Here we report a middle-aged healthy female who had acute fulminant perimyocarditis onset 2 days after the first dose of ChAdOx1 vaccine (AstraZeneca) without any other identified etiology. Detailed clinical presentation, serial ECGs, cardiac MRI, and laboratory data were included in the report. Possible mechanisms of acute myocarditis after adenoviral vaccine was reviewed and discussed. To our knowledge, a few cases of myocarditis after Ad26.COV2.S vaccine were reported, and this is the first case report after ChAdOx1 vaccine.

Keywords: COVID-19, vaccine, adenovirus, ChAdOx1, myocarditis

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INTRODUCTION

Growing evidence has shown that acute myocarditis is a rare complication after mRNA COVID-19 vaccinations, with an estimated incidence of ∼2 per 100,000 persons after BNT162b2 mRNA vaccine (1, 2), and the risk is higher in adolescent males. Typically, acute myocarditis occurs within 5 days after mRNA vaccination, and the mechanism is still unclear. Myocarditis after adenovirus or protein-based vaccines has seldom been reported. Here, we report the case of a 44-year-old female who had acute fulminant perimyocarditis following the first dose of ChAdOx1 nCoV-19 vaccine with no other identified etiology.

CASE DESCRIPTION

A previously healthy 44-year-old Taiwanese female hairdresser (153 cm, 63 kg), without any documented systemic disease, received first dose of ChAdOx1 nCoV-19 vaccine (AstraZeneca) on August 6, 2021. She denied taking any long-term or short -term medication, and had no fever, sore throat, or other symptoms suggesting viral infection within 2 weeks before vaccination. She started to feel persistent stabbing chest pain and breathless approximately 48 h after vaccination. Because the symptoms progressed, she visited the emergency department at another hospital on August 11. Initial troponin I was 17 ng/mL and D-dimer was 1020 ng/mL FEU. ECG showed diffuse low QRS voltage and 1 mm convex ST elevation over V1 and V2 (**Figure 1A**). Coronary angiography revealed patent coronary arteries, and no pulmonary embolism was found on enhanced CT. She had nausea, vomiting, and abdominal distension after admission. Hypotension developed on August 12, and echocardiography showed poor left ventricular function. Norepinephrine was infused, and she was transferred to our intensive care unit for further management on August 13.

On arrival, her vital signs included temperature 37.2°C, heart rate 108/min, blood pressure 96/77 mmHg (under norepinephrine 0.3 μg/kg/min), respiration 20/min, and O2 saturation 93% under O2 nasal cannula. Fine crackles were heard over bilateral basal lung fields and there was no audible pleural or pericardial friction rub. ECG showed sinus tachycardia, diffuse low QRS voltage, and convex ST elevation over V1 to V3 (**Figure 1B**). Chest X-way revealed acute pulmonary edema, and echocardiography showed left ventricular diameter 47/39 mm, left ventricular ejection fraction (LVEF) about 35%, and small amount of pericardial effusion. Initial laboratory data on August 13 showed elevated troponin I (8.1 ng/mL), BNP (399 pg/mL), D-dimer (3,815 ng/mL FEU), and ALT (100 U/L). Her creatinine (0.6 mg/dL) and lactate (19 mg/dL) were normal. Complete blood cell count showed leukocytosis (WBC 11,700/μL with segment 88%) with normal hemoglobin (12.4 g/dL) and platelets (251 K/μL). Other relevant in-hospital laboratory results were presented in **Supplementary Table 1**.

We checked COVID-19, influenza A/B, adenovirus, coxsackievirus, mycoplasma, CMV, EBV, HIV, and markers for autoimmune disease. The results were all negative except for reactive CMV IgG with negative CMV IgM and low C3 66 mg/dL (reference 90∼180). Myocardial biopsy was suggested but she refused. Because D-dimer level increased from 3,815 to 6,433 ng/mL FEU and history of ChAdOx1 vaccination, anti-PF4 antibody level was checked on August 16, and it was 0.15 optical density (normal < 0.4 OD). There was no detectable venous thrombosis by chest CT and peripheral Doppler. Post-vaccine acute fulminant myocarditis is impressed. Since there is no established treatment protocol for post-vaccine myocarditis, we offered the patient standard therapy for heart failure and perimyocarditis.

Initial medication included furosemide, ivabradine, colchicine, and norepinephrine to keep mean arterial pressure above 65 mmHg. After above treatment for 2 days, her appetite and orthopnea gradually improved, and norepinephrine was discontinued on August 16. Her pulmonary edema resolved and troponin I level decreased (daily troponin I 8.1, 6.8, 5.6, 2.1 mg/mL from August 13 to 16). Spironolactone was added and she was transferred to ward on August 18.

Cardiac MRI on August 19 showed global LV hypokinesia with LVEF 41.6% and markedly increased LV T1 and T2 signal values (**Figure 2**). Late Gadolinium enhancement (LGE) images depict the patchy enhancements sparsely distributed in the mid-layer and subepicardium, and subendocardial enhancement in the antero-septal subendocardium of LV mid-cavity. On August 23, her LVEF was 45% by echocardiography and ECG showed evolutionary changes including higher QRS voltage and diffuse T wave inversion (**Figure 1C**). She was discharged on August 24 with colchicine, losartan, ivabradine, and spironolactone. She had mild dyspnea on exertion and tingling chest pain at discharge, and the symptoms gradually disappeared after discharge. Her latest echocardiography on January 17 2022 showed normal LV diameter (45/31 mm), LVEF 60%, and no pericardial effusion. ECG showed normal sinus rhythm without ST-T changes (**Supplementary Figure 3**). There was a complete recovery of her fulminant perimyocarditis.

DISCUSSION

Acute perimyocarditis is an uncommon side effect after vaccination in the pre-COVID-19 era. In the US Vaccine Adverse Event Reporting System (VAERS), total 708 reports met the definition as perimyocarditis from 1990 to 2018 (3). It occurs more commonly in males (79%) than in females, and the most frequently reported vaccines are smallpox (59%), anthrax (23%), and typhoid (13%) vaccines. There is growing evidence that myocarditis is a rare side effect of mRNA vaccines against COVID-19 (1, 2, 4–6). Considering the background incidence of viral myocarditis [about 10–22 per 100,000 individuals per year (7)], a nationwide study in Israel reported a calculated risk ratio of 2.35-fold of acute myocarditis between BNT162b2 (Pfizer) vaccinated and unvaccinated persons (2), and the risk ratio was higher in adolescent males. Most cases of myocarditis occurred within 5 days (median 2 days) following the second dose (1, 2, 8).

While clinical and basic researchers are working on the relationship between myocarditis and mRNA vaccines, myocarditis after adenovirus or protein-based COVID-19 vaccines has seldom been reported. In a recent review of post-COVID-19 vaccination myocarditis (9), only one of the 61 cases received Ad26.COV2.S adenoviral vaccine (Johnson and Johnson) while the other cases all received mRNA vaccine. In another case report of fulminant myocarditis after Ad26.COV2.S vaccine, the patient expired within 24 h despite of ECMO support (10). Autopsy revealed lymphohistocytic myocarditis. In our report, because the patient refused myocardial biopsy, the diagnosis of myocarditis is based on diagnostic criteria from European Society of Cardiology Working Group on Myocardial and Pericardial Diseases (**Table 1**) (11). All diagnostic criteria include abnormal ECG and echocardiography, elevated Troponin I, and myocardial damage by cardiac MRI were met and coronary angiography showed patent coronary arteries. Because her symptoms onset 2 days after the first dose of ChAdOx1 nCoV-19 vaccine without any other identified etiology, vaccine-related myocarditis was highly suspected. Currently there is no established test to confirm the causal relationship. According to the report from VAERS, rates of post-vaccine myocarditis for females aged 40–49 years was 0.1/1.1 per 1 million doses after first/second dose of BNT162b2 and 0.2/1.4 after first/second dose of mRNA-1273 vaccine (Moderna) (12). The reported incidence of myocarditis after mRNA vaccines is quite low at her age as well. A phase 3 study of ChAdOx1 nCoV-19 vaccine enrolled 32,451 participants, and the number was still underpowered to detect uncommon side effects such as vaccine-induced immune thrombotic thrombocytopenia (VITT). Although no myocarditis was reported in either group, two cases with cardiac disorders were reported as medically attended adverse events in the ChAdOx1 group compared to 0 events in the placebo group (13).

Our patient had negative anti-PF4 antibody, so the pathophysiology was different from VITT. There are several possible mechanisms that may lead to myocarditis after ChAdOx1 vaccination. First, adenovirus is an established cause of acute myocarditis (14). Adenovirus can enter cardiomyocytes by binding to a common transmembrane receptor [coxsackievirus

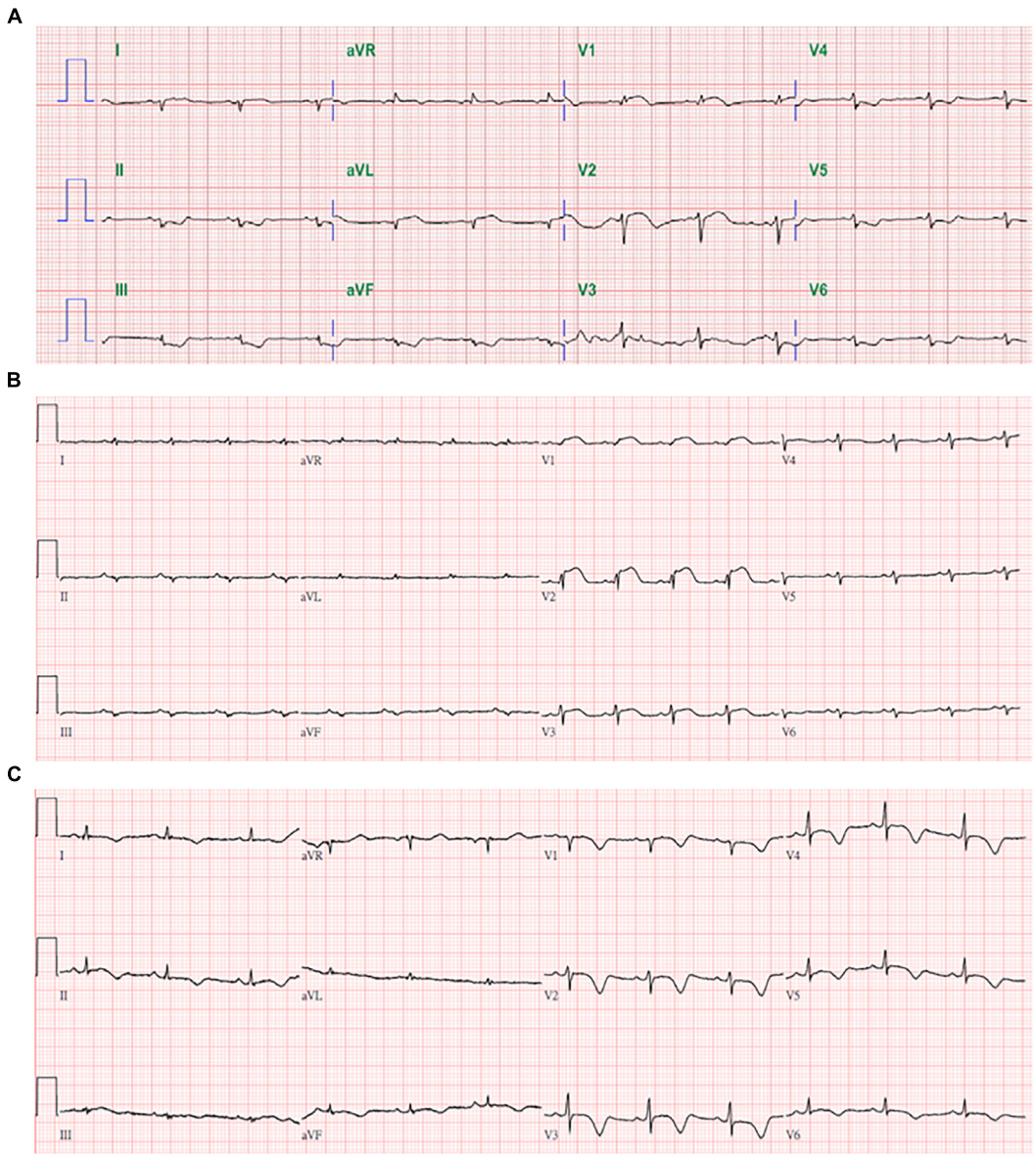

FIGURE 1 | Serial in-hospital ECGs on 8/11 **(A)** from other hospital, 8/13 **(B)**, and 8/24 **(C)**.

and adenovirus receptor (CAR)], induce direct myocardial injury, and trigger an uncontrolled immune response even after viral clearance (15). The genes of dsDNA adenovirus are classified into early genes (E 1–4) which encode proteins for DNA replication and late genes (L 1–5) which encode structural proteins. The viral vector of ChAdOx1 vaccine is a chimpanzee adenovirus (ChAd), which can evade pre-existing human immunity. The ChAd was vectorized by deleting E1/E3 and modifying E4 to reduce virulence and replication in human body (16). In an animal study on rhesus macaques, virus replication in the respiratory tract was limited after vaccination with ChAdOx1 (17). This may explain why a throat swab for adenoviral antigen was negative in our patient.

Another potential mechanism is the molecular similarity between SARS-CoV-2 spike protein and human antigens. Commercially available mouse monoclonal antibodies against SARS-CoV-2 spike protein have been shown to cross-react with some human protein sequences, including α-myosin and actin (18). Repeated antigen exposure may also trigger a dysregulated host response in certain individuals, resulting in polyclonal B-cell expansion, immune complex formation, and inflammation. Induction of anti-idiotype antibodies (antibody 2 against antibody 1) is another possible mechanism for myocarditis after SARS-CoV-2 infection or vaccination (19). Post-vaccination myocarditis bears some similarities to anti-idiotype antibody related myocarditis

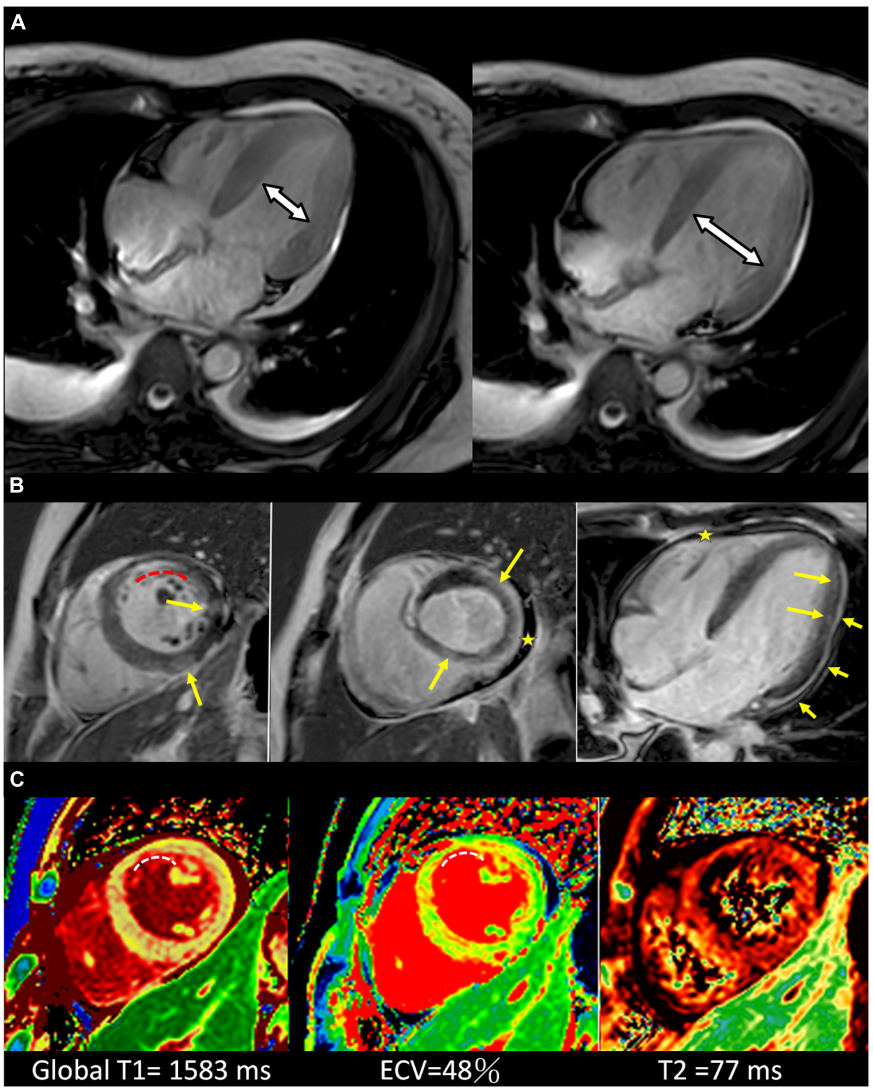

FIGURE 2 | Cardiac MRI of the patient. **(A)** Cardiac MR 4-chamber cine end-systolic (left) and end-diastolic (right) images show the limited LV dimensional change, indicative of the impaired LV systolic function. **(B)** Cardiac MR late Gadolinium enhancement images of short-axis (left, middle) and 4-chamber (right) view. Yellow arrows depict the patchy enhancements sparsely distributed in the mid-layer and subepicardium in a non-ischemic pattern, arrowheads depict pericardial enhancement and stars depict pericardial effusion. The curved dashed line depicts the subendocardial enhancement in the antero-septal subendocardium of LV mid-cavity which is within the LAD territory. **(C)** T1 map (left), ECV map (middle) and T2 map (right) in short-axis views show elevated T1, ECV, and T2 values, indicating acute myocardial injury (global T1 = 1,583 ms, ECV = 48%, T2 = 77 ms; institution-specific cut-off values for abnormal myocardium: T1 global $\geq$ 1,250 ms, T2 global $\geq$ 60 ms). CMR findings meet updated 2018 Lake Louise criteria for acute myocarditis (25). The curved dashed lines depict the focally elevated T1 and ECV values in the antero-septal subendocardium, equivalent to the enhanced area depicted in **(B)**.

after viral infections (20). These autoimmune hypotheses can explain the higher incidence of myocarditis after second dose comparing to first dose.

The cardiac MRI in our patient showed increased LV T1 and T2 signal values, indicating acute myocardial injury. Patchy enhancements in the mid-layer and subepicardium by LGE can be observed in the infarction-caused fibrosis and also myocardial damage/necrosis such as myocarditis. These changes are similar to the finding from other myocarditis cases after mRNA vaccination (9). An unusual finding is the enhancement in the antero-septal subendocardium of LV by LGE image, and the pattern is compatible with myocardial infarction with non-obstructive coronary arteries (MINOCA) (21). Common causes of MINOCA are coronary dissection, coronary artery or microvascular spasm, Takotsubo cardiomyopathy, and myocarditis (22). The MRI abnormalities may be related to the degree of myocardial damage, but cannot explain the etiology. Clinically, most cases of myocarditis following mRNA vaccination have been reported to be mild. In a report from Israel, 41 of the 54 cases were mild, one case received ECMO support, and one case died of unknown cause after discharge (1).

TABLE 1 | Diagnostic criteria for clinically suspected myocarditis from European society of cardiology working group on myocardial and pericardial diseases (11).

Clinical presentations
1. Acute chest pain, pericarditic, or pseudo-ischemic.
2. New-onset (days up to 3 months) or worsening of: dyspnea at rest or exercise, and/or fatigue, with or without left and/or right heart failure signs
3. Sub-acute/chronic (> 3 months) or worsening of: dyspnea at rest or exercise, and/or fatigue, with or without left and/or right heart failure signs
4.Palpitation, and/or unexplained arrhythmia symptoms and/or syncope, and/or aborted sudden cardiac death
5. Unexplained cardiogenic shock
Diagnostic criteria
1. ECG/Holter/stress test: Newly abnormal 12 lead ECG and/or Holter and/or stress testing, any of the following: I to III degree atrioventricular block, or bundle branch block, ST/T wave change (ST elevation or non ST elevation, T wave inversion), sinus arrest, ventricular tachycardia or fibrillation and asystole, atrial fibrillation, reduced R wave height, intraventricular conduction delay (widened QRS complex), abnormal Q waves, low voltage, frequent premature beats, supraventricular tachycardia.
2. Myocardiocytolysis markers: Elevated TnT/TnI
3. Functional and structural abnormalities on cardiac imaging (Echo/Angio/CMR): New, otherwise unexplained LV and/or RV structure and function abnormality (including incidental finding in apparently asymptomatic subjects): regional wall motion or global systolic or diastolic function abnormality, with or without ventricular dilatation, with or without increased wall thickness, with or without pericardial effusion, with or without endocavitary thrombi
4. Tissue characterization by CMR: Edema and/or LGE of classical myocarditic pattern
Clinically suspected myocarditis if ≥ 1 clinical presentation and ≥ 1 diagnostic criteria from different categories, in the absence of: (1) angiographically detectable coronary artery disease (coronarystenosis ≥ 50%); (2) known pre-existing cardiovascular disease or extra-cardiac causes that could explain the syndrome (e.g., valve disease, congenital heart disease, hyperthyroidism, etc.). Suspicion is higher with higher number of fulfilled criteria. *If the patient is asymptomatic ≥ 2 diagnostic criteria should be met.*

According to Australian Guidance on Myocarditis and Pericarditis after mRNA COVID-19 Vaccines (23), initial evaluation of post-vaccine myocarditis/pericarditis was similar to that of typical myocarditis, including history taking, 12-lead ECG, chest X-ray, and Troponin level. Suspected cases require referral to a cardiologist for further investigations including echocardiogram, coronary angiography, and cardiac MRI. Endomyocardial biopsy is rarely indicated, as determined by cardiologist. Often supportive treatment is all that is required. Another important issue is about the subsequent COVID-19 vaccines after post-vaccine myocarditis. According to a recent report about the risk of a second COVID-19 vaccine in 40 patients with VITT after first dose of ChAdOx1 nCoV-19 vaccine (5 patients received ChAdOx1 nCoV-19 again, 2 received mRNA-1273, and 33 received BNT162b2), none of the 40 patients had relapse of symptoms or severe adverse reactions (24). To date, there is no published report about the risk of subsequent vaccine on patients with post-vaccine myocarditis. The Canadian National Advisory Committee on Immunization recommends that individuals who had myocarditis/pericarditis after a first dose of mRNA vaccine should wait to receive a second dose until more information is available. In our case, the patient decided to postpone the schedule of second vaccine.

CONCLUSION

Acute pericarditis/myocarditis is a rare but existing side effect after mRNA COVID-19 vaccination, and the incidence is higher among young and adolescence males. Our report demonstrated the possibility of acute myocarditis after ChAdOx1 nCoV-19 vaccine, and the pathophysiology is different from VITT. The risk of post-vaccine myocarditis has affected the public policy in some countries. For example, Finland and Sweden have limited the use of mRNA-1273 vaccine in young people since October 2021. Although myocarditis is potentially lethal, benefits of COVID-19 vaccination (9) still far outweigh this uncommon side effect. Without appropriate evidences, policies about vaccine should be made carefully. Further information about the mechanism and long-term clinical outcome of post-vaccine myocarditis is needed for physicians to manage and give advice about subsequent vaccination on these affected individuals.

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

C-TW took care of the patient and wrote the report. S-CC performed cardiac MRI and provided the image. P-HC revised the report. 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.2022.856991/full#supplementary-material

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SYSTEMATIC REVIEW
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Quantifying the Excess Risk of Adverse COVID-19 Outcomes in Unvaccinated Individuals With Diabetes Mellitus, Hypertension, Ischaemic Heart Disease or Myocardial Injury: A Meta-Analysis

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Background: More than 80% of individuals in low and middle-income countries (LMICs) are unvaccinated against coronavirus disease 2019 (COVID-19). In contrast, the greatest burden of cardiovascular disease is seen in LMIC populations. Hypertension (HTN), diabetes mellitus (DM), ischaemic heart disease (IHD) and myocardial injury have been variably associated with adverse COVID-19 outcomes. A systematic comparison of their impact on specific COVID-19 outcomes is lacking. We quantified the impact of DM, HTN, IHD and myocardial injury on six adverse COVID-19 outcomes: death, acute respiratory distress syndrome (ARDS), invasive mechanical ventilation (IMV), admission to intensive care (ITUadm), acute kidney injury (AKI) and severe COVID-19 disease (SCov), in an unvaccinated population.

Methodology: We included studies published between 1st December 2019 and 16th July 2020 with extractable data on patients ≥18 years of age with suspected or confirmed SARS-CoV-2 infection. Odds ratios (OR) for the association between DM, HTN, IHD and myocardial injury with each of six COVID-19 outcomes were measured.

Results: We included 110 studies comprising 48,809 COVID-19 patients. Myocardial injury had the strongest association for all six adverse COVID-19 outcomes [death: OR 8.85 95% CI (8.08–9.68), ARDS: 5.70 (4.48–7.24), IMV: 3.42 (2.92–4.01), ITUadm: 4.85 (3.94–6.05), AKI: 10.49 (6.55–16.78), SCov: 5.10 (4.26–6.05)]. HTN and DM were also significantly associated with death, ARDS, ITUadm, AKI and SCov. There was substantial heterogeneity in the results, partly explained by differences in age, gender, geographical region and recruitment period.

Conclusion: COVID-19 patients with myocardial injury are at substantially greater risk of death, severe disease and other adverse outcomes. Weaker, yet significant associations are present in patients with HTN, DM and IHD. Quantifying these associations is

important for risk stratification, resource allocation and urgency in vaccinating these populations.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/, registration no: CRD42020201435 and CRD42020201443.

Keywords: COVID-19, cardiovascular risk factors, myocardial injury, ischaemic heart disease, diabetes, hypertension, adverse outcomes

INTRODUCTION

The global coronavirus disease 2019 (COVID-19) pandemic has impacted healthcare systems and economies worldwide. It has laid bare health inequalities and magnified unequal effects of public health measures implemented across the world, with associated ramifications on global health. This is well-exemplified by the global COVID-19 vaccine inequality, where only 14.4% of individuals in low-income countries have received one dose of COVID-19 vaccine, as of March 2022 (1).

Low and middle-income countries (LMICs) are plagued by the difficult decision between strict non-pharmaceutical interventions such as national lockdowns and their socioeconomic impact, particularly on the urban poor. Moreover, these countries suffer from increased COVID-19 associated mortality owing to insufficient healthcare resources and poorly funded emergency response programmes (2, 3).

While high-income countries (HICs) have made significant progress in vaccination rollout programmes, LMICs continue to lag behind. Importantly, studies from HIC populations have suggested the COVID-19 incidence and hospitalization rates among unvaccinated individuals are approximately 2 and 5-times that of vaccinated (but without a booster) individuals respectively (4).

Multiple observational studies have shown associations between cardiovascular (CV) risk factors such as hypertension (HTN), diabetes mellitus (DM), previous ischaemic heart disease (IHD), myocardial injury and outcomes such as mortality or severe disease due to COVID-19 (SCov). Other studies have challenged these findings, showing heterogeneous associations between these risk factors and COVID-19 related death (5, 6). In addition, there remains a lack of consensus on the impact of myocardial injury and CV risk factors on other important COVID-19 adverse outcomes such as acute respiratory distress syndrome (ARDS), invasive mechanical ventilation (IMV) and intensive care admission (ITUadm).

Therefore, quantifying the risk of COVID-19-related adverse outcomes attributable to CV risk factors, IHD and myocardial injury in unvaccinated persons is essential, not only for patient-specific care but also for risk stratification and planning of healthcare delivery in already stretched LMICs. This is especially relevant where the greatest burden of cardiovascular disease is amongst LMICs. In this meta-analysis, we quantify the association between CV risk factors, IHD and myocardial injury, and specific adverse clinical outcomes in unvaccinated adults with COVID-19 infection.

METHODS

The protocol for this meta-analysis with pre-specified aims and objectives was prospectively registered on PROSPERO (CRD42020201435 and CRD42020201443). The aim of the meta-analysis was to study the impact of pre-specified cardiovascular risk factors (HTN, DM, previous IHD, and presence of myocardial injury) on adverse COVID-19 outcomes [all-cause mortality, ARDS, IMV, admission to intensive care (ITUadm), AKI, and study-defined severe COVID-19 disease (SCoV)]. We included studies published (in print or pre-print version) during the early phase of the COVID-19 pandemic, prior to widespread use of COVID-19 vaccinations.

Population Selection

We included studies that reported prevalence of pre-existing cardiovascular risk factors in adult patients ($\geq$18 years of age) with suspected or confirmed COVID-19 disease and any one of the COVID-19 related adverse outcomes.

Search Strategy

We searched databases of published (MEDLINE, CINAHL, Embase, EMCARE, British Nursing Index) and pre-print (medRxiv) articles without language restrictions, between 1st December 2019 and 16th July 2020. We also searched data from the COVID-19 specific World Health Organization (WHO) global research database. Duplicate studies were identified and removed initially through a Mendeley folder (AT) followed by manual de-duplication by two authors working independently (JP and SMN). The final study list was agreed by consensus. Three authors (JP, KM, SMN) screened references of full-text studies, review articles and existing meta-analyses for additional studies. **Appendix A** gives the full search strategy.

Selection Criteria

Studies were included if they had extractable data on (i) patients $\geq$ 18 years of age with suspected or confirmed SARS-CoV-2 infection (COVID-19); (ii) pre-existing CV risk factors, specifically HTN and DM, IHD, or evidence of myocardial injury (defined as serum troponin level above the 99th percentile upper reference limit); and (iii) COVID-19 related outcomes, in particular all-cause mortality, ARDS, IMV, admission to intensive care (ITUadm), AKI, and study-defined severe COVID-19 disease (SCoV). Data from Chinese studies were translated and extracted by JP. Studies reporting only on special populations (e.g., dialysis patients, pregnant women, elderly patients or

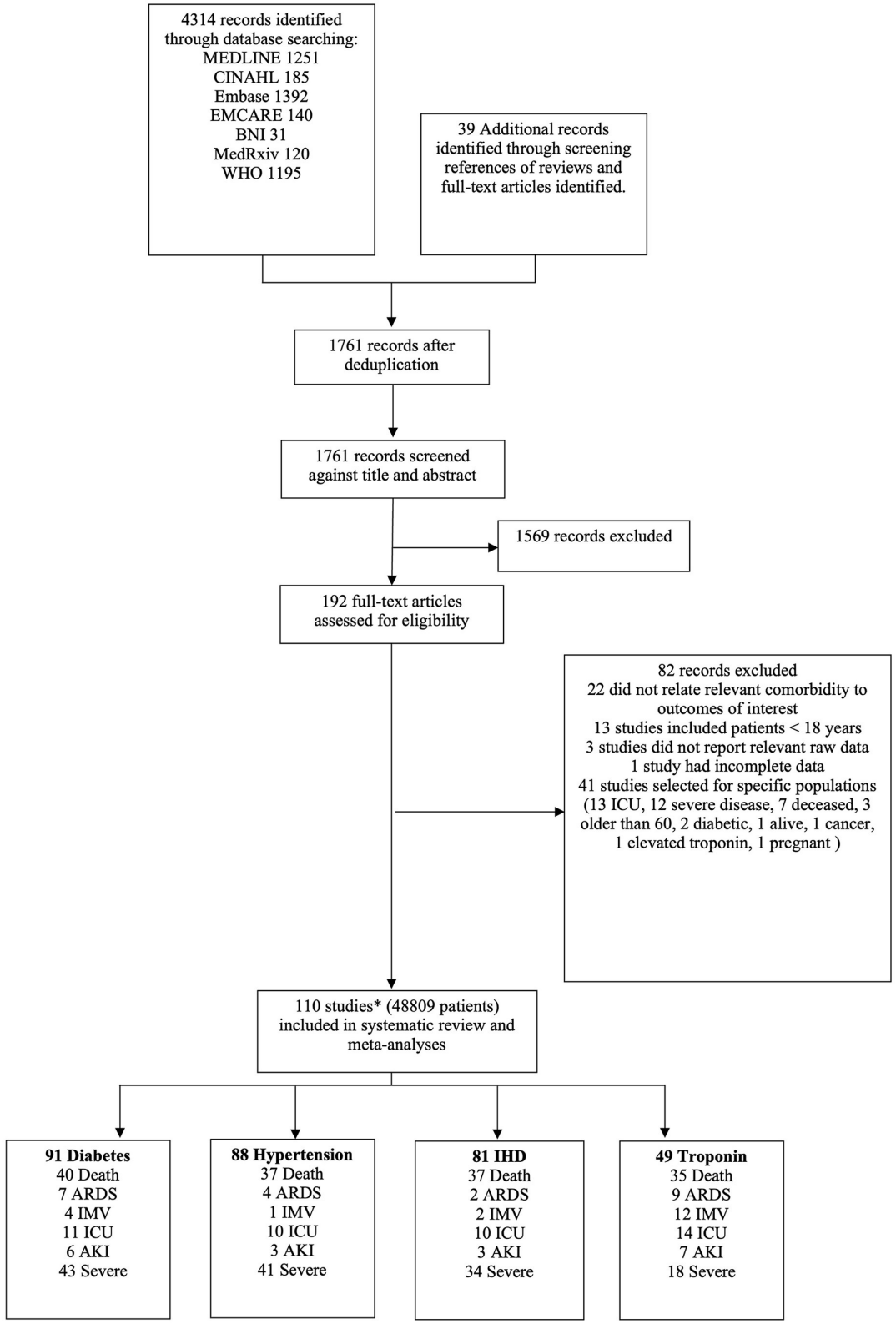

FIGURE 1 | PRISMA flow chart of study selection. *Three studies reported different outcomes on the same cohort. Therefore, 208 patients have been excluded from the total number of patients as they were considered to be a duplicate cohort.

children) were excluded (**Appendix C**). Case reports, case series, review articles and meta-analyses were also excluded. **Figure 1** shows the PRISMA flow chart for study selection.

Three authors (KM, JP, SMN) independently screened all titles and abstracts, reviewed full text articles, extracted data onto pre-specified forms and performed risk of bias assessments. Disagreements were resolved by consensus. Risk of bias assessment for individual studies was performed using the Newcastle-Ottawa Scale (NOS) (**Appendix D**). The quality of body of evidence for each outcome was assessed using Grading of Recommendations, Assessment, Development and Evaluation (GRADE) working group approach (**Appendix E**).

Statistical Analysis

Associations between disease status and COVID-19 outcomes were quantified using odds ratios (OR) which were combined using fixed-effects meta-analysis (7). Random-effects analysis was also performed as an alternative assessment of the impact of heterogeneity on the analyses. Results presented were derived from the fixed-effects model if no notable difference from the random-effects analysis was identified. Heterogeneity around the fixed-effects (inverse-variance weighted) average effect was assessed using the I^2 statistic (8) and where larger numbers of studies were present, fixed-effects meta-regression. Meta-regression was used to assess the effect-modification of disease status on COVID-19 outcomes by age, geographic region, date of last recruitment and proportion of male participants. In sensitivity analyses to address miscalibration of inference due to small sample sizes, we compared meta-analysis results to those with higher-order accuracy (9).

RESULTS

We identified 110 studies comprising 49,017 patients with COVID-19. The full list of included and excluded studies are detailed in **Appendices B, C**. As three studies reported on the same cohort of patients, we excluded 208 individuals from the meta-analysis, leaving 48,809 patients (**Figure 1**). The mean age was 56.7 years and 57% were male. A median of three risk factors and outcomes were reported per study. In total 20% had DM, 37% had HTN, 10% had IHD and 12% had evidence of myocardial injury. Death and severe COVID-19 disease were the commonest reported outcomes; overall, there were 7,150 deaths, 2,180 cases of ARDS, 3,162 individuals needing IMV, 2,950 admissions to intensive care, 3119 patients with AKI and 4,804 severe COVID-19 cases. **Table 1** summarizes the characteristics of included studies, categorized by risk factors.

Presence of Diabetes, Hypertension and Ischaemic Heart Disease and COVID-19 Outcomes

Table 2 summarizes the associations between the four studied risk factors and six outcomes of interest.

Presence of DM (40 studies, 18,979 patients, 3,791 with DM), HTN (37 studies, 17,995 patients, 6,695 with HTN) or IHD (37 studies, 19,968 patients, 2,619 with IHD) was strongly associated with death from COVID-19 [DM: OR 2.16 (95% confidence interval, CI: 1.97–2.36), HTN: 2.72 (2.51–2.97) and IHD: 3.29 (3.00–3.63), respectively]. These cardiovascular risk factors were also associated with severe COVID-19 disease (**Figure 2**).

Fewer studies explored the association between DM, HTN and IHD with ARDS, IMV, ITUadm and AKI. Nevertheless, we observed significant associations between these CV risk factors, IHD and ITUadm as well as AKI. While DM and HTN were significant risk factors for developing ARDS, a similar association was not observed in patients with pre-existing IHD [OR 1.42 (0.49–4.10), $p = 0.30$], though only 2 studies involving 310 patients (15 with IHD) were included in this meta-analysis. Only one study (393 patients) explored the association between HTN and IMV, finding no significant association [OR 1.25 (0.82–1.90)].

There was some discrepancy between the fixed-effects and random-effect analysis of the association between IHD and ITUadm. However, the odds ratio and confidence intervals from the fixed-effects model are entirely within the confidence intervals from the random-effect analysis. This would be expected when a random-effect analysis is simply a less efficient estimate of the same parameter or a numerically similar one estimated by the fixed-effects analysis.

Presence of Myocardial Injury and COVID-19 Outcomes

Forty-nine studies reported on the risks of death and other adverse outcomes associated with the presence of myocardial injury at baseline in patients with COVID-19. Presence of myocardial injury was associated with all six adverse COVID-19 outcomes (**Figure 2**). There was a near 9-fold increase in the risk of death [OR 8.85; (8.08–9.68)] amongst those with myocardial injury (vs. those without) in 35 studies including 21,707 patients, where 5,225 had myocardial injury and 2,197 patients died. In a meta-analysis of 7 studies comprising 1,777 patients, those with myocardial injury had more than 10-fold increased risk of AKI [OR 10.5; (6.55–16.8)]. Similarly, myocardial injury was also associated with ARDS [OR 5.70; (4.48–7.24)], IMV [3.42; (2.91–4.01)], ITUadm [4.85; (3.93–6.05)] and SCov [OR 5.10 (4.26–6.05)]. Apart from the association between myocardial injury and acute kidney injury, there was substantial heterogeneity in association between studies for all other outcomes (10).

The mean NOS score for all studies combined was 6.95, indicating that they were of satisfactory quality (**Appendix D**). Moderate to substantial heterogeneity was observed in the majority of meta-analyses. Only four meta-analyses had insignificant heterogeneity ($I^2 < 10\%$), all with small numbers of included studies.

We performed a meta-regression to assess the effect modification by age, gender, publication date and geographic region, on the association of cardiovascular risk factors and myocardial injury on death and severe COVID-19 disease. Overall, advanced age, studies conducted in Asia and male gender showed stronger association between cardiovascular risk factors, ischaemic heart disease and myocardial injury with

TABLE 1 | Characteristics of included studies reporting COVID-19 related outcomes, categorized by risk factors.

Outcome by risk factor	Number of studies	Total number of patients	N patients with risk factor* No. (%)	N patients with outcome** No. (%)	Mean age	Male no. (%)	Region of study	N patients with risk factor and outcome No. (%)	% of patients exposed to risk factor reaching outcome
Death									
Diabetes mellitus	40	18,979	3,791 (20)	3,194 (17)	60	61	24 Asia, 11 Europe, 5 USA	984 (5)	26
Hypertension	37	17,995	6,695 (37)	3,063 (17)	59.1	60	22 Asia, 10 Europe, 5 USA	1,698 (9)	25
Ischaemic heart disease	37	19,968	2,619 (13)	3,521 (18)	60.3	60	21 Asia, 11 Europe, 5 USA	928 (5)	35
Myocardial injury	35	21,707	5,225 (24)	3,259 (15)	58.2	54	26 Asia, 5 Europe, 4 USA	2,197 (10)	42
ARDS									
Diabetes mellitus	7	1,428	257 (18)	404 (28)	57	750 (53)	6 Asia, 1 Europe	112 (8)	44
Hypertension	4	476	154 (32)	172 (36)	56.5	287 (60)	3 Asia, 1 Europe	65 (14)	42
Ischaemic heart disease	2	310	15 (5)	137 (44)	53	187 (60)	2 Asia	8 (3)	53
Myocardial injury	9	2,189	584 (27)	615 (28)	57.8	1,128 (52)	6 Asia, 2 Europe, 1 USA	348 (16)	60
IMV									
Diabetes mellitus	4	1,345	275 (20)	214 (16)	58.7	1,376 (52)	3 Asia, 1 USA	65 (5)	24
Hypertension	1	393	197 (50)	130 (33)	61.5	238 (61)	1 USA	70 (18)	36
Ischaemic heart disease	2	8,831	777 (9)	689 (8)	59.6	4,782 (54)	2 USA	109 (1)	14
Myocardial injury	12	10,424	2,796 (26)	836 (8)	57.3	5,625 (54)	9 Asia, 1 Europe, 2 USA	553 (5)	20
ITU									
Diabetes mellitus	11	2,487	482 (19)	432 (17)	56.9	1,376 (55)	7 Asia, 3 Europe, 1 USA	133 (5)	28
Hypertension	10	1,891	761 (40)	394 (21)	57.2	1,089 (58)	6 Asia, 3 Europe, 1 USA	211 (11)	28
Ischaemic heart disease	10	1,891	259 (14)	394 (21)	57.2	1,089 (58)	6 Asia, 3 Europe, 1 USA	63 (3)	24
Myocardial injury	14	2,753	698 (25)	644 (23)	56	1,487 (54)	9 Asia, 4 Europe, 1 USA	309 (11)	44
AKI									
Diabetes mellitus	6	7,018	2,124 (30)	2,205 (31)	59.6	4,081 (58)	4 Asia, 2 USA	914 (13)	43
Hypertension	3	6,066	3,332 (55)	2,140 (35)	61.5	3,618 (60)	1 Asia, 2 USA	1,419 (23)	43

(Continued)

TABLE 1 | Continued

Outcome by risk factor	Number of studies	Total number of patients	N patients with risk factor* No. (%)	N patients with outcome** No. (%)	Mean age	Male No. (%)	Region of Study	N patients with risk factor and outcome No. (%)	% of patients exposed to risk factor reaching outcome
Ischaemic heart disease	3	6,066	673 (11)	2,140 (35)	61.5	3,618 (60)	1 Asia, 2 USA	318 (5)	47
Myocardial injury	7	1,777	410 (23)	153 (9)	57.4	914 (51)	5 Asia, 1 Europe, 1 USA	113 (6)	28
Severe disease									
Diabetes mellitus	43	11,495	2,171 (19)	3,444 (30)	52.3	6,215 (54)	41 Asia, 2 USA	959 (8)	44
Hypertension	41	10,653	3,774 (35)	3,206 (30)	50.8	5,800 (54)	39 Asia, 2 USA	1,602 (15)	42
Ischaemic heart disease	34	10,149	1,325 (13)	3,001 (30)	53.3	5,531 (54)	32 Asia, 2 USA	644 (6)	48
Myocardial injury	18	4,731	925 (20)	1,461 (31)	53.2	2,456 (52)	16 USA, 1 Europe, 1 USA	549 (12)	59

**Risk factors are defined as presence of diabetes, hypertension, ischaemic heart disease or myocardial injury. ARDS, acute respiratory distress syndrome; IMV, invasive mechanical ventilation; ITU, admission to intensive care; AKI, acute kidney injury. **Total number of patients with specific outcomes presented in this table include only studies which reported outcomes according to risk factors of interest. Reported outcomes that were not explicitly associated with risk factors are not included in this table. Therefore, these numbers may not reflect the total number of adverse COVID-19 outcomes in all included studies.*

TABLE 2 | Odds Ratio [Confidence Intervals] for COVID-19 adverse outcomes according to risk exposure.

	COVID-19 adverse outcome Odds ratio [95% confidence interval]																	
Risk exposure	**Death**	***p*-value**	I^2	**ARDS**	***p*-value**	I^2	**IMV**	***p*-value**	I^2	**ITU**	***p*-value**	I^2	**AKI**	***p*-value**	I^2	**Severe Disease**	**p-value**	I^2
Diabetes mellitus	2.15 [1.97, 2.36]	<0.001	68.6	2.48 [1.82, 3.32]	<0.001	43.9	1.77 [1.25, 2.51]	0.0014	66.3	1.65 [1.26, 2.16]	<0.001	60.1	1.84 [1.65, 2.05]	<0.001	0.0	1.80 [1.63, 1.99]	<0.001	48.0
Hypertension	2.72 [2.51, 2.97]	<0.001	67.8	1.68 [1.07, 2.63]	0.025	0.0	1.24 [0.82, 1.90]	0.30	N/A	1.55 [1.20, 1.99]	<0.001	66.3	1.90 [1.70, 2.12]	<0.001	88.5	2.14 [1.93, 2.34]	<0.001	73.4
Ischaemic heart disease	3.29 [3.00, 3.63]	<0.001	51.2	1.42 [0.49, 4.10]	0.49	7.1	1.99 [1.58, 2.48]	<0.001	0.0	1.51 [1.05, 2.16]	0.024	69.3	1.75 [1.49, 2.05]	<0.001	37.6	2.20 [1.93, 2.51]	<0.001	40.2
Myocardial injury	8.85 [8.08, 9.68]	<0.001	77.1	5.70 [4.48, 7.24]	<0.001	82.7	3.42 [2.92, 4.01]	<0.001	70.1	4.85 [3.94, 6.05]	<0.001	75.7	10.49 [6.55, 16.78]	<0.001	10.8	5.10 [4.26, 6.05]	<0.001	73.5

ARDS, acute respiratory distress syndrome; IMV, invasive mechanical ventilation; ITU, admission to intensive care; AKI, acute kidney injury.

the COVID-19 outcomes of interest. Gender did not appear to affect the association between myocardial injury and death whereas age did not modify the association between myocardial injury and COVID-19 disease severity. All four modifiers also partly accounted for the observed heterogeneity between studies. Importantly, the GRADE assessment shows low to moderate levels of certainty for associations studied (**Appendix E**). We did not identify apparent publication bias in our meta-analyses (**Appendix F**).

DISCUSSION

The co-existence of HTN, DM, IHD and/or myocardial injury among patients with COVID-19 has been considered to be a harbinger of adverse clinical outcomes. By undertaking this meta-analysis, we confirmed a significant association between four risk factors (HTN, DM, IHD and myocardial injury) and six important adverse COVID-19 outcomes: death, ARDS, IMV, ITUadm, AKI and SCoV. Furthermore, we demonstrated the differential impact of these risk factors on individual COVID-19 outcomes, with myocardial injury emerging as the most adverse indicator of all. These findings may be considered when risk-stratifying unvaccinated patients and unexposed individuals for potential of severe unfavorable outcomes of COVID-19, as well as prioritization of vaccination rollout programmes.

It is worth reviewing the pathogenesis of COVID-19 disease to better understand the detrimental effects of cardiovascular risk factors and myocardial injury on COVID-19 related outcomes. Entry of SARS-CoV-2 into host cells relies on the surface glycoprotein, spike (S) protein, which has a receptor-binding domain (RBD) mediating direct contact with angiotensin-converting enzyme 2 (ACE2). In addition, the betacoronavirus also contains an S1/S2 polybasic cleavage site that is cleaved by cellular transmembrane protease serine 2 (TMPRSS2) and cathepsin L, which further facilitate viral entry. Whilst the predominant tissue tropism of SARS-CoV-2 is that of the alveolar epithelial cells, ACE2 is widely expressed in other organs such as the gastrointestinal tract, myocardium, kidneys and vascular endothelial cells (11). The latter likely contributes to the extrapulmonary manifestations commonly seen in severe COVID-19 disease. Viral replication within alveolar pneumocytes results in the activation of immune cells and release of inflammatory cytokines resulting in a cytokine storm. This is further exacerbated by the downregulation of ACE2 on cell surface membranes, which have a lung-protective and anti-inflammatory effect via the PIP3/Akt signaling pathway. Overall, the propagation of pro-inflammatory cytokine release accelerates clinical deterioration resulting in severe respiratory complications such as ARDS and multiorgan dysfunction (12).

Cardiovascular Risk Factors, IHD and COVID-19 Outcomes

Pre-existing DM, HTN and IHD were associated with a higher risk of death and severe COVID-19 disease in our meta-analysis, each comprising data from more than 30 studies. These findings concur with pre-existing studies showing worse COVID-19 outcomes in patients with DM and HTN. We further quantified risks of developing specific COVID-19 adverse outcomes, namely death, ARDS, IMV, ITUadm, SCov and AKI in patients with these co-morbidities.

Whilst patients with DM were at an increased risk of developing all six adverse outcomes, the strongest association was seen between DM and the development of ARDS. The pathogenic mechanism underlying severe respiratory complications in patients with DM and COVID-19 is speculated to be due to alveolar-capillary microangiopathy and interstitial fibrosis, resulting from overactive pro-inflammatory pathways and vascular inflammation. A proposed key player to the ongoing inflammation and endothelial damage in DM is interleukin-6 (IL-6), a pro-inflammatory cytokine suggested as a severity predictor of lung disease in DM (13, 14). These pathophysiological changes have previously been associated with obstructive and restrictive lung pathology in patients with DM.

The development of a cytokine storm in patients with severe COVID-19 is well-described (15). Previous studies have found elevated levels of IL-6 in COVID-19 patients, which were independent predictors of COVID-19 disease severity (16). Thus, it is plausible that increased oxidative stress resulting from higher IL-6 levels can lead to rapid progression of microvascular and macrovascular complications in DM patients with pre-existing low-grade vascular inflammation, resulting in increased risk of ARDS and COVID-19 mortality in this cohort (17). This, in part, may account for the observed beneficial effects of IL-6 inhibitor, tocilizumab in hospitalized patients with COVID-19. One could extrapolate the increased odds of IMV and ITUadm in patients with DM and COVID-19 to be due to the requirement for respiratory support in context of ARDS. In addition, multi-organ dysfunction as a consequence of a cytokine storm in DM may contribute further to the need for organ support in intensive care units, especially considering the increased risk for myocardial injury and AKI in patients with DM and SARS-CoV-2 infection. The pre-existing low-grade inflammation coupled with dysregulated immunomodulation in DM patients raises the question of whether a lower threshold or earlier use of IL-6 inhibitors should be considered in this at-risk cohort.

Similarly, patients with pre-existing HTN are at increased odds of COVID-19 related death, SCov and AKI. This may reflect the interlink between SARS-CoV-2 cell entry via angiotensin converting enzyme 2 (ACE2) binding, the renin-angiotensin-aldosterone system (RAAS) and the ubiquity of ACE2 in multiple organs including the lungs, myocardium, kidneys and gastrointestinal tract. Chronic mechanical stress on the vascular wall as a result of increased intraluminal pressure in hypertension leads to endothelial dysfunction, release of reactive oxygen species and a pro-coagulant state. In conjunction with RAAS dysfunction following SARS-CoV-2 infection, this facilitates a pro-inflammatory state, cytokine release syndrome and progression to multi-organ involvement in COVID-19 resulting in more severe disease and adverse outcomes (18).

Whilst also at increased odds of respiratory complications such as ARDS and requirement for IMV, patients with HTN and COVID-19 appear to be at a lower risk of these complications when compared to DM patients. Here, it may be worth raising whether there is a protective role of regular antihypertensives such as ACE-inhibitors and angiotensin

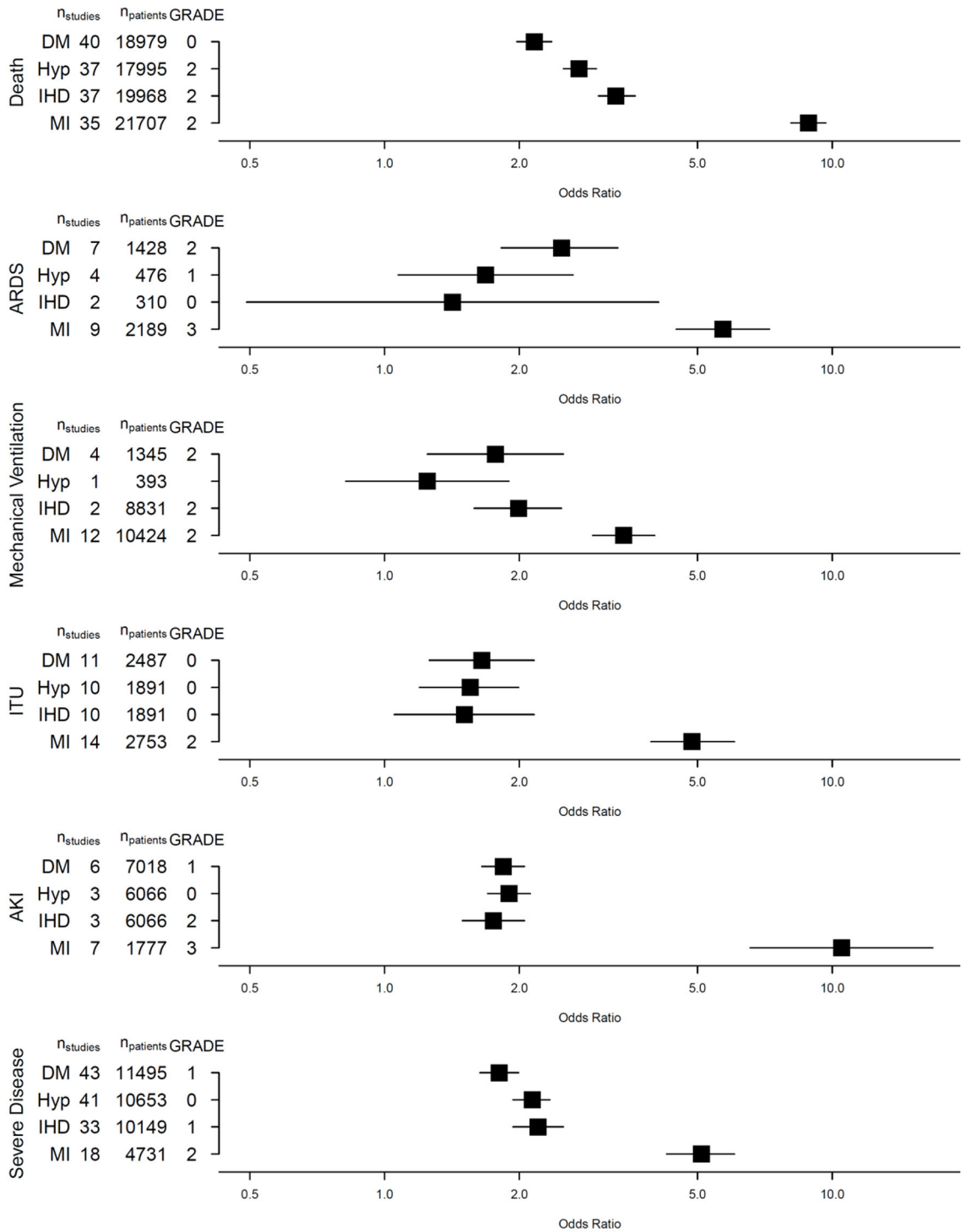

FIGURE 2 | Summary of evidence.

receptor blockers (ARB). The reduction in angiotensin-2 levels and suppression of angiotensin-2 binding to angiotensin-I receptors (AT1R) by ACE-inhibitors and ARBs, respectively, may in fact prevent the downstream pro-inflammatory and vasoconstrictive effects of angiotensin-2, lowering the risk of respiratory complications such as ARDS. Indeed, large cohort-based population studies have suggested a protective effect of ACE-inhibitors and ARBs (as compared to calcium channel blockers) against SCoV, death and IMV in patients with hypertension (19).

Another commonly raised question is whether optimal glycaemic control in DM or blood pressure management in HTN have a protective role against adverse COVID-19 outcomes. Certainly, infection with SARS-CoV-2 leads to dysregulated glucose metabolism that can result in elevated IL-6 levels as compared to normoglycaemic patients. Optimal glucose control significantly lowers levels of pro-inflammatory cytokines with improved outcomes in COVID-19 patients with or without diabetes (20). Further studies have also demonstrated adverse effects of hyperglycaemia on COVID-19 outcomes and reduction in the effectiveness of tocilizumab in patients with COVID-19 and hyperglycaemia (21). What remains unclear is whether prior glycaemic control in patients with known diabetes affect outcomes in COVID-19. Population-based studies in the UK have shown an increased risk of COVID-19 mortality with higher glycated hemoglobin (HbA1c) levels (22). This is in contrast to hospital-based cohort studies that have not demonstrated an association between prior glycaemic control with COVID-19-related mortality or invasive mechanical ventilation (23). In our opinion, the increased risk of COVID-19 adverse outcomes in patients with DM are likely reflective of the associated chronic end-organ microvascular and macrovascular complications exacerbated by acute infective sequelae such as increased insulin resistance and an exaggerated inflammatory response. The potential role of antidiabetic medications such as dipeptidylpeptidase-4 (DPP4) inhibitors and glucagon-like peptide 1 (GLP-1) analogs for optimal glycaemic control and their anti-inflammatory properties should be explored in both the acute and chronic stages of COVID-19 infection.

Whilst one may assume patients with poorly controlled blood pressure to be at increased risk of adverse COVID-19 outcomes, this was interestingly not the case in a large observational study of more than 45,000 symptomatic COVID-19 patients with hypertension. Sheppard et al. (24) observed that patients with recent uncontrolled blood pressure had lower odds of COVID-19 related mortality as compared to patients with well-controlled blood pressure. This may suggest a greater role of chronic hypertensive end-organ damage as risk factors for worse COVID-19 outcome.

Importantly, it is worth remembering that many patients have more than one of these studied risk factors. The combined effect of DM and HTN, as well as other components of the "metabolic syndrome" such as dyslipidaemia and obesity is likely greater than its individual components with regards to increased odds of adverse COVID-19 outcome, as demonstrated in specific meta-analyses studying the risk of metabolic syndrome on SCov and death (25). Other comorbidities beyond CV risk factors may play a significant role in adverse COVID-19 outcomes also, as suggested through a prior meta-analysis finding association between cerebrovascular disease and chronic liver disease with IMV in COVID-19 (26).

Myocardial Injury and COVID-19 Outcomes

Of the four studied risk predictors, myocardial injury had the strongest association with all six adverse COVID-19 outcomes. One could argue this may be a marker of multi-organ involvement and disease severity, rather than a direct pathogenic mechanism. Also, pre-existing cardiovascular diseases such as HTN and IHD increase odds of developing myocardial injury in context of COVID-19 disease (27). Autopsy studies have also suggested an upregulation of ACE2 expression in cardiomyocytes of patients with DM, increasing susceptibility to SARS-CoV-2 entry and myocardial injury in this patient cohort (28). As such, it is not be unreasonable to assume that myocardial injury, as indicated by raised serum troponin levels may simply be a surrogate to the increased odds of adverse COVID-19 outcomes in patients with DM, HTN and IHD. However, Shi et al. (29) and Chen et al. (30) have previously demonstrated that myocardial injury is an independent predictor of mortality in COVID-19. Subsequent multivariable analyses by Wang et al. (31) however showed that this association was only significant on univariate analysis. To date, it remains unclear whether myocardial injury represents a cause or consequence of severe COVID-19 disease.

In our study, we found that 67% (2,197/3,259) of the deceased patients had evidence of myocardial injury, which was associated with a near 9-fold increased odds of COVID-19 related death. Further, our study explores the association between myocardial injury and AKI, demonstrating patients with COVID-19 and myocardial injury are at 10-fold increased odds of developing AKI. 28% of patients with myocardial injury developed AKI as compared to 2.4% of patients without myocardial injury.

The exact mechanism linking myocardial injury with ARDS and AKI in COVID-19 infection is poorly understood. Several hypotheses include a bystander process with cytokine storm and hyperinflammation in severe COVID-19 disease as the driver of multi-organ involvement, endothelial damage and thrombo-inflammation secondary to ACE2-mediated entry of SARS-CoV-2 into endothelial cells of multiple vascular beds and right ventricular dysfunction secondary to increased afterload from raised pulmonary artery pressures in ARDS, thereby causing dysregulated renal blood flow (32, 33).

Strengths and Limitations

Our meta-analysis pools data from studies across the world and focuses on COVID-19 outcomes in the early phase of the pandemic, before effective evidence-based treatments and vaccines were commonplace. The geographical diversity of included studies overcomes concerns regarding generalisability of earlier meta-analyses, while avoiding biases introduced by novel therapies and vaccines in later stages of the pandemic. Our meta-analysis comprehensively explores the multiple associations between CV risk factors, IHD and myocardial injury with specific COVID-19 outcomes and complications such as AKI and ARDS alongside more commonly reported outcomes such as mortality and IMV.

The generalisability of findings from our meta-analysis was enhanced by not setting language restrictions in our search strategy and by including studies from conventional as well as novel databases (e.g., medRxiv and the WHO COVID-19 database). Moreover, risk of bias was minimized by excluding studies involving specific population cohorts or pre-selected COVID-19 outcomes. By undertaking meta-regression analyses, we were able to explore sources of heterogeneity including age, gender, study recruitment date and geographic region.

Our study has several limitations. Firstly, it is limited in its scope, focusing on outcomes of COVID-19 in a relatively unexposed population in the immediate months following its initial outbreak. Its applicability to populations with high vaccination or herd immunity rates may be limited. Indeed, the emergence of variants with significant mutations affecting virulence features such as transmissibility and pathogenesis may alter aspects of the natural history of COVID-19. At the same time, variants could affect effectiveness of current vaccines (34). In order to delineate the associations between cardiometabolic risk factors and myocardial injury with significant outcomes of COVID-19, we focused on the pre-vaccine phase of the pandemic when rates of adverse outcomes were high and bias arising from access to treatments and vaccines was comparably less pronounced. A necessary trade-off limiting broader applicability to vaccinated populations and potential future variants was accepted.

In addition, the majority of included studies explored adverse outcomes in hospitalized COVID-19 patients. This introduces an inherent selection bias as patients presenting with mild symptoms were likely excluded. Unvaccinated, non-hospitalized individuals with mild COVID-19 symptoms remain an important cohort to study, particularly with the changing trends of disease severity with different SARS-CoV-2 variants.

As our meta-analysis focused specifically on the pre-vaccination phase of the pandemic, we are unable to comment on the association between DM, HTN, IHD and myocardial injury with biomarkers of disease severity including coagulation indicators such as fibrinogen degradation products, prothrombin time, D-dimer and platelets, as these were infrequently reported in earlier studies included. Indeed, the pro-coagulation state in COVID-19 disease is now well-recognized and biomarkers such as D-dimer are used to guide routine use of anticoagulants in COVID-19 patients (35). Whilst findings from the REMAP-CAP trial have not shown a protective effect of antiplatelets in patients with critically-ill patients with COVID-19, it would be interesting to further stratify whether empirical antiplatelet and anticoagulant therapy in patients with different risk profiles (e.g., DM vs. non-DM) will improve overall outcomes (36).

The inclusion of studies conducted at different centers across geographical regions also results in considerable between-study heterogeneity. This could be due to multiple reasons including different sociodemographic factors, varying definitions of outcomes (e.g., ARDS, severe COVID-19 disease) and variations in clinical practice between the countries (we therefore assessed for the effect of geographical variation through meta-regression). For outcomes with fewer studies (ARDS, IMV and AKI), the level of certainty of evidence is low owing to the small study size and wide confidence intervals, limiting the veracity of these findings.

Notwithstanding the above limitations, our meta-analysis quantifies the excess risk of adverse COVID-19 outcomes in unvaccinated patients with pre-existing CV risk factors, IHD and myocardial injury. In addition, we demonstrate the differential impact of these factors on six important adverse COVID-19 outcomes. Our findings will help inform clinicians, policymakers and patients in terms of risk prediction, stratification and resource allocation. Our meta-analysis contributes to the expanding body of evidence reporting on risk factors for poor COVID-19 outcomes, which could inform public health advice regarding social isolation guidelines and vaccine prioritization strategies, especially relevant for LMICs.

Future studies evaluating the altered impact of cardiovascular risk factors such as HTN, DM and IHD on COVID-19 outcomes in a post-vaccination population are required. In addition, the immunogenicity of different COVID-19 vaccines in these patient groups remain poorly elucidated and should be better characterized to guide design of vaccination programmes and choice of vaccine.

CONCLUSION

In summary, our meta-analysis demonstrates a significant association between myocardial injury and adverse clinical outcomes in COVID-19 patients. To a lesser extent, we also found that DM, HTN and IHD predict poorer outcomes, especially for death and severe disease. These findings provide comprehensive quantitative data that can be used in risk prediction and risk stratification by clinicians as well as policymakers. It also provides the underpinning evidence for the vaccination policies targeting vulnerable 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.

AUTHOR CONTRIBUTIONS

SN, SK, VK, and AG designed the study. SN, JP, and KM identified studies and extracted data. KR performed the statistical analysis. SN, JP, KM, SK, VK, KR, and AG 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.2022.871151/full#supplementary-material

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ECG Utilization Patterns of Patients With Arrhythmias During COVID-19 Epidemic and Post-SARS-CoV-2 Eras in Shanghai, China

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Background: The COVID-19 pandemic has led to concerns around its subsequent impact on global health.

Objective: To investigate the health-seeking behavior, reflected by ECG utilization patterns, of patients with non-COVID-19 diseases during and after COVID-19 epidemic.

Methods: Taking advantage of the remote ECG system covering 278 medical institutions throughout Shanghai, the numbers of medical visits with ECG examinations during the lockdown (between January 23 and April 7, 2020), post-lockdown (between April 8 and December 31, 2020) and post-SARS-CoV-2 (between January 23 and April 7, 2021) periods were analyzed and compared against those during the same periods of the preceding years (2018 and 2019).

Results: Compared with the same period during pre-COVID years, the number of medical visits decreased during the lockdown (a 38% reduction), followed by a rebound post-lockdown (a 17% increase) and a fall to the baseline level in post-SARS-CoV-2 period. The number of new COVID-19 cases announced on a given day significantly correlated negatively with the numbers of medical visits during the following 7 days. Medical visit dynamics differed for various arrhythmias. Whereas medical visits for sinus bradycardia exhibited a typical decrease-rebound-fallback pattern, medical visits for atrial fibrillation did not fall during the lockdown but did exhibit a subsequent increase during the post-lockdown period. By comparison, the volume for ventricular tachycardia remained constant throughout this entire period.

Conclusion: The ECG utilization patterns of patients with arrhythmias exhibited a decrease-rebound-fallback pattern following the COVID-19 lockdowns. Medical visits for diseases with more severe symptoms were less influenced by the lockdowns, showing a resilient demand for healthcare.

Keywords: epidemic, COVID-19, health-seeking behavior, arrhythmias, atrial fibrillation

INTRODUCTION

The Coronavirus disease 2019 (COVID-19) is a highly contagious viral infection and has spread around the world by multiple transmission modes. (1) The outbreak of COVID-19, caused by SARS-CoV-2, led to an unprecedented global public health crisis, and resulted in a large death-toll and long-term side effects for the survivors of the disease. The Chinese government imposed lockdown measures in Wuhan, the epicenter of the outbreak, from January 23 to April 7, 2020, to fight against SARS-CoV-2 in China. Since March 11, 2020, the daily number of new confirmed cases of COVID-19 has decreased significantly in China. (2) With the epidemic under control, China's prevention and control strategy were gradually adjusted to facilitate the recovery of normal economic production and life in China (3–5).

Not only did patients with COVID-19 suffer serious health damage during epidemic, but the shortage of medical resources and the strict preventive measures necessary to deal with the outbreak have also posed challenges to the routine management of non-COVID-19 diseases such as cardiovascular diseases (6–8), which has been reported in a Danish Nationwide Cohort Study regarding all-cause mortality and location of death in patients with established cardiovascular disease in COVID-19 epidemic. (9) Previous studies, mostly based on large medical centers (10), showed that the number of hospital visits decreased during the lockdowns, but research on the health-seeking behavior of populations at large have been less reported. One study from Israel showed a decrease in hospital admissions for myocardial infarction during the early stage of the pandemic, as well as a rebounding increase as the first wave of the pandemic faded (11). This research focused, however, on one specific disease and does not reflect the health-seeking dynamics of other cardiovascular diseases. The current evidence focusing on the association between COVID-19 and cardiovascular diseases is based on small, disease-specific studies and lacks quantitative backing, which highlights the importance of our study regarding the health-seeking dynamics associated with various cardiovascular diseases during and following the epidemic.

As a widely adopted routine examination characterized by its easy access and low cost, electrocardiograms (ECG) act as the diagnostic method for various types of cardiac arrhythmias. Therefore, changes to the number of medical visits with ECG examinations might reflect changes in the medical-seeking behavior of patients with cardiac arrhythmias. Taking advantage of the largest remote ECG platform in China, we were able to glimpse how COVID-19 affected the health-seeking behavior of patients with various arrhythmias during and after the epidemic.

METHODS

Study Population

This study was approved by the ethics committee at Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. All data used for this research were derived from the Siwei (Shanghai Siwei Medical, Shanghai, China) remote ECG diagnosis system. (12) As the largest ECG diagnostic system in China, the platform collected ECG data from 1,320 medical institutions across 13 provinces in China, with a volume of more than one million medical visits involving ECGs per year (**Figure 1A**). As approximately 85% of this ECG data was collected from Shanghai (**Supplementary Table 1**), which covered 278 medical institutions from almost all administrative districts (**Figure 1B** and **Supplementary Table 2**), the present study analyzes ECG data in Shanghai between January 1, 2018 and April 7, 2021.

Research Design

The numbers of medical visits with ECG examinations (ECG visits) during the lockdown (between January 23 and April 7, 2020), post-lockdown (between April 8 and December 31, 2020) and post-SARS-CoV-2 (between January 23 and April 7, 2021) periods were compared with those of the same periods during the two years prior to the COVID-19 outbreak (averages taken from 2018 and 2019). Subgroup analyses were then performed according to sex (male or female), age ($\leq$ 59 years old, 60–79 years old or $\geq$ 80 years old) and the tier of medical institution (academic hospitals or community clinics).

The number of new COVID-19 cases, new deaths, new discharged cases and existing confirmed cases in the Chinese mainland were then analyzed based on daily updates from China's Center of Disease Control (CDC) between January 11, 2020, and April 7, 2021. (2, 13) Spearman's rank correlation was analyzed for the COVID-19 data and the number of medical visits concerning various arrhythmias.

ECG-Based Diagnoses of Arrhythmias

ECG diagnoses, derived from a combination of artificial intelligence (AI) assistance and clinician diagnosis (details in **Supplementary Methods**), were adopted for the following diseases: sinus bradycardia, sinus tachycardia, atrial extrasystole, atrial tachycardia, atrial flutter, atrial fibrillation, ventricular extrasystole, ventricular tachycardia, paroxysmal supraventricular tachycardia(SVT), first-degree AV block (AVB), severe AVB (including second-degree type 2, high-degree and third-degree AVB), right bundle branch block (RBBB), left bundle branch block (LBBB) and left anterior fascicular block. Other abnormal ECG readings, such as myocardial infarction and ST segment depression, were not analyzed specifically for the inaccuracy of diagnosis due to lack of sufficient medical information.

Statistical Analysis

The numbers of daily medical visits involving ECG examinations were regarded as continuous variables, and the Mann-Whitney non-parametric test was used to compare the changes in the number of ECG visits between the different time periods or subgroups. Chi-square tests were used to compare the differences in the proportions of various arrhythmias between academic hospitals and community clinics. Spearman correlation analyses were used to reveal the correlations between the number of COVID-19 cases and the number of medical visits on following days. Statistical analyses were performed using IBM SPSS Statistics 24 (SPSS Inc., Chicago, IL). A two-sided p value of < 0.05 was considered significant.

RESULTS

Medical Visits During the Pre-COVID-19 Years (2018 and 2019)

The number of medical visits with ECG examinations remained stable during the two consecutive years preceding the COVID-19 pandemic. ECG visits in Shanghai were 696,800 and 702,989 in 2018 and 2019, respectively. Averages from 2018 and 2019 were taken as a pre-COVID baseline for subsequent comparison in this study.

The number of the ECG examinations were lower in the Jan-Feb and higher in May and June (**Figure 2**), different from the common sense that the cardiac disease patients are more prevalent in the Winter, the underlying reason remains unclear, and we checked the data from previous years. This pattern could also be observed in 2016, 2017, 2018, and 2019 (**Supplementary Figure 1**), future analysis is needed to clarify this issue.

Medical Visits During and After the Epidemic

Table 1 shows that the number of medical visits with ECG examinations decreased from 86,232 to 53,246 (a 38% reduction) during the lockdowns, after which it then increased from 591,661 to 657,774 (an 11% increase) during the post-lockdown period. Finally, this figure returned to its baseline level during the post-SARS-CoV-2 period (87,178 compared to the baseline of 86,232). When compared with the same periods during pre-COVID years, there were fewer monthly medical visits between February and June 2020, an equal number in July 2020, and higher-than-baseline levels between August and December 2020 (**Figure 2** and **Supplementary Tables 3, 4**). In addition, the yearly peak in the volume of medical visits occurred in May or June between 2016 and 2019, while in 2020, this peak was postponed to July (**Supplementary Figure 1**).

Relationship Between Medical Visits in Shanghai and Prevalence of COVID-19 in China

We analyzed the relationship between the number of new confirmed cases per day during the lockdowns in Chinese mainland and the number of medical visits in Shanghai. During the lockdowns, the number of new confirmed cases per day was negatively correlated with the number of medical visits during the following three days ($r = -0.765$, $p < 0.001$), seven days ($r = -0.873$, $p < 0.001$), 14 days ($r = -0.804$, $p < 0.001$), 21 days ($r = -0.693$, $p < 0.001$), 28 days ($r = -0.615$, $p < 0.001$), 35 days ($r = -0.544$, $p < 0.001$) and 42 days ($r = -0.506$, $p < 0.001$) in Shanghai (**Figure 3**, **Supplementary Figure 2** and **Supplementary Table 5**). This correlation coefficient exhibited a U-shaped curve, with a nadir at 7 days. Similarly, negative correlations with a U-shaped correlation coefficient curve were detected between the number of new COVID-related deaths in China and the number of medical visits in Shanghai during the lockdowns (**Figure 3**, **Supplementary Figure 2** and **Supplementary Table 5**). Such a correlation was not detected between the number of new discharged cases or existing confirmed cases and medical visits in Shanghai during the lockdown (**Supplementary Table 6**). No correlations between medical visit in Shanghai and COVID-19 prevalence in China were detected during the post-lockdown and post-SARS-CoV-2 periods (**Supplementary Table 5** and **Supplementary Figure 3**).

We further analyzed the relationship between medical visits in Shanghai and COVID-19 prevalence before and after the Spring Festival to exclude the impact of migration during the Spring Festival (January 23 and February 2, 2020). A similar negative correlation was observed even after factoring in for the Spring Festival migration (**Supplementary Table 7**).

Subgroup Analyses of Medical Visits in Shanghai

The impact of COVID-19 on health-seeking behavior related to cardiac arrhythmias varied by tier of medical institution (**Figure 4**). Compared with the same period during pre-COVID years, the number of medical visits to academic hospitals did not decrease during the lockdowns but increased during the post-lockdown (a 58% increase) and post-SARS-CoV-2 periods (a 30% increase). ECG visits to community clinics, however, decreased drastically during the lockdowns (a 51% reduction) followed by rebounding growth post-lockdowns and a subsequent recovery to the baseline level during the post-SARS-CoV-2 period.

In addition, medical visits from different sexes and above the age of 60 years generally followed the same decrease-rebound-fallback pattern, which was most prominent in patients between 60 and 79 years old. Medical visits by patients under 60 years of age, however, remained at a relatively constant level throughout the entire period (**Table 1**, **Supplementary Tables 3**, **4** and **Supplementary Figures 4**, **5**).

Similar to the results for the entire population, subgroup analyses based on age, sex and tier of medical institution revealed negative correlations between daily new cases or deaths with medical visits during the following 21 days during the lockdowns but not during the post-lockdown and post-SARS-CoV-2 periods (**Supplementary Tables 5**, **6**).

Disease-Specific Medical Visits During and Following the Epidemic

The number of medical visits were further analyzed according to different arrhythmias during the lockdown, post-lockdown, and post-SARS-CoV-2 periods. During the lockdowns, the number of medical visits related to arrhythmias with severe symptoms, such as atrial flutter ($p = 0.230$), atrial fibrillation ($p = 0.172$) and severe AVB ($p = 0.816$) were comparatively similar with those from the same period during the pre-COVID years, revealing a high degree of rigidity in health seeking demand. By comparison, medical visits for diseases with little to no symptoms, such as sinus bradycardia ($p = 0.002$), ventricular extrasystole ($p = 0.004$) and RBBB ($p = 0.001$) significantly fell during the lockdown period. No matter the diagnosis, the number of medical visits exceeded baseline levels during the post-lockdown period, followed by a gradual fall to baseline levels during the post-SARS-CoV-2 period. Medical

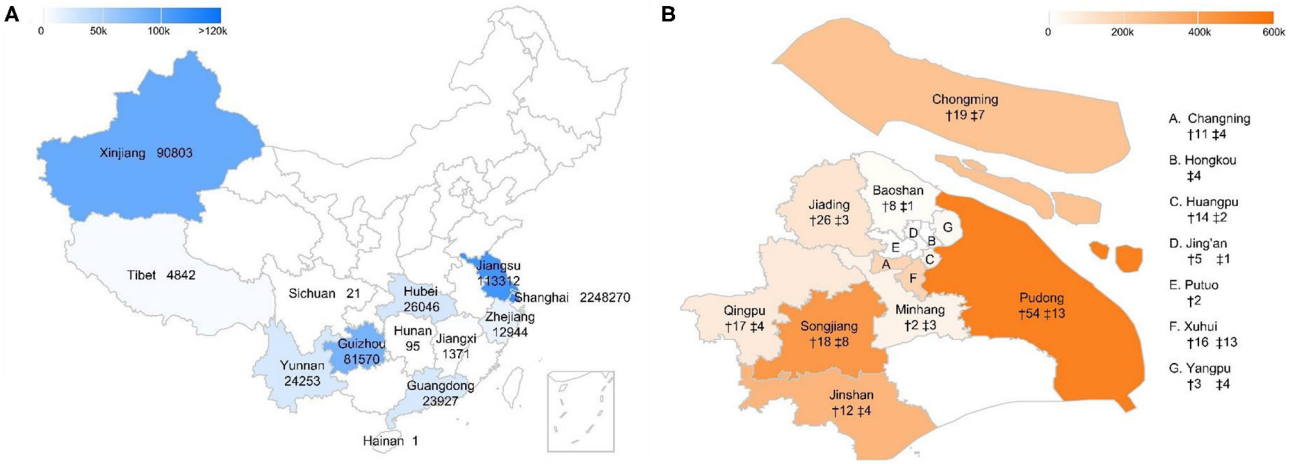


FIGURE 1 | Medical institutions covered by the ECG platform in China and Shanghai. **(A)** Regions covered by the remote ECG platform in China. The number following the province name shows the number of medical visits with ECG examinations in that province between Jan 1, 2018 and Apr 7, 2021. **(B)** Number of medical institutions covered by the ECG platform in Shanghai. † and ‡ indicate the number of community clinics and academic hospitals in that district.

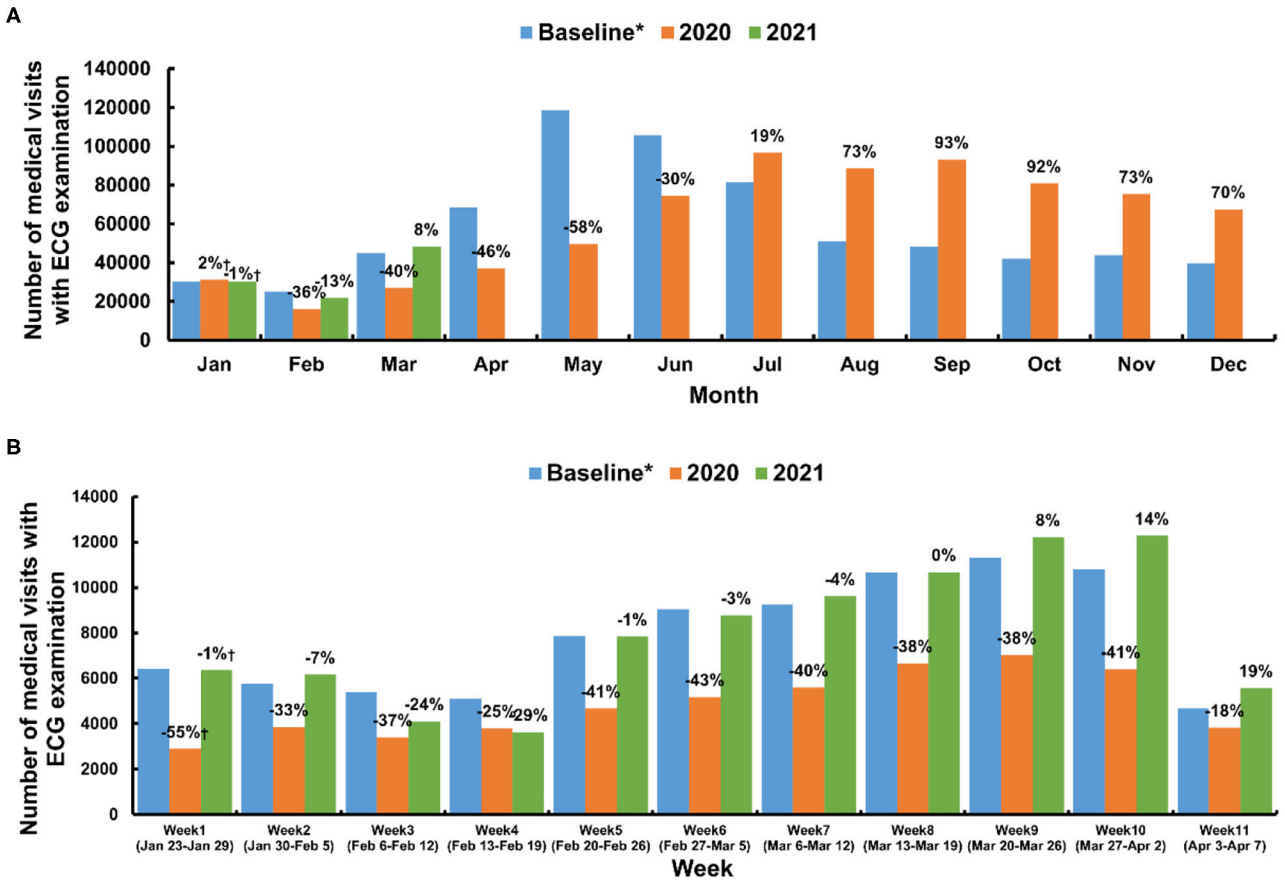


FIGURE 2 | Medical visits with ECG examination in Shanghai. **(A)** Monthly medical visits with ECG examinations of the baseline, 2020, and 2021. **(B)** Weekly medical visits during the lockdown (between January 23 and April 7, 2020) and the same period from the preceding years (baseline) and 2021. *Average number taken from 2018 and 2019.† The percentage difference compared with the baseline.

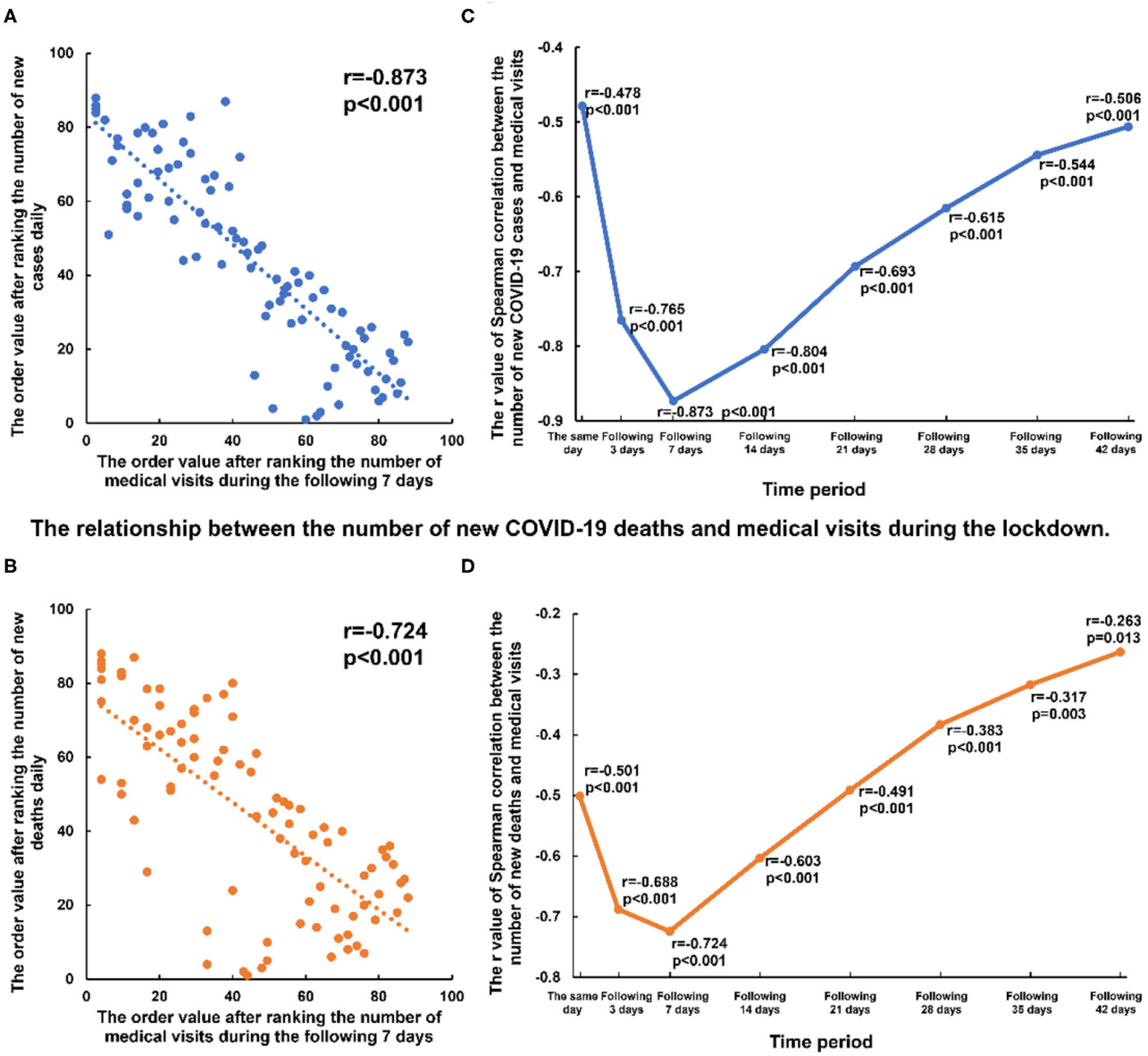


FIGURE 3 | Correlation between the number of new COVID-19 cases or deaths and medical visits of the following days during the lockdown. **(A,B)** the negative correlation between the new COVID-19 cases or deaths and the number of medical visits with ECG examination of the following 7 days during the lockdown. **(C,D)** the correlation coefficient (r) between the new COVID-19 cases or deaths and the number of medical visits of the same day and the following 3–42 days.

visits for ventricular tachycardia remained at a relatively constant level throughout the entire period analyzed (**Figure 5**, **Table 2**, **Supplementary Table 8** and **Supplementary Figures 6**, 7).

During the lockdowns, there were statistically significant but weak correlations or no correlations between new COVID-19 cases and medical visits related to diseases with severe symptoms, such as severe AVB ($r = -0.495$, $p < 0.001$) and ventricular tachycardia ($r = -0.055$, $p = 0.614$). Negative correlations became prominent in diseases with mild symptoms, such as ventricular extrasystole ($r = -0.816$, $p < 0.001$) and sinus bradycardia ($r = -0.877$, $p < 0.001$). But these correlations disappeared during the post-lockdown and post-SARS-CoV-2 periods (**Supplementary Table 9** and **Supplementary Figure 8**).

DISCUSSION

Based on a large volume of ECG data, we investigated the impact of the COVID-19 epidemic on the health-seeking behavior of patients with cardiac arrhythmias in Shanghai, China. The main findings of this study are as follows. First, the health-seeking behavior of patients with suspected cardiac arrhythmias, reflected by medical visits with ECG examinations, exhibited a decrease-rebound-fallback pattern during the period

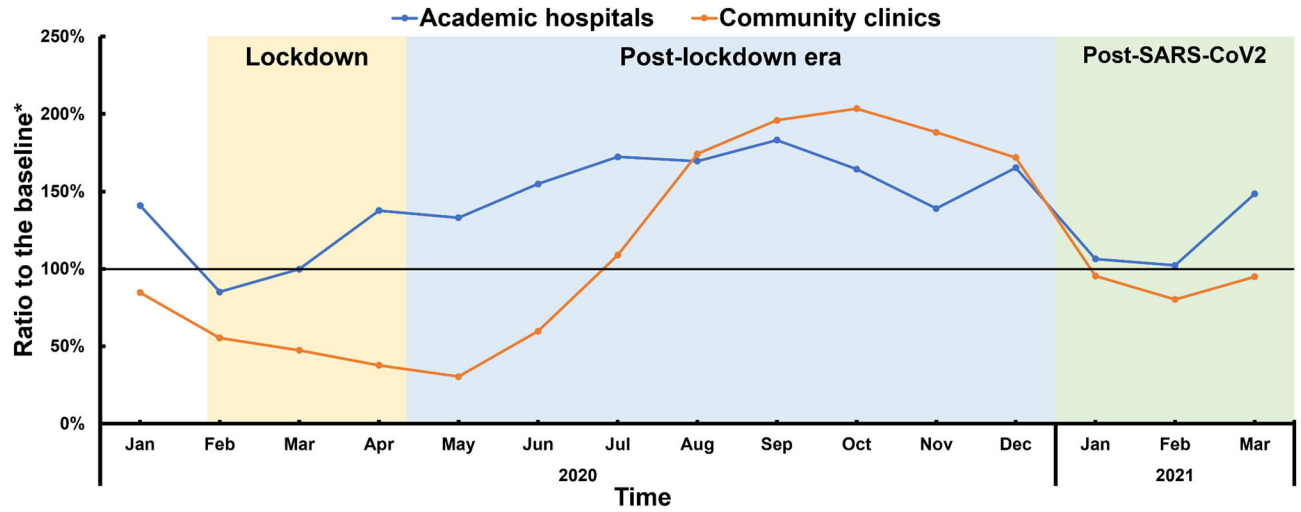
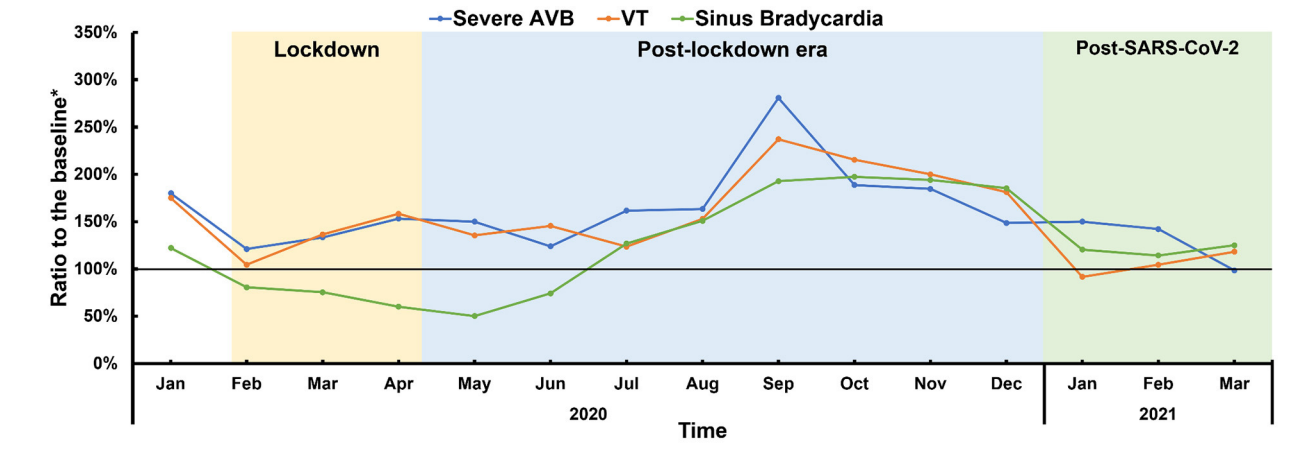


FIGURE 4 | Medical visits to academic hospitals and community clinics. *Ratio to the average number of medical visits between 2018 and 2019.



p value†	2020 vs. baseline												2021 vs baseline					
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Lockdown	Post-lockdown	Post-SARS-CoV-2
Severe AVB	0.52	0.63	0.57	0.25	0.25	0.39	0.19	0.037	0.00094	0.032	0.035	0.48	0.15	0.53	0.90	0.82	<0.0001	0.68
VT	0.35	0.53	0.55	0.71	0.93	0.38	0.56	0.68	0.21	0.31	0.19	0.20	0.44	0.69	0.73	0.31	0.047	0.88
Sinus bradycardia	0.00011	0.085	0.0036	0.00057	<0.0001	0.0050	0.021	0.00040	<0.0001	0.0011	<0.0001	<0.0001	0.043	0.39	0.027	0.0021	0.00030	0.094

FIGURE 5 | Different patterns of dynamics in disease-specific medical visits, represented by severe AVB, VT and sinus bradycardia. Three patterns of disease-specific medical visits dynamics during and following the pandemic were noticed, according to the ratio to the medical visits of the pre-COVID period. Pattern 1 (stable-increase-stable pattern) is represented by severe AVB. Pattern 2 (stable-stable-stable) is represented by ventricular tachycardia. Pattern 3 (decrease-rebound-fallback) is represented by sinus bradycardia. *Baseline was determined by the average number from 2018 and 2019. †The comparisons were obtained by a Mann-Whitney test. AVB, atrioventricular block; VT, ventricular tachycardia. The severe AVB includes second-degree type 2, high-degree and third-degree AVB.

starting from the COVID-19 lockdowns. This decrease in visits during the lockdown period was largely attributed to reduced patient volume at community clinics, whereas visits to academic hospitals were less affected. Second, a negative correlation was found between the number of new COVID-19 cases or deaths and medical visits on the same day as well as during the following six weeks during the lockdowns; these correlations were most prominent within the seven days after the report of new COVID-19 cases or deaths. Third, the impact of COVID-19 on health-seeking behavior varied with the types of the arrhythmias, and the medical demand for arrhythmias with potentially severe symptoms was less affected by the epidemic.

Decrease-Rebound-Fallback Pattern Following COVID-19 Lockdowns

During the initial phase of the COVID-19 outbreak, in the absence of vaccines or effective treatment protocols, self-isolation, as a standard quarantine measure, proved to be the most effective non-medical means to stop the spread of the virus. As a surging number of COVID-19 patients overwhelmed

TABLE 1 | Dynamics of medical visits of subgroups during different periods.

Groups	All year round			Lockdown			Post-lockdown			Post-SARS-CoV-2	
	Baseline *, n	2020, n	Percentage change †	Baseline *, n	2020, n	Percentage change †	Baseline *, n	2020, n	Percentage change †	2021, n	Percentage change †
Groups											
Males	321669	331239	3%	38834	23305	−40%	273130	295456	8%	40413	4%
Females	378226	407128	8%	47398	29941	−37%	318531	362318	14%	46765	−1%
≤59y	206975	214259	4%	32354	19285	−40%	165738	184151	11%	33855	5%
60-79y	389963	420944	8%	38971	23503	−40%	341967	386286	13%	40660	4%
≥80y	102957	103164	0%	14907	10458	−30%	83957	87337	4%	12663	−15%
Academic hospitals	139083	205164	48%	23273	22231	−4%	108788	171500	58%	30157	30%
Community clinics	560812	533203	−5%	62959	31015	−51%	482874	486274	1%	57021	−9%
Total	699895	738367	5%	86232	53246	−38%	591661	657774	11%	87178	1%

*Average number of 2018 and 2019.
†Compared with baseline.

medical resources during the early stage of the epidemic, the Chinese government called for non-COVID-19 patients with mild symptoms to stay at home to reserve the capacity of medical institutions for patients with COVID-19 or for patients with severe diseases and symptoms. Medical institutions also provided online consultations with doctors to help patients identify urgent situations.

Similar to the findings of previous studies in China and other countries (10, 14–18) medical visits in Shanghai, an area outside the epidemic's epicenter, also decreased by approximately 40% during the Wuhan lockdown period. But the suppressed health-seeking demand from non-COVID-19 diseases was released after the lockdown period, forming a drastic post-lockdown surge. This fall-rebound pattern was also reported by a study from Israel in that noted a decrease in hospital admissions for myocardial infarction was observed during the early stage of the epidemic, as well as a rebounding increase upon the receding end of the first wave of the epidemic (11) Another study from Korea also showed that the number of outpatient visits in internal medicine decreased during the COVID-19 pandemic and tended to rebound during the second half of the year (19) Subsequently, medical visits gradually fell back to the baseline level of prior years, reflecting the normalization of health-seeking demand during the post-SARS-CoV-2 period in China.

New cases and deaths reported daily exerted a negative influence on the health-seeking behavior of patients over the following 6 weeks, and this trend was especially strong the first week after the report. It is also of note that this negative correlation between reported new cases or deaths with medical visits was detected based on new cases or deaths reported for the whole of China rather than just Shanghai locally (**Supplementary Table 10** and **Supplementary Figure 9**), which reflects the impact of uniform policy on the behavior pattern of patients across China. As new cases per day dropped into single digits and as Wuhan lifted outbound travel restrictions, the correlation between COVID-19 cases and medical visits weakened or even disappeared, reflecting a shift of public focus and a return to normal daily life.

In addition, the general decrease in medical visits during the lockdowns was largely attributed to the decrease in visits to community clinics rather than to academic hospitals. Considering the hierarchical medical system in China, community clinics are mainly responsible for the long-term management of chronic diseases with mild symptoms, whereas patients with critical conditions and severe symptoms are usually treated at academic hospitals. This hierarchical separation of functions at different medical institutions was supported by our data regarding medical visits concerning cardiac arrhythmias during the pre-COVID-19 years (**Supplementary Table 11**). Such health-seeking preferences regarding academic hospitals and community clinics were amplified during the COVID-19 epidemic, suggesting that urgent conditions drove patients to admit themselves to academic hospitals regardless of the suggestions to isolate at home; medical visits by arrhythmic patients with mild symptoms or chronic conditions, however, largely decreased due to concerns over nosocomial infection by COVID-19.

TABLE 2 | Dynamics of medical visits of various ECG events during different periods.

Category of ECG events	All year round	Lockdown	Post-lockdown	Post-SARS-CoV-2							
	Baseline *, n	2020, n	Percentage change †	Baseline *, n	2020, n	Percentage change †	Baseline *, n	2020, n	Percentage change †	2021, n	Percentage change †
Normal ECG	310113	317929	3%	37617	20207	-46%	263332	286963	9%	37689	2%
Sinus bradycardia	64264	69573	8%	6393	4876	−24%	58051	64796	12%	7872	23%
Sinus tachycardia	28500	29462	3%	7075	5866	−17%	20126	22821	13%	5870	−17%
Atrial extrasystole	44217	45725	3%	6822	5167	−24%	36416	39526	9%	6249	−8%
Atrial tachycardia	3574	3840	7%	681	567	−17%	2779	3130	13%	570	−16%
Atrial flutter	1527	2204	44%	279	354	27%	1193	1802	51%	303	9%
Atrial fibrillation	20387	22060	8%	3466	3367	−3%	16334	18374	12%	3093	−11%
Ventricular extrasystole	26625	28982	9%	4371	3652	−16%	21667	24812	15%	4297	−2%
Ventricular tachycardia	141	206	47%	26	32	23%	110	168	53%	31	19%
Paroxysmal SVT	1168	1391	19%	203	231	14%	940	1167	24%	216	7%
First-degree AVB	25299	29582	17%	3188	2924	−8%	21664	26068	20%	3707	16%
Severe AVB	348	495	42%	61	78	28%	282	413	47%	72	18%
RBBB	36882	40705	10%	4890	4023	−18%	31337	35997	15%	5181	6%
LBBB	3309	3810	15%	476	427	−10%	2800	3324	19%	486	2%
Left anterior fascicular block	6863	6452	−6%	1000	692	−31%	5741	5656	−1%	904	−10%

**Average number of 2018 and 2019.*

† Compared with baseline.

SVT, supraventricular tachycardia; atrioventricular junctional tachycardia; AVB, atrioventricular block; RBBB, right bundle branch block; LBBB, left bundle branch block.

The severe AVB includes second-degree type 2, high-degree and third-degree AVB.

Behavioral Differences Exhibited Among Various Arrhythmias

Health-seeking behavior differed among various arrhythmias regarding the number of medical visits during and following the COVID-19 epidemic. Three patterns were detected. Pattern 1 was named the "stable-increase-stable" pattern and is represented by diseases such as severe AVB and atrial fibrillation (**Supplementary Figure 6**). The number of such medical visits remained stable during the lockdowns, followed by a post-lockdown increase and a return to baseline levels during the post-SARS-CoV-2 period. No prominent cosine-like curve in medical visits was observed. Patients with diseases conforming to Pattern 1 often manifested with severe symptoms and urgent conditions, and they tended to seek health services regardless of their concerns about nosocomial infection with COVID-19, thus reflecting a rigid medical demand. Pattern 2 was named the "stable-stable-stable" pattern and is represented by VT. This pattern corresponded to a relatively constant level of medical visits during and following the epidemic. As the ECG diagnostic system did not differentiate between non-sustained VT as short as three beats and sustained VT, which can cause hemodynamic disorders, it is possible that this "stable" level represents the mixed effects of situations of different clinical severities. Pattern 3 was named the "decrease-rebound-fallback" pattern, i.e., a cosine-like curve, and is represented by sinus bradycardia and the conditions in **Supplementary Figure 7**. In this pattern, patients with arrhythmias exhibiting mild symptoms tended to follow home isolation suggestions during the lockdowns. Medical demand, however, was not suppressed after the lockdown, as a rebound in medical visits occurred. As COVID-19 was further controlled in China, health-seeking behavior returned to the rational levels of prior years. Taken together, these health-seeking behaviors during and following the epidemic were not uniform for different diseases. A rigid medical demand for some diseases, such as severe AVB and VT, was revealed by unchanging or increased medical visits during the epidemic. Our results thus highlight the importance of medical institutions coping with non-COVID-19 -but nonetheless severe - diseases during the epidemic, as well as the importance of preparing for a surge in medical visits for various arrhythmias during the post-SARS-CoV-2 period.

Implications

China's anti-COVID-19 measures included city-wide lockdowns, transportation freezes or controls in hard-hit areas, the timely release of COVID-19 information, the prevention of social gatherings and infections, thorough community screening, the quarantining of suspected individuals, the early admission and treatment of confirmed cases, extensive epidemiological investigations and a tremendous number of other efforts aimed at controlling the epidemic, such as the manufacture of sufficient medical products and vaccine research and development (20–25) As COVID-19 exerted negative effects on non-COVID diseases (7, 10) the early control of COVID-19 was also of great significance in improving the prognoses of patients with cardiac arrhythmias.

Considering that COVID-19 is still prevalent around the world and that China is now facing a new wave of Omicron variants recently, the results of our research may have important implications for China and other countries in planning the allocation of medical resources during these new epidemic and post-SARS-CoV-2 periods. Our results show that the number of medical visits will exhibit a post-SARS-CoV-2 surge that might last for nearly half a year. As an economically developed area in China, Shanghai properly handled the prior post-SARS-CoV-2 surge in medical demand. There might be an imbalance between medical supply and demand, however, during another post-SARS-CoV-2 period in less-developed regions and rural areas with fewer medical resources. Given the rapidly expanding vaccination process, the COVID-19 pandemic is expected to be taken under control around the world in the near future. Governments and medical institutions should pay great attention to preemptively coping with post-COVID surges in medical demand among patients with cardiac arrhythmias. In addition, this might also be true for patients with other diseases.

Limitations

As the corresponding clinical, laboratory and imaging data were lacking, diagnoses made by ECG alone will result in a certain number of misdiagnoses and missed diagnoses, as well as conditions beyond arrhythmias. However, the diagnoses of cardiac arrhythmias highly relied on ECG. Second, a 30-second ECG is not able to discriminate between subtypes or between the severity of symptoms of specific arrhythmias, such as non-sustained and sustained VT, and atrial fibrillation with a fast or normal range of ventricular rate. Sinus bradycardia in this system follows the ECG criteria in which the sinus rate must be slower than 60 beats per minute. But according to new clinical guidelines, sinus bradycardia is now defined by a heart rate of $<$50 beats per minute (26) Also, ECG examination could be avoided to prevent contact-related infection after COVID-19, especially during the lockdown periods, which might be more prominent in community clinics and in patients with mild symptoms. These factors might bias the findings of our study.

CONCLUSIONS

The number of medical visits related to cardiac arrhythmias exhibited a decrease-rebound-fallback pattern during the period starting from the COVID-19 lockdown in Shanghai. During this lockdown period, the severity of the epidemic, reflected by daily new cases or deaths, exerted a negative 6-week impact on the patients' behaviors in their seeking of medical services, and this impact was most prominent during the week following the daily report of new cases or deaths. Medical visits for arrhythmias with potentially severe symptoms, such as severe AVB and VT, were not negatively affected by the epidemic, reflecting the rigid medical demand of these 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.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CL, MuC, and ML reviewed literature, analyzed the data, drafted, revised the manuscript, and designed or coded the figures and tables. HWa and XH collected the data, conducted a literature review, and interpreted the data. YZhu, BZ, and XQ developed and maintained the platform for the remote ECG diagnosis system. QW, JS, and MY critically revised the diagnosis of ECG for further analyses. MiC, XL, YZha, MS, and JH provided critical feedback on data sources. LL and PL provided guidance and support for statistical methods. HWu provided support for the development of the ECG platform. YG-L designed the study, acquired funding and managed the project. All authors had final responsibility for the decision to submit this paper 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.2022.829679/full#supplementary-material

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Reduction of Cardiac Autonomic Modulation and Increased Sympathetic Activity by Heart Rate Variability in Patients With Long COVID

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Although several clinical manifestations of persistent long coronavirus disease (COVID-19) have been documented, their effects on the cardiovascular and autonomic nervous system over the long term remain unclear. Thus, we examined the presence of alterations in cardiac autonomic functioning in individuals with long-term manifestations. The study was conducted from October 2020 to May 2021, and an autonomic assessment was performed to collect heart rate data for the heart rate variability (HRV) analysis. The study participants were divided into the long COVID clinical group, the intragroup, which included patients who were hospitalized, and those who were not hospitalized and were symptomatic for different periods (≤3, >3, ≤6, and >6 months), with and without dyspnoea. The control group, the intergroup, comprised of COVID-free individuals. Our results demonstrated that the long COVID clinical group showed reduced HRV compared with the COVID-19-uninfected control group. Patients aged 23–59 years developed COVID symptoms within 30 days after infection, whose diagnosis was confirmed by serologic or reverse-transcription polymerase chain reaction (swab) tests, were included in the study. A total of 155 patients with long COVID [95 women (61.29%), mean age 43.88 ± 10.88 years and 60 men (38.71%), mean age 43.93 ± 10.11 years] and 94 controls [61 women (64.89%), mean age 40.83 ± 6.31 and 33 men (35.11%), mean age 40.69 ± 6.35 years] were included. The intragroup and intergroup comparisons revealed a reduction in global HRV, increased sympathetic modulation influence, and a decrease in parasympathetic modulation in long COVID. The intragroup showed normal sympathovagal balance, while the intergroup showed

reduced sympathovagal balance. Our findings indicate that long COVID leads to sympathetic excitation influence and parasympathetic reduction. The excitation can increase the heart rate and blood pressure and predispose to cardiovascular complications. Short-term HRV analysis showed good reproducibility to verify the cardiac autonomic involvement.

Keywords: autonomic nervous system, coronavirus infection, long COVID, heart rate, heart rate variability

INTRODUCTION

Coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), manifests numerous clinical symptoms, ranging from mild to severe (1). In Brazil, 21,478,546 confirmed cases, 598,152 accumulated deaths, and 20,462,345 recovered cases were reported as of October 4, 2021. In the state of Pará, 591,872 COVID-19 cases and 16,667 deaths were registered (2).

Some patients who do recover present with symptoms that persist longer than 3–4 weeks. According to Sher (3), this post-COVID condition may be called "post-COVID syndrome," "long COVID," or "post-acute COVID-19." The persistent symptoms of patients with long-term COVID-19 include dyspnoea, fatigue, myalgia, and joint pain (3, 4). The cardiovascular effects of prolonged COVID-19 are still under debate but they may include the lack of clinical symptoms, biomarker (high-sensitivity cardiac troponin I) abnormalities, or an increased risk of myocarditis. Coronavirus infection the potential to affect the cardiovascular system. SARS-CoV-2 is not considered a cardiotropic virus, although the virus causes non-specific cytokine-mediated cardiotoxicity (5, 6). Long COVID is found to be associated with autonomic dysfunction due to neurotropism because the systemic inflammatory state can occur acutely or chronically for up to 1 year (7). Dysautonomia can thus be assessed based on heart rate variability (HRV). A reduction in HRV is a predictor of cerebrovascular and cardiovascular events and an indicator of the risk of death (8). HRV as a non-invasive index of autonomic control may reflect both sympathetic and parasympathetic effects. The HRV indicates the variations in the duration of cardiac cycles and the RR intervals. HRV can be analyzed using linear algorithms for the time and frequency domains and also by non-linear analyses (9, 10).

Changes in cardiac autonomic modulation can occur in patients with COVID-19 and, through HRV, detect autonomic dysregulation. Patients with COVID-19 with low HRV are indicated for intensive care unit admission in the first week after hospitalization, regardless of age and chronic heart disease status (11). In this context, the aim of this study was to investigate the autonomic changes in the heart among patients with long COVID.

METHODS

Study Design and Ethics

This observational, analytical, controlled, quantitative, and descriptive study was approved by the Research Ethics Committee of the Pará State University (approval number 4.252.664). Participants consented to be included in the study by signing an informed consent form; the study was performed following the Strengthening the Reporting of Observational Studies in Epidemiology guidelines for observational studies and in accordance with the principles of the Declaration of Helsinki.

The patients were followed up at the laboratory of infectious and cardiopulmonary diseases at UEPA. The study participants were divided into a long COVID clinical group (intragroup), which comprised both patients who were hospitalized and those who were not hospitalized but were symptomatic for $\leq$3, >3, $\leq$6, and >6 months, with and without dyspnoea, as well as a control group (intergroup), which comprised COVID-free individuals. As it was a cardiopulmonary program, patients with dyspnoea and respiratory symptoms such as shortness of breath were required to adhere to the program. The following patients were included in the study: those aged between 23 and 59 years, those who underwent assessment 30 days after diagnostic confirmation and onset of COVID-19 symptoms, and those whose diagnosis was confirmed by reverse-transcriptase polymerase chain reaction (PCR) or serology tests to identify the type of antibodies [immunoglobulin (Ig) M and/or IgG]. Patients who used medication that altered the HRV (such as beta-blockers, beta-mimetics, and theophylline), those who developed chronic obstructive pulmonary disease, those who had persistent lung changes, those who showed persistent desaturation, those with anemia, and those who used pacemakers were excluded. Patients with long COVID underwent regular medical examinations.

From October 2020 to May 2021, 4,100 patients with complaints of long-term symptoms (like fatigue, breathlessness, cough, joint pain, chest pain, muscle aches, and headaches) that could not be attributed to any other cause were enrolled in the clinic's database. However, only 155 patients met the inclusion criteria.

Assessment of HRV

Study participants in both groups were instructed to refrain from consuming caffeine or caffeine derivatives, smoking, and eating heavy meals at least 24 h prior to the test. In preparation for the examination, the volunteers rested for 15 min, while the patients were placed in a supine position for 10 min to measure the heart rate (HR). The patients were instructed to avoid talking or moving to prevent interference during the test. The environment temperature was maintained between 22 and 24°C, while the air humidity was maintained between 40 and 60%. The temperature

and relative humidity were measured using a thermo-hygrometer (São Paulo, Brazil).

The Heart Rate (HR) was recorded using a Polar® RS800CX (Kempele, Finland) that captured the R wave on the electrocardiogram with a sampling rate of 500 Hz. The temporal distance between two consecutive peaks of the R wave was considered the iRR (the fluctuations in the intervals between consecutive heartbeats). The data series displayed on the monitor were exported using the Polar Pro Trainer 5 software (Polar Electro Ou, Finland). Subsequently, linear and nonlinear analyses were performed using the Kubios HRV version 3.1 software (Kuopio, Finland). Variability analysis was performed within a short period of time (12) to visually check the distribution of the iRRs for erroneous and absent R waves to determine the stretch with greater stability within 5 min for 256 consecutive beats. Then, the data collected during the first 30 s and final 30 s were discarded (10).

Linear Analysis

In the linear analysis of the HRV in the time domain, the following indices were included: mean iRRs, standard deviation of all normal RR intervals (SDNN), and the square root of the mean square of the differences between adjacent normal iRRs within an interval (RMSSD). SDNN indicates the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) activities, whereas RMSSD indicates the PNS (13) activity. To obtain the indices in the frequency domain, a fast Fourier transform analysis was performed and showed the components with high frequency (HF = 0.15–0.4 Hz), low frequency (LF = 0.04–0.15 Hz), and very low frequency (VLF = 0.003–0.04 Hz), as indicated in equations 1 and 2 (13, 14).

The three frequency bands (HF, LF, and VLF) were expressed in powers (ms^2) and in normalized units (n.u), which is the relative power of the LF or HF band after subtracting the VLF power from the total power. The LF/HF ratio and LF and HF bands were also obtained (15). LF and HF in normalized units represent the balance between the two autonomic nervous systems. LF demonstrates indirect sympathetic activity, while HF demonstrates the parasympathetic influence. The LF/HF ratio was reported as a marker of sympathovagal balance (10).

$$HF\ (n.u) = 100\ x\ \frac{\text{HF}}{(\text{full power} - \text{VLF})} \quad (1)$$

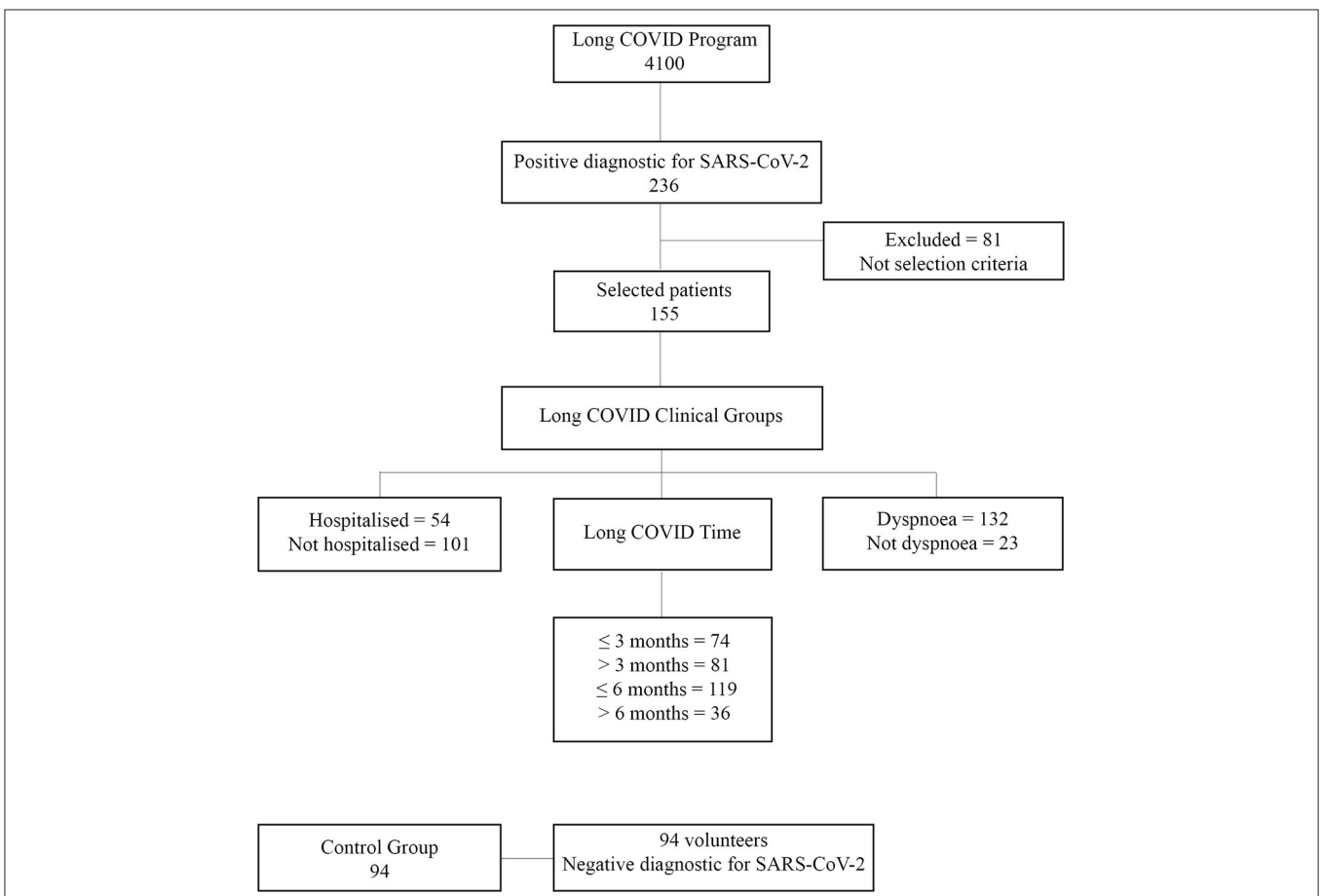

FIGURE 1 | Flowchart of selection and recruitment of patients with long COVID. SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; COVID, coronavirus disease.

$$LF\ (n.u) = 100\,x\,\frac{\text{LF}}{(\text{full power} - \text{VLF})}. \quad (2)$$

Nonlinear Analysis

For the nonlinear analysis, geometric methods, such as the Lorenz plot or Poincaré plot, were used to obtain the HRV measurements. These were performed by measuring the dispersions of interval RRs which analyse the HRV quantitatively, by calculating the beat-to-beat standard deviation (SD). With this, the short term changes in RRs in the PNS index of the sinoatrial node control (SD1) and the long-term standard deviation of continuous iRRs (SD2), which are influenced by the PNS and SNS were measured, and the relationship between the short- term and the long-term intervals was defined as SD1/SD2 (15, 16).

In addition, other nonlinear methods based on approximate entropy (ApEn) show the degree of irregularity and complexity of the signal as the iRRs and the complexity increase (16, 17). Meanwhile, simple entropy (SampEn) shows the regularity of the selected iRR series; higher values indicate a healthy condition, while lower values indicate heart failure (10, 18).

Statistical Analysis

The information collected was stored in MS Excel 2010™ (Washington, United States) and analyzed using GraphPad Prism version 5.0™ (San Diego, United States). D'Agostino's test was used to assess the normality of the distribution to compare the measured values between the different study groups. The Student's *t*-test was used for variables with a normal distribution, whereas the Mann–Whitney *U* test was used for non-normally distributed variables. For dichotomous or nominal variables, Fisher's exact test was used. A two-tailed *P*-value of <0.05 was considered significant.

RESULTS

General Characteristics of the Study Patients

After screening 236 patients, 155 were included in the study, as shown in **Figure 1**. The demographic characteristics and comorbidities of the 155 patients with long COVID symptoms are listed in **Table 1**. Most participants were women with a mean age of over 40 years; dyspnoea was one of the most prevalent symptoms of long COVID, accounting for more than 30% of hospitalized cases.

TABLE 1 | Demographic characteristics and comorbidities of the study population and symptoms of long COVID.

Variables	Patients ($n = 155$)	Group control ($n = 94$)
Female, No (%)	95 (61.29%)	61 (64.89%)
Male, No (%)	60 (38.71%)	33 (35.11%)
Age (years), mean ± SD	43.88 ± 10.03	40.69 ± 6.35
Height (cm), mean ± SD	1.63 ± 0.08	1.64 ± 0.08
Weight (kg), mean ± SD	79.95 ± 17.84	73.73 ± 15.43
BMI, mean ± SD	30.06 ± 7.31	27.04 ± 4.30
Smoker (No, %)	3 (1.93%)	N/A
Former smoker (No, %)	28 (18.06%)	94 (100%)
Long COVID symptoms (No, %)		
Dyspnoea, No (%)	132 (85.16%)	N/A
Chest pain, No (%)	93 (60%)	N/A
Muscle weakness, No (%)	112 (72.25%)	N/A
Fatigue, No (%)	118 (76.12%)	N/A
Myalgia, No (%)	103 (66.45%)	N/A
Insomnia, No (%)	87 (56.12%)	N/A
Lower members Oedema, No (%)	58 (37.41%)	N/A
Comorbidities (No, %)		N/A
Asthma (No, %)	24 (15.48%)	N/A
DM (No, %)	13 (8.38%)	N/A
SAH (No, %)	34 (21.93%)	N/A
Obesity (No, %)	72 (46.45%)	28 (29.78%)
Hospital admission (*n*, %)	54 (34.83%)	
Length of hospital stay, mean ± SD	17.25 ± 15.96	N/A
≤10 days, (*n*, %)	19 (35.18%)	N/A
>10 days, (*n*, %)	35 (64.82%)	N/A
Long COVID period, mean ± SD		N/A
≤3 months, (*n*, %)	74 (47.74%)	N/A
>3 months, (*n*, %)	81 (52.26%)	N/A
≤6 months, (*n*, %)	119 (76.77%)	N/A
>6 months, (*n*, %)	36 (23.23%)	N/A
Dyspnoea	132 (85.16%)	N/A
Not dyspnoea	23 (14.84%)	N/A

BMI, body mass index; DM, diabetes mellitus; SAH, systemic arterial hypertension; SD, standard deviation.

Intragroup Comparison

Intragroup comparisons were performed between the long COVID clinical groups. The mean age of the groups was >40 years, with >40% of them admitted to the hospital for 3 months or longer, while 80% experienced dyspnoea. The demographic characteristics, comorbidities, and symptoms of the long COVID groups are shown in **Table 2**.

Regarding the HRV data, patients who experienced symptoms for >3 and >6 months had higher HRV, those who experienced symptoms for ≤3 months had reduced parasympathetic modulation and increased sympathetic modulation influence of, and those who were hospitalized had a reduction in the sympathovagal balance (**Table 3**).

Intergroup Comparison

Major changes were observed in the intergroup comparison between the long COVID clinical groups and the control group. The clinical groups showed a reduction in global HRV (RR, SDNN, SD2, and SD1/SD2), increased the influence of sympathetic modulation (LF, LF/HF), decreased parasympathetic modulation (RMSSD, SD1, and HF), and decreased sympathovagal balance of the heart (LF/HF) in

TABLE 2 | Demographic characteristics, comorbidities, and symptoms of the study population considering the long COVID clinical group.

Variables	Hospitalised (*n* = 54) No, (%)	Not hospitalised (*n* = 101) No, (%)	*P*-Value	≤3 months (*n* = 74) No, (%)	>3 months (*n* = 81) No, (%)	*P*-Value	≤6 months (*n* = 119) No, (%)	>6 months (*n* = 36) No, (%)	*P*-Value	Dyspnoea (*n* = 132) No, (%)	Not dyspnoea (*n* = 23) No, (%)	*P*-Value
Sex												
Female	24 (44.44%)	71 (70.29%)	*0.002	38 (51.35%)	57 (70.37%)	*0.020	67 (56.30%)	28 (77.77%)	*0.020	83 (62.87%)	12 (52.17%)	0.359
Male	30 (55.56%)	30 (29.71%)		36 (48.65%)	24 (29.63%)		52 (43.70%)	8 (22.23%)		49 (37.13%)	11 (47.83%)	
Mean age (years)	44.27 ± 9.22	43.67 ± 10.47	0.837	42.17 ± 10.77	45.44 ± 9.09	0.074	43.78 ± 10.61	44.19 ± 7.90	0.940	43.43 ± 9.56	46.47 ± 12.30	0.180
Stature	1.64 ± 0.09	1.63 ± 0.08	0.441	1.64 ± 0.09	1.62 ± 0.08	0.221	1.63 ± 0.09	1.63 ± 0.08	0.739	1.63 ± 0.08	1.63 ± 0.09	0.938
Weight	86.80 ± 18.41	76.29 ± 16.49	*0.000	82.52 ± 19.21	77.60 ± 16.26	0.118	79.79 ± 17.80	80.49 ± 18.24	0.581	79.53 ± 17.78	82.35 ± 18.42	0.396
BMI	32.23 ± 7.11	28.90 ± 7.18	*0.005	30.70 ± 7.81	29.48 ± 6.82	0.361	29.94 ± 7.19	30.48 ± 7.80	0.684	29.89 ± 7.22	31.07 ±7.88	0.422
Smoker												
Yes	0	3 (2.97%)	0.314	1 (1.35%)	2 (2.46%)	1.00	3 (2.52%)	3 (8.33%)	0.138	2 (1.51%)	1 (4.34%)	0.381
Not	54 (100%)	98 (97.03%)		73 (98.65)	79 (97.54%)		116 (97.48%)	33 (91.67%)		130 (98.49%)	22 (95.66%)	
Ex-smoker												
Yes	12 (22.22%)	6 (5.94%)	*0.006	5 (6.75%)	12 (14.81%)	0.128	10 (8.40%)	7 (19.44%)	0.123	17 (12.87%)	3 (13.04%)	0.986
Not	42 (77.78%)	95 (94.06%)		69 (93.25%)	69 (85.19%)		109 (91.6%)	29 (80.56%)		115 (87.13%)	20 (86.96%)	
Long COVID symptoms (*n*, %)												
Dyspnoea	47 (87.03%)	85 (84.15%)	0.813	60 (81.08%)	72 (88.88%)	0.183	97 (81.51%)	35 (97.22%)	*0.016	132 (100%)	0	0
Chest pain	31 (57.40%)	62 (61.38%)	0.731	43 (58.10%)	50 (61.72%)	*0.041	68 (57.14%)	25 (69.44%)	*0.021	78 (59.09%)	15 (65.21%)	0.649
Fatigue	40 (74.07%)	78 (77.22%)	0.695	52 (70.27%)	66 (81.48%)	0.131	86 (72.26%)	32 (88.88%)	*0.045	103 (78.03%)	15 (65.21%)	0.192
Muscle weakness	46 (85.18%)	66 (65.34%)	*0.008	55 (74.32%)	57 (70.37%)	0.595	81 (68.06%)	31 (86.11%)	*0.035	96 (72.72%)	16 (69.56%)	0.802
Myalgia	40 (74.07%)	63 (62.37%)	0.157	52 (70.27%)	51 (62.96%)	0.395	78 (65.54%)	25 (69.44%)	0.130	86 (65.15%)	17 (73.91%)	0.480
Insomnia	36 (66.66%)	51 (50.49%)	0.062	42 (56.75%)	45 (55.55%)	1.00	67 (56.30%)	20 (55.55%)	1.00	75 (56.81%)	12 (52.17%)	0.820
Lower members Oedema	28 (51.85%)	30 (29.70%)	*0.008	26 (35.13%)	32 (39.50%)	0.620	40 (33.61%)	18 (50%)		48 (36.36%)	10 (43.47%)	0.641
Comorbidities												
Asthma	3 (5.55%)	21 (20.79%)	*0.011	8 (10.81%)	16 (19.75%)	0.181	20 (16.80%)	4 (11.11%)	0.450	20 (15.15%)	4 (17.39%)	0.756
DM	7 (12.96%)	6 (5.94%)	0.221	6 (8.10%)	7 (8.64%)	1.00	11 (9.24%)	2 (5.55%)	0.733	12 (9.09%)	1 (4.34%)	0.693
SAH	11 (20.37%)	23 (22.77%)	0.839	18 (24.32%)	16 (19.75%)	0.561	28 (23.52%)	6 (16.66%)	0.492	28 (21.21%)	6 (26.08%)	0.591
Obesity	33 (61.11%)	39 (38.61%)	*0.010	37 (50%)	35 (43.20%)	0.423	53 (44.53%)	19 (52.77%)	0.447	61 (46.21%)	11 (47.82%)	1.00
Hospital admission (*n*, %)												
Yes	54 (100%)	0	0	32 (43.24%)	22 (27.16%)	*0.043	40 (33.61%)	14 (38.88%)	0.690	47 (35.60%)	7 (30.43%)	0.081
Not	0	101 (100%)		42 (56.76%)	59 (72.83%)		79 (66.69%)	22 (61.12%)		85 (64.40%)	16 (69.57%)	
Mean length of stay (days)												
≤10 days (*n*, %)	19 (35.18%)	0	0	11 (34.37%)	8 (9.87%)	1.00	15 (12.60%)	4 (28.57%)	0.747	16 (34.04%)	3 (42.85%)	0.686
>10 days (*n*, %)	35 (63.22%)	0		21 (65.63%)	14 (90.13%)		25 (87.4%)	10 (71.43%)		31 (65.96%)	4 (57.15%)	

BMI, body mass index; MMII, lower member; DM, diabetes mellitus; SAH, systemic arterial hypertension; SD, standard deviation.
**P significant value.*

TABLE 3 | Analysis of HRV considering the long COVID clinical group.

Variables	Hospitalised (*n* = 54) Mean ± SD	Not hospitalised (*n* = 101) Mean ± SD	*P*-Value	≤3 months (*n* = 74) Mean ± SD	>3 months (*n* = 81) Mean ± SD	*P*-Value	≤6 months (*n* = 109) Mean ± SD	>6 months (*n* = 28) Mean ± SD	*P*-Value	Dyspnoea (*n* = 132) Mean ± SD	Not dyspnoea (*n* = 23) Mean ± SD	*P*-Value
RR (Ms)	794.16 ± 132.22	822.28 ± 126.72	0.228	806.93 ± 129.25	847.24 ± 138.30	*0.002	806.93 ± 129.25	830.86 ± 127.96	*0.002	812.97 ± 131.03	809.69 ± 118.88	0.925
SDNN (Ms)	28.47 ± 28.46	42.58 ± 119.92	0.171	39.84 ± 111.68	46.83 ± 133.77	0.122	39.84 ± 111.68	30.46 ± 19.96	0.571	39.81 ± 106.10	25.35 ± 20.63	0.208
RMSSD (Ms)	31.48 ± 37.22	35.05 ± 30.59	0.132	33.61 ± 34.34	38.25 ± 35.68	*0.043	33.61 ± 34.34	34.45 ± 28.44	0.529	34.57 ± 33.17	29.42 ± 32.35	0.438
SD1 (Ms)	22.29 ± 26.36	24.82 ± 21.66	0.133	23.80 ± 24.32	27.09 ± 25.27	*0.042	23.80 ± 24.32	24.40 ± 20.14	0.523	24.48 ± 23.48	20.82 ± 22.83	0.432
SD2 (Ms)	32.88 ± 31.12	35.27 ± 21.96	0.175	34.27 ± 26.80	35.93 ± 23.32	0.210	34.27 ± 26.80	35.00 ± 20.69	0.435	35.47 ± 26.30	28.52 ± 19.25	0.157
SD1/SD2	0.61 ± 0.25	0.66 ± 0.22	0.193	0.65 ± 0.24	0.69 ± 0.24	*0.011	0.65 ± 0.24	0.64 ± 0.20	0.876	0.64 ± 0.23	0.67 ± 0.23	0.604
ApEn	1.12 ± 0.15	1.14 ± 0.12	0.613	1.14 ± 0.13	1.12 ± 0.13	*0.007	1.14 ± 0.13	1.11 ± 0.15	0.176	1.14 ± 0.13	1.09 ± 0.16	0.079
SampEn	1.58 ± 0.37	1.63 ± 0.32	0.560	1.62 ± 0.34	1.59 ± 0.32	0.150	1.62 ± 0.34	1.59 ± 0.34	0.659	1.62 ± 0.33	1.55 ± 0.39	0.569
LF (n.u)	54.39 ± 21.54	48.83 ± 16.71	0.073	51.18 ± 19.26	47.29 ± 18.33	*0.014	51.18 ± 19.26	49.39 ± 16.66	0.533	50.39 ± 19.02	52.94 ± 16.60	0.572
HF (n.u)	45.54 ± 21.52	51.04 ± 16.84	0.079	48.69 ± 19.23	52.60 ± 18.33	*0.014	48.69 ± 19.23	50.56 ± 16.65	0.519	49.50 ± 18.99	46.97 ± 16.58	0.586
LF/HF	6.33 ± 30.60	1.26 ± 1.15	*0.032	1.73 ± 1.59	4.22 ± 25.06	*0.024	1.56 ± 1.56	7.90 ±37.51	0.887	3.30 ± 19.64	1.46 ± 1.12	0.657

SD, standard deviation; RR, average of RR intervals; N.u, normalized units; SDNN, standard deviation of all normal RR intervals; RMSSD, square root of the mean square of the differences between adjacent normal RR intervals in a time interval; SD1, rapid changes in RR intervals in parasympathetic nervous system index; SD2, long-term changes; SD1/SD2, short term ratio for long-term range variation; ApEn, approximate entropy, complexity, regularity of the RR interval series and signal complexity; SampEn, simple entropy, regularity of the RR interval series; LF, low-frequency components, ranging from 0.004 to 0.15 hertz; HF, high-frequency components, ranging from 0.15 to 0.004 hertz; LF/HF, low/high frequency components (normal range 1.5 to 2.0).

**P significant value.*

relation to the control group that did not manifest COVID-19. Data are shown in **Tables 4**, **5**.

DISCUSSION

In this study, patients with long COVID had persistent symptoms of dyspnoea, fatigue, muscle weakness, and chest pain and were mostly women. Long COVID clinical groups with increased sympathetic activity influence of, less parasympathetic activity, and reduced sympathovagal balance were compared. When the participants in the clinical groups with long COVID were compared with the COVID-19-uninfected control group, they demonstrated a decreased sympathovagal balance in the heart. When linear and nonlinear analyses were performed, this population showed changes in HRV, thus suggesting changes in the autonomic control of cardiac function.

The HRV changes observed in the long-term COVID population suggest the need for non-invasive assessments and the early detection of possible changes. The study by Mol et al. (11) demonstrated that higher HRV might predict greater chances of survival in older patients with COVID-19, independent of prognostic factors. Moreover, low HRV predicted ICU admission in the first week after hospitalization. Therefore, HRV measurements may be useful not only for monitoring patients with COVID-19 but also in the early identification of patients with long COVID at risk of clinical deterioration.

The majority of the people infected with SARS-CoV-2 (mild, moderate, or severe) demonstrated chronic signs and symptoms for weeks or months after the infection, lasting 12 weeks or more (19, 20). These signs of potential chronicity were observed in our study of patients who had chronic symptoms for 12 weeks or more.

Carfì et al. (20) reported the occurrence of persistent symptoms (37%) in 179 patients (53 women) for an average of 60 days after the onset of symptoms. Fatigue (53.1%) and dyspnoea (43.4%) persistently occurred in 87.54% of patients with COVID-19. The proportion of females and the prevalence of symptoms were similar to those in our study.

The prevalence of fatigue amongst women in the present study was also confirmed in a study by Kamal et al. (21), which analyzed 287 patients (64.1% women) and reported several persistent manifestations of long COVID and a higher prevalence of fatigue in women (72.8%).

In the present study, those with persistent symptoms were closer to the beginning of COVID-19 recovery, that is, ≤3–6 months of recovery. Al-Aly et al. (22) studied patients with COVID-19, who recovered at least 30 days after their diagnosis for 6 months. This is because the first 30 days or more of the illness after the diagnosis is associated with an increased risk of death; this results from the occurrence of several respiratory, neurological, and cardiovascular disorders, as well as malaise; fatigue; and musculoskeletal pain.

Persistent post-COVID-19 symptoms within 3–6 months after "recovery" from COVID-19 were also described by González-Hermosillo et al. (23), who analyzed 130 patients. Of these, 91.5% reported at least one symptom prior to the onset of infection. The

TABLE 4 | Analysis of HRV duration of long COVID and dyspnoea in the hospitalization groups based on the control group.

Variable	Hospitalised (*n*= 54) Mean ± DP	Control group (*n* = 94) Mean ± SD	*P*-Value	Not hospitalised (*n*= 101) Mean ± SD	Control group (*n* = 94) Mean ± SD	*P*-Value	≤3 months (*n*= 74) Mean ± SD	Control group (*n* = 94) Mean ± SD	*P*-Value	>3 months (*n*= 81) Mean ± SD	Control group (*n* = 94) Mean ± SD	*P*-Value
RR (Ms)	794.16 ± 132.22	865 ± 121	*0.001	822.28 ± 126.72	865 ± 121	*0.010	806.93 ± 129.25	865 ± 121	*<0.0001	847.24 ± 138.30	865 ± 121	0.366
SDNN (Ms)	28.47 ± 28.46	46.50 ± 29.20	*<0.0001	42.58 ± 119.92	46.50 ± 29.20	*<0.0001	39.84 ± 111.68	46.50 ± 29.20	*<0.0001	46.83 ± 133.77	46.50 ± 29.20	*<0.0001
RMSSD (Ms)	31.48 ± 37.22	54.90 ± 40.64	*<0.0001	35.05 ± 30.59	54.90 ± 40.64	*<0.0001	33.61 ± 34.34	54.90 ± 40.64	*<0.0001	38.25 ± 35.68	54.90 ± 40.64	*0.000
SD1 (Ms)	22.29 ± 26.36	39.89 ± 28.39	*<0.0001	24.82 ± 21.66	39.89 ± 28.39	*<0.0001	23.80 ± 24.32	39.89 ± 28.39	*<0.0001	27.09 ± 25.27	39.89 ± 28.39	*0.000
SD2 (Ms)	32.88 ± 31.12	51.52 ± 31.79	*<0.0001	35.27 ± 21.96	51.52 ± 31.79	*<0.0001	34.27 ± 26.80	51.52 ± 31.79	*<0.0001	35.93 ± 23.32	51.52 ± 31.79	*<0.0001
SD1/SD2	0.61 ± 0.25	0.76 ± 0.324	*0.002	0.66 ± 0.22	0.76 ± 0.324	*0.028	0.65 ± 0.24	0.76 ± 0.324	*0.000	0.69 ± 0.24	0.76 ± 0.324	0.160
ApEn	1.12 ± 0.15	1.07 ± 0.130	*0.023	1.14 ± 0.12	1.07 ± 0.130	*0.000	1.14 ± 0.13	1.07 ± 0.130	*<0.0001	1.12 ± 0.13	1.07 ± 0.130	*0.046
SampEn	1.58 ± 0.37	1.47 ± 0.383	0.066	1.63 ± 0.32	1.47 ± 0.383	*0.003	1.62 ± 0.34	1.47 ± 0.383	*0.002	1.59 ± 0.32	1.47 ± 0.383	0.059
LF (n.u)	54.39 ± 21.54	44.65 ± 20.71	*0.007	48.83 ± 16.71	44.65 ± 20.71	*0.006	51.18 ± 19.26	44.65 ± 20.71	*0.001	47.29 ± 18.33	44.65 ± 20.71	0.377
HF (n.u)	45.54 ± 21.52	55.28 ± 20.69	*0.007	51.04 ± 16.84	55.28 ± 20.69	*0.006	48.69 ± 19.23	55.28 ± 20.69	*0.001	52.60 ± 18.33	55.28 ± 20.69	0.370
LF/HF	6.33 ± 30.60	1.26 ± 1.42	*0.002	1.26 ± 1.15	1.26 ± 1.42	0.099	1.56 ± 1.56	1.26 ± 1.42	*0.001	4.22 ± 25.06	1.26 ± 1.42	0.235

SD, standard deviation; RR, average of RR intervals; N.u, normalized units; SDNN, standard deviation of all normal RR intervals, RMSSD, square root of the mean square of the differences between adjacent normal RR intervals in a time interval; SD1, rapid changes in RR intervals is an SNP index; SD2, long-term changes; SD1/SD2, short-term ratio for long-term range variation; Approximate entropy, ApEn complexity, approximate entropy, regularity of the RR interval series and signal complexity; SampEn, simple entropy, regularity of the RR interval series; LF, low-frequency components, ranging from 0.04 to 0.15 Hz; HF, high-frequency components, ranging from 0.15 to 0.4 HZ; LF/HF, low/high frequency components (normal range 1.5 to 2.0).

**P significant value.*

TABLE 5 | Analysis of HRV considering the duration of long COVID-19 and dyspnoea in the control group.

Variables	≤6 months (*n* = 119) Mean ± DP	Control group (*n* = 94) Mean ± DP	*P*-Value	>6 months (*n* = 36) Mean ± DP	Control group (*n* = 94) Mean ± DP	*P*-Value	Dyspnoea (*n* = 132) Mean ± DP	Control group (*n* = 94) Mean ± DP	*P*-Value	Not dyspnoea (*n* = 23) Mean ± DP	Control group (*n* = 94) Mean ± DP	*P*-Value
RR (Ms)	806.93 ± 129.25	865 ± 121	*0.001	830.86 ± 127.96	865 ± 121	0.159	812.97 ± 131.03	865 ± 121	*0.002	809.69 ± 118.88	865 ± 121	0.051
SDNN (Ms)	39.84 ± 111.68	46.50 ± 29.20	*<0.0001	30.46 ± 19.96	46.50 ± 29.20	*0.000	39.81 ± 106.10	46.50 ± 29.20	*<0.0001	25.35 ± 20.63	46.50 ± 29.20	*<0.0001
RMSSD (Ms)	33.61 ± 34.34	54.90 ± 40.64	*<0.0001	34.45 ± 28.44	54.90 ± 40.64	*0.002	34.57 ± 33.17	54.90 ± 40.64	*<0.0001	29.42 ± 32.35	54.90 ± 40.64	*0.000
SD1 (Ms)	23.80 ± 24.32	39.89 ± 28.39	*<0.0001	24.40 ± 20.14	39.89 ± 28.39	*0.001	24.48 ± 23.48	39.89 ± 28.39	*<0.0001	20.82 ± 22.83	39.89 ± 28.39	*<0.0001
SD2 (Ms)	34.27 ± 26.80	51.52 ± 31.79	*<0.0001	35.00 ± 20.69	51.52 ± 31.79	*0.001	35.47 ± 26.30	51.52 ± 31.79	*<0.0001	28.52 ± 19.25	51.52 ± 31.79	*<0.0001
SD1/SD2	0.65 ± 0.24	0.76 ± 0.324	*0.004	0.64 ± 0.20	0.76 ± 0.324	*0.050	0.64 ± 0.23	0.76 ± 0.324	*0.002	0.67 ± 0.23	0.76 ± 0.324	0.211
ApEn	1.14 ± 0.13	1.07 ± 0.130	*<0.0001	1.11 ± 0.15	1.07 ± 0.130	0.213	1.14 ± 0.13	1.07 ± 0.130	*<0.0001	1.09 ± 0.16	1.07 ± 0.130	0.501
SampEn	1.62 ± 0.34	1.47 ± 0.383	*0.003	1.59 ± 0.34	1.47 ± 0.383	0.111	1.62 ± 0.33	1.47 ± 0.383	*0.002	1.55 ± 0.39	1.47 ± 0.383	0.294
LF (n.u)	51.18 ± 19.26	44.65 ± 20.71	*0.016	49.39 ± 16.66	44.65 ± 20.71	0.221	50.39 ± 19.02	44.65 ± 20.71	*0.031	52.94 ± 16.60	44.65 ± 20.71	0.077
HF (n.u)	48.69 ± 19.23	55.28 ± 20.69	*0.015	50.56 ± 16.65	55.28 ± 20.69	0.222	49.50 ± 18.99	55.28 ± 20.69	*0.029	46.97 ± 16.58	55.28 ± 20.69	0.076
LF/HF	1.56 ± 1.56	1.26 ± 1.42	*0.017	7.90 ±37.51	1.26 ± 1.42	0.063	3.30 ± 19.64	1.26 ± 1.42	*0.022	1.46 ± 1.12	1.26 ± 1.42	*0.033

SD, standard deviation; RR, average of RR intervals; N.u, normalized units; SDNN, standard deviation of all normal RR intervals, RMSSD, square root of the mean square of the differences between adjacent normal RR intervals in a time interval; SD1, rapid changes in RR intervals is an SNP index; SD2, long-term changes; SD1/SD2, short-term ratio for long-term range variation; Approximate entropy, ApEn complexity, approximate entropy, regularity of the RR interval series and signal complexity; SampEn, simple entropy, regularity of the RR interval series; LF, low-frequency components, ranging from 0.04 to 0.15 Hz; HF, high-frequency components, ranging from 0.15 to 0.4 HZ; LF/HF, low/high frequency components (normal range 1.5 to 2.0).

**P significant value.*

symptom of fatigue persisted among those aged between 40 and 50 years who had long COVID for 3–6 months. As in our study, in the same age group, women were more likely to experience long-term symptoms.

The mechanism of COVID-19 development, immune system response, and Autonomic Nervous System (ANS) are complex subjects. SARS-CoV-2 can activate the innate and adaptive immune responses, generating inflammatory responses that can lead to local and systemic damage (24). Autonomic dysfunction may be mediated by the virus itself. However, during the cytokine storm, vagal stimulation induces an anti-inflammatory response, while sympathetic activation induces the release of pro-inflammatory cytokines. Some studies reported the association between autonomic dysfunction and the short and long-term neurotropism of SARS-CoV-2 (25, 26).

Increase in the influence of sympathetic activity at rest, which can generate an increase in premature deaths, remains of great concern (27). This alteration may increase the HR, while the emergence of cardiovascular diseases predisposes the patient to systemic arterial hypertension and incorrect adaptations of the ANS in response to this (28), thus impairing cardiac regulation.

Heart rate variability has been used to diagnose autonomic regulation, and sympathetic and parasympathetic imbalance occurs in dysautonomia. It is unclear, how dysautonomia with HRV dysregulation occurs in patients with COVID-19 and long COVID. This could be due to neurotropism, hypoxia, and inflammation caused by the autonomic-virus pathway or immune-mediated processes after viral exposure (29). Cardiovascular dysautonomia frequently occurs in patients who recover from COVID-19. There is a reduction in the HRV components (rMSSD and SDNN) when compared with that in uninfected individuals. Despite the scarcity of HRV data, some researchers have been investigating autonomic dysfunction in patients with long COVID to improve disease management and prognosis and limit the progression of the disease (30). Further, the adverse effects of viral infection can generate an increase the sympathetic tone influence, thus preventing the balance in parasympathetic modulation in patients with long COVID.

We reported an increase influence of in the resting sympathetic tone, a decrease in parasympathetic tone, and significant changes in RMSSD and SDNN in long COVID clinical groups compared with those in long-term COVID groups and reduced LF/HF compared with that in the COVID-19-uninfected control group. A previous cross-sectional study conducted by Kaliyaperumal et al. (31) analyzed 106 patients treated for COVID-19 (asymptomatic or mildly to moderately symptomatic). Of these, 63 (59.4%) had COVID-19, while 43 (40.6%) were healthy. The authors demonstrated high rates of autonomic imbalance in patients with COVID-19. Parasympathetic modulation (rMSSD and SDNN) increased in the patients with COVID-19 independent of age, sex, and comorbidities, while the HRV components in LF and HF potencies decreased in COVID-19 patients, when compared with the healthy uninfected individuals.

The parasympathetic activity (RMSSD, SD1, and HF) decreased in long COVID clinical groups; this finding suggests that parasympathetic changes may be associated with mediation of the inflammatory process. However, in a meta-analysis of 159 studies by Williams et al. (32), a negative association was found between HRV and vagal indices (e.g. HF), SDNN, and inflammation markers. SDNN was strongly associated with inflammatory markers and had greater effects in women than in men.

Other decreases in parasympathetic modulation were reported by Gifford et al. (33), who examined the autonomic function and HRV after extreme resistance exercise. They reported that healthy women have a lower sympathetic profile; an increase in HRV within 15 days after performing exercise showed better parasympathetic activity (RMSSD, SD1, and HF), increased global HRV (SD1/SD2), and increased SampEn.

In the present study, changes in ApEn and SampEn entropies were not observed in the long COVID clinical groups compared with that in the COVID-19-free control group. However, Bajic, Đajić, and Milovanović (34), when analyzing the different entropies (Apen, SampEn, binary, sample, and multiscale) of 116 patients with COVID-19 (mild to severe) and 77 healthy controls, only found significant cross-entropies in heart rate signals and systolic pressure. Most of the patients with COVID-19 had lower SampEn values compared with those in the control group. Considering that ANS dysfunctions are associated with COVID-19 severity, we believe that signal acquisition is complex; moreover, no difference was found in the entropies between patients with COVID-19 and controls.

Heart rate variability has been assessed in other studies to determine autonomic functions (35). Linear and nonlinear methods were used to analyse HRV to assess cardiac modulation (36). Our study demonstrated HRV alterations in the long COVID population with cardiac autonomic dysfunction; increased influence of sympathetic activity at rest was associated with increased HR and blood pressure levels, cardiovascular problems, poorer prognosis, and sudden death. However, this finding still needs to be extensively explored to understand the mechanisms leading to these alterations. In addition, the usefulness of this tool in clinical practice should be evaluated.

Strengths and Limitations

Heart rate variability analysis was performed using a cardiofrequency meter, which is influenced by individual and behavioral factors. The study was performed at a single center and had a small sample size. The sample was representative of the population studied; few studies described in the scientific literature used HRV analysis for assessing cardiac modulation in the long COVID population. However, further studies need to be conducted to understand the repercussions of long COVID in different body organs and on breathing controls to understand whether long COVID impacts cardiac autonomic modulation. The results collected in this study will be fundamental for the initial understanding of cardiac autonomic alterations in patients with long COVID.

CONCLUSIONS

Our results demonstrated that the long COVID clinical groups showed reduced HRV compared with the COVID-19-uninfected control group. Short-term linear and nonlinear methods demonstrated good precision in this population. Therefore, changes in long COVID should be monitored to understand its involvement in cardiac autonomic modulation and detect possible cardiovascular changes for short- or long-term prevention. In particular, increased influence of sympathetic activity may be linked with cardiovascular imbalances, chronic disease, and sudden death. Hence, further tests and clinical trials should be conducted to understand the after-effects of long COVID on cardiac autonomous modulation. Although changes in the ANS were observed, it is unclear, whether the changes were caused directly or indirectly by infection or systemic inflammatory state in patients who recovered from COVID-19.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee of the Pará State University (UEPA) opinion number 4.252.664. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JQ and LF: project administration, support, supervision, review, and scientific collaboration. MS and RR: support, review, and edition. CS and ST: investigation, data collection, and written. KM: investigation, data collection, written, and edition and review. All authors read and approved the final manuscript.

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Serial Cardiovascular Magnetic Resonance Studies Prior to and After mRNA-Based COVID-19 Booster Vaccination to Assess Booster-Associated Cardiac Effects

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Background: mRNA-based COVID-19 vaccination is associated with rare but sometimes serious cases of acute peri-/myocarditis. It is still not well known whether a 3rd booster-vaccination is also associated with functional and/or structural changes regarding cardiac status. The aim of this study was to assess the possible occurrence of peri-/myocarditis in healthy volunteers and to analyze subclinical changes in functional and/or structural cardiac parameters following a mRNA-based booster-vaccination.

Methods and Results: Healthy volunteers aged 18–50 years (n = 41; m = 23, f = 18) were enrolled for a CMR-based serial screening before and after 3rd booster-vaccination at a single center in Germany. Each study visit comprised a multi-parametric CMR scan, blood analyses with cardiac markers, markers of inflammation and SARS-CoV-2-IgG antibody titers, resting ECGs and a questionnaire regarding clinical symptoms. CMR examinations were performed before (median 3 days) and after (median 6 days) 3rd booster-vaccination. There was no significant change in cardiac parameters, CRP or D-dimer after vaccination, but a significant rise in the SARS-CoV-2-IgG titer ($p < 0.001$), with a significantly higher increase in females compared to males ($p = 0.044$). No changes regarding CMR parameters including global native T1- and T2-mapping values of the myocardium were observed. A single case of a vaccination-associated mild pericardial inflammation was detected by T2-weighted CMR images.

Conclusion: There were no functional or structural changes in the myocardium after booster-vaccination in our cohort of 41 healthy subjects. However, subclinical pericarditis was observed in one case and could only be depicted by multiparametric CMR.

Keywords: COVID-19, vaccination, CMR, MRI, myocarditis, t1-mapping, t2-mapping

INTRODUCTION

Without any doubt, COVID-19 vaccines are a blessing and prevented many millions of people world-wide from becoming either very ill or even dying due to a COVID-19 infection. Nevertheless, various reports showed that particularly mRNA-based COVID-19 vaccines are associated with rare but sometimes serious cases of acute peri-/myocarditis (1, 2). We still need to better understand why some people show cardiac adverse events following vaccination.

Magnetic resonance imaging (MRI) is the non-invasive gold standard in the diagnosis of myocardial inflammation (3). In this context, some impressive case reports presented severe myocardial damage on cardiovascular magnetic resonance imaging (CMR) even without functional impairment (4, 5), and the true number of COVID-19 vaccine-associated peri-/myocarditis may even be underreported since CMR is still not widely available.

So far, there are only limited data available regarding the frequency of vaccine-associated peri-/myocarditis following a 3rd booster vaccination for COVID-19 and regarding the value of CMR (6, 7). Hence, the aim of this prospective study was (a) to assess the possible occurrence of peri-/myocarditis following a mRNA-based booster vaccination in healthy volunteers and (b) to also analyze whether there are subclinical changes in functional and/or structural cardiac parameters possibly triggered by the preceding booster vaccination.

METHODS

Healthy volunteers aged 18–50 years were enrolled for a CMR-based serial screening before and after 3rd booster vaccination in the CMR-Center of University Hospital Muenster, Germany. Each study visit comprised a CMR scan, blood analyses with cardiac markers, markers of inflammation and SARS-CoV-2-IgG antibody titers, resting ECGs and a questionnaire regarding clinical symptoms. After their baseline examination, the study subjects received their 3rd booster dose of a mRNA-based COVID-19 vaccine with either mRNA-1273 (Moderna) or BNT162b2 (Pfizer-BioNTech) within 1–10 days. The follow-up examination was performed 4–10 days after booster vaccination.

Cardiovascular magnetic resonance imaging was performed on a 1.5 T-scanner (Philips Healthcare, Best, Netherlands) with a modified standard protocol used in clinical practice for suspected myocarditis (8). The protocol included high resolution cine, native T1- as well as T2-mapping, T2-STIR imaging and flow measurements. Contrast agent administration with additional late-gadolinium-enhancement (LGE) imaging was only intended if the native scan showed clear signs of active myocardial inflammation. Native T1- and T2- times were measured on three short axis views using pixelwise maps. All subjects gave their written informed consent to the study.

Skewed variables are expressed as median and interquartile range (IQR). Categorical variables are expressed as frequency with percentage. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Between November 2021 and January 2022, we prospectively examined 41 healthy, individuals with a median age of 35 years before (median 3 days) and after (median 6 days) their 3rd booster vaccination. The subjects (56% male) had no history of any cardiac disease or prior COVID-19 infection. There was one loss of follow up, because one participant experienced a COVID-19 infection in the interval between the third vaccination and the follow-up appointment. 30% of the subjects received mRNA-1273 (Moderna) and 70% received BNT162b2 (BioNTech) for booster (**Table 1**). No association between the subjective burden of symptoms and the respective increase in SARS CoV-2-IgG titer was observed.

There was no pathological elevation and no significant change in serum markers such as CK, CK-MB, high-sensitive troponin T, NT-proBNP, CRP or D-dimer before and after the 3rd booster vaccination (**Table 2**). As expected, there was a highly significant rise in the SARS-CoV-2-IgG titer ($p < 0.001$) in our study population. In addition, females showed a significantly higher increase in SARS-CoV-2-IgG titer ($p = 0.044$) compared to males.

In general, the assessment of both functional as well as structural CMR parameters showed highly consistent and reproducible values when the respective CMR parameters measured before and after the booster vaccination were compared. In particular, there was no change in biventricular function and volumes, in global longitudinal strain and in myocardial mass (**Table 2**). Moreover, the global native T1- and T2-mapping values remained unchanged (988 vs. 983 ms in T1

TABLE 1 | Subject characteristics.

Parameter			
Age, years	35 (31–38)		
Male/female, *n* (%)	23/18 (56%/44%)		
BMI, kg/m^2	23.2 (22.2–24.4)		
Time between BL-CMR and booster vaccination, days	3.0 (1.3–6.0)		
Time between FUP-CMR and booster vaccination, days	(5.3–7.8)		
Vaccine for 3rd vaccination			
- BioNTech (BNT162b2)	28 (70%)		
- Moderna (mRNA-1273)	12 (30%)		
Allergies, *n* (%)	14 (34%)		
Symptoms after 3rd vs. 2nd vaccination	***n***	***n***	***p****
- Local	34	22	**0.008**
- Fever	9	10	0.08
- Palpitations	4	1	0.27
- Chest pain	1	1	0.31
- Dyspnea	2	1	0.65
- Fatigue	22	24	0.67
- No symptoms	6	12	**0.011**

*If not stated otherwise all data are expressed as median (interquartile range). *Calculated for duration of symptoms not frequency among subjects. BMI, body mass index; BL-CMR, cardiac magnetic resonance at baseline; FUP-CMR, cardiac magnetic resonance at follow-up. The variables in bold show the significant correlations at a significance level of p.*

TABLE 2 | Cardiac magnetic resonance (CMR)-findings, laboratory, and ECG parameters.

Parameter	Pre booster	Post booster	*p*-Value
➢ **CMR findings**			
LV-EF,%	60 (55–63)	61 (57–64)	0.05
LV-EDVi, ml/m^2	87 (80–95)	86 (90–94)	0.24
LV- GLS,%	−16.2 (−17.2 to −15.0)	−15.9 (−17.1 to −14.6)	0.75
RV-EF,%	55 (50–59)	54 (51–57)	0.85
RV-EDVi, ml/m^2	92 (83–102)	91 (80–100)	0.48
LV-mass, g/m^2	47 (42–54)	47 (42–54)	0.61
Global native T1, ms	988 (964–1,011)	983 (970–1,024)	0.90
No. of segments with elevated T1 Mapping value > 1,060 ms, *n* (%)	0 (0%)	0 (0%)	
Global T2, ms	50 (49–51)	50 (49–51)	0.40
No. of segments with elevated T2 Mapping value > 59 ms, *n* (%)	0 (0%)	0 (0%)	
Presence of edema in T2 weighted images,			
- myocardial, *n* (%)	0 (0%)	0 (0%)	
- pericardial, *n* (%)	2 (1.6%)	3 (2.6%)	
➢ **Biochemistry marker**			
CK, U/l	113 (83–187)	99 (78–133)	0.07
CK-MB, U/l	14 (12–16)	14 (12–17)	0.50
Troponin T, ng/l	3.0 (3.0–4.7)	3.0 (3.0–4.3)	0.59
NT-pro-BNP, pg/ml	30 (13–58)	21 (11–52)	0.26
D-dimer, mg/l	0.27 (0.27–0.27)	0.27 (0.27–0.28)	0.39
CRP, mg/dl	0.5 (0.5–0.5)	0.5 (0.5–0.5)	0.32
SARS CoV-2-IgG, AU/ml	1,319 (681–1,788)	16,077 (10,312–32,540)	**<0.001**
SARS CoV-2-IgG in male, AU/ml	1,807 (601–2,485)	15,643 (9,129–19,650)	**<0.001**
SARS CoV-2-IgG in female, AU/ml	2,076 (691–1,717)	24,271 (11,092–40,000)	**<0.001**
Δ SARS CoV-2-IgG, AU/ml	*Male*	*Female*	
	13,388 (8,873–15,927)	20,640 (9,332–38,637)	**0.044**
➢ **ECG parameters**			
Heart rate, bpm	67 (60–75)	71 (64–78)	0.06
ST-elevation			
- minor < 0,1 mV, *n*	14 (34%)	12 (30%)	
- significant ≥ 0,1 mV, *n*	0 (0%)	0 (0%)	
ST-depression ≥ -0,1 mV, *n*	0 (0%)	0 (0%)	

If not stated otherwise all data are expressed as median (interquartile range); All biochemistry marker (with exception of SARS CoV-2-IgG) are in normal range of values. LV-EF, left ventricular ejection fraction; LV-EDVi, indexed left ventricular end-diastolic volume; LV-GLS, left ventricular global longitudinal stain; RV-EF, right ventricular ejection fraction; RV-EDVi, indexed right ventricular end-diastolic volume; CK, creatinine kinase; CK-MB, creatinine kinase isoenzyme MB; NT-pro BNP, N-terminal -pro brain natriuretic peptide; CRP, C-reactive protein; SARS CoV-2-IgG, anti-severe acute respiratory syndrome coronavirus 2 -immunoglobulin G. Δp – significance between the changes among IgG rise in male and female. $p < 0.05$ is considered as significant. The variables in bold show the significant correlations at a significance level of p.

and each 50 ms in T2). There was one female who demonstrated a new "pericardial" T2-STIR-weighted hyperintensity in the basal to midventricular inferolateral pericardium and also a new pleural effusion (**Figure 1**). In the absence of any symptoms or signs of other diseases, we interpreted these findings as a vaccination-associated form of very mild pericardial inflammation. There was no known clinical characteristic or laboratory parameter that could provide a predisposition to pericarditis in this case.

DISCUSSION

Although the pivotal approval studies, sponsored by the respective pharmaceutical companies, did not show an increased risk of myocarditis following COVID-19 vaccination (9, 10), today there is no doubt that mRNA-based COVID-19 vaccination can cause peri-/myocarditis particularly in young males (1, 11, 12). It has also been shown that the risk of myocarditis is predominantly increased after the second vaccination dose. Assuming an autoimmune-mediated process, it is still unknown whether a 3rd booster vaccination is also associated with a non-neglectable risk of peri-/myocarditis.

To the best of our knowledge, our present study is the first one that used multi-parametric serial CMR studies prior to and after mRNA-based COVID-19 booster vaccination to carefully assess potential booster-associated cardiac effects. Our major findings can be summarized as follows:

First, the present data show that no relevant myocardial changes could be observed by CMR following the 3rd booster vaccination. Our data support current recommendations regarding booster vaccinations that should not be withheld for fear of adverse cardiac events in healthy subjects aged <50 years (considering that there is no vaccination with mRNA-1273

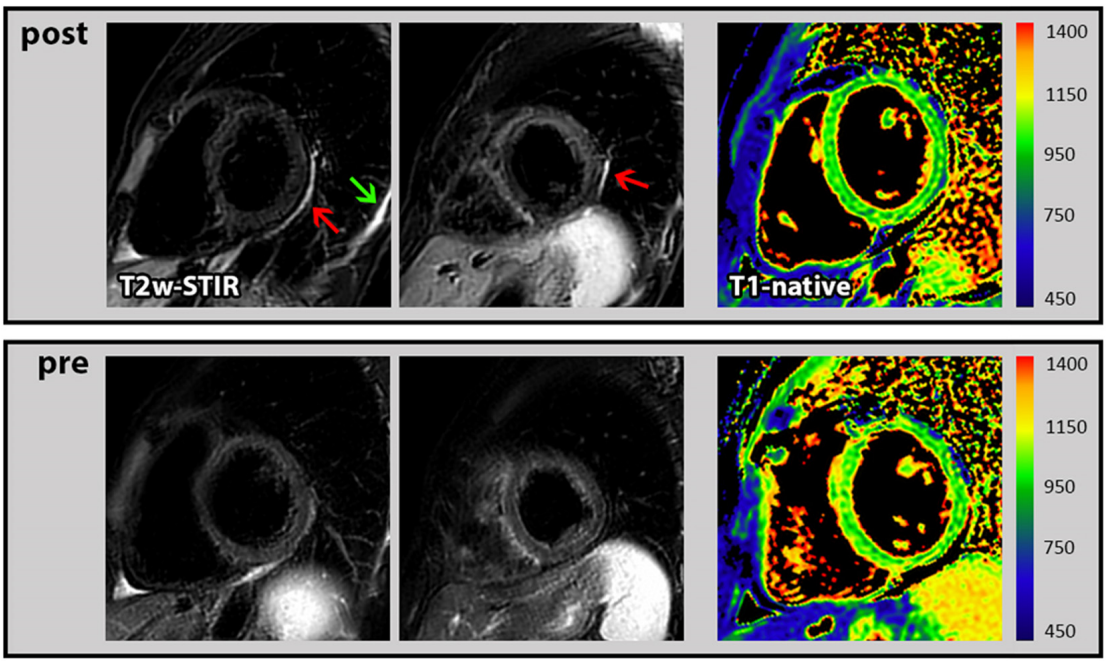

FIGURE 1 | Cardiac magnetic resonance (CMR) images of pericarditis. *First row*: T2-STIR-weighted short-axis images with the occurrence of pericardial hyperintensity as indication for edema/mild pericardial inflammation (red arrow) and a new pleural effusion (green arrow) following the 3rd COVID-19 vaccination. In addition, corresponding T1 mapping without signs of myocardial impairment. *Second row*: Corresponding images at baseline (prior to 3rd COVID-19 vaccination) from the same subject without any pathological findings.

(Moderna) in subjects <30 years since cases of peri-/myocarditis were more frequently observed after Moderna vaccination in this age group).

Second, subclinical pericarditis was observed in 1 out of 40 subjects following a 3rd booster vaccination whereas no cases of myocarditis were observed in the present study. Importantly, multi-parametric CMR imaging was the only diagnostic modality that allowed to depict such a mild pattern of pericardial inflammation. In line with this findings, a large descriptive study, based on reports to the VEARS (Vaccine Adverse Event Reporting System) reported only 17% of abnormal findings based on echocardiograms (in the cohort of myocarditis patients younger than 30 years), but abnormalities were reported in >70% by CMR (1).

Today, CMR is well-known and robust modality for the non-invasive diagnosis of myocarditis that does not only detect regional dysfunction, but allows also to depict edema and other subtle structural changes based on elevated T1- and T2-mapping values and/or characteristic patterns of LGE (13, 14). Therefore, the Lake Louise criteria for CMR-based diagnosis of myocardial inflammation were already established in 2009 and updated in 2018 (15, 16). Since the diagnosis of vaccine-associated myocarditis is important for symptom management, for exercise recommendations, for further cardiomyopathy monitoring and future (e.g., booster) vaccination decisions (3), physicians should be aware of the potential of multi-parametric CMR.

Last but not least, our present data clearly show that a 3rd booster vaccination leads to a substantial increase in the SARS CoV-2-IgG antibody titer – and interestingly to a higher increase in females compared to males. Hence, gender-based differences should be evaluated more carefully in future studies.

CONCLUSION

The present serial CMR data support current recommendations regarding the safety of 3rd booster vaccinations since no functional or subtle structural changes were observed in the myocardium – as long as current vaccination recommendations are followed. However, subclinical pericarditis was observed in one case and could only be depicted by multiparametric CMR.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical approval was not provided for this study on human participants because written consent of participants were obtained and no identifiable images or data are presented in the study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CM devised the project, performed CMR examinations, performed major analyses of the CMR data, and drafted the manuscript. DK performed the CMR analyses and participated in the interpretation of results. MB participated in the design of the study, performed CMR examinations, and statistical analyses. BC participated in the design of the study, CMR exams, and critically reviewed the manuscript. SD participated in the CMR exams and in the analysis of the CMR data. VV and PS performed CMR examinations and participated in the analysis of clinical data. AY provided the main conceptual idea, supervised the work, provided critical feedback and helped shape the research, analysis, and manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Mechanisms of Cardiovascular System Injury Induced by COVID-19 in Elderly Patients With Cardiovascular History

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The coronavirus disease-2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2), represents a great threat to healthcare and socioeconomics worldwide. In addition to respiratory manifestations, COVID-19 promotes cardiac injuries, particularly in elderly patients with cardiovascular history, leading to a higher risk of progression to critical conditions. The SARS-CoV-2 infection is initiated as virus binding to angiotensin-converting enzyme 2 (ACE2), which is highly expressed in the heart, resulting in direct infection and dysregulation of the renin-angiotensin system (RAS). Meanwhile, immune response and hyper-inflammation, as well as endothelial dysfunction and thrombosis implicate in COVID-19 infection. Herein, we provide an overview of the proposed mechanisms of cardiovascular injuries in COVID-19, particularly in elderly patients with pre-existing cardiovascular diseases, aiming to set appropriate management and improve their clinical outcomes.

Keywords: COVID-19, cardiovascular system injuries, renin-angiotensin system (RAS), inflammation, immune dysregulation, endothelial injury

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INTRODUCTION

In December 2019, pneumonia of unknown cause was reported in Wuhan, China (1). By early January 2020, sequencing analysis indicated the pathogen as a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the related clinical syndrome was named coronavirus disease-2019 (COVID-19) (2). It had spread rapidly throughout the world and on 11 March, 2020, the World Health Organization (WHO) declared COVID-19 a global pandemic. This infection has brought a great threat to healthcare and socioeconomics worldwide.

Whereas COVID-19 is characterized by respiratory symptoms, Huang et al. reported that 12% of patients present acute cardiac injury, defined as an ejection fraction decline and troponin I elevation (3), with a wide spectrum of clinical manifestations ranging from an acute coronary syndrome, myocarditis, arrhythmia, to cardiac dysfunction. Accumulating evidence reveals that acute cardiovascular injury is associated with increased severity and mortality of COVID-19 (4). Recent literature on long-term sequelae of COVID-19 shows prolonged cardiovascular damage in a large proportion of post-COVID-19 patients (5), for example, parasympathetic overtone and increased heart rate variability (6).

People with underlying cardiovascular diseases are prone to develop severe conditions, even in pediatric patients. The COVID-19-infected children with congenital heart disease represent worse clinical courses when compared to healthy control (7). Likewise, the elderly people with

pre-existing cardiovascular comorbidities, who are more susceptible to cardiac injuries of COVID-19, are at a higher risk of poorer prognosis. In this review, we emphasize the pathogenesis of COVID-19-induced cardiovascular injury, particularly in elderly patients with underlying cardiovascular diseases.

THE ANGIOTENSIN-CONVERTING ENZYME 2 RECEPTOR AND RENIN-ANGIOTENSIN SYSTEM

As with SARS-CoV, ACE2 has been established as the dominant route of entry for SAR-CoV-2 upon binding of the viral spike protein (S protein) (8). In the process, furin, a proprotein convertase, cleaves S protein into two activated subunits, namely, S1 and S2. The S1 domain binds the ACE2 receptor through its receptor-binding domain (RBD), while the S2 subunit is necessary for virus-cell fusion after further processed by transmembrane serine protease 2 (TMPRSS2) (9). Compared to SARS-CoV, SARS-CoV-2 exhibits potent binding to ACE2 and immune evasion because of greater affinity of RBD, protease pre-activation of the spike, and hidden RBD, all of which in turn results in higher transmissibility of SARS-CoV-2 (9, 10). Until now, SARS-CoV-2 has undergone substantial evolution, for instance, SARS-CoV-2 Delta variant, one of the predominant circulating strains, exhibits higher infectivity, on a basis of increased ACE2 interaction owing to more RBD-up states and Delta T478K substitution (11). The virus-ACE2 binding actuates the virus-cell fusion, virus replication, and ACE2 loss at the same time.

Angiotensin-converting enzyme 2, a type I integral membrane protein, acts as a transmembrane protein or a soluble catalytic ectodomain *in vivo* (12). The transmembrane ACE2 can be measured as ACE2 expression, and studies indicate that the transmembrane ACE2 is abundant in lungs, heart, and endothelial cells (ECs) (13, 14). Chen et al. delineate ACE2 expression in cardiac resident cells, particularly in pericytes, a type of perivascular mural cells. Pericytes support capillary EC function and are associated with myocardial microcirculation (15). Pathology analysis of COVID-19 infections reveals direct viral infection and diffuses inflammation of the ECs, which may attribute to coronary plaque disruption and thrombosis (16). In this regard, direct viral infection of cardiac tissue implicates the cardiac complications of COVID-19 infection. A disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) mediates cleavage and shedding of the soluble ACE2 ectodomain. ACE2 ectodomain, also known as soluble ACE2, can be detected as plasma ACE2 activity (12). The soluble ACE2 has been recently recognized to help in controlling SARS-CoV-2 infection *via* inhibiting their interaction with cell-bound ACE2 (17). The circulating ACE2 has been shown to correlate with cardiac remodeling, endothelial dysfunction, and is a predictor of major adverse cardiovascular events (18).

Beyond the host receptor in SAR-CoV-2 infection, ACE2 is an important component of the RAS. The circulating RAS system is finely controlled by complex feedback to maintain blood volume. Commonly, the action of Angiotensin (Ang) II on Ang II type 1 receptor (AT_1R) stimulates aldosterone secretion regulated by renin in response to homeostatic demand. On the other hand, tissue angiotensin system has been identified since prorenin expression was found in many organs, tissues, such as heart, lungs, and brain. Thereafter, other biological effects of RAS have been recognized. Prorenin, firstly activated through proteolytic enzymes or unfolded by binding with (pro)renin receptor, converts the substrate angiotensinogen (AGT) to Ang I (10, 17). Ang I is cleaved to Ang II, the most active agent in RAS by ACE or chymase, the major catalytic enzyme in the heart (19). Ang II acts on two G-protein coupled receptors, AT_1R and type 2 receptor (AT_2R). Ang II/AT_1R binding exerts vasoconstriction, pro-inflammatory, pro-fibrotic, and proliferative effects through various intracellular protein signaling pathways, such as tyrosine kinases, serine/threonine kinases, mitogen-activated protein kinase (MAPK) family, and various protein kinase C isoforms, while Ang II/AT_2R interaction, with a much less affinity when compared to Ang II/AT_1R action, activates various protein phosphatases, the nitric oxide (NO)/cyclic GMP system, and phospholipase A_2, counteracting AT_1R actions (20–22). ACE2 mediates Ang (1–7) generation from Ang II. Ang (1–7) acts *via* AT_2R, Mas receptor (MasR), and Mas-related G protein-coupled receptor D (MgrD) and performs protective actions of anti-inflammation, vasodilation, and anti-fibrotic effects (10, 23). As such, Ang II degradation and Ang 1–7 generation accelerate cardiovascular protection. Previous animal studies showed that ACE2 inactivation was correlated with reduced cardiac contractility, coronary vasoconstriction, microvascular dysfunction, and less myocardial blood flow (24, 25).

Virus-ACE2 internalization in COVID-19 infection leads to ACE2 destruction. The ACE2 deficiency modulates the imbalance of Ang II/Ang (1–7) and thus amplifies Ang II/AT_1R actions (**Figure 1A**). Moreover, aging-related RAS alteration contributes to cardiac dysfunction in COVID-19 infection. A previous study discovers increased cardiac Ang II formation in a chymase-driven manner in aged rats (26). Ang II level *per se* mediates ACE and ACE2 expression, with higher ACE and lower ACE2 production, which in turn leads to outweighed Ang II/AT_1R interaction (27). In addition, animal studies display upregulation of AT_1R in both the aging heart and vasculature. On the other hand, AT_2R was highly expressed in fetal tissues but dropped to a comparatively low level in adulthood. The altered ratio of AT_1R and AT_2R might increase blood pressure and induce inflammation (22). Elderly patients with pre-existing cardiovascular diseases reported the increased ACE2 expression, promoting vulnerabilities to COVID-19 and direct viral damage. Noteworthy, RAS inhibitors, frequently medicated to older patients with cardiovascular diseases, are safe, despite increased membrane-bound ACE2 expression (28).

IMMUNITY AND INFLAMMATION

After initial infection with the virus, the innate immune signaling activates as the first-line defense, mediating virus recognition, killing virus-infected cells, stimulating inflammation, and adaptive immunity. The adaptive immune system, consisting of

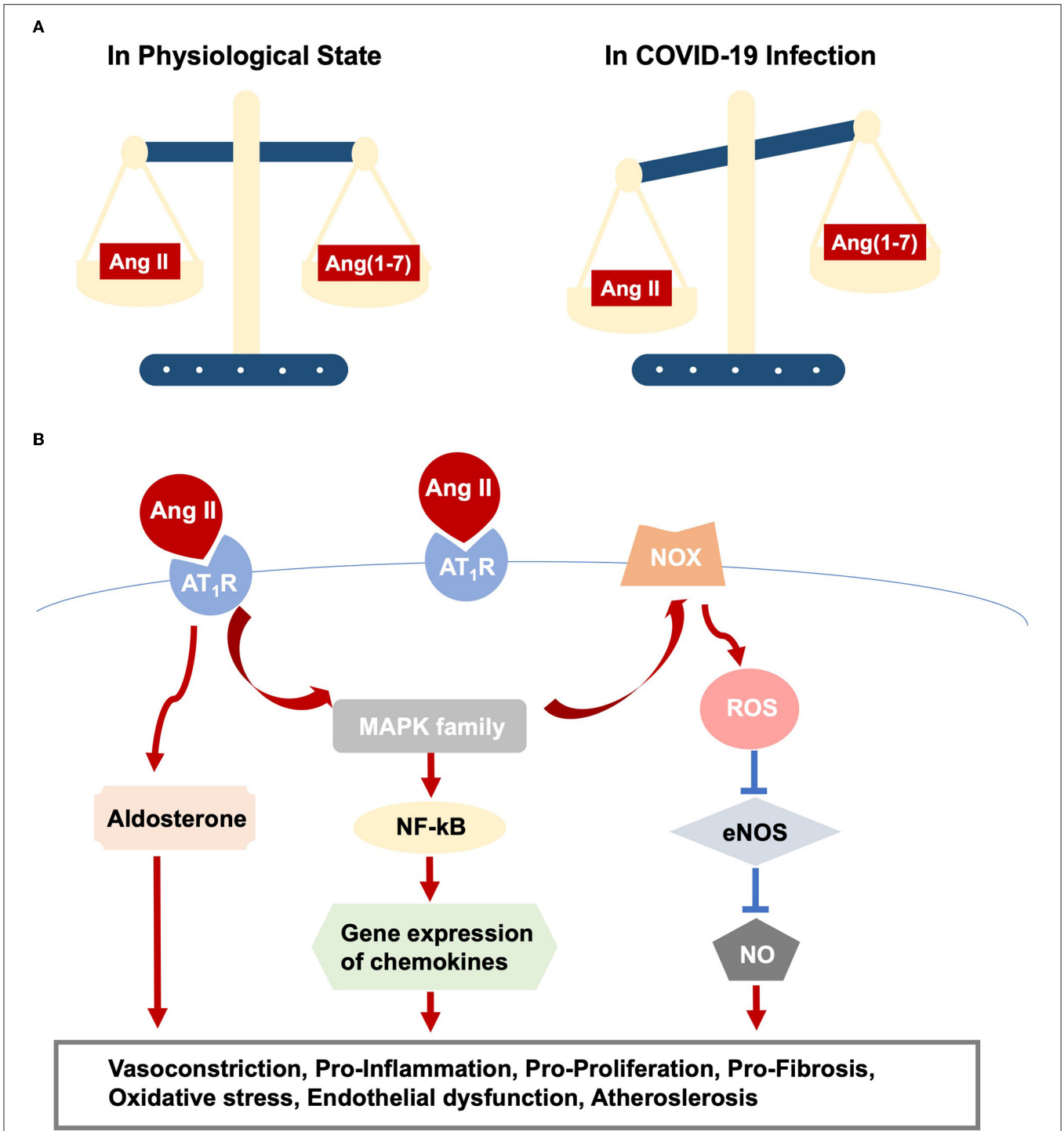

FIGURE 1 | RAS dysfunction in COVID-19 infection. **(A)** Outlines imbalance between Ang II/AT_1R and Ang(1-7)/Mas action. **(B)** Elucidates intracellular pathways upon Ang II/AT_1R interaction, which ultimately result in various cardiac and vascular injuries.

T and B cells, neutralizes viral particles, clears the virus, and sets long-term immunity.

Upon COVID-19 infection, pattern recognition receptors (PRRs) of antigen-presenting cells (APCs) detect pathogen-associated molecular patterns (PAMPs), namely, viral RNA and spike proteins as the main PAMPs in the case of SARS-CoV-2. Interaction of PAMPs with PRRs, such as membrane-bound Toll-like Receptors (TLRs), or cytosolic RIG-I-Like Receptors (RLRs), alongside the recruitment of cytoplasmic molecular adapters, such as MyD88, stimulates

a variety of signaling cascades, mediating cytoplasmic transcription factors, such as nuclear factor kappa B (NF-KB), interferon regulatory transcription factor 3 (IRF3) translocating toward nuclear (29, 30). NF-KB facilitates the expression of genes in innate and adaptive immune response, as well as the development of cytokine storm (31, 32).

Subsequently, the immune cells produce cytokines, such as interferons (IFNs), interleukins (ILs), chemokine, and tumor necrosis factor (TNF), exerting broad antiviral effects (33). Type 1 IFNs, produced at the early stage of SARS-CoV-2 infection, exhibit pivotal antiviral effects by promoting apoptosis of virus-infected cells and antigen presentation to T cells *via* the induced expression of major histocompatibility complex class I (MHC I) (34, 35). However, SARS-CoV-2 produces multiple interferon antagonists and impairs IFN actions, resulting in viral replication, inflammation, and hypercytokinemia, which are considered the main causes of COVID-19 severity (32).

The adaptive immune response also plays a pivotal role in virus defense. B cells release virus-specific antibodies with the help of CD4+ T cells while CD8+ T cells mediate direct apoptosis of virus-infected cells (36). In the process, antigen presentation by APCs is essential for the adaptive immune response of T and B lymphocytes. However, COVID-19 infection is characterized by lymphopenia (37). Probably, SARS-CoV-2 exerts immune evasion through impaired maturation of dendritic cells, leading to hampered dendritic cells (DCs) homing to lymph nodes and failure of T lymphocytes activation (38).

In addition to direct viral infection, exacerbated inflammatory drivers and dysregulated cell-mediated response contribute to cardiovascular injuries in COVID-19 infection. Noteworthy, the elderly population are vulnerable to cardiovascular injuries and poor prognosis, partly attributed to the age-related changes of the immune system. Aging is well-characterized by chronic inflammatory responses in the absence of infection, also called inflammaging (39). Proper inflammation is necessary for pathogen clearance and tissue repair, whereas inflammaging is associated with tissue damage and disease. Meanwhile, with age, there is a decline in both the count and functionalities of immune cells. The DCs from aged mice and humans are less efficient to migrate, secret cytokines, and prime T cells in viral defense (40, 41). Less production of new lymphocytes was observed in the aged population (42). Sex hormones participate in immune activities directly, through the expression of estrogen or testosterone receptors on immune cells, such as lymphocytes and macrophages (17). The hormonal changes with aging may, to some extent, elucidate the age-related changes of the immune response.

ENDOTHELIOPATHY AND COAGULOPATHY

Endothelial dysfunction and coagulopathy are hallmarks of COVID-19 infection. The increased levels of von Willebrand factor (VWF) antigen, D-dimers, and tissue plasminogen activator are reported in the COVID-19 group, substantiating endothelial damage and pro coagulation in COVID-19 infection (43, 44). Autopsy cases identify lymphocytic endotheliitis, frequent microthrombi as well as venous and arterial thromboembolism (16, 45, 46). A recent study indicates persistent endothelial damage in post-COVID-19 patients; on the other hand, Charfeddine et al. demonstrate that the lasting endothelial dysfunction is an independent risk factor of long COVID-19 syndrome (47, 48).

The wide distribution of ACE2 on ECs makes it a direct target for SARS-CoV-2 entry (49). Virus-cell binding downregulates membrane ACE2, resulting in reduced degradation of Ang II and decreased production of Ang (1–7). Subsequently, the mounting Ang II/AT_1R interaction exhibits pro-inflammatory cytokines secretion and pro-thrombotic actions by limiting NO and prostacyclin release. Otherwise, ACE2 regulates the kinin-kallikrein systems and participates in the inactivation of circulating bradykinin (BK). In this regard, ACE2 loss in COVID-19 infection leads to an increased level of BK, which induces EC activation and dysfunction, together with increased vascular permeability (50, 51). Exposure to SARS-CoV-2 spike protein *in vitro* stimulates caspase and apoptosis in ECs, whereby the loss of endothelial integrity triggers hypercoagulation (51).

The vascular endothelium participates in immune response and inflammation. Cytokines, such as IL-6, activate ECs, and in turn, the activated ECs express plenty of adhesion molecules, i.e., intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), resulting in the recruitment of leukocytes and platelets. In addition, ECs express different TLRs, mediating PAMPs recognition and antigen presenting to T cells (52). Regulated EC activation helps in limiting pathogen invasion, whereas the hyperinflammatory profile, often seen in the severe COVID-19 cases, promotes profound endothelial dysfunction and damage, contributing to multiple organ failure (46).

Resting ECs also participate in the dynamic interplay between coagulation and fibrinolysis. Direct SAR-CoV-2 infection induces endothelium injury and apoptosis, decreasing its antithrombotic activity. In addition, in the setting of inflammation, inflammatory molecules or injured ECs stimulate coagulation by increasing tissue factor (TF) expression by monocytes and ECs *in vitro* (53). TF and its downstream activated factors ultimately stimulate the coagulation cascade and produce clots (54). In addition, SARS-CoV-2 infection, together with SARS and Middle East respiratory syndrome (MERS), is correlated with thrombocytopenia (55). One explanation is that platelets are hyper-activated in these viral-infected patients, probably owing to hypoxia, immune responses, and endothelial dysfunction in the case of COVID-19 (16, 55). The activated platelets interact with leukocytes, contributing to the leukocyte cytokine release, such as CC-chemokine ligand 2 (CCL2), CCL3, IL-1β, and the release of neutrophil extracellular traps (NETs) wrapped with TFs, which in turn activates the extrinsic coagulation cascade resulting in thrombin formation (56, 57). Terminal complement components, such as the C5b-9 (membrane attack complex) and the C4d, have been discovered in the microvasculature, suggesting the association of complement system with microvascular injury in COIVID-19 (58).

Indeed, cardiovascular complications of COVID-19 are highly prevalent and contain acute cardiac injury, myocarditis, and a hypercoagulable state, all of which may be influenced by endotheliopathy and coagulopathy. Age is the main risk factor for COVID-19-related death and intensive care unit (ICU) admission. Age-associated EC dysfunction might be the reason for the poor prognosis in the elderly, leading to vascular pathologies and cardiovascular diseases. Abundant evidence demonstrates that the impaired endothelium-dependent NO-mediated vasodilation is associated with cardiovascular events, and the findings that endothelial nitric oxide synthase (eNOS)-deficient mice display a premature cardiac aging phenotype together with early mortality indicate the critical role of endothelium-derived NO on cardiovascular protection in aging (59, 60). The reduced bioavailability of NO contributes to age-associated impairment of angiogenesis, leading to ischemic tissue injury, such as myocardial ischemia and infarction (61). Csiszar et al. (62) show with advancing age, coronary arteries undergo pro-inflammatory alterations, age-related decline in NO bioavailability as well as upregulation of TNFα and caspase 9, promoting endothelial apoptosis.

COVID-19-RELATED CARDIOVASCULAR COMPLICATIONS IN ELDERLY

A variety of cardiovascular complications are documented in COVID-19, ranging from myocardial injury, myocarditis, arrhythmia, to cardiac dysfunction and heart failure. The crosstalk between RAS, hyper-inflammation, endotheliopathy, and coagulopathy accounts for the mechanism of cardiovascular involvement in COVID-19 (**Figure 2**).

- Direct viral infection induces myocardial injury, whereas virus-ACE2 binding brings overactive Ang II/AT1R actions, resulting in vasoconstriction and increased blood pressure because of its role as an endocrine regulator. Additionally, activated Ang II/AT_1R interacts with multiple intracellular signaling, for example, the MAPK family (63), and regulates the inflammatory process. In this sense, AT_1R triggers NF-kB activation, which promotes the gene expression of chemokines, cytokines, and adhesion molecules (64). The immune response and hyper-inflammation concerning SARS-CoV-2 infection, partly owing to Ang II/AT_1R action, lead to cardiac and vascular remodeling, as well as atherosclerotic plaque growth and rupture (63, 65) (**Figure 1B**).
  - Likewise, Ang II promotes oxidative stress and endothelial dysfunction *via* action on AT_1R and the downstream phagocytic nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) and reactive oxygen species (ROS) signaling (66), promoting lipid oxidation, macrophage uptake of lipids, and monocyte recruitment, leading to vascular inflammation and atherosclerosis (43, 67) (**Figure 1B**).
- In turn, previous studies show that the recruitment of immune cells, i.e., monocytes and macrophages, into the vascular wall strengthens the Ang II-induced endothelial dysfunction and inflammation (68, 69). At the same time, ECs participate in the SARS-CoV-2-induced immune response and hyper-inflammation, which in turn trigger endothelial injury as we discussed above.
- In addition, vascular smooth muscle cells (VSMCs), responsible for vascular homeostasis, also play a key role in disease progressions, such as hypertension and atherosclerosis. In the process, phenotypic switching of VSMCs has been considered of fundamental importance, transforming the contractile VSMCs to synthetic phenotypes, i.e., macrophage-like genotypes. Activated EC-VSMC interaction *via* inflammatory cytokines promotes the transition of VSMCs to macrophage-like phenotypes. Macrophage-like VSMCs acquire inefficient phagocytic functions and express different scavenger receptors, for example, low-density lipoprotein receptor-related protein 1, facilitating the influx of low-density lipoprotein, thus attributing to the formation of VSMC-derived foam cells and subsequent atherosclerotic plaque growth (70). Meanwhile, Ang II/AT1R action mediates proliferation and migration of VSMCs through phosphatidylinositol 3-kinase (PI3K)/Akt and MAPKs, affecting atherogenesis (71, 72). Therefore, we are assuming that VSMCs modulate COVID-19 progression and the relevant cardiovascular complications. To support that, more investigations are needed.

Aging is associated with dysregulated RAS, inflammaging, and endothelial dysfunction as we described above. Therefore, we speculate that RAS activation, immune and hyper-inflammatory actions, endotheliopathy, and coagulopathy, all of which mutually reinforce each other, together with the pre-existing aging-related dysregulations, unfold the underlying mechanisms of COVID-19 infection, and the contaminant cardiovascular complications in the elderly.

The incidence of COVID-19-related stroke, one of the most important primary cardiovascular outcomes, ranges from 1 to 6% in hospitalized patients with COVID-19 (73). Concerningly, stroke in patients with COVID-19 is associated with a poorer prognosis when compared to COVID-19 negative stroke patients (74). Moreover, COVID-19-related stroke is more often in the elderly population, particularly those with pre-existing disorders, such as hypertension, atherosclerosis, and atrial fibrillation (75). The pathogenesis of ischemic stroke, the dominant subtype of strokes, is multifactorial and similar to other arterial thromboses, it is developed in COVID-19. In this regard, the interplay of inflammation, coagulopathy, endotheliopathy, and platelet activation, together with cardioembolism, contribute to COVID-19-related ischemic stroke (73).

COMORBIDITIES

The elderly people often have to deal with various comorbidities, i.e., diabetes mellitus (DM), chronic kidney disease (CKD), dyslipidemia, all of which are risk factors of cardiovascular disease. In the case of COVID-19 infection, the interplay between the viral infection and the concomitant comorbidities might exacerbate COVID-19 outcomes, such as cardiovascular injuries.

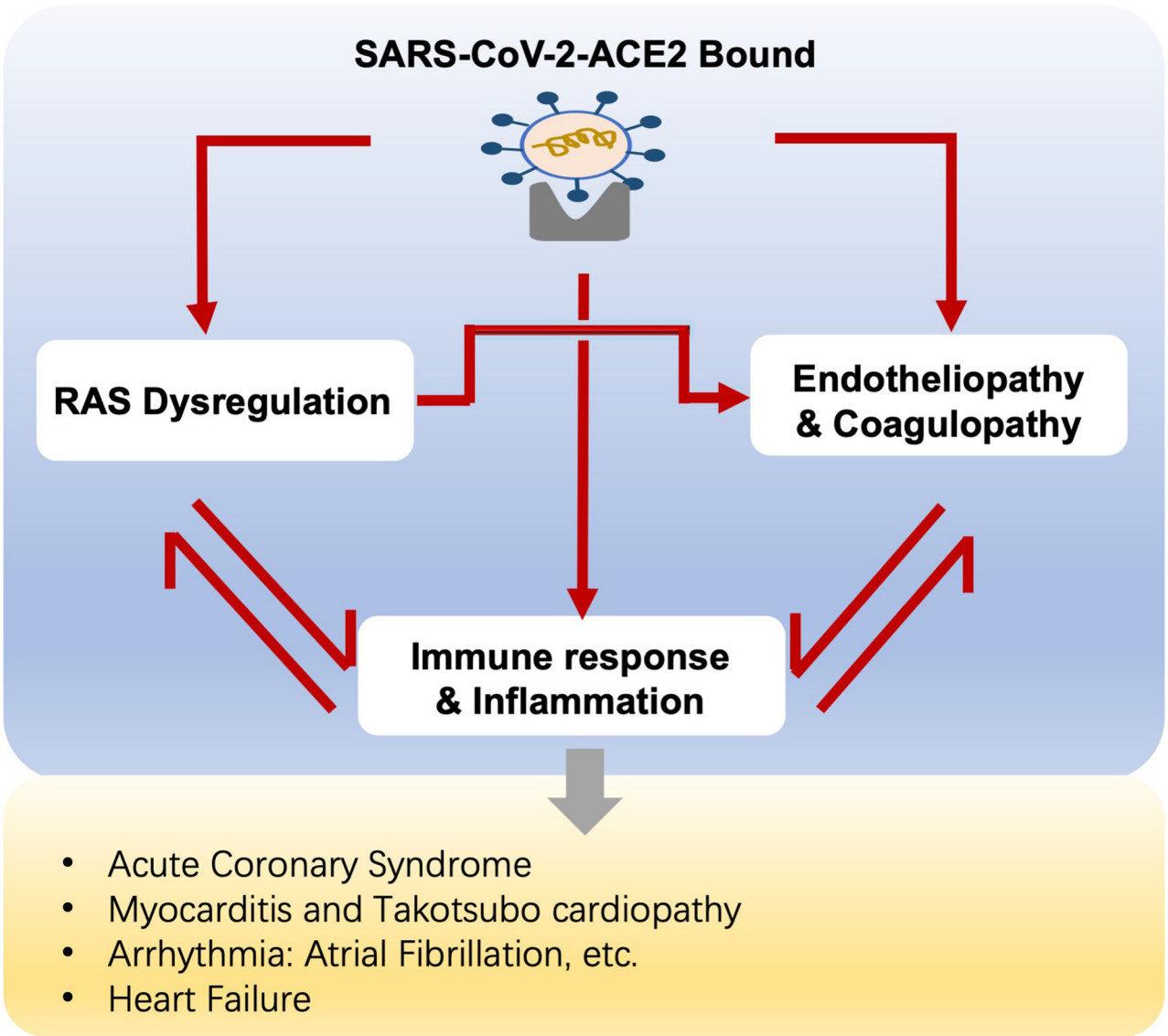

FIGURE 2 | Potential mechanisms of cardiovascular complications in COVID-19 patients.

Diabetes mellitus increases the risk of hospitalization, mortality, and need for critical care in COVID-19. The DM group with pre-existing systemic endothelial and microvascular dysfunction undergoes extra endothelial and microvascular impairment in COVID-19 infection and the "double-killing" results in worse prognosis and multiple organ failure (76).

The incidence of CKD increases with age, and 38% of the patients with CKD are more than 65 years old (77). Cardiovascular causes are recognized as the leading cause of death, accounting for 50%of the mortality in the CKD population (78). Therefore, it is of great importance to investigate the cardiovascular injuries in the elderly with CKD induced by the pandemic COVID-19 infection. A comprehensive review reveals the effect of CKD on increased hospitalization and mortality of COVID-19, perhaps owing to immune dysfunction and increased susceptibility to infections (77, 79).

Accumulating studies demonstrate that lipid disorders are associated with an increased risk of COVID-19 progression by 39% (80, 81). Although Petrilli et al. (82) show no correlation between dyslipidemia and prognosis of COVID-19. Cholesterol is an essential factor in lipid rafts, which are involved in the entry of SARS-CoV-2. Therefore, increased cholesterol level increases susceptibility to SARS-CoV-2 (83). On the other hand, COVID-19 alters lipid metabolism, characterized by a decrease in total cholesterol, high-density lipoprotein, low-density lipoprotein, and an increase in triglycerides (83).

CONCLUSION

The COVID-19 pandemic has swept the world and brought significant loss of health, life, and livelihoods, especially in the aged and those with underlying cardiovascular diseases. To our current knowledge, the COVID-19 is initiated as the viral-ACE2, the dominant host receptor interaction, and the subsequent effects on RAAS signaling, immune system, endothelium, and thrombosis confer to the complex pathologies in the viral infection. The findings of ACE/ACE2 imbalance, dysregulation of immune responses, endothelial dysfunction, and angiogenesis impairment in the elderly might explain the more severe conditions and cardiovascular involved in the old patients of COVID-19 infection.

Consequently, during the course of treatment for COVID-19, medical experts/clinicians must pay particular attention to protecting the cardiovascular system. Elderly patients with cardiovascular disease will be encountered significant healthcare disparities that exist in their management, when compared with younger counterparts. While making therapeutic decisions, age should not be considered in isolation but rather as one of many factors in the comprehensive assessment model, keeping in mind patients' overall health, frailty, cognition, quality of life, estimated life expectancy, and above all preferences. We should pay close attention to the comorbidities, balance the risk of ischemia and bleeding, and carefully adjust the medication dose. Overall, elderly patients with a history of cardiovascular disease remain undertreated with evidence-based therapies, experience worse outcomes, and represent an opportunity for enhancing and mitigating healthcare disparities. Scientists have developed vaccines for the coronavirus, which bring promise to tackle the global pandemic of COVID-19, especially for elderly patients. In addition, close monitoring of cardiac function in elderly patients with COVID-19 can prevent, or at least limit, myocardial injury, thereby reducing mortality. Further studies are urgently needed to more clearly elucidate the pathophysiology, host/pathogen interactions, the host immune response, and heart phenotype characteristics of COVID-19-infected elderly patients. The underlying mechanisms of myocardial injury, diagnosis, related effective medical treatment strategies, and follow-up are required to advance targeted treatments and improve patient prognosis.

AUTHOR CONTRIBUTIONS

YY searched and selected the references, and wrote the first draft of the review. YY and MY contributed towards literature review and interpretation of the manuscript. MY helped to determine the content and structure of the review, and contributed to the writing and revision of the manuscript. All authors approved the final version of the paper.

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Acute Coronary Syndromes and SARS-CoV-2 Infection: Results From an Observational Multicenter Registry During the Second Pandemic Spread in Lombardy

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Background: COVID-19 had an adverse impact on the management and outcome of acute coronary syndromes (ACS), but most available data refer to March-April 2020.

Aim: This study aims to investigate the clinical characteristics, time of treatment, and clinical outcome of patients at hospitals serving as macro-hubs during the second pandemic wave of SARS-CoV-2 (November 2020-January 2021).

Methods and Results: Nine out of thirteen "macro-hubs" agreed to participate in the registry with a total of 941 patients included. The median age was 67 years (IQR 58-77) and ST-elevation myocardial infarction (STEMI) was the clinical presentation in 54% of cases. Almost all patients (97%) underwent coronary angiography, with more than 60% of patients transported to a macro-hub by the Emergency Medical Service (EMS). In the whole population of STEMI patients, the median time from symptom onset to First Medical Contact (FMC) was 64 min (IQR 30-180). The median time from FMC to CathLab was 69 min (IQR 39-105). A total of 59 patients (6.3%) presented a concomitant confirmed SARS-CoV-2 infection, and pneumonia was present in 42.4% of these cases. No significant differences were found between STEMI patients with and without SARS-CoV-2 infection in treatment time intervals. Patients with concomitant SARS-CoV-2 infection had a significantly higher in-hospital mortality compared to those without (16.9% vs. 3.6%, $P < 0.0001$). However, post-discharge mortality was similar to 6-month mortality (4.2% vs. 4.1%, $P = 0.98$). In the multivariate analysis, SARS-CoV-2 infection did not show an independent association with in-hospital mortality, whereas pneumonia had higher mortality (OR 5.65, $P = 0.05$).

Conclusion: During the second wave of SARS-CoV-2 infection, almost all patients with ACS received coronary angiography for STEMI with an acceptable time delay. Patients with concomitant infection presented a lower in-hospital survival with no difference in post-discharge mortality; infection by itself was not an independent predictor of mortality but pneumonia was.

Keywords: acute coronary syndrome, COVID-19, coronary angiography, hub, STEMI (myocardial infarction)

INTRODUCTION

From the beginning of 2020, the world has had to face the COVID-19 pandemic caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection. Italy has been one of the most affected countries in Europe with more than seven million infections and over one hundred thousand deaths (1). In addition to mortality directly caused by severe acute respiratory syndrome and viral interstitial pneumonia, the COVID-19 pandemic played an indirect adverse effect on overall mortality excess, mainly by the necessity to divert resources from the optimal treatment of time-dependent medical and surgical emergencies to COVID-19 cases as a consequence of the dramatic surge in hospital admissions due to SARS-CoV-2 infection (2). An excess in cardiovascular deaths has been observed during 2020 compared to 2019 (3), which could be related to several factors, including reduction of acute coronary syndrome (ACS) hospitalizations, delay in ST-elevation myocardial infarction (STEMI) hospital presentation, an increase of out-of-hospital cardiac arrests, reduction of coronary revascularization procedures, and reduction of outpatient surveillance (4–7). Moreover, direct cardiac involvement has been reported in patients with COVID-19, and patients with ACS and concomitant infection had the worst outcome compared to patients without (8–12). Most of the available data refer to the first spread of the SARS-CoV-2 pandemic that occurred during the first months of 2020, while a second wave of the pandemic was observed worldwide between the end of 2020 and the beginning of 2021.

Lombardy, the most densely populated region in Italy, has been dramatically affected both during the first and the second wave of infection. To guarantee an optimal time of treatment for clinical emergencies, the regional healthcare authorities applied, during the first spread, a model of centralization called "macro-hubs" that was organized according to the estimated patient transportation time and the geographical features of the region. A detailed description of this model has been previously described and a retrospective analysis of its application, during the first wave, found an acceptable time delay in the ACS treatment (13, 14) of patients. This centralization model was, hence, further adopted during the second pandemic wave.

In the present study, we aimed to investigate the clinical characteristics, time to treatment, and clinical outcome of patients hospitalized at the macro-hub centers identified by the healthcare authorities of Lombardy during the second pandemic wave of SARS-CoV-2, from November 2020 to January 2021. Moreover, we performed an exploratory assessment of the GRACE score predictive performance in the present pandemic context.

MATERIALS AND METHODS

This study presents a retrospective analysis of prospectively collected data from a multicenter observational registry of consecutive patients with diagnoses of ACS hospitalized during the second SARS-CoV-2 pandemic spread. The macro-hubs involved in the registry and the duration of data collection (from 2 November 2020 to 31 January 2021) were based on the application of the decrees by Lombardy health authorities. The decrees defining a macro-hub were: (a) to perform primary percutaneous coronary intervention (PPCI) to all incoming STEMI on a 24/7 basis; (b) to guarantee a PPCI team was available 24/7 in the hospital (rather than on-call); (c) to provide separate pathways for patients with ACS and suspected/diagnosed COVID-19 from triage to catheterization laboratory and isolated care unit to avoid the risk of cross-infections.

At each participating hospital, a principal investigator was responsible for data collection in a custom electronic database provided by the coordinating center (Cardiology Department, University of Milan, ASST Santi Paolo e Carlo, Milan, Italy). At the end of data collection, the completed databases were submitted to the coordinating center for data analysis.

The study complies with the Declaration of Helsinki and was approved by the local institutional review board of each participating center. Patients gave their informed consent at admission for data collection and future publications in anonymous studies.

Study Population

Eligible patients were included in the registry if they received a diagnosis of ACS during hospitalization. STEMI was defined as typical symptoms lasting at least 20 min and persistent ST-elevation of $\geq$ 2 mm in at least two contiguous leads or new or presumed new left bundle-branch block. NSTEMI was defined as new onset or worsening angina (or equivalent) and elevated biomarkers of myocardial necrosis (troponin I or T above the upper limits of normal at each study site) with or without associated electrocardiographic signs of ischemia (ST-depression, transient ST-elevation, or T-wave inversion). Unstable angina (UA) was defined by the absence of troponin elevation.

The diagnosis of SARS-CoV-2 infection was based on the positive nasopharyngeal swab, bronchoalveolar lavage, and a

pulmonary TAC diagnostic for interstitial pneumonia, as a single test or in combination.

Patients with either STEMI or high-risk non-ST-elevation ACS (NSTE-ACS) (presence of hemodynamic and/or electrical instability, recurrent or ongoing chest pain refractory to medical treatments, and/or relevant ST-T wave changes) were directly transferred to the catheterization laboratory with the execution of a nasopharyngeal swab. Patients with low- or intermediate-risk NSTE-ACS were evaluated in the emergency department (ED) and underwent nasopharyngeal swab immediately, deferring percutaneous coronary intervention (PCI) decision after swab results and clinical conditions. All patients, regardless of the immediate treatment decision, were admitted to different wards according to their molecular nasopharyngeal swab results.

Data Collection

For each patient, the following data were collected: demographic characteristics, cardiovascular risk factors, prior cardiac events or procedures, presence of cardiogenic shock, pulmonary edema or cardiac arrest on or before admission, site of STEMI at ECG, and echocardiographic left ventricular ejection fraction (LVEF). Moreover, blood hemoglobin, white blood cells, estimated glomerular filtration rate (eGFR) (CKD-EPI formula), and troponins values at admission were collected. Finally, the Global Registry of Acute Coronary Events (GRACE) score at admission was calculated (15). Data about in-hospital pharmacological treatments and interventional procedures had to be reported for all included patients.

For patients with STEMI, we analyzed the critical time intervals: "symptom-onset to first medical contact (FMC) (defined as the diagnosis by 12-lead electrocardiogram) and "FMC to arrival at catheterization laboratory (CathLab)."

As clinical adverse events, we considered the in-hospital occurrence of all-cause death, acute pulmonary edema, shock, cardiac arrest, acute kidney injury (AKI), major bleedings, pneumonia, and need for invasive and/or non-invasive ventilation. AKI was defined according to Kidney Disease Improving Global Outcomes (KDIGO) guidelines (16) and bleeding events were appraised according to Bleeding Academic Research Consortium (BARC) definitions (17). Total mortality was also collected at a 6-month follow-up.

Statistical Analysis

Categorical data are reported as absolute values and percentages and compared using the chi-square test; continuous variables are described as the median and interquartile range (IQR) and compared using the Mann–Whitney test. The associations between clinical variables and clinical events were investigated using univariate and multivariate logistic regression analysis. The GRACE score predictive performance for in-hospital and post-discharge mortality was assessed using the C-statistic and receiver operating characteristic curves. The software used for statistical analysis was MedCalc Statistical Software version 16.2.0 (MedCalc Software bvba, Ostend, Belgium) and the cut-off adopted for statistical significance was $P < 0.05$.

RESULTS

Nine out of thirteen "macro-hubs" of the Lombardy region agreed to participate in the registry during the second pandemic wave and a total of 941 consecutive patients were included.

The baseline demographic and clinical characteristics and in-hospital treatments of the overall population are summarized in **Table 1**. The median age was 67 years (IQR 58-77), 30% were ≥ 75 years old, and 26% were females. STEMI

TABLE 1 | Baseline characteristics of the overall population.

VARIABLE	*N* = 941
Age, years, median (IQR)	67 (58–77)
Age ≥ 75 years, n (%)	284 (30)
Females, n (%)	242 (26)
Arterial hypertension, n (%)	625 (66.4)
Diabetes mellitus, n (%)	225 (24)
Hyperlipidemia, n (%)	477 (51)
Active smoking, n (%)	237 (25)
Previous MI, n (%)	195 (20.7)
Previous PCI, n (%)	212 (22.5)
Previous CABG, n (%)	54 (5.7)
Clinical presentation	
STEMI, n (%)	507 (54)
NSTE-ACS, n (%)	434 (46)
LVEF,%, median (IQR)	50 (40–55)
GRACE score, median (IQR)	121 (100–143)
Acute pulmonary edema, n (%)	55 (5.8)
Shock, n (%)	37 (3.9)
Cardiac arrest, n (%)	40 (4.3)
SARS-CoV-2 infection, n (%)	59 (6.3)
Blood samples	
Hemoglobin at admission, gr/dl, median (IQR)	14 (13–15)
White blood cells at admission, n/mcl, median (IQR)	9.8 (7.6–12)
Troponin at admission, ng/dl, medin (IQR)	0.25 (0.04–1.75)
eGFR at admission, ml/min/1.73 mq, median (IQR)	79.9 (59–92.6)
Coronary angiography and revascularization	
Coronary angiography, n (%)	914 (97)
STEMI, n (%)	494 (97.4)
NSTE-ACS, n (%)	420 (96.8)
Radial artery access, n (%)	809 (88.5)
PCI, n (%)	762 (83.4)
CABG, n (%)	60 (6.5)
Complete revascularization, n (%)	574 (60)
IABP, n (%)	56 (6)
PMCS, n (%)	7 (0.7)
Drug therapy	
Aspirin, n (%)	857 (91)
P2Y12 inhibitors, n (%)	778 (82.6)
Glycoprotein IIb/IIIa inhibitors, n (%)	125 (13.3)
Inotropic drugs, n (%)	91 (9.7)

CABG, coronary artery by-pass grafting; eGFR, estimated glomerular filtration rate; IABP, intra-aortic balloon pump; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NSTE-ACS, non ST elevation acute coronary syndrome; PCI, percutaneous coronary intervention; PMCS, percutaneous mechanic circulatory support; STEMI, ST elevation myocardial infarction.

was the clinical presentation in 54% of the cases (anterior site in 52%). The GRACE score at admission was 121 (IQR 100-143). Overall, 97% of the patients underwent coronary angiography (97.4% of STEMI and 96.8% of NSTE-ACS patients). Multivessel coronary artery disease (CAD) was present in 51% of cases, and there was no significant angiographic CAD in 8% of cases. A PCI was performed in 83.4% of the cases (90.7% of patients with STEMI and 74.8% of patients with NSTE-ACS), and coronary artery by-pass grafting (CABG) was performed in 6.5% of cases. Complete revascularization was obtained in 60% of cases within index admission.

Sixty percent of the patients were transported to a macro-hub by the Emergency Medical Service (EMS), whereas 26% self-presented to the ED of a macro-hub and 12.8% were transferred from spoke centers; the remaining patients were already at the hospital at the time of ACS.

Patients With Concomitant SARS-CoV-2 Infection

A total of 59 patients (6.3%) had concomitant confirmed SARS-CoV-2 infection. **Table 2** shows the comparisons between demographic, baseline clinical characteristics,

TABLE 2 | Comparison between patients with and without SARS-CoV-2 infection.

VARIABLE	SARS-CoV-2 (*N* = 59)	No SARS-CoV-2 (*N* = 882)	*P*-value
Age, years, median (IQR)	69 (62–77)	67 (58–77)	0.29
Age ≥ 75 years, n (%)	19 (32.2)	265 (30)	0.72
Females, n (%)	11 (18.6)	231 (26.2)	0.19
Arterial hypertension, n (%)	44 (74.6)	581 (65.9)	0.17
Diabetes mellitus, n (%)	19 (32.2)	206 (23.4)	0.12
Hyperlipidemia, n (%)	27 (45.8)	450 (51)	0.43
Previous MI, n (%)	16 (27)	179 (20.3)	0.21
Previous PCI, n (%)	16 (27)	196 (22.2)	0.38
Clinical presentation			
STEMI, n (%)	33 (56)	474 (53.7)	0.74
NSTE-ACS, n (%)	26 (44)	408 (46.3)	
LVEF,%, median (IQR)	48 (38–55)	50 (40–55)	0.09
GRACE score, median (IQR)	139 (105–158)	121 (100–142)	0.02
Acute pulmonary edema, n (%)	4 (6.8)	51 (5.8)	0.75
Shock, n (%)	3 (5.1)	34 (3.9)	0.64
Cardiac arrest, n (%)	3 (5.1)	37 (4.2)	0.74
Pneumonia, n (%)	25 (42.4)	7 (0.8)	<0.0001
Blood samples			
Hemoglobin at admission, gr/dl, median (IQR)	13.9 (12.3–15.4)	14 (12.8–15.2)	0.57
White blood cells at admission, n/mcl, median (IQR)	9.04 (7.55–11.19)	9.81 (7.64–12.20)	0.18
Troponin at admission, ng/dl, median (IQR)	0.61 (0.13–2.14)	0.24 (0.04–1.67)	0.04
eGFR at admission, ml/min/1.73 mq, median (IQR)	74 (52–90)	80 (59–93)	0.27
Diagnostic and therapeutic procedures			
Coronary angiography, n (%)	58 (98)	856 (97)	0.57
No significant CAD, n (%)	6 (10.3)	67 (8)	0.25
SVD, n (%)	18 (31)	355 (41.5)	
MVD, n (%)	34 (58.6)	434 (50.7)	
PCI, n (%)	47 (81)	715 (83.5)	0.62
CABG, n (%)	4 (6.9)	56 (6.5)	0.90
Complete revascularization, n (%)	29 (49)	545 (64)	0.04
IABP, n (%)	5 (8.5)	51 (5.8)	0.39
PMCS, n (%)	0 (0)	7 (0.8)	0.78
NIV, n (%)	13 (22)	26 (2.9)	<0.0001
IMV, n (%)	1 (1.7)	13 (1.5)	0.89
Drug therapy			
Aspirin, n (%)	52 (88)	805 (91)	0.41
P2Y12 inhibitors, n (%)	47 (79.7)	731 (82.9)	0.53
Glycoprotein IIb/IIIa inhibitors, n (%)	11 (18.6)	114 (12.9)	0.21
Inotropic drugs, n (%)	6 (10.2)	85 (9.6)	0.89

CABG, coronary artery by-pass grafting; CAD, coronary artery disease; eGFR, estimated glomerula filtration rate; IABP, intra-aortic balloon pump; IMV, invasive mechanical ventilation; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MVD, multivessel disease; NIV, non -invasive ventilation; NSTE-ACS, non ST elevation acute coronary syndrome; PCI, percutaneous coronary intervention; PMCS, percutaneous mechanic circulatory support; STEMI, ST elevation myocardial infarction; SVD, single vessel disease.

TABLE 3 | Time to treatment in the overall STEMI population and separately in patients with and without SARS-CoV-2 infection.

	Overall STEMI *N* = 507	SARS-Cov-2 *N* = 33	No SARS-Cov-2 *N* = 474	*P*-value
Symptom onset-FMC, median (IQR)	64 (30–180)	77 (37–240)	60 (30–180)	0.40
FMC-CathLab, median (IQR)	69 (39.5–105)	65 (37–160)	70 (40–125)	0.98

FMC, first medical contact.

TABLE 4 | Clinical outcomes in the overall population and separately in patients with and without SARS-CoV-2 infection.

	Overall population	SARS-Cov-2	No SARS-Cov-2	*P*-value
Acute pulmonary edema, n (%)	38 (4)	1 (1.7)	37 (4.2)	0.34
Shock, n (%)	49 (5.1)	7 (11.9)	42 (4.8)	0.02
In-hospital cardiac arrest, n (%)	66 (7)	6 (10.2)	60 (6.8)	0.32
Major bleedings, n (%)	37 (3.9)	3 (5.1)	34 (3.8)	0.84
AKI, n (%)	91 (9.7)	10 (16.9)	81 (9.2)	0.13
In-hospital mortality, n (%)	42 (4.5)	10 (16.9)	32 (3.6)	<0.0001
Mortality at 6 months among hospital survivors, n (%)	36 (4.1)	2 (4.2)	34 (4.1)	0.98

AKI, acute kidney injury.

and in-hospital treatments of patients with and without SARS-CoV-2 infection.

In these patients, STEMI was the clinical presentation in 56% of cases (a rate comparable to that observed in patients without SARS-CoV-2 infection). The GRACE score was 139 (IQR 105-158), significantly higher than in patients without infection. Almost all patients (about 98%) underwent coronary angiography in both groups, and no significant differences were found in CAD extension; however, patients with SARS-CoV-2 infection presented a non-significant higher rate of no significant CAD (10.3 vs. 8%). PCI was performed in 81% of cases and CABG in 6.9%. Complete revascularization was obtained in 49% of cases, a significantly lower rate compared to that observed in patients without SARS-CoV-2 infection (64%, $P = 0.04$).

Pneumonia was present in 42.4% of patients with SARS-CoV-2 infection (vs.8% in patients without SARS-CoV-2 infection, $P < 0.0001$). Significantly more patients with COVID-19 underwent non-invasive ventilation (NIV) (22 vs. 2.9%, $P < 0.0001$), whereas no significant difference was observed regarding invasive mechanical ventilation utilization (IMV) between patients with and without COVID-19.

Diagnosis and Treatment Times

Table 3 shows treatment times in the overall STEMI population and patients with and without SARS-CoV-2 infection.

In the whole population, the median time from symptoms-onset to FMC was 64 min (IQR 30-180). The median time from FMC to CathLab was 69 min (IQR 39-105). No significant differences were found between STEMI patients with and without infection in both time intervals.

Clinical Outcomes

Table 4 summarizes the clinical outcomes observed in the overall population and separately in patients with and without SARS-CoV-2 infection.

Except for cardiogenic shock, which was higher in patients with SARS-CoV-2 infection (11.9 vs. 4.8%, $P = 0.02$), no significant differences were found in the incidence of the other adverse events. In-hospital mortality was 4.5% in the overall population and was significantly higher in patients aged $\geq$ 75 years (8.1 vs. 2.9%, $P = 0.004$) and in STEMI (5.9 vs. 2.8%, $P = 0.02$).

In patients with concomitant SARS-CoV-2 infection, in-hospital mortality was significantly higher than in patients without (16.9 vs. 3.6%, $P < 0.0001$). Although in the univariate logistic regression analysis the presence of infection was significantly associated with in-hospital mortality (OR 5.41, 95% CI 2.51–11.65, $P < 0.0001$), in the multivariate analysis it showed a weak and not significant association, whereas the presence of pneumonia showed an independent association but with a borderline statistical significance (**Table 5**).

Of the 899 patients discharged alive, mortality data at 6 months was available in 877 (98%). At this time point, mortality was 4.1% in the overall population and no significant difference

TABLE 5 | Regression coefficients and odds ratios from multivariate logistic regression analysis testing association between clinical variables and in-hospital mortality.

VARIABLE	Regression coefficient (SE)	*P*-value	Odds ratios (95% CI)
Age	0.046 (0.024)	0.05	1.04 (0.99–1.09)
Diabetes mellitus	0.130 (0.542)	0.79	0.87 (0.30–2.52)
STEMI	0.445 (0.550)	0.41	1.56 (0.53–4.58)
MVD	1.237 (0.359)	0.02	3.44 (1.17–10.15)
LVEF $\leq$ 35%	1.568 (0.526)	0.003	4.79 (1.71–13.46)
eGFR < 60 ml/min/1.73 mq	1.027 (0.530)	0.05	2.79 (0.98–7.90)
Cardiac arrest	1.327 (1.160)	0.25	0.26 (0.02–2.57)
Shock	2.537 (0.670)	0.0002	12.65 (3.39–47.10)
SARS-CoV-2 infection	1.415 (0.834)	0.08	4.11 (0.80–21.12)
Pneumonia	1.732 (0.901)	0.05	5.65 (0.96–33.06)

eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; MVD, multivessel disease; STEMI, ST elevation myocardial infarction. In the model were included all variables with P < 0.10 at the univariate analysis.

was found between patients with and without SARS-CoV-2 infection (4.2 vs. 4.1%, $P = 0.98$). Infection was not significantly associated with post-discharge mortality. In the multivariate regression analysis only age, LVEF $\leq$ 35% at discharge, and the diagnosis of pneumonia were independently associated with post-discharge mortality.

In order to evaluate the predictive performance of the GRACE score in the present pandemic context, with particular regard to SARS-CoV-2 patients, we tested the predictive accuracy of the GRACE score at admission both for in-hospital and post-discharge mortality. **Table 6** reports the results of the C-statistic. The score showed globally a good predictive performance for mortality, with higher C-statistic for in-hospital (0.85 95% CI.82–0.87, $p < 0.0001$) as compared to post-discharge mortality (0.75 95% CI.71–0.77, $p < 0.0001$), particularly with regard to in-hospital death in patients with concomitant SARS-CoV-2 infection (0.94 95% CI.82–0.98, $p < 0.0001$).

DISCUSSION

In the present article, we describe the presentation, time of care, and mortality data of patients with ACS managed at hospitals identified as "macro-hubs" in a specific geographical area during the second spread of SARS-CoV-2 infection with a modified network of assistance based on a model of centralization of care.

The main findings of our analysis are as follows: more than half of patients presented with STEMI and these were treated within the ESC-recommended time delay (18); patients with ACS and positive at SARS-CoV-2 had a higher baseline risk profile, as suggested by a significantly higher GRACE score, and significantly higher mortality compared to patients without infection. This excess mortality risk appears to be attributable to the presence of concomitant pneumonia.

A delay in STEMI treatment was one of the first observations reported as a consequence of the COVID-19 outbreak at the beginning of the pandemic (19); particularly, patients with STEMI and COVID-19 presented the longest time of assistance as a consequence of a prolonged time from symptom onset to hospital admission, mainly due to the lack of dedicated organization of the healthcare system and for the limited availability of EMS due to systemic overload (12).

TABLE 6 | Predictive values of the GRACE score for in-hospital and post-discharge mortality in the overall population and separately in patients with and without SARS-CoV-2 infection.

	C-statistic (95% CI)	Sens/Spec	*P*-value
In-hospital mortality			
Overall population	0.85 (0.82–0.87)	70/88	<0.0001
SARS-CoV-2 patients	0.94 (0.82–0.98)	100/88	<0.0001
NoSARS-CoV-2 patients	0.82 (0.79–0.85)	60/82	<0.0001
Post-discharge mortality			
Overall population	0.75 (0.71–0.77)	52/91	<0.0001
SARS-CoV-2 patients	0.82 (0.67–0.93)	100/62	<0.002
NoSARS-CoV-2 patients	0.73 (0.70–0.76)	50/90	<0.0001

The centralized model used in Lombardy did not show a negative impact on time to treatment; furthermore, as previously reported, the time from symptom onset to CathLab was significantly shorter during the second compared to the first spread of infection (February-May 2020) (20). In the present analysis, about 60% of STEMI were directly transported to a macro-hub by EMS. The STEMI care network available for 15 years in the Lombardy Region comprising 55 CathLabs, mostly performing 24/7 primary PCI, and a well territorially distributed EMS certainly contributed to this positive result. However, the application of standardized protocols for fast-tracking the treatment of STEMI during the pandemic was endorsed by scientific societies, (21) allowing healthcare workers to obtain results in terms of the time of reperfusion, clinical outcomes, and staff safety in line with those before pandemic (22).

Patients with concomitant infection presented a significantly higher rate of in-hospital death compared to patients without infection (16.9 vs. 3.6%), whereas post-discharge mortality was not affected (4.2 vs. 4.1%); furthermore, in the multivariate analysis, infection by itself was not an independent predictor of mortality, whereas pneumonia was, though with a borderline statistical significance. It has been previously reported that patients with ACS, particularly STEMI, and concomitant COVID-19 present worse outcomes: in the North American COVID-19 Myocardial Infarction Registry, the in-hospital mortality of these patients was 33% (11). In the present data, a significant difference between patients with and without infection was found only in the rate of pneumonia and in the need of non-invasive ventilation: therefore, it is likely that pulmonary complications continue to have an adverse prognostic impact on these patients during the acute phase, whereas for survivors no significant difference in mortality was found at mid-term follow-up. However, we have reported a higher rate of pneumonia during the first spread of COVID-19 (about 60%) in patients with ACS and concomitant infection (14) that has been reduced (but not erased!) in the second wave by early and specific treatment (e.g., steroids and ventilation strategies); furthermore, the wide availability of diagnostic tools led to the diagnosis of patients with less severe clinical infection.

Although the GRACE score is a well-established predictive tool for outcomes prediction in patients with ACS (15), to our knowledge little information exists about its usefulness during the COVID-19 pandemic. Based on this, we tried an explorative investigation on the predictive value of the GRACE score on mortality and we found a good value of C-statistic for the overall population that was even stronger for patients with SARS-CoV-2 infection. Although the GRACE score is used to predict clinical outcomes in patients with ACS beyond infections, the baseline value was higher in patients with SARS-Cov2. These observations suggest that patients with ACS and SARS-CoV-2 might have a worse baseline risk profile and that the GRACE score retains a good predictive power in these patients. In a similar study, a significant difference was not found for GRACE score between patients with and without infection but a value > 140 and the presence of COVID-19 were independent risk factors associated with higher in-hospital mortality (23).

Limitations

Small sample size, retrospective analysis, and lack of correction for covariates with consequent confounding bias can be considered as the main limitations of the present study. Furthermore, complete information on pharmacologic therapies was lacking. Finally, geographical differences do not allow definite conclusions and make our findings not necessarily representative of different areas in Italy or worldwide.

CONCLUSION

The main aim of our work was to offer an overall clinical picture of ACS population during the second pandemic wave of SARS-CoV-2 infection and to describe its prognosis within the macro-hub network implemented by the Lombardy region in order to cope with the COVID-19 pandemic (13). The present article adds further confirmation to what we observed previously (14, 20): a timely adequate treatment of STEMI patients was obtained and a better prognosis in overall patients with ACS, both with and without SARS-CoV-2 infection, was observed during the second pandemic wave, corroborating in our opinion the beneficial effect of the organizational strategy adopted. Moreover, patients with concomitant infection had lower in-hospital survival, whereas post-discharge mortality was similar; infection by itself was not an independent predictor of mortality, whereas pneumonia implied a higher mortality risk.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

SC, MF, and DC contributed to the conception and design of the study. DC, MF, and GF organized the database. DC performed the statistical analysis. MF and DC wrote the first draft of the manuscript. GM, MMo, SS, MD'U, CL, CC, MMa, and LV wrote the sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Overstimulation of the ergoreflex—A possible mechanism to explain symptoms in long COVID

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Long COVID refers to a multitude of symptoms that persist long after SARS-CoV-2 infection. Fatigue and breathlessness are the most common symptoms of long COVID across a range of studies. They are also cardinal symptoms of chronic heart failure (CHF). In this review, we propose that fatigue and breathlessness in patients with long COVID may be explained by skeletal muscle abnormalities, in a manner similar to patients with CHF. The ergoreflex is a cardiorespiratory reflex activated by exercise, which couples ventilation and cardiovascular function to exercise intensity. At least part of the symptomatology of CHF is related to abnormal skeletal muscle and an enhanced ergoreflex, resulting in heightened sympathetic, vasoconstrictor and ventilator drives. Similarly, SARS-CoV-2 infection results in a hyperinflammatory and hypercatabolic state. This leads to reduction in skeletal muscle mass and altered function. We postulate that the ergoreflex is chronically overstimulated, resulting in fatigue and breathlessness. Exercise training preserves muscle mass and function as well as reduces ergoreflex activation; therefore may have a role in improving symptoms associated with long COVID. Should the ergoreflex be proven to be an important pathophysiological mechanism of long COVID, tailored exercise interventions should be trialed with the aim of improving both symptoms and perhaps outcomes in patients with long COVID.

KEYWORDS

long COVID, ergoreflex, pathophyisology, breathlessness, fatigue, heart failure

Introduction

COVID-19 is a multisystem disease, affecting lungs, digestive tract, kidneys, heart, endocrine system and brain (1, 2). Long COVID refers to a multitude of symptoms that persist long after initial infection. Currently, National Institute for Health and Care Excellence (NICE) defines long COVID as ongoing signs and symptoms beyond 4 weeks after acute COVID-19; whilst the World Health Organisation (WHO) defines it as persistent symptoms 3 months following acute infection that last for at least 2 months and cannot be explained by an alternative diagnosis (3, 4).

Long COVID is a significant challenge for patients, physicians and society. Multiple mechanisms have been proposed to explain the pathophysiology of long COVID. These include viral persistence in certain tissues, immune dysregulation, SARS-CoV2 interactions with host microbiome/virome communities, chronic inflammation, prolonged prothrombotic state, and dysfunctional brainstem/vagus nerve signaling (1, 2). However, the exact etiology of long COVID remains unclear, and the patient profile and symptom patterns are variable and difficult to define with precision. Despite this, two symptoms—fatigue and breathlessness—are consistently the most common symptoms described in observation studies (5). The European Society for Clinical Microbiology and Infectious Diseases reports the prevalence of fatigue and breathlessness in long COVID to be 31–58% and 24–40%, respectively (6). Here, we propose that fatigue and breathlessness in patients with long COVID may be explained by skeletal muscle abnormalities, in a manner similar to patients with chronic heart failure (CHF).

Breathlessness and fatigue in chronic heart failure

To understand how skeletal muscle abnormalities may contribute to the development of breathlessness and fatigue in patients with long COVID, we will begin by examining how these symptoms arise in the context of CHF.

Fatigue and breathlessness are the dominant symptoms of patients with CHF. Traditionally, the pathophysiology underlying the symptoms was thought to be a consequence of inadequate cardiac pump function. Low cardiac output leads to abnormal muscle perfusion and signals are then transmitted to the brain which are interpreted as fatigue. In order to maintain cardiac output, the failing heart adapts by increasing left ventricular filling pressure. This leads to a rise in pulmonary venous pressure with pulmonary congestion often presenting as breathlessness. If this chain of events were to explain completely the symptoms of fatigue and breathlessness, then the severity of symptoms should be directly related to the severity of left ventricular systolic dysfunction. However, there is no relation between any measure of central hemodynamic function and exercise performance. In the last 30 years, a large body of research has demonstrated the importance of pathophysiological changes in the periphery as being responsible for the generation of fatigue and breathlessness.

The ergoreflex

The ergoreflex is a cardiorespiratory reflex activated by exercise which couples ventilation and cardiovascular function to exercise intensity. The existence of a reflex triggered by muscle activity was proposed in 1937 by Alam and Smirk (7). Healthy volunteers performed dynamic exercise while blood vessels draining the exercising limbs were occluded by a sphygmomanometer cuff. The exercise lasted for 4 min and circulatory occlusion was maintained for another 11 min. In the recovery period whilst circulatory occlusion was present, the rise in blood pressure reached during exercise was maintained and further increased after another 3–4 min. There was also a sustained increase in heart rate during circulatory occlusion. Blood pressure and heart rate dropped after removal of circulatory occlusion. It has been posited that a reflex triggered by accumulation of metabolites in exercising muscles is able to influence hemodynamic function; the "metaboreflex." The metaboreflex causes blood pressure to rise to ensure adequate perfusion of the exercising muscle. Animal models show that mechanical stimulation of muscles and tendons also leads to increase heart rate and blood pressure; this is known as the "mechanoreflex" (8).

The combination of the "mechanoreflex" and "metaboreflex" forms the ergoreflex. The mechanoreflex is activated at the beginning of exercise by mechanical stretching of the muscles and tendons. Afferent stimuli are transmitted rapidly *via* thinly myelinated group III fibers in the muscle interstitial space. The metaboreflex is activated by accumulation of metabolites in the exercising muscle, including lactate, hydrogen, and potassium ions, prostaglandins and bradykinin. These are sensed by receptors in the muscle interstitial space, e.g., acid-sensing ion channels, cannabinoid receptors and μ-opioid receptors. Afferent stimuli are transmitted *via* small non-myelinated group IV fibers with a period of latency. Signals from both components integrate with other peripheral and central signals (such as the chemoreflex and baroreflex) in the central nervous system. The efferent limb of the reflex results in increased ventilation and sympathetic activation, in turn causing peripheral resistance and cardiac output to rise, thereby maintaining systemic blood pressure (**Figure 1**) (8).

The ergoreflex and heart failure

At least part of the symptomatology of CHF is related to abnormal muscle and an enhanced ergoreflex. Skeletal muscle

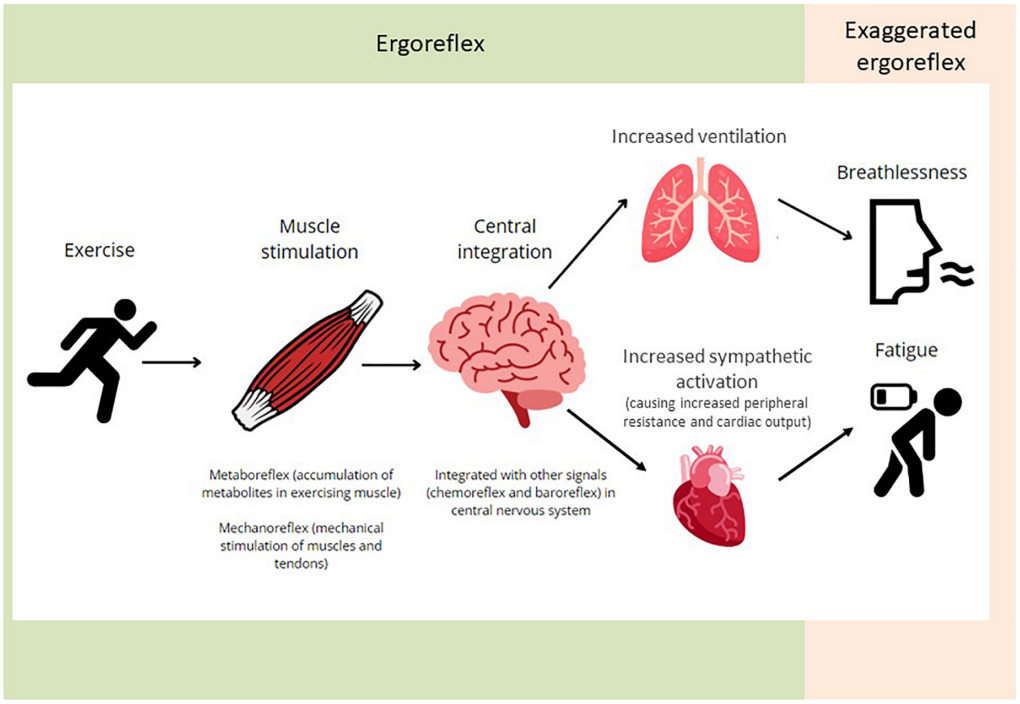

FIGURE 1
An illustration of the ergoreflex. Skeletal muscle exercise causes stimulation of the metabo- and mechano-receptors. The signals are integrated centrally and contribute to the ventilatory and cardiovascular responses to exercise. Exaggerated ergoreflex (e.g., heart failure) leads to excessive response relative to work performed, leading to the sensation of breathlessness and fatigue.

loss and dysfunction is common, resulting in sarcopenia and cachexia, both of which are associated with poor clinical outcomes (9). Histologically, patients with CHF have a shift in muscle fiber distribution from aerobic type I fibers to anaerobic type II fibers. Mitochondrial structure is also abnormal, with a reduction in the volume of cristae and fall in the enzymes of the Krebs cycle (10). Cardiac dysfunction leads to abnormal muscle physiology *via* several mechanisms including the release of proinflammatory cytokines, mitochondrial dysfunction, physical inactivity as a result of lower exercise tolerance, intestinal congestion and malnutrition. There is resistance to pro-anabolic hormones such as insulin. Skeletal myopathy increases ergoreflex sensitivity, leading to exertional breathlessness due to a greater ventilatory response to a given amount of exercise. Chronic sympathetic activation also results in peripheral vasoconstriction and increased cardiac afterload, leading to a vicious cycle of progressive muscle and cardiac dysfunction (10).

Piepoli et al. examined the ergoreflex in patients with CHF. Subjects performed handgrip exercise using their non-dominant arm by performing two 5-min handgrip manoeuvers at approximately 50% of pre-determined maximal contraction, in random order, separated by 30 min rest: one bout with circulatory occlusion during the last 10 s of exercise and the first 3 min of recovery ("clamp session") and one bout with no occlusion (**Figure 2**). Ergoreceptor sensitivity was quantified as the percentage of the ventilatory and hemodynamic response to exercise maintained by circulatory occlusion during the third minute of recovery, compared with the third minute of recovery without occlusion (11).

Patients with CHF had a much greater ergoreflex than controls with heightened sympathetic, vasoconstrictor and ventilatory drives (**Figure 2**). Interestingly, these abnormalities are potentially reversible. After 6 weeks of forearm training, there was a marked reduction in ergoreflex activity. These findings perhaps underlie some of the beneficial effects of exercise training in CHF: an improvement in muscle structure and function by exercise training can reduce ergoreflex sensitivity, thereby leading to a reduction in symptoms (11).

Skeletal muscle changes and ergoreflex activation in long COVID

Acute SARS-CoV-2 infection results in a hypercatabolic state (12, 13). Firstly, insulin resistance is common in patients with COVID-19 during acute infection (14). As a consequence of the RECOVERY trial, universal treatment with corticosteroids for patients hospitalized with COVID-19

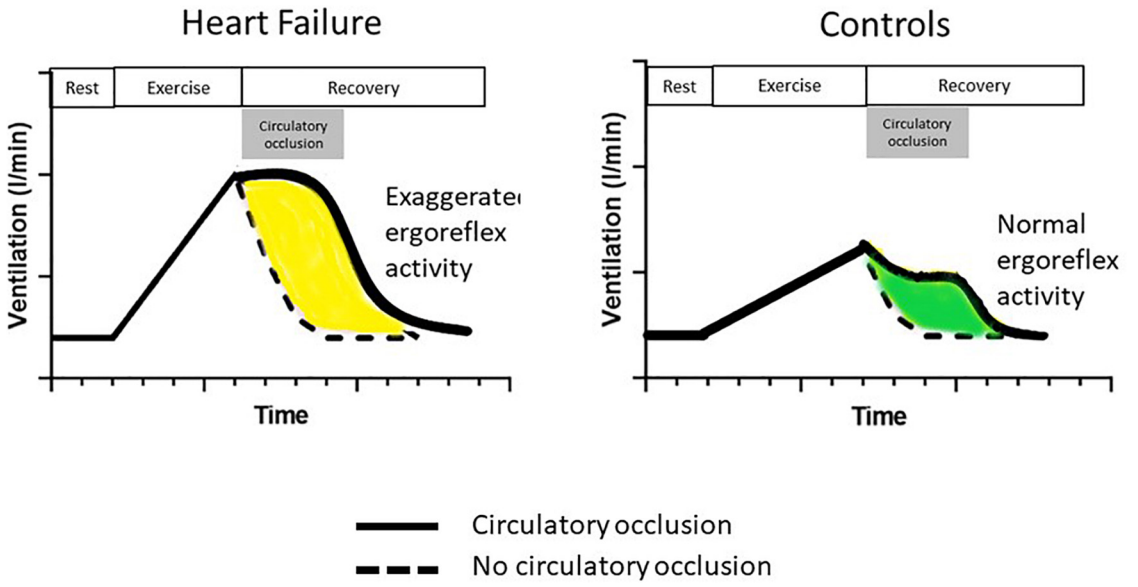

FIGURE 2
The ergoreflex in chronic heart failure. Following handgrip dynamometer exercise to exhaustion, a cuff is either inflated around the exercising arm at peak exercise (solid line) or not (dotted line). The cuff is deflated after 3 min (end of circulatory occlusion in gray box). Compared to controls, patients with heart failure have an exaggerated ergoreflex response, resulting in greater ventilatory response to exercise. Their ventilatory response is maintained at the same level as maximum exertion throughout circulatory occlusion. Adapted from Piepoli et al. (11).

requiring oxygen has decreased mortality from acute infection but also predisposed patients to the development of insulin resistance (15, 16). Insulin resistance and the development of diabetes are common in patients with long COVID, both of which are characterized by high circulating concentrations of insulin and normal fasting glucose (17). Secondly, a cytokine storm during COVID-19 infection also results in excessive cortisol secretion in the first 2 weeks of acute illness, causing sympathetic overactivation (18, 19). Thirdly, SARS-CoV-2 uses the angiotensin-converting enzyme-2 (ACE-2) to facilitate cell entry, which might cause stimulation of the renin-angiotensin-aldosterone system (RAAS), predisposing to chronic inflammation and hypercatabolism (20). The mechanism by which age, obesity, hypertension and diabetes are risk factors in COVID infection might relate to RAAS activation (21).

A chronic catabolic state in infected individuals may lead to long-term reduction in skeletal muscle mass and altered function, predisposing to the development of long COVID. In a study of 213 patients with COVID-19, during the acute phase of infection, 29% of patients lost over 5% of their body weight (median percentage weight loss = 8.1%, 95% confidence interval = 6.1–10.9%). The weight loss observed may be due to a combination of acute inflammatory state and disuse atrophy (22). Sarcopenia may develop within a matter of days or insidiously over months and years. Patients admitted to an intensive care unit due to COVID-19 have a median reduction of 30% in their rectus femoris cross sectional area and 19% in the thickness of the anterior compartment of the quadricep muscle between the first and tenth day of their intensive care admission (23). Sarcopenia may contribute to fatigue, the extent of which depends on disease severity. In a study of 807 people with long COVID 1 year following acute infection, 7 of 10 most common persistent symptoms could be explained by sarcopenia (fatigue, aching muscles, physically slowing down, breathlessness, joint swelling or pain, general pain and limb weakness). Proteomic analysis shows that increased inflammatory mediators of tissue damage and repair are associated with the most severe symptoms (24).

A unifying hypothesis to explain breathlessness and fatigue in patients with long COVID is that skeletal muscle becomes abnormal in some patients following acute infection, secondary to hyperinflammatory and hypercatabolic response (**Figure 3**). Excessive ergorflex activation leads to the sensations of fatigue and breathlessness.

Future research

To prove this hypothesis, future work should aim to characterize muscle changes in patients with prior COVID-19 infection, comparing those with and without long COVID. The prevalence and severity of sarcopenia in people with long COVID should be investigated and quantified in more detail. Widespread availability of dual energy X-ray absorptiometry

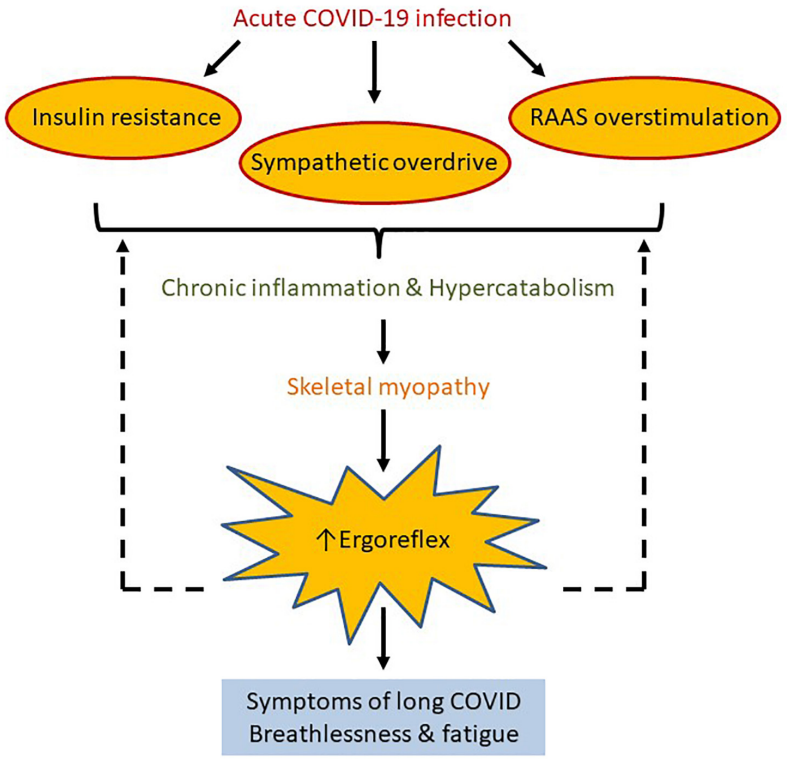

FIGURE 3
We propose ergoreflex overstimulation as a possible pathophysiological mechanism of long COVID. Acute COVID-19 infection predisposes to insulin resistance and sympathetic and renin-angiotensin-aldosterone system overstimulation, which may lead to chronic inflammation and hypercatabolism. This in turn can cause reduction in skeletal mass and function, which increases ergoreflex sensitivity, and perhaps explains the symptoms of long COVID such as breathlessness and fatigue. Solid and dotted arrows represent known and hypothetical relations, respectively.

scanning is a straightforward way to assess the problem of loss of muscle bulk. The ergoreflex itself can be directly examined using the protocol developed and standardized by Piepoli et al. (11).

Although COVID vaccination reduces the risk of developing COVID-19 and associated disease severity, its relation with long COVID is unknown (25). Comparing the muscle characteristics and ergoreflex response in vaccinated vs. non-vaccinated individuals may help understand whether vaccines could lower the risk of developing long COVID and severity of symptoms.

Importantly, the ergoreflex hypothesis supports the use of exercise training to improve symptoms in patients with long COVID. By preserving the bulk and functioning of large muscle groups, as well as reducing ergoreflex activation, we postulate that exercise training may improve symptoms associated with long COVID. Indeed, despite very severe left ventricular dysfunction, some patients with CHF have normal exercise responses (due to preserved muscle bulk) and are asymptomatic. Should the ergoreflex be proven to be an important pathophysiological mechanism of long COVID, tailored exercise interventions should be trialed with the aim of improving both symptoms and perhaps prognosis in patients with long COVID.

Author contributions

SS and DP conceived of the presented idea and drafted the manuscript. AM, CO, MP, IS, and AC provided feedback and commented on the manuscript. 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.

Publisher's note

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Early antithrombotic post-discharge therapy using prophylactic DOAC or dipyridamole improves long-term survival and cardiovascular outcomes in hospitalized COVID-19 survivors

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Introduction: Cardiovascular events are common in COVID-19. While the use of anticoagulation during hospitalization has been established in current guidelines, recommendations regarding antithrombotic therapy in the post-discharge period are conflicting.

Methods: To investigate this issue, we conducted a retrospective follow-up (393 ± 87 days) of 1,746 consecutive patients, hospitalized with and surviving COVID-19 pneumonia at a single tertiary medical center between April and December 2020. Survivors received either 30-day post-discharge antithrombotic treatment regime using prophylactic direct oral anticoagulation (DOAC; n = 1,002) or dipyridamole (n = 304), or, no post-discharge antithrombotic treatment (Ctrl; n = 440). All-cause mortality, as well as cardiovascular mortality (CVM) and further cardiovascular outcomes (CVO) resulting in hospitalization due to pulmonary embolism (PE), myocardial infarction (MI) and stroke were investigated during the follow-up period.

Results: While no major bleeding events occured during follow-up in the treatment groups, Ctrl showed a high but evenly distributed rate all-cause

mortality. All-cause mortality (CVM) was attenuated by prophylactic DOAC (0.6%, $P < 0.001$) and dipyridamole (0.7%, $P < 0.001$). This effect was also evident for both therapies after propensity score analyses using weighted binary logistic regression [DOAC: $B = -3.33$ (0.60), $P < 0.001$ and dipyridamole: $B = -3.04$ (0.76), $P < 0.001$]. While both treatment groups displayed a reduced rate of CVM [DOAC: $B = -2.69$ (0.74), $P < 0.001$ and dipyridamole: $B = -17.95$ (0.37), $P < 0.001$], the effect in the DOAC group was driven by reduction of both PE [$B-3.12$ (1.42), $P = 0.012$] and stroke [$B = -3.08$ (1.23), $P = 0.028$]. Dipyridamole significantly reduced rates of PE alone [$B = -17.05$ (1.01), $P < 0.001$].

Conclusion: Late cardiovascular events and all-cause mortality were high in the year following hospitalization for COVID-19. Application of prophylactic DOAC or dipyridamole in the early post-discharge period improved mid- and long-term CVO and all-cause mortality in COVID-19 survivors.

KEYWORDS

COVID-19, long COVID-19, direct anticoagulation, dipyridamole, cardiovascular disease in COVID-19

Introduction

Cardiovascular events including thromboembolisms due to coagulopathy represent frequent and serious complications in COVID-19 patients. Accordingly, high rates of stroke, pulmonary embolism and venous thromboembolisms have been reported in the context of COVID-19 disease. These events seem primarily driven by the profound inflammatory response, along with endothelial inflammation and dysfunction (1–3) which cause an increase in platelet adhesion and aggregation, thus promoting procoagulatory effects and thromboinflammatory processes (3, 4). Additionally, platelet activation itself further triggers the release of proinflammatory cytokines. As a consequence, elevated levels of fibrinogen and D-dimer have been reported as frequent finding of prognostic relevance in COVID-19 patients. Furthermore, occlusive thrombotic microangiopathy has been observed (5). While prothrombotic effects in acute COVID-19 disease seem evident, there is conflicting data in this context, as well as a lack of long-term follow-up evaluating the risk of cardiovascular events and death in the post-discharge period (6, 7).

In hospitalized patients, no beneficial effects of therapeutic anticoagulation was observed in critically ill COVID-19 (8, 9), while non-critically ill COVID-19 patients seem to benefit from this therapeutic approach (10–12). Since a higher inflammatory burden is present in critically ill patients, COVID-19-related vascular inflammation was discussed as a potential explanation for these controversial findings (13–16). Early studies investigating the use of antiplatelet agents in acute COVID-19 also showed promising results (17). However, these findings could not be confirmed in large, randomized trials (18, 19). On the other hand, smaller trials indicated a potentially beneficial effect of dipyridamole (20, 21). In addition, although COVID-19 also affects long-term cardiovascular outcomes (22), present antithrombotic guidelines for extended post-discharge thromboprophylaxis are conflicting, recommending either no routine thromboprophylaxis or an individualized approach (23, 24).

In mid-2020, dipyridamole or prophylactic direct anticoagulation (DOAC) were routinely prescribed in the early post-discharge period (30-days post-discharge) in several medical centers based on experts' recommendations. This approach was subsequently adopted in a nationwide class C guideline recommendation for prophylactic DOAC in September 2020 (25). The use of anticoagulants in the post-discharge regime following COVID-19 hospitalization seems to be supported by data from a US registry in the 90 day follow-up of post-discharge COVID-19 patients (7) as well as by recent results from Brazil indicating prophylactic rivaroxaban improves short-term (35 days) outcomes in high-risk patients (26). Nevertheless, to the best of our knowledge, the efficacy and safety of the described strategy have not been systematically or adequately evaluated, despite its routine use in clinical practice. Furthermore, longer follow-up data on cardiovascular outcomes in hospitalized COVID-19 survivors are also lacking. To investigate this issue, we assessed the incidence of all-cause death as well as cardiovascular mortality and hospitalizations for relevant cardiovascular outcomes including pulmonary embolism, stroke and myocardial infarction of 1,746 hospitalized COVID-19 survivors receiving post-discharge thromboprophylaxis using either prophylactic DOAC or dipyridamole or no thromboprophylaxis during

follow-up of 393 ± 87 days. We hypothesized, that the applied thromboprophylactic post-discharge strategy would affect incidence of cardiovascular events and thus potentially all-cause mortality rates.

Methods

The study was performed in accordance with the standards of good clinical practice and the principles of the Declaration of Helsinki, receiving approval by the ethics commission of the Bashkir State Medical University (N5, 2020).

For this single-center, retrospective study, 2,294 COVID-19 survivors were consecutively screened at discharge following hospitalization for COVID-19 disease at a tertiary medical center (Bashkir State Medical University Hospital, Bashkir State, Russian Federation) between April 2020 and December 2020 for moderate COVID-19 associated pneumonia, defined according to current WHO recommendations (27).

All included patients were 18 years or older and suffered from moderate COVID-19-related pneumonia requiring hospitalization. Exclusion criteria were defined as: requirement for therapeutic anticoagulation using Vitamin K antagonists or therapeutic DOAC therapy before or/and after enrollment, history of relevant thrombotic disorders requiring anticoagulation therapy. Furthermore, with respect to potential bleeding complication, according to our hospital standard of clinical care procedures, patients with requirement for combination therapy of DOAC and/or dipyridamole and/or any other additional antiplatelet therapies including acetylsalicylic acid, ticagrelor, prasugrel or clopidogrel were not considered for the investigated post-discharge antithrombotic regimes. Consequently, to avoid any bias, which might be associated with the described patients' selection, patients in Ctrl treated with antiplatelet medications including acetylsalicylic acid, ticagrelor, prasugrel or clopidogrel were also excluded from further analyses. In addition, to account for disease severity and associated potential thrombotic risk, patients requiring mechanical ventilation during their hospitalization were also excluded from further analyses (Figure 1).

Based on the primary inclusion and exclusion criteria, 2,073 qualifying COVID-19 patients were further stratified based upon the recommended anticoagulation post-discharge regime: anticoagulation using dipyridamole 75 mg TID or prophylactic DOAC (DOAC) for 30-days post hospital discharge (rivaroxaban 10 mg QD, dabigatran 110 mg BID, or apixaban 2.5 mg BID) or a no anticoagulation therapy group as the control group (Figure 1). Choice of the antithrombotic post-discharge therapy (Ctrl. or specific DOAC or dipyridamole) was based on the decision of the attending physician and implemented hospital-specific standard of care procedures. In all eligible patients, patient hospital data including demographics, medical history, laboratory examinations, comorbidities, complications, specific treatment measures, and outcomes were collected and analyzed. During follow-up, patients' outcome and survival were evaluated until October 1, 2021. The investigated outcomes were compromised of all-cause mortality and the need for hospitalization due to cardiovascular events including pulmonary embolism, myocardial infarction and stroke. We further analyzed the incidence of cardiovascular mortality defined as in hospital death due to cardiovascular causes or out of hospital death meeting the criteria of sudden cardiac death (28). In addition, patients were evaluated for relevant bleeding events requiring hospitalization. Major and non-major bleeding, were defined according to the International Society on Thrombosis and Haemostasis (ISTH) criteria (29). Follow-up was conducted with the help of the remote data capture system "ProMed" (Program for Medical Cases Monitoring). The program enables distant online monitoring of all hospitalization discharge notes of all regional hospital institutions as well as all death certificates.

At the time point of data collection (after October 1st 2021), all patients with confirmed recommendation for post-discharge anticoagulation were further contacted by phone. A standardized telephone interview was performed to verify the applied antithrombotic substance use and to confirm compliance to the DOAC or dipyridamole regime in the post-discharge setting (DOAC. including rivaroxaban, dabigatran and apixaban or dipyridamole). If a patient was deceased by the time of scheduled contact, a standardized telephone interview was performed with a close relative. Patients were excluded from further analyses, if the recommended anticoagulation regime was not taken by the patient or if collection of sufficient information about the therapy regime was not possible (n = 327). Follow-up outcomes in the remaining 1,002 patients with confirmed prophylactic Direct Oral Anticoagualtion (DOAC) intake and 304 patients with confirmed dipyridamole therapy intake was propensity-matched to the control group, in whom no anticoagulation regime was prescribed at hospital discharge (Figure 1).

Statistical analyses

Statistical analyses were conducted using R [version 4.0.2., R Core Team (2013), R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org/] and the packages "Rcmdr," "ggplot2," "pastecs," "Hmisc," "ggm," "polycor," "QuantPsyc," "glmnet," "twang," "survey," "stddiff," "survival" and "survminer," as well as, SPSS (Version 23.0, IBM, Armonk, New York, USA). Distribution of continuous data was assessed visually and using the Kolmogorov-Smirnov-test, kurtosis and skew were assessed visually. Since data were not normally distributed, median ± interquartile-range (IQR) are depicted. Medians were compared by Kruskal-Wallis test, whereas categorical data was analyzed using Fisher's exact test.

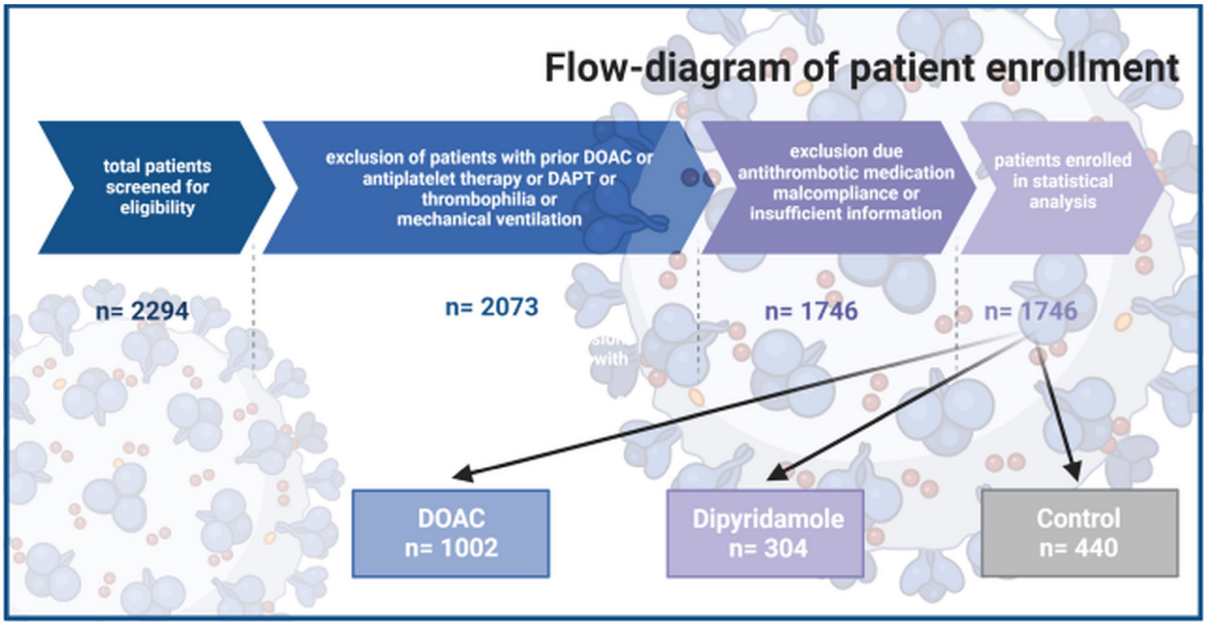

FIGURE 1
Flow chart of patients' inclusion

TABLE 1 Baseline characteristics of enrolled patients.

	DOAC ($n = 1,002$)		Dipyridamole ($n = 304$)		Control ($n = 440$)			
	%	*n*	%	*n*	%	*n*	*P*-value	std.diff.
Female sex	56.4	563	58.7	178	61.1	265	0.240	0.12
Arterial hypertension	39.0	391	35.9	109	30.8	135	0.012*	0.11
Diabetes mellitus	12.1	121	10.2	31	10.7	47	0.586	0.05
Chronic kidney disease	3.0	30	4.3	13	4.3	19	0.337	0.10
Coronary heart disease	8.50	85	8.60	26	7.7	34	0.894	0.02
Heart failure	7.7	77	7.9	24	8.4	37	0.882	0.07
COPD	2.9	29	3.0	9	3.9	17	0.593	0.07
In hospital therapy								
Corticosteroids	90.6	908	88.8	270	69.5	306	<0.0001*	0.64
Therapeutic anticoagulation	81.0	812	67.1	204	30.9	136	<0.0001*	1.27
JAK-inhibitors	8.4	84	9.2	28	5.2	23	0.064	0.12
IL6-antagonist	60.9	610	51.3	156	34.1	150	<0.0001*	0.58
Remdesivir	0.3	3	0	0	0.5	2	0.724	0.01
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	***P*-value**	
Age (years)	59	48–66	56	46–65	55	43–63	<0.0001	0.26
IMPROVE score	1	0–1	0	0–1	0	0–1	0.127	0.17
Creatinine (μmol/l)	89.40	80.60–100.00	91.10	80.90–104.60	90.30	79.95–103.83	0.085	0.08
CRP (mg/l)	26.00	6.00–58.75	26.15	0.00–58.23	18.00	0.00–48.00	0.002	0.32

Baseline characteristics of enrolled patients. COPD, chronic obstructive pulmonary disease; CRP, c-reactive protein; DOAC, direct oral anticoagulation; IL6, interleukine-6; IMPROVE, International Medical Prevention Registry on Venous Thromboembolism; JAK, Janus kinase; std. diff, standardized differences. *$p < 0.05$ using Kruskal-Wallis test or Fisher's exact test.

Survival probability is depicted using the Kaplan-Meier method, Cox proportional hazards analysis was performed to assess the association of applied therapies with mortality. To account for imbalances in baseline covariates with possible influence on outcome, standardized differences between the three groups were calculated. Covariates with statistically significant

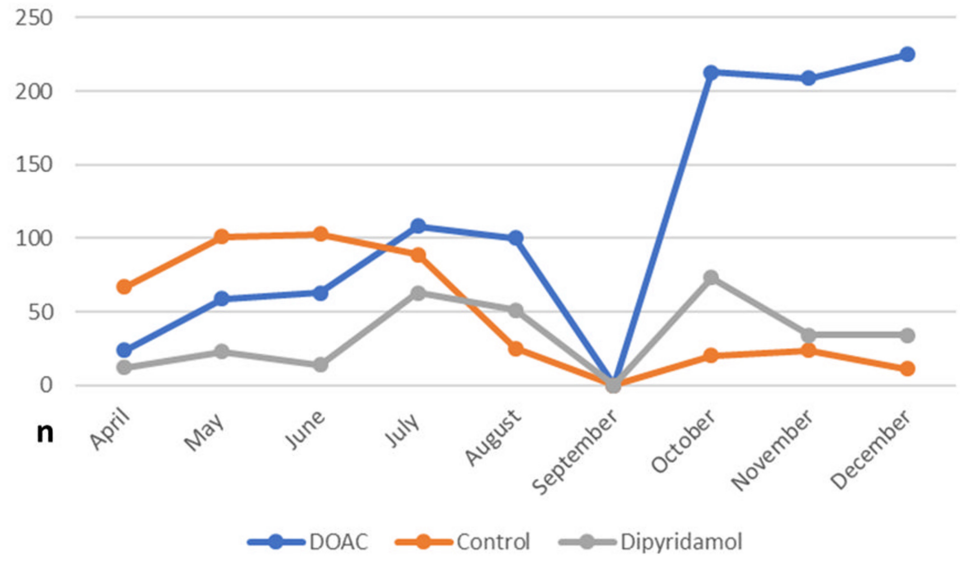

FIGURE 2
Frequency of prescribed post-discharge antithrombotic regimes in the study population during the study inclusion period (April to December 2020).

differences or standardized differences >0.25 between the groups (see Table 1; arterial hypertension, age, C-reactive protein, in-hospital treatment with corticosteroids, in-hospital treatment with anticoagulation, in-hospital treatment with IL-6 antagonists) were then included in propensity score weighting of the groups by Generalized Boosted Models (GBM) using the Average Treatment Effect on Treated (ATT) estimate (30). Prior to GBM, continuous data were transformed to z-scores to assure standardization and overlap concerns were checked by density plots of continuous data, as well as cross tabulations of nominal data. After balancing, weighted logistic regression analysis was performed for the predefined endpoints of the study using the "survey" package of R. A p-value of <0.05 was considered statistically significant.

Results

In total, 1,746 patients (100% Caucasian) were included in the final statistical analysis. As illustrated in Figure 2, a large number of patients enrolled in the control group were treated in the very early stage of the pandemic, while antithrombotic therapies including DOAC and dipyridamole have been routinely applied since July 2020. Of these, 57.4% (n = 1,002) received DOAC (rivaroxaban: 91.6% (918/1,002), 7.1% apixaban (71/1,002) 1.3% dabigatran (13/1,002), and 17.4% (n = 304) received dipyridamole. The control group consisted of 25.2% (n = 440) of the study population. Baseline characteristics and laboratory values at the time of enrollment are depicted in Table 1. During in-hospital period all patients were treated at least with prophylactic antithrombotic therapy using a heparinoid, the majority also received therapeutic anticoagulation. To note, patients in the DOAC group had a higher prevalence of arterial hypertension and were significantly older than patients in the other groups. Furthermore, patients in the DOAC group significantly more often received corticosteroids, anticoagulation and IL-6 antagonists during the hospital stay (see Table 1).

Outcome

Mean follow-up in the total cohort was 393 ± 87 days. Patients in the control group had significantly worse 30-day all-cause mortality [DOAC: 0% (n = 0), dipyridamole: 0% (n = 0), Ctrl.: 0.9% (n = 4), p = 0.005], 3-month all-cause mortality [DOAC: 0% (n = 0), dipyridamole: 0% (n = 0), Ctrl.: 2.7% (n = 12), p < 0.0001], 6-month all-cause mortality [DOAC: 0.1% (n = 1), dipyridamole: 0.7% (n = 2), Ctrl.: 3.9% (n = 17), p < 0.0001] and all-cause mortality at the end of follow-up [DOAC: 0.6% (n = 6), dipyridamole: 0.7% (n = 2), Ctrl.: 5.9% (n = 26), p < 0.001] than patients treated with DOAC or dipyridamole (see Figure 3; Table 2).

While there were no statistically significant differences in the prevalence of myocardial infarction between the three investigated groups, stroke occurred significantly more often in control group patients [DOAC: 0.3% (n = 3), dipyridamole:

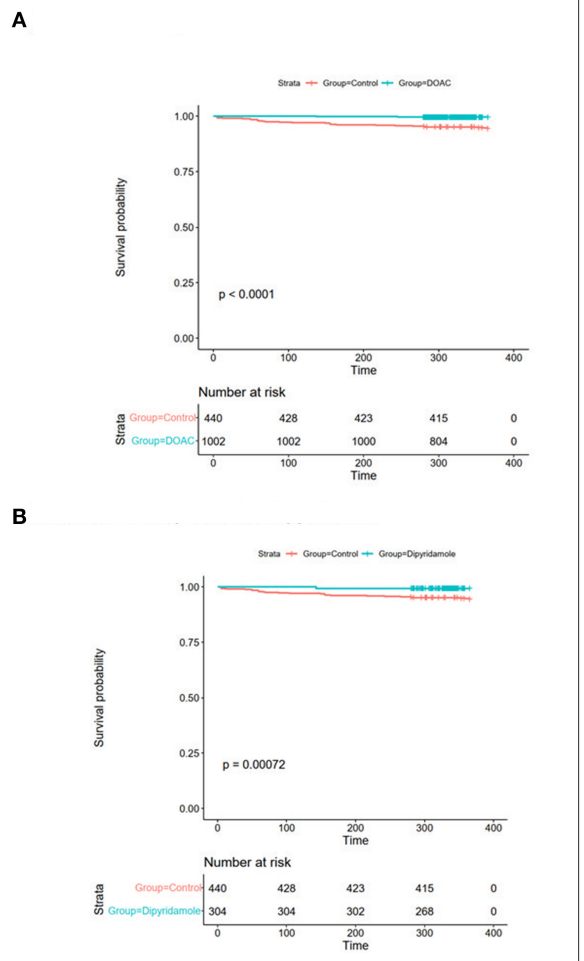

FIGURE 3
Kaplan-Meier plots of all-cause survival probability **(A)** DOAC: 1.1% vs. Ctrl.: 5.7%, OR 0.19 (95% CI 0.07–0.55), $p = 0.001$ and **(B)** Dipyridamole: 1.2%, Ctrl.: 5.7%, OR 0.20 (95% CI 0.05–0.86), $p = 0.023$.

0.3% ($n = 1$), Ctrl.: 1.6% ($n = 7$), $p = 0.014$] during follow-up. A trend toward higher prevalence of pulmonary embolisms was also observed in the control group [DOAC: 0.1% ($n = 1$), dipyridamole: 0% ($n = 0$), Ctrl: 0.7% ($n = 3$), $p = 0.081$; see Figure 5; Table 2], although not statistically significant. Furthermore, cardiovascular mortality was higher in the Crtl: 2.0% ($n = 9$) when compared to DOAC: 0.3% ($n = 3$) and dipyridamole: 0% ($n = 0$, $p = 0.001$, Figure 4; Table 2).

In univariate Cox proportional hazards analysis, both treatment with DOAC or dipyridamole was associated with a reduced risk of mortality [DOAC: HR 0.08 (95% CI 0.03–0.22), $p < 0.0001$; dipyridamole: HR 0.35 (95% CI 0.17–0.72), $p = 0.005$].

We performed Generalized Boosted Models (GBM) using the Average Treatment Effect on Treated (ATT) estimate for propensity score weighting of groups to account for covariate imbalances between the three groups, which might affect outcome. Covariates included were those with statistically significant differences and/or standardized differences >0.25 between the groups (see Table 1; arterial hypertension, age, C-reactive protein, in-hospital treatment with corticosteroids, in-hospital treatment with anticoagulation, in-hospital treatment with IL-6 antagonists; see Figure 6). After weighted binary logistic regression analysis, the association of treatment with DOAC or dipyridamole and reduced all-cause mortality remained statistically significant [Death during total follow-up: DOAC: B (SE) = −3.33 (0.60), $p < 0.0001$, dipyridamole: B (SE) = −3.04 (0.76), $p < 0.0001$]. In addition, weighted logistic regression revealed protective effects of treatment with DOAC or dipyridamole for cardiovascular mortality [DOAC: B (SE) = −2.69 (0.74), $P < 0.001$, dipyridamole: B (SE) = −17.95 (0.37), $P < 0.001$] as well as for pulmonary embolism [DOAC: B (SE) = −3.12 (1.42), $p = 0.028$, dipyridamole: B (SE) = −17.05 (1.01), $p < 0.0001$]. Treatment with DOAC was furthermore protective for stroke [DOAC: B (SE) = −3.08 (1.23), $p = 0.0122$, dipyridamole: B (SE) = 0.40 (1.23), $p = 0.743$; see also Table 3; Figures 7, 8].

Discussion

The post-hospital management of COVID-19 survivors remains a clinical challenge to date. The prothrombotic state, promoted by endothelial inflammation and dysfunction leading to increased platelet adhesion and aggregation as well as proinflammatory cytokine release (1–4) remains a central issue in COVID-19 disease. Meanwhile the rates of thromboembolic events and the use of thromboprophylaxis in hospitalized COVID-19 patients represent a topic of ongoing debate. Although, guidelines on anticoagulation during hospital stay have already been issued, recommendations regarding the antithrombotic treatment for extended post-discharge thromboprophylaxis are conflicting, suggesting either no routine thromboprophylaxis or an individualized approach (23, 24). Of note, existing recommendations focus mainly on anticoagulation, leaving out potential antithrombotic treatment options.

Interestingly, most studies conducted to date reported relatively low rates of thromboembolic events within the first 30–45 days after discharge of hospitalized COVID-19 patients, hence routine thromboprophylactic therapy is not recommended in this patient collective (6, 31, 32). In contrast, the CORE-19 study reported comparably higher rates of thromboembolisms in over three percent of the total patient collective (7). Accordingly, a 46% reduction of major thromboembolic events and death in the presence of (prophylactic) anticoagulation therapy was reported during the mean follow-up of 92 days (7).

Thus, to further investigate efficacy of post-discharge thromboprophylaxis following hospitalization with COVID-19,

TABLE 2 Outcome of patients enrolled in the three investigated groups.

	DOAC ($n = 1,002$)		Dipyridamole ($n = 304$)		Control ($n = 440$)		
	%	*N*	%	*N*	%	*N*	*P*-value
30-day all-cause mortality	0.0	0	0.0	0	0.9	4	0.005*
3-month all-cause mortality	0.0	0	0.0	0	2.7	12	<0.0001*
6-month all-cause mortality	0.1	1	0.7	2	3.9	17	<0.0001*
Outcomes during total follow-up (393 ± 87 days)							
All-cause mortality	0.6	6	0.7	2	5.9	26	<0.0001*
Cardiovascular mortality	0.3	3	0.0	0	2.0	9	0.001*
Myocardial infarction	1.5	15	0.7	2	1.1	5	0.532
Stroke	0.3	3	0.3	1	1.6	7	0.014*
Pulmonary embolism	0.1	1	0.0	0	0.7	3	0.081
Major bleeding	0.0	0	0.0	0	0.2	1	0.426

Outcome of patients enrolled in the three investigated groups. DOAC, direct oral anticoagulation. *$p < 0.05$ using Fisher's exact test.

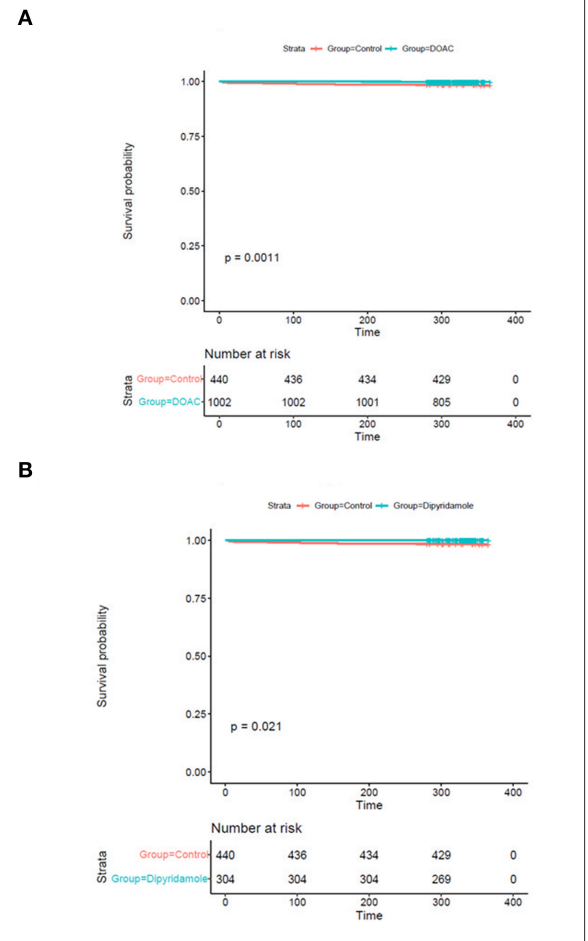

FIGURE 4
Kaplan-Meier plots of cardiovascular survival probability **(A)** cardiovascular mortality rates of controls vs. patients treated with DOAC, **(B)** cardiovascular mortality rates of controls vs. patients treated with dipyridamole.

we analyzed 30-day use of prophylactic DOAC or dipyridamole therapy compared to no anticoagulatory treatment following hospital discharge. To the best of our knowledge, the present study is the first of its kind to offer longer outcome (393 ± 87 days) data capturing extended post-discharge thromboprophylaxis in COVID-19 patients, including different anticoagulatory treatment regimens.

With respect to baseline characteristics, thromboembolic risk as indicated by the IMPROVE score was similar between groups. The control group however showed significantly lower rates of in-hospital corticosteroids, IL-6-inhibitors and therapeutic anticoagulation. This might be due in part to the comparably lower inflammatory burden, indicated by significantly lower baseline CRP-levels in the control group. With regards to concomitant disease, control patients were younger and had lower rates of arterial hypertension (Table 1). However, despite these findings, both DOAC and dipyridamole groups showed lower rates of cardiovascular events during follow-up when matched to untreated patients (Figures 4, 5; Table 2). Importantly, both therapies were associated with reduced all-cause mortality compared to controls, a finding which was consistent during follow-up (30 days, 3 months, 6 months, and overall follow-up; Table 2; Figure 3). Furthermore, cardiovascular mortality was also reduced during follow-up (Figure 4; Table 2). To account for the described differences between groups, propensity score weighting was conducted to account for covariate imbalances, which might affect outcome. As covariates displaying a statistically significant difference were included in the propensity score weighting, the depicted coefficients estimate the causal effects of DOAC or dipyridamole vs. controls assuming there are no unobserved confounders (Figure 6). Of note, the reduction in overall all-cause mortality but also cardiovascular mortality remained highly significant after propensity score weighting of groups (Table 3; Figure 7).

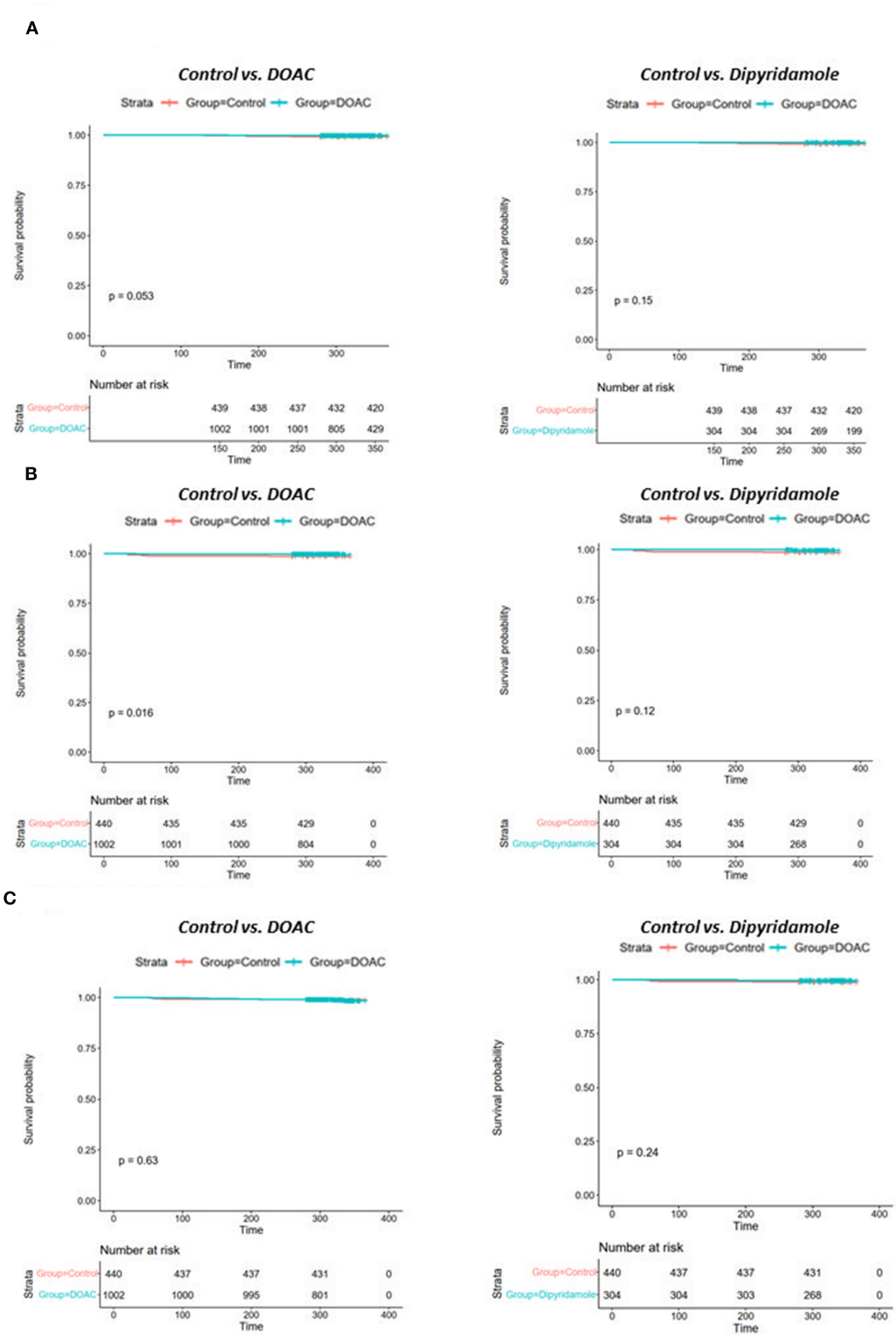

FIGURE 5
Kaplan-Meier plots of **(A)** pulmonary embolism rates, **(B)** stroke rates and **(C)** myocardial infarction rates of controls vs. patients treated with DOAC and dipyridamole.

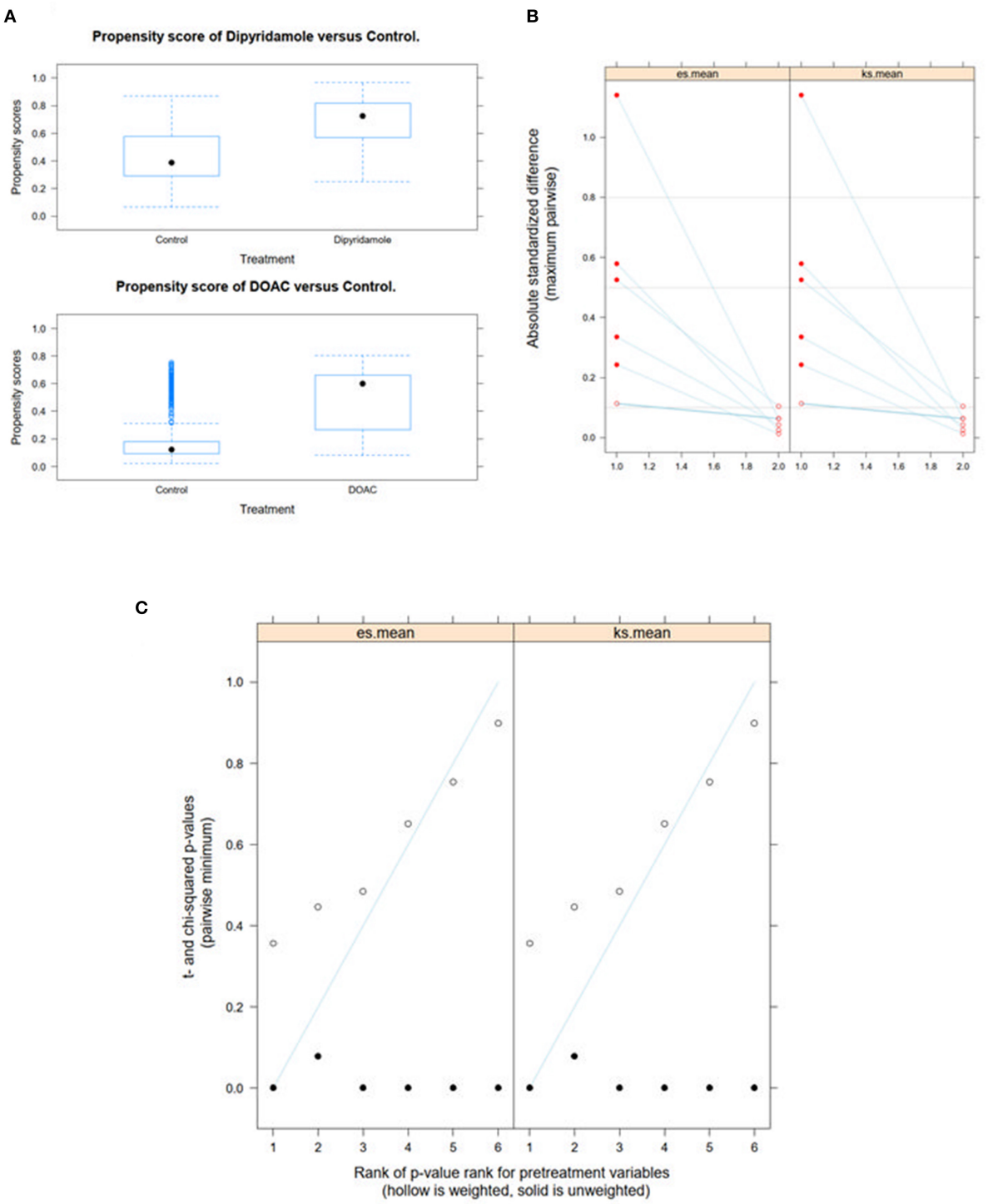

FIGURE 6
Propensity score weighting of groups was performed by applying Generalized Boosted Models (GBM) using the Average Treatment Effect on Treated (ATT) estimate. **(A)** depicts boxplots of the overlap of propensity score distribution between the three groups, **(B)** the comparison of the absolute standardized mean differences (ASMD) of the selected covariates between the groups before and after weighting and **(C)** the *t*-test and $\chi 2$ statistic before and after weighting.

Overall mortality in patients not receiving thromboprophylaxis was high reaching 5.9% during the total follow-up period (Table 2). Thus, mortality rates during follow-up resemble the in-hospital mortality of COVID-19 patients, indicating an ongoing disease process after hospital discharge. This finding could indicate potential severe long-term

TABLE 3 Data of weighted binary logistic regression regarding the predefined study endpoints.

Dependent variable	DOAC		Dipyridamole	
	B (SE)	*P*-value	B (SE)	*P*-value
Outcome during total follow-up (393 ± 87 days)				
All-cause mortality	−3.33 (0.60)	<0.0001*	−3.04 (0.76)	<0.0001*
Cardiovascular mortality	−2.69 (0.74)	<0.001*	−17.95 (0.37)	<0.0001*
Myocardial infarction	−0.31 (1.00)	0.757	−0.44 (0.65)	0.498
Stroke	−3.08 (1.23)	0.0122*	0.40 (1.23)	0.743
Pulmonary embolism	−3.12 (1.42)	0.028*	−17.05 (1.01)	<0.0001*

Data of weighted binary logistic regression regarding the predefined study endpoints. DOAC, direct oral anticoagulation; B, regression coefficient; SE, standard error. *$p < 0.05$ using weighted logistic regression analysis.

effects after COVID-19 disease requiring hospitalization. Similarly, the high mortality rates along with the observations of ongoing thromboembolic events during the complete follow-up period might support previously described theories of virus persistence with consequent inflammatory processes suspected in long-COVID disease. Thus, potential beneficial effects of anticoagulatory therapy after hospital-discharge seems plausible. Furthermore, both therapies were also associated with a reduction in several predefined cardiovascular outcomes indicating a link of all-cause mortality to cardiovascular pathologies (Table 3; Figures 7, 8).

While the treatment regime was only applied in the early phase after hospitalization, differences in relevant clinical outcomes were also observed after a longer follow-up. Therefore, it can be speculated that even after survived hospitalization, medical intervention might be crucial to minimize disease progression and improve cardiovascular outcomes as well as mortality rates. Our speculations are further supported by publications indicating an increase in the incidence of cardiovascular events even after mild COVID-19 disease (33) as well as previous reports describing longer virus persistence (34) and hints for inflammatory processes being persistent even during long-term follow-up in COVID-19 survivors (35).

Despite their comparable effects on all-cause mortality and, also cardiovascular mortality, different pathophysiologic effects of DOAC and dipyridamole therapy on predefined cardiovascular events have to be considered with regards to our findings and are potentially in part reflected in our study results.

After propensity score weighting, dipyridamole led to a significant reduction in pulmonary embolism while no significant associations with incidence of stroke and myocardial infarction were evident (Table 3; Figure 8). As dipyridamole acts as an inhibitor of platelet aggregation, a reduction of thrombotic events might be speculated. On the other hand, inflammation constitutes a key player in the pathophysiologic mechanisms leading to thromboembolic events in COVD-19. Sole inhibition of platelet aggregation seems an insufficient explanation on this regard. However, beside the inhibition of platelet aggregation, additional pleiotropic pharmacological actions leading to a broad range of potential beneficial effects in the context of COVID-19 have been reported for dipyridamole, including anti-inflammatory effects along with a significant reduction of D-dimer levels as well as a significant increase in lymphocyte and platelet count (21, 36). Accordingly, the anti-inflammatory effect of dipyridamole might be considered as a potential explanation for the significant reduction of thrombotic and thromboembolic events observed in our study. Additionally, dipyridamole was reported to suppress SARS-CoV-2 replication *in vitro* (21). This is of major importance with respect to the suspected virus persistence in the context of long-COVID-19, with chronically elevated levels of D-dimer and CRP (37). This theory might be supported by the incidence of late thrombotic and thromboembolic events during long-term follow-up after discharge in our study in the control group (Figure 5). Considering these effects, the combination of platelet inhibition, anti-inflammatory effects and a potential impact on virus replication might be speculated to contribute to the observed association between dipyridamole therapy and reduced cardiovascular events observed in post-discharge setting following COVID-19 infection. However, it remains unclear; as towhy no effect of dipyridamole treatment on stroke was observed. A potential explanation is that low-dose dipyridamole monotherapy might have a too small effect on stroke prevention. This is reflected by current recommendations and studies on secondary stroke prevention, in which a higher dose of 200 mg of dipyridamole is recommended only in a combination with acetylsalicylic acid (38). As venous thromboembolisms often occur in the context of COVID-19, potential benefits of dipyridamole therapy is likely decreased in the context of stroke (4).

A significant reduction of stroke and pulmonary embolism rates were observed in patients taking DOAC therapy post-discharge, while no significant association with rate of myocardial infarction was evident (Table 3; Figure 8). Interestingly, studies reported an impact on activation of coagulation in the cytokine storm associated with COVID-19 (14, 39). The thrombin-induced secretion of proinflammatory cytokines and growth factors represent the key factors

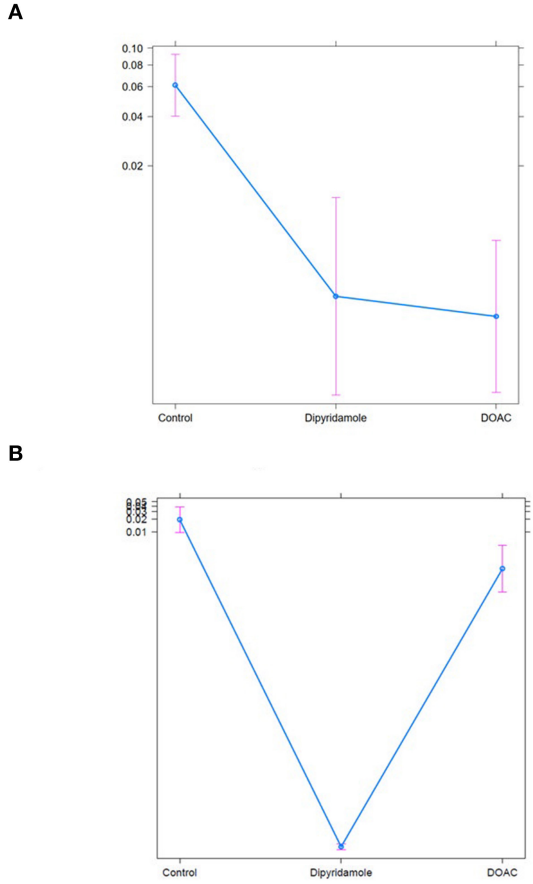

FIGURE 7
Treat effect plots of weighted binary logistic regression regarding the predefined study endpoints, depicted are predicted probabilities and 95% CI: **(A)** all-cause mortality (predicted probabilities: Ctrl.: 6.1% (95% CI 4.0–9.2) vs. Dipyridamole: 0.3% (95% CI 0.1–1.3) vs. DOAC: 0.2% (95% CI 0.1–0.7), **(B)** all-cause mortality [predicted probabilities: Ctrl.: 6.1% (95% CI 4.0–9.2) vs. Dipyridamole: 0.3% (95% CI 0.1–1.3) vs. DOAC: 0.2% (95% CI 0.1–0.7)], **(B)** cardiovascular mortlaity [predicted probabilities: Ctrl.: 0.02% (95% CI 0.01–0.4) vs. Dipyridamole <0.01% (95% CI 0.0–0.1) vs. DOAC: <0.01% (95% CI 0.0–0.1)].

in coagulation-induced inflammation (40). Consequently, anticoagulation might be helpful to attenuate the interaction between inflammation and thrombosis in COVID-19 (14, 39). However, it can be argued that while anticoagulation is recommended in non-critically ill patients, it failed to provide a clinical benefit in patients requiring intensive care treatment. Nevertheless, a preventive approach must be kept in mind on this regard. While anticoagulation might attenuate the vicious circle of thrombosis and inflammation, the process might be too far advanced in severe COVID-19, requiring intensive care treatment. Accordingly, the potential anti-inflammatory effect of anticoagulation therapy might be negligible in the context of advanced cytokine storm and high inflammatory burden. This may explain the failure of previous multicentre studies on therapeutic anticoagulation in intensive care COVID-19 patients. Of note, patients in the present study received prophylactic DOAC doses to counterbalance thromboembolic and bleeding risk.

While no significant differences in major bleeding were observed in the two treatment arms, one major bleeding was observed in the control group during follow-up (0.2%, $P = 0.426$; Table 2). However, it must be mentioned that minor bleeding events could not be assessed given the study design. Thus, the validity of our study findings with respect to the bleeding endpoint is limited.

In summary, the present study is the first to offer long follow-up (393 ± 87 days) of different thromboprophylactic treatment regimens after hospitalization for COVID-19. Mortality rates were significantly reduced by both 30-day regimes of dipyridamole and prophylactic DOAC treatment, emphasizing the ongoing thromboembolic and inflammatory burden in COVID-19 in the early post-discharge period following the acute phase of the disease. Accordingly, thromboprophylactic treatment might offer beneficial effects in the long-term treatment of COVID-19 patients. Therefore, further randomized trials are necessary to investigate the effects of these regimes in COVID-19 survivors.

Limitations

The present study has by design its limitations, mainly due to its single-center and retrospective design as well as lack of randomization and treatment arm blinding. Among others, this could bias the results due to hospital-specific standards of patient care. The overstrained medical system amidst the pandemic may have exacerbated cardiovascular events rates and mortality leading to an overestimation of the effects of the investigated medical regimes. On the other hand, rates of cardiovascular outcomes were based on hospitalized events only. Therefore, an underestimation of events is possible. This may be further aggravated by the unwillingness of patients to be hospitalized during the pandemic. While anticoagulatory regimes in the investigated center were used as the pandemic progressed, a large number of patients enrolled in the control group were treated in the very early stage. Therefore, limited accumulated clinical experience, the implementation of novel therapy regimes and the evolution of the viral genome could have affected disease management and therefore long-term outcomes. Nevertheless, to adjust for this bias, propensity score weighting of groups was performed, which did not significantly affect our results. Furthermore, since the first novel viral variants, B.1.1.7 and B.1.351 were declared a variant of concern on December 18th, 2020, followed by P.1 on January 11th, 2021 (41) differences in the viral genome seem improbable in our study cohort which was recruited between middle of April 2020 and December 2020. Based on our study design, we were only able to analyze bleeding events requiring hospitalization, which is a major limitation of our study. Nevertheless, the

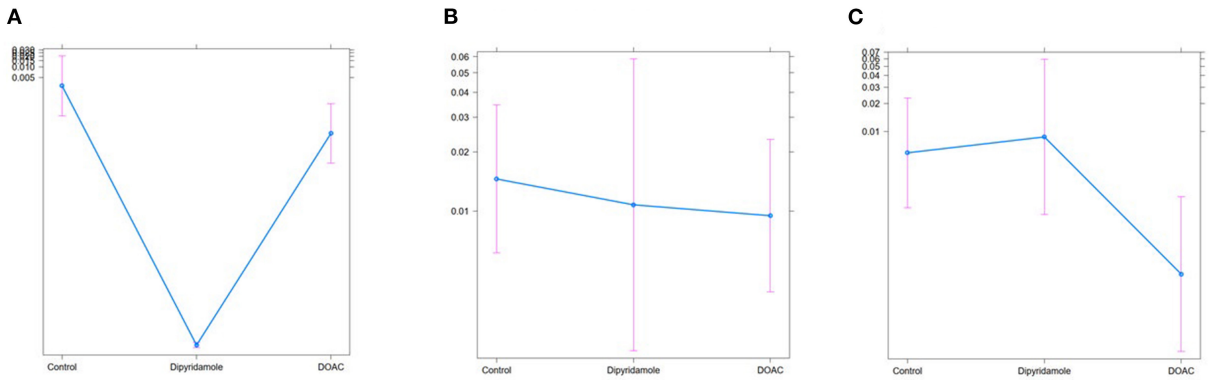

FIGURE 8
Treat effect plots of weighted binary logistic regression regarding the predefined study endpoints, depicted are predicted probabilities and 95% CI: **(A)** pulmonary embolism [predicted probabilities: Ctrl.: 0.03% (95% CI 0.0–0.2) vs. Dipyridamole: <0.01% (95% CI 0.0–0.1) vs. DOAC: <0.01% (95% CI 0.0–0.1)], **(B)** myocardial infarction [predicted probabilities: Ctrl.: 1.5% (95% CI 0.6–3.5) vs. Dipyridamole: 1.1% (95% CI 0.2–5.9) vs. DOAC: 0.8% (95% CI 0.4–2.3)], **(C)** stroke [predicted probabilities: Ctrl.: 0.6% (95% CI 0.1–2.3) vs. Dipyridamole 0.8% (95% CI 0.1–5.9) vs. DOAC: 0.02% (95% CI 0.0–0.1)].

low incidence of bleeding events observed in our trial, seems plausible, since it is comparable to results presented in the MICHELLE study, which applied a similar therapeutic regime in a comparable patient population (26). Moreover, it is important to emphasize that our findings only apply to patients hospitalized with moderate COVID-19 infection.

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 Bashkir State Medical University (N5, 2020). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

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

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.916156/full#supplementary-material

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Clinical and electrocardiographic outcomes evaluated by telemedicine of outpatients with clinical suspicion of COVID-19 treated with chloroquine compounds in Brazil†

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Aims: To evaluate clinical and electrocardiographic outcomes of patients with COVID-19, comparing those using chloroquine compounds (chloroquine) to individuals without specific treatment.

Methods: Outpatients with suspected COVID-19 in Brazil who had at least one tele-electrocardiography (ECG) recorded in a telehealth system were enrolled in two arms (Group 1: chloroquine and Group 2: without specific treatment) and one registry (Group 3: other treatments). Outcomes were assessed through follow-up calls (phone contact, days 3 and 14) and linkage to national mortality and hospitalization databases. The primary outcome was composed of: hospitalization, intensive care admission, mechanical ventilation, and all-cause death, and the ECG outcome was the occurrence of major abnormalities by the Minnesota code. Significant variables in univariable logistic regression were included in 4 models: 1-unadjusted; 2-adjusted for age and sex; 3-model 2 + cardiovascular risk factors and 4-model 3 + COVID-19 symptoms.

Results: In 303 days, 712 (10.2%) patients were allocated in group 1, 3,623 (52.1%) in group 2 and 2,622 (37.7%) in group 3; 1,969 had successful phone follow-up (G1: 260, G2: 871, and G3: 838). A late follow-up ECG was obtained for 917 (27.2%) patients [group 1: 81 (11.4%), group 2: 512 (14.1%), group 3: 334 (12.7%)]. In adjusted models, chloroquine was independently associated with greater chance of the composite clinical outcome: phone contact (model 4): OR = 3.24 (95% CI 2.31–4.54), $p < 0.001$. Chloroquine was also independently associated with higher mortality, assessed by phone + administrative data (model 3): OR = 1.67 (95% CI 1.20–2.28). However, chloroquine did not associate with the occurrence of major ECG abnormalities [model 3; OR = 0.80 (95% CI 0.63–1.02, $p = 0.07$)]. Abstracts with partial results of this work was accepted in the American Heart Association Scientific Sessions, November 2022, in Chicago, IL, USA.

Conclusion: Chloroquine was associated with a higher risk of poor outcomes in patients suspected to have COVID-19 when compared to those who received standard care. Follow-up ECGs were obtained in only 13.2% of patients and did not show any significant differences in major abnormalities amongst the three groups. In the absence of early ECG changes, other side effects, late arrhythmias or deferral of care may be hypothesized to explain the worse outcomes.

KEYWORDS

COVID-19, chloroquine, treatment, outcomes, prognosis, electrocardiogram, telemedicine

Introduction

The pandemic of the new coronavirus disease (COVID-19) caused by the SARS-CoV-2 was decreed by the World Health Organization (WHO) on 11 March 2020. Worldwide, as of 23rd December 2022, over 660 million cases and 6.7 million deaths have already been recorded (1). Brazil has been severely hit by the pandemic, ranking fifth in the absolute number of reported cases (over 36.1 million) and second in the number of deaths (over 692 thousand) (1). The Brazilian cumulative incidence rate is now approximately 16,750 cases per 100,000 inhabitants, with an accumulated mortality rate of around 322 deaths per 100,000 inhabitants (2). COVID-19 proved to be a challenging health condition, with high transmissibility, potential systemic involvement, and without well-established treatments. It led to the collapse of several health systems, noticeably in areas with limited health structure in the country (3).

The clinical presentation of COVID-19 is mainly respiratory, with mild involvement in approximately 80% of cases. The new coronavirus also affects the cardiovascular system, especially in severe cases and in patients with established cardiovascular disease. Acute myocardial injury, the most frequent cardiac abnormality (8–20% of patients), is associated with a worse prognosis (4–6). Furthermore, there is an association of COVID-19 with acute coronary syndromes, venous thromboembolism, heart failure—as a consequence of a deterioration of underlying heart disease or induced by viral myocarditis—and a myriad of rhythm disturbances (7). Ventricular tachycardia, a marker of disease severity, is associated with increased serum troponin levels (8) and QT prolongation, observed in approximately 13% of infected patients (8, 9).

Given the absence of well-established pharmacological treatments and the limited knowledge about the natural history of the disease and predictors of worse outcomes—especially in high-risk groups—there were several recommendations for the use of off-label drugs, based on limited scientific evidence, noticeably *in vitro* and observational studies (10, 11). This occurred especially—but not exclusively—in the beginning of the pandemic. Chloroquine compounds (hydroxychloroquine/chloroquine) (namely chloroquine)—associated or not with azithromycin—and ivermectin were among these drug schemes. However, larger-scale observational studies and, more recently, Brazilian randomized trials (12, 13) failed to demonstrate any benefit. Although relatively well tolerated, chloroquine compounds can induce cardiovascular side effects, such as QT interval prolongation and potentially fatal arrhythmias (14, 15), which may further increase the patients' cardiovascular risk. Epidemiological studies assessing cardiac effects of chloroquine for the treatment of COVID-19, with real-life data and broad inclusion criteria, are still limited. We aimed to evaluate the clinical and electrocardiographic outcomes of outpatients with suspected COVID-19, comparing those using chloroquine with individuals without specific treatment and a parallel registry of individuals using other drug classes.

Materials and methods

The procedures and methods of this study will be made available for replication upon reasonable request directed to the corresponding author. The Institutional Review Board of Universidade Federal de Minas Gerais approved the study under CAAE number 37228120.9.0000.5149.

This is a comparative observational study with prospective data collection. The sample consisted of two arms and one parallel registry, and clinical and electrocardiographic outcomes were assessed remotely. The project was funded by the Brazilian Ministry of Health and conducted by the Telehealth Center of Hospital das Clínicas, Universidade Federal de Minas Gerais (Belo Horizonte, MG, Brazil). Remote data collection occurred in health units connected to the Teleassistance Network of Minas Gerais (Rede de Teleassistência de Minas Gerais–RTMG), and the tele-electrocardiography (ECG) system for COVID-19, in all Brazilian regions.

During the COVID-19 pandemic, RTMG adapted its mobile ECG application to provide clinical decision support for COVID-19 cases in health units, especially in primary care, with demographic and clinical data collection, and ECGs for remote interpretation. It was recommended by health authorities that an ECG be obtained before and following the initiation of drugs for COVID-19. ECGs were captured by commercial equipment linked to specific proprietary software, which allows for getting the ECG signal and clinical data, and transmitted by internet to a central server at the Telehealth Center. The requesting healthcare provider collected baseline history, demographic and clinical data. ECGs were centrally analyzed by a team of experienced cardiologists, utilizing specific semi-automated software with measurement and magnification tools, with visual inspection and subsequent classification by the Minnesota code. Minnesota is the most widely used ECG classification system in the world, developed in the 1950s by Dr. Henry Blackburn, which utilizes a defined set of measurement rules to assign specific numerical codes according to the severity of findings (16, 17). In the presence of a discrepancy between automated reports and the cardiologist's

interpretation, exams were audited by the study team, composed of three previously trained investigators. All ECGs of patients with suspected COVID-19 in the study period were eligible for this analysis and stored in a specific database.

Inclusion criteria

Adult patients (≥ 18 years) of both sexes, seen by health professionals in outpatient units with clinical suspicion or laboratory confirmation of COVID-19, whose clinical data and baseline ECG were transmitted through the RTMG app, were screened. This same set of patients was divided into two study groups and one registry, based exclusively on the treatment informed in the online data collection system, as follows:

- Group 1: Patients submitted, at some point during clinical management, to drug therapy with chloroquine, in schemes recommended by the Brazilian Ministry of Health (18): chloroquine diphosphate D1: 500 mg every 12 h (300 mg of chloroquine base) and D2 to D5: 500 mg every 24 h (300 mg of chloroquine base) or hydroxychloroquine sulfate: D1: 400 mg every 12 h and D2 to D5: 400 mg every 24 h.
- Group 2: Patients under standard/supportive clinical treatment for unspecific respiratory syndrome (flu-like syndrome), without any specific drugs for COVID-19.
- Registry (Group 3): Patients submitted, at some point during clinical management, to drug therapy with ivermectin, antibiotics, antivirals, or other specific drugs proposed for COVID-19 at any recommended doses or chloroquine in dose schemes different from those recommended by the Brazilian Ministry of Health.

Exclusion criteria

Exclusion criteria were insufficient baseline clinical data entered in the ECG app; failure to transmit an interpretable digital ECG; refusal to sign the electronic informed consent form and to participate in the 3- and 14-day telephone clinical follow-up; impossibility to collect minimally comprehensible information during clinical follow-up, from the patients or relatives/companions.

Evaluation of outcomes

Study arms: Study groups were continuously identified from data entered in the mobile application (prescription of chloroquine or other specific treatments for COVID-19) and during phone follow-up.

a) Clinical follow-up: Clinical outcomes were systematically assessed on the 3rd (−1 or +2 days) and 14th days (±2 days) after the transmission of the first ECG through standardized phone calls. Contacts were made by trained non-medical professionals at the Telehealth Center or remotely, with at least four attempts per patient, using contact data provided in the mobile app. In case of failure in the 3- and 14-day calls, the patient returned to the study queue for late additional attempts. The link to the electronic informed consent was sent to all patients by short message system (SMS), and clinical follow-up was only initiated after its electronic signature. Throughout the study, messages were sent by SMS and messaging app, with information about the study and reminders about follow-up calls and scheduled ECGs. Considering the small number of patients answering follow-up calls, the study protocol was amended. Clinical outcomes were administratively collected through linkage to national mortality and hospitalization databases: Mortality Information System (Sistema de Informação Sobre Mortalidade—SIM), Influenza Epidemiological Surveillance Information System (Sistema de Informação da Vigilância Epidemiológica da Gripe–SIVEP-Gripe), and COVID-19 Notification System (e-SUS Notifica) (**Supplementary material 1**). Patient-level data on mortality, hospital admissions, and occurrence of severe acute respiratory syndromes were collected for the whole sample after full-access authorization by the Institutional Review Boards and health authorities, and combined with follow-up data.

b) Electrocardiographic data: All patients with adequate clinical data and at least 1 ECG transmitted to the RTMG system were included in the electrocardiographic study. For the assessment of electrocardiographic outcomes, patients in group 1 (chloroquine) were included only when at least one ECG was performed after drug initiation. For group 2, all registered patients were included. Outcomes were recorded if present in any of the ECGs, at baseline (initiation of treatment, at study entry), or follow-up ECGs.

Outcomes of interest

The following clinical and electrocardiographic outcomes were measured:

Clinical follow-up: The composite primary clinical outcome consisted of: (a) all-cause hospitalization; (b) admission to intensive care unit (ICU); (c) need for invasive mechanical ventilation; (d) all-cause death. The secondary outcomes were individual components of the primary outcome. For the analysis of outcomes including administrative data, the exportation of the raw database was crosslinked by name, date of birth, mother's name, and social security number. When necessary, source documents were requested.

Electrocardiographic data: The composite primary electrocardiographic outcome consisted of the occurrence of any new major electrocardiographic abnormalities by the Minnesota coding system in baseline (after treatment initiation) or follow-up (14 days or later) ECGs, confirmed by audit when indicated. When more than 1 ECG was available, abnormalities observed in any of them were considered. The list of major abnormalities considered for the primary outcome is detailed in **Supplementary Table 1**.

Statistical analysis

Statistical analysis was performed using the R software version 1.4.1717-3 [The R Foundation for Statistical Computing, Vienna,

Austria (19)]. The distribution pattern of the variables was evaluated with the Shapiro–Wilk test. Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR), as appropriate. Categorical variables were expressed as absolute values and percentages. Considering the 7.2% case fatality reported in Italy (20), and a 1.3 hazard ratio for mortality in the chloroquine group (21), a sample of 5,830 patients was needed, with a 1:1 distribution between treatment and control groups and at least 463 events, with 80% power to detect the difference in all-cause death. Comparison between treatment groups (group 1, group 2, group 3) was performed using the analysis of variance (ANOVA) *t*-test for continuous variables with normal distribution and the Kruskal–Wallis test for those with non-normal distribution (Mann–Whitney U test for pairwise comparisons). Categorical variables were compared using the chi-square test, and the Fisher's exact test for pairwise comparisons.

Multivariable logistic regression was used to examine the association between COVID-19 treatment groups and the primary outcome for each study arm separately. Significant variables ($p < 0.10$) in univariate analyses were included in multivariate models. The models (4) for clinical outcomes, with data from phone follow-up, were adjusted as follows: Model 1 unadjusted, Model 2, adjusted for age and sex; Model 3, adjusted for model 2 variables plus cardiovascular risk factors, collected in a clinical interview (asthma, diabetes, heart disease, hypertension, stroke, heart failure, other lung diseases, kidney disease, overweight/obesity); Model 4, adjusted for variables in model 3 plus clinical variables related to the severity of COVID-19 at presentation (defined as dyspnea and persistent fever) collected during a clinical interview. For clinical outcomes, combining phone contact and administrative databases, model 4 was not applied—as data on disease severity was not available through the ECG app for most patients. The same models (1 to 3) utilized for ECG outcomes were adjusted as follows: Model 1 unadjusted, Model 2, adjusted for age and sex; Model 3, adjusted for model 2 variables plus cardiovascular risk factors, collected through the ECG app (hypertension, diabetes, dyslipidemia, coronary artery disease, previous stroke, smoking, chronic kidney disease, Chagas disease, chronic lung disease). A two-tailed significance level of 0.05 was considered statistically significant.

Results

During 303 days, a total of 6,957 eligible patients had at least one ECG and clinical data submitted to the online system and were included, with a median age of 49.0 (IQR 38.0–62.0) years, 57% women. Of these, 712 (10.2%) were allocated to group 1 (chloroquine), 3,623 (52.1%) to group 2 (control) and 2,622 (37.7%) to group 3 (other treatments). The baseline demographic and clinical characteristics of the study population are detailed in **Table 1**. Groups were relatively similar; however, group 1 had a lower proportion of women and a lower prevalence of cardiovascular risk factors: hypertension, diabetes, and dyslipidemia. Groups 1 and 3 had a lower prevalence of Chagas disease (**Table 1**). In terms of drug therapy, in group 1 100% of the patients used chloroquine compounds at recommended doses, 13.6% corticosteroids, 30.9% antibiotics, and 25.0% antiparasitics; in group 2, 6.0% used corticosteroids and 13.2% antibiotics (at regimens not specific for COVID-19); in group 3, 10.8% used corticosteroids, 25.2% antibiotics, 15.3% antiparasitics, 1.7% chloroquine at non-recommended doses and 40% used other drug classes or associations recommended for COVID-19. No patients received antivirals.

Throughout the study period, there was a decreasing trend in daily entries, with no significant increase during the 2nd peak of the COVID-19 pandemic in Brazil (April to June 2021) (**Supplementary Figure 1**). A late (≥ 14 days) follow-up ECG was obtained for 917 (13.2%) patients [group 1: 81 (11.4%), group 2: 512 (14.1%), group 3: 334 (12.7%)].

Regarding individual components of the primary outcome, crude rates of hospitalization, ICU admission, mechanical ventilation and all-cause death were higher in groups 1 (chloroquine) and 3 (other treatments) compared to group 2 (control). A total of 462 deaths were recorded. Rates of the primary ECG endpoint, however, were similar (**Table 1**).

Clinical outcomes—phone follow-up

At total, 1,969 (28.3%) patients responded the clinical phone follow-up: 260 (13.2%) patients in group 1, 871 (44.2%) in group 2 and 838 (42.6%) in group 3. Baseline demographic and clinical characteristics of this subpopulation, as well as COVID-19 symptoms at presentation, are detailed in **Table 2**. The groups were overall similar, except for the significantly higher proportion of women in group 2, younger age and lower prevalence of hypertension in group 1, lower prevalence of lung diseases in groups 1 and 2, and higher incidence of severe COVID-19 symptoms (fever and dyspnea) in groups 1 and 3, compared to controls (group 2) (**Table 2**).

Among individual clinical outcomes, higher hospitalization rates were observed in groups 1 (38.5%) and 3 (34.2%) compared to the control group (2) (18.0%), $p < 0.001$, in addition to higher ICU admission rates in group 3 (7.0%) compared to groups 1 (3.1%) and 2 (3.1%), $p < 0.001$. Mechanical ventilation and death rates were similar (**Table 2**). The composite primary endpoint occurred more frequently in groups 1 (38.5%) and 3 (34.2%) compared to group (2) (18.0%), $p < 0.001$ (**Table 2**).

In logistic regression models, prescription of chloroquine was independently associated with a 2.8-fold greater chance of the primary composite outcome, compared with the control group, in the unadjusted model [OR: 2.84 (95% CI 2.10–3.85), $p < 0.001$], as well as in models: 2 [adjusted for sex and age; OR: 3.17 (95% CI 2.31–4.35), $p < 0.001$]; 3 [adjusted for sex, age and risk factors; OR: 3.23 (95% CI 2.34–4.45), $p < 0.001$] and 4 [adjusted for sex, age, risk factors and variables of COVID-19 presentation; OR: 3.24 (95% CI 2.31–4.54), $p < 0.001$]. A similar association was observed for group 3, which also associated with a 2.4-fold greater risk of occurrence of the primary outcome in the unadjusted model 1 [OR: 2.37 (95% CI 1.90–2.97), $p < 0.001$], as well as in the adjusted models (2, 3, and 4) (**Table 3**).

Clinical outcomes—phone follow-up plus national administrative databases

When outcome data from phone follow-up and administrative databases were combined (N = 6,957), the composite primary endpoint also occurred more frequently in groups 1 (22.8%) and 3 (21.4%) compared to controls (2) (13.9%), $p < 0.001$ (**Table 1**). Chloroquine was again associated with a 1.8-fold greater chance of

TABLE 1 Baseline characteristics and rates of clinical outcomes of all patients included in the study (at least 1 ECG and clinical data entered in the online system), combining phone and administrative follow-up data, and comparison between treatment groups.

Variable	Total[1] (*N* = 6,957)	Group 1 (Chloroquine)[1] (*N* = 712)	Group 2 (Control)[1] (*N* = 3,623)	Group 3 (Registry)[1] (*N* = 2,622)	*p*-value[2]	*post hoc*[3]
Sex, male (%)	3,022 (43.4%)	364 (51.1%)	1,476 (40.7%)	1,182 (45.1%)	**<0.001**	group 1 > group 3 > group 2 (0.000) (0.001)
Age (years), median (IQR)	49.0 (38.0, 62.0)	48.0 (38.0, 60.0)	49.0 (38.0, 63.0)	50.0 (38.0, 62.0)	0.142	–
Days to latest ECG, median (IQR)	22.0 (7.0, 95.0)	9.0 (4.0, 19.0)	49.0 (11.0, 129.0)	16.0 (7.0, 68.5)	**<0.001**	group 1 < group 3 < group 2 (0.000) (0.000)
Hypertension, *N* (%)	3,187 (45.8%)	261 (36.7%)	1,707 (47.1%)	1,219 (46.5%)	**<0.001**	group 1 < group 2 = group 3 (0.000) (0.626)
Diabetes, *N* (%)	1,116 (16.0%)	84 (11.8%)	573 (15.8%)	459 (17.5%)	**<0.001**	group 1 < group 2 = group 3 (0.001) (0.077)
Dyslipidemia, *N* (%)	421 (6.1%)	22 (3.1%)	258 (7.1%)	141 (5.4%)	**<0.001**	group 1 < group 3 < group 2 (0.000) (0.001)
Coronary artery disease, *N* (%)	181 (2.6%)	12 (1.7%)	101 (2.8%)	68 (2.6%)	0.240	–
Previous stroke, *N* (%)	148 (2.1%)	12 (1.7%)	82 (2.3%)	54 (2.1%)	0.592	–
Smoking, *N* (%)	408 (5.9%)	37 (5.2%)	223 (6.2%)	148 (5.6%)	0.507	–
Chronic kidney disease, *N* (%)	58 (0.8%)	4 (0.6%)	28 (0.8%)	26 (1.0%)	0.452	–
Chagas disease, *N* (%)	79 (1.1%)	3 (0.4%)	54 (1.5%)	22 (0.8%)	**0.009**	group 1 = group 3 < group 2 (0.221) (0.003)
Chronic lung disease, *N* (%)	63 (0.9%)	7 (1.0%)	32 (0.9%)	24 (0.9%)	0.965	–
Outcomes						
Major ECG abnormality, *N* (%)*	1,020 (14.7%)	89 (12.5%)	547 (15.1%)	384 (14.6%)	0.201	–
Major ECG abnormality (follow-up)*	156 (17.0%)	12 (14.8%)	98 (19.5%)	46 (13.8%)	0.082	–
Hospital admission, *N* (%)	1,014 (14.6%)	139 (19.5%)	399 (11.0%)	476 (18.2%)	**<0.001**	group 1 = group 3 > group 2 (0.406) (0.000)
ICU admission, *N* (%)	269 (3.9%)	31 (4.4%)	98 (2.7%)	140 (5.3%)	**<0.001**	group 1 = group 3 > group 2 (0.281) (0.000)
Mechanical ventilation, *N* (%)	148 (2.1%)	21 (2.9%)	56 (1.5%)	71 (2.7%)	**0.002**	group 1 = group 3 < group 2 (0.729) (0.000)
Death, *N* (%)	462 (6.6%)	56 (7.9%)	200 (5.5%)	206 (7.9%)	**<0.001**	group 1 = group 3 > group 2 (0.994) (0.000)
Composite clinical outcome, *N* (%)	1,225 (17.6%)	162 (22.8%)	503 (13.9%)	560 (21.4%)	**<0.001**	group 1 = group 3 > group 2 (0.425) (0.000)

[1] *n* (%); median (IQR). [2] Pearson's Chi-squared test; Kruskal–Wallis rank sum test. [3] Fisher's exact test. *For ECGs obtained at baseline and/or follow-up. Number of patients with late follow-up ECG: 917 (13.2%) patients [group 1: 81 (11.4%), group 2: 512 (14.1%), group 3: 334 (12.7%), $p = 0.004$]. Bold values represent a *p*-value < 0.05.

the primary outcome compared to the control group (2), in the unadjusted model [OR: 1.83 (95% CI 1.49–2.23), $p < 0.001$], with similar effects observed in models 2 [adjusted for sex and age; OR: 1.96 (95% CI 1.59–2.41), $p < 0.001$] and 3 [adjusted for sex, age and risk factors; OR: 1.99 (95% CI 1.61–2.44), $p < 0.001$]. Group 3 was also independently associated with the primary outcome, although with a lesser magnitude (Table 4).

In the analysis of secondary outcomes, chloroquine was also independently associated with higher all-cause mortality, with a 1.7-fold increase in the final adjusted model (3) (Supplementary Table 2).

Electrocardiographic outcomes

The detailed comparison between electrocardiographic characteristics between groups, at baseline and follow-up, is depicted in Supplementary Table 2. The occurrence of the primary ECG outcome ($N = 6,957$) was similar between groups (group 1: 12.5%; group 2: 15.1%; group 3: 14.6%, $p = 0.201$). In multivariable logistic models, the use of chloroquine was not associated with a higher occurrence of the primary ECG endpoint in the unadjusted model, nor in the models adjusted for sex, age and risk factors (Table 4). Likewise, the prescription of other specific treatments for COVID-19 (group 3) was also not associated with the occurrence of the primary ECG endpoint during COVID-19 treatment (Table 4).

Discussion

Our study, with a large sample of outpatients with clinical suspicion of COVID-19 in different regions of Brazil, showed that the prescription of chloroquine did not increase major ECG abnormalities compared to patients without specific treatment.

TABLE 2 Baseline characteristics and rates of outcomes of interest of patients included in the phone clinical follow-up, and comparison between treatment groups.

Variable	Total[1] (*N* = 1,969*)	Group 1 (Chloroquine)[1] (*N* = 260)	Group 2 (Control)[1] (*N* = 871)	Group 3 (Registry)[1] (*N* = 838)	*p*-value[2]	*post hoc*[3]
Sex, male (%)	869 (44.1%)	135 (51.9%)	353 (40.5%)	381 (45.5%)	**0.003**	group 2 > group 1 = group 3 (0.004) (0.069)
Age (years), median (IQR)	47.0 (37.0, 59.0)	45.0 (35.0, 55.0)	47.0 (37.0, 60.0)	47.0 (37.0, 59.8)	**0.022**	group 1 < group 2 (0.046) group 1 < group 3 (0.020)
Asthma, *N* (%)	55 (2.8%)	3 (1.2%)	23 (2.6%)	29 (3.5%)	0.134	–
Diabetes, *N* (%)	269 (13.7%)	31 (11.9%)	115 (13.2%)	123 (14.7%)	0.460	–
Cardiac disease, *N* (%)	136 (6.9%)	19 (7.3%)	55 (6.3%)	62 (7.4%)	0.652	–
Hypertension, *N* (%)	770 (39.1%)	77 (29.6%)	338 (38.8%)	355 (42.4%)	**0.001**	group 1 < group 2 = group 3 (0.001) (0.134)
Myocardial infarction, *N* (%)	23 (1.2%)	0 (0.0%)	11 (1.3%)	12 (1.4%)	0.125	–
Heart failure, *N* (%)	5 (0.3%)	0 (0.0%)	2 (0.2%)	3 (0.4%)	0.843	–
Other lung diseases, *N* (%)	60 (3.0%)	4 (1.5%)	20 (2.3%)	36 (4.3%)	**0.018**	group 1 = group 2 < group 3 (0.441) (0.006)
Overweight/obesity, *N* (%)	286 (14.5%)	44 (16.9%)	117 (13.4%)	125 (14.9%)	0.342	–
Kidney failure and/or dialysis, *N* (%)	10 (0.5%)	0 (0.0%)	6 (0.7%)	4 (0.5%)	0.457	–
Other kidney diseases, *N* (%)	34 (1.7%)	4 (1.5%)	11 (1.3%)	19 (2.3%)	0.279	–
Shortness of breath, *N* (%)	820 (41.6%)	117 (45.0%)	319 (36.6%)	384 (45.8%)	**<0.001**	group 2 < group 1 = group 3 (0.000) (0.816)
Fever, *N* (%)	968 (49.2%)	132 (50.8%)	390 (44.8%)	446 (53.2%)	**0.002**	group 2 < group 1 = group 3 (0.001) (0.489)
Prostration, *N* (%)	745 (37.8%)	103 (39.6%)	326 (37.4%)	316 (37.7%)	0.812	
Outcomes						
Hospital admission, *N* (%)	544 (27.6%)	100 (38.5%)	157 (18.0%)	287 (34.2%)	**<0.001**	group 2 < group 1 = group 3 (0.000) (0.216)
ICU admission, *N* (%)	94 (4.8%)	8 (3.1%)	27 (3.1%)	59 (7.0%)	**<0.001**	group 3 > group 1 = group 2 (0.000) (0.985)
Mechanical ventilation, *N* (%)	33 (1.7%)	3 (1.2%)	14 (1.6%)	16 (1.9%)	0.758	–
Death, *N* (%)	24 (1.2%)	3 (1.2%)	8 (0.9%)	13 (1.6%)	0.533	–
Composite clinical outcome, *N* (%)	544 (27.6%)	100 (38.5%)	157 (18.0%)	287 (34.2%)	**<0.001**	group 2 < group 1 = group 3 (0.000) (0.216)

[1] *n* (%); median (IQR). [2] Pearson's Chi-squared test; Kruskal–Wallis rank sum test. [3] Fisher's exact test. *Patients who answered the phone follow-up. Bold values represent a *p*-value < 0.05.

On the other hand, chloroquine was associated with higher rates of adverse outcomes, noticeably hospitalization. This effect was consistent after adjustments for multiple variables, including clinical comorbidities and severity of COVID-19 at presentation.

Since the inception of the COVID-19 pandemic, given the great transmissibility and high mortality of the disease—especially in individuals with high cardiovascular risk—there has been great interest in the investigation of specific treatments for early and advanced stages aimed at the control of the exaggerated inflammatory response. In this context, chloroquine emerged as a potential option, and, despite the absence of robust evidence on its effectiveness and safety, there were recommendations for its widespread use in several countries (22). Chloroquine belongs to the class of quinoline antimalarials and blocks the fast-activating delayed rectifier potassium current (23), coded by the human ether-related gene (hERG), in a concentration and time-dependent manner. Such inhibition of the hERG K+ channel can lead to prolongation of the action potential duration and, consequently, of the QT interval on the ECG, potentially triggering ventricular arrhythmias (24). The cardiovascular risk is theoretically further potentialized by the higher incidence of cardiac arrhythmias and acute myocardial injury including myocarditis—a pro-arrhythmogenic condition (25, 26)—in severe COVID-19 phenotypes, markedly in individuals requiring intensive care. Therefore, drugs that prolong the QTc can presumably exacerbate the risk of underlying arrhythmia (27).

Doubts about the real benefits of chloroquine in COVID-19, associated with its potential risks and the absence of other treatments with an impact on mortality, prompted the design of several clinical studies involving thousands of patients from different continents in a variety of disease presentations and stages. There was particular interest in the so-called "early treatment," with the hypothesis that chloroquine, administered after contact with a confirmed case or in the early stages of the flu-like syndrome, could prevent progression to severe forms. One of the most extensive studies, with 2,314 contacts of COVID-19 patients randomized between

TABLE 3 Multivariate risk model for the composite primary outcome (hospitalization/ICU admission/mechanical ventilation/death) assessed by phone follow-up, adjusted for demographic, clinical, and COVID-19 presentation-related variables.

Variables (N = 1,969)	OR (95% CI)	P-value
Model 1		
(Intercept)	0.22 (0.18–0.26)	**<0.001**
Group 1 (chloroquine)	2.84 (2.10–3.85)	**<0.001**
Group 3 (registry: other treatments)	2.37 (1.90–2.97)	**<0.001**
Model 2		
(Intercept)	0.03 (0.02–0.05)	**<0.001**
Group 1 (chloroquine)	3.17 (2.31–4.35)	**<0.001**
Group 3 (registry: other treatments)	2.40 (1.91–3.04)	**<0.001**
Sex (Male)	1.78 (1.45–2.20)	**<0.001**
Age	1.03 (1.03–1.04)	**<0.001**
Model 3		
(Intercept)	0.03 (0.02–0.05)	**<0.001**
Sex (Male)	1.88 (1.52–2.33)	**<0.001**
Age	1.03 (1.02–1.04)	**<0.001**
Group 1 (chloroquine)	3.23 (2.34–4.45)	**<0.001**
Group 3 (registry: other treatments)	2.37 (1.88–3.01)	**<0.001**
Asthma	0.82 (0.40–1.59)	0.572
Diabetes	2.00 (1.49–2.69)	**<0.001**
Cardiac disease	1.10 (0.73–1.63)	0.651
Hypertension	1.06 (0.83–1.34)	0.646
Myocardial infarction	1.14 (0.46–2.76)	0.776
Heart failure	3.62 (0.52–30.44)	0.193
Other lung diseases	2.15 (1.22–3.76)	**0.008**
Kidney disease and/or dialysis	1.22 (0.24–4.8)	0.788
Overweight/Obesity	1.57 (1.16–2.09)	**0.003**
Model 4		
Sex (Male)	1.81 (1.44–2.27)	**<0.001**
Age	1.03 (1.03–1.04)	**<0.001**
Group 1 (chloroquine)	3.24 (2.31–4.54)	**<0.001**
Group 3 (registry: other treatments)	2.21 (1.73–2.85)	**<0.001**
Asthma	0.64 (0.30–1.28)	0.217
Diabetes	1.85 (1.36–2.53)	**<0.001**
Cardiac disease	1.02 (0.66–1.55)	0.933
Hypertension	1.03 (0.80–1.32)	0.837
Heart failure	4.24 (0.57–35.32)	0.144
Other lung diseases	1.7 (0.94–3.07)	0.076
Kidney disease and/or dialysis	1.00 (0.18–4.60)	0.998
Overweight/Obesity	1.27 (0.93–1.73)	0.131
Shortness of breath	3.75 (2.99–4.73)	**<0.001**
Fever	2.03 (1.61–2.55)	**<0.001**

Bold values represent a p-value < 0.05.

hydroxychloroquine (its less toxic hydroxylated metabolite) and usual care, demonstrated similar rates of symptomatic COVID-19 (hydroxychloroquine 5.7% vs. placebo 6.2%, p = NS) and disease transmission (18.7 vs. 17.8%, p = NS), with non-severe side effects reported in the treatment arm (28). Another large-scale randomized trial showed similar results (COVID-19 incidence 11.8 vs. 14.3%, p = NS), with higher rates of adverse effects in the hydroxychloroquine group (29). Similarly, hydroxychloroquine also did not result in less positive diagnostic tests at 28 days in an open study including 150 patients, again with higher rates of side effects in those receiving the drug, including two serious events (30). In none of these studies adverse electrocardiographic outcomes were reported in subjects using chloroquine, although this was not the outcome of interest.

Our sample reflects the context of outpatient treatment of COVID-19, predominantly at early stages and in primary care or emergency public units. Similar to the findings of most primary studies, we observed no clinical benefit of chloroquine; conversely, the drug was independently associated with higher rates of the primary composite outcome, especially at the expense of higher hospitalization rates [adjusted OR: 3.24 (95% CI 2.31–4.54)]. The findings were reinforced by the combination of phone follow-up and administrative data for outcome assessment. However, this trend should be cautiously analyzed, considering the study's methodology (observational, non-randomized, and with secondary data) and the enrollment strategy, based on clinical suspicion of COVID-19—not necessarily confirmed—often raised in units with limited technical resources. However, a meta-analysis of 28 randomized and non-randomized studies published until October 2020, involving over 13,000 patients with COVID-19, showed comparable results, with increased mortality (consistent across all sensitivity analyses) with hydroxychloroquine (OR: 1.11, 95% CI 1.02–1.20, I^2 = 0%) and a similar trend, with smaller sample size, for chloroquine (OR: 1.77, 95% CI 0.15–21.13, I^2 = 0%) (31). Among other factors, this effect may be associated with the dose regimens of chloroquine, especially at the beginning of the pandemic, since interim analyzes of clinical trials showed an increase in both ECG abnormalities (especially prolonged QTc) and lethality with the prescription of higher doses (32). In our protocol, the chloroquine group consisted of patients using low doses standardized by the Brazilian Ministry of Health (18). However, inaccuracies in filling data in the ECG app and difficulties inherent to secondary—and frequently retrospective—data collection through phone contact may have led to the inclusion of different and potentially risky therapeutic regimens (32). It may also be hypothesized that the prescription of chloroquine as a presumable effective treatment may have delayed or deferred the access to guideline-driven supportive therapies or even to hospital and intensive care.

In trials in other clinical scenarios involving patients in late and more severe stages of COVID-19, including those in the ICU, chloroquine also did not improve clinical status during hospitalization, nor did they have an impact on mortality (33), with a trend toward clinical deterioration in some studies (34). Likewise, different studies failed to demonstrate any benefit of combining such compounds with other specific treatments—including antibiotics such as azithromycin, antiparasitics such as ivermectin, and antivirals—in moderate to severe COVID-19 (12, 13, 35). Although clinical benefit was null, no robust data suggest an increase in serious adverse effects and unfavorable outcomes with these drugs at their usual doses. This also contrasts, in a way, with

TABLE 4 Multivariate risk model for the composite primary outcome (hospitalization/ICU/mechanical ventilation/death) assessed by phone follow-up plus administrative databases, and for the primary electrocardiographic outcome (major ECG abnormalities by the Minnesota code), adjusted for demographic and clinical variables.

Variable (N = 6,957)	OR (95% CI)	P-value	OR (95% CI)	P-value
Outcome	Hospitalization/ICU admission/ mechanical ventilation/death		Major ECG abnormalities*	
Model 1				
(Intercept)	0.16 (0.15–0.18)	**<0.001**	0.18 (0.16–0.19)	**<0.001**
Group 1 (chloroquine)	1.83 (1.49–2.23)	**<0.001**	0.80 (0.63–1.02)	0.074
Group 3 (registry: other treatments)	1.68 (1.48–1.92)	**<0.001**	0.96 (0.84–1.11)	0.620
Model 2				
(Intercept)	0.02 (0.01–0.02)	**<0.001**	0 (0–0.01)	**<0.001**
Group 1 (chloroquine)	1.96 (1.59–2.41)	**<0.001**	0.85 (0.65–1.09)	0.203
Group 3 (registry: other treatments)	1.72 (1.50–1.97)	**<0.001**	0.93 (0.80–1.09)	0.389
Sex (Male)	1.51 (1.33–1.72)	**<0.001**	1.82 (1.58–2.11)	**<0.001**
Age	1.04 (1.03–1.04)	**<0.001**	1.06 (1.06–1.07)	**<0.001**
Model 3				
(Intercept)	0.02 (0.01–0.02)	**<0.001**	0 (0–0.01)	**<0.001**
Sex (Male)	1.52 (1.34–1.73)	**<0.001**	1.9 (1.64–2.21)	**<0.001**
Age	1.04 (1.03–1.04)	**<0.001**	1.06 (1.05–1.06)	**<0.001**
Group 1 (chloroquine)	1.99 (1.61–2.44)	**<0.001**	0.92 (0.71–1.19)	0.542
Group 3 (registry: other treatments)	1.70 (1.48–1.96)	**<0.001**	0.97 (0.83–1.13)	0.704
Hypertension	1.00 (0.86–1.15)	0.965	1.48 (1.25–1.74)	**<0.001**
Diabetes	1.38 (1.17–1.63)	**<0.001**	0.95 (0.79–1.14)	0.607
Dyslipidemia	0.88 (0.68–1.14)	0.351	1.18 (0.91–1.52)	0.214
Coronary artery disease	1.04 (0.71–1.50)	0.844	3.13 (2.23–4.38)	**<0.001**
Previous stroke	1.39 (0.93–2.05)	0.102	1.05 (0.69–1.57)	0.819
Smoking	0.84 (0.64–1.10)	0.223	0.95 (0.70–1.26)	0.713
Chronic kidney disease	1.33 (0.70–2.42)	0.364	1.04 (0.49–2.06)	0.924
Chagas disease	1.27 (0.72–2.13)	0.391	3.76 (2.29–6.14)	**<0.001**
Chronic lung disease	1.21 (0.64–2.16)	0.544	0.76 (0.38–1.46)	0.429

ECG: electrocardiogram; ICU: intensive care unit. *For ECGs obtained at baseline and/or follow-up. Number of patients with late follow-up ECG: 917 (13.2%) patients (group 1: 81 (11.4%), group 2: 512 (14.1%), group 3: 334 (12.7%), $p = 0.004$). Bold values represent a p-value < 0.05.

our results, since the use of other specific COVID-19 treatments—excluding recommended chloroquine doses—(parallel registry) was also associated with worse outcomes [adjusted OR: 2.21 (95% CI 1.73–2.85)]. Again, this finding should be carefully interpreted in light of the methodology applied and—especially for the registry—considering the heterogeneity of the treatments in terms of drug classes, doses, and associations. Despite the consistency of the effect after multiple adjustments, biases resulting from such a degree of heterogeneity may have impacted the results.

Regarding ECG data, the lack of association between chloroquine prescription and major ECG abnormalities or arrhythmias was consistent across different model adjustments, and no association was observed between other treatments (registry) and the primary ECG outcome. Despite the challenges for the acquisition of longitudinal electrocardiographic data in a nationwide study carried out by a public telemedicine network, this finding is in agreement with most studies published so far (36). Despite the potential risk of cardiac arrhythmias associated with antimalarial drugs, in addition to the cardiac involvement in severe COVID-19 and the preliminary case reports of potentially fatal arrhythmias in patients with the disease (37), studies with chloroquine in both hospital and outpatient settings have not consistently shown an increase in major ECG changes, despite the myriad of incident side effects in the treatment groups (36, 38). Data heterogeneity should also be considered for this analysis, especially about the timing of the baseline ECG versus the peak incidence of electrocardiographic outcomes following chloroquine administration (first 72 h) and the considerable loss of electrocardiographic follow-up. As the majority of ECGs analyzed preceded the peak of the drug's arrhythmogenic effect, with very limited 3-day and 14-day tracings, definite causal inferences cannot be drawn from our study. On the other hand, there are no consistent evidences of late electrocardiographic effects of chloroquine, especially after discontinuation. Furthermore, major ECG abnormalities were associated with variables as age, gender, hypertension, coronary artery disease and Chagas' disease—all known risk factors for cardiac pathologies. Thus, they may possibly be due to underlying cardiovascular disease, and not directly related to COVID-19 or chloroquine.

Even with the aforementioned difficulties for the acquisition of longitudinal data, tele-ECG emerged as a promising tool to support risk stratification and decision-making during a pandemic. Although the strategy may not be feasible in certain areas—noticeably remote locations with limited connection—worldwide studies suggest that tele-ECG may help guide effective control and interventions, including from low- and middle-income countries where documentation of cardiovascular abnormalities and risk factors in COVID-19 patients is scarce (39). Furthermore, other technology-based solutions as wearable devices and smartwatches hold promise for individuals with respiratory diseases. They have been successfully tested to predict the onset of COVID-19 through early changes in heart rate variability, to track the effects of vaccination on the body and to monitor normalization of heart rate after SARS-CoV-2 infection, as a surrogate for long COVID-19 (40, 41). This opens up a route for clinical application of biometric data.

Despite the challenges of conducting large-scale population-based research, with clinical and ECG data collection in a continental country, our study represents the largest Latin American outpatient sample with real-life data. Although outcomes should be parsimoniously analyzed, mainly due to the high rate of loss-to-follow-up—requiring cross-linkage with administrative databases—and the possibility of selection (greater response to phone contacts in families who experienced severe cases, with closer connection with health services) and treatment (drugs most often administered to severe cases) biases, the final models are consistent. Even after detailed adjustment for demographic and clinical variables, comorbidities, and COVID-19 symptoms at presentation, the association between chloroquine and unfavorable outcomes—especially hospitalization—remained broadly significant, with OR > 3.0. Furthermore, the effect was strengthened by the inclusion of administrative data, suggesting that these findings should be considered for therapeutic decision-making. On the other hand, robust ECG data suggest the safety of chloroquine and other drug regimens in terms of the induction of rhythm disturbances and major abnormalities, even with the increased risk associated with the potential severity of the disease, inflammatory response, cardiac involvement, and coexistence of cardiovascular disease. In the absence of ECG changes explaining the worse outcomes among treated patients, it may be hypothesized that late incident arrhythmias, or other severe side effects may have accounted for higher rates of clinical events. Thus, continuous efforts should be made to mitigate the risks of cardiac and systemic toxicity (42).

Limitations

Our study has several limitations, which should be considered to interpret the findings. First, data collection was performed indirectly, through telephone contact, information entered into the ECG app by the provider, or cross-linkage with national mortality and hospitalization databases. Despite the pragmatic research protocol, there may have been some imprecision in data collection, especially regarding details and timing of outcomes. Markedly for the phone contact arm, there is potential bias related to the precision of outcome assessment as well as to misinterpretation of clinical questions by patients and families. Patient literacy—not systematically evaluated in the study—may have contributed to this issue. Second, there was a great difficulty in the completion of telephone follow-up, underpowering this specific analysis and possibly selecting individuals with access to communication and mobile devices and better acceptance of the approach by the research team. The heterogeneous timing of the late phone follow-up, noticeably when several attempts were needed, may have also affected outcome rates. Although this sample with detailed clinical information was limited, the magnitude of the observed effects was robust and maintained despite several adjustments. Third, the rates of ECG follow-up were extremely low. Although treatment-related ECG abnormalities usually develop early (being detectable in the 3-day ECG), our data do not allow for longitudinal analyses of the incidence of ECG changes during COVID-19. Fourth, the inclusion criteria were broad, and patients were enrolled regardless of laboratory diagnosis of COVID-19. As COVID-19 tests were not broadly available in Brazil in the beginning of the pandemic, data on positivity and type of test was not available. Finally, detailed information about causes of hospitalization and death was not possible by remote contact nor by the non-qualified databases, limiting inferences about underlying and associated conditions. Despite these limitations, to the best of our knowledge, this is the largest study with clinical and electrocardiographic follow-up of outpatients with COVID-19 in Latin America. The results are representative of real-life patient care during the pandemic. Added to available data, these findings may help consolidate evidence-based recommendations for the treatment of COVID-19.

Conclusion

Chloroquine was associated with a higher risk of poor outcomes in patients suspected to have COVID-19 when compared to those who received standard care. The utilization of other specific treatments for COVID-19 in the parallel registry was also associated with an equally higher risk. Follow-up ECGs were obtained in only 13.2% of patients and did not show any significant differences in major abnormalities amongst the three groups. In the absence of early incident ECG abnormalities, other side effects of chloroquine, late arrhythmias or deferral of medical care may explain the worse outcomes.

Such data add to the evidence on the non-efficacy and potential risk of treating COVID-19 with chloroquine. However, limitations inherent to the observational study design and the remote and indirect collection of non-randomized data preclude definite causal inferences.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of Universidade Federal de Minas Gerais, CAAE number 37228120.9.0000.5149. The

patients/participants provided their written informed consent to participate in this study.

Author contributions

BN, AR, and GP: conception and design of the research. LT, AA, DP, LR, PG, and MM: acquisition of data. AR, BN, and GP: analysis and interpretation of data. MP and BN: statistical analysis. BN, GP, and AR: writing of the manuscript. BN, AR, and GP: responsible for the overall content as guarantors. All authors contributed critical revision of the manuscript for intellectual content and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2023.1028398/full#supplementary-material

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Acute changes in myocardial tissue characteristics during hospitalization in patients with COVID-19

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Background: Patients with a history of COVID-19 infection are reported to have cardiac abnormalities on cardiovascular magnetic resonance (CMR) during convalescence. However, it is unclear whether these abnormalities were present during the acute COVID-19 illness and how they may evolve over time.

Methods: We prospectively recruited unvaccinated patients hospitalized with acute COVID-19 (n=23), and compared them with matched outpatient controls without COVID-19 (n=19) between May 2020 and May 2021. Only those without a past history of cardiac disease were recruited. We performed in-hospital CMR at a median of 3days (IQR 1–7days) after admission, and assessed cardiac function, edema and necrosis/fibrosis, using left and right ventricular ejection fraction (LVEF, RVEF), T1-mapping, T2 signal intensity ratio (T2SI), late gadolinium enhancement (LGE) and extracellular volume (ECV). Acute COVID-19 patients were invited for follow-up CMR and blood tests at 6months.

Results: The two cohorts were well matched in baseline clinical characteristics. Both had normal LVEF (62±7 vs. 65±6%), RVEF (60±6 vs. 58±6%), ECV (31±3 vs. 31±4%), and similar frequency of LGE abnormalities (16 vs. 14%; all p>0.05). However, measures of acute myocardial edema (T1 and T2SI) were significantly higher in patients with acute COVID-19 when compared to controls (T1=1,217±41ms vs. 1,183±22ms; p=0.002; T2SI=1.48±0.36 vs. 1.13±0.09; p<0.001). All COVID-19 patients who returned for follow up (n=12) at 6months had normal biventricular function, T1 and T2SI.

Conclusion: Unvaccinated patients hospitalized for acute COVID-19 demonstrated CMR imaging evidence of acute myocardial edema, which normalized at 6 months, while biventricular function and scar burden were similar when compared to controls. Acute COVID-19 appears to induce acute myocardial edema in some patients, which resolves in convalescence, without significant impact on biventricular structure and

function in the acute and short-term. Further studies with larger numbers are needed to confirm these findings.

KEYWORDS

SARS-COV2, COVID-19, cardiovascular magnetic resonance imaging, T1-mapping, myocardial edema, T2-weighted images

Introduction

Early studies of COVID-19 survivors at 3–6 months post-infection found a high prevalence (26–78%) of cardiac abnormalities when evaluated using cardiovascular magnetic resonance imaging (CMR), mainly reporting high myocardial T1 and T2 signals (1–3). However, a case–control study found no excess in CMR abnormalities at 6 months after mild COVID-19 (4). These discrepancies may be multi-factorial, including heterogeneity in the severity of COVID-19 and prevalence of past cardiac disease, scan timing, and protocols (T1 and T2 assessments did not offer full heart coverage in most published studies). Additionally, there was also a lack of systemic testing for biochemical evidence of acute myocardial injury (Troponin rise) at the time of acute illness and lack of control groups (2). Importantly, it is unclear if the myocardial T1 and T2 abnormalities found during convalescence were present during the acute COVID-19 illness. Elevated T1 and T2 indicate elevated myocardial water content, and has been observed in patients with other acute non-cardiac systemic illnesses (5); these may not necessarily indicate histopathologic myocarditis, but may reflect acute myocardial edema. Thus, in this prospective study, we investigated the cardiac findings during the acute and recovering phases of COVID-19 using CMR, in patients without a history of cardiac disease, and compared them to controls without COVID-19 but with matching cardiovascular risk factors.

Materials and methods

Two cohorts of participants (total $n = 42$) were prospectively recruited near the start of the COVID-19 pandemic, between May 2020 and May 2021: (1) patients hospitalized for acute COVID-19 ($n = 23$) and (2) outpatient controls without COVID-19 but with cardiovascular risk factors and no cardiac symptoms ($n = 19$). Ethical approval was obtained (REC:10/H0408/24); all participants gave written informed consent. The acute COVID-19 cohort included patients admitted with hypoxia requiring oxygen and/or steroid therapy. COVID-19-negative controls were prospectively recruited from the community to match the clinical characteristics (age, gender, and cardiovascular risk factors) in the acute COVID-19 cohort. Exclusion criteria included documented history of pre-existing cardiac disease or cardiac symptoms (verified by electronic patient records), previous COVID-19 and/or vaccination (verified on history and with dedicated antibody testing), and hemodynamic or respiratory instability (i.e. escalating oxygen support) at the point of scanning. Cardiac Troponin levels (cTnI) were measured before the CMR scan. Normal Troponin level at our institution is <34 ng/L. Patients with a clinical diagnosis of acute myocardial infarction (MI) or a clinical indication for CMR were excluded; this was done to investigate the incidental cardiac findings on CMR during acute COVID-19. COVID-19 severity was graded on the World Health Organization (WHO) four-point scale on chest imaging (6). All patients were recruited after they became clinically and hemodynamically stable, and underwent blood sampling and CMR, per our prospective research protocol. Safety precautions, including the wearing of full personal protection equipment (PPE) by researchers, were implemented in line with hospital-wide guidance at the time of the study.

The CMR (3-Tesla) methods included whole-heart slice-matched cine, native T1-mapping (ShMOLLI), bright-blood T2-weighted imaging (T2 signal intensity ratio of myocardial vs. skeletal muscle; T2SI), late gadolinium enhancement (LGE) imaging and extracellular volume (ECV) quantification (7–10). Acute COVID-19 patients were invited for a 6-month follow-up CMR and blood tests. Image analysis was performed on CVI_{42} (Circle Cardiovascular Imaging Inc., Canada) by three experts (MS, MB, and AB). Abnormally high myocardial T1 and T2SI were defined as T1 > 1,244 ms (>2SD above mean in healthy volunteers; 1,184 ± 30 ms) and T2SI > 1.4 (>2SD above mean in healthy volunteers and rounded up; 1.2 ± 0.1), respectively.

Statistical analysis was performed on SPSS v25 (SPSS Inc., United States). Categorical values are presented as frequencies and percentages. Continuous values are presented as mean ± SD or median (IQR), where applicable. Between-group comparisons were performed using independent and paired *t*-tests, Wilcoxon signed-rank test, Kruskal-Wallis test, Fisher's exact test or McNemar's test, as appropriate.

Results

Acute COVID-19 patients and controls had similar baseline characteristics, although there was a trend toward higher proportion of smokers in the acute COVID-19 cohort (11 vs. 40%, $p = 0.075$; Table 1). 65% of acute COVID-19 patients had ≥ WHO grade 2 disease severity on chest imaging; 22% had received non-invasive ventilation; and 13% had a diagnosis of pulmonary embolism prior to the CMR scan. CTnI was elevated in 8 (35%) patients with acute COVID-19, which were considered by the clinical care team to be mild and part of their acute illness (median rise of 2.4-fold above the normal threshold), and thus these patients were not on treatment for acute coronary syndrome or myocarditis. None of the patients had any documented arrhythmia prior to recruitment.

Median time between admission for COVID-19 infection and CMR was 3 days (1–7). On CMR imaging, controls and acute COVID-19 patients had similar left and right ventricular ejection fraction (LVEF 62 ± 7 vs. 65 ± 6%; $p = 0.103$, RVEF 60 ± 6 vs. 58 ± 6%; $p = 0.373$). They also had similar ECV (31 ± 3 vs. 21 ± 4%; $p = 0.0804$) and frequency of LGE abnormalities (16 vs. 14%; $p = 0.981$). Non-ischemic LGE, suggestive of myocarditis, was observed in two participants each from the control (11%) and acute COVID-19 (9%) cohorts. Myocardial infarction was present in one each from the two cohorts (Table 2).

TABLE 1 Baseline characteristics and blood test results.

	Control (n=19)	Acute COVID-19 (n=23)	p-value
Age (years)	57 ± 12	58 ± 14	0.838
Gender (Male %)	63	69	0.748
Ethnicity (White Caucasian %)	74	70	1
Past history of cardiac disease	0	0	1
Diabetes (%)	0	9	0.492
Hypertension (%)	16	35	0.291
Smoker (%)	11	40	0.075
Temperature	37.13 ± 0.5	37.96 ± 0.71*	<0.001
Oxygen saturation (%)	97.37 ± 1.8	90.13 ± 4.61*	<0.001
Systolic blood pressure (mmHg)	136 ± 16	103 ± 23*	<0.001
Heart rate (bpm)	67 ± 7	98 ± 17*	<0.001
White blood cell count (10^9/L)	5.63 ± 1.9	7.1 ± 3.9*	0.143
Lymphocyte count (10^9/L)	1.59 (1.42–1.94)	0.69 (0.48–1.00)*	<0.001
Hematocrit	0.43 ± 0.03	0.40 ± 0.04	0.031
D-dimer (ng/ml)	227 (167–317)	1,609 (769–2,723)*	<0.001
C-reactive protein (mg/L)	1 (0.60–1.5)	120 (60–160)*	<0.001
Troponin-I (ng/ml)	1.99 (1.99–3)	9 (5–62)*	<0.001
Elevated Troponin (>34 ng/ml; %)	0	35	0.005
NT-pro-BNP (pg/ml; normal <400 pg/ml)	53 (25–77)	217 (70–365)*	0.001
COVID-19 severity score ≥ 2 on CT	N/A	65	N/A
Pulmonary embolism diagnosed (%)	N/A	13	
Treatment with steroids (%)	N/A	57	
Treatment with Remdesevir (%)	N/A	48	
Treatment with antibiotics (%)	N/A	74	
Non-invasive ventilation (%)	N/A	22	

Baseline demographics, vital signs, blood test results (* worst values during hospitalization for COVID-19 patients), and treatment administered and CMR findings. Categorical values are presented as frequencies in percentage (%). Continuous values are presented as mean ± SD or median (IQR) where applicable. CTnI levels reported as < 2 ng/ml in the laboratory assay are presented as 1.99 ng/ml. NT-pro-BNP, N-Terminal prohormone of brain natriuretic peptide; CT, computed tomography.

Compared to controls, patients with acute COVID-19 had significantly higher myocardial T1 (1,183 ± 22 vs. 1,217 ± 41 ms; p = 0.002) and T2SI (1.13 ± 0.09 vs. 1.47 ± 0.36; p < 0.001; Figure 1). Whilst all control subjects had normal T1 and T2SI, abnormally high T1 and T2SI were present in 26 and 50% of acute-COVID patients, respectively. Those with abnormally high T1 had significantly higher LVEF compared to patients with normal T1 (68 ± 8 vs. 62 ± 5%; p = 0.04), while there was no significant difference in the LVEF between patients with abnormally high and normal T2SI (65 ± 7 vs. 63 ± 6%; p = 0.519). There were no significant differences in the T1, T2SI, or ECV between patients treated with and without corticosteroids [1,220 ± 42 vs. 1,213 ± 41 ms (p = 0.703), 1.43 ± 0.34 vs. 1.53 ± 0.40 (p = 0.543), and 32 ± 4 vs. 30 ± 4% (p = 0.303), respectively].

The acute COVID-19 patients with mildly elevated CTnI had similar CMR findings compared to acute COVID-19 patients with normal CTnI levels; the T1 was 1,217 ± 28 vs. 1,205 ± 45 ms (p = 0.357) and the T2SI was 1.51 ± 0.33 vs. 1.37 ± 0.45 (p = 0.274), respectively (Figure 2). Pathological LGE was seen in 2/8 patients with raised CTnI and 1/15 patients with normal CTnI levels, leading to further cardiology referrals, and coronary revascularization in one patient. The CMR findings did not change the clinical management of other patients.

Twelve acute COVID-19 patients returned for follow-up at around 6 months (median 166, IQR 116–184 days) after the acute CMR scan. At follow up, patients had normal inflammatory [CRP 1.4 (1–2.2) mg/L] and cardiac biomarkers [Troponin-I 4.1 (1.99–7.3 ng/L); NT-proBNP 83 (36–230) pg./ml; all p < 0.05 when compared to blood tests during the hospitalization; Table 2]. All patients demonstrated normal myocardial T1 (1,180 ± 36 ms) and T2SI (1.24 ± 0.09) on the 6-month scan (Table 2; Figure 3). There were no new LGE abnormalities at follow up.

Discussion

For the first time, we describe the acute cardiac findings using CMR in hospitalized patients with acute COVID-19, and compared them to matched controls and performed follow-up scans at 6 months. We found that acute COVID-19 was associated with significant myocardial edema in some patients, as demonstrated by significantly elevated myocardial T1 and T2 signals compared to controls (Figure 4). Otherwise, both cohorts had normal biventricular systolic function, similar ECV fraction and frequency of LGE findings. Given the similar ECV fraction between acute COVID-19 patients and controls, we propose that the mechanism of raised T1 and T2 signals in acute COVID-19 may, in part,

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TABLE 2 CMR and blood test findings in controls, acute COVID-19 patients, and at follow up.

	Controls (*n*=19)	Acute COVID-19 (*n*=23)	*p*-value *(vs controls)*	Follow up COVID-19 (*n*=12)	*p*-value *(vs controls)*	*p*-value *(vs acute; paired analysis)*
Time of CMR scan after admission (days)	N/A	3 (1–7)	N/A	166 (116–184)	N/A	N/A
Heart rate during scan (bpm)	67 ± 7	72 ± 15	0.077	65 ± 11	0.571	0.199
LVEDV indexed to BSA (ml/m^2)	74 ± 13	67 ± 15	0.103	75 ± 13	0.920	0.761
Left ventricular ejection fraction (%)	62 ± 7	65 ± 6	0.272	65 ± 7	0.397	0.615
RVEDV indexed to BSA (ml/m^2)	77 ± 15	69 ± 15	0.118	72 ± 14	0.356	0.975
Right ventricular ejection fraction (%)	60 ± 6	58 ± 6	0.373	61 ± 6	0.490	0.085
Global LV myocardial T1 (ms)	1,183 ± 22	1,217 ± 41	**0.002**	1,180 ± 36	0.435	0.177
Global myocardial T2 Signal Intensity ratio (T2SI)	1.13 ± 0.09	1.47 ± 0.36	**<0.001**	1.24 ± 0.09	**0.006**	0.127
Global myocardial extracellular volume (ECV) (%)	31 ± 3	31 ± 4	0.804	29 ± 3	0.073	0.234
Presence of pathological LGE[$], *n* (%)	3 (16)	3 (14)	1	0 (0)	N/A	N/A
Presence of ischemic LGE, *n* (%)	1 (5)	1 (5)	0.981	0 (0)	N/A	N/A
Presence of non-ischemic LGE, *n* (%)	2 (11)	2 (9)		0 (0)	N/A	N/A
C-reactive protein (mg/L)	1 (0.60–1.5)	120 (60–160)	**<0.001**	1.4 (1–2.2)	0.104	**0.005**
Troponin-I (ng/ml; normal <34 ng/ml)	1.99 (1.99–3)	9 (5–62)	**<0.001**	4.1 (1.99–7.3)	0.085	**0.004**
NT-pro-BNP (pg/ml; normal <400 pg/ml)	53 (25–77)	217 (70–365)	**0.005**	83 (36–230)	0.346	**0.033**

Baseline CMR findings. Categorical values are presented as frequencies in percentage (%). Continuous values are presented as mean ± SD or median (IQR) where applicable. LVEDV, left ventricular end diastolic volume; BSA, body surface area; ECV, extracellular volume. Myocardial T2 signal intensity ratio (T2SI) was compared to skeletal muscle. LGE, late gadolinium enhancement. Other abbreviations as per Table 1 [$]Presence of LGE in the ventricular insertion points was not considered pathological. The patterns of non-ischemic LGE seen in each group: two control subjects had sub-epicardial LGE and two COVID-19 patients had mid-wall fibrosis. *p* values less than <0.05 are in bold. N/A - Not applicable.

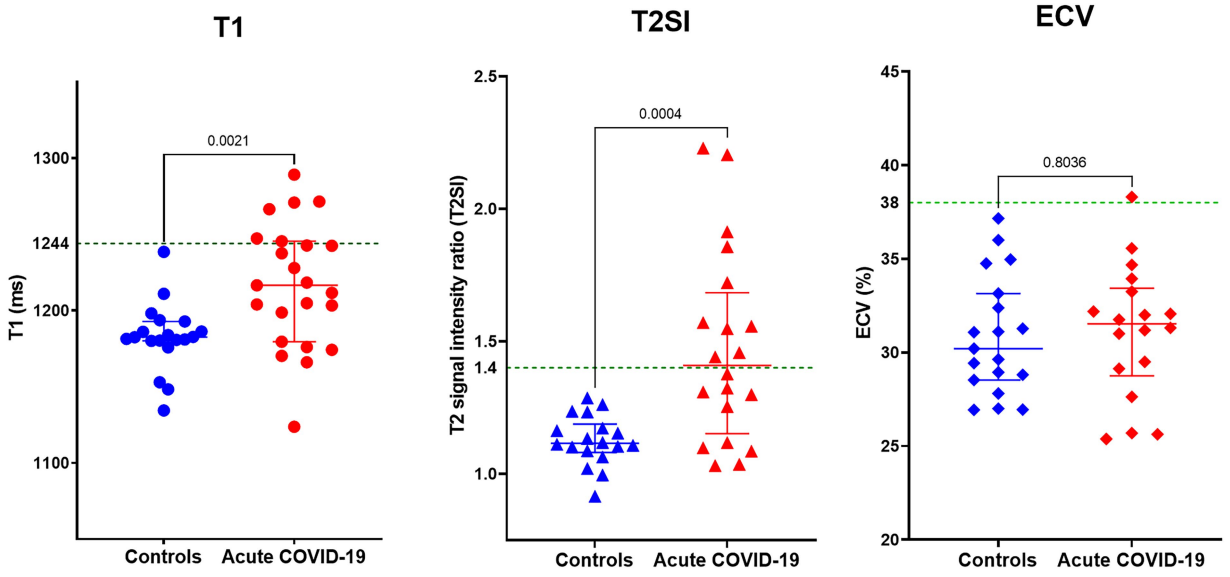

FIGURE 1
Myocardial tissue characteristics on cardiovascular magnetic resonance (CMR) during acute COVID-19 in hospitalized patients compared to controls without COVID-19 from the community matched for cardiovascular risk factors. Comparison of global myocardial T1, T2 signal intensity (T2SI) ratio, and extracellular volume (ECV) fraction between outpatient controls without COVID-19 and patients with acute COVID-19 during their hospitalization. Green dotted line in the graphs denotes the upper limit of normal ranges for T1, T2SI ratio, and ECV in the 3T MR scanner used for this study.

be attributed to the presence of intracellular edema. Findings from the follow up CMR scans suggest that the acute myocardial edema tend to normalize over time during the convalescent phase, with preserved biventricular function.

SARS-CoV2 has been reported to induce acute myocardial injury and heart failure, similar to other cardiotropic viruses (11, 12). Earlier studies of COVID-19 survivors in the community reported significantly elevated myocardial T1 and T2 signals on CMR in convalescence; but the pathophysiologic origins of these imaging findings were unclear, especially in the absence of evidence for acute myocardial injury (troponin rise and fall) during the acute illness and the inclusion of patients with previous history of cardiac disease (2, 13). T1 and T2

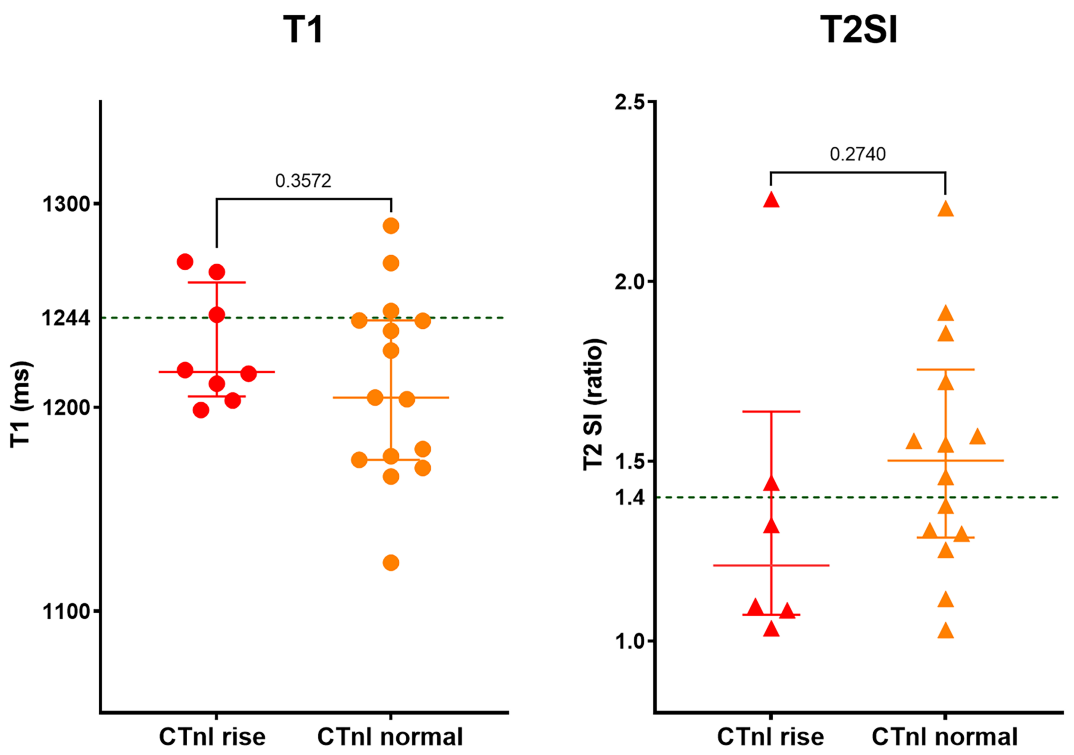

FIGURE 2
Myocardial tissue characteristics on CMR during acute COVID-19 in hospitalized patients with and without mild cardiac Troponin (CTnI) rise. Out of 23 patients, eight had mildly elevated CTnI levels (on average 2.4-fold above normal range). Green dotted line in the graphs denotes the upper limit of normal range for T1 and T2SI ratio in the 3T MR scanner used for this study.

signals can be prolonged due to increased myocardial water content, regardless of injury to the cardiomyocytes or histopathological myocarditis. Such a change in water content can take place in either or both intracellular or extravascular (including coronary arteries) compartments and be attributed to physiological changes during stress (14, 15). Therefore, it is possible that similar alterations may occur during a systemic inflammatory condition such as acute COVID-19, leading to elevation in T1 and T2 values, which then regress to normal during convalescence.

To our knowledge, the only study to-date to describe findings in acutely ill COVID-19 patients was performed by Chen et al. (16): it was retrospective study of a young cohort (median age 23 years) of well COVID-19 patients ($n = 25$) with suspected myocardial injury [Troponin rise ($n = 8$) or ECG changes or cardiac symptoms] at ~6 days after symptoms onset, and who had a clinical indication for CMR. They observed higher T1, T2, and ECV fraction when compared to healthy controls. In contrast, given the association of older age with negative clinical outcomes (17), our prospective study recruited older (mean age 56 years) and sicker acute COVID-19 patients without a clinical indication for CMR. Furthermore, to reduce the effects of confounding factors, we excluded patients with a history of cardiac disease, and included a control group with similar cardiovascular risk factors, measured cardiac troponin levels acutely, investigated their associations with the imaging findings, and performed follow-up CMR imaging.

We observed that myocardial T1 and T2SI abnormalities in acute COVID-19 patients with mildly elevated cTnI levels were not different from acute COVID-19 patients who had normal cTnI levels. This is similar to the observation made by Chen et al. (16). This suggests that myocardial edema in COVID-19 can occur independently of troponin rise. Moreover, we found no differences in the frequency of LGE abnormalities between controls and acute-COVID patients (who were recruited on the basis of no documented history of cardiac disease). This suggests that the LGE abnormalities described in recent studies of patients during the recovering phase of COVID-19 could have been pre-existing, pre-dating the acute COVID-19 illness; thus longitudinal studies of healthy subjects who had CMR scans performed pre-pandemic are needed (2). Furthermore, while patients in our study with mild rise in cTnI exhibited similar CMR findings to those with normal CTnI, in clinical practice, the magnitude of cTnI and clinical context, especially the presence of past history of cardiovascular disease, need to be carefully evaluated. Moreover, we did not detect any arrhythmias, a potential cause of Troponin rise, in our cohort during the hospital admission; but previous reports have documented arrhythmias during acute COVID-19 (18, 19).

The findings from our follow-up of acute COVID-19 patients on their 6-month CMR suggest that the myocardial tissue abnormalities (T1, T2) improved (and normalized) in convalescence, in keeping with previous reports (20, 21). The preservation of global biventricular systolic function during both the acute and recovery phases of COVID-19 in our study supports evidence from other reports that T1 and T2 abnormalities were not correlated with biochemical or imaging evidence of heart failure at the time of assessment during the recovery phase (1, 2, 22). Furthermore, the presence of higher LVEF in patients with abnormally high

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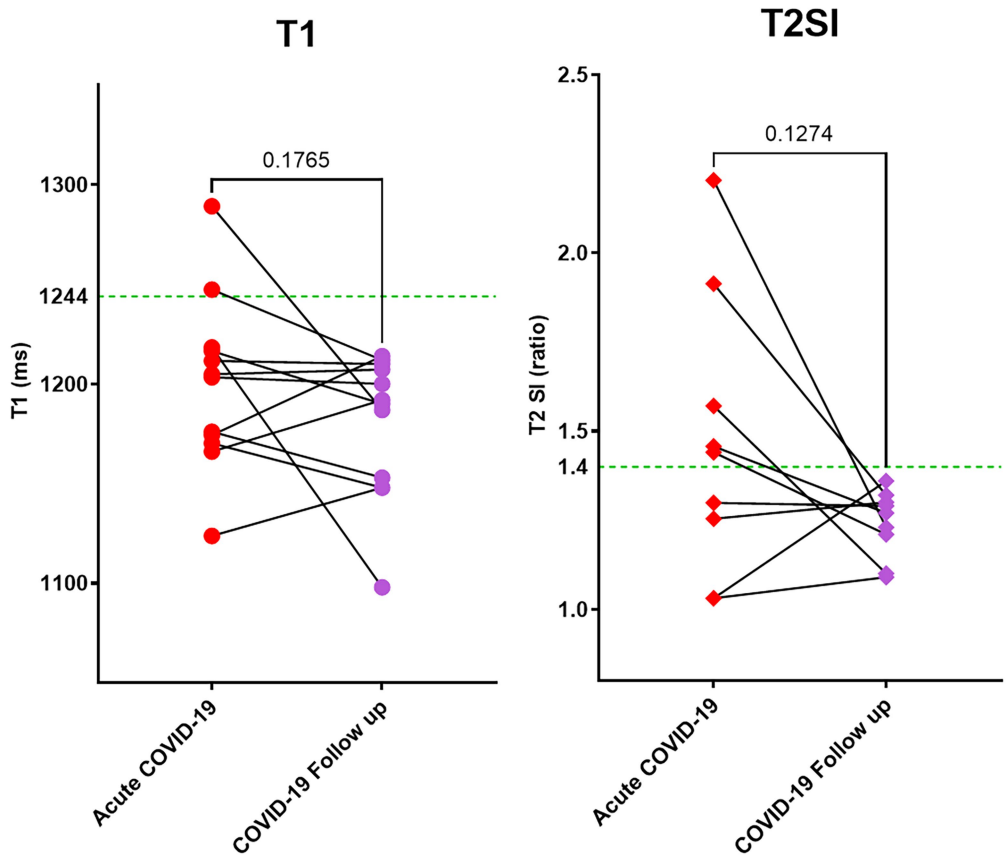

FIGURE 3
Myocardial tissue characteristics as measured by CMR T1 mapping and T2 weighted imaging. During acute COVID-19 illness and at follow up (at 6months). Out of 23 patients scanned during acute COVID-19, 12 returned for follow up during the pandemic. All patients had normal T1 and T2SI ratio at follow up. Green dotted line in the graphs denote the upper limit of normal range for T1 and T2SI ratio in the 3T MR scanner used for this study.

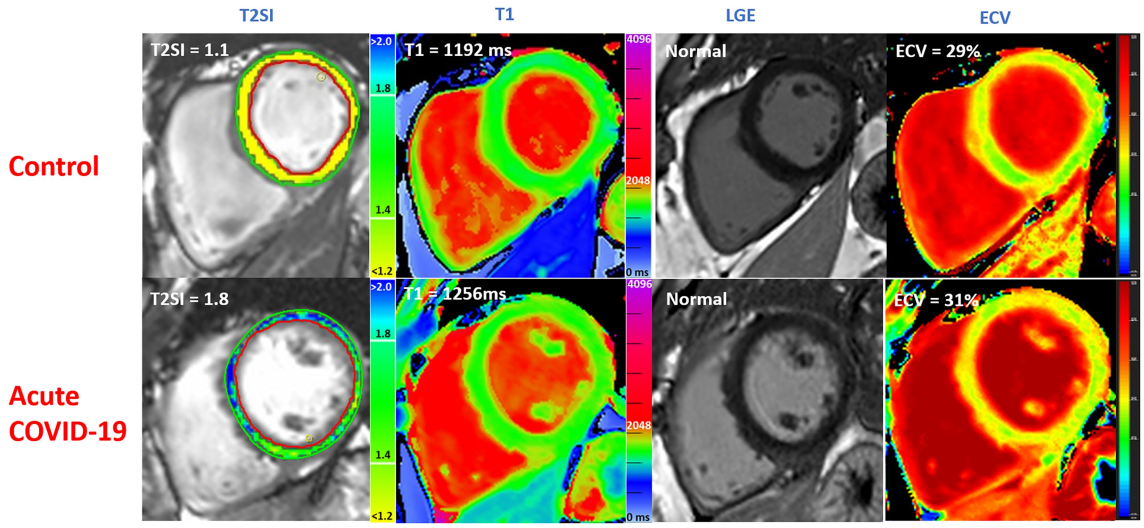

FIGURE 4
Multiparametric CMR imaging in 3T MRI scanner comparing acute COVID-19 patients during their hospitalization with matched controls without COVID-19 from the community. T2SI=T2 signal intensity ratio (myocardium: skeletal muscle). LGE, late gadolinium enhancement; ECV, extra cellular volume fraction. Normal values: T1≤1,244ms, T2≤1.4. All values correspond to single slice have been shown.

myocardial T1 raises the possibility that acute COVID-19 leads to a hyperdynamic state resulting in increased myocardial blood volume (plasma and red blood cells), which may have been detected as higher myocardial T1 (15). Such a hyperdynamic state would be expected to return to baseline after the resolution of the acute illness resulting in normalization of T1.

Limitations

Our study has some limitations. This is a small study that was confined to hospitalized acute COVID-19 patients and conducted before the launch of mass vaccination programs; thus, the findings may not be applicable to those managed in the community or those who have had the COVID-19 vaccine. Due to pandemic restrictions and patient preferences, we were unable to complete follow-up assessments in all COVID-19 patients, which may have under-powered some of the results; such as the lack of statistical significance in the differences of T1 and T2SI between the acute and follow-up time points. The thinness of the RV free wall renders it almost impossible to accurately quantify its T1 and T2 characteristics; thus, important pathophysiological changes in the RV myocardium secondary to acute COVID-19, which can increase the RV afterload, cannot be ascertained here (23). The influences of the differences in pathophysiology (e.g., hypoxia) and treatment (e.g., oxygen therapy and steroids) in acute COVID-19 on myocardial T1 and T2 signals were not explored in this study. On average, we scanned our patients at ~3 days into their hospitalization with acute COVID-19; it is unclear if the infection may lead to different or more myocardial abnormalities at a later time point during the acute illness. Whilst 65% of our patients had moderate or severe grade of COVID-19 illness on chest imaging (Table 2), we recruited relatively stable patients and thus our findings may not be generalizable to a sicker cohort. It is not yet clear if myocardial edema associated with SARS-COV2 illness is unique or whether similar changes happen with other viral illnesses. Thus, our study's findings should be viewed as hypothesis-generating, and may inform future study designs with a larger sample of patients with the ability to compare between vaccinated and unvaccinated patients, as well other cohorts of patients with different viral illnesses.

Conclusion

In this prospectively conducted study of participants with no history of cardiac disease or COVID-19 vaccination, patients hospitalized for acute COVID-19 demonstrated acute myocardial edema on CMR when compared to controls with similar cardiovascular risk factors, which normalized at 6 months. Biventricular systolic function was normal and scar burden was low and similar between the two groups. Acute COVID-19 appears to induce acute myocardial edema in some patients, which resolves in convalescence, without significant impact on biventricular structure and function in the acute and short-term. Further studies with larger numbers are needed to confirm these findings.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by NHS Health Research Authority, NRES Committee South Central—Oxford C. REC reference number: 10/H0408/24. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MS, VF, and KC conceived and designed the study. MS, YN, FM, and RK recruited study participants and performed blood sampling and analysis. AM helped to design the study and blood analysis. ME performed blood analysis and data collection and curation. MS, MB, VD, CX, and ABo performed image analysis. BR, SN, and ET provided scientific advice. VF, KC, and SP provided supervision of the project. MS and ABa performed statistical analysis. MS and VF prepared the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

SP has patent authorship rights for U.S. patent 9285446 B2 [systems and methods for Shortened Look Locker Inversion Recovery (Sh-MOLLI) cardiac gated mapping of T1], granted March 15, 2016; IPs are owned and managed by Oxford University Innovations.

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

Publisher's note

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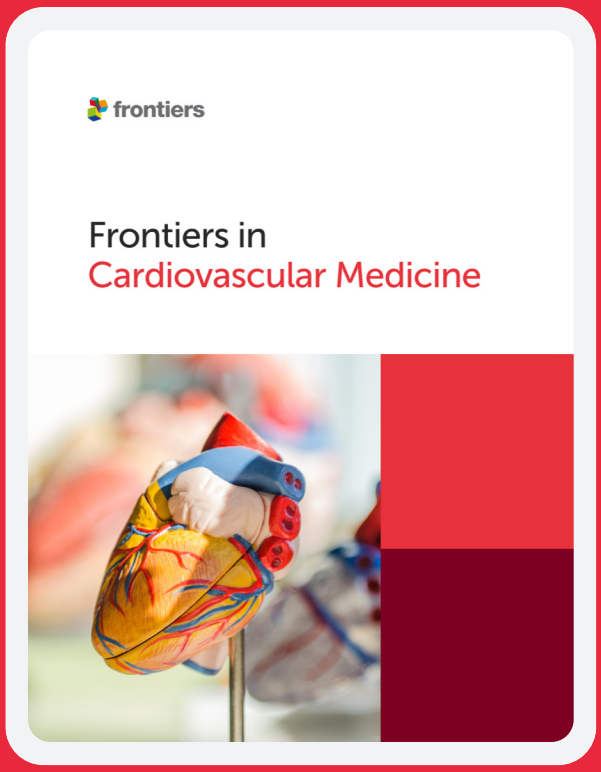

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