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Contemporary causes of acute myocarditis and pericarditis: Diagnosis by advanced imaging techniques and therapeutic strategies

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Contemporary causes of acute myocarditis and pericarditis: Diagnosis by advanced imaging techniques and therapeutic strategies

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Editorial: Contemporary causes of acute myocarditis and pericarditis: diagnosis by advanced imaging techniques and therapeutic strategies

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cardiac magnetic resonance (CMR), late gadolinium enhancement (LGE), multimodal imaging, tissue characterization, COVID-19, vaccination, perimyocarditis

Editorial on the Research Topic
Contemporary causes of acute myocarditis and pericarditis: diagnosis by advanced imaging techniques and therapeutic strategies

The incidence of myocarditis was estimated to be ~22/100,000 patients annually before the beginning of the COVID-19 pandemic in 2020 (1, 2). A large proportion of patients with acute myocarditis may develop dilated cardiomyopathy with symptomatic heart failure, which can also be associated with future adverse outcomes and high mortality rates (3).

The clinical symptoms of myocarditis are angina, dyspnea, and fatigue. In addition, malignant arrhythmias causing sudden cardiac death are feared, especially in younger individuals (4). Since the beginning of the pandemic, the number of patients affected by myocarditis has increased, both due to SARS-COVID infections and due to vaccination-induced myocarditis, the latter occurring more commonly in young male individuals who received mRNA-related vaccines (5, 6).

Based on contemporary guidelines, cardiac magnetic resonance (CMR) plays a central role in the diagnosis of myocarditis, however, endomyocardial biopsy should only be used for select patients due to its invasive character. CMR is an established non-invasive imaging technique, aiding the diagnosis of myocarditis by late gadolinium enhancement (LGE), which provides evidence of focal fibrosis or oedema due to inflammation and by T1&T2 mapping, which provides evidence of diffuse fibrosis of the extracellular space and myocardial edema, respectively. In this regard, CMR does not only aid diagnostic classification and risk stratification of such patients but may also influence important clinical decisions, such as tailoring heart failure therapy, indication for ICD or wearable CD, duration of immobilization, and time point for a return to work or play.

The role of CMR and myocardial strain for the detection of classical myocarditis was investigated by Motevalli et al. in 133 patients. The myocardial strain was assessed by feature tracking imaging. In particular, the results indicated that the global longitudinal strain (GLS) was a strong predictor of major adverse cardiac events during the follow-up period.

Numerous studies have investigated patients with COVID-19 vaccine associated myocarditis and pericarditis. Jahnke et al. describe a series of four young male individuals (18–42 years.) who presented with signs of myocarditis in temporal association with SARS-CoV-2 vaccines and abnormal CMR findings. In another study, Vago et al. investigated 16 young male patients (22 ± 7 years.), who presented with vaccine related myocarditis. CMR depicted myocardial oedema by T2 weighted and mapping images, and myocardial necrosis by LGE, which decreased or disappeared during follow-up. Interestingly, mostly individuals with predisposing immunologic factors including previous myocarditis were affected, exhibiting increased T-cell response compared to controls. Similar CMR findings were demonstrated by Kravchenko et al. who investigated vaccine associated myocarditis patients with CMR at baseline and at ~6 months of follow-up. Myocardial oedema disappeared during follow-up, areas of LGE were smaller compared to baseline, and clinical symptoms were resolved in most patients. Additionally, Evertz et al. investigated a series of 10 young male patients with COVID-19 mRNA vaccine-associated myocarditis and an age-matched group of patients suffering from classical viral myocarditis. Using CMR, they found that vaccine-related myocarditis shows similar patterns of myocardial oedema and LGE compared to regular viral myocarditis. Furthermore, Ochs et al. described an interesting series of 5 patients, who presented with clinical symptoms related to myocarditis but showed isolated pericarditis by CMR diagnostic work-up, which included LGE, T1, and T2 mapping. The patients were older (median of 55 years., IQR = 43–76) than the patients with vaccine related myocarditis described in other series, but the underlying pathophysiologic mechanisms require further evaluation in future studies.

Three further studies of this Research Topic focus on patients with COVID-19 related myocarditis, partly comparing CMR related findings to patients with classical viral or with SARS-CoV-2 vaccine associated myocarditis. Tanacli et al. performed baseline and part follow-up CMR examinations in 32 patients with persistent cardiac symptoms after COVID-19 infection. Interestingly, 10 (31%) of the patients with COVID-19 showed evidence of myocardial injury by CMR, while the number was reduced to only 3 (9%), considering the updated Lake Louise criteria. In addition, none of the COVID-19 patients exhibited histologic findings of acute or chronic inflammation by endomyocardial biopsy. In the same direction, Groschel et al. investigated 34 patients with subacute and 63 with post-COVID-19 infection by CMR. In addition, 44 patients with vaccine-related myocarditis were included in their study. The investigators found that patients after COVID-19 infection exhibited more focal fibrosis, primarily having a non-ischemic subepicardial pattern, compared to patients with vaccine-related myocarditis. However, LV and RV-function and diameters were within the normal range both in post-COVID and vaccine-related myocarditis cases. In addition, Zhang et al. conducted an autopsy study on the hearts of 26 deceased patients who had been hospitalized in intensive care units due to COVID-19 infection in Wuhan, China. Active myocarditis was present in 4 (15.4%) patients, who also exhibited higher interleukin values, higher CRP values at admission, and higher troponin values during hospitalization, compared to the remaining 22 patients. Finally, Xu et al. provide an interesting overview of studies related to COVID-19 associated cardiac disorders, including myocarditis and heart failure. This article demonstrates how initial research in this area was often focused on pneumonia. Then, with the spread of the disease, the infection was found to trigger an inflammatory response that resulted in cardiac injury. Therefore, the keywords myocarditis, heart failure, and cardiac troponins have become increasingly prominent.

In conclusion, the role of CMR as a central tool for the diagnosis of classical viral myocarditis has been reestablished within the COVID pandemic era. Several studies and case series reported in this article collection demonstrate how the ability to diagnose it can enable better support and clinical management in patients with COVID-19 and vaccination-related myocarditis and pericarditis. With the end of the ongoing pandemic hopefully approaching, future studies need to focus on longer-term follow-up examinations and care for these patients.

Author contributions

All authors contibuted significantly to this editorial, in terms of writing, reviewing and editing. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

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ORIGINAL RESEARCH
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COVID-19 vs. Classical Myocarditis Associated Myocardial Injury Evaluated by Cardiac Magnetic Resonance and Endomyocardial Biopsy

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Background: Despite the ongoing global pandemic, the impact of COVID-19 on cardiac structure and function is still not completely understood. Myocarditis is a rare but potentially serious complication of other viral infections with variable recovery, and is, in some cases, associated with long-term cardiac remodeling and functional impairment.

Aim: To assess myocardial injury in patients who recently recovered from an acute SARS-CoV-2 infection with advanced cardiac magnetic resonance imaging (CMR) and endomyocardial biopsy (EMB).

Methods: In total, 32 patients with persistent cardiac symptoms after a COVID-19 infection, 22 patients with acute classic myocarditis not related to COVID-19, and 16 healthy volunteers were included in this study and underwent a comprehensive baseline CMR scan. Of these, 10 patients post COVID-19 and 13 with non-COVID-19 myocarditis underwent a follow-up scan. In 10 of the post-COVID-19 and 15 of the non-COVID-19 patients with myocarditis endomyocardial biopsy (EMB) with histological, immunohistological, and molecular analysis was performed.

Results: In total, 10 (31%) patients with COVID-19 showed evidence of myocardial injury, eight (25%) presented with myocardial oedema, eight (25%) exhibited global or regional systolic left ventricular (LV) dysfunction, and nine (28%) exhibited impaired right ventricular (RV) function. However, only three (9%) of COVID-19 patients fulfilled updated CMR–Lake Louise criteria (LLC) for acute myocarditis. Regarding EMB, none of the COVID-19 patients but 87% of the non-COVID-19 patients with myocarditis presented

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histological findings in keeping with acute or chronic inflammation. COVID-19 patients with severe disease on the WHO scale presented with reduced biventricular longitudinal function, increased RV mass, and longer native T1 times compared with those with only mild or moderate disease.

Conclusions: In our cohort, CMR and EMB findings revealed that SARS-CoV-2 infection was associated with relatively mild but variable cardiac involvement. More symptomatic COVID-19 patients and those with higher clinical care demands were more likely to exhibit chronic inflammation and impaired cardiac function compared to patients with milder forms of the disease.

Keywords: COVID-19, myocarditis, Lake Louise Criteria, CMR, biopsy, inflammation

BACKGROUND

The systemic (immune) response to a SARS-CoV-2 infection varies widely, ranging from asymptomatic or mildly symptomatic respiratory infection to a systemic life-threatening condition with multiple organ failure. A three-phase model of pathogenesis of COVID-19 has been proposed (1) where a significant minority of patients progress to a critical hyperinflammation phase characterized by a systemic host response with elevated IL-2, IL-6, IL-7, TNF-α, C-reactive protein, and D-dimer levels. Several studies (2–4) demonstrated cardiac pathologic modifications reflected by elevated troponin and N-terminal pro B-type natriuretic peptide in 10–28% of COVID-19 patients, requiring hospitalization. In a large multicentre study (5), myocardial injury was diagnosed in 62% of cases presenting with troponin elevation and was associated with higher percentage of abnormal echocardiographic findings and higher mortality. Moreover, patients with cardiovascular comorbidities are more likely to develop severe forms of COVID-19 (2, 3).

Several pathogenic mechanisms may explain the specific cardiac findings post-COVID-19: triggered pan-endotheliitis (4) or macrophage activation (6) precipitating acute plaque rupture and coronary events (7), imbalanced activation of helper T cells leading to cytokine storm and direct myocardial injury (3), sepsis, or hypoxia-induced myocyte apoptosis (8).

A recent cardiac magnetic resonance (CMR) cross-sectional study (9) suggested that COVID-19 might be responsible for a sustained subacute or chronic inflammatory state of the myocardium comparable with cases of viral myocarditis and prone to cause long-term cardiac impairment by downstream activation of ventricular remodeling and fibrosis. However, the clinical relevance of these findings has been discussed controversially, given the lack of a matched comparison group (10). To date, studies comparing CMR findings including late gadolinium enhancement (LGE) and mapping with histology are limited, and longitudinal analyses are completely missing in this context (11).

In this study, we used an advanced CMR protocol to examine potential effects of COVID-19 on cardiac function and structural remodeling in consecutive patients with a recent SARS-CoV-2-infection using endomyocardial biopsy (EMB) data as the reference standard. In addition, we sought to compare these findings to healthy volunteers and a cohort of patients with "classic" myocarditis. Follow-up CMR assessment was performed in patients with COVID-19 and in those with "classic" myocarditis. To compare histological and/or immunohistological findings between patients with COVID-19 and patients with myocarditis, available EMB samples were evaluated according to the current diagnostic criteria for myocarditis (12).

METHODS

Study Population

All post-COVID-19 patients referred to our clinic with a clinical indication for CMR (13) were asked for consent to be included in our observational study. Healthy control subjects were identified from an existing database available at our institution (13). This study was reviewed and approved by the Charité–Universitätsmedizin Berlin Ethics Committee and complies with the Declaration of Helsinki.

In total, 32 patients with a previous COVID-19 infection were included in the study. For comparison, we retrospectively included 22 patients with a clinically confirmed diagnosis of acute non-COVID-19 myocarditis and an available baseline CMR scan, along with 16 healthy volunteers. Ten of the post-COVID-19 patients underwent a clinically indicated follow-up scan. Thirteen of the 22 patients with acute myocarditis had an available follow-up CMR on file. Inclusion criteria for the post-COVID-19 patients were as follows: (I) a previously diagnosed (14) SARS-CoV-2 infection with COVID-19 disease; (II) clinical indication for CMR such as suspected myocardial injury (elevated troponin), reduced LVEF and/or presence of pericardial effusion on echocardiogram, persistent arrhythmia, persistent dyspnoea, reduced exercise capacity, or fatigue; and

Abbreviations: AHA, American Heart Association; AUC, area under curve; bSSFP, balanced steady state free precession; CMR, cardiac magnetic resonance; COVID-19, Coronavirus disease 2019; DNA, desoxyribonucleic acid; ECV, extracellular volume; EF, ejection fraction; EMB, endomyocardial biopsy; ESC, European Society of Cardiology; FT, feature tracking; GCS, global circumferential strain; GLS, global longitudinal strain; HF, heart failure; LA, left atrium/atrial; LGE, late gadolinium enhancement; LLC, Lake Louise Criteria; LV, left ventricle/ventricular; LVM, left ventricular mass; NT-proBNP, N-terminal pro-B type natriuretic peptide; RNA, ribonucleic acid; RV, right ventricle/ventricular; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TE, echo time; TR, repetition time.

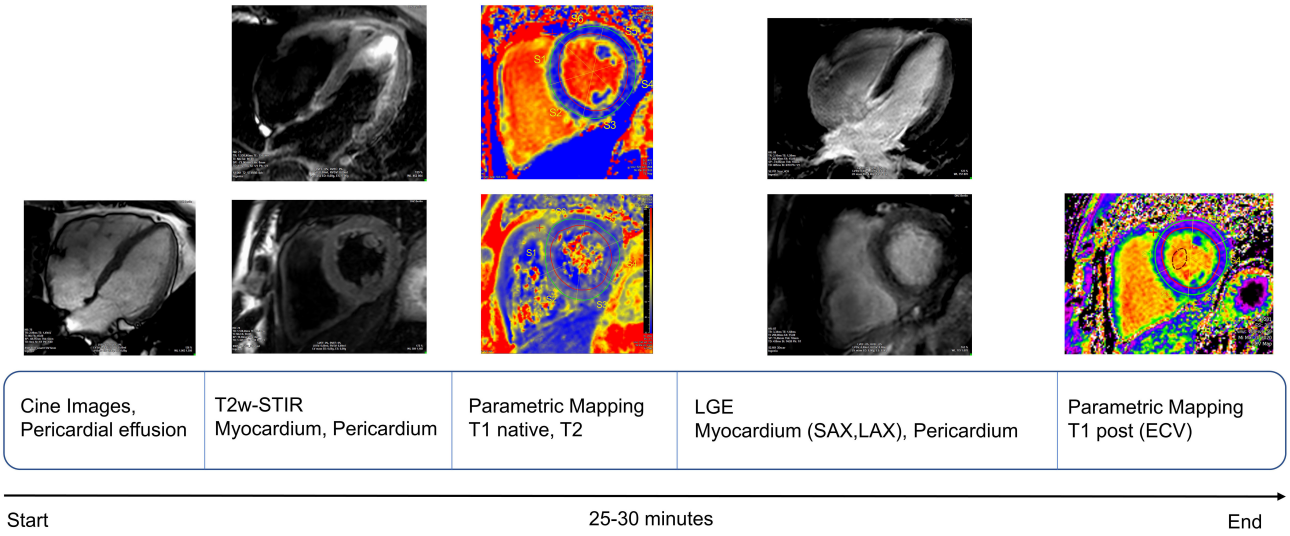

FIGURE 1 | Schematic representation of cardiac magnetic resonance (CMR) protocol workflow used for our study, in keeping with recent SCMR recommendations for evaluation of patients with COVID-19 (18).

(III) resolution of acute COVID-related symptoms to allow the end of self-isolation or a confirmation of a negative PCR test. Exclusion criteria were absolute contraindications to CMR and impossibility to obtain consent. The non-COVID-19 myocarditis cases were retrospectively identified from our local database and inclusion criteria followed the most recent ESC recommendations (15). Exclusion criteria were as follows: coexistence of underlying cardiac pathology (myocardial infarction, cardiomyopathy, and/or haemodynamically relevant valvulopathy) (16).

WHO Score Description

World Health Organization (WHO) guidance on clinical management of COVID-19 (https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-2) was used to define disease severity as follows: mild and moderate disease–symptomatic patients meeting the case definition for COVID-19 without evidence of viral pneumonia, hypoxia, or some clinical signs of pneumonia (fever, cough, dyspnoea, and/or fast breathing) without other criteria of severity and $SpO_2 \geq 90\%$ on room air. Severe and critical disease were defined as presence of additional severity signs and respiratory distress as follows: respiratory rate > 30 breaths/min; severe respiratory distress; or SpO2 $< 90\%$ at room air or presence of acute respiratory distress syndrome or specific signs on radiograph, CT scan, or lung ultrasound (e.g., bilateral opacities, not fully explained by volume overload, lobar or lung collapse, or nodules).

Cardiac Magnetic Resonance

Cardiac magnetic resonance (CMR) images of the following patients were acquired using three clinical MRI scanners: 25 post-COVID-19 patients, 18 patients with myocarditis, and all 16 healthy controls with a clinical 3T system (Ingenia, Philips Healthcare, Best, the Netherlands), four post-COVID-19 patients, four patients with myocarditis with a 1.5T system (Achieva, Philips Healthcare, Best, the Netherlands), and three post-COVID-19 patients with a 1.5T system (Magnetom Aera, Siemens Inc.). All study participants were scanned with a comprehensive imaging protocol and appropriate local receiver coil arrays in accordance with the Society for Cardiovascular Magnetic Resonance (SCMR) guidelines (17) (**Figure 1**).

All CMR scans were acquired with ECG gating or retrospective gating (cine) in one breath hold (8–15 s). Typical imaging parameters are summarized as follows:

Long-axis cine-imaging (Philips 3T): balanced steady state free precession (bSSFP), TR = 2.9 ms, TE = 1.45 ms, flip angle = 45°, acquisition voxel size = 1.9 × 1.9 × 6.0 mm^3, acquired/reconstructed heart phases = 27/40, parallel imaging (SENSE) acceleration = 2.

Long axis cine-imaging (Philips 1.5T): TR = 3.4 ms, TE = 1.7 ms, flip angle = 60°, acquisition voxel size = 1.6 × 1.6 × 6.0 mm^3, acquired/reconstructed heart phases = 25/50, parallel imaging (SENSE) acceleration = 2.

T2-weighted short tau inversion recovery STIR (Philips 1.5T / 3T): black blood prepared turbo spin echo (TSE) imaging, TE = 100 ms, flip angle 90°, refocusing angle 160°, acquisition voxel size = 1.5 × 1.5 × 8.0 mm^3, half-Fourier factor 0.65, acquisition every other heart beat to allow for optimal blood signal suppression.

T2 mapping: (Philips 1.5T / 3T): black blood prepared gradient and spin echo (GraSE) imaging, flip angle 90°, refocusing angle 180°, 9 echoes, echo times = n × 8.8 ms, EPI-factor 7, acquisition voxel size = 2.0 × 2.0 × 10.0 mm^3, parallel imaging (SENSE) acceleration = 2.3.

Siemens 1.5T Magnetom Aera: repetition time (TR) = 40.8 ms, echo time (TE) = 1.07 ms, flip angle = 55°, acquisition voxel size = 1.9 × 1.9 × 8.0 mm^3 (19).

TABLE 1 | Demographics clinical parameters.

	Healthy Control *N* = 16	COVID-19 *N* = 32	Myocarditis *N* = 22	P ANOVA	P Control vs. COVID-19	P Control vs. Myocarditis	P COVID-19 vs. Myocarditis
Patient characteristics							
Age, years	24 ± 5	48 ± 14	32 ± 15	***<0.001***	***<0.001***	***0.031***	***0.004***
Male, *N (%)*	8 (50)	19 (59)	17 (77)		0.54	0.08	0.17
BMI	22 ± 3	26 ± 5	26 ± 5	***0.005***	***0.004***	***0.030***	0.75
Hypertension, *N (%)*	0 (0)	13 (42)	3 (19), *N* = 16		***0.012***	0.12	0.14
Diabetes, *N (%)*	0 (0)	1 (3)	1 (6), *N* = 16		0.48	0.31	0.22
Hypercholesterolemia, *N (%)*	0 (0)	8 (26)	2 (13), *N* = 16		***0.029***	0.14	0.31
Known CAD, *N (%)*	0 (0)	3 (10)	1 (6), *N* = 16		0.21	0.31	0.71
Smoking, *N (%)*	0 (0)	13 (41)	5 (31), *N* = 16		***0.003***	***0.015***	0.53
COPD or asthma, *N (%)*	0 (0)	3 (10)	2 (13), *N* = 16		0.21	0.14	0.74
Systolic Blood pressure, mm Hg	112 ± 17	119 ± 15	114 ± 19		0.50	0.92	0.72
Diastolic Blood pressure, mm Hg	68 ± 11	73 ± 11	66 ± 10		0.26	0.90	0.09
Heart rate, beats per min	65 ± 5	78 ± 15	88 ± 22		0.83	0.94	0.91
Blood test results							
High-sensitivity CRP, mg/dL		4 ± 9, *N* = 23	8 ± 12, *N* = 18				0.17
Elevated hsCRP, *N (%)*		5 (22), *N* = 23	10 (56), *N* = 18				***0.033***
Elevated Troponin, *N (%)*		9 (45), *N* = 20	10 (71), *N* = 14				0.17
CK, U/L		70 ± 37, *N* = 22	343 ± 396, *N* = 20				***<0.001***
CK-MB, U/L		18 ± 5, *N* = 15	37 ± 25, *N* = 20				***<0.001***
NT-proBNP, pg/mL		1291 ± 2484, *N* = 23	2194 ± 2360, *N* = 17				0.19
Elevated NT-proBNP, *N (%)*		6 (24), *N* = 25	15 (88), *N* = 17				***<0.001***
eGFR, mL/min		83 ± 24, *N* = 26	95 ± 16, *N* = 17				***0.045***
Medication		***N* = 32**	***N* = 16**				
Oral Anticoagulants, *N (%)*		4 (13)	2 (13)				0.70
Statins, *N (%)*		3 (9)	1 (6)				0.51
β-blockers, *N (%)*		14 (44)	8 (50)				0.59
Diuretics, *N (%)*		12 (38)	6 (38)				0.43
Nitrates, *N (%)*		0 (0)	0 (0)				0.99
ACE inhibitors, *N (%)*		10 (31)	3 (19)				0.14
Sartans, *N (%)*		5 (16)	1 (6)				0.20
Calcium Antagonists, *N (%)*		2 (6)	0 (0)				0.23
Symptoms		***N* = 32**	***N* = 16**				
Initial Presentation							
Fever, *N (%)*		19 (59)	5 (31)				0.07
Chest pain, *N (%)*		8 (25)	9 (56)				***0.033***
Dyspnea, *N (%)*		20 (63)	9 (56)				0.68
Arrythmia, *N (%)*		1 (3)	7 (44)				***<0.001***
Cough, *N (%)*		24 (75)	1 (6)				***<0.001***
Nausea/vomiting/diarrhea, *N (%)*		11 (34)	3 (19)				0.26
Fatigue/weakness, *N (%)*		24 (75)	11 (69)				0.65
Amnesia, *N (%)*		10 (31)	5 (31)				0.99
Lack of taste or smell, *N (%)*		21 (66)	0 (0)				***<0.001***

(Continued)

TABLE 1 | Continued

	Healthy Control $N = 16$	COVID-19 $N = 32$	Myocarditis $N = 22$	P ANOVA	P Control vs. COVID-19	P Control vs. Myocarditis	P COVID-19 vs. Myocarditis
Persistent							
Fatigue/weakness, *N (%)*		9 (28)	0 (0)				***0.019***
Amnesia, *N (%)*		2 (6)	0 (0)				0.31
Lack of taste or smell, *N (%)*		2 (6)	0 (0)				0.31
Arrythmia, *N (%)*		9 (28)	3 (19)				0.48

BMI, body mass index; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; COVID-19 coronavirus disease; hsCRP, high sensitivity C-reactive protein; CK, creatin-kinase; NT- proBNP, N-terminal pro-b type natriuretic peptide; eGFR, estimated glomerular filtration rate; ACE, acetyl coenzyme A.
For comparison of the continuous variables, ANOVA and post-hoc Tukey's tests were used, for categorial variables Chi-square or Fischer tests test were used, a $P < 0.05$ was considered significant. For incomplete set of data, N represents the number of subjects included in the analysis. Statistically significant p-values are indicated in bold.

Native and 15 min post-contrast T1-mapping were performed using modified Look-Locker imaging (MOLLI) in two left-ventricular short-axis slices (basal and mid-ventricle), as described previously (17). Patients received.15 mmol/kg of gadolinium-based contrast agent (Gadobutrol 1.0 mmol/ml, Gadovist®, Bayer AG, Leverkusen, Germany). Segmented inversion-recovery fast gradient–echo imaging was used to assess late gadolinium enhancement (LGE) 10 min after the administration of contrast substance (19). mDixon-imaging was used to differentiate pericardial enhancement from fat (20).

Image Analysis

All images were analyzed offline by two cardiologists with more than 10 years of experience in CMR and are certified SCMR Level 3. We used commercially available software (Medis Suite, version 3.1, Leiden, The Netherlands) in accordance to a recent consensus paper for the quantification of left ventricular (LV) function in CMR (16) and our internal standard operating procedures (MRI Core Lab, German Heart Center, Berlin, Germany). To assess whether the updated Lake Louise Criteria (LLC) for myocarditis were fulfilled, the proposed updated analysis algorithm (21) was scrupulously followed.

Global myocardial longitudinal (GLS) and circumferential (GCS) strain was assessed at the level of 2 distinct myocardial layers: Endo (subendocardial layer) and Myo (midwall layer) as previously described (17). Similarly, right ventricular (RV) GLS at Endo and Myocardium levels was determined through drawing RV endocardial and epicardial contours in 4Ch cine images with automatic propagation over the whole cardiac cycle using QStrain (22). Similarly, left atrial (LA) strain was measured in 4Ch and 2Ch views and these values averaged. Mapping parameters were measured using QMap RE version 2.0 (Medis Medical Imaging Systems bv, Leiden, the Netherlands). For parametric imaging, pre- and post-contrast MOLLI images were manually adjusted for in-plane motion and T1 native and post-contrast relaxation times were determined using nonlinear fitting with a maximum likelihood estimator (17). Extracellular volume (ECV) was computed from pre- and post-contrast T1 and hematocrit values as previously described (23). Given the inherent variability of normal values in parametric imaging between different field strengths and magnets (24), we only present the parametric values acquired on the 3T Ingenia scanner. Healthy controls received no contrast and for comparison, ECV reference values (24) corresponding to the same model of scanner and magnetic field strength were used. Global T1 native, ECV, and T2 values were calculated by averaging individual segmental values derived for each patient from mapping of two distinct ventricular short-axis (SA) slices at basal and mid-ventricular levels (17). The presence of LGE was established by visual assessment of two experienced readers (consensus read, both CMR-level-III certified), and evaluated in all the slices of the short axis stack and three long axis views.

Biopsy Samples

In all patients, including those with COVID-19 undergoing EMB, myocarditis was clinically suspected, following the recent guideline recommendations (15). Myocardial biopsy was performed in all COVID-19 and myocarditis cases as described previously. Briefly, endomyocardial samples were collected through femoral vein access using either a 7F long-sheath with angulated tips (from the RV surface of the interventricular septum) or a 7F long-sheath without angulation using a retrograde approach (from the LV surface of the interventricular septum). At least four pieces per patient were collected with which Fluoroscopic guidance was used to identify the region of interest. Vital parameters and ECG were closely monitored during the procedure. A routine echocardiogram was performed at the end of the procedure to exclude iatrogenic pericardial effusion. Analysis of endomyocardial biopsy (EMB) samples was performed in specialized laboratories by experienced cardio-pathologists as described previously (25). Myocardial inflammation was considered to be present when $\geq$ 20 infiltrating immune cells/mm^2 were observed (CD3 T-lymphocytes and/or CD68 macrophages). Additionally, enhanced HLA class II expression in antigen-presenting immune cells was evaluated. Screening for viral genomes was performed after extraction of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) with Proteinase-K digestion and phenol/chloroform. Reverse transcriptase-polymerase chain reaction was used subsequently to detect virus presence within cardiac tissue samples, e.g., COVID-19 and, respectively, viruses frequently involved in myocarditis, such as enteroviruses

TABLE 2 | Cardiac magnetic resonance imaging findings. baseline.

	Healthy Control *N* = 16	COVID−19 *N* = 32	Myocarditis *N* = 22	P ANOVA	P Control vs. COVID-19	P Control vs. Myocarditis	P COVID-19 vs. Myocarditis
Left Ventricle							
ED volume, mL/m2	82 ± 9	78 ± 25	104 ± 31	***0.001***	0.94	***0.022***	***<0.001***
ES volume, mL/m2	33 ± 4	32 ± 20	54 ± 34	***0.003***	0.98	***0.023***	***0.004***
Stroke volume, mL/m2	49 ± 7	48 ± 8	50 ± 11	0.66			
Ejection fraction, %	60 ± 4	62 ± 10	52 ± 16	***0.006***	0.77	0.09	***0.005***
Cardiac Index, L/min/m2	3.5 ± 0.8	3.6 ± 0.9	3.7 ± 0.9	0.80			
Endo Longitudinal Strain %	−26.0 ± 2.1	−24.2 ± 4.4	−20.1 ± 7.0	***0.001***	0.61	***0.003***	***0.008***
Myo Longitudinal Strain %	−24.5 ± 2.0	−22.3 ± 4.1	−18.3 ± 5.8	***<0.001***	0.25	***<0.001***	***0.005***
Endo Circumferential Strain %	−31.5 ± 4.4	−32.0 ± 7.2	−24.0 ± 9.3	***<0.001***	0.96	***0.009***	***<0.001***
Myo Circumferential Strain %	−20.9 ± 2.7	−20.9 ± 4.2	−17.3 ± 6.9	***0.025***	0.99	0.08	***0.030***
LV Mass (g/m2)	50 ± 7	55 ± 19	72 ± 23	***<0.001***	0.70	***0.001***	***0.003***
Left Atrium							
LA max Vol mL	37 ± 9	36 ± 8	41 ± 11	0.26			
LA emptying fraction %	70 ± 6	61 ± 12	54 ± 17	***0.003***	0.07	***0.002***	0.19
LA strain %	44 ± 11	39 ± 12	28 ± 16	***0.001***	0.42	***0.001***	***0.011***
Right Ventricle							
ED volume, mL/m2	87 ± 10	77 ± 15	86 ± 26	0.10			
ES volume, mL/m2	38 ± 7	36 ± 10	42 ± 19	0.26			
Stroke volume, mL/m2	49 ± 8	41 ± 9	44 ± 12	***0.040***	***0.031***	0.30	0.51
Ejection fraction, %	56 ± 6	54 ± 8	53 ± 11	0.55			
Endo RV longitudinal strain %	−29.7 ± 7.1	−28.2 ± 7.8	−27.1 ± 7.3	0.56			
Myo RV longitudinal strain %	−27.9 ± 6.5	−26.0 ± 7.4	−25.2 ± 6.8	0.81			
RV Mass (g/m2)	13 ± 1	13 ± 4	16 ± 3	***0.006***	0.87	***0.013***	***0.014***
Right Atrium							
RA max Vol mL	40 ± 7	37 ± 12	43 ± 11	0.15			
RA emptying fraction %	56 ± 9	54 ± 11	47 ± 13	***0.027***	0.80	***0.039***	0.07
RA strain %	50 ± 16	42 ± 13	36 ± 13	***0.012***	0.16	***0.009***	0.25
Parametric Imaging							
T1 native	1236 ± 21 (*N* = 16)	1271 ± 50 (*N* = 25)	1352 ± 113 (*N* = 12)	***<0.001***	0.21	***<0.001***	***0.003***
ECV	26 ± 4* (ref)	25 ± 4 (*N* = 24)	30 ± 8 (*N* = 12)	***0.044***	0.59	0.06	***0.035***
T2	43 ± 2 (*N* = 16)	48 ± 6 (*N* = 25)	61 ± 10 (*N* = 15)	***<0.001***	***0.026***	***<0.001***	***<0.001***
		***N* = 31**	***N* = 22**				
LGE No. (%)							
Ischaemic	n/a	1 (3)	3 (14)				0.17
Nonischaemic	n/a	5 (16)	19 (86)				***<0.001***
Pericardial	n/a	3 (10)	4 (18)				0.37
* Reference Value							

ED, end diastolic; ES, end systolic; Endo, subendocardial layer; Myo, mid-myocardial layer; LV, left ventricular, RV, right ventricular; LA, left atrial; RA, right atrial; ECV, extracellular volume; LGE, late gadolinium enhancement.

For comparison of the continuous variables, ANOVA and post-hoc Tukey's tests were used, for categorial variables Chi-square or Fischer tests test were used, a P < 0.05 was considered significant. For incomplete set of data, N represents the number of subjects included in the analysis.

**Reference Values for ECV in healthy controls was taken from Dabir et al., (24). Statistically significant p-values are indicated in bold.*

(including coxsackieviruses of group A and B and echoviruses), parvovirus B19 (PVB19), adenoviruses, human cytomegalovirus, Epstein-Barr virus, and human herpesvirus type 6 (HHV6). Oligonucleotide sequences were chosen from the glyceraldehyde-3-phosphate-dehydrogenase gene as a control for successful extraction of DNA and RNA. Negative and positive controls were included in each PCR reaction. Automatic DNA sequencing was used to confirm the specificity of all viral amplification products. Masson trichrome staining was used for histological examination of various types of fibrosis (multifocal fibrosis/scarring without

inflammation/diffuse collagen deposition). Congo-red staining was used to exclude amyloid deposition.

Statistical Analysis

All the data within the text, tables, or figures is presented as mean ± SD unless stated otherwise. The Shapiro-Wilk test was used to assess normal distribution of data for every dataset included. To compare the three subgroups, a one-way ANOVA for normally distributed data or Kruskal-Wallis for non-normally distributed data was performed followed by Tukey *post-hoc* tests to compare differences among subgroups. The Wilcoxon test was used to assess the differences between baseline and follow-up groups. The Welch correction was applied for unequal sample sizes or if the assumption of homogeneity of variance was infirmed. For differences between categorical variables, Fisher's exact and χ^2 test were used. A two-tailed *p*-value below.05 was considered statistically significant. All statistical tests were performed using SPSS version 27.0.

RESULTS

Study Population Characteristics

Demographics, clinical, and biochemical characteristics of the patients included in our study are presented in **Table 1.** Twelve (38%) of 32 patients with COVID-19 and 17 (77%) of 22 patients with non-COVID-19 "classic" myocarditis required hospitalization. According to the WHO disease severity criteria for COVID-19, 20 out of 32 (63%) patients with COVID-19 had mild or moderate, seven (22%) had severe, and five (16%) had critical disease. While fever, cough, and loss of taste or smell were dominant in COVID-19 patients, some of them also suffered from palpitations (1, 3%), chest pain (8, 25%), arrhythmia (9, 28%), or had elevated troponin (9, 28%) or NT-proBNP (6, 24%). During the convalescence phase, fatigue persisted in 9 (28%), loss of taste or smell in 2 (6%), and amnesia in two (6%) patients with COVID-19.

Post-COVID-19 patients were scanned for a baseline visit at 95 ± 59 days after a first positive test and received a follow-up scan 68 ± 40 days after the baseline scan. Patients with myocarditis were scanned shortly after the onset of symptoms had a longer and more variable follow-up interval of 156 ± 124 days after baseline. There was an age discrepancy between the healthy control, post-COVID-19. and myocarditis groups (24 ± 5 vs. 48 ± 14 vs. 32 ± 15 years, $p < 0.001$). Healthy controls had a lower BMI than post-COVID-19 and patients with myocarditis. The risk factors were similar in the post-COVID-19 and myocarditis groups (**Table 1**).

CMR Parameters: Comparison Between COVID-19, Myocarditis, and Healthy Volunteers

Cardiac magnetic resonance (CMR) parameters for each of the three groups are presented in **Table 2.** In one of the

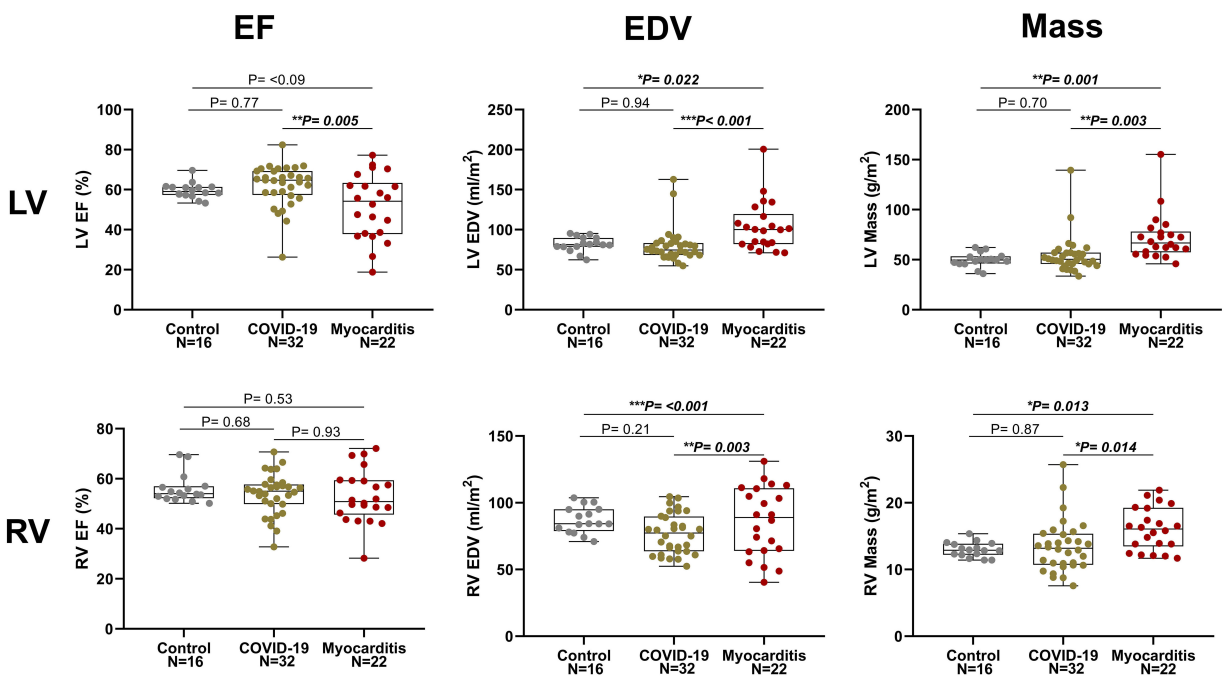

FIGURE 2 | Left ventricle (LV, upper row) and right ventricular (RV, lower row) ejection fraction (EF), end diastolic volume (EDV), and myocardial mass in three pathology groups (from left to right: Control, COVID-19, and Myocarditis). There is no difference on average between patients with COVID-10 and Controls. However, there is significant dysfunction and remodeling in some of these patients that overlaps with the myocarditis spectrum. A $p < 0.05$ was considered statistically significant, and indicated as follows: * < 0.05, ** < 0.01. *** < 0.001.

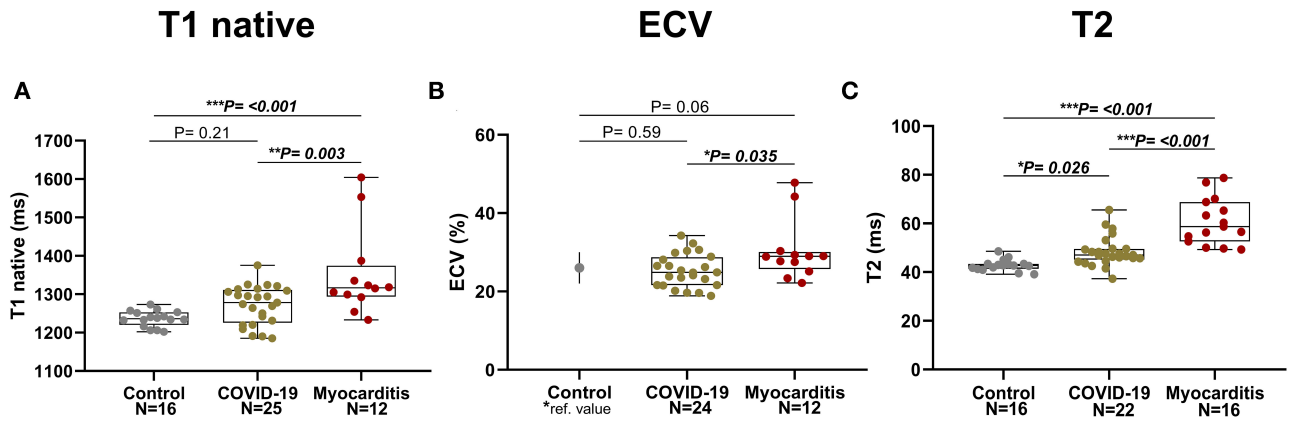

FIGURE 3 | Parametric Imaging in three pathology groups (from left to right: Control, COVID-19, Myocarditis): **(A)** T1 native, **(B)** Extracellular volume (ECV)–for the Control it is represented the mean and SD referenced in literature [Dabir et al., (24)] corresponding to similar manufacturer and magnet field strength, **(C)** T2. A $p < 0.05$ was considered statistically significant, and indicated as follows: * < 0.05, ** < 0.01, *** < 0.001.

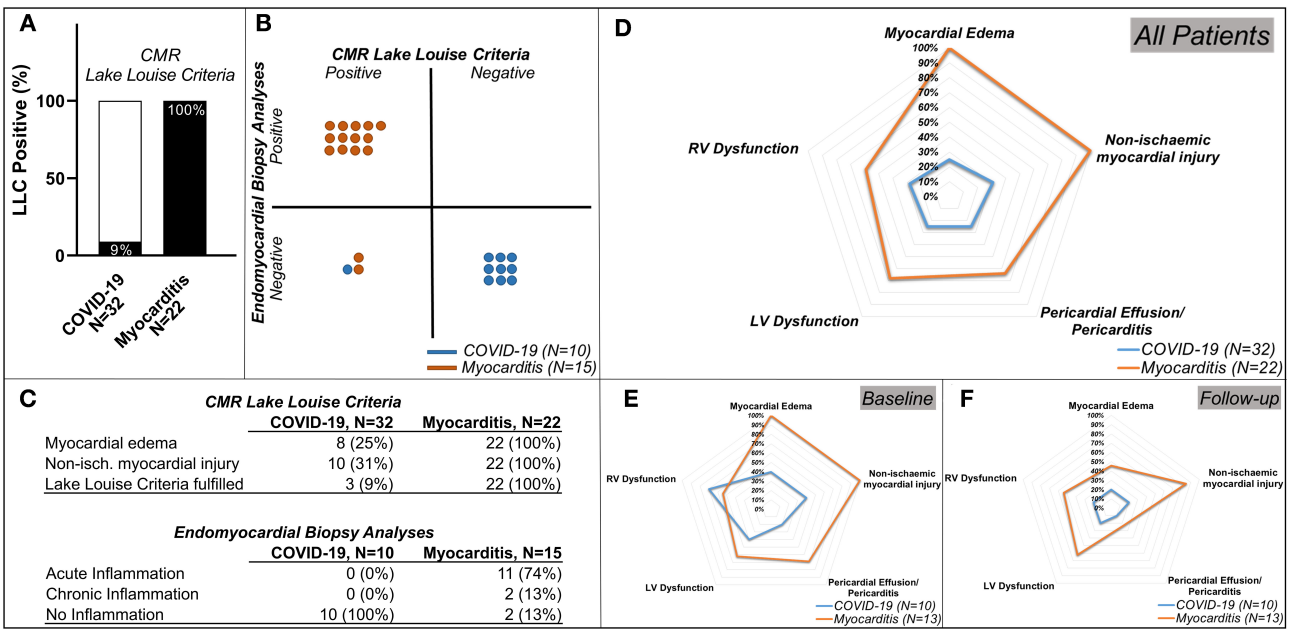

FIGURE 4 | Lake Louise Criteria (LLC) and Functional Impairment in patients with COVID-19 and patients with Myocarditis. **(A)** Comparison of the CMR LLC and endomyocardial biopsy (EMB) findings. Three out of 32 (9%) patients with COVID-19 and all 22 (100%) of the patients with myocarditis have positive LLC, **(B)** from the patients in which endomyocardial biopsy (EMB) was available: none of the patients with COVID-19 and 13 (87%) out of 15 patients with myocarditis with available EMB had histological findings in keeping with this diagnosis, 1 (10%) of the patients with COVID-19, and 2 (13%) of the patients with myocarditis have positive LLC but negative EMC diagnostic criteria. **(C)** tables with numeric values for CMR LLC (upper rows) and Endomyocardial Biopsy Analyses (lower rows) **(D)** spidernet representation of myocardial inflammation, injury, pericardial involvement, LV, and RV dysfunctions in all COVID-19 (blue polygons) and all Myocarditis (red polygons) patients, **(E)** (baseline), **(F)** (follow-up)–spidernet representations of myocardial inflammation, injury, pericardial involvement, LV, and RV dysfunctions in patients with COVID-19 (blue polygons) and Myocarditis (red polygons) with an available follow-up scan.

COVID-19 patients, no contrast agent was administered due to claustrophobia and distress during the scan. Hence, an abbreviated protocol was used in this case. Given the discrepancies between the normal range of values for parametric mapping (T1, ECV, T2 values) between different field strengths and scanners, only the patients and volunteers scanned in the 3T Philips Ingenia scanner were included in the analysis (specific numbers per group are specified in brackets in **Table 2**).

There were no significant differences between post-COVID-19 and controls for any of the CMR parameters apart from T2 values, which were significantly higher in the COVID-19

(48 ± 6 vs. 43 ± 2 ms, $p = 0.026$). Compared to controls, RV stroke volume was significantly lower in the COVID-19 group (41 ± 9 vs. 49 ± 8 ml/m^2, $p = 0.031$). There were no differences between LV function, RV function, and mass between COVID-19 patients and healthy controls (**Figure 2**).

Patients with COVID-19 had a normal left ventricular ejection fraction (LVEF), compared to patients with myocarditis who had significantly reduced LVEF (61 ± 11 vs. 51 ± 17%, $p = 0.016$). LV dysfunction in myocarditis was also reflected by lower values of longitudinal and circumferential strain, which were normal in patients with COVID-19. LV and RV mass were both increased in myocarditis but not in COVID-19 compared with controls (**Figure 3**). Atrial function, measured as LA and RA emptying fractions and strain, was impaired in myocarditis compared with controls and COVID-19. T1 native, ECV and T2 values were all higher in myocarditis compared with controls and COVID-19 (see **Figure 3**, **Table 2**). Myocardial and pericardial LGE was present in all patients with myocarditis, while only 6 (19%) of the patients with COVID-19 had myocardial and 6 (19%) had pericardial LGE (**Table 2**).

CMR–Lake Louise Criteria and EMB Histological Analyses

In total, only three (9%) post-COVID-19 patients fulfilled the updated LLC for acute myocarditis, four (13%) presented signs of myocardia oedema (areas of elevated signal on T2 maps or T2-weighted fat suppressed images), and 10 (31%) had signs of myocardial injury (abnormal T1, ECV or LGE). Additionally, eight (25%) had LV wall motion abnormalities, nine (28%) had an impaired RV function, and eight (25%) had evidence of pericardial effusion and/or pericarditis (**Figures 4A,C**, **Table 3**).

All myocarditis cases (100%) fulfilled the updated LLC with evidence of myocardial oedema and non-ischaemic myocardial injury. Eight (25%) showed signs of pericarditis or pericardial effusion. Fifteen (68%) had systolic LV dysfunction and 13 (59%) LV dilatation. Impairment of RV function was present in 13 (59%) and RV dilatation in nine (41%) (**Figures 4A,C**, **Table 3**).

Endomyocardial biopsy (EMB) was available in 10 out of 32 post-COVID-19 patients. None of these samples presented evidence of acute or chronic myocarditis, as usually observed in viral myocarditis (**Figure 4**). In five (50%) of these patients, traces of previous myocardial

TABLE 3 | Summary of Lake Louise Criteria and Ventricular dysfunction.

All patients, baseline

A	COVID-19 (*N* = 32)	Myocarditis (*N* = 22)
2018 Lake Louise for myocarditis fulfilled N (%)	3 (9)	22 (100)
Myocardial edema (T2-mapping or T2W images)	8 (25)	22 (100)
Non-ischaemic myocardial injury (abnormal T1, ECV or LGE)	10 (31)	22 (100)
Pericarditis (effusion in cine images or abnormal LGE, T2 STIR)	8 (25)	14 (64)
Systolic LV dysfunction (regional and or global WMA)	8 (25)	15 (68)
Depressed LVEF, *N* (%)	6 (19)	11 (50)
LV dilatation, *N* (%)	3 (9)	13 (59)
LV increased wall thickness, *N* (%)	7 (22)	4 (18)
Depressed RVEF, *N* (%)	9 (28)	13 (59)
RV dilatation, *N* (%)	4 (13)	9 (41)

Only patients will follow-up

B	COVID-19 (*N* = 10)		Myocarditis (*N* = 13)	
	Baseline	Follow-up	Baseline	Follow-up
2018 Lake Louise for myocarditis fulfilled, *N* (%)	2(20)	1(10)	13 (100)	5 (38)
Myocardial edema (T2-mapping or T2W images)	4 (40)	2 (20)	13 (100)	6 (46)
Non-ischaemic myocardial injury (abnormal T1, ECV or LGE)	4 (40)	2 (20)	13 (100)	11(85)
Pericarditis (effusion in cine images or abnormal LGE, T2 STIR)	2 (20)	1 (10)	9 (69)	3 (23)
Systolic LV dysfunction (regional and or global WMA)	4 (40)	2 (20)	8 (62)	8 (62)
Depressed LVEF, *N* (%)	3 (30)	1 (10)	5 (38)	4 (31)
LV dilatation, *N* (%)	1 (10)	0 (0)	7 (54)	4 (31)
LV increased wall thickness, *N* (%)	3 (30)	3 (30)	2 (15)	2 (15)
Depressed RVEF, *N* (%)	7 (70)	2 (20)	7 (54)	7 (54)
RV dilatation, *N* (%)	2 (20)	1 (10)	6 (46)	5 (38)

***A**: All patients with COVID-19 (N = 32) and Myocarditis (N = 22), **B**: only patients with follow-up with COVID-19 (N = 10) and Myocarditis (N = 13).*
T2W, T2 weighted; STIR, Short-TI Inversion Recovery; LGE, late gadolinium enhancement; WMA, wall motion abnormalities; LV, left ventricle; RV, right ventricle; EF, ejection fraction.

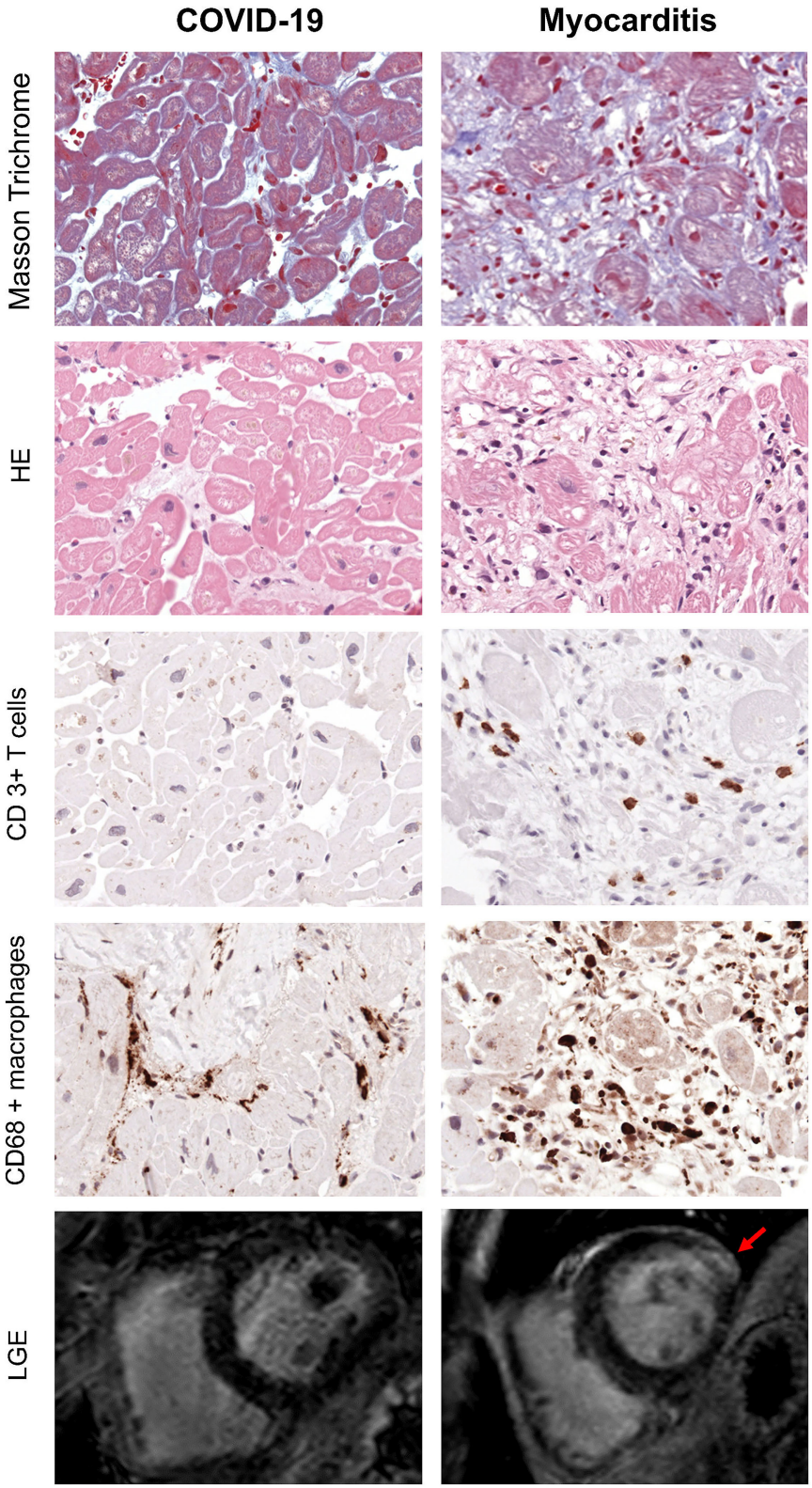

FIGURE 5 | Exemplary EMB and CMR findings in COVID-19 (left) and Myocarditis (Right). First four rows from top represent respectively: Masson Trichrome, hematoxylin/eosin and immunohistological stainings of CD3+ T cells and CD68+ macrophages. In myocarditis, numerous T cells and macrophages are detected in

(Continued)

FIGURE 5 | presence of myocyte necrosis and fibrosis. In contrast, the majority of patients with COVID-19 often show fibrosis but no myocyte necrosis or significant T cell infiltration. However, the number of macrophages is enhanced (x400). Bottom row represents CMR short-axis late gadolinium enhancement (LGE) images: there is evidence of fibrosis in the lateral wall (indicated by the red arrow) in patients with myocarditis (right), while in COVID-19 there are no LGE positive areas in keeping with the absence of active inflammation and fibrosis indicated by the histology.

inflammation were identifiable without evidence of ongoing inflammation *(*<14 infiltrating cells/mm^2) (12). EMB was available in 15 out of 22 patients with classic myocarditis, 11 of 15 (74%) showed evidence of acute inflammation, and 2 of 15 (13%) had signs of chronic inflammation (**Figure 5**). In two patients with classic myocarditis on CMR, no histological evidence of inflammation was present (**Figure 4B**).

Of note, CMR LLC were positive in one patient (10%) with COVID-19 and in two patients (13%) with clinical suspicion of classic myocarditis, in whom EMB samples were negative (**Figure 4B**).

CMR Parameters and WHO Classification

We compared the CMR parameters between patients with mild and moderate forms of the disease (as defined by WHO) and complicated or critical stages, respectively. Both LV and RV longitudinal systolic deformation is lower, and RV Mass and LV T1 native values are higher in patients with COVID-19 with complicated or critical disease compared with those with mild or moderate disease. LA and RA strains were lower in more severely affected patients. Additionally, there were trends for higher LVM and longer T2 times in complicated or critical compared with mild or moderate disease. Detailed results are presented in **Table 4**.

Comparison of CMR Parameters Upon Follow-Up

A complete description of these data is presented in **Table 5**. In post-COVID-19 patients, the difference in LVEF and Endo GCS (-31.7 ± 8.9 vs. -34.2 ± 8.6 %, $p = 0.21$) was not significant but showed a trend for improvement (60 ± 11 vs. 64 ± 8 %, $p = 0.13$). There was, however, significant improvement in Endo GLS (-23.4 ± 3.8 vs. -26.5 ± 3.7 %, $p = 0.034$) and in right ventricular ejection fraction (RVEF, 48 ± 7 vs. 54 ± 5 %, $p = 0.032$), whereas LV mass and RV mass decreased significantly ($p = 0.002$ and $p = 0.040$, respectively) (**Figure 6**).

Similar differences in terms of GLS improvement and LV mass reduction were detected in the myocarditis subgroup (**Figure 6**).

In addition, a significant reduction of native T1 values ($1{,}306 \pm 39$ vs. $1{,}257 \pm 94$ ms, $p = 0.033$, $n = 8$) but not of ECV or T2 values was seen with COVID-19, whereas, in the myocarditis group, there was a significant reduction in T2 values (58 ± 10 vs. 50 ± 5 ms, $p = 0.043$, $n = 10$) and non-significant trends for reduction in native T1 values and ECV (**Figure 7**). Two examples of patients with COVID-19 and one with myocarditis to illustrate the complexity of structural modifications induced by the disease and the variability in recovery at follow-up are shown in **Figure 8**.

Dichotomized data (**Figures 4D–F**, **Table 3**) indicated that LLC criteria, fulfilled in two (20%) of the patients with COVID-19 at baseline, were fulfilled only in one (10%) at follow-up interval, with improvements in both myocardial oedema and myocardial injury from 4 (40%) to 2 (20%). Pericardial involvement was present in two (20%) of the COVID-19 patients, and, at follow-up, persisted only in 1(10%). Importantly, the RV function, impaired in seven (70%) of patients, remained impaired only in two (20%) of these patients at the follow-up visit. In patients with myocarditis, there was a marked improvement of myocardial inflammation at follow-up in majority of patients [from 13 (100%) to 6 (46%)] with five (38%) still fulfilling the LLC criteria at follow-up. The LV dysfunction [8 (62%)] and RV impairment [7 (54%)] persisted in all patients initially affected. Pericardial pathology improved in six patients [from 9 (69%) at baseline to 3 (23%) at follow-up] (**Figures 4D–F**, **Table 3**).

DISCUSSION

Our findings can be summarized as follows:

1. Patients with cardiac symptoms and a recent COVID-19 infection of varying severity showed only subtle changes in cardiac structure and function. On average, standard LV-ejection fraction and mass did not differ from controls, however, significant differences were observed with slightly elevated mean T2 relaxation times and decreased RV stroke volumes in COVID-19 patients.
2. In comparison, the morphological changes observed in COVID-19 patients were less pronounced than in patients with "classic" lymphocytic virus-associated myocarditis or eosinophilic myocarditis, the latter exhibiting marked myocardial and pericardial inflammation and injury in EMB and impaired RV- and LV-function on CMR.
3. In COVID-19 patients, a more severe clinical presentation (WHO severe or critical disease) was associated with lower biventricular longitudinal function, increased native T1 values and higher RV mass.
4. Only three (9%) of the COVID-19 patients fulfilled the diagnostic CMR criteria for acute myocarditis. More frequently, supportive criteria such as pericarditis and pericardial effusion (25%), LV (25%) and RV (28%) dysfunction were present and suggest a *sui generis* "myocarditis-like" pattern, the prognostic implications of which are yet to be established.
5. On EMB analyses, none of COVID-19 patients presented evidence of acute or persistent inflammation, in contrast, the majority of myocarditis patients [13 of 15 (87%)] showed ongoing myocardial inflammation.
6. In COVID-19 patients, LV GLS, LVM, RV EF, T1 values mildly improved at follow-up while T2 values remained elevated.

Although we found that cardiac function on the whole was unaffected in patients who recovered from a SARS-CoV-2 infection, we found significant functional impairment in a small

TABLE 4 | WHO criteria of disease severity.

COVID–19 Patients (*N* = 32)	WHO Disease Severity Scale		
	WHO mild, moderate disease (*N* = 20)	WHO severe disease (*N* = 12)	*P*
Initial Presentation			
Fever, *N (%)*	9 (45)	10 (83)	***0.033***
Chest pain, *N (%)*	6 (30)	2 (17)	0.40
Dyspnea, *N (%)*	12 (60)	8 (67)	0.71
Arrythmia, *N (%)*	0 (0)	1 (8)	0.19
Cough, *N (%)*	14 (70)	10 (83)	0.40
Nausea/Vomiting/Diarrhea, *N (%)*	8 (40)	3 (25)	0.39
Fatigue, weakness, *N (%)*	17 (85)	7 (58)	0.09
Amnesia, *N (%)*	6 (30)	4 (33)	0.99
Lack of taste or smell, *N (%)*	16 (80)	5 (42)	***0.027***
Persistent			
Fatigue/weakness, *N (%)*	9 (45)	0 (0)	***0.006***
Amnesia, *N (%)*	1 (5)	1 (8)	0.99
Lack of taste or smell, *N (%)*	2 (10)	0 (0)	0.26
Arrythmia, *N (%)*	6 (30)	4 (33)	0.84
Left Ventricle			
ED volume, mL/m2	78 ± 27	79 ± 23	0.85
ES volume, mL/m2	31 ± 22	34 ± 16	0.71
Stroke volume, mL/m2	50 ± 6	46 ± 10	0.19
Ejection fraction, %	64 ± 10	59 ± 11	0.23
Cardiac index, L/min/m2	3.5 ± 0.8	3.7 ± 1.2	0.50
Endo longitudinal strain %	–26.3 ± 4.1	–21.6 ± 4.3	***0.004***
Myo longitudinal strain %	–24.0 ± 3.3	–19.5 ± 3.8	***0.001***
Endo circumferential strain %	–32.8 ± 6.1	–31.0 ± 8.9	0.49
Myo circumferential strain %	–21.8 ± 3.8	–19.4 ± 4.5	0.13
LV Mass (g/m2)	50 ± 12	62 ± 25	0.07
Left Atrium			
LA max Vol mL	37 ± 7	36 ± 11	0.84
LA emptying fraction %	65 ± 8	54 ± 15	***0.030***
LA strain %	42 ± 11	33 ± 12	***0.029***
Right Ventricle			
ED volume, mL/m2	77 ± 14	77 ± 17	0.97
ES volume, mL/m2	34 ± 8	39 ± 13	0.27
Stroke volume, mL/m2	43 ± 9	38 ± 7	0.16
Ejection fraction, %	55 ± 7	51 ± 9	0.14
Endo RV longitudinal strain %	–30.5 ± 5.9	–24.2 ± 9.1	***0.048***
Myo RV longitudinal strain %	–28.3 ± 5.3	–22.1 ± 8.9	***0.044***
RV Mass (g/m2)	12 ± 3	16 ± 5	***0.011***
Right Atrium			
RA max Vol mL	37 ± 12	37 ± 12	0.85
RA emptying fraction %	53 ± 11	55 ± 11	0.65
RA strain %	46 ± 11	36 ± 14	***0.034***
Parametric Imaging			
T1 native	1258 ± 55 (N=16)	1296 ± 30 (N=9)	***0.035***
ECV	26 ± 5 (N=16)	27 ± 3 (N=9)	0.64
T2	47 ± 4 (N=16)	51 ± 7 (N=9)	0.06
	(N=20)	(N=11)	P
LGE No. (%)			
Ischaemic	0 (0)	1 (9)	0.19
Nonischaemic	3 (15)	2 (18)	0.90
Pericardial	3 (15)	0 (0)	0.16
	(N=20)	(N=12)	
Lake Louise Criteria			
2018 LLC fulfilled, *N (%)*	0 (0)	3 (19) 5 (42) 4 (33)	
Myocardial edema	3 (15)		
Non–ischaemic myocardial injury	6 (30)		
Pericarditis/ pericardial effusion	3 (15)	5 (42)	
Systolic LV Dysfunctio*N (*global or regional)	2 (10)	6 (50)	
Depressed LVEF, *N (%)*	1 (5)	5 (42)	
LV dilatation, *N (%)*	2 (10)	1 (8)	
LV increased wall thickness, *N (%)*	2 (10)	5 (42)	
Depressed RVEF, *N (%)*	2 (5)	7 (58)	
RV dilatation, *N (%)*	2 (10)	2 (17)	

WHO, World Health Organization; ED, end diastolic; ES, end systolic; Endo, subendocardial layer; Myo, mid–myocardial layer; LV, left ventricular; RV, right ventricular; LA, left atrial; RA, right atrial; ECV, extracellular volume; LLC, 2018 updated Lake Louise Criteria for acute myocarditis.

For comparison of the continuous variables, ANOVA and post–hoc Tukey's tests were used, for categorial variables Chi–square or Fischer tests test were used, a P < 0.05 was considered significant.

For incomplete set of data, N represents the number of subjects included in the analysis. Statistically significant p-values are indicated in bold.

subset of patients. Out of 32 patients, eight (25%) showed global or regional LV dysfunction, nine (28%) showed depressed RV function, 10 (31%) showed structural myocardial alterations, and eight (25%) showed pericardial effusion or pericarditis on CMR. Although these changes seem to recede over time and some of might have partially resolved at the time of the first scan after the acute phase of a COVID-19 infection, pathologic cardiac findings could be more severe in patients with pre-existing cardiac disease, in particular, heart failure (26). Particularly, RV remodeling, diagnosed and assessed with transthoracic echocardiography,

TABLE 5 | Cardiac magnetic resonance imaging findings follow–up.

	COVID–19 *N* = 10		*P*	Myocarditis *N* = 13		*P*
	Baseline	**Follow–up**		**Baseline**	**Follow–up**	
Left ventricle						
ED volume, mL/m2	76 ± 11	75 ± 16	0.88	105 ± 35	94 ± 24	***0.042***
ES volume, mL/m2	31 ± 10	28 ± 10	0.10	52 ± 39	46 ± 25	0.24
Stroke volume, mL/m2	45 ± 8	47 ± 7	0.40	52 ± 13	48 ± 11	0.13
Ejection fraction, %	60 ± 11	64 ± 8	0.13	54 ± 16	53 ± 12	0.79
Cardiac index, L/min/m2	3.5 ± 1.0	3.2 ± 0.4	0.32	3.8 ± 1.0	3.1 ± 1.0	***<0.001***
Endo longitudinal strain %	–23.4 ± 3.8	–26.5 ± 3.7	***0.034***	–20.4 ± 7.4	–23.8 ± 4.3	***0.039***
Myo longitudinal strain %	–21.3 ± 3.0	–23.6 ± 3.2	0.07	–18.8 ± 6.5	–22.0 ± 3.9	***0.022***
Endo circumferential strain %	–31.7 ± 8.9	–34.2 ± 8.6	0.21	–25.4 ± 9.5	–25.8 ± 5.6	0.79
Myo circumferential strain %	–20.1 ± 4.0	–21.1 ± 4.1	0.35	–18.2 ± 7.1	–17.3 ± 3.7	0.56
LV mass (g/m2)	55 ± 7	50 ± 8	***0.002***	74 ± 27	59 ± 13	***0.005***
Left atrium						
LA max Vol mL	35 ± 9	33 ± 10	0.53	42 ± 13	38 ± 11	0.13
LA emptying fraction %	55 ± 15	65 ± 6	0.10	52 ± 20	60 ± 12	***0.026***
LA strain %	39 ± 14	39 ± 9	0.99	27 ± 18	32 ± 13	0.29
Right ventricle						
ED volume, mL/m2	82 ± 17	82 ± 20	0.87	85 ± 28	92 ± 16	0.44
ES volume, mL/m2	43 ± 12	38 ± 12	0.05	37 ± 14	44 ± 9	0.13
Stroke volume, mL/m2	39 ± 7	44 ± 8	***0.032***	44 ± 14	48 ± 11	0.22
Ejection fraction, %	48 ± 7	54 ± 5	***0.032***	53 ± 12	52 ± 8	0.60
Endo RV longitudinal strain %	–24.7 ± 8.0	–29.0 ± 5.9	0.32	–27.4 ± 8.6	–28.9 ± 5.5	0.41
Myo RV longitudinal strain %	–23.2 ± 8.1	–28.2 ± 6.1	0.26	–25.5 ± 8.1	–25.8 ± 5.3	0.84
RV Mass (g/m2)	16 ± 4	12 ± 3	***0.040***	16 ± 3	14 ± 3	0.06
Right atrium						
RA max Vol mL	37 ± 13	40 ± 17	0.27	45 ± 12	42 ± 11	0.34
RA emptying fraction %	55 ± 11	56 ± 9	0.94	48 ± 15	44 ± 9	0.46
RA strain %	39 ± 14	42 ± 13	0.70	36 ± 15	30 ± 10	0.14
Parametric imaging						
T1 native	1306 ± 39 (*n* = 8)	1257 ± 49 (*n* = 8)	***0.033***	1294 ± 24 (*n* = 5)	1224 ± 42 (*n* = 5)	***0.015***
ECV	26 ± 4 (*n* = 10)	25 ± 2 (*n* = 10)	0.27	28 ± 5 (*n* = 5)	26 ± 3 (*n* = 5)	0.31
T2	48 ± 6 (*n* = 8)	50 ± 5 (*n* = 8)	0.66	58 ± 10 (*n* = 10)	50 ± 5 (*n* = 10)	***0.043***
LGE No. (%)						
Ischaemic	1(10)	1 (10)	0.99	2 (15)	2(15)	0.99
Nonischaemic	2(20)	0 (0)	0.14	11 (85)	4 (31)	***0.015***
Pericardial	1 (10)	0 (0)	0.30	11 (92)	2 (15)	0.20

ED, end diastolic; ES, end systolic; Endo, subendocardial layer; Myo, mid–myocardial layer; LV, left ventricular; RV, right ventricular; LA, left atrial; RA, right atrial; ECV, extracellular volume; LGE, late gadolinium enhancement.

For comparison of the continuous variables, paired Student t–test with Welch correction and, for categorial variables Chi–square test were used, a P < 0.05 was considered significant. Statistically significant p-values are indicated in bold.

increased the mortality risk in patients with COVID-19 by more than 100% (27). Using CMR, we showed that decreased RV function is present in about one in four post-COVID-19 patients [9 (28%)]. In half of whom [4 (13%)], RV dilatation is also present.

In agreement with recent data (9), we found that elevated myocardial T2 relaxation times in patients with COVID-19 did not recede at follow-up. This is possibly related to a certain degree of reactive myocardial inflammation triggered by an abnormal immune response that persists even months after an infection (28). However, since almost all other functional markers improve over time, the clinical significance of this finding remains unclear and merits further investigation in future long-term studies.

Previous reports signal the particular targeting of the endothelium by the SARS-CoV2 (4, 29), especially in cases with systemic severe disease. Reduced longitudinal function of the heart is a primary hallmark of a dysfunctional myocardial microvasculature (30). Our findings indicate that both LV and RV long-axis deformation are decreased in more severe forms of COVID-19. This corresponds to increased values of native

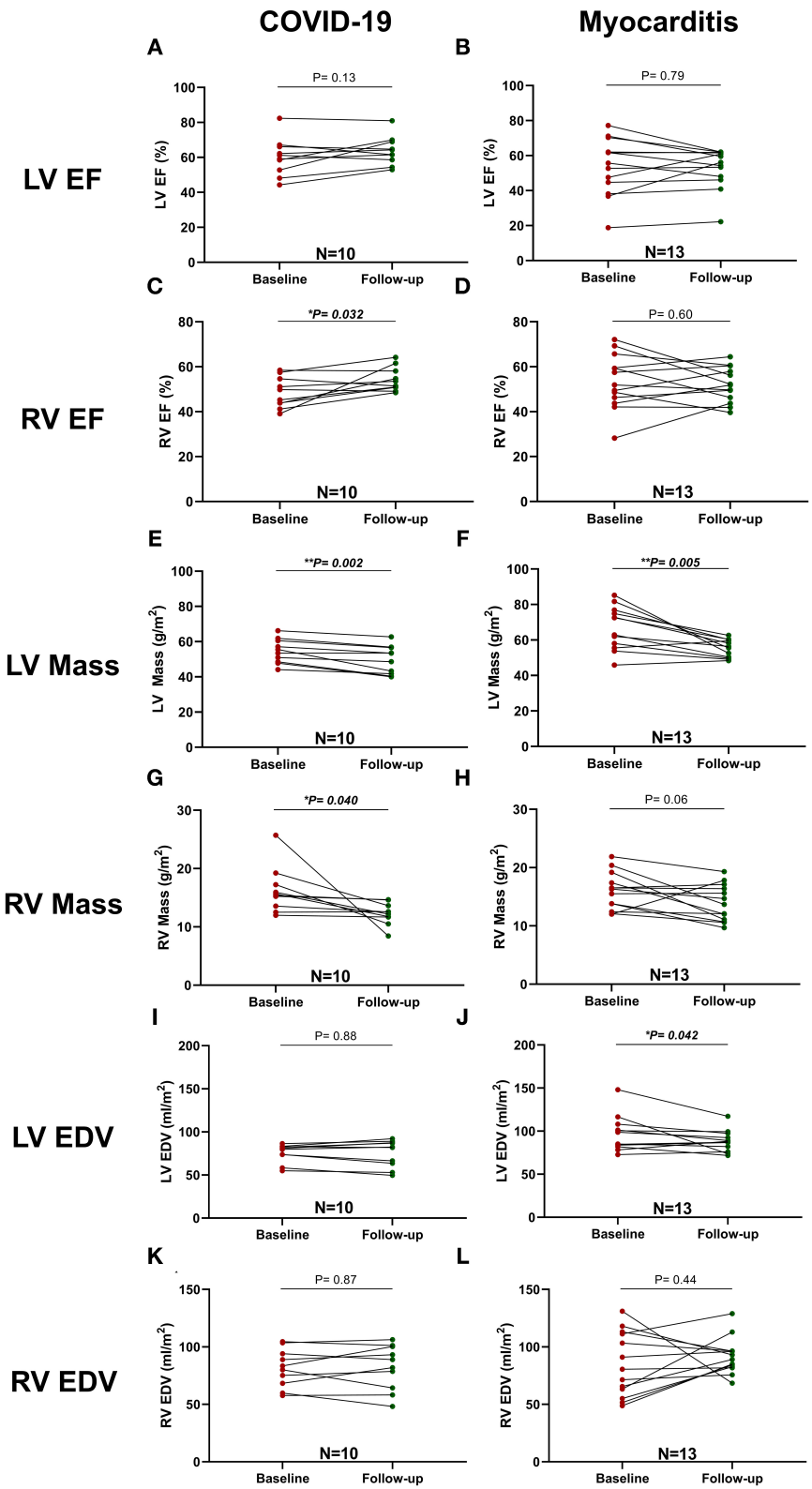

FIGURE 6 | Comparison between LV and RV function and mass at baseline and follow-up in patients with COVID-19 and Myocarditis. On the left column, patients with COVID-19: **(A)** LV EF, **(C)** RV EF, **(E)** LV Mass, **(G)** RV Mass, **(I)** LV EDV, **(K)** RV EDV, and, on the right column, patients with Myocarditis: **(B)** LV EF, **(D)** RV EF, **(F)** LV Mass, **(H)** RV Mass, **(J)** LV EDV, **(L)** RV EDV. A $p < 0.05$ was considered statistically significant and indicated as follows: * < 0.05, ** < 0.01, *** < 0.001.

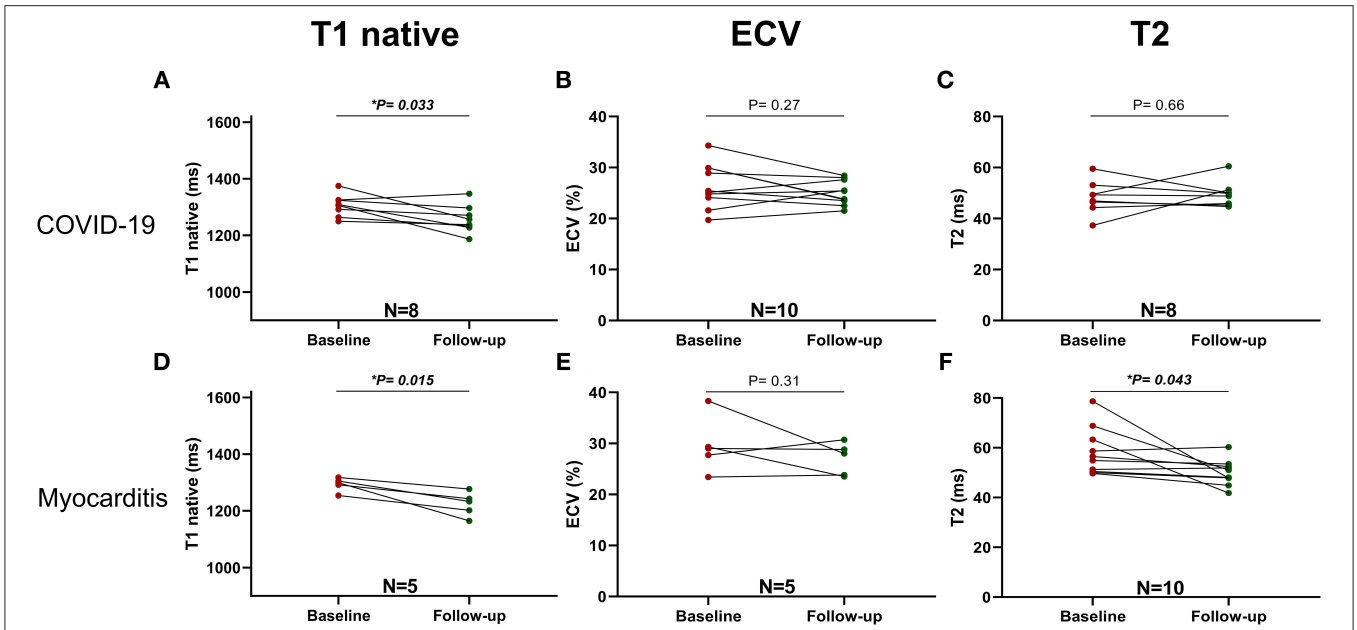

FIGURE 7 | Parametric Imaging at baseline and follow-up in COVID-19 (upper row): **(A)** T1 native, **(B)** ECV and Myocarditis (lower row): **(C)** T2, **(D)** T1 native, **(E)** ECV, **(F)** T2. A $p < 0.05$ was considered statistically significant, and indicated as follows: $^{*} < 0.05$, $^{**} < 0.01$, $^{***} < 0.001$.

T1 relaxation times in these patients, which is a marker of persistent inflammatory response that is possibly accompanied with diffuse structural changes. Taken together, these findings may provide a link to capillary endothelial damage in patients with more severe forms of COVID-19. All patients with a recent COVID-19 infection included in this study improved clinically over time and attended the CMR examinations in an ambulatory outpatient setting. Thus, more pronounced myocardial injury may be present in a more acute stages of the disease in older patients and in patients with underlying cardiac conditions. Impaired RV function that can be observed, in particular, in patients with COVID-19 with a more severe initial presentation or clinical evolution (WHO Score of 3 or 4) may be due to a persistent lung inflammation with microvascular congestion and retrogradely elevated pulmonary arterial pressure (PAP), which could not be excluded by our study where contemporaneous high resolution CT chest imaging was not available.

In a multi-center analysis of 68 hospitalized patients that succumbed to COVID-19 in Wuhan, China, extensive myocardial damage was identified as the main cause of death in 5 (7%) of the cases (31). In contrast, the largest whole heart study to date (32) examined explanted hearts from 39 patients who died following a COVID-19 infection identified the intra-myocardial presence of the virus in 24 samples and signs of viral replication in five samples but failed to demonstrate the presence of any acute inflammatory infiltration of the myocardium even in patients with clinically significant viral load (>1,000 copies). In a similar study, an unexpectedly high density of macrophages was identified in the cardiac tissue of a majority of patients who died of COVID-19, and overt lymphocytic myocarditis was identified in 14% (14) We also observed increased amounts of CD68+ macrophages but not of CD3+ lymphocytes or other specific immune cells in our post-COVID-19 patients. However, it remains uncertain if these dire consequences are the direct effects of viral penetration of the cardiac structures and intra-myocardial viral replication or rather part of an exacerbated systemic response, such as autoimmune virus-triggered cytokine storm or sepsis (33).

In our study, none of the EMB samples obtained from patients with COVID-19 showed any sign of acute or chronic lymphocytic inflammation or viral RNA in the myocardium. In contrast, 13 (87%) out of 15 patients with myocarditis showed histologic evidence of acute inflammation or clinically relevant virus presence in the myocardial tissue in EMB. However, importantly, one (10%) of the post-COVID-19 patients and two (13%) of the patients with myocarditis showed positive CMR LLC criteria but had a negative EMB sample (**Figures 4A,B**). Our results are in agreement with a recently published meta-analysis (34), scrutinizing 277 post-mortem histopathology reports in COVID-19 cases, which identified a very low prevalence of myocarditis if strict diagnostic criteria were applied. However, some myocardial abnormalities were present in as many as half of the cases.

Our study provides further evidence for the role of CMR in the diagnosis of cardiac complications in patients with COVID-19. Yet, due to the small number of included patients, the results of our study should be interpreted with caution (11). Nonetheless, we believe that our study shows that a greater number of post-COVID patients would benefit from a comprehensive CMR (13) work-up and should ideally be included in multi-center, national, or international CMR COVID-19 databases with stringent long-term follow-up. Patients included in these longitudinal studies should include patients with pre-existing cardiovascular disease and risk factors, in whom myocardial injury and dysfunction may prove to be more severe, recovery slower, and long-term sequelae more pronounced. In addition, our study also underlines the complex and incomplete overlap of CMR and EMB criteria in the

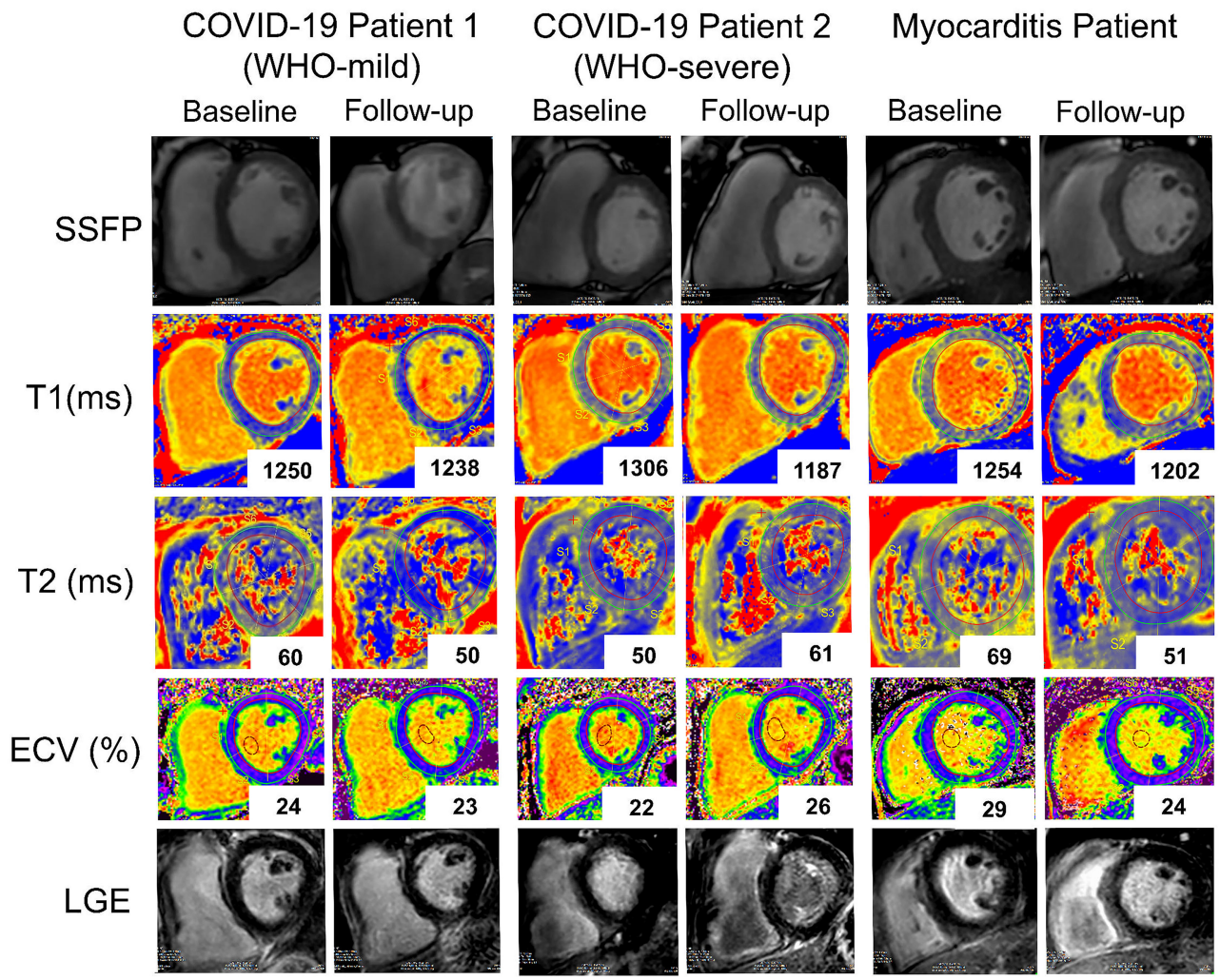

FIGURE 8 | Representative baseline and follow-up CMR images of two patients with COVID-19 (COVID-19 patient 1 with a WHO-score: mild and COVID-19 patient 2 with a WHO-score: severe and a patient with Myocarditis. Rows (from top to bottom): SSFP-cine images, T1 native maps, T2 maps, Extracellular volume maps, late gadolinium enhancement images. While in the first patient with COVID-19 (first and second columns from the left), T1 native (from 1,250 to 1,238ms), and T2 (from 60 to 50 ms) signals improved at follow-up in the second patient with COVID-19 (third and fourth columns) T1 native signal improved (from 1,306 to 1,187 ms) while T2 (from 50 to 61ms) and ECV (from 22 to 26%) worsened. In the patient with myocarditis (fifth and sixth columns) there is at follow-up a marked improvement in all the parameters.

diagnosis of acute myocarditis and suggests their complementary diagnostic role (33).

Several studies (35–39) agree on the fact that acute myocarditis or myocarditis-like traits are present after anti-SARS-CoV2 vaccination in a minority of subjects with CMR findings similar to those observed in patients with COVID-19. However, the mechanisms of these change as the mechanisms of post-COVID-19 myocardial modification remain largely unknown. In addition, while it is legitimate to suppose that pathophysiological similarities related to the specific immune response elicited SARS-CoV2 viral particles, more focused studies are warranted to demonstrate such correlations.

Immunosuppressive therapy was shown to be effective in the partial recovery of cardiac function in patients with chronic myocarditis or HF resulting from resolved acute myocarditis. (40) RECOVERY Trial demonstrated the beneficial effect of Dexamethasone in severely affected patients hospitalized for COVID-19 (41) and several other immunomodulatory interventions (42–44) mostly done in an acute setting and in very ill patients which improved mortality rate and clinical course of the disease. HEAL-COVID clinical trial (https://clinicaltrials.gov/ct2/show/NCT04801940), which commenced in April 2021, aims to recruit subjects recovered from COVID-19 who experience longer-term complications of the disease. So far, the lack of consensus regarding the molecular pathophysiology of these changes and their reversibility hampers a more targeted pharmacological approach. With the paradigm of long-COVID now widely accepted, we expect to see an

increase in the number of clinical trials including incompletely recovered patients, including those with persistent myocardial or pericardial disease.

LIMITATIONS

COVID-19 is an ongoing global pandemic, and study findings to support clinical guidelines and decision making are urgently needed. Our study was primarily designed to assess what appears to be the most important alteration observed with CMR in post-COVID-19 patients, namely, parametric mapping (T1, T2 relaxation times). T1 and T2 values notoriously vary between scanners from different manufacturers and field strengths. Thus, in order to increase the sensitivity, the comparison of parametric mapping between the three groups included only patients and volunteers scanned with the same 3T Philips Ingenia scanner. To comply with these inclusion criteria, the final number of patients included in the study and the number of these patients who underwent a follow-up scan were relatively reduced. Importantly, we acknowledge the age disparity between healthy controls, post-COVID-19, and myocarditis groups. There was more variability in the follow-up interval within in the myocarditis group compared to the post-COVID-19 group due to a more complex clinical management, frequently involving hospitalization, and clinically indicated multiple scans. Despite the best of our efforts, some of the datasets remain incomplete, in particular, clinical data collected retrospectively from patients with COVID-19 and patients with myocarditis. To overcome this limitation, we clearly indicated the exact number of datasets available per subgroup. As our study design did not permit the examination of hyper-acute cardiac manifestations of COVID-19, our main purpose was to investigate whether persistent cardiac changes, as proposed previously, can induce structural or functional remodeling of the heart and impede complete recovery.

CONCLUSIONS

In our cohort, CMR and EMB findings revealed that a SARS-CoV-2 infection shows relatively mild but variable cardiac involvement. More symptomatic patients and those with higher clinical care demands are more likely to exhibit impaired myocardial function and chronic inflammation compared to patients with "classic" acute myocarditis during the acute and convalescent phases. Our study highlights the importance of collecting large multicentre cardiac imaging data from patients with and recovering 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.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Charité – Universitätsmedizin Berlin Ethics Committee and comply with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

RT and SK designed the study. RT, PD, CG, VZ, JW, and JE participated in data collection and analysis. PD, AF, AB, SK, DG, and TC recruited the patients and supervised the CMR scans. KK and CT supervised the histology analyses and reviewed the EMB results. GK, TK, TC, PS, MW, KK, SV, BP, CT, and SK reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Case Series of Potential Cardiac Inflammation Associated With Various SARS-CoV-2 Vaccinations Assessed by Cardiac MRI

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Serious adverse events associated with new vaccines targeting SARS-CoV-2 are of high interest to the public and to public health as a worldwide mass immunization campaign has been initiated to contain the ongoing COVID-19 pandemic. We describe a series of 4 individuals with signs of a myocarditis/pericarditis according to cardiac MRI results in temporal association with currently in the European Union authorized SARS-CoV-2 vaccines. We found mild abnormal MRI results independent of the type of SARS-CoV-2 vaccine. There is a need of continuing monitoring outcomes of myocarditis cases after COVID-19 vaccination as recently published cases suggest an uncomplicated short-term course whereas the long-term implications are not yet known but taking the available evidence into account the benefits of using COVID-19 vaccines still clearly outweigh the risks.

Keywords: CMR, COVID-19, SARS-CoV-2 vaccination, myocarditis, pericarditis

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BACKGROUND

Before the COVID-19 pandemic, there have only been a few reports of myocarditis and pericarditis as an adverse event following immunization with the exception of cases following live-attenuated smallpox vaccine (1, 2).

Serious adverse events associated with new vaccines targeting SARS-CoV-2 are of high interest to the public and to public health as a worldwide mass immunization campaign has been initiated to contain the ongoing COVID-19 pandemic.

SARS-CoV-2 vaccines currently authorized for use in the European Union by the European Pharmacy Agency include the messenger RNA (mRNA) vaccine Comirnaty (Pfizer-BioNTech), Spikevax (Moderna) and the vector-based vaccines Vaxzevria (AstraZeneca), and Vaccine Janssen (Johnson and Johnson) (3).

We describe a series of 4 individuals with signs of a myocarditis/pericarditis according to cardiac MRI results in temporal association with SARS-CoV-2 vaccination to investigate any differences regarding the phenotype.

MATERIALS AND METHODS

For this report we retrospectively reviewed cardiac MRI exams performed at our institution between 07/01/2021-09/06/2021 for MRI findings of cardiac inflammation such as myocarditis or pericarditis associated with SARS-CoV-2 Vaccination. We reviewed the medical records

regarding the timing of COVID-19 or SARS-CoV-2 vaccination and the vaccine used. All available demographic, clinical or laboratory information were documented (**Table 1**).

The study complies with the declaration of Helsinki. Approval was obtained from the ethics committee of Charite – Unversitätsmedizin Berlin. All examinations have been clinically indicated.

Cardiac MRI was performed at 1.5T/3T [Philips Healthcare, Best, Netherlands] and evaluated using a standardized diagnostic protocol as described previously (4). The protocol included cine, T1 and T2 mapping, and late gadolinium enhancement (LGE) images. Cutoffs for elevated T1 values (normal 903 to 1,085 ms at 1.5 Tesla and 1,173 to 1,334 ms at 3 Tesla) and T2 values (normal 41 to 57 ms at 1.5 Tesla and 35 to 51 at 3 Tesla) were based on 2 standard deviations above the respective means in a healthy reference group examined on the same scanners. Clinical cardiac MRI reports were reviewed by three cardiologists experienced in cardiovascular imaging in consensus.

CASES

Patient 1

Patient 1, a healthy 21-year-old male, received his second vaccination dose of Spikevax (Moderna). The following day the patient complained about chest pain and discomfort, shortness of breath, limited physical capacity and malaise. At presentation to the hospital the electrocardiogram showed no pathological findings. The serum levels for C-reactive protein and NT-proBNP were normal. High-sensitive Troponin T was elevated up to 526 ng/l (normal <14 ng/l). A coronary angiography was performed with exclusion of a coronary artery disease. CT pulmonary angiography excluded a pulmonary embolism. Transthoracic echocardiography showed normal myocardial function without wall motion abnormalities or relevant valvular heart disease.

Cardiac MRI at 3 Tesla showed a normal left and right ventricular size with normal left and right ventricular ejection fraction and normal values for the global longitudinal strain. T2 weighted images indicated a regional edema anterolateral/inferolateral (basal) with corresponding elevated quantitative myocardial T2 mapping parameters up to 70 ms (normal 35 to 51 ms at 3 Tesla) (**Figure 1**). Corresponding patchy subepicardial LGE indicating inflammatory myocardial necrosis (**Figure 1**). Pericardial enhancement in the LGE and T2 weighted images in corresponding locations indicated a pericardial involvement (**Figure 1**). The global T1 relaxation time (1,227 ms, normal 1,173 to 1,334 ms at 3 Tesla) and global T2 relaxation time (43 ms, normal 35 to 51 ms at 3 Tesla) were normal. The patient was discharged after 6 days with stable cardiopulmonary parameter and improved symptoms with an anti-inflammatory therapy with ibuprofen and a supportive therapy with ACE-inhibitor and outpatient follow-up appointments.

Patient 2

Patient 2, a healthy 42-year-old male, received the second vaccination dose of Comirnaty (Pfizer-BioNTech). Two days later, the patient presented to the emergency room of a referring hospital with chest pain and discomfort, shortness of breath and a decreased physical capacity.

The electrocardiogram on admission showed no pathological findings. The serum levels for C-reactive protein (59 mg/l, normal <5.0 mg/l) and cardiac necrosis marker were elevated with a highsensitive Troponin-I level of 4,868 pg/ml (normal <34.1 pg/ml) and creatinin kinase of 581 U/l (normal 190 U/l). The levels for D-Dimer and BNP were normal. A coronary angiography was performed with exclusion of a coronary artery disease. CT pulmonary angiography excluded a pulmonary embolism. Transthoracic echocardiography showed normal myocardial function without wall motion abnormalities or relevant valvular heart disease.

Cardiac MRI at 3 Tesla showed a normal left and right ventricular size with normal left and right ventricular ejection fraction but reduced values for the global longitudinal strain with −17.8% (normal −28.5 to −20.5% according to local reference values). T2 weighted images indicated a regional edema inferior/inferolateral (basal) with corresponding elevated quantitative myocardial T2 mapping parameters up to 53 ms (normal 35 to 51 ms at 3 Tesla) and corresponding subepicardial LGE in this region (**Figure 1**). The global T1 relaxation time (989 ms, normal 903 to 1985 ms at 1.5 Tesla) was normal. In summary, this provided evidence for acute myocarditis without functional limitation. An anti-inflammatory therapy with ibuprofen was started. During hospitalization, the patient complained of left lower leg pain. Duplex sonography of the veins showed thrombosis of a collateral vein in the region of the posterior tibial artery. Compression therapy and oral anticoagulation were started. The patient was discharged after 6 days with improved symptoms with recommendation to continue the anti-inflammatory, compression and oral anticoagulation therapy.

Patient 3

Patient number 3 was an 18-year-old healthy and athletic young man. The patient reported that shortly after a vaccination with Janssen (Johnson and Johnson), he initially experienced an episode of fever and limb pain. The initial symptoms subsided significantly after 3 days. However, a marked limitation of physical capacity and a feeling of chestpain and discomfort at rest and under physical stress remained. After the complaints persisted even for 2 months after vaccination, an outpatient presentation was made for further diagnostics. The electrocardiogram showed no pathological findings. Transthoracic echocardiography showed normal myocardial function without wall motion abnormalities or relevant valvular heart disease. Cardiac MRI at 1.5 Tesla showed a normal left and right ventricular size with normal left and right ventricular ejection fraction and normal values for global longitudinal strain. The images showed a mild pericardial effusion in the area of the free RV wall and the basal posterior LV wall up to a maximum of 4 mm with evidence of inflammatory changes of the pericardium in the T2 weighted images and the LGE images in the area of the lateral LV wall. The global T2 relaxation time (49 ms, normal 35 to 51 ms at 1.5 Tesla) and T1 relaxation time (1,071 ms, normal 903 to 1,085 ms at 1.5 Tesla) were normal. Assuming a discrete pericarditis of the LV-lateral area, a native MRI follow-up after 3 months was recommended.

TABLE 1 | Four patients diagnosed with signs of myocarditis/pericarditis in temporal relation to a SARS-CoV-2 vaccination.

Clinical data	Patient 1	Patient 2	Patient 3	Patient 4
Demographic data	21 years old/male	42 years old/male	18 years old/male	18 years old/male
Type of vaccine	2nd dose Spikevax (Moderna)	2nd dose Comirnaty (Pfizer-BioNTech)	Janssen (Johnson and Johnson)	Vaxzevria (AstraZeneca)
Symptoms	Chest pain and discomfort, malaise, dyspnea, limited physical capacity	Chest pain and discomfort, dyspnea, limited physical capacity	Chest pain and discomfort, dyspnea, limited physical capacity	Chest pain and discomfort, limited physical capacity
Vaccination–symptoms (days)	1	2	1	12
Vaccination–Cardiac MRI (days)	6	8	57	68
Troponin (ng/ml)	Troponin-T-hs 526 (normal <14 ng/l)	Troponin-I-hs 4,868 (normal <34.1 ng/l)	NA	Troponin-I-hs <5.1 (normal<34.1 ng/l)
NT-pro-BNP (ng/l)	79 (normal <97 ng/l)	40 (normal <100 ng/l)	NA	NA
Coronary angiography	No pathological findings	No pathological findings	NA	No pathological findings
Pulmonary angiography	No pathological findings	NA	NA	NA
Cardiac MRI results				
LV-EF (%) (normal 57 to 77%)	58	64	60	60
RV-EF (%) (normal 52 to 72%)	59	NA	55	62
GLS (%) (normal −28.5 to −20.5%)	−21. 2	−17.8	−23.2	−24
ECV (%) (normal ≤ 30%)	21	25	26	25
Wall motion abnormalities	+	−	−	−
Local T2w signal abnormality	+	+	+	−
Elevated global T2 relaxation time	−	−	−	−
Elevated global T1 relaxation time	−	−	−	−
Pericardial effusion	−	+	+	+
Local LGE	+	−	+	−

LV-EF, Left ventricular ejection fraction; RV-EF, Right ventricular ejection fraction; GLS, Global longitudinal strain; ECV, Extracellular volume; LGE, Late gadolinium enhancement; NA, Not available; NT-pro-BNP, N-terminal pro-B-type natriuretic peptide.

Patient 4

Patient number 4, a healthy 18-year-old male received the first dose of Vaxzevria (AstraZeneca) at the end of June 2021. The patient reported new episodes of chestpain and discomfort and exercise limitation ∼10 days after the vaccination. Initially, no medical presentation was made in the expectation that the symptoms would disappear. After a clear increase of the symptoms in the course of time, the patient presented to the emergency department of a referring hospital. The electrocardiogram on admission showed ST segment elevation in the inferior leads (II, III, and aVF). A coronary angiography was performed with exclusion of a coronary artery disease. The serum levels for C-reactive Protein [<1.0 mg/l, normal < 5.0 mg/l)] and highsensitive Troponin-I [<5.1 pg/ml, normal<34.1 pg/ml)] were normal. Creatinin kinase was increased with a serum level of 255 U/l (normal <190 U/l). Transthoracic echocardiography showed normal myocardial function without wall motion abnormalities or relevant valvular heart disease.

Cardiac MRI at 1.5 Tesla showed a normal left and right ventricular size with normal left and right ventricular ejection fraction and normal values for global longitudinal strain. The examination showed a mild to moderate pericardial effusion up to 11 mm in the mid-posterior wall of the LV. There was no evidence of acute cardiac inflammation in the T2 weighted and LGE images. The global T2 relaxation time (52 ms, normal 35 to 51 ms at 1.5 Tesla) and T1 relaxation time (979 ms, normal 903 to 1,085 ms at 1.5 Tesla) were normal. In the absence of signs of acute cardiac inflammation in the T2 weighted images and the LGE images, the pericardial effusion was considered as a possible residual of an expired pericarditis/myocarditis.

DISCUSSION

Myocarditis is an inflammation of the heart muscle in the absence of ischemia (5). If it is accompanied by pericarditis, an inflammation of the pericardium, it is referred to as myopericarditis. Myocarditis is predominantly mediated by viral infection, but can also be induced by bacterial, protozoal or fungal infections as well as systemic immune-mediated diseases and a variety of toxic substances and certain drugs as well as vaccine exposures (5).

For vaccine associated myocarditis the underlying mechanisms are not fully understood either. Molecular mimicry between the spike protein of SARS-CoV-2 and self-antigens, trigger of preexisting dysregulated immune pathways, immune response to mRNA, and activation of immunologic pathways, and dysregulated cytokine expression have recently been proposed (6).

Vaccine associated myocarditis is still overall rare and more common in males and the young population (7). The

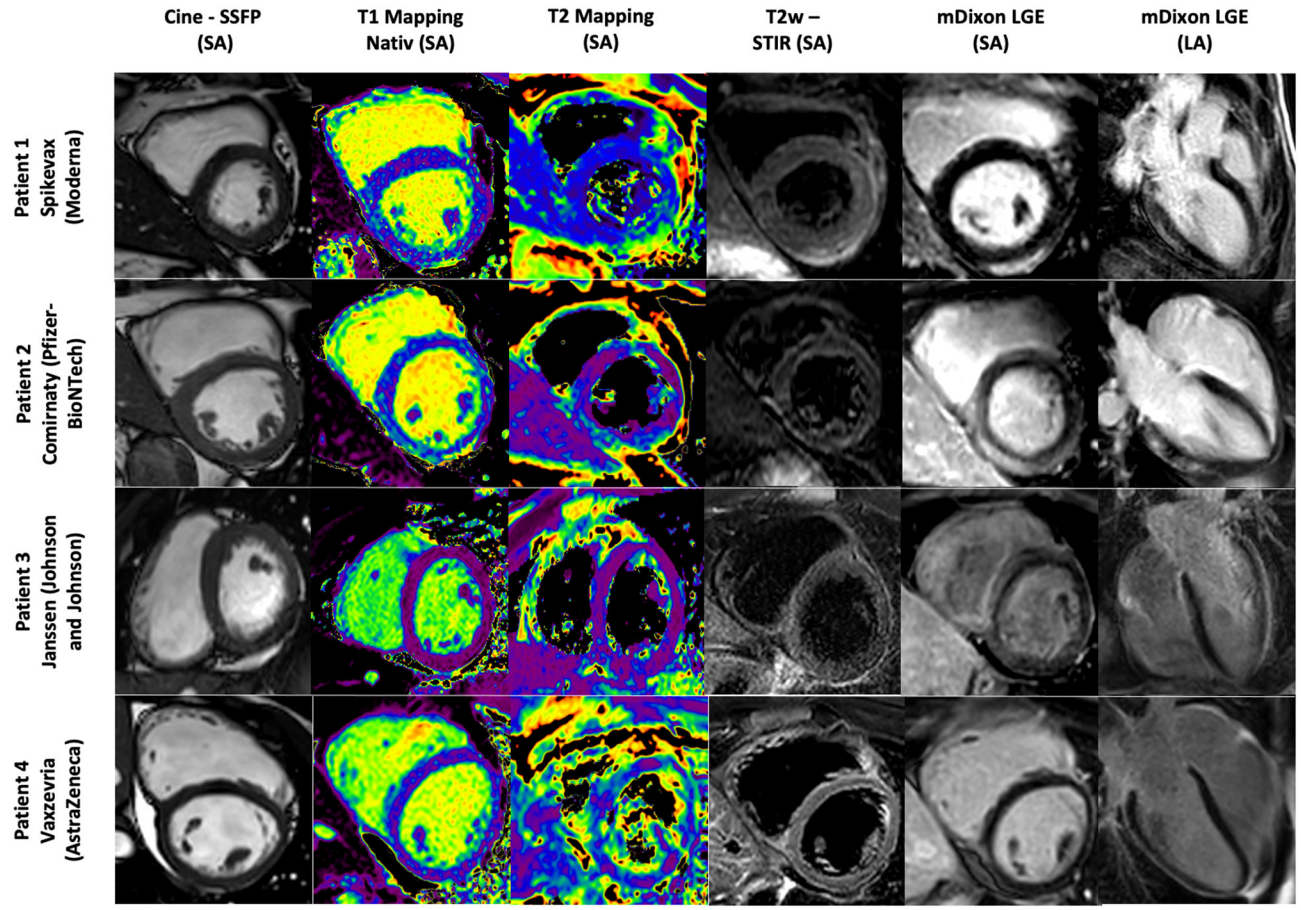

FIGURE 1 | Cardiac MR images of Patient 1–4. Patient 1 with signs of a myopericarditis after mRNA SARS-CoV-2 vaccination with Spikevax (Moderna). Patient 2 with signs of a myocarditis after mRNA SARS-CoV-2 vaccination with Comirnaty (Pfizer-BioNTech). Patient 3 with signs of a discrete pericarditis in the LV-lateral area after SARS-CoV-2 vaccination with Janssen (Johnson and Johnson). Patient 4 with a mild to moderate pericardial effusion up to 11 mm in the medial posterior wall of the LV as a possible residual of an expired pericarditis/myocarditis after SARS-CoV-2 vaccination with Vaxzevria (AstraZeneca). SSFP, Stady state free precession; T2w FSE, T2-weighted fast spin echo; mDixon, Single breath-hold three-dimensional (3D) ECG-gated multi-echo chemical shift-based sequence; T1 relaxation were calculated from single breath-hold two-dimensional (2D) modified Look-Locker inversion recovery (MOLLI) sequence LA, long axis; SA, short axis.

Advisory Committee on Immunization Practices (ACIP) recently published an incidence of 40.6 cases per million second doses of mRNA SARS-CoV-2 vaccinations in a population of males aged 12–29 years compared to 2.4 per million second doses administered to males aged ≥30 years (7).

The reasons for male predominance is unknown, but theories relate to sex hormone differences in immune response and myocarditis and underdiagnosis of cardiac disease in women (6).

Severity and clinical presentation of myocarditis or pericarditis vary among patients. Symptoms might include dyspnea, chest pain or palpitations, although especially in younger children other symptoms might be present (8). The clinical diagnostic evaluation might show elevated cardiac injury marker, pathological findings on electrocardiogram, echocardiogram, or as shown cardiac MRI results.

As also seen in our patients the clinical course of SARS-Cov2 vaccination associated myocarditis is typically mild and self-limited (2). Data published by the Israeli Ministry of Health showed 148 cases of myocarditis among 10.4 million vaccinated Israelis (9). Most cases occurred within 30 days after the second dose of a mRNA vaccination. Most cases required a hospitalization up to 4 days but were considered mild (9).

Regarding the guidelines for a mild and uncomplicated myocarditis/pericarditis a myocardial biopsy or viral serology was not performed in our patients. According to the guidelines the management depends on supportive therapy with targeted cardiac and anti-inflammatory medications and specific interventions if necessary (10, 11). Exercise restriction is recommended until the heart recovers (10, 11).

We found mild abnormal MRI results independent of the type of SARS-CoV-2 vaccine. We hypothesize, that abnormal findings might be present independent of vaccine and potentially might also be present when patients are flu-vaccinated. Future research work should also focus on this aspect. A more pragmatic approach might be to look first for cardiac abnormalities in cases of cardiac symptoms after vaccination, such as elevated lab values as troponin and NTproBNP or abnormalities at

echocardiography as previously described from our group post COVID-19 (12).

In conclusion clinicians should be aware of vaccine-induced myocarditis as a possible adverse event after SARS-CoV-2-vaccination.

There is a need of continuing monitoring outcomes of myocarditis cases after COVID-19 vaccination as recently published cases suggest an uncomplicated short-term course whereas the long-term implications are not yet known.

Taking into account the available evidence including the risks of myocarditis and pericarditis, it can be determined that the benefits of using COVID-19 vaccines still clearly outweigh the risks. There is a need for a continued educational campaign for the public regarding the risk of COVID-19 and the benefits and risks of a SARS-CoV-2 vaccination.

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 Charite Unversitätsmedizin Berlin. 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

The first draft of the manuscript was written by CJ and all authors commented on previous versions of the manuscript. All authors contributed to the study conception and design, read, and approved the final manuscript.

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ORIGINAL RESEARCH
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Different Impacts on the Heart After COVID-19 Infection and Vaccination: Insights From Cardiovascular Magnetic Resonance

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Introduction: Myocarditis-like findings after COVID-19 (coronavirus disease 2019) infection and vaccination were reported by applying cardiovascular magnetic resonance (CMR). These results are very heterogenous and dependent on several factors such as hospital admission or outpatient treatment, timing of CMR, and symptomatic load. This retrospective study aimed to identify differences in myocardial damage in patients with persistent symptoms both after COVID-19 infection and vaccine by applying CMR.

Materials and Methods: This study entails a retrospective analysis of consecutive patients referred for CMR between August 2020 and November 2021 with persistent symptoms after COVID-19 infection or vaccination. Patients were compared to healthy controls (HC). All patients underwent a CMR examination in a 1.5-T scanner with a scan protocol including: cine imaging for biventricular function and strain assessment using feature tracking, T2 mapping for the quantification of edema, and T1 mapping for diffuse fibrosis and late gadolinium enhancement (LGE) for the detection and quantification of focal fibrosis. Patients were divided into a subacute COVID-19 (sCov) group with symptoms lasting < 12 weeks, post-COVID-19 (pCov) group with symptoms > 12 weeks, and patients after COVID-19 vaccination (CovVac).

Results: A total of 162 patients were recruited of whom 141 were included for analysis. The median age in years (interquartile range (IQR)) of the entire cohort was 45 (37–56) which included 83 women and 58 men. Subgroups were as follows (total patients per subgroup, median age in years (IQR), main gender): 34 sCov, 43 (37–52), 19 women; 63 pCov, 52 (39–58), 43 women; 44 CovVac, 43 (32–56), 23 men; 44 HC (41 (28–52), 24 women). The biventricular function was preserved and revealed no differences between the groups. No active inflammation was detected by T2 mapping. Global T1 values were higher in pCov in comparison with HC (median (IQR) in ms: pCov 1002ms (981–1023) vs. HC 987ms (963–1009; $p = 0.005$) with other parings revealing no differences. In 49/141 (34.6%) of patients, focal fibrosis was detectable with the majority having a non-ischemic pattern (43/141; 30.4%; patients) with the subgroups after infection having

more often a subepicardial pattern compared with CovVac (total (% of group): sCov: 7/34(21%); pCov 13/63(21%); CovVac 2/44(5%); $p = 0.04$).

Conclusion: Patients after COVID-19 infection showed more focal fibrosis in comparison with patients after COVID-19 vaccination without alterations in the biventricular function.

Keywords: cardiovascular magnetic resonance, mapping, late gadolinium enhancement, COVID-19, vaccination, fibrosis

INTRODUCTION

COVID-19 (coronavirus disease 2019) can virtually impact any organ ranging from the respiratory tract to the kidneys, the central nervous system, and the cardiovascular system (1). Similar to the broad range of organ involvement, the specific organ-related pathophysiologic changes can also show a wide array of patterns. Acute and mid-term myocardial tissue changes after COVID-19 infection have been described with varying degrees and frequencies, depending on various co-factors, such as hospitalization (2) or ambulatory recovery (3), timing between event and cardiovascular magnetic resonance (CMR) (3–5), and each individual's risk factor profile (6–8). Taking the time between the acute event and CMR into consideration, patients can have reduced left (LV) and right ventricular (RV) function if examined within 2–3 months (2) or no biventricular impairment if CMR is performed 5 months after the initial event (9). **Figure 1** visually integrates and compares this study with other published work regarding the time interval between CMR and acute infection or vaccination. Another factor to consider is the presence of symptoms, as evidence is expanding that they can have a high longevity even after the acute phase of the infection has subsided (10). Based on these findings, the terms subacute COVID-19 or long-COVID-19 for symptom persistency after 4 weeks of the infection and post-COVID-19 with ongoing symptoms for more than 12 weeks have been introduced by Nalbandian et al. in 2021 (10). From a cardiologic perspective, this is relevant as symptoms warranting further dedicated cardiologic work-up, such as fatigue, palpitations, and chest pain, are fairly common in these patients (11). One study recently reported findings in a patient cohort with ongoing symptoms, such as exertional dyspnea, fatigue, and palpitations, for more than 30 days after initial COVID-19 diagnosis (4). The studied population underwent a CMR examination at a median of 103 days revealing no signs of active myocardial inflammation in comparison with a healthy cohort. This raises the question how responsible structural myocardial impairment could actually be in terms of the symptom load or whether the etiology is more centered around a chronic fatigue syndrome with a complex neurological background. The first reports describe diagnostic criteria fulfillment for chronic fatigue syndrome in about half of the patients with ongoing symptoms after COVID-19 infection (12). CMR has been characterized as the non-invasive modality of choice for the detection of acute myocarditis (13) and is listed as a mandatory test in patients with heart failure and suspected myocarditis by the European Society of Cardiology (14). Even beyond the acute stages dominated by myocardial inflammation and edema, CMR can further deduce whether there is a complete recovery or whether changes might be persistent as marked by chronic replacement fibrosis detected on late gadolinium enhancement imaging (LGE) (15). Parametric techniques, such as T1 mapping and extracellular volume (ECV), might further identify potential diffuse fibrotic processes (13). Therefore, CMR might be useful in the assessment of patients after COVID-19 infection at different phases (16).

Along with the development of messenger RNA-based vaccines targeting the COVID-19 virus, reports on post-vaccination myocarditis followed (17, 18). A recent study of 15 patients undergoing CMR for clinically diagnosed post-vaccination myocarditis revealed findings similar to viral myocarditis. The patient cohort had a good clinical outcome (19). This was supported by another recent study demonstrating that patients with COVID-19 vaccination-associated myocarditis had no adverse outcomes and good clinical recovery (20). In comparison with patients with COVID-19 myocarditis and other viral myocarditis cases, the patients after COVID-19 vaccination showed less extensive LGE.

The focus of the study was on continuously symptomatic patients after COVID-19 infection or vaccination who were referred in an ambulatory setting for CMR. The aim of the investigation was to detect alterations in myocardial function and tissue structure with an intergroup comparison of patients with subacute COVID-19, post-COVID-19, and after COVID-19 vaccination.

Abbreviations: COVID-19, Coronavirus disease 2019; CMR, cardiovascular magnetic resonance; HC, healthy controls; LV, left ventricle; RV, right ventricle; LGE, late gadolinium enhancement; ECV, extracellular volume; sCov, subacute COVID-19; pCov, post-COVID-19; CovVac, COVID-19 vaccination; SAX, short axis; STIR, short T1 inversion recovery; FT, feature tracking; IQR, interquartile range; hs, high sensitive; EF, ejection fraction; SENC, strain-encoded magnetic resonance.

MATERIALS AND METHODS

Study Patients

For this exploratory, retrospective study, all patients undergoing CMR examinations between August 2020 and November 2021 with persistent symptoms after either COVID-19 infection or vaccination were included. Patients were referred by primary care physicians or cardiologists. For the purposes of cohort characterization, the electronic health records were searched. Symptoms were systematically recorded before every scan by

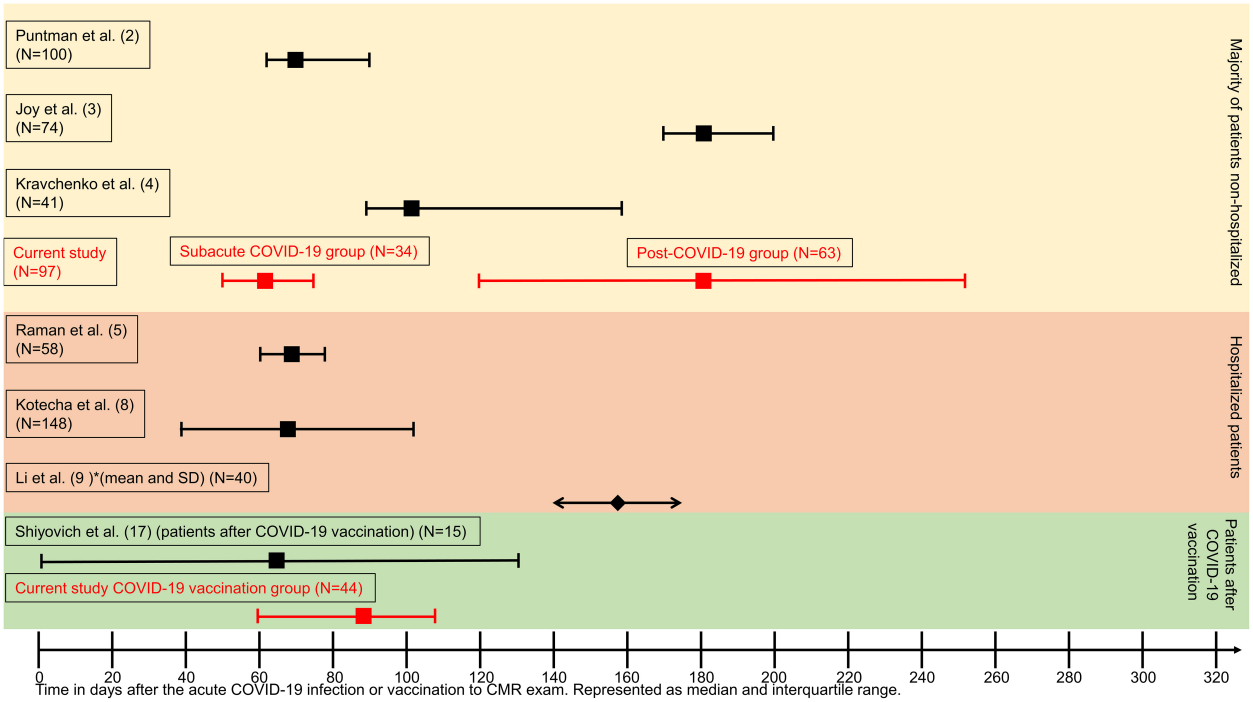

FIGURE 1 | Graphical overview representing median time between COVID-19 infection or vaccination and cardiovascular magnetic resonance (CMR) examination. Time is represented as days on the X-axis. Data are given as median (squares) and interquartile range (whiskers indicate 25th and 75th percentile, respectively) except for Li et al. (9), which are represented as mean (diamond) and standard deviation (arrows pointing outward). Red colors highlight the time ranges of this study.

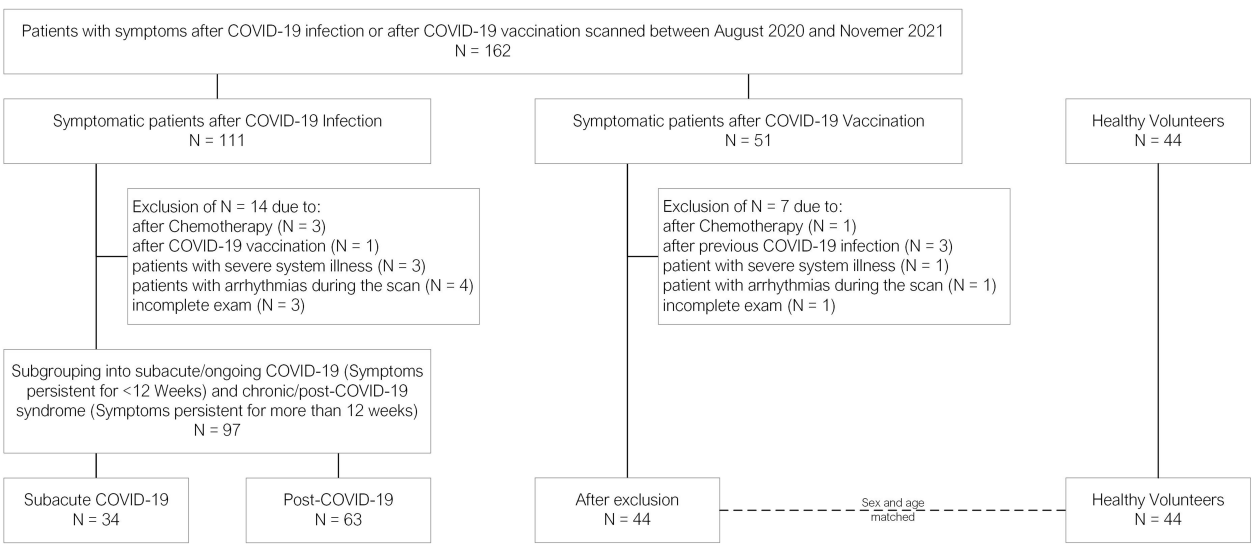

FIGURE 2 | Flowchart detailing the patients excluded for each subgroup.

the attending physician on a standardized patient information sheet. After the inclusion, patients were subdivided into a cohort after COVID-19 infection with symptoms lasting between 4 and 12 weeks after infection (subacute COVID-19; sCov), with symptoms lasting > 12 weeks (post-COVID-19; pCov), and symptomatic patients after COVID-19 vaccination (CovVac). The time of the acute event was defined by the first positive polymerase chain reaction test or the time of the last dose of vaccination before symptom onset. Patients were excluded from the final analysis if severe systemic illnesses including systemic autoimmune disease, malignancies, cardiomyopathies, previous myocarditis, or previous chemotherapy were known. Similarly,

patients who were vaccinated after COVID-19 infection were excluded and vice versa. Finally, if arrhythmias during the scan impaired image acquisition or the examination was incomplete, patients were excluded. A flowchart is shown in **Figure 2**. A healthy cohort (HC), recruited in previous studies before the outbreak of COVID-19, was age- and gender-matched to the CovVac patients and gender-matched to the sCov and pCov groups (15, 21). As only in a minority of HC contrast medium was applied, post-contrast image analysis was not carried out in the HC.

Ethics Statement

This study complied with the Declaration of Helsinki and was approved by the institutional ethics committee. Parts of the study were carried out under the PA-COVID study approval (clinicaltrials.gov: NCT04747366). The remaining patients were examined with the requirement for written informed consent being waived due to the retrospective study design (EA1/042/22).

Cardiovascular Magnetic Resonance Imaging Protocol

All patients underwent a CMR examination on a 1.5-T scanner (AvantoFit®, Siemens, Erlangen, Germany) with ECG gating and a 32-channel surface phased-array coil. For the biventricular function assessment, balanced steady-state free precession cine images were acquired in four long-axis views including a four-, two-, three-chamber view as well as a RV view and one short-axis (SAX) stack, covering the entire ventricle without a gap. Parametric T2 and T1 mapping was acquired in multiple SAX slices covering the entire ventricle. T2-mapping acquisition was based on a motion-corrected balanced steady-state free precession sequence. In addition, T2-weighted imaging with a STIR (short T1 inversion recovery) sequence was carried out. Native T1 mapping was based on a motion-corrected modified Look-Locker inversion recovery technique using a 5–3–3 scheme. Synthetic ECV was calculated from T1-mapping pre- and post-contrast media application based on a prototype sequence in basal and midventricular slices. LGE imaging was acquired by a phase-sensitive inversion recovery sequence, 10–15 min after the application of 0.2mmol/kg of contrast media (gadoteridol, Prohance®, Bracco Imaging, Konstanz, Germany). LGE images were acquired in four-, two-, and three-chamber views as well as one SAX stack. **Supplementary Material E1** shows a graphical representation of the full coverage approach for mapping and LGE acquisitions. Details about the sequence parameters are given in **Supplementary Material E2**.

Cardiovascular Magnetic Resonance Image Analysis

Two readers [one with 6 years of experience in CMR (YB) and one with 2 years of experience (JG)] performed image analysis by using CVI42® (version 5.13.0, Circle Cardiovascular Imaging, Calgary, Canada). The biventricular function assessment was executed on cine SAX images according to current recommendations (22). For the LV function assessment, papillary muscles were attributed to the total myocardial mass in diastole and systole. Left atrial function was assessed in cine four- and two-chamber views with a biplanar approach. Myocardial deformation assessment by feature tracking (FT) was carried out as published recently (23). STIR images were visually analyzed for myocardial edema. Quantitative mapping analysis was carried out with endo- and epicardial border delineation in each slice to obtain both global and segmental values, according to the 17-segment American Heart Association model, omitting the apical cap. Slice locations were allocated in the respective segment and level by delineating the extent of the LV. Slices with visible LV-outflow tract were excluded. Similarly, apical slices with no blood pool or thin myocardial walls were excluded. Institutional reference values for parametric mapping are as follows: native T1 (in ms) $>$ 1018 (range 1018–1051), T2 (in ms) 52 (range 52–54), and ECV (in %) $>$ 24 (24–30). Based on these cutoffs, the mean values and segmental values were categorized as normal or abnormal to assess differences in rates of abnormal mean and affected segments. A qualitative survey ensured to exclude segments with artifacts as well as focal fibrosis detected by LGE in order to properly assess diffuse fibrosis without confounding by focal replacement fibrosis. Focal scars were assessed visually by LGE analysis by both readers independently regarding the presence and location of scars. In case of uncertainties, a consensus read was performed. For LGE quantification, a semi-automated signal threshold versus reference mean method was chosen as previously described (24). Given the high frequency of non-ischemic scar burden, a 5-standard-deviation approach was applied (9, 25).

Statistical Analysis

Continuous variables are expressed as mean and interquartile range. Categorical variables are given as absolute frequencies and percentages. Normal distribution was assessed by the Shapiro–Wilk test. Continuous variables were compared using either the Kruskal–Wallis method or one-way ANOVA. The correlation was based on the Spearman's correlation coefficient given non-normal distribution. Categorical variables were compared using chi-square or Fisher's exact test. A mixed model was used to assess differences regarding the rates of affected segments between the groups. In case of a significant global test, pairwise comparisons were performed. As all analyses were regarded exploratory, a significance level of 5% was regarded as a strong trend and was followed up by pairwise comparison with appropriate tests dispending adjustments for multiple comparisons. Intra- and interobserver agreement was assessed by Bland–Altman analysis based on 10 randomly chosen cases by JG and a third reader (MF; 4 years of experience in CMR), respectively. A p-value $<$ 0.05 was considered statistically significant. Statistical calculations were performed using SPSS Statistics (version 27.0.0, IBM, Armonk, NY, United States) and SAS (version 9.4, SAS Institute Inc., Cary, NC, United States).

RESULTS

Patient Characteristics

A total of 162 patients were recruited of whom 141 could be included for analysis (median age [interquartile range (IQR)],

45 (37–56); 83 women; 34/141 sCov, 43 (37–52); 19 women; 63/141 pCov, 52 (39–58); 43 women; 44/141 CovVac, 43 (32–56), 23 men; and 44 HC, 41 (28–52); 24 women; **Table 1**). Based on the group allocation on symptom duration, the time between infection and CMR was longer in the pCov group in comparison with the sCov group (median and interquartile range: pCov 180 (124–253) days vs. sCov 61 (50–76) days ($p = < 0.001$). This was similarily observable for the pCov and CovVac groups (pCov vs. CovVac 88 (60–107) days ($p = < 0.001$)). There was a difference regarding age between sCov and HC ($p = 0.03$) as well as pCov and HC ($p = 0.01$). In comparison with the HC, the three patient cohorts showed higher weight (sCov vs HC $p = 0.047$; pCov vs HC $p = 0.03$; CovVac vs HC $p = 0.003$) and body mass index (sCov vs HC $p = 0.02$; pCov vs HC $p = < 0.001$; CovVac vs HC $p = 0.001$). Comorbidities were equally distributed among the patient groups showing no differences, with arterial hypertension being the most common. In comparison with the CovVac group, patients after COVID-19 infection presented more often with ongoing fatigue (sCov 19/34 patients (56%) vs. CovVac 14/44 patients (32%; $p = 0.03$); pCov 38/63 patients (60%) vs. CovVac ($p = 0.003$); sCov vs. pCov ($p = 0.07$)) and palpitations (sCov 13/34 patients (38%) vs. CovVac 8/44 patients (18%; $p = 0.047$); pCov 26/63 patients (41%) vs. CovVac ($p = 0.02$); sCov vs. pCov ($p = 0.77$)). Systolic and diastolic blood pressure measurements during the scan revealed no differences. Higher heart rates were detected for pCov and CovVac patients in comparison with HC (pCov 74 (67–80) vs. HC 69 (61–75); $p = 0.001$); CovVac 74 (66–83) vs. HC ($p = 0.02$)). Dyspnea was observed more often in the pCov group compared with the CovVac patients (pCov 43/63 patients (68%) vs. CovVac 16/44 patients (36%; $p = 0.001$); sCov 17/34 patients (50%) vs. CovVac ($p = 0.23$); pCov vs. sCov ($p = 0.77$)). In total, 25 laboratory results for NT-pro-BNP and high-sensitive (hs) troponin-T were available: 8 in the sCov group (mean NT-pro-BNP in ng/L (IQR) 91 (32–103), mean hs troponin-T in ng/L (IQR) 6 (3–10)); 11 in the pCov group (NT-pro-BNP 66 (37–90), hs troponin-T 6 (3–6)); and 6 in the CovVac group (NT-pro-BNP 15 (4–22), hs troponin-T 4 (3–5)). There were no differences between hs troponin-T values but significant differences between sCov and CovVac ($p = 0.01$) as well as pCov and CovVac ($p = < 0.001$) regarding NT-pro-BNP levels. Patient characteristics are given in **Table 1**. In total, 14 patients were excluded from the COVID-19 infection group and seven from the CovVac group (see flowchart in **Figure 2**). No patient required hospitalization for ongoing symptoms. In the infection groups, 3/97 (3%) required hospitalization and one patient had to be admitted to the intensive care unit during the acute phase. None of the patients from the CovVac group required hospitalization. In the CovVac group, 40/44 patients (91%) received a messenger RNA-based vaccine and 4/44 (9%) received a vector-based vaccine. Of the 40 patients receiving an mRNA vaccine, 37/44 (84%) received BNT162b2 mRNA vaccine (Pfizer-BioNTech) and 3/44 (7%) received mRNA-1273 vaccine (Moderna). The majority of patients (37/44; 84%) presented after the first vaccination dose and 7/44 patients (16%) after the second dose.

Cardiovascular Magnetic Resonance Results

The biventricular function was within normal ranges for the entire studied population with no differences in LV ejection fraction (EF; sCov 61.6% (56.8–65.6); pCov 62.6% (59.2–65.7); CovVac 61.7% (56.7–63.9); HC 62.3% (58–66.2; $p = 0.46$)) and RV-EF (sCov 53.8% (50.6–56.7); pCov 53.6% (48.3–57.6); CovVac 52.1% (47.3–55.7); HC 52.9% (50.1–58.9; $p = 0.43$)). Global radial and circumferential strains were lower in the patient cohorts in comparison with the HC (see **Table 2**), but after exclusion of patients with focal scars on LGE, no differences between the groups were detectable for global radial strain (sCov 25.9% (24.1–30.7); pCov 26.1% (23.7–29.3); CovVac 26.2% (22.3–28.7); HC 29.1% (26–30.3; $p = 0.07$)) and global circumferential strain (sCov –16.7% (−18.7 – (−16)); pCov −16.8%(−18.1 – (−15.8)); CovVac −16.8% (−17.7 – (−15)); HC −18% (−18.5 – (−16.7); $p = 0.07$)). Global longitudinal strain values did not show significant differences between the groups (sCov −18.6 (−20.4 – (−16.4)); pCov −18 (−19.3 – (−16.4)); CovVac −17.5 (−19.5 – (−15.4)); HC −18 (−19.1 – (−16.9); $p = 0.52$)). T2-weighted imaging revealed no myocardial edema. Pericardial effusions were detected in 50 patients (sCov 15/34 (44%); pCov 25/63 (40%); CovVac 10/44 (23%; $p = 0.09$)). None of them were hemodynamically relevant.

Global native T1 mapping did not differ between HC, sCov, and CovVac, whereas pCov patients showed higher global values in comparison with HC (pCov 1002 ms (981–1023) vs. HC 987 ms (963–1009; $p = 0.005$); **Table 3**). Basal native T1 values were higher in the pCov and CovVac groups in comparison with HC (pCov 1008 ms (990–1022) vs HC 993 ms (972–1014; $p = 0.005$); CovVac 1006 ms (975–1032) vs HC ($p = 0.02$)). Admittedly, no patients presented with signs of active inflammation, but differences were found between pCov patients and the HC for global T2 times (pCov 48.8 ms (47.9–49.8) vs. HC 50.4 ms (48.5–51.2; $p = 0.001$)), basal (pCov 48.2 ms (47.1–49.3) vs. HC 50.1 ms (47.6–50.8; $p = 0.01$)), and midventricular T2 slices (pCov 48.7 ms (47.8–49.6) vs. HC 50.2 ms (48.3–51.2; $p = 0.001$)). ECV showed no differences between the patient groups. **Figure 3** visually represents the mapping findings. Based on the reference values given in methods section, we did not find a statistical difference for the rates of T1 involvement between the groups (patients with T1 above cutoff/total patients in the group (%): sCov 10/34 (29%); pCov 18/63 (29%); CovVac 15/44 (34%); and HC 5/44 (11%); $p = 0.07$). No statistically significant differences were found between the patient groups regarding ECV (patients with ECV above cutoff/total patients in the group (%): sCov 10/34 (29%); pCov 18/63 (29%); and CovVac 13/44 (30%) $p = 0.99$). Regarding rates of affected segments for T1, we found statistically significant differences between the groups for all segments ($p = 0.02$), basal ($p = 0.04$), and midventricular segments ($p = 0.03$). In a pairwise comparison, the differences were between sCov and HC for midventricular segments (rate difference of affected segments 0.105; $p = 0.04$), between pCov and HC for basal segments (rate difference of affected segments 0.09; $p = 0.045$), and between CovVac and HC for

TABLE 1 | Summary of patient characteristics.

Parameter	All patients after COVID-19 infection (*N* = 97)	Subacute COVID-19 (*N* = 34)	Post-COVID-19 (*N* = 63)	COVID-19 vaccination (*N* = 44)	Healthy controls (*N* = 44)	*p* value*	Pairings with significant differences
Gender (F/M)	62/35	19/15	43/20	21/23	24/20	0.18	n.a.
Age (years)	48 (38–56)	43 (37–52)	52 (39–58)	43 (32–56)	41 (28–52)	0.02	**sCov vs. HC; pCov vs. HC**
Height (cm)	171 (164–180)	173 (166–181)	170 (163–180)	175 (167–182)	173 (168–180)	0.34	n.a
Weight (kg)	77 (65–86)	75 (67–85)	77 (64–86)	82 (65–97)	70 (63–78)	**0.02**	**sCov vs. HC; pCov vs. HC; CovVac vs. HC**
Body mass index (kg/m^2)	25.3 (22.9–28.7)	24.9 (22.8–27.5)	25.5 (23.5–29.3)	25.8 (22.7–30.2)	22.9 (21–25.2)	**0.001**	**sCov vs. HC; pCov vs. HC; CovVac vs. HC**
Event to CMR (days)	141 (80–231)	61 (50–76)	180 (124–253)	88 (60–107)	n.a.	**<0.001**	**sCov vs. pCov; pCov vs. CovVac**
Heart rate (beats per minute)	74 (66–80)	72 (64–81)	74 (67–80)	74 (66–83)	69 (61–75)	**0.035**	**pCov vs. HC; CovVac vs. HC**
Systolic blood pressure (mmHg)	126 (115–132)	125 (118–130)	126 (115–134)	129 (117–137)	119 (113–135)	0.38	n.a.
Diastolic blood pressure (mmHg)	75 (70–84)	81 (72–89)	72 (70–83)	73 (68–80)	72 (67–77)	0.13	n.a.
Symptoms							
Fatigue	57 (58%)	19 (56%)	38 (60%)	14 (32%)	n.a.	**0.01**	**sCov vs. CovVac; pCov vs. CovVac**
Dyspnea	59 (60%)	17 (50%)	43 (68%)	16 (36%)	n.a.	**0.004**	**pCov vs. HC**
Chest pain	33 (34%)	13 (38%)	24 (38%)	21 (48%)	n.a.	0.43	n.a
Palpitations	36 (37%)	13 (38%)	26 (41%)	8 (18%)	n.a.	**0.04**	**sCov vs. CovVac; pCov vs. CovVac**
Comorbidities							
Arterial hypertension	27 (28%)	7 (21%)	20 (32%)	15 (34%)	n.a.	0.34	n.a.
Diabetes mellitus	4 (4%)	1 (3%)	3 (5%)	3 (7%)	n.a.	0.79	n.a.
Hyperlipidemia	8 (8%)	3 (9%)	5 (8%)	4 (9%)	n.a.	0.99	n.a.
Congestive heart failure	1 (1%)	1 (3%)	0 (0%)	2 (5%)	n.a.	0.17	n.a.
Coronary artery disease	3 (3%)	2 (6%)	1 (2%)	0 (0%)	n.a.	0.24	n.a.
Mild/moderate systemic disease	5 (5%)	1 (3%)	4 (6%)	1 (2%)	n.a.	0.66	n.a.
Chronic lung disease	4 (4%)	2 (6%)	2 (3%)	1 (2%)	n.a.	0.72	n.a.
Valvular heart disease	2 (2%)	1 (3%)	1 (2%)	3 (7%)	n.a.	0.44	n.a.
Chronic kidney disease	0 (0%)	0 (0%)	0 (0%)	1 (2%)	n.a.	0.55	n.a.

Data are median and interquartile ranges for continuous and number with percentages in brackets for continuous variables. $p < 0.05$ is considered to indicate a statistically significant difference.
COVID-19, coronavirus disease 2019; sCov, subacute COVID-19; pCov, post-COVID-19; CovVac, COVID-19 vaccination; HC, healthy controls.
**p-values given for tests between subacute COVID-19, post-COVID-19, COVID-19 vaccination, and healthy controls.*
Bold text represents statistically significant differences.
n.a., not applicable.

all T1 segments (rate difference of affected segments 0.142; $p = 0.002$), basal (rate difference of affected segments 0.144; $p = 0.004$), and midventricular segments (rate difference of affected segments 0.136; $p = 0.005$). We separately compared 14 older HC controls (age 54 years (49–63) to 14 age-, gender-, weight-, and height-matched pCov patients (age 56 years (49–64). No statistically significant differences were found for T1 times (pCov median 1014ms (982–1037); older HC median 994ms (977–1010); $p = 0.09$) and T2 times (pCov median 48.8 ms (48.1–50.7); and older HC median 50.8ms (50.1–51.2); $p = 0.1$). Details about the number of slices analyzed and segments excluded for parametric assessment are given in **Supplementary Material (E3)**. Visual Bland–Altman revealed good intra- and interobserver agreement for functional and parametric assessment (**Supplementary Material E4**).

Visual LGE analysis revealed focal scars in 49/141 patients (34.6%). There was no statistically significant difference between the groups regarding the rate of patients with LGE findings (sCov 10/34 (29%); pCov 26/63 (41%); CovVac 13/44 (30%; $p = 0.34$)). A non-ischemic pattern dominated in the entire study with 43/49 (88%) being either subepicardial, intramyocardial, or RV insertion point fibrosis (non-ischemic scars/total scars: sCov 9/10 (90%); pCov 22/26 (85%); CovVac 12/13 (92%). For sCov (7/10 (70%)) and pCov (13/26 (50%), a subepicardial

TABLE 2 | Cardiac function parameters derived from cardiovascular magnetic resonance (CMR).

Parameter	All patients after COVID-19 infection (*N* = 97)	Subacute COVID-19 (*N* = 34)	Post-COVID-19 (*N* = 63)	COVID-19 vaccination (*N* = 44)	Healthy controls (*N* = 44)	*p* value*	Pairings with significant differences
LV-EDV (ml)	141.8 (121.6–168.2)	143.6 (124.9–172.8)	137.6 (118.8–167.2)	162.1 (126.6–193.2)	138.6 (119.7–162.8)	0.16	n.a.
LV-ESV (ml)	53.6 (43.4–66.8)	54 (45–75.1)	51.9 (42.3–62.5)	59 (47.8–74.1)	52.2 (43.7–64.8)	0.15	n.a.
LV-SV (ml)	87.7 (75.1–103.1)	88.2 (73.9–103.9)	86.6 (75.1–104.3)	97.5 (79.3–110.6)	84.6 (74.4–100.8)	0.64	n.a.
LV-EF (%)	62.3 (58.5–65.6)	61.6 (56.8–65.6)	62.6 (59.2–65.7)	61.7 (56.7–63.9)	62.3 (58–66.2)	0.46	n.a.
LVM (g)	80.8 (66.5–103.5)	81.1 (68.5–108)	80.8 (65.7–102.8)	97.7 (74.6–115.6)	82.3 (69.4–99.8)	0.09	n.a.
RV-EDV (ml)	151.5 (132.4–183.5)	154.6 (134.4–193.6)	151.3 (129.8–178.9)	174.4 (132–204.7)	160.4 (138.9–182.9)	0.58	n.a.
RV-SV (ml)	82.3 (71.4–95.5)	83.2 (72–103.4)	78.8 (71.3–95.1)	92.1 (72.2–102.4)	83.4 (73.4–99.2)	0.53	n.a.
RV-EF (%)	53.6 (49.6–57.1)	53.8 (50.6–56.7)	53.6 (48.3–57.6)	52.1 (47.3–55.7)	52.9 (50.1–58.9)	0.43	n.a.
LA (cm^2)	20 (17.4–22.3)	20 (16.7–22.4)	20 (17.5–22.6)	20.7 (18.6–23.2)	20.9 (18.7–22)	0.71	n.a.
LA-EF (%)	65.1 (59–70.4)	68.6 (58.1–72.9)	63.6 (59.1–67.8)	63.9 (60.4–71.6)	61.7 (58.3–69.2)	0.06	n.a.
LA-EDV (ml)	60 (49.5–72.7)	58.8 (48.9–71.6)	60.1 (49–73.7)	64.8 (53.4–75.1)	61.9 (51.6–68.9)	0.53	n.a.
LA-SV (ml)	38.3 (31.2–47.9)	38.7 (31–50.4)	37.5 (30.9–47.8)	42.9 (33.5–50)	39.4 (31.6–43)	0.32	n.a.
GLS (%)	−18.3 (−19.8–(−16.4))	−18.6 (−20.4–(−16.4))	−18 (−19.3–(−16.4))	−17.5 (−19.5–(−15.4))	−18 (−19.1–(−16.9))	0.52	n.a.
GRS (%)	25.7 (23–28.7)	25.9 (22.5–29.4)	25.7 (23.1–28.5)	25.7 (22–28.8)	29.1 (26–30.3)	**0.004**	**sCov vs. HC; pCov vs. HC; CovVac vs. HC**
GCS (%)	−16.7 (−17.9–(−15.4))	−16.7 (−18–(−15.2))	−16.7 (−17.8–(−15.5))	−16.7 (−17.6–(−15))	−18 (−18.5–(−16.7))	**0.005**	**sCov vs. HC; pCov vs. HC; CovVac vs. HC**
GRS (%) without LGE + patients	26.1 (24–29.4) (*N* = 50)	25.9 (24.1–30.7) (*N* = 23)	26.1 (23.7–29.3) (*N* = 37)	26.2 (22.3–28.7) (*N* = 28)	29.1 (26–30.3) (*N* = 44)	0.07	n.a.
GCS (%) without LGE + patients	−16.8 (−18.1–(−16)) (*N* = 50)	−16.7 (−18.7–(−16) (*N* = 23)	−16.8 (−18.1–(−15.8)) (*N* = 37)	−16.8 (−17.7–(−15)) (*N* = 28)	−18 (−18.5–(−16.7)) (*N* = 44)	0.07	n.a.

Data are median and interquartile ranges. $p < 0.05$ is considered to indicate a statistically significant difference.
COVID-19, coronavirus disease 2019; LV-EDV, left ventricular end-diastolic volume; LV-ESV, left ventricular end-systolic volume; LV-SV, left ventricular stroke volume; LV-EF, left ventricular ejection fraction; LVM, left ventricular mass; RV-EDV, right ventricular end-diastolic volume; RV-ESV, right ventricular end-systolic volume; RV-SV, right ventricular stroke volume; RV-EF, right ventricular ejection fraction; LA, left atrium; LA-EF, left atrial ejection fraction; LA-EDV, left atrial end-diastolic volume; LV-SV, left atrial stroke volume; GLS, global longitudinal strain; GRS, global radial strain; GCS, global circumferential strain; sCov, subacute COVID-19; pCov, post-COVID-19; CovVac, COVID-19 vaccination; HC, healthy controls.
**p-values given for tests between subacute COVID-19, post-COVID-19, COVID-19 vaccination, and healthy controls.*
Bold text represents statistically significant differences.
n.a., not applicable.

pattern was most commonly encountered, whereas CovVac patients most often displayed an intramyocardial pattern (7/13 (54%)). In comparison with the CovVac group, patients after COVID-19 infection had more focal subepicardial findings (subepicardial fibrosis/patients per group: sCov 7/34 (21%) vs. CovVac 2/44 (5%; $p = 0.04$); pCov 13/63 (21%) vs. CovVac ($p = < 0.001$)); however, no differences were found between the subgroups after an infection ($p = 0.99$; **Figure 4**; details in **Supplementary Material E5**). In the sCov group, 6/7 (86%) of subepicardial scars were located in the basal segments with one in the anterolateral wall (1/7; 14%), four in the inferolateral wall (4/7; 57%), and one in the lateral wall (1/7; 14%). One subepicardial scar was found in the medial-lateral wall (1/7; 14%). The intramyocardial scars were in the middle ventricular section with one being in the septal and one in the lateral wall. For the pCov groups, all LGE findings were located in the basal part. Of the 13 subepicardial scars, six were found in the lateral segments (6/13; 46%), five in the

TABLE 3 | Parametric mapping quantification derived by CMR.

Parameter	Subacute COVID-19 (*N* = 34)	Post-COVID-19 (*N* = 63)	COVID-19 vaccination (*N* = 44)	Healthy controls (*N* = 44)	*p* value*	Pairings with significant differences
T1 global (ms)	1001 (977–1029)	1002 (981–1023)	999 (968–1030)	987 (963–1009)	**0.046**	**pCov vs. HC**
T1 basal (ms)	1003 (980–1030)	1008 (990–1022)	1006 (975–1032)	993 (972–1014)	**0.04**	**pCov vs. HC; CovVac vs. HC**
T1 mid (ms)	1001 (976–1025)	999 (982–1027)	995 (973–1029)	987 (966–1010)	0.10	n.a.
T1 apical (ms)	987 (957–1034)	996 (969–1027)	992 (951–1038)	985 (962–1009)	0.66	n.a.
T2 global (ms)	48.7 (47–51.2)	48.8 (47.9–49.8)	49.2 (47.8–50.3)	50.4 (48.5–51.2)	**0.03**	**pCov vs. HC**
T2 basal (ms)	48.5 (46.6–50.3)	48.2 (47.1–49.3)	49.1 (47.5–50.3)	50.1 (47.6–50.8)	**0.03**	**pCov vs. HC**
T2 mid (ms)	48.8 (47–51)	48.7 (47.8–49.6)	49 (47.6–51)	50.2 (48.3–51.2)	**0.04**	**pCov vs. HC**
T2 apical (ms)	49.7 (47.2–52.2)	50 (48.4–51.1)	50.3 (48.3–52.7)	51.1 (48.5–52.1)	0.33	n.a.
ECV global (%)	23.2 (20.8–24.4)	23.1 (21.8–24.7)	22.5 (20.9–24.5)	n.a.	0.54	n.a.
ECV basal (%)	22.6 (20.8–24.4)	23 (21.5–24.3)	22.6 (20.6–24.3)	n.a.	0.47	n.a.
ECV mid (%)	22.9 (20.6–24.4)	23.4 (21.9–24.8)	22.8 (20.9–24.8)	n.a.	0.39	n.a.

Data are median and interquartile ranges. $p < 0.05$ is considered to indicate a statistically significant difference.
COVID-19, coronavirus disease 2019; ECV, extracellular volume; sCov, subacute COVID-19; pCov, post-COVID-19; CovVac, COVID-19 vaccination; HC, healthy controls.
**p-values given for tests between subacute COVID-19, post-COVID-19, COVID-19 vaccination, and healthy controls.*
Bold text represents statistically significant differences.
n.a., not applicable.

inferolateral segments (5/13; 39%), and two in the inferior segments (2/13; 15%). Similarly, the intramyocardial scars were in a majority of cases in the lateral wall (2/3; 66%) with one in the inferolateral wall (1/3; 33%). The CovVac group had in total more intramyocardial scars with three in the inferior basal segment (3/7; 43%), three in the inferolateral basal segment (3/7; 43%), and one in the lateral basal segment (1/7; 14%). The two subepicardial findings were equally distributed in the basal part with one each in the lateral (1/2; 50%) and inferolateral segments (1/2; 50%). Statistically, there were no differences regarding the lateral ($p = 0.34$), inferolateral ($p = 0.81$), and inferior ($p = 0.16$) segments regarding the expected frequency of distribution between sCov, pCov, and CovVac groups. Of the six patients with ischemic scars, only one had a previous medical history of coronary artery disease. The majority of ischemic LGE lesions were found in the pCov group (4/6; 67%). In the sCov group, one patient (1/34; 3%) had a lateral subendocardial scar covering the basal to early apical segments. Of the four patients with ischemic scar burden in the pCov group (4/63; 6%), two had an anterior basal location, one had an inferior lateral pattern in the basal part, and the remaining patient had a small but visible scar in the apical region. One patient from the CovVac group had a lateral subendocardial scar in the basal segments (1/44; 2%). LGE quantification showed no difference between the groups, neither for total enhanced mass ($p = 0.95$) nor for enhanced percentage ($p = 0.52$; **Table 4**).

No correlation between overall symptom load, defined as the sum of the symptoms (fatigue, dyspnea, chest pain, and palpitations), and markers of myocardial involvement, especially the presence of LGE (r (Spearman's correlation coefficient) $= 0.07$), mean native T1 ($r = 0.03$), mean T2 ($r = -0.17$), and mean ECV ($r = 0.13$), was found. Similarly, no statistical differences were found between patients with no symptoms and patients with at least one symptom considering the entire patient cohort (mean native T1 $p = 0.56$; mean T2 $p = 0.11$; mean ECV $p = 0.27$).

DISCUSSION

The ongoing COVID-19 pandemic remains to be a burden for healthcare systems around the globe with symptoms persisting for more than half a year after an acute infection in some patients (11). In this retrospective analysis, we identified a higher focal fibrotic burden in patients with persistent symptoms after COVID-19 infection in comparison with patients after COVID-19 vaccination.

CMR analysis revealed normal biventricular function and no active myocardial inflammation. Global T2 times were lower in the pCov group compared with the HC. Regarding this finding, we can only speculate about its implication. Potential discrepancies in oxygen delivery to the myocardial tissue or a complex interaction between fibrosis and myocardial inflammation might be involved (26). Another explanation for the lower T2 times together with the higher native T1 times in pCov in comparison with HC could be the higher age in the pCov cohort. Previous studies on T1 values have reported an increase of around 12–15 ms per decade (27). In our subgroup comparison between the pCov and the older HC, we found no differences for T1 and T2 underlining these results. However, the subgroup only entails 14 cases of both groups limiting the generalizability of this non-significant finding. In addition, comparing the absolute values of T1 in the older subgroup (994 ms (range 977–1010) to the entire HC (987 ms (963–1009), the absolute differences are marginal. Next to age, other potential confounders could include the difference in weight and BMI between the groups as recent studies found significant associations between T1 times and weight (28). The overall small differences for T1 and T2 times are well within the limits of the intra- and interobserver

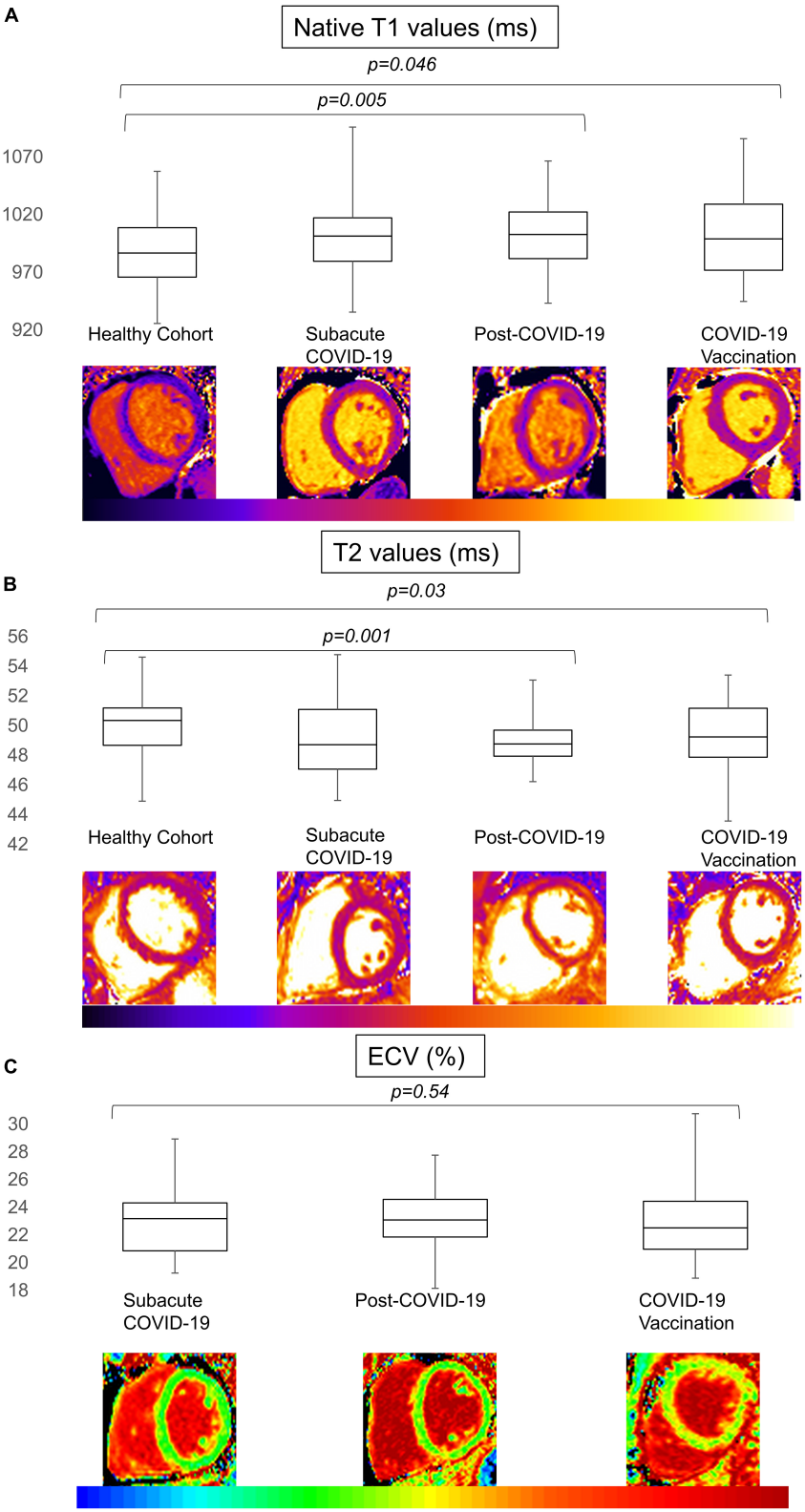

FIGURE 3 | T1, T2, and extracellular volume (ECV) values for the patient cohort and healthy controls. Boxplot representation of the mapping values for T1 in ms **(A)**, T2 in ms **(B)**, and ECV in% **(C)** for patients after COVID-19 infection (subacute and post-COVID-19), after COVID-19 vaccination, and healthy controls (from left to right in each panel). Whiskers represent minimal and maximal values with boxes representing 25th percentile, median, and 75th percentile (from bottom to top). Significant values for general tests were followed by subgroup comparison. A p-value of < 0.05 was regarded as statistically significant.

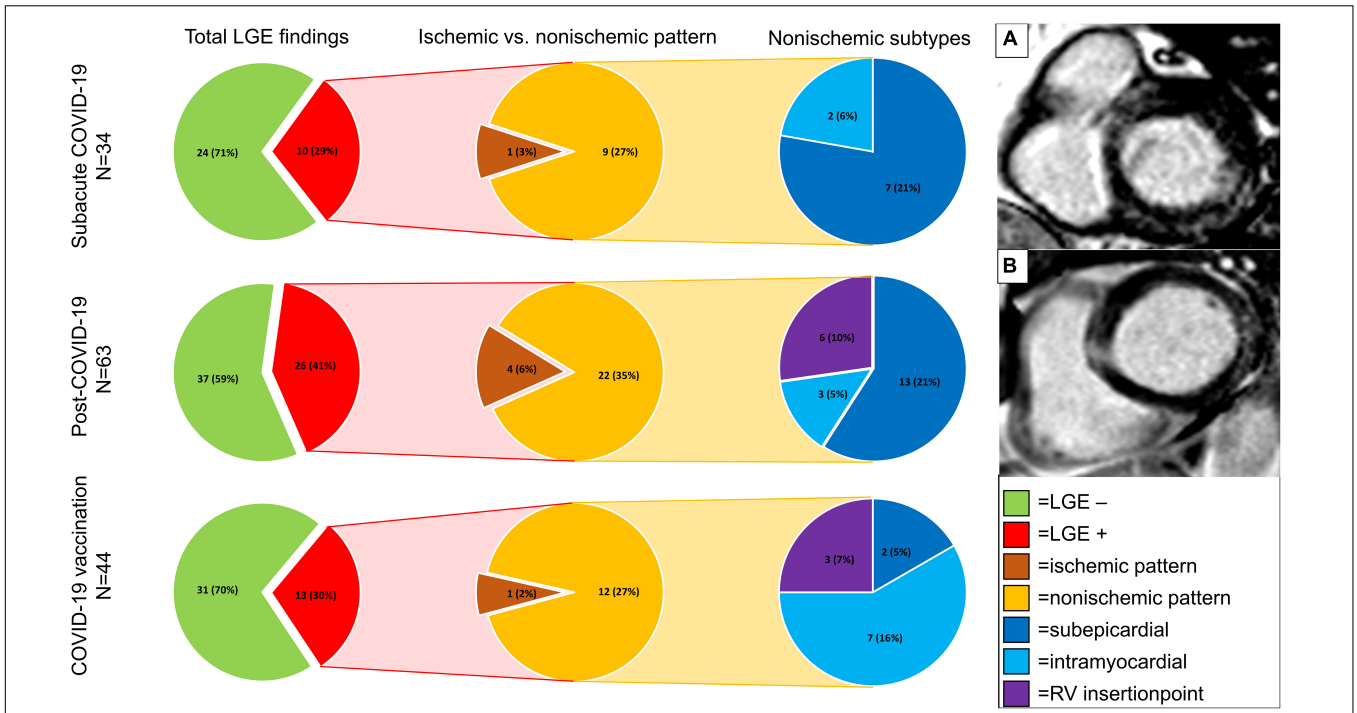

FIGURE 4 | Focal fibrosis detected by late gadolinium enhancement imaging in the patient cohorts. Presented are total and percentages of findings (findings/cohort size) in pie charts. Different subtypes of late gadolinium enhancement (LGE) patterns are indicated by colors with a legend on the lower right side (lime green = no LGE; red = LGE positive; brown = ischemic pattern; orange = non-ischemic pattern; dark blue = subepicardial LGE; light blue = intramyocardial LGE; purple = RV insertion point). Significant differences were found between subepicardial LGE findings in the subacute COVID-19 group and the COVID-19 vaccination group ($p = 0.04$) and between the post-COVID-19 group and the COVID-19 vaccination group ($p = < 0.001$). No differences were found between the infection subgroups ($p = 0.99$) for subepicardial LGE. Other pairings revealed no differences. **(A)** Subepicardial scar in the basal inferolateral part. **(B)** Subepicardial scar in the basal lateral part.

TABLE 4 | Quantitative late gadolinium enhancement (LGE) findings.

Parameter	Total ($N = 48$)	All patients after COVID-19 infection ($N = 35$)	Subacute COVID-19 ($N = 10$)	Post-COVID-19 ($N = 25$)	COVID-19 vaccination ($N = 13$)	*p* value*	Pairings with significant differences
Total enhanced volume (ml)	1.4 (0.5–2.4)	1.4 (0.5–2.1)	0.9 (0.5–2.7)	1.7 (0.6–2)	1 (0.3–2.6)	0.94	n.a.
Total enhanced mass (g)	1.5 (0.5–2.5)	1.5 (0.6–2.2)	1 (0.5–2.8)	1.6 (0.6–2.1)	1.1 (0.3–2.7)	0.95	n.a.
Enhanced volume (%)	2 (0.9–4.1)	2.1 (1–4.1)	1.7 (0.9–5.9)	2.3 (1.3–4.1)	1.2 (0.4–4.5)	0.52	n.a.

Data are given as median and interquartile range. $p < 0.05$ is considered to indicate a statistically significant difference.
COVID-19, coronavirus disease 2019; sCov, subacute COVID-19; pCov, post-COVID-19; CovVac, COVID-19 vaccination; HC, healthy controls.
**p-values given for tests between subacute COVID-19, post-COVID-19, and COVID-19 vaccination.*
n.a., not applicable.

limits of agreement (**Supplementary Material E4**). Therefore, these findings require further investigation in follow-up studies as well as multicenter studies to understand their full clinical impact. One other potential explanation might be that segments without chronic replacement fibrosis are undergoing long-lasting more subtle and diffuse changes that evolve over months. Several studies reported dynamics of T1 relaxation times over a time course of 6 months after an acute viral myocarditis (15, 29). It was shown that for viral myocarditis, T2 times might be elevated even up to 5 weeks after the acute event, but return to normal within 6 months, with T1 times behaving similarly with the exception that they might be elevated beyond the 6-month time frame (15). It is not clear yet whether the pathophysiologic and myocardial injury pattern after a COVID-19 infection differs from a classic viral myocarditis or whether the course is comparable. The current evidence is conflicting with one study reporting reduced T1 and elevated T2 times at follow-up examinations 68 days after the baseline scan (29). This contrasts with others, who reported no signs of active myocardial inflammation in patients with persistent symptoms (4). The latter findings are in line with ours as we also did not find evidence for an acute inflammatory process at the time of the CMR examination. The large Hamburg City Health Study COVID program reported findings in patients 9 months after the first positive test, comparing this group to healthy matched controls (30). They did not find any differences between

patients and the healthy controls for T1 and T2 times. LGE findings were more predominant in the group after an infection but did not reach statistical significance (30). Similar to the conflicting evidence regarding T1 and T2, ECV findings also differ substantially. One group described persistently elevated ECV values (9), whereas Filipetti et al. showed that during follow-up, ECV as well as T1 times significantly decreased (31). Both studies analyzed patients after hospital admissions. We observed no difference in the sCov and pCov groups. Depending on the severity of the initial symptoms and the requirement for hospitalization, there might be either an improper immune response with persistent inflammation (2, 29) or a more subtle and diffuse process (9) that drives the changes after COVID-19. We did not, however, find a correlation between symptom load and myocardial tissue changes visualized by CMR for any patient group. This finding is supported by other studies which also did not find any correlation between reported symptoms and tissue changes (32, 33).

For the basal part, we found higher native T1 values for pCov and CovVac in comparison with the HC. These findings could potentially indicate a diffuse focal interstitial process. This is underlined by finding a higher rate of segmental involvement in all groups in comparison with healthy volunteers. Interestingly, in the CovVac cohort not only basal and midventricular slices were more often focally affected, but also the overall segmental affection rate was higher. In studies including patients after COVID-19 vaccination, findings were similar to a viral myocarditis but less pronounced (19, 20). One group reported a normal LV-EF, elevated T1 times in 46%, and LGE findings in 87% (19). The majority of LGE findings were found in the basal inferolateral region (19). The population in this study was clinically diagnosed and scanned at a median of 65 days (range 3–130) after the second dose. Fronza et al. presented findings for patients after COVID-19 vaccination myocarditis and COVID-19 infection with a mixed patient profile of hospitalized and non-hospitalized patients (20). Patients after the vaccination had higher LV-EF and lower native T1 values. In a short-term follow-up, LV-EF was further improving and no clinical adverse events were observed (20). In contrast to the above-mentioned studies, our population was scanned at a median of 88 days (IQR 60–107) after receiving a vaccination, likely reflecting a different stage. This is also shown by normal T2 times and the prevalence of LGE findings in our CovVac cohort with non-ischemic scars in 12/44 patients (27%). In comparison with the groups after COVID-19 infection, CovVac presented with less focal subepicardial scars. The frequency of subepicardial involvement in our study is higher than that of Kravchenko et al. (5%; all patients non-hospitalized) (4) but similar to Puntmann et al. (20%, 67% patients recovered at home) (2) and Kotecha et al. (22%, hospitalized patients) (8). The main segments involved were the inferior and inferolateral ones. This is in accordance with Wang et al. who, despite a more scattered pattern, found in 10 out of 12 patients subepicardial or intramyocardial LGE in these segments (34). It should be noted that these patterns are commonly described in cases with viral myocarditis (15, 35). As mentioned in the editorial by Lim and Bluemke (36), it has yet to be shown how the presence of LGE findings in symptomatic patients after acute COVID-19 infection might influence prognosis or relate to symptom load. Similarly, this holds true for symptomatic patients after COVID-19 vaccination. Strain analysis might potentially help to better understand myocardial dynamics after COVID-19 infection. One study with follow-up CMR performed at 3 months also detected reduced global circumferential strain in patients with LGE findings (34). It has been shown that strain assessment by FT correlates significantly with the ECV burden in patients with non-ischemic cardiomyopathies (37), potentially being a non-contrast dependent tissue marker for myocardial fibrosis. Strain values can also aid in risk stratification with decreased strain values being associated with worse outcomes (38, 39). Similar results have been observed by the application of strain-encoded magnetic resonance (SENC) tagging acquisitions (40). In contrast to strain analysis by FT, SENC relies on the additional acquisition of images. However, recent advances have reduced the necessary time to a single heartbeat with the possibility of free-breathing acquisitions in a technique called fast-SENC (41). Bucius et al. showed that despite a significant difference between FT and fast-SENC for the assessment of global strain values, there is an excellent agreement between these techniques (41). It should be noted that in the same study, FT had the lowest segmental inter-study agreement. Therefore, only global strain values are reported as regional strain values vary depending on number of slices, contouring, and post-processing software as described recently (23). Studies presenting follow-up data are required to further cohesively understand the pathophysiologic changes in the myocardium after acute COVID-19 infection and its sequelae and should base the results on the same standardized image analysis conditions.

Although there are significant differences regarding the NT-pro-BNP levels between the groups after infection in the CovVac group, we want to underline that first, the sample size is small compared with the entire cohort and second, all values are below the laboratory cutoff values/thresholds (NT-pro-BNP < 500 ng/dl and hs troponin-T < 15 ng/dl).

LIMITATIONS

Our study has some limitations. First, there was a selection bias as only patients with symptoms were referred for CMR, omitting asymptomatic patients after COVID-19 infection or vaccination. Second, given the retrospective nature of our study, laboratory tests were available for only a minority of patients. Hence, the analysis of the laboratory tests only covers a subgroup. Similarly, no information was available regarding the medication at the time of the scan. Third, no intraindividual follow-up data can be presented at this time point. Fourth, the age difference between the healthy cohort and the two patient groups after COVID-19 infection could have potentially influenced the mapping results, as shown by the subgroup comparison. Finally, ECV and LGE cannot be provided in the healthy cohort as the application of contrast media was limited due to concerns from the ethical board.

CONCLUSION

In summary, we conclude that all patients had a normal biventricular function, but more diffuse fibrosis was detectable in symptomatic patients after COVID-19 infection with symptom persistence for more than 12 weeks. This mandates further research into pathophysiologic and histopathological changes connected with COVID-19. In comparison with symptomatic patients after COVID-19 vaccination, more focal subepicardial scars were detected in patients after an infection with the COVID-19 virus.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of German data privacy laws. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Charité's Ethics Committee (EA1/042/22). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JG and JS-M were the guarantors of integrity of entire study. JG, MF, and JS-M were involved in the literature research and were involved in the statistical analysis. All authors were involved in the study concepts/study design or data acquisition or data analysis/interpretation, manuscript drafting or manuscript revision for important intellectual content, approval of the final version of submitted manuscript, and manuscript editing and agreed to ensure any questions related to the work are appropriately resolved.

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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.2022.916922/full#supplementary-material

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Cardiac Involvement in COVID-19: A Global Bibliometric and Visualized Analysis

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Objective: Coronavirus disease 2019 (COVID-19), which was caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), had already resulted in widespread epidemics worldwide and millions of people's deaths since its outbreak in 2019. COVID-19 had also been demonstrated to affect people's cardiac function. However, the specific mechanism and influence of this damage were not clear yet. The purpose of the present study was to provide a bibliometric analysis of the current studies related to cardiac involvement after SARS-CoV-2 infection.

Methods: A bibliometric literature search was performed on the web of science. The number and type of publications, countries, institutional sources, journals, and citation patterns were analyzed. In addition, qualitative and quantitative evaluations were carried out to visualize the scientific achievements in this field by using the VOSviewer software.

Results: Web of science had recorded 2,24,097 documents on COVID-19 at the time of data collection (May 12, 2022). A total of 2,025 documents related to cardiac involvement were recorded at last. The countries with the most published articles were the United States of America (USA) (n =747, 36.9%), Italy (n =324, 16%), and England (n =213, 10.5%). Although the countries and institutions that published the most articles were mainly from the USA, the top three authors were from Germany, England, and Poland. Frontiers in Cardiovascular Medicine was the journal with the most studies (65 3.2%), followed by ESC Heart Failure (59 2.9%) and Journal of Clinical Medicine (56 2.8%). We identified 13,739 authors, among which Karin Klingel and Amer Harky had the most articles, and Shaobo Shi was co-cited most often. There existed some cooperation between different authors, but the scope was limited. Myocarditis and heart failure (HF) were the main research hotspots of COVID-19 on cardiac dysfunction and may be crucial to the prognosis of patients.

Conclusions: It was the first bibliometric analysis of publications related to COVID-19-associated cardiac disorder. This study provided academics and researchers with useful information on the most influential articles of COVID-19 and cardiac dysfunction. Cooperation between countries and institutions must be strengthened on myocarditis and HF during COVID-19 pandemic.

Keywords: COVID-19, cardiac, heart, bibliometric, myocarditis, heart failure

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), which leads to a global pandemic, is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Up to now, COVID-19 has caused millions of deaths, which resulted in a catastrophic impact on global systems. COVID-19 mainly attacks the respiratory system. The most common symptoms in the early period are fever, dry cough, and shortness of breath (1). With the increase in the number of infected people, more and more clinical evidence showed that COVID-19 had a serious effect on various systems and multiple organ injuries in severe patients aggravated the difficulty of treatment (2). Previous studies also suggested that COVID-19 patients with cardiovascular disease were often more severely ill and had a higher risk of death, especially in elderly patients (3). In addition, doctors from many countries had reported that compared with the general population, patients with cardiovascular disease had a worse prognosis for SARS-CoV-2 infection. Early literature reported that COVID-19 can directly or indirectly cause a series of cardiovascular damage, ranging from acute myocardial injury, myocarditis, cardiomyopathy, acute coronary syndrome, and myocardial infarction (4). It can be suggested that COVID-19 and cardiovascular diseases affect each other and together lead to acute and malignant adverse events. Therefore, it is particularly vital to pay special attention to the pathological characteristics, clinical manifestations, disease process, and prognosis of cardiac damage caused by COVID-19.

Bibliometrics was first proposed by American bibliographers in 1969. Bibliometrics belongs to the discipline that applies mathematical and statistical methods to the study of books or other communication media (5). A bibliometric study is aimed to introduce this topic uniquely and comprehensively and provide evidence-based practice. Bibliometrics major in related papers of a specific field by statistical analysis and the results can reflect the research status of a specific topic, including main countries, research institutions/organizations, researchers, and the main journals that published related literature. As the pandemic continued in the world, bibliometric assessments on a wide range of issues were published in COVID-19 (6, 7). Although there were many reports on the cardiovascular disorder caused by COVID-19, the previous literature had not systematically described and summarized the effect yet. At the same time, as the epidemic situation had a serious impact on daily communication, it was necessary to summarize the experience due to the different epidemic prevention policies taken by the various countries and regions. Although vaccines are available, we still need 70% to 80% of the population with active immunity through infection or vaccines to cut down the disease chain. Therefore, bibliometric analysis was adopted to guide future research priorities by evaluating the most relevant scientific research on COVID-19 and cardiac dysfunction. This study significantly contribute to the allocation and refinement of future cardiac research caused by COVID-19.

TABLE 1 | Summary of all literature initially included in the study.

Publication type	Counts	%
Article	4,300	60.0
Review	1,351	18.8
Editorial material	429	6.0
Meeting abstract	375	5.2
Letter	360	5.0
Early access	310	4.3
Revised	26	0.4
Book Chapter	11	0.2
News item/Conference papers/Retraction/Data paper	9	0.1

METHODS

Study Design

Bibliometric techniques were used to perform a descriptive cross-sectional analysis of publications relevant to cardiac involvement in COVID-19.

Database Used

SCI-E and SSCI of the core database of the document information index database Web of Science (WOS) were selected for source document retrieval. The search formula was set to TS = ("cardiac" OR "heart" OR "cardiomyocyte") AND TS = ("SARS-CoV-2" OR "COVID-19" OR "2019-nCoV"), and the literature search time of the present study was from the earliest time of publication in the database to the latest time of literature publication (May 12, 2022).

Data Analysis

VOSviewer was used to analyze the exported articles. VOSviewer displayed a map based on the construction of the co-occurrence matrix. The similarity matrix was calculated to refer to the co-occurrence matrix and the map was visualized by a special VOS mapping technique. The term co-occurrence graph in VOSviewer only includes terms that appear in the title and abstract at least 50 times under the binary count (8). The algorithm can make it possible that the terms occur more frequently entitled with larger bubble images and that terms with high similarity are close to each other (9).

RESULTS

General Description of the Retrieved Publications

Web of science has published 2,24,097 documents on COVID-19 in all study fields at the time of data collection [TS=("SARS-CoV-2" OR "COVID-19" OR "2019-nCoV")]. A total of 7,171 documents related to cardiac involvement were primarily retrieved by the corresponding mesh terms. Of these, 4,300 (60%) were research articles, 1,351 (18.8%) were reviews, 429 (6%) were editorial materials, and 375 (5.2%) were meeting

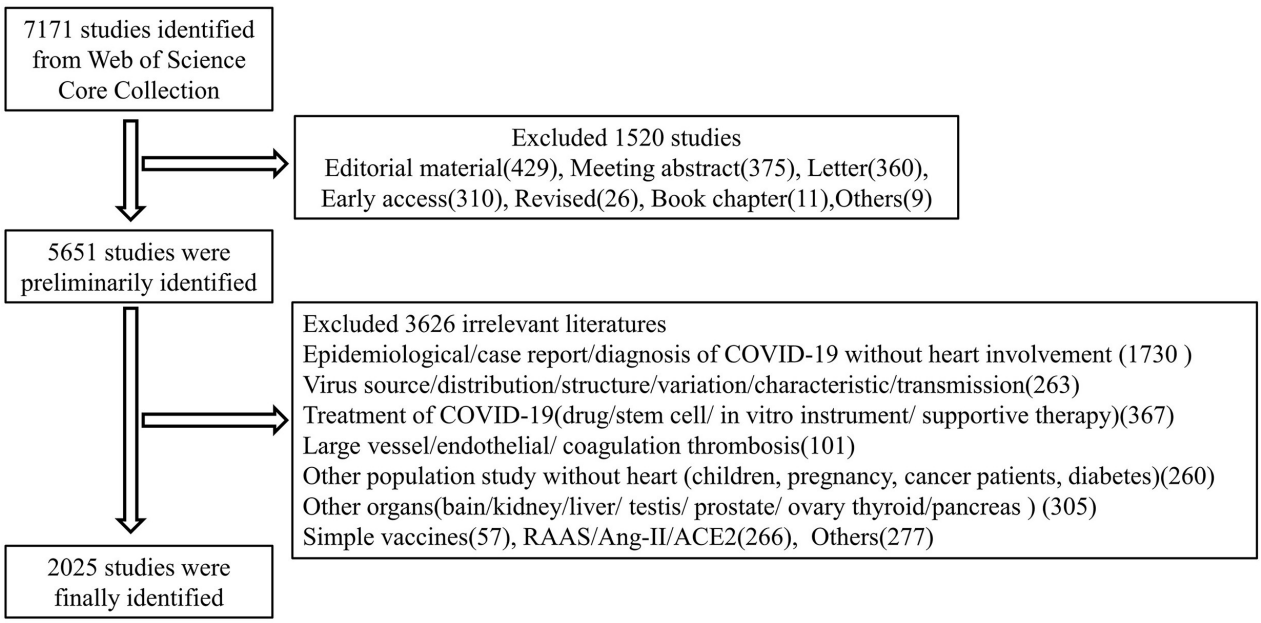

FIGURE 1 | The detailed information of the screening process. All of the relevant studies of COVID-19 and heart were primarily recorded by the initial retrieval in the web of science. Only articles and reviews were selected for the second analysis. We eliminated invalid documents of which the themes were not related to COVID-19 and heart. Finally, a total of 2025 records were used as the dataset in the final study.

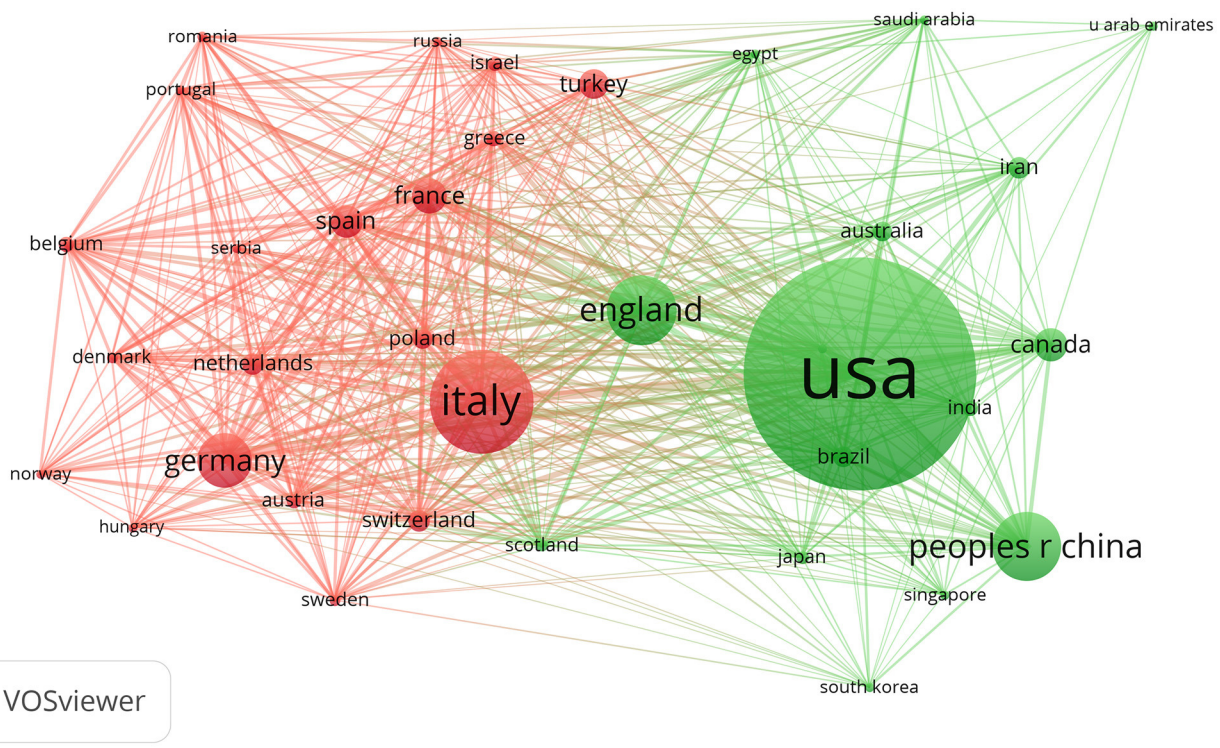

FIGURE 2 | The detailed information of country distribution. The terms were represented by nodes, while links (lines) connected the nodes. The size of the circle was proportional to its number of publications, while the width of the line between the two items was related to the magnitude of their collaboration. Items of the same color belonged to the same cluster, indicating that they cooperated closely in this field. The more clusters there were, the more decentralized the cooperation was. The USA, Italy, and England were the top three countries that published the most articles. They were mainly divided into two clusters as shown in this figure. Cluster 1 included the USA, China, England, Canada, and Australia. Cluster 2 included Italy, German, France, Spain, Greece, and Turkey.

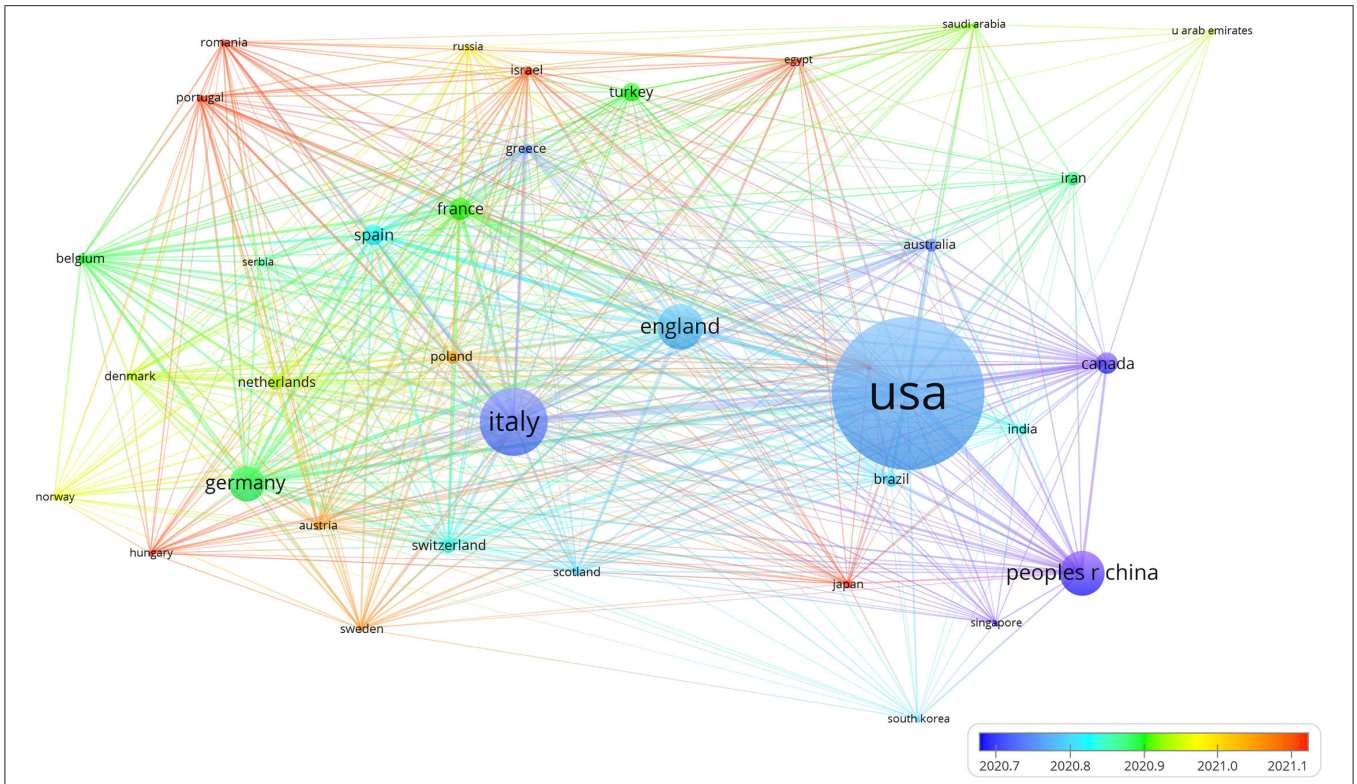

FIGURE 3 | The timeline view of the country distribution. Different colors represented different years. The warmer the color was, the closer the year. The distribution of cardiac disorders also reflected the dynamic change of critically ill patients in different countries. China, Italy, Canada, Singapore, USA, and England mainly carried out research on the cardiac involvement of COVID-19 in the early stage. With the epidemic of COVID-19, Spain, Switzerland, France, Germany, Israel, Russia, and other countries also began to focus on the cardiac disorder in COVID-19.

TABLE 2 | Detailed information of the top ten countries and organizations.

Rank	Source	Publications	Citations	Rank	Source	Country	Publications	Citations
1	USA	747	15,694	1	Harvard Medical School	USA	67	2,553
2	Italy	324	8,414	2	Columbia University	USA	56	1,270
3	England	213	3,997	3	Mayo Clinic	USA	43	1,704
4	China	211	10,409	4	University of Milan	Italy	42	1,483
5	Germany	165	3,440	5	Huazhong University of Science and Technology	China	39	1,952
6	France	104	2,048	6	Massachusetts General Hospital	USA	34	1,087
7	Canada	99	1,690	7	King's College London	England	33	384
8	Spain	96	1,173	8	Cleveland Clinic	USA	32	732
9	Turkey	87	612	9	Stanford University	USA	29	1,085
10	Netherlands	64	1,050	10	Icahn School of Medicine at Mount Sinai	USA	29	793

abstracts. Detailed information of the articles was presented in **Table 1**. To obtain more accurate information about the impact of COVID-19 on the heart, we further screened all of the included literature by the title and abstract. A total of 3,626 articles were removed without specific cardiac involvement and 2,025 studies were included in the final analysis. The detailed information of the screening process was shown in **Figure 1**.

Distribution of Countries

All of the documents were from 102 countries and 4,020 organizations published before May 12, 2022. The countries that had published the most articles on COVID-19 with heart were the United States of America (USA) ($n = 747$, 36.9%), Italy ($n = 324$, 16%), and England ($n = 213$, 10.5%). This finding suggested that the study of COVID-19 influence on the heart in these countries may have played a critical role in cardiovascular research and

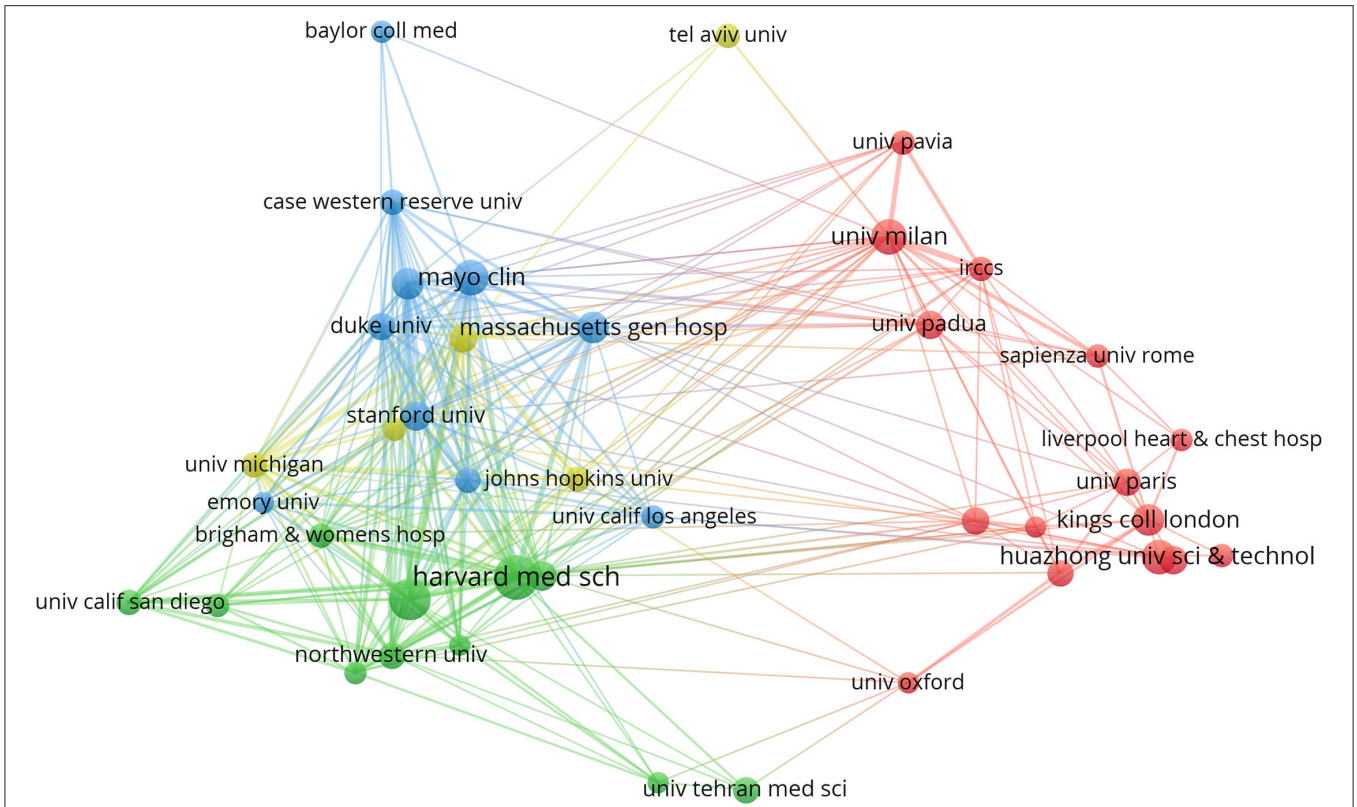

FIGURE 4 | Detailed information of organization distribution. The size of the circle represented its number of publications. Harvard Medical School had published the highest number of research publications in this field followed by the Columbia University and Mayo Clinic. Various institutions cooperated with each other, however, mutual exchanges and cooperation were relatively limited. Harvard Medical School, Columbia University, and Stanford University cooperated with each other most frequently. Huazhong University of Science and Technology, King's College London, and Wuhan University cooperated more frequently.

TABLE 3 | Detailed information of the top ten authors.

Rank	Author	Country	Documents	Citations	Average citation/ Publication
1	Karin Klingel	Germany	12	229	19.1
2	Amer Harky	England	12	187	15.6
3	Lukasz Szarpak	Poland	9	19	2.1
4	Matteo Cameli	Italy	8	54	6.8
5	Serafina Valente	Italy	8	56	7.0
6	Dao Wen Wang	China	8	1,092	136.5
7	Mina K Chung	USA	8	324	40.5
8	Ehtisham Mahmud	USA	8	235	29.4
9	Gianluca Pontone	Italy	8	140	17.5
10	Nir Uriel	USA	8	198	24.8

USA was in a leading position in the field, which may benefit from the contributions of scientific research. The visualization map of the country was generated by the VOSviewer software. Each node represented a country, and the size of the node was proportional to the number of articles published. The lines between nodes represented cooperation between countries and denser lines corresponded to closer cooperation. These countries cooperated and exchanged with each other. They were mainly divided into two clusters as shown in **Figure 2**. Cluster 1 included the USA, China, England, Canada, and Australia who carried out research on the cardiac effects caused by COVID-19 in the early stage. Cluster 2 included Italy, German, France, Spain, Greece, and Turkey. As only critically ill patients were associated with cardiac involvement, the impact of COVID-19 on the heart can also reflect the changes in critically ill patients' distribution in different countries. With the progress of time, Spain, France,

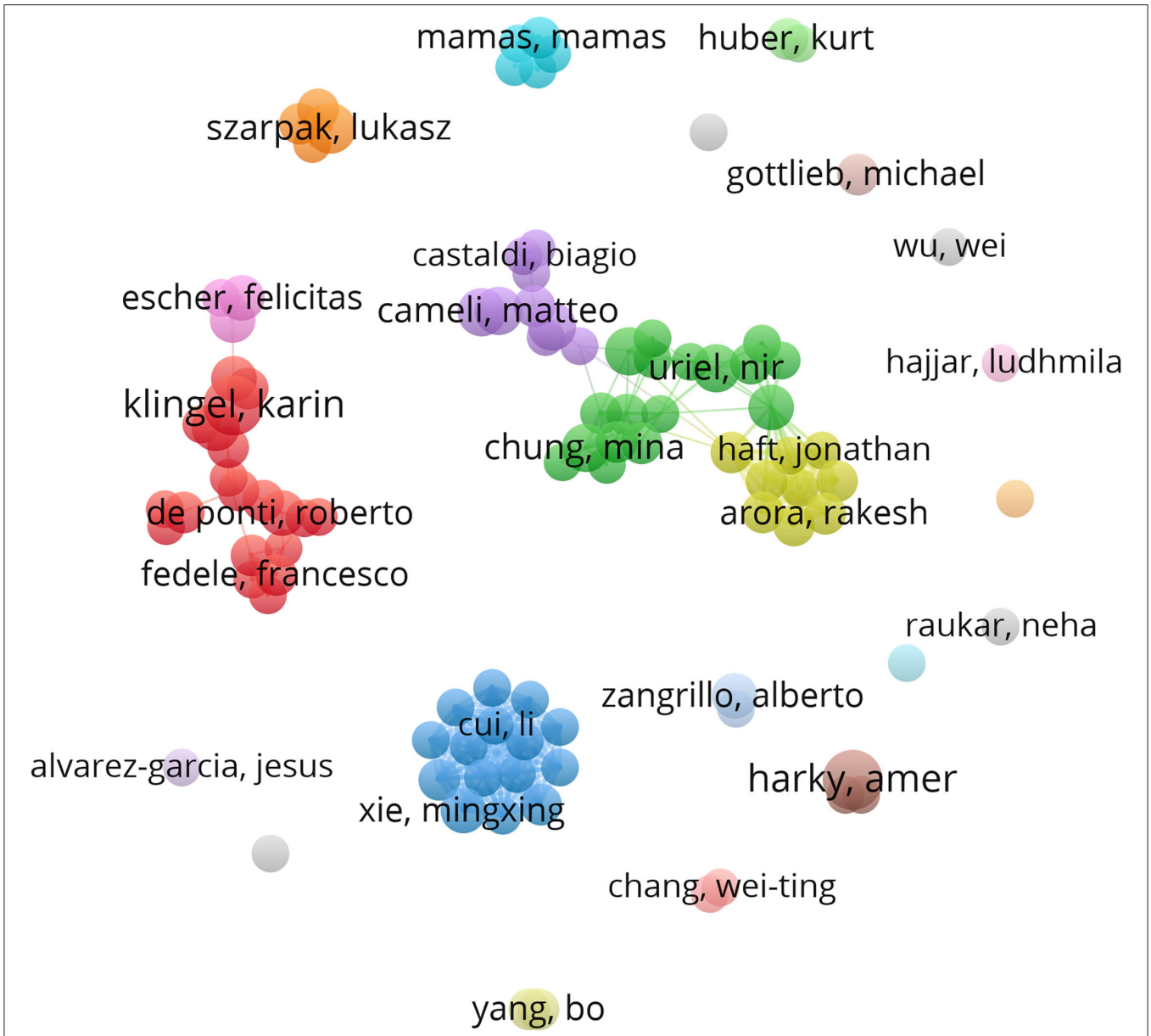

FIGURE 5 | The detailed information of the authors' distribution. Each node represented an author, with larger nodes representing more published articles. Different colors referred to clusters of close cooperation. Karin Klingel and Amer Harky had the greatest number of published papers. Most authors were scattered and lacked stable and intensive cooperation and communication. Some authors mainly cooperated on a small scale in this research area. Karin Klingel only cooperated with DaoWen Wang, Enrico Ammirati, Burkert M. Pieske, and Carsten Tschoepe. Amer Harky only cooperated with Aung Oo and Ana Lopez-Marco.

Germany, and other countries also began to focus on the cardiac disorder in COVID-19 (**Figure 3**).

Distribution of Institutions

The institution with the highest number of research publications in this field was the Harvard Medical School with a quantity of 67, followed by the Columbia University with a quantity of 56 and Mayo Clinic with a quantity of 43 (**Table 2**). Furthermore, we analyzed the cooperative relationships of major institutions and found that various institutions cooperated with each other. However, mutual exchanges and cooperation were relatively limited. Harvard Medical School, Columbia University, and Stanford University cooperated most frequently. Huazhong University of Science and Technology and King's College London and Wuhan University cooperated more frequently, which demonstrated that further cooperation was needed to a larger extent. Detailed information of organization distribution was shown in **Figure 4**. The research institutions

TABLE 4 | Top 10 journals of COVID-19-mediated cardiac disorder.

Rank	Source	IF	Publications	Citations	Average citation/ Publication
1	Frontiers in Cardiovascular Medicine	6.050	65	184	2.8
2	Esc Heart Failure	4.411	59	442	7.5
3	Journal of Clinical Medicine	4.241	56	340	6.1
4	Journal of Cardiac Surgery	1.620	37	327	8.8
5	Cardiology in the Young	1.093	32	50	1.6
6	Journal of the American Heart Association	5.501	25	247	9.9
7	American Journal of Emergency Medicine	2.469	24	642	26.8
8	Scientific Reports	4.379	24	49	2
9	JAMA Cardiology	14.676	20	7,746	387.3
10	Circulation	29.690	19	2,217	116.7

with active cooperation were the University of Pittsburgh, Stanford University, Harvard University, Brigham and Women's Hospital, and Harvard Medical School.

Distribution of Authors

A total of 13,739 authors published articles on COVID-19 in cardiac involvement (**Table 3**). Karin Klingel and Amer Harky from cardio-pathology, University Hospital Tuebingen, Germany, and the department of cardiothoracic surgery, Liverpool Heart and Chest, UK, had the greatest number of published papers (12, 0.6%), followed by Lukasz Szarpak (9, 0.4%), Matteo Cameli (8, 0.39%), Serafina Valente (8, 0.39%), Dao Wen Wang (8, 0.39%), and so on. Although the countries and institutions that published the most articles were almost from the USA, the authors with the largest number of articles were not mainly from the USA. The detailed information of the authors was shown in **Figure 5**. Each node represented an author, with larger nodes representing more published articles. Thicker lines implied closer cooperation between authors. Different colors referred to clusters of close cooperation. As shown in **Figure 5**, most authors were scattered and lacked stable and intensive cooperation. Some authors mainly communicated with each other on a small scale in this area. Karin Klingel, the author with the largest number of published articles, only cooperated with DaoWen Wang, Enrico Ammirati, Burkert M Pieske, and Carsten Tschoepe. Amer Harky only cooperated with Aung Oo and Ana Lopez-Marco. It was suggested that research on the cardiac disorder caused by COVID-19 was still relatively limited, lacking in-depth communication and cooperation.

Distribution of Journals

The top 10 journals published 361 articles, accounting for 17.8% of the total literature (**Table 4**). The Frontiers in Cardiovascular Medicine (65, 3.2%) had the highest number of outputs, followed by the ESC Heart Failure (59; 2.9%) and the Journal of Clinical Medicine (56; 2.8%). Most journals mainly belong to the cardiovascular field. Among the top 10 journals, Circulation and JAMA Cardiology had the highest impact factor, and more in-depth research was still needed in this field.

Citation Analysis/Co-cited Authors and Journals

Co-citation analysis is designed to measure the degree of relationship between articles. The influence of journals depends on the number of times they are co-cited, which reflects whether the journal has a significant influence in a particular research field. Among the 26,451 authors, 68 authors had been cited more than 100 times. Shaobo Shi from Renmin hospital of Wuhan University ranked as the first co-cited author with 665 citations followed by Fei Zhou from Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China, and Tao Guo from the department of cardiology, Zhongnan Hospital of Wuhan University, Wuhan, China. Almost most of the top co-cited authors were from China and two were from the city of Wuhan. Moreover, all the co-cited authors often cooperated with each other from **Figure 6**. Among 7,563 co-cited journals, 7 journals were cited over 2,000 times. As shown in **Table 5**, the New England Journal of Medicine (3,390) was the most frequently cited journal, followed by Circulation (3,268) and JAMA Cardiology (2,814). Among the top 15 journals, the New England Journal of Medicine had the highest impact factor (IF) (91.245), followed by the Lancet with an IF of 79.321. According to the journal citation reports partition in 2021, all of the top 10 co-cited journals were distributed in the Q1 region.

Co-cited References and Top-Cited Articles

Co-citation analysis indicated that two references appeared in the reference list of a third citation article, and then the two references formed a co-citation relationship. We listed the top 10 most frequently cited references related to the research. Among the 39,581 co-cited references, 40 references were cited more than 100 times, and the references listed in the top three were all cited more than 500 times (**Table 6**). The most frequently cited reference topic was closely associated with cardiac injury and mortality in hospitalized COVID-19 patients in Wuhan, China. Almost all the co-cited literature of COVID-19 combined with cardiac injury were mainly from the year 2020 and was reported by articles and case reports. It was suggested that

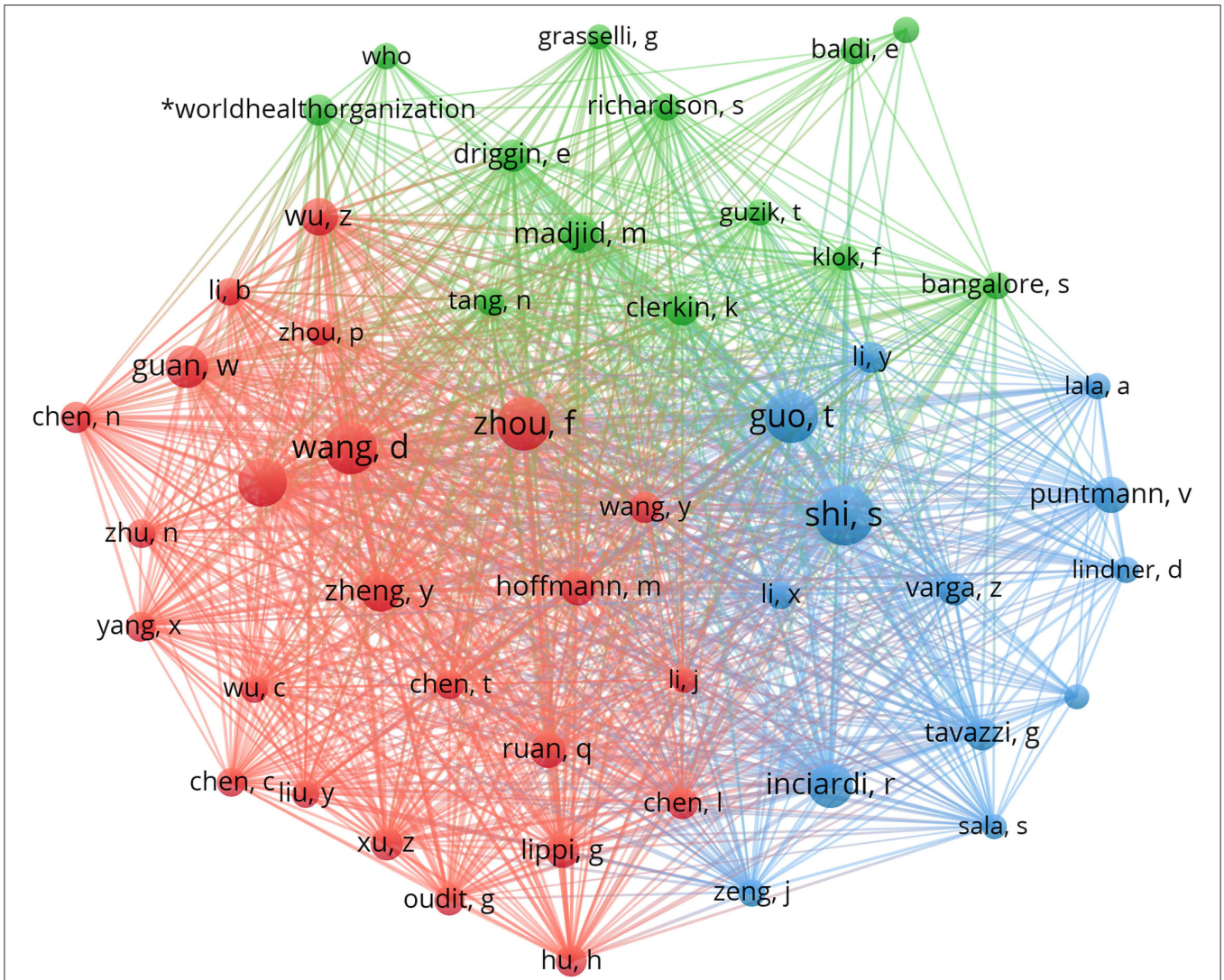

FIGURE 6 | The distribution of all the co-cited authors. VOSviewer is a reliable tool for displaying the intellectual base and frontiers of a certain research field by performing co-citation analysis and burst detection. When two references were cited by the third reference at the same time, these two references constituted a co-citation relationship. The strength of the co-citation relationship between the two cited articles was proportional to the similarity of their research contents and the more times they were cited at the same time, the stronger the co-citation relationship was. There were two major clusters. Cluster 1 (blue) included Shaobo Shi, Tao Guo, Zsuzsanna Varga, and Riccardo M Inciardi; while cluster 2 (red) included Daowei Wang, Fei Zhou, Chaolin Huang, and Wei-Jie Guan. These authors occupied a pivotal position in this field.

articles published in the early stage of the epidemic deserved high citations.

Analysis of Hotspots and Main Research Directions

Keywords summarize the research topics. Through the analysis of keywords, we can understand the research hotspots in specific fields. **Table 7** displayed the high-frequency keywords. Among these keywords, myocarditis, heart failure (HF), and myocardial injury ranked as the top three cardiac injury terms, suggesting that COVID-19 had a substantial effect on cardiac function. We used the VOSviewer software to cluster the keywords. The circle and label form an element, the color identified the cluster to which it belongs. **Figure 7** displayed the clusters of red, blue, and green, indicating three research directions. Red clusters were composed of SARS-CoV-2, coronavirus, ace2, pneumonia, inflammation, Wuhan, and heart. The keywords of the green cluster were COVID-19, management, heart failure, pandemic, and cardiovascular disease. The keywords of the blue cluster were myocardial injury, mortality, echocardiography, cardiac injury, and troponin. The timeline view was designed based on the interaction and mutual relationship between keywords in a particular field, and it was used to explore the evolutionary track and stage characteristics in the field. **Figure 8** displayed a timeline chart of COVID-19 mediated cardiac involvement based on VOSviewer software; it visually reflected the phased

TABLE 5 | Top 10 co-cited authors and journals.

Rank	Author	Citations	Country	Rank	Journal	IF (2021)	Citations	JCR (2021)
1	Shaobo Shi	665	China	1	New England Journal of Medicine	91.245	3,390	Q1
2	Fei Zhou	537	China	2	Circulation	29.690	3,268	Q1
3	Tao Guo	533	China	3	JAMA Cardiology	14.676	2,814	Q1
4	Dawei Wang	511	China	4	Lancet	79.321	2,775	Q1
5	Chaolin Huang	446	China	5	Journal of the American College of Cardiology	24.094	2,482	Q1
6	Riccardo M Inciardi	376	Italy	6	European Heart Journal	29.983	2,472	Q1
7	Weijie Guan	337	China	7	JAMA-Journal of the American Medical Association	56.272	2,263	Q1
8	Yingying Zheng	290	China	8	Nature	49.962	727	Q1
9	Mohammad Madjid	276	USA	9	European Journal of Heart Failure	15.534	726	Q1
10	Zunyou Wu	256	China	10	Circulation Research	17.367	672	Q1

hotspots and epidemic status of severe COVID-19 from the time dimension. The initial research mainly focused on pneumonia caused by SARS-CoV2 through ACE2 receptor in Wuhan. With the progress of the disease, it was found that severe pneumonia with an obvious inflammatory response mediated by SARS-CoV2 can cause cardiac injury. With the global pandemic of COVID-19, the keywords referring to myocardiac injury, including HF, myocarditis, and troponin had become increasingly prominent. With further understanding of the disease, identification of HF and myocarditis through echocardiography was of great significance for the diagnosis and prognosis in severe COVID-19 patients. As only severe patients were complicated with heart injury, the situation of severe patients can also be estimated by cardiac injury. The timeline view also reflected the fluctuations in patients with severe pneumonia in various countries to some extents.

DISCUSSION

COVID-19 caused by the SARS-CoV-2 was reported in Wuhan, China, in December 2019 and had spread across the whole globe and adversely affected the livelihood of millions of people (10). Previous studies had demonstrated that a substantial majority of patients hospitalized developed an acute COVID-19 cardiovascular syndrome, which manifested with a variety of clinical presentations ranging from acute cardiac injury with cardiomyopathy, ventricular arrhythmias, and hemodynamic instability in the absence of obstructive coronary artery disease (11). Early studies had also shown that almost all patients with severe illness had severe myocardial damage, which almost reached 100% in critically ill patients and 70–80% in severely ill patients. However, the specific role of cardiac involvement in COVID-19 had not been elucidated yet. Bibliometric can not only offer a quantitative and statistical analysis of publications in specific fields but also accurately reflect the most representative studies (12, 13). In addition, by presenting numerous data in the form of knowledge maps, researchers can comprehensively analyze the development of a discipline and understand the frontier trends.

Our study discovered that about half of the countries and regions in the world had reported SARS-CoV-2 combined with the cardiac disorder, suggesting that cardiac involvement caused by severe COVID-19 was not uncommon. The USA was the country that had published the most articles on SARS-CoV-2 combined with cardiac in the world, which was almost corresponding to the high mortality in America according to the world health organization (https://covid19.who.int/?mapFilter=deaths). Italy and England ranked as the second and third countries in the number of articles which was also consistent with the order of the mortality rate in Europe by the world health organization (WHO). In addition to the country with the most published articles, the scientific research institutions or organizations with the most published articles were also mainly from the USA. However, Karin Klingel and Amer Harky, the authors with the most published articles were not from the USA. Karin Klingel mainly majored in SARC-CoV-2 mediated myocarditis, while Amer Harky worked as a surgeon and majored in cardiac surgery which suggested that COVID-19 had also a significant influence on cardiac surgery application. Although some scholars had cooperation to some extents, most cooperation was relatively limited and require greater and deeper improvement. Moreover, although many literature had reported that COVID-19 was complicated by heart injury, most of the articles belong to journals with a medium level of impact factor. The main reason may due to that most scholar mainly focused on the lung damage caused by SARC-CoV-2 and the number of severe patients may decrease with the continuous variation of the virus and the development of vaccines and anti-viral drugs.

Co-citation analysis can demonstrate the weight of authors and journals in a specific research field. Among the 26,451 co-cited authors, 68 authors had been cited more than 100 times. Shaobo Shi from Renmin hospital of Wuhan University

TABLE 6 | Top 20 co-cited articles.

Rank	Articles	Author	Journal	Year	Type	Occurrences	Total link strength
1	Association of Cardiac Injury With Mortality in Hospitalized Patients With COVID-19 in Wuhan, China	Shaobo Shi	JAMA Cardiology	2020	Article	559	2,802
2	Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study	Fei Zhou	Lancet	2020	Article	532	2,790
3	Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19)	Tao Guo	JAMA Cardiology	2020	Article	524	2716
4	Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China	Chaolin Huang	Lancet	2020	Article	376	2,113
5	Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China	Dawei Wang	JAMA	2020	Article	337	1,980
6	Cardiac Involvement in a Patient With Coronavirus Disease 2019 (COVID-19)	Riccardo M Inciardi	JAMA Cardiology	2020	Case Report	289	1,717
7	Clinical Characteristics of Coronavirus Disease 2019 in China	Wei-Jie Guan	New England Journal of Medicine	2020	Article	275	1,468
8	COVID-19 and the cardiovascular system	Ying-Ying Zheng	Nature Reviews Cardiology	2020	comment	271	1,530
9	Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention	Zunyou Wu	JAMA	2020	Article	245	1,302
10	Outcomes of Cardiovascular Magnetic Resonance Imaging in Patients Recently Recovered From Coronavirus Disease 2019 (COVID-19)	Valentina O Puntmann	JAMA Cardiology	2020	Article	233	892
11	Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China	Qiurong Ruan	Intensive Care Medicine	2020	Letter	217	1,437
12	SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor	Markus Hoffmann	Cell	2020	Article	205	1,223
13	COVID-19 and cardiovascular disease	Kevin J. Clerkin	Circulation	2020	Review	202	1,072
14	Cardiovascular Considerations for Patients, Health Care Workers, and Health Systems During the COVID-19 Pandemic	ElissaDriggin	Journal of the American College of Cardiology	2020	Review	197	1,127
15	Myocardial localization of coronavirus in COVID-19 cardiogenic shock	Guido Tavazzi	European Journal of Heart Failure	2020	Case Report	195	1,164
16	Pathological findings of COVID-19 associated with acute respiratory distress syndrome	Zhe Xu	Lancet Respiratory Medicine	2020	Case Report	191	1,223
17	Endothelial cell infection and endotheliitis in COVID-19	Zsuzsanna Varga	Lancet	2020	Case Report	179	990
18	Potential Effects of Coronaviruses on the Cardiovascular System	Mohammad Madjid	JAMA Cardiology	2020	Review	177	934
19	Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study	Nanshan Chen	Lancet	2020	Article	172	1,005
20	A Novel Coronavirus from Patients with Pneumonia in China, 2019	Na Zhu	New England Journal of Medicine	2020	Article	158	763

TABLE 7 | Top 20 keywords related to COVID-19-mediated cardiac involvement.

Rank	keyword	Occurrences	Total link strength	Rank	Keyword	Occurrences	Total link strength
1	COVID-19	1,297	2,034	11	Heart	123	312
2	SARS-CoV-2	434	1,008	12	Disease	119	294
3	Coronavirus	255	637	13	Infection	118	358
4	Mortality	197	427	14	Inflammation	116	323
5	Myocarditis	175	421	15	Management	116	263
6	Heart failure	170	385	16	Risk	116	250
7	Ace2	157	450	17	Coronavirus disease 2019	108	196
8	Myocardial injury	128	385	18	Echocardiography	100	193
9	Pneumonia	127	364	19	Cardiovascular disease	89	227
10	Outcome	127	325	20	Heart-failure	83	202

ranked as the first co-cited author with 665 citations followed by Fei Zhou from the Chinese Academy of Medical Sciences, Peking Union Medical College, China, and Tao Guo from the department of cardiology, Zhongnan Hospital of Wuhan University, China. Shaobo Shi had demonstrated that cardiac injury was a common phenomenon among hospitalized patients with COVID-19 in Wuhan, and it was closely associated with a higher risk of mortality (14), while Fei Zhou had demonstrated that the potential risk factors of older age, high sequential organ failure assessment (SOFA) score, and d-dimer could predict patients with poor prognosis. Prolonged viral shedding offered the rationale strategy of isolation of infected patients and proper antiviral interventions (15). The third co-cited author Tao Guo discovered that myocardial injury was significantly associated with the fatal outcome of COVID-19, while the prognosis of patients without underlying cardiovascular diseases was relatively favorable. Myocardial injury was associated with cardiac dysfunction and arrhythmias. Aggressive treatment may be considered for patients at high risk of myocardial injury (16). All the three studies were clinical studies from China, which respectively elaborated the impact of SARS-CoV2 from different aspects and heart damage caused by SARS-CoV2 in the early stage. The three articles had been published in international top journals, which were worthy of high reference.

The presentation and distribution of keywords and hotspots can help us quickly identify the frontier and directions of a certain research field. After capturing the hot spots and forewords of SARS-CoV2-mediated cardiac injury, we found that myocarditis and HF were the main complications of SARS-CoV2 mediated heart injury. Previous literature also reported that myocarditis and HF were also one of the main reasons for death in severe patients.

Myocarditis

Although myocardial injury occurred in 20–30% of hospitalized patients with COVID-19 infection, cardiovascular complications contributed to approximately 40% of all COVID-19-related deaths according to a previous study (17). SARS-CoV-2-mediated myocarditis ranged from ordinary myocarditis with slightly elevated myocardial enzymes to severe myocarditis accompanied by hemodynamic changes, HF, and even cardia shock. Most cases of myocarditis related to COVID-19 infection occurred in the initial phase of infection and were self-limited. The risk of death was significantly increased in patients with severe myocarditis. In the early stage, many studies had reported the injury of myocarditis mediated by SARS-CoV-2 (18, 19). Although the virus continued to mutate and its virulence decreased with the virus mutation, there were still reports of scattered severe myocarditis. Clinical myocarditis during the acute phase of illness had been reported in only 1.4–7.2% of cases in autopsy studies (20). Delayed acute myocarditis with COVID-19 infection had also been reported recently. Alistair Thomson had recently reported a 39-year-old female with no significant previous medical history and confirmed delta variant COVID-19 infection. Endomyocardial biopsy discovered diffuse interstitial macrophage infiltration and small vessel thrombosis. Despite treatment with tocilizumab, high-dose steroids, and intravenous immunoglobulin, she eventually died due to disease-related complications (21). Although myocarditis was mainly secondary to acute inflammatory disease of the lung, approximately 60% to 80% of patients who recovered from COVID-19 were found evidence of myocarditis by cardiac magnetic resonance imaging at a median of 70 days from infection (22). Mahmoud Ismayl described a case of delayed-onset fulminant myocarditis that developed 5 weeks after mild COVID-19 infection resulting in cardiogenic shock and the need for mechanical circulatory support (23). The direct infiltration of SARS-CoV-2 and the infiltration of immune cells mediated by systemic inflammatory response were the main pathogenesis of viral myocarditis. The incidence of acute and delayed acute myocarditis was consistent with the study that SARS-CoV-2 may be associated with a postinfectious, immune-mediated myopathy (24). Another COVID-19-associated myocarditis was virus vaccine-mediated myocarditis. Myocarditis following mRNA COVID-19 vaccination predominantly occurred among young males in their teens or twenties a few days later of the second dose of the vaccine (25). According to the US Centers for Disease Control and Prevention, myocarditis/pericarditis rates

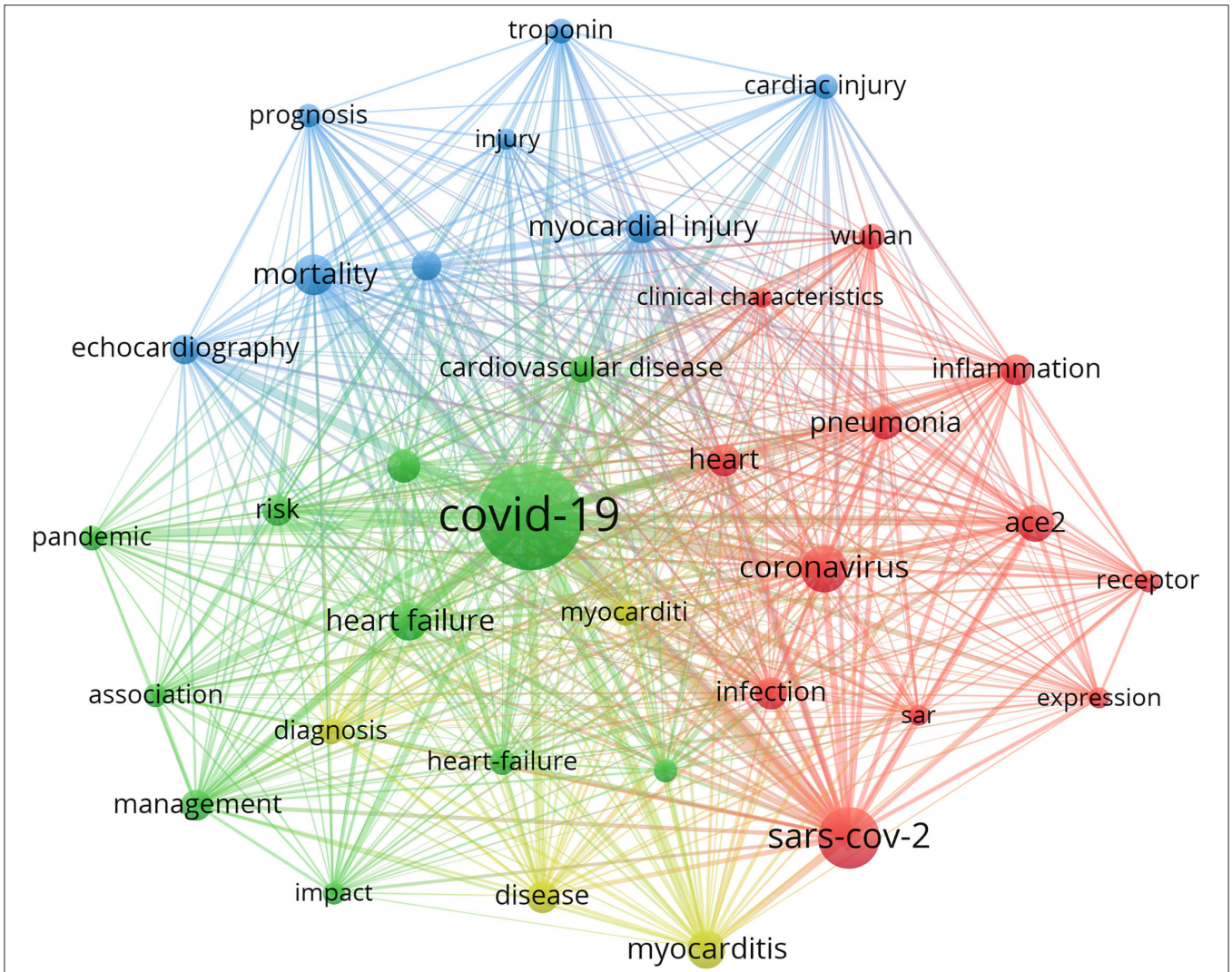

FIGURE 7 | The network mapping on keywords of cardiac involvement caused by COVID-19. The size of each circle represented the weight of a keyword. The distance between the two circles indicated the relatedness between the two circles. The stronger the relatedness, the shorter the distance. The color of the circles represented the respective cluster class. This figure displayed three clusters of red, blue, and green, indicating three research directions. Red clusters were composed of SARS-CoV2, coronavirus, ace2, pneumonia, inflammation, Wuhan, and heart. The keywords of the green cluster were COVID-19, management, heart failure, pandemic, and cardiovascular disease. The keywords of the blue cluster were myocardial injury, mortality, echocardiography, cardiac injury, and troponin.

were almost 12.6 cases per million after second-dose mRNA vaccine among individuals 12–39 years of age (26). Almost all the clinical symptoms were mild, and this young population exhibited a good prognosis.

Heart Failure

Another hotpot related to COVID-19-associated cardiac dysfunction was HF. HF was a common disease state that can be encountered at different stages during COVID-19. New or pre-existing HF in the setting of COVID-19 can present challenges that can be encountered in presentation, management, and prognosis. Lessons from the previous coronavirus and influenza epidemics implied that viral infections can exacerbate a pre-existing HF, with multiple studies showing increased mortality during influenza-like illness seasons (27). With the more aggressive COVID-19 infection, HF patients were considered at a higher risk of acute deterioration, and multiple mechanisms may be responsible for triggering and aggravating this process. It was also reported that the virus almost led to 15–29% kidney impairment in COVID-19 patients (28), which may result in volume overload that may exacerbate a pre-existing chronic HF. Instead of aggravating the pre-existing cardiac disease, new onset of HF was observed in a quarter of hospitalized COVID-19 patients and as much as one-third of those patients admitted to the intensive care unit (ICU) did not have a history of HF (29).

Acute HF was suspected to be a direct consequence of COVID-19, with a dramatic impact on mortality. During COVID-19 hospitalization, about one-third of patients with previous HF had an acute decompensation of HF (30); however, acute HF can be triggered not only as decompensation of

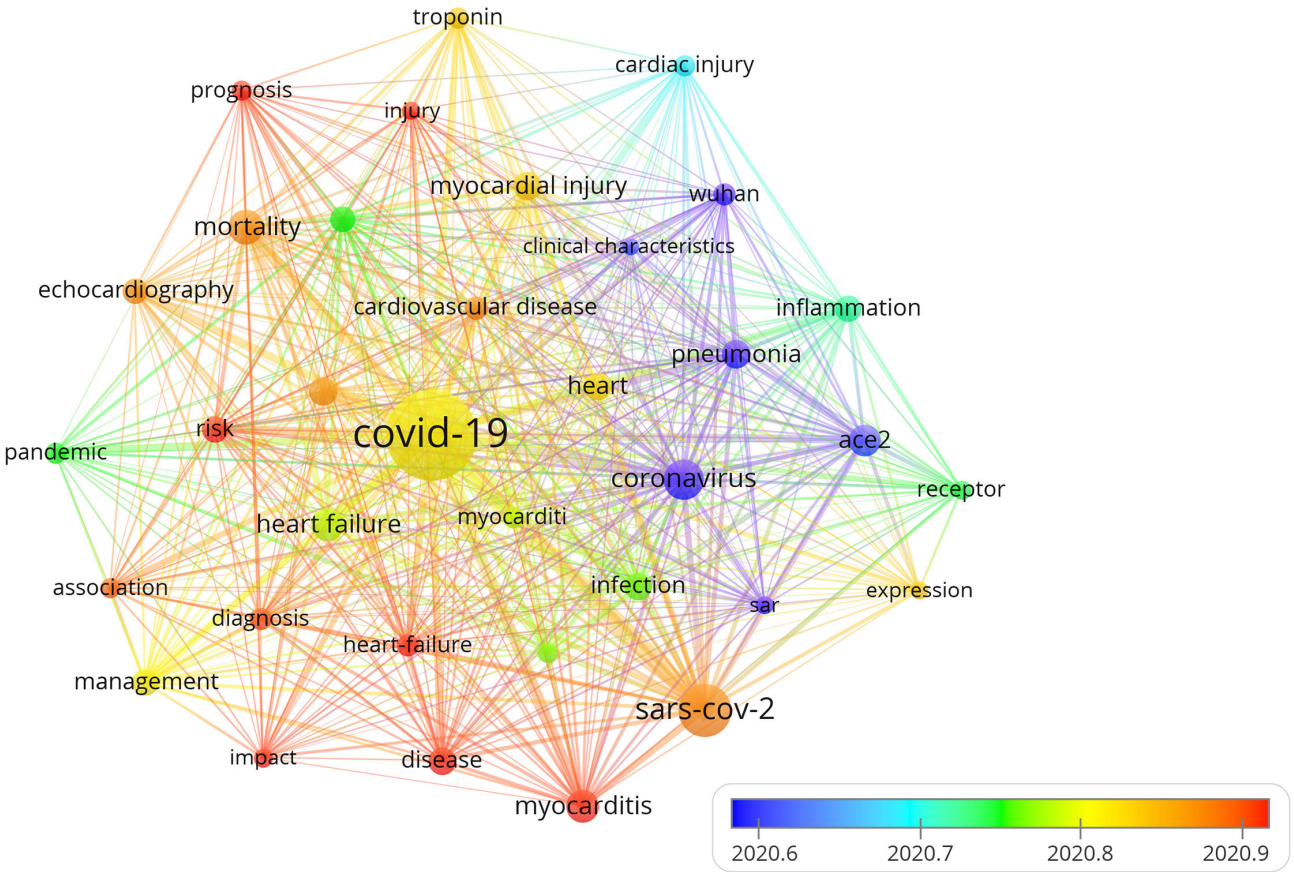

FIGURE 8 | The timeline visualization map of keywords clustering analysis on cardiac involvement in COVID-19. Different colors represented different years. The warmer the color was, the closer the year. This figure displayed a timeline chart of COVID-19-mediated cardiac involvement based on VOSviewer software. The figure visually reflected the phased hotspots and epidemic status of severe COVID-19 from the time dimension. The initial research mainly focused on pneumonia caused by coronavirus through the receptor of ACE2 in Wuhan. With the spread of the disease, it was found that severe pneumonia infection with an obvious inflammatory response mediated by SARS-CoV-2 can lead to cardiac injury. With the global pandemic of COVID-19, the keywords referring to myocadiac injury, including HF, myocarditis, and troponin had become increasingly prominent. With the deepening understanding of the disease, further identification of HF and myocarditis through echocardiography was of great significance for the prognosis in severe COVID-19 patients.

chronic HF but also as a new-onset HF (31). In an Italian multicenter study, acute HF occurred in 9.1% of patients during hospitalization, and almost half of them were new-onset HF with no previous HF history (30). The main reasons for COVID-19-induced HF included virus directly induced infiltration of inflammatory cells, system pro-inflammatory cytokines releasing syndrome, endothelial injury coupled with micro-thrombosis, and ARDS or respiratory failure that could lead to HF due to severe hypoxia (32). In another study of 131 patients who died of COVID-19, 49% of all deaths were attributed to HF in patients without a previous history of cardiovascular disease (33). We can speculate that respiratory failure including ARDS, cardiac injury, and HF were the most common sequelae of COVID-19. Since SARS-CoV2 was still evolving, the extent of the degree of cardiac involvement was still elusive.

In COVID-19 patients presenting acute HF, left ventricular (LV) systolic function was not usually reported; on the contrary, impairment of right ventricular (RV) systolic function and LV diastolic function can be detected more commonly (34). In one study of 100 patients hospitalized for COVID-19, 32% were reported to have normal echocardiography, whereas 39% presented RV dilatation and dysfunction and 16% LV diastolic dysfunction, while less than 10% of patients were reported with LV ejection fraction disorder (35). Accordingly, LV diastolic impairment with elevated LV filling pressures (E/e' ratio) could be observed in a quarter of patients with COVID-19. Consistently, patients hospitalized with COVID-19 showed a high likelihood of preserved ejection fraction (HFpEF) as compared with patients without COVID-19 according to the score of the Heart Failure Association (HFA) of the European Society of Cardiology (ESC), and HFpEF was regarded as the main cardiac structural and functional alterations and myocardial injury (36).

The link between COVID-19 and HF was complex. First of all, the COVID-19 pandemic had an obvious impact on HF management and increased mortality due to HF had been shown

during the pandemic. Several studies showed a reduction in HF hospitalizations, ranging from 30 to 66% in different countries. Second, pre-existing HF was a risk factor for a more severe condition of clinical course in COVID-19 and an independent predictor of in-hospital mortality. Patients with a history of chronic HF were prone to develop into acute decompensated HF after a COVID-19 diagnosis (37). Third, patients hospitalized for COVID-19 may aggravate into acute decompensation of chronic HF and *de-novo* HF as a consequence of myocardial injury. In a word, HF was closely associated with cardiac injury in COVID-19 and deserved further study.

Limitation

VOSviewer cannot fully represent the original distribution and wholly replace system retrieval. The uneven quality of collected literature data and cumbersome document screening process can result in reduced credibility of atlas drawing. The constant updates of data also lead to the retrieval results different from the actual number of included articles. Therefore, a more accurate literature analysis should be based on the knowledge map constructed by the VOSviewer combined with specific literature. Nevertheless, literature analysis based on visualization also helps the scholars quickly understand the research hotspots and development trends of COVID-19 in cardiovascular science to some extents.

CONCLUSION

The research on the cross-talk between COVID-19 and cardiac involvement revealed that cardiac disorder was common in the world, and the USA, Italy, England, and China were the leading countries in this research by using the VOSviewer software for visual analysis. Among the research organization, Harvard Medical School was the institution with the highest influence on achievements. Different countries and institutions need to strengthen cooperation and exchange with each other. At present, the research on COVID-19-related cardiac disorders should concentrate on myocarditis and HF, especially left ventricular diastolic dysfunction and right ventricular systolic dysfunction which will also be the focus of future research.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

SX, WW, and SZ designed the research. SX and XZ collected the primary data. XZ and HX preliminary screened out the irrelevant literature. SX, XZ, and HX checked and sorted out the literature again and analyzed the data. SX wrote the primary manuscript. WW and SZ wrote and revised the final manuscript. All authors listed have read and approved it for publication.

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Case report: Case series of isolated acute pericarditis after SARS-CoV-2 vaccinations

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During the worldwide ongoing immunization campaign against SARS-CoV-2, growing data on very rare but potentially harmful side effects of such vaccines arise since approval trials have not been adequately powered to detect those events. Besides the already reported vaccine-related myocarditis, which primarily occurs in young male individuals, our attention was recently drawn to a series of older male and female patients, who were referred to our institutions with isolated acute pericarditis without myocardial damage, shortly after SARS-CoV-2 vaccination. We describe a series of five adult patients presenting with chest pain, shortness of breath and isolated pericarditis with and without pericardial effusion after SARS-CoV-2 vaccination. All patients underwent echocardiography and cardiac magnetic resonance, and the corresponding findings, including late gadolinium enhancement (LGE) and T1 and T2 mapping are reported herein. To our knowledge, such cases have not been systematically reported in the current literature so far.

KEYWORDS

isolated pericarditis after SARS-CoV-2 vaccination, SARS-CoV-2 vaccination, acute isolated pericarditis, cardiac troponins, late gadolinium enhancement, T1 and T2 mapping

Introduction

More than 11.4 billion doses of SARS-CoV-2 vaccines have been administered worldwide by March 2022 during the largest immunization campaign in human history (1). Studies have undoubtedly proven the benefits of European Union authorized SARS-CoV-2 vaccines in terms of mortality and morbidity, thus outweighing the potential risks of this clearly life-saving strategy (2–4). However, clinical trials were typically underpowered to detect very rare adverse events after SARS-CoV-2 vaccination. Therefore, the continuous evaluation of potential side effects after SARS-CoV-2 vaccination, addressing risk-benefit evaluations, which may in the future guide our vaccination strategy is of major medical and scientific interest. While vaccine-related myocarditis was recently identified as a very rare adverse event in predominantly young men (5, 6), reports on the prevalence and characteristics of acute isolated pericarditis after SARS-CoV-2 vaccination have been limited so far.

In this dual-center study, we systematically report the demographic and clinical characteristics of five consecutive patients, who presented with cardiac symptoms related to possible perimyocarditis after SARS-CoV-2 vaccination and were diagnosed with isolated pericarditis after review of clinical data, laboratory markers, ECG changes, echocardiography, and cardiac magnetic resonance (CMR) findings, including late gadolinium enhancement (LGE) and T1 and T2 mapping. In all cases cardiac troponins were within normal range, and myocardial involvement was not detected by LGE or by mapping techniques.

Materials and methods

In this dual-center report of two German cardiac imaging centers at the GRN Hospital, Weinheim and the Theresien Hospital, Mannheim we systematically reviewed our clinical data management systems, including patients who presented with cardiac symptoms, suggestive of acute perimyocarditis after SARS-CoV-2 immunization, using European Union authorized vaccines. All available clinical data on timing and type of the administered SARS-CoV-2 vaccine, potential prior SARS-CoV-2 infections, previous medical history, laboratory results and demographics were extracted from medical records and analyzed. Study participants individually consented for the anonymized data analysis as approved by our local ethics board (S-526-2016), and in accordance with the declaration of Helsinki.

All examinations were performed using 1.5 T MR systems (Siemens MAGNETOM Aera, Siemens Healthcare Erlangen, Germany). A standard protocol was used, according to previous recommendations (7). In short, an overview the of thorax is acquired (T2-HASTE–half-Fourier acquisition single shot turbo-spin echo), followed by three scout images. The cine acquisitions (steady state free precession) were performed in a stack of short axis covering the entire length of left ventricle (LV) and three long axes. T1 and T2 mapping were performed using standard short-axis mid-ventricular acquisition–MOLLI 5(3)3 and trueFISP sequences, respectively. The late gadolinium enhancement (LGE) acquisitions were performed in three long axis and multiple short axis covering the entire length of the left ventricle after the administration of (Dotarem® - gadoterate meglumine in a dosage of 0.1 mmol/kg) (7). Consensus interpretation was performed by at least two experienced cardiologists, specialized and board certified for CMR.

Case series

A total of 44 patients were referred to our departments between December 2021 and February 2022 for CMR due to clinical suspicion of myocardial injury after SARS-CoV-2 vaccination, which comprises 12.5% of all CMR examinations performed in both centers during this time.

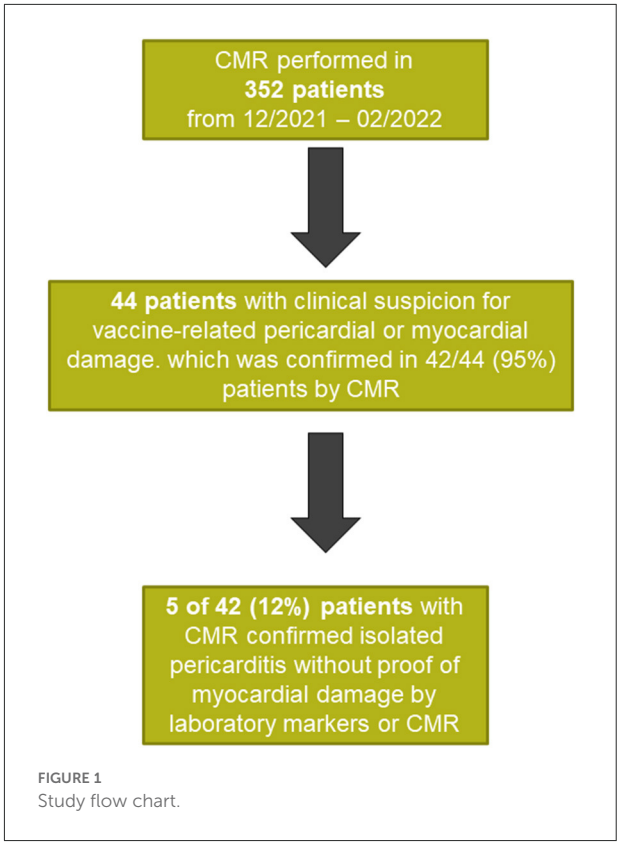

FIGURE 1
Study flow chart.

Five patients with a mean age of 55years (range 43–76 years) were diagnosed with vaccine associated isolated pericarditis, without proof of myocardial damage. A corresponding flow chart is presented in Figure 1.

Patients reported increasing chest pain and dyspnea or fatigue, whereas one patient also suffered from palpitations. In all cases, symptoms were associated with the second or third dose of mRNA SARS-CoV-2 vaccines with a time range of 2.8–7.0 days (median of 3.0 days) between the vaccination and onset of symptoms. Booster vaccination was performed using m-RNA vaccines with BioNTech in 4/5 patients (80%) or BioNTech and Moderna in one patient (20%) (Table 1).

Four of 5 patients had no atherogenic risk factors, whereas one had history of type 2 diabetes mellitus (Table 1). Patients #1–3 presented with acute symptoms within 2 weeks, whereas patients #4 and #5 presented 1–2 months after onset of cardiac symptoms. None of the patients have been pre-treated with non-steroidal anti-inflammatory drugs (NSAID), corticosteroids or colchicine before their initial presentation in our departments. The time range between symptom onset and presentation in our departments was 11–41 days (median of 14 days). ECG showed abnormalities in 80% of the patients (negative T-waves in II, III, and aVF or in V3-V6), whereas troponin elevation

TABLE 1 Patient characteristics.

1	Case #1	Case #2	Case #3	Case #4	Case #5	Median and ranges
Age (years)	75	55	78	42	43	55 (43–76)
Gender	Male	Female	Female	Male	Female	2/5 male
Risk factors	None	None	None	Type 2 DM	None	
BMI (kg/m²)	31.2	23.1	24.9	31.8	20.1	24.9 (22.4–31.4)
Type of vaccine	1. Astra Zeneca 2. BioNTech 3. Moderna	3*BioNTech	3*BioNTech	3*BioNTech	3*BioNTech	100% including m-RNA vaccines
Cardiac symptoms	Chest pain and dyspnea	Chest pain and dyspnea	Chest pain and dyspnea	Chest pain and dyspnea	Fatigue and dyspnea	(4/5) 80% chest pain and dyspnea
Days between last vaccination and symptoms	2	3	3	7	7	3.0 (2.8–7.0)
Days between symptoms onset and presentation	6	12	14	58	35	14 (11–41)
Highly sensitive troponin T (ng/L)	15.1	9.9	12.1	8.4	3.0	9.9 (7.1–12.9)
C-reactive protein (mg/L) (normal range <5)	73.9	224.8	237.4	4.8	0.5	73.9 (3.7–227)
White blood cell count (1,000/μL)	8.3	24.0	11.0	7.4	8.4	8.4 (8.0–14.2)
Pleural effusion (bilateral)	yes	yes	yes	no	no	(3/5) 60%
Pericardial effusion	yes	yes	yes	no	no	(3/5) 60%
ECG changes	Negative T-waves in V3-V6	Negative T-waves in I. II and aVF	None	Negative T-waves in V3–6	Negative T-waves in II. III& aVF	4/5 (80%) with significant ECG changes
LVEF (%)	52	71	68	61	65	65.0 (58.8–68.8)
T1/T2 values (ms)	1,000/42	1,050/49	1,030/53	990/46	995/47	1,000 (993–1,035) for T1
Normal ranges T1/T2 (900–1,080 ms/44–62 ms)						47 (45–50) T2
Pericardial LGE	Diffuse/circular	Diffuse/circular	Diffuse/circular	Anterior and apical	Anterior and lateral	Diffuse in 3/5 cases Regional in 2/5 cases
Myocardial LGE	None	None	None	None	None	0/5
Treatment	Colchicine (3 months) and ibuprofen (2 weeks)	Colchicine (3 months). pericardial paracentesis. Cortisone	Colchicine (3 months). Ibuprofen (2 weeks). Cortisone	Colchicine (3 months)	Colchicine (3 months)	Colchicine in all. Paracentesis in 1/5. Cortisone in 2/5

Five adults (age 58 ± 15 years) presented with clinical symptoms of acute pericarditis, all exhibiting pericardial late gadolinium enhancement (LGE) by CMR. The complaints began with all patients in close temporal association to the third dose of SARS-CoV-2 vaccines. None of the patients had evidence of acute myocardial damage by troponins or LGE. Four of five patients were treated with immunosuppressive therapy. In a single case urgent pericardiocentesis was necessary due to pericardial tamponade.

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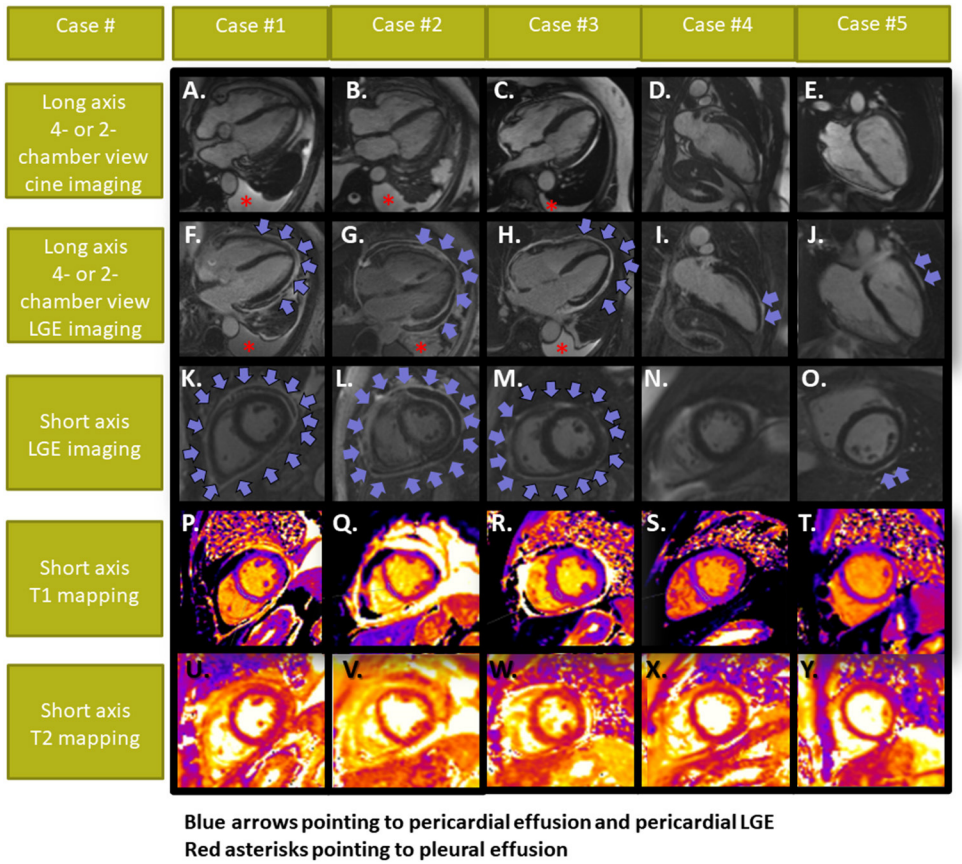

FIGURE 2
Cardiac MRI images of patients #1–5. Cine images are displayed in **(A–E)**. All patients showed pericardial LGE, which was either diffuse [**(F,K)** in patient #1; **(G,L)** in patient #2 and **(H,M)** in patient #3] or focal [**(I, N)** in patient #4 and **(J,O)** in patient #5], whereas myocardial LGE or elevated T1- and T2-values **(P–Y)** were not present with any of our patients. Pericardial and pleura effusion was present in patients #1–3. Patient #2 developed signs of a pericardial tamponade and underwent urgent pericardiocentesis (arrows depicting pericardial effusion and LGE in cases #1–3 and pericardial LGE without effusion in cases #4,5; asterisks pointing to the pleura effusions in cases #1–3).

was not present with any of the patients [median highly sensitive troponin (hs-TnT) of 9.9 ng/L, range 7.1–12.9 ng/L]. Inflammatory values like C-reactive protein (CRP) on the other hand, were markedly elevated in patients #1–3 (median CRP of 73.9 mg/dl, range 4–227 mg/dl), who presented within 2 weeks after the onset of symptoms but were normal in patients #4–5. No infectious cause for the elevated CRP could be identified in patients #1–3. COVID-19 swab testing using polymerase chain reaction was negative in all patients.

Echocardiography and pleural sonography revealed pericardial and pleura effusion in patients #1–3, while left-ventricular (LV)-function was normal with all patients. All patients underwent CMR, which confirmed diagnosis of polyserositis with pericardial and pleura effusion in patients #1–3. LV-ejection fraction was normal in all patients (median LV-ejection fraction of 65%, range 59–69%). Using late gadolinium enhancement (LGE), pericardial enhancement was observed in all patients, being diffuse in patients #1–3 and regional in patients #4–5 (Figure 2). T1 and T2 values were within normal range in all 5 patients. In addition, epicardial, intramyocardial or endocardial LGE was not present in any of the patients. Treatment with colchicine was administrated in all patients, resulting in clinical improvement in patients #1–5. In patients #2 and #3, who presented with more severe symptoms, additional treatment with corticosteroids was necessary in both patients, whereas in patient #2 paracentesis of the pericardial effusion was necessary due to hemodynamic instability and compression of the right ventricle at admission. CMR was performed after paracentesis in this patient. The pericardial effusion was serous, rich in neutrophilic granulocytes and without tumour cells. All patients so far exhibited good mid-term outcomes without major adverse events at 3–6 months of follow-up, all remaining under close surveillance.

Discussion

Our case series reports on five patients who presented with acute pericarditis without myocardial damage in close temporal association to the administration of SARS-CoV-2 vaccines. Although, the present data does not definitely prove causality between the vaccine and the observed acute pericarditis, it should raise the awareness throughout the clinical scientific community to carefully register such potential adverse side effects. Epidemiological studies would be necessary in this context to prove the statistical probability of such adverse effects after SARS-CoV-2 vaccination.

For the diagnosis of acute pericarditis, several clinical, ECG, echocardiography and CMR data were considered in our study. Since CMR alone may be inconclusive for the diagnosis of isolated pericarditis, current guidelines recommend the consideration of multiple diagnostic modalities and of the clinical presentation of the patients, to establish the correct diagnosis (8, 9). Despite these considerations, diagnosis of an isolated acute pericarditis may still be challenging or even remain controversial in some cases. In this regard, the subsequent clinical course and response to anti-inflammatory treatments may further help supporting the initial suspicion. Finally, it underlies the clinical judgement of the treating physician to establish the final diagnosis of this clinical entity.

The current cases series may serve as hypothesis generating for patient characteristics prone to develop acute isolated pericarditis after SARS-CoV-2 vaccination. In this respect, all affected patients had multiple doses of various vaccines and were older than those with vaccine-related myocarditis in current reports (5, 6). If multiple vaccine doses and increasing age are true risk factors for SARS-CoV-2 vaccination isolated pericarditis merits further investigation in future epidemiological studies. Interestingly, pericarditis was frequently associated by concomitant pleura effusion in our case series. Possibly the vaccination seems to have triggered a systemic inflammatory syndrome, manifesting as a secondary polyserositis, which can also be caused by severe systemic inflammation, for e.g., due to SARS-CoV-2 infection (10, 11). It should be noted, however, that pericardial abnormalities by CMR were more prominent in patients #1-3 compared to #4-5, where changes were regional and subtle. This may by attributed to the longer duration between onset of symptoms and presentation of the patients, as well as the milder form of clinical manifestation.

Even if future studies confirm the occurrence of acute isolated pericarditis or polyserositis after SARS-CoV-2 vaccination, such adverse effects can be considered as rare. However, clinicians need to be aware of such potential adverse effects since such patients benefit from prompt diagnosis and anti-inflammatory treatment. Thus, our patients could be treated successfully, and in all cases without short-term residues. Regarding these aspects, the risk of such rare and possibly reversible adverse effects should be balanced against the benefits of protecting against severe COVID-19 related complication and seem to clearly outweigh such risks in this context.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by University of Heidelberg. 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

MO and GK designed the study, performed the analysis, wrote, reviewed the manuscript, and provided important intellectual input. SG performed the acquisitions, reviewed the manuscript, and provided important intellectual input. SG, MH, MO, GK, and SH reviewed the manuscript and provided important intellectual input. 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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Cardiovascular magnetic resonance imaging patterns of acute COVID-19 mRNA vaccine-associated myocarditis in young male patients: A first single-center experience

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Background: The risk of myocarditis after mRNA vaccination against COVID-19 has emerged recently. Current evidence suggests that young male patients are predominantly affected. In the majority of the cases, only mild symptoms were observed. However, little is known about cardiac magnetic resonance (CMR) imaging patterns in mRNA-related myocarditis and their differences when compared to classical viral myocarditis in the acute phase of inflammation.

Methods and results: In total, 10 mRNA vaccination-associated patients with myocarditis were retrospectively enrolled in this study and compared to 10 patients suffering from viral myocarditis, who were matched for age, sex, comorbidities, and laboratory markers. All patients (n = 20) were hospitalized and underwent a standardized clinical examination, as well as an echocardiography and a CMR. Both, clinical and imaging findings and, in particular, functional and volumetric CMR assessments, as well as detailed tissue characterization using late gadolinium enhancement and T1 + T2-weighted sequences, were compared between both groups. The median age of the overall cohort was 26 years (group 1: 25.5; group 2: 27.5; p = 0.57). All patients described chest pain as the leading reason for their initial presentation. CMR volumetric and functional parameters did not differ significantly between both groups. In all cases, the lateral left ventricular wall showed late gadolinium enhancement without significant differences in terms of the localization or in-depth tissue characterization (late gadolinium enhancement [LGE] enlargement: group 1: 5.4%; group 2: 6.5%; p = 0.14; T2 global/maximum value: group 1: 38.9/52 ms; group 2: 37.8/54.5 ms; p = 0.79 and p = 0.80).

Conclusion: This study yielded the first evidence that COVID-19 mRNA vaccine-associated myocarditis does not show specific CMR patterns during the very acute stage in the most affected patient group of young male patients. The observed imaging markers were closely related to regular viral myocarditis in our cohort. Additionally, we could not find any markers implying adverse outcomes in this relatively little number of patients; however, this has to be confirmed by future studies that will include larger sample sizes.

KEYWORDS

COVID-19, myocarditis, mRNA-related, vaccination, cardiac magnetic resonance

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has been a global challenge for the economic and medical systems. As of 22 May 2022, 525.4 million people have been infected by SARS-CoV-2 and more than 6 million people have died worldwide according to Johns Hopkins University (1, 2).

As a result of great efforts, 10 billion doses of newly developed COVID-19 vaccines have been administrated just within 2.3 years after the initial onset of the pandemic (1, 2). The European Medicine Agency (EMA) has authorized the use of five different vaccines, two of them are mRNA-based vaccines [Comirnaty by BioNTech and Spikevax (previously COVID-19 Vaccine Moderna) by Moderna], further two are the vector-based vaccines [Vaxzevria (previously COVID-19 Vaccine AstraZeneca) by AstraZeneca and Janssen by Johnson and Johnson], and one is protein based (Novaxovid by Novavax). While the advantages of the vaccination exceeded the potential side effects by far, the vaccine-associated myocarditis was called out as a threat and affecting young male patients, in particular (3, 4).

Myocarditis is defined as an injury of the heart muscle caused by inflammation in the absence of underlying ischemia (5). While the clinical presentation can be very heterogeneous and include unspecific symptoms, potential complications are associated with a poor outcome as myocarditis represents the major cause of cardiogenic shock in young adults (6, 7). Even though numerous myocarditis etiologies have been described, viral infections, such as SARS-CoV-2, are the most common ones (5). However, vaccinations for Smallpox and Influenza were previously also allocated with a potential risk to induce myocardial inflammation (8). This attributable risk of a vaccine-induced myocarditis is in accordance with the most recently described side effects of mRNA-based COVID-19 vaccinations (9–12) and was listed as a rare but potentially life-threatening side effect by the EMA and the USA Food and Drug Administration (FDA).

Current evidence suggests mRNA vaccination-related myocarditis as a condition that is predominantly affecting young male patients (9, 10–13), which usually occurs within days after the second vaccination dose (4–14).

While COVID-19-related myocarditis did not show major differences when compared to acute myocarditis of other causes, recent data demonstrated that particularly in COVID-19-related myocarditis, uncommon patterns of edema and late gadolinium enhancement (LGE) enhancement in contrast to COVID-19 vaccination-associated myocarditis were present (15, 16). However, COVID-19 vaccine-induced myocarditis on the other hand was compared with myocarditis of other causes and found to share clinical and imaging appearances in a heterogeneous cohort of different age and sex groups (17, 18). Consequently, this study is aimed to particularly discriminate patterns of acute COVID-19 vaccine-induced myocarditis in the primarily affected patient group of young male patients.

Materials and methods

Study population

In total, 20 male participants were retrospectively enrolled after an initial hospitalization due to one of the following diagnoses. Group 1: confirmed vaccination-associated myocarditis (between June and December 2021); group 2: non-vaccination-associated myocarditis (between September 2018 and October 2021). Myocarditis was considered as vaccination related to the cases within 2 weeks after COVID-19 vaccination and no other explanation was found, especially no other vaccination was given within the last month and no other symptoms of an infectious disease were present within 30 days prior to clinical presentation. Patients with classical viral myocarditis were primarily matched according to their age and sex in the first step. Secondly, patients within group 2 were matched with regard to cardiovascular risk factors and other comorbidities (cardiovascular risk factors and atrial fibrillation) of patients within group 1. For the remaining patients, we

sought to balance laboratory markers as accurate as possible within both groups.

The initial referral of patients allocated to group 1 was for further evaluation of a suspected myocarditis due to COVID-19 vaccination or due to chest pain episodes with suspected myocardial ischemia during first diagnostic evaluations. Patients of group 2 were admitted with chest pain symptoms to our emergency department for ischemia rule-out. Diagnoses were based on clinical judgment, such as the clinical presentation, changes in electrocardiogram (ECG), and laboratory findings indicating myocardial damage. Furthermore, imaging findings had to be in accordance with the updated Lake Louise Criteria (19). The following criteria were defined as reasons for exclusion: (1) age < 18 years and > 40 years, (2) an active SARS-CoV-2 infection at the time of the scan or within 4 weeks prior to the scan [detected by polymerase chain reaction (PCR) test], (3) other COVID-19 vaccination than mRNA-based ones, and (4) a history of coronary artery disease.

The study was approved by the local ethics committee. Due to the retrospective design of the study, the need for informed consent was waived.

Diagnostic workup

Patients underwent a standardized clinical evaluation, including a detailed medical history, a physical examination, an ECG at rest, transthoracic echocardiography (TTE), cardiac magnetic resonance (CMR), and blood testing. ECG and TTE were performed in concordance to the ESC position paper for myocardial and pericardial diseases (20). TTE measurements included visual estimation of left ventricular ejection fraction (LVEF), tricuspid annular plane systolic excursion (TAPSE), and the visual assessment of the presence of pericardial effusion or wall motion abnormalities. Blood testing included high sensitive troponin T or I [due to the fact of different normal values, troponin levels are expressed as multiple times increment above the upper limit of normal (ULN)], creatine kinase (CK), creatine kinase muscle and brain (CK-MB), and c-reactive protein (CRP). Further diagnostic workup was based on the results of the latter tests.

Cardiac magnetic resonance

Cardiac magnetic resonance was performed using a dedicated myocarditis protocol on a 3T Magnetom Vida (Siemens Healthcare GmbH, Erlangen, Germany) with a 32-channel anterior receiver coil in all patients. The protocol included a long- and short-axis stack of balanced steady state-free precession (bSSFP) slices with an in-plane resolution of 1.41 mm^3 $\times$ 1.41 mm^3 $\times$ 6 mm^3 and a slice gap of 6 mm. LGE assessments were performed in phase-sensitive inversion recovery (PSIR) short-axis image stacks starting 15 min after injection of Gadobutrol (0.15 mmol/kg body weight) (Gadovist®, Bayer Healthcare, Berlin, Germany) (21). For quantitative T1 and T2 mapping, a single midventricular short-axis slice was obtained using a Modified Look-Locker Inversion Recovery (MOLLI) technique for T1 maps and a T2-Prep Fast Low Angle Shot (FLASH) at the same slice position for T2 maps, respectively (22, 23). Inline motion correction and the generation of pixel-based maps were automatically executed by the scanner.

Image analysis

Postprocessing analyses were performed by an experienced observer blinded to all previously documented clinical information using commercially available Software (QMass® and QStrain®, version 3.2.36.4, Medis Medical Imaging Systems, Leiden, Netherlands). Functional and volumetric parameters were assessed using semi-automated contouring detection with manual correction if necessary following established standards (24). Feature tracking strain analysis was based on three independently repeated measurements (25). Global longitudinal strain (GLS) was assessed from bSSFP image data in all three long-axis views (26, 27). The presence of LGE was visually evaluated by the reader followed by a quantification using the full-width half density method and was later displayed in absolute mass (grams) and its relation to the total left ventricular mass (percentage) (28, 29). T1 and T2 maps were screened for artifacts prior to analysis and affected segments were excluded from the analysis. During segmentation, the blood pool and right ventricular insertion point were carefully avoided. Furthermore, two specific regions of interest (ROIs) were defined as the septal region and the region with maximum values based on the color maps. Both ROIs were manually delineated. As suggested by the Society for Cardiovascular Magnetic Resonance (SCMR) and the European Association for Cardiovascular Imaging (EACVI), abnormal values were defined as T1 $>$ 1,289 ms and T2 $>$ 46 ms at the local facility (30). Extra cellular volume (ECV) was calculated as suggested by the SCMR with hematocrit obtained on the day before scanning. Abnormal values were defined as $>30\%$ at the local facility (30).

Statistics

Statistical analysis was performed using IBM SPSS Statistics version 27 for Windows (International Business Machines Corporation (IBM® Corp., Armonk, NY, United States). Continuous data were expressed as median $\pm$ interquartile range (IQR). Normal distribution for continuous data was tested using the Shapiro-Wilk test. In consequence,

statistical significance was tested using Student's *t*-test and the Mann-Whitney *U* test as appropriate. An alpha level of ≤0.05 was considered as statistically significant.

Intergroup comparison of categorical variables was performed using the χ^2 test, and results were presented as absolute numbers and percentages. Nominal values were presented in percentages. Again, an alpha level of ≤0.05 was considered as statistically significant.

Results

Participant's demographics

Patients' characteristics are displayed in **Table 1**. Matching was performed successfully with a median age of 26.0 [21.3–32.8] years (group 1: 25.5 [21.8–33.5]; group 2: 27.5 [19.5–36.5]; $p = 0.574$). Cardiac risk factors and comorbidities were equally distributed across both groups (all $p > 0.100$). As by study design, predefined levels of troponin and creatinine kinase (CK, CK-MB) did not differ between both groups. The same was true for leucocytes and CRP (all $p > 0.200$; **Table 2**).

In group 1, all patients received mRNA vaccinations; with six of them vaccinated with Spikevax by Moderna and four patients with Comirnaty by BioNTech. All patients in both groups had chest discomfort as the main clinical symptom at the initial presentation (**Table 2**). Among the patients who received COVID-19 vaccination, two patients (20%) received the first dose and eight patients received (80%) the second. In group 2, myocarditis was consistently caused by non-COVID viral infections according to the medical records. The time between symptom onsets after vaccination in group 1 was 5.0 [3.5–7.3] days. CMR was performed promptly after symptom onset within 3.0 [1.0–5.5] days in group 1 and 2.0 [2.0–3.0] days in group 2, respectively ($p = 0.239$).

There was no clinical evidence of an underlying autoimmune disorder in any of the patients in group 1 or group 2.

Electrocardiogram and transthoracic echocardiography

Electrocardiogram and TTE were obtained in all patients. The most prevalent ECG abnormality was ST elevation in 80% of group 1 and 40% of group 2. ST depression (group 1: 10%, group 2: 0%) and T wave changes (group 1: 20%, group 2: 20%) were less frequently present. No statistically significant differences between both groups could be observed (**Table 2**).

Left ventricular ejection fraction estimated by TTE was within the normal rage in most patients (group 1: LVEF 55% [50–55]; group 2: LVEF 55% [51.3–58.8] and without significant intergroup differences ($p = 0.695$). Right ventricular function measured by TAPSE was normal (above 16 mm) in all patients with no significant differences within both groups ($p = 0.355$). Furthermore, no statistically significant differences could be found for the presence of pericardial effusion ($p = 0.136$) or wall motion abnormalities ($p = 0.329$; **Table 3**).

Cardiac magnetic resonance findings

Cardiac magnetic resonance results are presented in **Tables 4**, **5**. Volumetric cardiac measurements for both ventricles were within normal range without any statistically significant differences (group 1: left ventricular end-diastolic volume index [LVEDVI] 91.0 ml/m^2 [81.8–97.8], left ventricular end-systolic volume index [LVESVI] 40.0 ml/m^2 [33.8–42.0]; group 2: LVEDVI 93.5 ml/m^2 [78.8–99.5], LVESVI 38.0 ml/m^2 [34.5–43.0]; $p = 0.796$ and $p = 0.561$). In addition, no differences were found in terms of functional measurements, e.g., left and right ventricular ejection fractions (group 1: LVEF 58.0% [52.0–64.5], RVEF 50.0% [46.8–53.3]; group 2: LVEF 58.0% [63.6–60.0], RVEF 54.0% [46.8–57.3]; $p = 0.796$ and $p = 0.143$). Furthermore, there were no differences in GLS (group 1: GLS −20.2 [−19.3 to −21.2]; group 2: GLS −20.4 [−18.2 to −22.5]; $p = 0.912$) on CMR.

In all patients, LGE was present within the subepicardial layers without statistical differences regarding its relative enlargement within the myocardium (group 1: LGE 5.4%; group 2: LGE 6.5%; $p = 0.143$). Myocarditis affected the lateral segment in all cases, with partial involvement of the inferior segments in some of the patients (group 1: 40%; group 2: 20%; $p = 0.329$). A detailed overview is provided in **Table 5**.

One patient in each group showed artifacts within the anterior region of the myocardium in the T1 map. The affected segments were excluded from further analysis. Global T1 values were increased above the ULN for patients with both, vaccine-associated myocarditis and viral myocarditis (group 1: 1,311 ms; group 2: 1,316 ms). No significant differences in-between both groups could be observed ($p = 0.719$).

Segments with the highest T1 values were 23% above the global T1 in group 1 and 24% above the global T1 in group 2, respectively, without significant differences between both groups ($p = 0.853$).

Global T2 times were within normal ranges within both groups (group 1: 38.9 ms; group 2: 37.8 ms) with no significant differences ($p = 0.787$). Segmental T2 values at their maximum were numerically but not significantly higher within patients with viral myocarditis when compared to patients suffering from vaccine-associated myocarditis (group 1: 52.0 ms; group 2: 54.5 ms; $p = 0.796$). The latter values were 34% above the global T2 times in group 1 and 45% in group 2. Segments with the maximum T2 values were above the reference range in both groups. ECV was within the normal range in both groups (group 1: 24.8%; group 2: 26.3%; $p = 0.293$). An illustration of typical CMR findings for both groups is presented in **Figure 1**.

TABLE 1 Baseline characteristics.

Variable	All patients	Vaccine associated myocarditis	Non-vaccine associated myocarditis	*P*-value
Age (years)	26.00 (21.3–32.8)	25.50 (21.8–33.5)	27.5 (19.5–36.5)	0.574
Male [*n* (%)]	20 (100)	10 (100)	10 (100)	
Height (cm)	182 (176–187)	180 (174–187)	182 (179–188)	0.554
Weight (kg)	86 (68–93)	80 (67–90)	86 (68–97)	0.692
BMI (kg/m^2)	24 (22–30)	24 (22–27)	24 (20–31)	0.740
Comorbidities				
Hypertension [*n* (%)]	1 (5)	1 (10)	0 (0)	0.305
Dyslipidaemia [*n* (%)]	1 (5)	0 (0)	1 (10)	0.305
Atrial fibrillation [*n* (%)]	2 (10)	1 (10)	1 (10)	1

Data are expressed as median (interquartile range), numbers, and percentage. Comparisons of vaccine-associated myocarditis and non-vaccine-associated myocarditis were performed. Continuous parameters were tested for normal distribution using the Shapiro-Wilk test and compared using the Mann-Whitney *U* test or *t*-test as appropriate. Categorical parameters were tested using a χ^2 test. BMI, body mass index.

TABLE 2 Clinical presentation, blood test, and electrocardiogram (ECG) results at baseline.

Variable	All patients	Vaccine associated myocarditis	Non-vaccine associated myocarditis	*P*-value
Symptoms at presentation				
Chest pain [*n* (%)]	20 (100)	10 (100)	10 (100)	
Breathlessness [*n* (%)]	4 (20)	2 (20)	2 (20)	1
Palpitation [*n* (%)]	0 (0)	0 (0)	0 (0)	
Blood tests				
Troponin (x-fold above ULN)	92.9 (22.9–450.5)	125.3 (22.9–450.6)	92.90 (19.4–948.0)	0.418
CK (IU/l)	640.0 (248.5–829.5)	690.5 (508.25–886.50)	259.0 (120.0–745.0)	0.211
CK-MB (IU/l)	65.6 (31.78–90.5)	87.0 (58.6–95.5)	53.0 (23.5–99.8)	0.277
CRP (mg/l)	33.5 (6.8–65.2)	26.5 (13.9–45.5)	47.2 (4.9–101.0)	0.681
White blood cells (/μl)	9.4 (6.4–11.0)	8.2 (6.0–10.8)	9.5 (6.2–9.5)	0.499
ECG results				
ST-elevation [*n* (%)]	12 (60)	8 (80)	4 (40)	0.068
ST-depressions [*n* (%)]	1 (5)	1 (10)	0 (0)	0.279
T wave changes [*n* (%)]	4 (20)	2 (20)	2 (20)	1

Data are expressed as median (interquartile range), numbers, and percentage. Comparisons of vaccine-associated myocarditis and non-vaccine-associated myocarditis were performed. Continuous parameters were tested for normal distribution using the Shapiro-Wilk test and compared using the Mann-Whitney *U* test or *t*-test as appropriate. Categorical parameters were tested using a χ^2 test. ULN, upper limit of normal; CK, creatine kinase; CK-MB, creatine kinase muscle and brain; CRP, c-reactive protein; ECG, electrocardiogram.

TABLE 3 Echocardiographic characterization of the study population.

Variable	All patients	Vaccine associated myocarditis	Non-vaccine associated myocarditis	*P*-value
LVEF (%)	55 (51–55)	55 (50–55)	55 (51.3–58.8)	0.695
TAPSE (mm)	24.0 (20.2–29.4)	22.5 (19.6–27.3)	27.8 (20.6–29.9)	0.355
Wall motion abnormalities [*n* (%)]	6 (30)	4 (40)	2 (20)	0.329
Pericardial effusion [*n* (%)]	2 (10)	2 (20)	0 (0)	0.136

Data are presented as median (interquartile range), numbers and percentage. Comparisons of vaccine-associated myocarditis and non-vaccine-associated myocarditis were performed. Continuous parameters were tested for normal distribution using the Shapiro-Wilk test and compared using the Mann-Whitney *U* test or *t*-test as appropriate. Categorical parameters were tested using a χ^2 test. LVEF, left ventricular ejection fraction; TAPSE, tricuspid annular plane systolic excursion.

Further diagnostic workup

In total, four patients underwent a CT scan to rule out a pulmonary embolism (group 1: three patients; group 2: one patient; $p = 0.264$) and in seven patients, invasive coronary angiography was performed (group 1: four patients; group 2: three patients; $p = 0.639$).

Follow-up at discharge

In both groups, myocarditis-related symptoms, such as chest pain, were improved ($n = 2$; 10%) or even resolved ($n = 18$; 90%) at the time of discharge. The mean time of the hospital stay was 5 [3.8–6.3] days in group 1 when compared to 6 [4.8–7.0] days in group 2 ($p = 0.653$). Patients of both groups required

TABLE 4 Cardiac magnetic resonance (CMR) volumetric results.

Variable	All patients	Vaccine associated myocarditis	Non-vaccine associated myocarditis	*P*-value
Time symptom to CMR (days)	2.0 (1.0–4.0)	3.0 (1.0–5.5)	2.0 (2.0–3.0)	0.239
Left ventricle				
LVMI (g/m^2)	81.7 (68.5–94.4)	75.3 (56.5–100.8)	82.0 (81.5–89.6)	0.503
LVEDVI (ml/m^2)	93.5 (80.0–98.5)	91.0 (81.8–97.8)	93.5 (78.8–99.5)	0.796
LVESVI (ml/m^2)	39.5 (34.3–42.0)	40.0 (33.8–42.0)	38.0 (34.5–43.0)	0.561
LV-SVI (ml/m^2)	53.5 (56.0–63.0)	58.0 (44.5–67.8)	53.0 (44.3–57.3)	0.436
LVEF (%)	58.0 (52.3–62.3)	58.0 (52.0–64.5)	58.0 (52.5–60.0)	0.796
GLS (%)	−20.2 (−21.9 to −18.6)	−20.2 (−21.2 to −19.3)	−20.4 (−22.5 to 18.2)	0.912
Right ventricle				
RVEDVI (ml/m^2)	87.0 (78.5–94.0)	86.5 (69.8–94.0)	88.5 (81.5–99.8)	0.280
RVESVI (ml/m^2)	43.0 (37.0–47.8)	45.0 (40.5–50. 8)	39.5 (36.8–47.3)	0.315
RV-SVI (ml/m^2)	47.0 (42.3–49.8)	46.5 (38.0–48.5)	47.5 (43.5–50.8)	0.660
RVEF (%)	52.0 (48.3–54.8)	50.0 (46.8–53.3)	54.0 (46.8–57.3)	0.143

Data are presented as median (interquartile range). Comparison of vaccination-associated myocarditis and non-vaccine-associated myocarditis was performed. Continuous parameters were tested for normal distribution using Shapiro-Wilk test and compared using the Mann-Whitney *U* test or *t*-test as appropriate. LVMI, left ventricular muscle index; LVEDVI, left ventricular end-diastolic volume index; LVESVI, left ventricular end-systolic volume index; LV-SVI, left ventricular stroke volume index; LVEF, left ventricular ejection fraction; GLS, global longitudinal strain; RVEDVI, right ventricular end-diastolic volume index; RVESVI, right ventricular end-systolic volume index; RV-SVI, right ventricular stroke volume index; RVEF, right ventricular ejection fraction.

TABLE 5 Cardiac magnetic resonance (CMR) tissue characterization.

Variable	All patients	Vaccine associated myocarditis	Non-vaccine associated myocarditis	*P*-value
Myocardial injury localization				
Anterior [*n* (%)]	4 (20)	1 (10)	3 (75)	0.264
Septal [*n* (%)]	1 (5)	0 (0)	1 (10)	0.305
Lateral [*n* (%)]	20 (100)	10 (100)	10 (100)	
Inferior [*n* (%)]	6 (30)	4 (40)	2 (20)	0.329
LGE presence [*n* (%)]	20 (100)	10 (100)	10 (100)	
Subendocardial [*n* (%)]	0 (0)	0 (0)	0 (0)	
Mid-wall [*n* (%)]	5 (25)	2 (20)	3 (30)	0.606
Subepicardial [*n* (%)]	20 (100)	10 (100)	10 (100)	
Transmural [*n* (%)]	0 (0)	0 (0)	0 (0)	
LGE (g)	5.3 (3.1–6.3)	4.4 (2.3–5.7)	6.0 (3.7–6.6)	0.089
LGE (%)	6.1 (4.6–7.0)	5.4 (3.7–6.7)	6.5 (5.2–7.9)	0.143
ECV global mean (%)	25.2 (23.5–28.4)	24.8 (23.3–26.7)	26.3 (23.5–29.9)	0.293
T1 native global mean (ms)	1,315 (1,276–1,349)	1,311 (1,282–1,342)	1,316 (1,261–1,369)	0.719
T1 post Gd global mean (ms)	502.6 (484.6–549.4)	506.6 (485.5–534.5)	496.8 (483.5–560.4)	0.797
T2 global mean (ms)	38.4 (36.1–39.7)	38.9 (35.8–39.8)	37.8 (36.2–39.19)	0.787
Maximum T1 native (ms)	1,625 (1,541–1,720)	1,618 (1,519–1,720)	1,633 (1,594–1,728)	0.684
High T1 native [*n* (%)]	20 (100%)	10 (100)	10 (100)	
Maximum T1 post Gd (ms)	581.0 (547.8–599.5)	582.0 (544.3–598.5)	575.0 (547.8–612.0)	0.912
Maximum T2 (ms)	53.0 (50.0–59.3)	52.0 (49.0–62.8)	54.5 (49.8–58.0)	0.796
High T2 [*n* (%)]	20 (100)	10 (100)	10 (100)	
Maximum T1 native/T1 native global mean	1.26 (1.16–1.31)	1.28 (1.14–1.31)	1.25 (1.20–1.31)	0.853
Maximum T1 post Gd/T1 post Gd global mean	1.1 (1.1–1.2)	1.1 (1.1–1.2)	1.1 (1.1–1.2)	0.796
Maximum T2/T2 global mean	1.39 (1.31–1.49)	1.35 (1.28–1.57)	1.39 (1.33–1.50)	0.724
Pericardial effusion	6 (30)	4 (40)	2 (20)	0.329

Data are presented as median (interquartile range), numbers and percentage. Comparisons of vaccine-associated myocarditis and non-vaccine-associated myocarditis were performed. Continuous parameters were tested for normal distribution using the Shapiro-Wilk test and compared using the Mann-Whitney *U* test or *t*-test as appropriate. Categorial parameters were tested using χ^2 test. ULN: upper limit of normal; LGE: late gadolinium enhancement; ECV: extra cellular volume; Gd: gadolinium.

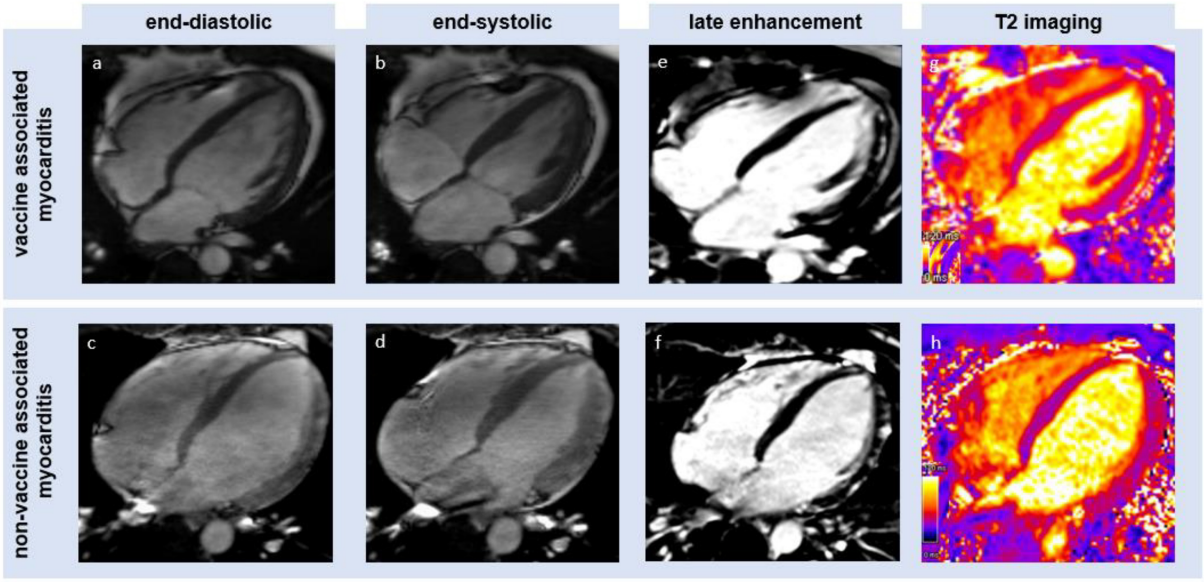

FIGURE 1
Illustration of cardiac magnetic resonance (CMR)-derived imaging (four chamber view) of an mRNA-based vaccination-associated myocarditis (upper row) and a non-vaccine-associated myocarditis (lower row). Panels **(a–d)** show left ventricular systolic function at the lower limit of normal. Panels **(e,f)** show a typical subepicardial late gadolinium enhancement. Panels **(g,h)** show a high signal on T2 mapping imaging as the result of edema.

non-steroidal anti-inflammatory drugs (group 1: 50%; group 2: 60%; $p = 0.653$) in equal partitions.

Discussion

The main findings of the study are summarized by the following points:

1. COVID-19 vaccine-associated myocarditis and regular viral myocarditis share the same CMR patterns of inflammation during the acute phase in a small, carefully matched cohort of young male patients, representing the most affected patient group.
2. In particular, the CMR measurements did not reveal any differences in terms of morphological and functional data when compared to the matched control group suffering from regular viral myocarditis. This is in concordance with a similar clinical presentation, ECG changes, and assimilable echocardiographic findings in patients of both groups.
3. Late gadolinium enhancement was the predominant pathological marker of myocarditis in this study. No differences in the spatial arrangement of the affected regions were found and the underlying tissue differentiation using T1, T2, and ECV mapping techniques showed highly comparable results.
4. The observed increased T1 times rather seem to reflect a state of an acute inflammation than myocardial fibrosis, considering normal ECV values. In fact, both groups of patients showed focal edema pertaining to the inflamed area suggested by increased T2 values.

Since some evidence suggests distinct differences between vaccination-associated myocarditis and cardiac COVID-19-related involvement (so-called COVID-19 myocarditis), we now add further data on comparing vaccination-associated myocarditis and classical myocarditis. In opposite to COVID-19-related cardiac injury, our data suggest that vaccine-associated myocarditis and regular viral myocarditis show the same CMR patterns of inflammation (15).

In addition to comparable functional and morphological parameters in CMR, both groups had a similar clinical presentation. In our cohort, all patients suffered from chest pain as the main symptom, which was previously described in other populations with COVID-19 vaccination-related myocarditis, already (4–17).

Both types of myocarditis involved an equivalent amount of myocardium within the inflammation and in keeping with findings in other causes of myocarditis, COVID-19 vaccination-related myocarditis predominantly affected the subepicardial layers of the lateral wall of the left ventricle (17–19).

As increased T2 value within the inflamed areas might demarcate small focal edema, the global T2 values were below the upper threshold of abnormality, which suggests

that no global edema was present in this very acute state. These observations agree with established knowledge of the progression of regular myocarditis and can be associated with minor myocardial damage.

Interestingly, imaging patterns of the viral and non-viral forms of myocarditis share the lateral wall as a predominant area of demarcation regardless of their distinct pathophysiology. This agrees with previous studies on both, viral myocarditis and COVID-19 vaccination-related myocarditis (31). In contrast to this, viral COVID-19 myocarditis was observed to show more diffusely distributed inflammation within the myocardium or a non-typical demarcation at the right ventricular insertion point (15–31). The pattern of lateral damage even in non-infectious causes, such as vaccination-related myocarditis, indicates a common ground lying pathophysiology, which may be related to immunologic reactions, which should be further investigated in future basic and translational research.

The similarity between the patterns of the vaccine-associated myocarditis and the regular viral myocarditis might be a reason for the fact that our control group was mostly balanced by laboratory markers, such as CK and troponin. Even if median troponin and CK levels in group 1 were higher as compared to group 2 without reaching statistical significance, both had analogical upper CK levels, which might suggest a comparable myocardial damage in both groups. However, it remains unclear, if the vaccine-associated myocarditis shows a similar progression as compared to other forms of myocarditis in general. Potentially, specific differences would have been shown up if fulminant forms of myocarditis would have been included. However, our findings agree with previously published data (4–32).

While the diagnosis of acute myocarditis is based on various parameters using T1- and T2-based imaging techniques (19), however, LGE, in particular, has been shown to be an important marker for risk stratification in non-ischemic cardiac myopathy (33). Myocardial deformation imaging, such as feature tracking or strain encoded (SENC) imaging, may provide additional capabilities for prognostication in non-ischemic cardiomyopathy and other patterns of myocardial injury (34–38), while being able to identify late-gadolinium-enhanced myocardial layers and even viable areas outside the directly affected regions (39–41). Furthermore, tissue tracking showed good agreement with ECV maps for the detection of myocardial fibrosis (42, 43). This offers a potential non-contrast-dependent diagnostic tool for tissue differentiation in the future. However, as different deformation imaging methods had a significantly varying agreement between the distinct techniques, global strain measurements showed the best reproducibility within each method (44). Therefore, we decided to just report global strain values for our study group, while the variability in-between the different techniques must be considered for interstudy comparisons and follow-ups.

In our matched study cohort, no differences could be observed with regard to volumetric and functional data on both echo and CMR. In contrast to this, a study by Fronza et al. recently described differences in functional parameters, such as GLS and LVEF, between COVID-19 vaccine-associated myocarditis and myocarditis of other causes with a trend of impaired left ventricular function in the non-vaccination-associated myocarditis group (17). As various aspects might impact this mismatch, it must be considered that the other study cohort was more heterogeneous, including women and older people, and CMR imaging was performed at a later timepoint after symptom onset. The combination of those factors might be a reason for the observed differences. In both studies, the presence of LGE was the parameter to majorly define the pathological presence of myocarditis and is in line with smaller case series (13).

Arguably, however, further differences might occur during later stages of myocarditis potentially offering a detailed insight into specific discrepancies of both forms of myocarditis and must be addressed in future prospective trials.

Notably, all patients with COVID-19 vaccination-related myocarditis have been found to be free of symptoms at the point of discharge already. While this observation is implying a promising outcome of vaccine-related myocarditis in young male patients as shown in earlier studies (10–13), a fast hospitalization and treatment after diagnosis might have been crucial to those results in our cohort.

Even though, we could not observe any adverse outcomes in our study cohort of young male patients with acute vaccine-associated myocarditis, this finding is limited by the small sample size. However, little is known about long-term follow-up data in patients suffering from mRNA vaccination-associated myocarditis. While in some cases, no pathological CMR patterns (increased T1 time and reduced LVEF) were resolved in the follow-up scan (45), other patients showed persistent LGE, even though initially abnormal global T1 times normalized and ECV values were decreased (18–46). As CMR shows promising capabilities for risk stratification in myocarditis, those preliminary results encourage future outcome studies, such as larger patient groups (47).

Limitations

It must be taken into account that the study cohort was small and retrospectively matched. A subsequent selection bias cannot be fully excluded due to this study design. It should be part of future research work to sample a comprehensive cohort of all vaccinated people minimizing these limitations.

We have focused on the most affected group in the early stage of rare COVID-19 vaccine-associated myocarditis in a small number of patients. Therefore, our findings might

not apply to the general population or other groups within vaccinated patients.

As exams at a later point of the disease's progression might detect further specific patterns of myocarditis, our collective was sampled at an acute point after symptom onset. This agrees with the current guidelines, however, a prospective trial with follow-up surveys is highly desirable to address this (48).

Mapping was performed using only one midventricular short-axis slice. Therefore, any inflammation or fibrosis in more basal or apical segments could have been missed. However, measurements were performed equally in both patient groups and in accordance with available published literature on this research topic (17).

Finally, even if we could not find any evidence of an infection, an ischemic or autoimmune disease, the association of the myocarditis with the vaccination cannot be proved with absolute certainty. It remains a diagnosis by exclusion.

Conclusion

COVID-19 mRNA vaccine-associated myocarditis does not show specific CMR patterns during the very acute stage in the most affected patient group of young men. The observed imaging findings are closely related to regular viral myocarditis and did not yield any evidence implying adverse outcomes in the investigated patient group.

Data availability statement

All relevant data are within the article and all data underlying the findings are fully available without restriction and can be accessed at the University Medical Center Göttingen by researchers who meet the criteria for access to confidential data.

Ethics statement

The studies involving human participants were reviewed and approved by Local Ethics Committee of the University Medical Center Göttingen. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

RE, AlS, and AnS: conceptualization, investigation, and writing – original draft preparation. RE and AlS: formal analysis. RE, AlS, TL, SB, DV, JK, SH, GH, and AnS: methodology, writing – review and editing, read, and agreed to the published version of the manuscript.

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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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The crucial role of cardiac MRI parameters in the prediction of outcomes in acute clinically suspected myocarditis: A functional and feature-tracking study

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Background: The definitive diagnosis of myocarditis is made by endomyocardial biopsy, but it is an invasive method. Recent investigations have proposed that cardiac MRI parameters have both diagnostic and prognostic roles in assessing myocarditis. We aimed to evaluate the role of functional and feature-tracking (FT)-derived strain values in predicting major adverse cardiovascular events (MACE) in patients with acute myocarditis.

Methods and results: We evaluated 133 patients with acute myocarditis (74.4% men) between January 2016 and February 2021. During a mean follow-up of 31 ± 16 months, sixteen patients (12.03%) experienced MACE: three deaths (2.3%), nine ICD implantations (6.76%), and five cardiac transplantations (3.8%). The left ventricular ejection fraction (LVEF), the LV end-diastolic volume index (EDVI), and the LV global longitudinal strain (GLS) were the strongest predictors of MACE. Each 1-unit decline in LVEF and LVGLS or 1-unit rise in LVEDVI resulted in a 5, 24, and 2% increase in MACE, respectively. LVEF ≤36.46% and LVGLS ≤9% indicated MACE with 75% sensitivity and 74.4 and 73.5% specificity, respectively.

Conclusions: In a group of acute myocarditis patients with evidence of myocardial edema and late Gadolinium enhancement, LVEF and GLS were the strongest predictors of adverse cardiac events.

KEYWORDS

acute myocarditis, major adverse cardiovascular events (MACE), cardiac MRI, feature tracking (FT), myocardial strain

Background

Myocarditis is an inflammatory myocardial disorder diagnosed by clinical, imaging, histological, immunological, and immunohistochemical criteria. Myocardial involvement is typically due to systemic viral infections, consisting of systemic inflammatory diseases and toxins; nonetheless, various infectious and non-infectious causes can result in myocarditis (1, 2).

It is difficult to ascertain the exact frequency of myocarditis due to the wide variety of clinical signs and symptoms. It is assumed that its incidence is 1–10 cases per 100 000 persons. Clinical history ranges from mild symptoms to findings of acute decompensation of heart failure. Feldman et al. classified myocarditis as follows: fulminant myocarditis, acute myocarditis, chronic active myocarditis, and chronic persistent myocarditis (3). Endomyocardial biopsy confers a definitive diagnosis of myocarditis, but it has considerable drawbacks. It is an invasive method and is prone to sampling error (4). The Lake Louise criteria (LLC) in cardiac magnetic resonance (CMR) examination consist of myocardial edema, hyperemia, and late gadolinium enhancement (LGE), and the updated LLC with mapping techniques, which constitute the cornerstone for diagnosing myocarditis, obviates the requirement for endomyocardial biopsy (2, 5).

Although the cardiac condition of most patients with myocarditis improves over time, some patients progress to devastating consequences. Accordingly, finding measures to predict prognosis is of utmost importance. Various CMR studies have derived different results on the predictive role of CMR parameters in patients with myocarditis (6, 7).

Strong evidence characterizes the role of echocardiographic strain especially global longitudinal strain (GLS) in evaluating different myocardial disorders (8, 9).

Recent research has underscored the value of CMR-derived myocardial strain measurement in the assessment of different types of cardiomyopathies (10–12). Evaluation of traditional CMR markers of the ejection fraction (EF) and LGE has a prognostic effect; nevertheless, little is known about the role of novel CMR methods, including feature tracking (FT) and mapping values (13).

Some previous studies have shown a significant association between cardiac prognosis and decreased ventricular strain values in myocarditis and other cardiomyopathies (6, 14–16).

In the present study, we aimed to evaluate the role of functional and FT-derived CMR strain values in predicting major adverse cardiovascular events (MACE) in patients with the diagnosis of acute myocarditis.

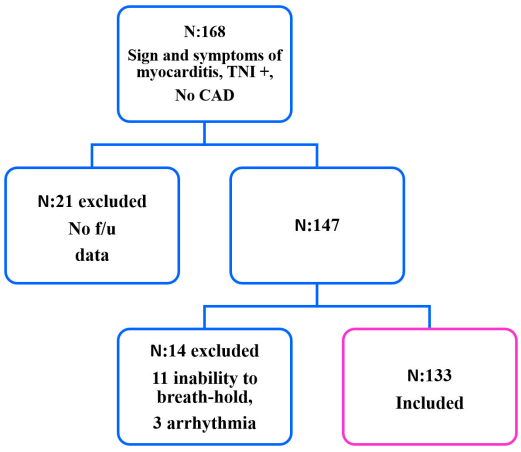

FIGURE 1
Flowchart of myocarditis patient selection. •f/u, follow-up; TNI, troponin I; CAD, coronary artery disease.

Methods

Study design and population

We evaluated all CMR examinations with a final diagnosis of acute myocarditis between January 2016 and February 2021. Based on electronic reports, the selection criteria were among patients who had all these four criteria: (1) History of acute symptoms (including dyspnea and chest pain) within a few days before admission. (2) Increased troponin levels. (3) Normal coronary angiography (no coronary artery disease) or CT angiography results during hospitalization. (4) CMR images with evidence of myocardial edema as well as mid-wall and subepicardial LGE (17). We found 168 CMR reports compatible with these myocarditis criteria. Among these reports, in 21 patients, the follow-up data was unavailable; in 14 subjects, the image quality was not acceptable (significant image artifact due to the patient's inability to breathe-hold in 11 patients and arrhythmia in three patients). Finally, 133 patients were selected with a diagnosis of acute myocarditis based on mentioned criteria (Figure 1). The exclusion criteria comprised the presence of a single criterion of myocardial edema or only being LGE + in the CMR study, subendocardial pattern of myocardial fibrosis, more-than-mild valvular disease, other cardiac pathologies including cardiomyopathy, and ischemic heart disease. An expert cardiologist with at least 6 years of experience in cardiac imaging conducted all measurements.

MACE was defined as the incidence of cardiac death, an implantable cardioverter-defibrillator (ICD) implantation, or heart transplantation.

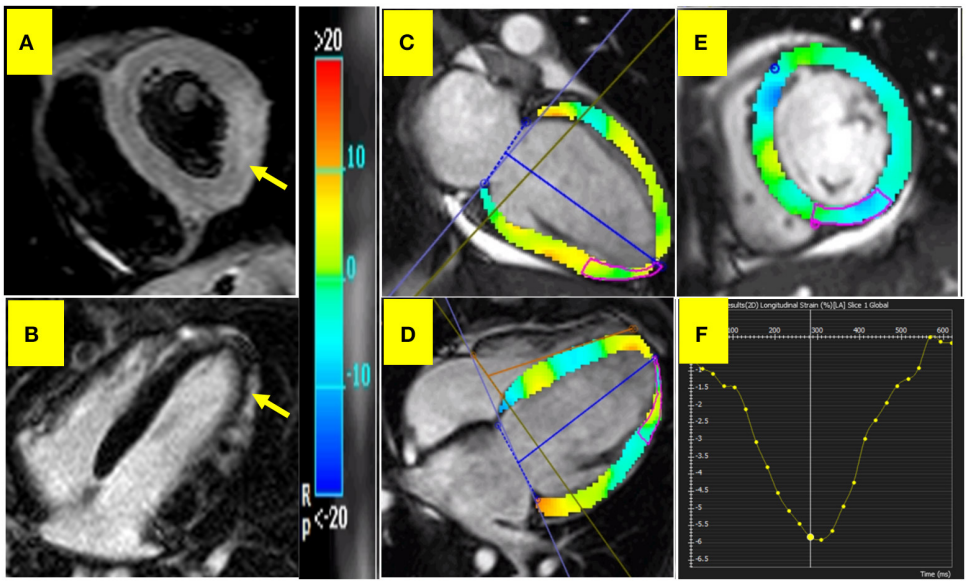

FIGURE 2
CMR in acute myocarditis. **(A)** myocardial inflammation. **(B)** Subepicardial LGE in LV inferolateral wall. **(C–E)** Depict feature tracking myocardial strain in 2, 4-chamber, and short-axis views, respectively. **(F)** Depicts longitudinal strain curve.

Follow-up

All the patients were assessed for the possible existence of MACE during a follow-up time of 10–69 months (mean ± SD: 31 ± 16 mon). We extract the follow-up data from patients' electronic records and telephone calls.

CMR examination

CMR was accomplished using a 1.5-Tesla scanner (Siemens Avanto, Erlangen, Germany) with an 8-element cardiac-phased array receiver surface coil.

CMR images

All ECG-gated cine functional sequences were done during an end-expiratory breath-hold. Left ventricular (LV) 2, 3, and 4-chamber views, as well as a stack of contiguous short-axis with LV coverage from inflow to apex, were acquired with sequence parameters, including a field of view (FOV) of 300 mm, an imaging matrix of 156 × 192, a slice thickness of 8 mm, no interslice gaps for short-axis images, and repetition time/ echo time of 31/1.2 ms. LV volume and LV systolic function were analyzed by tracing end-diastolic and end-systolic endocardial borders on cine short-axis images.

Myocardial inflammation was assessed utilizing the short tau inversion recovery (STIR) series as a ratio of myocardial-to-skeletal muscle signal intensity of more than 1.9 (Figure 2A).

LGE extent was determined visually by a 17-segment model; the presence and absence of LGE in each LV myocardial segment were scored as 1 and 0, respectively, and the sum of the scores (maximum: 17) was reported as the LGE-visual presence score (LGE-VPS) (18).

Due to the retrospective nature of our study, the mapping technique was unavailable. Therefore, we selected the myocarditis patients according to the old LLC, including 2 of 3 positive findings of myocardial hyperemia, edema, and LGE (Figure 2B).

Feature-tracking CMR method

CMR images were analyzed offline utilizing the FT software CVI 42 (Circle Cardiovascular Imaging, Calgary, Alberta, Canada), version 5.6.2 (634). All endocardial and epicardial borders were manually traced in the end-diastolic phase and then propagated to the whole cardiac cycle in short-axis stacks and 2-, 3-, and 4-chamber planes to identify 3D global longitudinal, circumferential, and radial strains (GLS, GCS, and GRS, respectively). Additionally, 2D right ventricular GLS was extracted from the 4-chamber view and RVGCS and RVGRS from

TABLE 1 Demographic and baseline CMR parameters of the study population.

Variables	Frequency (*n*)*	Percent %
Gender (male)	99	74.4
Cardiac death	3	2.3
ICD	9	6.76
Cardiac transplantation	5	3.8
Variables	**Mean**	**SD**
AGE (year)	36.5	16
LVEF (%)	41	14
RVEF (%)	46	13
LA volume (ml)	65	37
RA volume (ml)	51	47
LVEDVI (ml/m^2)	94	48
RVEDVI (ml/m^2)	83	31
LVGLS %	11.5	4.3
LVGCS %	13.5	5.1
LVGRS %	27.7	13.9
RVGLS %	19.6	7.2
RVGCS %	8.6	4
RVGRS %	14.3	7.9
LSSR 1/sec	−0.78	0.88
CSSR 1/sec	−0.88	0.51
RSSR	+1.9	1.2
LDSR	+0.89	1.8
CDSR	+0.86	0.38
RDSR	−1.86	1.17

*n, number; SD, standard deviation; LV, left ventricle; ICD, implantable cardioverter defibrillator; RV, right ventricle; LV, left ventricle; EF, ejection fraction; LA, left atrium; RA, right atrium; EDVI, end-diastolic volume index; ESVI, end-systolic volume index.
GLS, global longitudinal strain; GCS, global circumferential strain.
GRS, global radial strain; SV, stroke volume; LSSR, longitudinal systolic strain rate; CSSR, Circumferential systolic strain rate; RSSR, LSSR; Radial systolic strain rate; LDSR, longitudinal diastolic strain rate; CDSR, Circumferential diastolic strain rate; RDSR, Radial systolic strain.

the short-axis view. Image brightness was adjusted to discriminate between the endocardium and the blood pool. Longitudinal strain depicts longitudinal myocardial fiber shortening from the base to the apex, while circumferential strain shows the percentage of myocardial shortening around the perimeter (Figures 2C–F). On the other hand, radial strain denotes the percentage of myocardial wall thickening. All the strain values were expressed as absolute values.

Myocardial biopsy

All patient's electronic medical records were evaluated for myocardial biopsy data.

TABLE 2 Comparisons of CMR parameters between LVEF groups.

	LVEF ≥40% *n*: 81	LVEF <40% *n*: 52	
Variables	**Frequency (*n*)/ percent %**	**Frequency (*n*)/ percent %**	***P*-value**
Gender (male)	63 (77.8)	36 (69.2)	0.3
MACE	4 (4.94)	12 (23.08)	0.002
Cardiac death	1 (1.23)	2 (3.85)	0.56
ICD	2 (2.5)	7 (13.5)	0.02
Cardiac transplantation	1 (1.23)	4 (7.7)	0.07
Variables	**Mean ± SD**	**Mean ± SD**	
AGE (year)	33.8 ± 14.49	40.7 ± 17.7	0.01
LVEF (%)			
RVEF (%)	51.07 ± 7.99	39.1 ± 15.8	<0.001
LA volume (ml)	55.91 ± 27.09	79.6 ± 45.2	0.001
RA volume (ml)	44.67 ± 22.07	59.6 ± 69.9	0.14
LVEDVI (ml/m^2)	82.68 ± 28.0	112.2 ± 64.4	0.003
RVEDVI (ml/m^2)	79.31 ± 26.1	89.17 ± 37.62	0.1
LGE-VPS	2.44 ± 1.37	2.7 ± 2.4	0.4
LVGLS %	13.87 ± 3.1	7.9 ± 3.24	<0.001
LVGCS %	16.3 ± 3.3	9.21 ± 4.25	<0.001
LVGRS %	34.7 ± 12.0	16.84 ± 8.84	
RVGLS %	21.4 ± 6.3	16.7 ± 7.53	<0.001
RVGCS %	10.0 ± 3.63	6.41 ± 3.83	<0.001
RVGRS %	16.4 ± 6.8	10.89 ± 8.35	<0.001
LSSR 1/sec	−0.91 ± 1.08	−0.59 ± 0.28	0.03
CSSR 1/sec	−0.99 ± 0.54	−0.70 ± 0.42	0.001
RSSR	2.2 ± 1.1	1.20 ± 0.86	<0.001
LDSR	1.0 ± 2.26	0.65 ± 0.43	0.2
CDSR	0.99 ± 0.38	0.66 ± 0.30	<0.001
RDSR	−2.3 ± 1.1	−1.13 ± 0.79	<0.001

n, number; SD, standard deviation; LV, left ventricle; ICD, implantable cardioverter defibrillator; RV, right ventricle; LV, left ventricle; EF, ejection fraction; LA, left atrium; RA, right atrium; EDVI, end-diastolic volume index; ESVI, end-systolic volume index.
LGE-VPS, late gadolinium enhancement-Visual presence score.
GLS, global longitudinal strain; GCS, global circumferential strain.
GRS, global radial strain, SV, stroke volume; LSSR, longitudinal systolic strain rate; CSSR, Circumferential systolic strain rate; RSSR, Radial systolic strain rate; LDSR, longitudinal diastolic strain rate; CDSR, Circumferential diastolic strain rate; RDSR, Radial systolic strain.

Statistical analysis

The SPSS software, version 22.00, was utilized for analyses. Continuous variables were presented as the mean ± SD, while categorical variables were demonstrated as frequencies and percentages. Considering the sample size discrepancy between the MACE positive and MACE negative groups, we applied the Mann–Whitney *U* test to compare the parameters between the 2 groups. Independent sample *t*-test was to compare CMR parameters between groups with LVEF≥ 40 and <40%.

Significantly different variables were entered in the stepwise logistic regression analysis to find the significant variables in the regression model to reveal the occurrence of MACE. The receiver operating characteristic (ROC) analysis was utilized to find the cutoff values of CMR parameters for predicting MACE. The area under the curve (AUC), sensitivity, and specificity were reported. A cutoff value of 0.05 was considered to denote statistically significant results.

Results

The study population was composed of 133 patients, including 74.4% men, at a mean ± SD age of 37 ± 16 years diagnosed with myocarditis between January 2016 and February 2021. Table 1 demonstrates the demographic characteristics and CMR parameters of the study participants. All patients had normal coronary CT angiography/angiography results during hospitalization. The mean ± SD interval between CT angiography/angiography and CMR was: 3 ± 1.41 days. All CMR parameters were compared between groups with LVEF ≥40% and LVEF <40%. The results are depicted in Table 2.

Sixteen out of the 133 patients (12.03%) experienced MACE during a mean ± SD follow-up of 31 ± 16 months: three deaths (2.3%), nine ICD implantations (6.76%), and five cardiac transplantations (3.8%). One patient (0.75%) underwent ICD implantation and heart transplantation.

As we can evaluate the previous reports among patients with history of ICD implantation, seven out of nine patients had LVEF< 36%, and two out of nine patients had a history of recurrent VT that resulted in ICD implantation.

The Mann–Whitney *U* test revealed a significant difference in the following parameters in patients with and without MACE: biventricular EF and end-diastolic volume index (EDVI), left atrial (LA) volume, biventricular GLS, LVGCS, LVGRS, LV global longitudinal systolic strain rate and LV global circumferential diastolic strain rate (Table 3).

All significantly different variables were entered in the stepwise logistic regression analysis in the next step. Only LVEF and LVEDVI were meaningful in the model with an odds ratio of 0.946, confidence interval (CI): 0.904–0.989 (P = 0.01) and 1.017 and CI: 1.006–1.028 (P = 002), respectively. In other words, each 1-unit decrease in LVEF and increase in LVEDVI resulted in a 5 and 2% rise in the incidence of MACE, respectively.

The biventricular deformation parameters were entered in the stepwise logistic regression analysis. LVGLS was the meaningful variable with an odds ratio of 0.760 and CI:0.655–0.882 (P < 0.001). That is to say, each 1% decrease in LVGLS resulted in a 24% increase in the incidence of MACE.

The ROC curve was drawn upon to determine cutoff values (Figure 3). A maximum LVEF of 36.46% indicated MACE with 75% sensitivity and 74.4% specificity (AUC: 0.774, P<0.001). Furthermore, a maximum LVGLS of 9% predicted MACE with 75% sensitivity and 73.5% specificity (AUC: 0.784, P < 0.001).

TABLE 3 Comparison of age and CMR parameters between MACE negative and positive groups.

Variable	Median (IQR) MACE negative N = 117	Median (IQR) MACE positive N = 16	*P*-value
AGE (year)	37 (29)	35.5 (20)	0.2
LVEF (%)	44.14 (16.92)	23.00 (27.71)	<0.001
RVEF (%)	50.47 (13.09)	36.09 (27.54)	0.003
LA volume (ml)	53.00 (40.07)	69.39 (70.62)	0.04
RA volume (ml)	40.00 (30.67)	50.00 (39.32)	0.3
LVEDVI (ml/m^2)	81.42 (26.95)	108.34 (107.02)	0.01
RVEDVI (ml/m^2)	77.00 (33.38)	88.40 (67.00)	0.04
LVGLS %	12.00 (6)	7.39 (7)	<0.001
LVGCS %	14.63 (6)	7.24 (10)	0.001
LVGRS %	28.21 (19)	12.95 (23)	0.01
RVGLS %	21.23 (9)	13.06 (12)	0.003
RVGCS %	8.35 (5)	6.64 (8)	0.1
RVGRS %	13.00 (10)	10.12 (17)	0.3
LSSR 1/sec	−0.80 (0.39)	−0.49 (0.36)	0.001
CSSR 1/sec	−0.96 (0.35)	−0.70 (0.68)	0.07
RSSR	1.93 (1.17)	0.97 (2.51)	0.06
LDSR	0.73 (0.45)	0.56 (0.51)	0.1
CDSR	0.87 (0.48)	0.61 (0.50)	0.04
RDSR	−1.77 (1.38)	−1.31 (1.16)	0.1
LGE-VPS	2.00 (3)	2.00 (3)	0.5

SD, standard deviation; IQR, interquartile range; LV, left ventricle; RV, right ventricle; LV, left ventricle; EF, ejection fraction; LA, left atrium; RA, right atrium; EDVI, end-diastolic volume index; ESVI, end-systolic volume index.
GLS, global longitudinal strain; GCS, global circumferential strain.
GRS, global radial strain; SV, stroke volume; LSSR, longitudinal systolic strain rate; CSSR, Circumferential systolic strain rate, RSSR, Radial systolic strain rate, LDSR, longitudinal diastolic strain rate; CDSR, Circumferential diastolic strain rate; RDSR, Radial systolic strain; VPS, Visual presence score.

Based on the patient's electronic medical records, myocardial biopsy data were found in 11 (8.2%) cases. In two patients, the specimen was inadequate. Nine patients had evidence in favor of active myocarditis (myocardial inflammation and necrosis). The mean value and SD of the CMR parameters are depicted in Table 4. We assessed the MACE among these nine biopsy-proven patients and found three cases with ICD implantation, one with death, and three with heart transplantation. All patient had evidence of myocardial edema and fibrosis. LGE-VPS was evaluated, among them two patients had score five and nine, but others had score one.

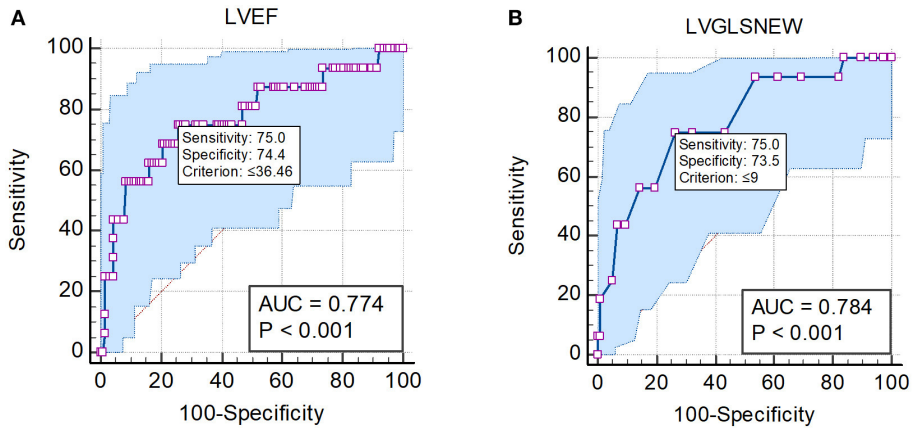

FIGURE 3
ROC analysis results for **(A)** LVEF. **(B)** LVGLS in patients with acute myocarditis.

Discussion

This retrospective cohort analysis reviewed the CMR findings of 133 patients with the diagnosis of acute myocarditis referred for CMR at the first presentation. We assessed the relationship between cardiac prognosis and functional and FT-derived strain parameters. A summary of our study results is as follows:

1. The strongest predictors of MACE were LVEF and LVEDVI.
2. The ROC analysis showed a sensitivity of 75% and a specificity of 74% for a maximum LVEF of 36.5% in the prediction of MACE. Each 1-unit decrease in LVEF resulted in a 5% increase in the incidence of MACE.
3. Among deformation parameters, LVGLS was the most potent predictor of MACE. A maximum LVGLS of 9% could predict MACE with a sensitivity of 75% and a specificity of 74%. A 1% decline in LVGLS resulted in a 24% increase in the incidence of MACE.

In our study population, the incidence rate of MACE was about 12%. In a prospective analysis of 539 unselected patients with 37 cases of myocarditis performed by Mordi et al. MACE occurred in 8.1% over a mean follow-up time of 2.2 years. (7). In our investigation, we selected patients with the diagnosis of acute myocarditis who were positive for both myocardial edema and LGE in CMR images. We showed that basic CMR measurements, including LVEF and LVEDVI, were the most powerful predictors of MACE. These parameters could be simply derived in routine CMR examination and provide important prognostic information. Moreover, we found that the LGE extent estimated by VPS was not different between MACE positive and negative groups. Similarly, Sanguineti et al. showed that different CMR parameters, including myocardial inflammation and the extent of LGE, were not predictive of the outcome in patients with a CMR-derived diagnosis of myocarditis without severe hemodynamic compromise. However, the initial alteration of LVEF was the only independent CMR predictor of adverse clinical outcomes (19).

In the present investigation, we included LGE + patients with acute myocarditis and found that LV function played an essential role in predicting the outcome even in patients with morphologically altered myocarditis. Our results also showed that LVEF with a cutoff value of 36.5% was able to predict MACE. It is logical to assume that moderate LV systolic dysfunction is the strongest predictor of adverse cardiac events.

Our analysis of the correlation between strain parameters and adverse cardiac events revealed that LVGLS with a cutoff value of 9% was the strongest deformation parameter to predict mortality, ICD implantation, and heart transplantation within the follow-up. Similarly, in a study by Garcia-Ropero et al. FT-derived LVGLS was an independent predictor of prognosis and disease severity among patients with acute myocarditis (20). In another investigation by Vos et al. (21) unlike RV strain and LA functional values, LV deformation parameters were the independent predictors of prognosis in patients with acute myocarditis. Porcari et al. (22) demonstrated that LVGLS was an independent prognostic factor superior to LGE in acute myocarditis patients with an LVEF exceeding 50%.

It is assumed that GLS represents the function of longitudinally oriented subendocardial myocardial fibers (23), while GCS widely depicts the function of the mid-myocardial circumferential fibers. Different pathologies

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TABLE 4 mean and SD of CMR parameters in nine myocarditis patients who underwent endomyocardial biopsy.

Variables	Mean	SD (±)
LGEVPS	2.81	2.48
LVEF	29.43	15.37
RVEF	36.23	16.09
LVEDVI	121.06	59.30
RVEDVI	97.83	34.06
LVGLS %	8.60	4.79
LVGCS %	10.28	6.40
RVGLS %	14.10	7.66
RVGCS %	7.32	5.27
LVGRS	20.62	16.88
RVGRS	12.61	9.62
LVGLS %	8.60	4.79
RSSR 1/sec	1.489	1.21
RDSR 1/sec	−1.50	0.95
CSSR 1/sec	−0.783	0.43
CDSR 1/sec	0.7418	0.42
LSSR 1/sec	−0.6245	0.36
LDSR 1/sec	0.8945	0.77

SD, standard deviation; LV, left ventricle; RV, right ventricle; LV, left ventricle; EF, ejection fraction; LA, left atrium; RA, right atrium; EDVI, end-diastolic volume index; ESVI, end-systolic volume index.
GLS, global longitudinal strain; GCS, global circumferential strain.
GRS, global radial strain; SV, stroke volume; LSSR, longitudinal systolic strain rat; CSSR, Circumferential systolic strain rate; RSSR, LSSR, Radial systolic strain rate; LDSR, longitudinal diastolic strain rate; CDSR, Circumferential diastolic strain rate; RDSR, Radial systolic strain.

can affect subendocardial longitudinal and mid-myocardial myofibers. Fischer et al. analyzed the association between prognosis and the CMR features of 455 patients and the diagnosis of myocarditis during a median follow-up of 3.9 years. FT-derived LVGLS provided cumulative prognostic value over clinical features, LVEF, and LGE. Still, they found no association between cardiac events and LVGCS (6). However, we demonstrated that CMR parameters, including LVEF and LVEDVI, had a more predictive power than strains. It seems logical to presume that patients with worse LV function and dilated LV are the subgroups more prone to the presence of cardiac complications during follow-up. Among global LV strain parameters, GLS had the strongest predictive role for adverse outcomes, which could be because abnormalities within radial and circumferential myocardial fibers are common in patients with acute myocarditis, but damage to longitudinal myocardial fibers represents more severe involvement of the LV myocardium.

Interestingly, we found that each 1% decrease in absolute LVGLS value caused a 24% increase in the incidence of MACE. It confirms the meaningful role of minor LV longitudinal strain alterations in demonstrating hard cardiac events in patients with acute myocarditis. We believe that FT-CMR strain measurement will play a more prominent role in the management of patients with myocarditis in the future.

We divided the study population into two groups according to LVEF> or <40%. All global strain values, EF and LVEDVI, differed between the two groups. We noticed no difference in the LGE extent between the patients with and without MACE or LVEF> or <40%. This research investigated myocarditis patients with edema and LGE in the acute phase MRI, which may represent underlying inflammation to a great extent and not precisely reflect the fibrosis. Thus, we suppose that the initial LGE extent probably is not a potent predictor for adverse outcomes.

A dearth of follow-up information forced us to select only hard events (i.e., cardiac mortality, ICD implantation, and heart transplantation) as outcomes, making these prognostic factors more invaluable. Functional and FT strain parameters can be calculated without prescribing a gadolinium contrast agent or requiring a unique cardiac sequence. In addition, they can select patients with acute myocarditis who need intense follow-up visits.

Based on electronic medical records, we found eleven patients with cardiac biopsy results consisting of nine with evidence in favor of acute myocarditis. Interestingly, seven out of nine patients had MACE during follow-up. It may refer to selecting patients with a more acute nature of the disease for myocardial biopsy, but prospective studies focusing on the role of cardiac tissue sampling are required.

In this research, we encountered some limitations. Firstly, we had no access to prognosis in a minority of patients, prompting their exclusion from the study and reducing the sample size. Future larger-scale multicentric investigations could address this drawback. Secondly, a lack of imaging data in a significant number of the studied patients precluded us from employing novel mapping methods. Additionally, we reported a relatively higher incidence of MACE. We think it is somehow related to referral bias; Many low-risk young myocarditis patients do not refer for cardiac MRI. In our study, almost all events happened during the first 3 months after the acute event suggesting more severe cases. Further prospective studies are required to determine the exact incidence of MACE. Additionally, due to the retrospective design of our study, we could only access the limited data regarding the patient's history and risk profile, and for many other cardiac complications, we did not have a clear recording; therefore, we had to omit them from the study. Furthermore, we suppose that different experts' functional measurements, including strain values, and reporting the interobserver variability may improve the results' reliability. Last but not least, we included only patients with acute myocarditis;

however, we suppose that a detailed CMR evaluation of other myocarditis categories and investigation of the prognostic role of measured parameters in each group will be of great value.

Conclusions

The present study evaluated the role of functional and deformation parameters in predicting outcomes in patients with acute myocarditis. LVEF and LVEDVI had a significant role in indicating cardiac mortality, heart transplantation, and ICD implantation. In addition, LVGLS had a strong association with MACE among deformation parameters.

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 Rajaie Cardiovascular Medicine and Research Center. 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.

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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Immunological response and temporal associations in myocarditis after COVID-19 vaccination using cardiac magnetic resonance imaging: An amplified T-cell response at the heart of it?

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Introduction: Although myocarditis after anti-SARS-CoV-2 vaccination is increasingly recognized, we have little data regarding the course of the disease and, consequently, the imaging findings, including the tissue-specific features. The purpose of this study is to describe the clinical, immunological, and cardiac magnetic resonance (CMR) features of myocarditis after COVID-19 immunization in the acute phase and during follow-up. We aimed to compare the trajectory of the disease to myocarditis cases unrelated to COVID-19.

Methods: We assembled a CMR-based registry of potentially COVID-19 vaccination-related myocarditis cases. All patients who experienced new-onset chest pain and troponin elevation after COVID-19 vaccination and imaging confirming the clinical suspicion of acute myocarditis were enrolled in our study. Participants underwent routine laboratory testing and testing of their humoral and cellular immune response to COVID-19 vaccination. Clinical and CMR follow-up was performed after 3–6 months. We included two separate, sex- and age-matched control groups: (1) individuals with myocarditis unrelated to COVID-19 infection or vaccination confirmed by CMR and (2) volunteers with similar immunological exposure to SARS-CoV-2 compared to our group of interest (no difference in the number of doses,

types and the time since anti-SARS-CoV-2 vaccination and no difference in anti-nucleocapsid levels).

Results: We report 16 CMR-confirmed cases of myocarditis presenting (mean ± *SD*) 4 ± 2 days after administration of the anti-SARS-CoV-2 vaccine (male patients, 22 ± 7 years), frequently with predisposing factors such as immune-mediated disease and previous myocarditis. We found that 75% received mRNA vaccines, and 25% received vector vaccines. During follow-up, CMR metrics depicting myocardial injury, including oedema and necrosis, decreased or completely disappeared. There was no difference regarding the CMR metrics between myocarditis after immunization and myocarditis unrelated to COVID-19. We found an increased T-cell response among myocarditis patients compared to matched controls ($p < 0.01$), while there was no difference in the humoral immune response.

Conclusion: In our cohort, myocarditis occurred after both mRNA and vector anti-SARS-CoV-2 vaccination, frequently in individuals with predisposing factors. Upon follow-up, the myocardial injury had healed. Notably, an amplified cellular immune response was found in acute myocarditis cases occurring 4 days after COVID-19 vaccination.

KEYWORDS

myocarditis, SARS-CoV-2 immunization, cardiovascular magnetic resonance, immunological response, vaccination, inflammation

Introduction

Increasing evidence links coronavirus disease 2019 (COVID-19) vaccination to rare cases of myocarditis and myopericarditis, primarily in the young adult (1) and adolescent (2) male population (3, 4). The connection between novel mRNA vaccines and these cases has been made. However, earlier data show that post-vaccination myocarditis may occur after a variety of vaccinations, including the smallpox vaccine that contains live virus (5).

Cardiac magnetic resonance (CMR) imaging is the method of choice for noninvasive visualization of myocardial injury (6–8). Case reports and case series demonstrated the role of CMR in the confirmation of myocarditis after anti-SARS-CoV-2 immunization. Importantly, these cases describe vaccine-induced myocarditis associated with mRNA vaccines, particularly after the second dose of the BNT162b2 mRNA-Pfizer-BioNTech and mRNA-1273-Moderna vaccines (9–12). An extensive cohort study from Israel based on hospital reporting systems described clinical follow-up data, but measures of cardiac function were not available (13). Therefore, we have little data regarding the course of the disease and, consequently, the CMR findings, including the tissue-specific features of myocarditis.

The underlying mechanism of the evolution of vaccination-related myocarditis is largely unclear. The proposed concepts include triggering of preexisting immune pathways and accelerated innate immunogenic reactions (4). Previously, it was also suspected that spike reactive mimicry might also play a role; however, this hypothesis has since been refuted by Marram et al. (14). However, these are primarily theoretical notions, as the immune response of myocarditis patients after COVID-19 vaccination has not been described (4).

The purpose of this study was to describe the clinical, CMR imaging and immunological features of different types of myocarditis after COVID-19 immunization in the acute phase and during follow-up. Second, we aimed to illustrate the features of myocarditis potentially linked to the COVID-19 vaccine in the context of myocarditis cases where vaccination or any contact with COVID-19 disease did not occur. Third, we describe the immunological response to COVID-19 immunization in patients with myocarditis and matched controls.

Methods

Study population

This is a retrospective CMR-based registry of myocarditis cases following COVID-19 immunization. We contacted all Hungarian institutions performing CMR scans ($n = 19$) between December 2020 and September 2021. All participants must exhibit the following inclusion criteria, to be admitted to the study: (1) COVID-19 vaccination not more than 21 days before the acute presentation; (2) presence of one or more of the following symptoms: new-onset chest pain, dyspnea,

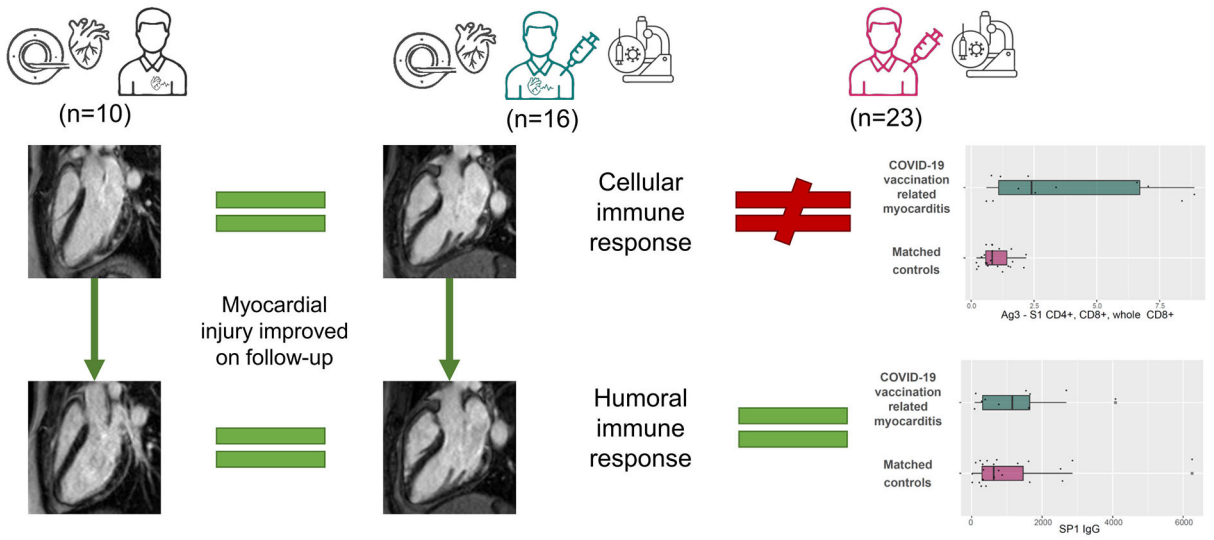

FIGURE 1
Visual abstract. We compared the CMR findings of myocarditis patients after COVID-19 vaccination (middle) to those of patients with myocarditis unrelated to COVID-19 immunization or infection (left). We did not find a difference between the groups in the acute (upper images) or follow-up (lower images) scans, but the myocardial injury improved. We compared the immune response of myocarditis patients after COVID-19 vaccination to COVID-19 immunization status-matched controls (right). There was no difference regarding the humoral immune response. In contrast, the cellular immune response was amplified in the myocarditis group.

or palpitation or syncope; (3) troponin elevation as per the local laboratory; and (4) CMR imaging confirming the clinical suspicion of acute myocarditis. Based on our criteria, four centers reported myocarditis cases after COVID-19 vaccination.

Study protocol

All participants completed a questionnaire regarding their acute symptoms and previous medical history, including their history of cardiovascular and immunological diseases. Cardiac biomarker levels, laboratory test results and 12-lead ECG results were recorded. Echocardiography and CMR examination were performed. Immunological tests were carried out in all acquiescent participants. Symptomatic patients (e.g., ongoing chest pain) were admitted to intensive/coronary care units (ICU/CCU) with continuous bedside monitoring. Asymptomatic patients with elevated cardiac troponin or patients discharged from ICU/CCU to general wards were monitored using telemetry. Follow-up examinations and CMR scans were carried out 3–6 months after the acute presentation in patients who consented. The study design is shown on Figure 1.

Ethical approval

Ethical approval was obtained from the National Public Health Center under the ethical standards laid out in the 1964 Declaration of Helsinki and its later amendments. IV/2568-1/2021/EKU. All participants or their legal guardian gave their written informed consent for the analysis.

Myocarditis comparator group

We included a group of myocarditis patients confirmed by CMR to illustrate the potential similarities and differences from the myocarditis patients after COVID-19 vaccination. The CMR comparator group was sex- and age-matched, retrospectively selected from the Semmelweis University CMR database according to the following criteria: (1) troponin elevation, (2) CMR examination confirming acute myocarditis was completed <2 weeks after the acute presentation, (3) CMR examination before the first reported case of SARS-CoV-2 infection in Hungary (2020.03.04.) OR negative PCR excluding the infection, and (4) follow-up CMR was carried out between 3 and 6 months after the acute scan. All control CMR scans were performed using a Siemens Magnetom Aera 1.5 T scanner. A comprehensive CMR protocol was carried out, including cine movies, T2-weighted spectral presaturation with inversion recovery (SPIR), T2 mapping using T2-prep balanced steady-state free precession (b-SSFP), T1 mapping using long-T1 5(3)3 and short-T1 5(3)3 modified look-locker inversion recovery (MOLLI) and late gadolinium enhancement (LGE) imaging. Functional evaluation was performed using b-SSFP cine sequences in four-chamber, two-chamber, and three-chamber long-axis views and a short-axis stack from the cardiac

base to apex with full coverage of the left ventricle and right ventricle. None of the myocarditis patients had a history of immune checkpoint inhibitor treatment.

CMR protocol

Overall, four Hungarian centers reported myocarditis cases after SARS-CoV-2 vaccination. CMR scans were performed on 1.5 T scanners (Siemens Magnetom Aera, Siemens Magnetom Amira, GE SIGNA Voyager, Phillips Ingenia). The CMR protocol had to include the following sequences regardless of the institution: cine sequence covering the whole heart for functional assessment, T2 weighted images or T1 mapping depicting myocardial oedema and LGE or T1 mapping showing necrosis or fibrosis. The protocol of the acute and control CMR scans was similar in most cases, although we accepted control CMR scans without T2-weighted images. If a control CMR scan was not possible in the original institution, the participant was offered a CMR scan slot at the Semmelweis University Heart and Vascular Center ($n = 2$). Mapping sequences were available from 3 institutions ($n = 13/16$). LGE images were acquired using segmented inversion recovery sequences 10–15 min after administration of an intravenous bolus of gadolinium-based contrast agent (gadobutrol in 0.15 ml/kg, or gadoteric acid in 0.4 ml/kg) at a rate of 2–3 ml/s through an antecubital intravenous line. The inversion time was adjusted to provide optimal suppression of normal myocardium.

CMR analysis

CMR scans were collected in raw DICOM format, and all post-processing analyses were conducted in a core CMR laboratory using the Medis Suite Software (Medis Medical Imaging Software, The Netherlands) to minimize observer-related variance. LV and RV volumes, function and mass were calculated from the SA stack using artificial intelligence-based automated contour detection (autoQ module) with manual adjustments if necessary. Short-axis LGE images were contoured manually, and then the LGE mass and LGE% were quantified using the 5SD technique with manual adjustments if required in the Medis QMass module. Myocardial native T1 and T2 relaxation times were consequently measured in the midventricular or basal septum (15) (if the midventricular images were technically inadequate for analysis) of the myocardium using motion-corrected images. One further ROI was manually drawn to the affected area guided by visual inspection (15). The comparison regarding mapping values was carried out in participants who underwent their CMR examination and at Semmelweis University Heart and Vascular Center ($n = 9$). Elevated T1 and T2 values were defined based on sequence-specific cut-offs of 2 standard deviations (SDs) above the respective means of the healthy male controls (T1: 1,000 ms, T2: 49 ms).

Acute myocarditis was defined as per the modified Lake Louise criteria (LLC) (7). Specifically, at least two positive main LLC criteria in corresponding locations were necessary for the diagnosis. At least one positive criteria for oedema visualization (T2-weighted images, T2 mapping or T1 mapping) and at least one positive criteria for necrosis visualization

TABLE 1 Baseline characteristics.

Age, years	22 ± 7
Sex, male %	16 (100)
BMI	26 ± 4
SARS-CoV-2 vaccine type *n*, (%)	
mRNA	
- Pfizer (BNT162b2 mRNA-Pfizer- BioNTech)	10 (62.5)
- Moderna (mRNA-1273-Moderna)	2 (12.5)
Vector vaccine	
- Sputnik V (Gam-COVID-Vac)	4 (25)
SARS-CoV-2 vaccine dose *n*, (%)	
- First dose	2 (12.5)
- Second dose	13 (81.2)
- Third dose	1 (6.2)
First complaint after vaccination, days	1.8 ± 1.6
Chest pain after vaccination, days	3.8 ± 1.9
Previous SARS-CoV-2 infection yes, *n* %	2 (12.5)
Previous myocarditis yes, *n* %	2 (12.5)
Positive immunological history	4 (25)
- Crohn's disease, *n* %	1 (6.2)
- Asthma, *n* %	1 (6.2)
- Psoriasis, *n* %	1 (6.2)
- Allergy, *n* %	1 (6.2)
Cardiovascular risk factors	
- Hypertension, *n* %	2 (12.5)
- Diabetes, *n* %	0 (0)
- Smoking, *n* %	4 (25)
- Obesity, *n* %	3 (18.8)
Intense physical activity after vaccination	4 (25)
- Sport activity	3 (18.8)
- Physically demanding job	1 (6.2)
Elevated troponin level *n*, %	16 (100)
CKMB (U/L) *Cut-off: ≥ 25 U/L*	31 [26, 62]
C-reactive protein (mg/L) *Cut-off: ≥ 5 mg/L*	23 [13, 43]
NTproBNP (pg/ml) *Cut-off: ≥ 125 pg/ml*	351 [223, 677]
Thrombocyte count (Giga/L) *Normal range: 150–400 Giga/L*	214 [199, 229]
White blood cell count (Giga/L) *Normal range: 4.0–10.0 Giga/L*	7.9 [5.7, 9.5]
Eosinophil count (Giga/L) *Cut-off: >0.5 Giga/L*	0.10 [0.07, 0.17]

Baseline characteristics.
CKMB, Creatine kinase-MB; NTproBNP, N-terminal pro B-type natriuretic peptide; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2.

(LGE or T1 mapping). The interpretation of CMR scans was standardized: the presence and pattern of myocardial oedema and LGE was visually defined independently by two EACVI certified observers (VH EACVI level 3-certified CMR specialists with more than 15 years of experience in CMR reporting and LS completed her EACVI written certification and has 3.5 years of experience reporting CMR). In case of disagreement between the observers, a third level 3 EACVI-certified CMR specialist (AT) with more than 15 years of experience in CMR reporting was consulted for consensus. Non-ischaemic LGE was defined as midmyocardial and/or subepicardial myocardial LGE confirmed in two perpendicular views.

Control group for immunological studies

The immune response of the study participants was compared with that of 23 sex- and age-matched controls from the Semmelweis University database. Subjects included in the control group were comparable to the myocarditis group regarding the doses and type of anti-SARS-CoV-2 vaccine they received and the time elapsed since their vaccination. We objectively quantified SARS-CoV-2 exposure using anti-nucleocapsid protein levels, which showed no difference between myocarditis patients after COVID-19 vaccination and controls. This matching step was crucial, as more participants reported previous SARS-CoV-2 infection in the control group than in the myocarditis group.

Laboratory protocol

Participants underwent routine laboratory testing for biomarkers including troponin, CKMB, CRP, white blood cell count, and eosinophil cell count. Antinuclear antibodies (ANAs), extractable nuclear antigen antibodies (ENAs), antineutrophil cytoplasmic antibodies (ANCAs) and serum immunoglobulin (IgG, IgM, IgA) levels were also measured from myocarditis samples ($n = 10$). A subgroup of myocarditis patients after COVID-19 vaccination ($n = 12$) and all immunization-matched controls ($n = 23$) underwent an evaluation of humoral and cellular immune responses at Semmelweis University. The immunology protocol and their interpretation were standardized to allow meaningful comparisons. Enzyme immunoassay providing semiquantitative *in vitro* determination of human antibodies of the immunoglobulin class IgG and IgM against modified nucleocapsid protein (NCP) of SARS-CoV-2 in serum or plasma has been obtained (referred to in the text as NCP-IgG and NCP-IgM). The results are given as a ratio (extinction of the sample/extinction of calibrator). The results below 0.8 are considered negative, the results equal to or above 0.8 and below 1.1 are considered borderline, and the results equal to or above 1.1 are considered positive due to the test description. SARS-CoV-2-specific antibodies (referred to in the text as S1 Ig) were analyzed using an Elecsys Anti-SARS-CoV-2 S immunoassay (Roche Diagnostics International Ltd, Switzerland) on a Cobas e6000 machine. The test detects antibodies specific to the SARS-CoV-2 spike (S) protein receptor-binding domain (RBD) in human serum and plasma. The method uses electrochemiluminescence to quantitatively determine antibodies based on the double-antigen sandwich principle. The test cut-off was $\geq$0.8 as per the manufacturer. The detailed immunoglobulin response was determined using the ELISA test, and the sample dilution was performed manually; further steps were carried out automatically using an Elite Lite (DAS, Italy) device. We will refer to the IgG and IgA immunoglobulins recognizing the S1 domain of the spike protein determined by ELISA as SP1 IgG and IgA for transparency. We quantified immunoglobulin levels in a quantitative (SP1 IgG) or semiquantitative (SP1 IgA) manner (16). The T-cell response was assessed via the QuantiFERON SARS-CoV-2 assay, an interferon-gamma release assay described in detail elsewhere (17). In short, this assay consists of three antigen tubes, SARS-CoV-2 Ag1, Ag2 and Ag3, that use a combination of proprietary antigen peptides specific to SARS-CoV-2 to stimulate lymphocytes involved in cell-mediated immunity in heparinized whole blood. The Ag1 tube contains CD4+ epitopes derived from the S1 subunit RBD of the spike protein. The Ag2 tube contains CD4+ and CD8+ epitopes from the S1 and S2 subunits of the spike protein. The Ag3 tube consists of CD4+ and CD8+ epitopes from S1 and S2 and immunodominant CD8+ epitopes derived from the whole SARS-CoV-2 genome.

Data management and statistical analysis

Statistical analysis and data visualization were performed using MedCalc software V.18.11 (Belgium) and RStudio (Version 1.3.1.093, RFoundation, Austria). The Shapiro–Wilk test was applied to test the normality of our data. Continuous variables showing a normal distribution are presented as the mean and SD, and those showing a non-normal distribution are reported as medians and IQRs. Categorical variables are presented as frequencies and percentages. Acute and follow-up examinations were compared using paired sample *t* tests and Wilcoxon tests. We applied analysis of covariance (ANCOVA) to formally test the difference between the trajectory of myocarditis after SARS-CoV-2 vaccination and myocarditis unrelated to COVID-19. Chi tests were applied to compare the distributions of categorical data. Comparisons between the immunological response of myocarditis patients after SARS-CoV-2 vaccine and the comparator group were conducted using independent samples *t* tests and Mann–Whitney *U* tests as appropriate. Associations were assessed using Spearman's rank correlation

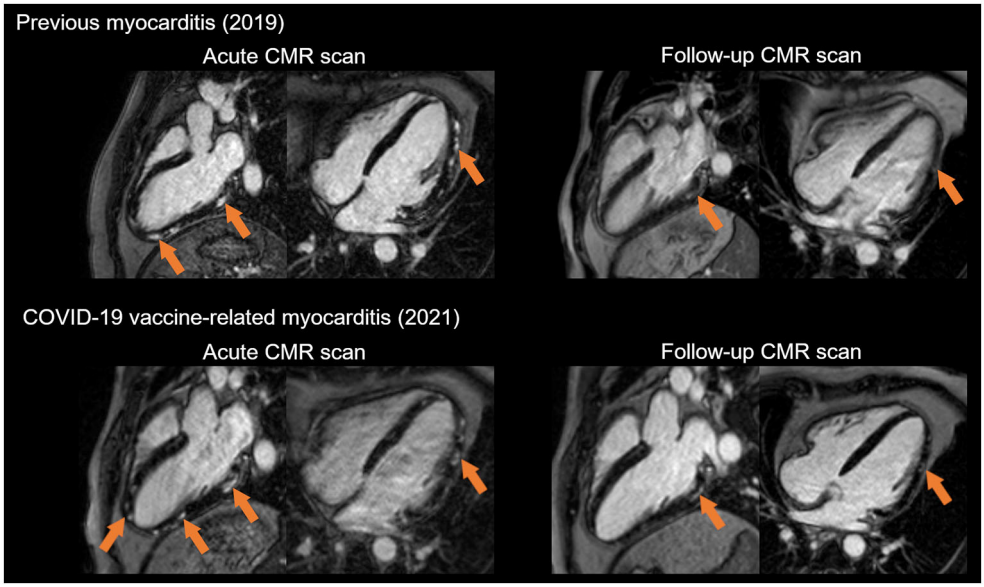

FIGURE 2
Recurrent myocarditis in a young male patient after the second dose of anti-COVID-19 vector vaccine. Our patient had prior myocarditis in 2019. At the time, he presented with chest pain preceded by gastrointestinal infection and fever. He had elevated troponin levels, and the CT coronary angiogram was negative. The acute CMR showed patchy subepicardial oedema and late gadolinium enhancement (LGE) (orange arrows). Three months later, on his follow-up scan, the oedema disappeared, and the LGE shrank. In 2021, the patient experienced fever and recurrent chest pain 2 days after the second dose of the COVID-19 vaccine. His acute CMR imaging showed LGE in a similar pattern as during the first acute myocarditis episode. Notably, signs of myocardial injury resolved on the follow-up scan.

analyses. Probability values were two-sided, and p values of <0.05 were considered significant. All data are available on reasonable request.

Results

Description of clinical characteristics

A total of four centers reported 16 CMR-confirmed cases of myocarditis following SARS-CoV-2 immunization, with chest pain presenting a mean of 4 ± 2 days after vaccination. Patient characteristics are included in Table 1. All of them were young (five were <18 years, mean age 22 ± 7 years, between 13 and 36 years) male patients and generally presented after their second dose of COVID-19 immunization (13, 81%). Most of them received mRNA vaccines (75%), while 25% presented with myocarditis after receiving a vector vaccine. Three patients reported prior SARS-CoV-2 infection, and one of them developed acute myocarditis after the first dose of vaccine. Two participants had acute myocarditis in their previous medical history confirmed by CMR imaging (Figure 2). In these cases, the time elapsed from the prior myocarditis to vaccination was 2 and 4 years, respectively. Four patients reported immune-mediated diseases, including Crohn's disease, psoriasis, asthma and allergies. None of the patients received systemic corticosteroid therapy. Overall, four participants reported intensive physical activity directly after vaccination (intensive sport activity, heavy physical labor), and

TABLE 2 Peak troponin value for myocarditis patients after COVID-19 vaccination.

Case no	Cardiac troponin type	Local cut-off	Peak value
1	hs troponin T (ng/L)	>14 ng/L	1,159
2	hs troponin T (ng/L)	>14 ng/L	1,007
3	hs troponin T (ng/L)	>14 ng/L	376
4	hs troponin T (ng/L)	>14 ng/L	1,366
5	hs troponin T (ng/L)	>14 ng/L	3,018
6	hs troponin T (ng/L)	>14 ng/L	144
7	hs troponin I (pg/ml)	>19 gp/ml	11,907
8	hs troponin I (μg/L)	>0.0198 μg/L	4.067
9	hs troponin T (ng/L)	>14 ng/L	2,136
10	hs troponin T (ng/L)	>14 ng/L	212
11	hs Troponin I (pg/ml)	>34.2 pg/ml	7,665
12	hs troponin T (ng/L)	>14 ng/L	220
13	hs troponin T (ng/L)	>14 ng/L	2,431
14	Troponin I (ng/L)	>19 ng/L	4,047
15	hs troponin I (pg/L)	>30 gp/ml	3,976
16	hs troponin T (ng/L)	>14 ng/L	228

Maximal troponin values for each participants is reported according to the local laboratory. hs, high-sensitive.

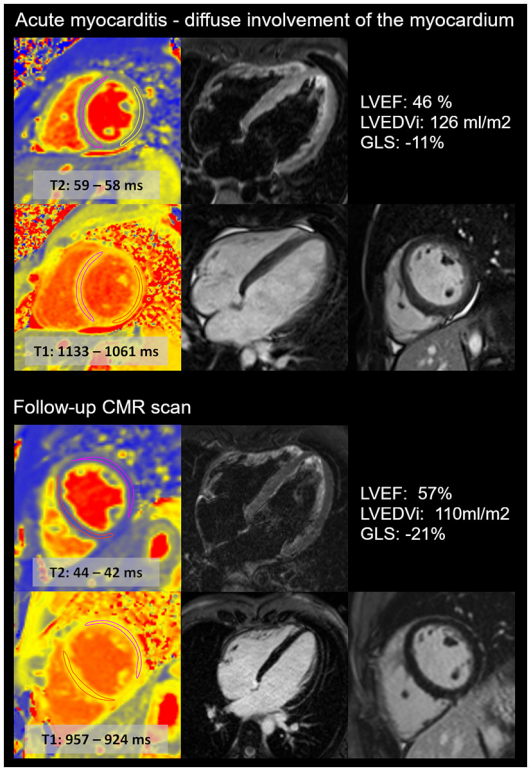

FIGURE 3
Diffuse acute myocarditis after the second dose of anti-COVID-19 mRNA vaccine in a young athlete. CMR images show the acute (upper images) and follow-up (lower images) scans of a young, highly trained athlete (national team member). The first CMR scan confirmed acute myocarditis with diffuse involvement of the myocardium, with elevated T2 and T1 mapping and diffuse myocardial oedema. The left ventricular ejection fraction (LVEF) was mildly decreased, and global longitudinal (GLS) strain was decreased during the acute scan. The follow-up scan revealed the normalization of T2 and T1 mapping values and left ventricular systolic function. The LVEDVi decreased. No LGE was present. The patient was prohibited from participation in sports activity for the first 3 months, and then he gradually returned to sports activity. Currently, the athlete performs a high level of sports activity and does not report recurrent or persisting symptoms.

one individual noted heavy alcohol consumption following immunization. The first systemic symptoms (fever, shivering) developed within 2 days, and chest pain presented a mean of 4 days after vaccination in all patients. ECG alterations were documented in seven patients (ST elevation in 6, negative T wave in 1). The initial troponin level was elevated in all study participants (Table 2), and we frequently noted CKMB, CRP and proBNP elevation as well. The white blood cell count, eosinophil count, and other markers remained in the normal range. During the acute phase, there were no heart failure symptoms, syncope, or documented sustained brady- or tachyarrhythmias.

CMR features of acute myocarditis after COVID-19 immunization

CMR was performed on average 4 ± 2 days (between 1 and 8 days) after the onset of acute chest pain. The majority of the cases showed a localized pattern of myocarditis, mainly affecting the lateral wall of the left ventricle with signs of subepi-midmyocardial oedema and necrosis (Figure 2). In one case, we found diffuse myocarditis with elevated T2, T1 and ECV values (Figure 3) caused by the mRNA vaccine. The left ventricular ejection fraction (LVEF) was in the normal range for most cases, except for two patients whose LVEF was mildly decreased (46 and 47%). Notably, these two patients had no previous history of acute myocarditis. There was no definitive pericardial involvement in any patients.

Clinical status and CMR changes during follow-up

During our follow-up, one patient experienced a recurrent episode of acute myocarditis (3 months after the vaccine), preceded by gastrointestinal infection. Other patients did not report symptom recurrence. The hs Troponin T (6[4, 7] ng/L), CKMB (2[2, 11] U/L), CRP (2[1, 3] mg/L) and proBNP (29[12,49] pg/ml) values returned to the normal range. Follow-up CMR was carried out 112 ± 27 days after the baseline scan ($n = 14$). We found that the LVEF marginally increased upon follow-up, and LVEDVi slightly decreased, both remaining in the normal range (Table 3). Elevated T2 values depicting local oedema in the affected area were resolved. The native T1 value and ECV measured in the affected area also decreased; however, ECV remained slightly elevated. The LGE area shrank in all participants and disappeared completely in 31% (4/13) of cases. The highly trained athlete in whom all signs of acute myocarditis disappeared on follow-up (Figure 3) was able to gradually return to sports activity. He restarted exercising 3 months ago and did not experience recurrent or persisting symptoms.

Myocarditis after SARS-CoV-2 immunization vs. myocarditis unrelated to COVID-19

The considering the effect of both follow-up time and myocarditis group, the ANCOVA test showed no difference between the trajectory of cardiac volumes, function, mass, oedema and LGE between myocarditis patients immunization and age- and sex-matched myocarditis patients unrelated to COVID-19 vaccination or infection (male patients, 22 ± 7 vs. 23 ± 6 years). Notably, we found a marginal difference between

TABLE 3 Comparison between acute and follow-up CMR scans of myocarditis patients after COVID-19 vaccination.

	Acute myocarditis after COVID-19 vaccination ($n = 16$)	Follow-up myocarditis after COVID-19 vaccination ($n = 14$)	Acute *vs.* follow-up CMR, myocarditis after COVID-19 vaccination (P values)
Elapsed time, days	4 ± 2	112 ± 27	NA
LVEF, %	58 ± 6	60 ± 3	0.042
LVEDVi, ml/m^2	87 ±13	83 ± 9	0.046
LVSVi, ml/m^2	50 ± 7	50 ± 6	0.961
LVMi, g	53 ± 10	51 ± 7	0.228
GLS, %	−20.5 [−22.5, −19]	−21 [−22, −20]	0.083
RVEF, %	58 ± 4	57 ± 5	0.559
RVEDVi, ml/m^2	83 ± 10	84 ± 9	0.722
RVSVi, ml/m^2	48 ± 6	48 ± 6	0.489
T1 mapping septal, ms	966 [951, 1,016]	957 [950, 965]	0.578
T1 mapping affected area, ms	1,056 [1,038, 1,113]	976 [953.5, 1,018]	0.031
T2 mapping septal, ms	43 [43, 44]	43 [42, 43]	0.375
T2 mapping affected area, ms	51 [50, 55]	44 [43, 47.5]	0.016
ECV septal, %	26 [24, 28]	25.5 [23.5, 27.5]	0.125
ECV affected area,%	38 [35, 41.5]	30.5 [28, 35]	0.016
LGE g	6 [3, 10]	2 [0.5, 4]	0.001
LGE %	7 [3, 12]	3 [1, 4]	0.001

Comparison between acute and follow-up CMR scans myocarditis after COVID-19 immunization. Continuous variables showing a normal distribution are presented as the mean and standard deviations (± SD), and those showing a non-normal distribution are reported as medians and interquartile ranges [IQRs]. Acute and follow-up examinations were compared using paired sample t tests and Wilcoxon tests.

CMR, cardiac magnetic resonance; ECV, extracellular volume; EDVi, left ventricular end diastolic volume index; EF, ejection fraction; ESVi, end systolic volume index; GLS, global longitudinal strain; Mi, mass index; NA, not applicable; LGE, late gadolinium enhancement; LV, left ventricular; RV, right ventricular; SVi, left ventricular stroke volume index.

T1 mapping (Table 4). Figure 4 illustrates the trajectory of CMR metrics between acute and follow-up scans in the both groups.

Assessment of the immunological response

Markers of the SARS-CoV-2 immune response were obtained for 12 patients. The test was performed a mean of 109 and 86 days after the first and second doses, respectively. Similarly, immunological testing was ascertained for the control group at a mean of 108 and 81 days after the first and second doses of anti-SARS-CoV-2 vaccine. The main difference between myocarditis patients and the comparator population was in terms of their history of previous SARS-CoV-2 infection (25 vs. 91%); however, anti-NCP (IgG, IgM) testing showed no difference between the two groups. There was no significant difference in the humoral immune response of myocarditis patients after SARS-CoV-2 immunization and those of sex- and age-matched controls (male patients, 22 ± 7 vs. 22 ± 6 years) (Table 5). In contrast, we found an increased T-cell response in myocarditis patients compared to controls ($P < 0.01$). We found that S1 IgG and IgA values negatively correlated with the time elapsed since the first vaccination (Supplementary Figure 1). Markers of the humoral immune response showed higher values after the mRNA vaccine than after the vector vaccine. At the same time, there was no difference regarding the cellular immune response between the two groups (Supplementary Table 1).

Notably, there was no difference in the immune response of myocarditis patients with or without predisposing factors (Supplementary Table 2).

Finally, there was no correlation between the humoral immune response (S Ig, SP1 IgG, SP1 IgA) and LVEF. In contrast, we found that the T-cell response parameters showed a negative correlation with the marker of systolic function (Figure 5).

Discussion

Summary of findings

The present data confirm and extend previous observations regarding the association of COVID-19 vaccination with myocarditis. This study of myocarditis patients after COVID-19 immunization confirmed by CMR makes the following contributions. First, in a cohort of acute myocarditis presenting a mean of 4 days after COVID-19 vaccination, we found

TABLE 4 Assessment of the trajectory of myocarditis patients after SARS-CoV-2 immunization and myocarditis patients unrelated to COVID-19 immunization or infection over the acute phase and follow-up using analysis of covariance.

CMR metricss	Effects	ANCOVA test *P*
LVEF, %	Group	0.476
	Group:Time	0.613
	Time	0.013
LVEDVi, ml/m2	Group	0.752
	Group:Time	0.445
	Time	0.044
LVSVi, ml/m2	Group	0.954
	Group:Time	0.599
	Time	0.641
LVMi, g	Group	0.676
	Group:Time	0.548
	Time	0.051
GLS, %	Group	0.318
	Group:Time	0.812
	Time	0.102
RVEF, %	Group	0.701
	Group:Time	0.384
	Time	0.924
RVEDVi, ml/m2	Group	0.435
	Group:Time	0.501
	Time	0.253
RVSVi, ml/m2	Group	0.601
	Group:Time	0.795
	Time	0.527
T1 mapping septal	Group	0.171
	Group:Time	0.382
	Time	0.002
T1 mapping affected area	Group	0.513
	Group:Time	0.04
	Time	<0.001
T2 mapping septal	Group	0.278
	Group:Time	0.741
	Time	0.075
T2 mapping affected area	Group	0.467
	Group:Time	0.175
	Time	<0.001
ECV septal	Group	0.041
	Group:Time	0.852
	Time	0.112
ECV affected area	Group	0.035
	Group:Time	0.92
	Time	<0.001
LGE g	Group	0.32
	Group:Time	0.554
	Time	<0.001
LGE %	Group	0.164
	Group:Time	0.438
	Time	<0.001

(Continued)

Analysis of covariance (ANCOVA) test results are shown for each CMR metrics, taking into account the effect of the patient group (myocarditis patients after SARS-CoV-2 vaccination vs. myocarditis not linked to SARS-CoV-2 infection) and time of the CMR scan (acute vs. follow-up CMR scan) and the combination of these effects. Models are unadjusted.

CMR, cardiac magnetic resonance; ECV, extracellular volume; EDVi, left ventricular end diastolic volume index; EF, ejection fraction; ESVi, end systolic volume index; GLS, global longitudinal strain; Mi, mass index; LGE, late gadolinium enhancement; LV, left ventricular; RV, right ventricular; SVi, left ventricular stroke volume index.

that 75% had received mRNA vaccines and 25% vector vaccines. Second, on the follow-up visit, a mean of 112 days after the acute presentation, CMR abnormalities depicting myocardial injury, decreased, or completely disappeared. Third, there was no apparent difference regarding CMR metrics between myocarditis cases potentially associated with COVID-19 vaccination and myocarditis unrelated to COVID-19. Finally, we found an increased T-cell response among myocarditis patients after vaccination compared to matched controls.

Comparison with existing literature

Our patients invariably presented with fever followed by chest pain and elevated troponin levels, typically 2–4 days after the second dose of the COVID-19 vaccine. This finding is consistent with previous reports (1, 12, 18). There was no evidence of ongoing SARS-CoV-2 infection or other viral infection in any of the participants. While most of our patients presented after the mRNA vaccine, similar to what studies from the US and Israel found (1, 13), 25% of all cases presented after receiving the Sputnik V vaccine. In Hungary, ∼40% of the population between the ages of 16 and 35 received a vector anti-SARS-CoV-2 vaccine (19), suggesting that myocarditis after COVID-19 vaccine might be less skewed toward mRNA vaccines than previously reported (20). Notably, at the time of our study, only the Pfizer-BioNTech vaccine was authorized to immunize the adolescent population ($n = 5$ in our cohort), who seem to be more prone to this adverse effect (4). This might limit meaningful comparison of the risk of myocarditis associated with different COVID-19 vaccines. Interestingly, a study based on the Vaccine Adverse Events Reporting System (VAERS) already cautioned against using mRNA vaccines among those with a higher risk for myocarditis and encourages vector vaccines as a safer alternative (20). However, a passive reporting

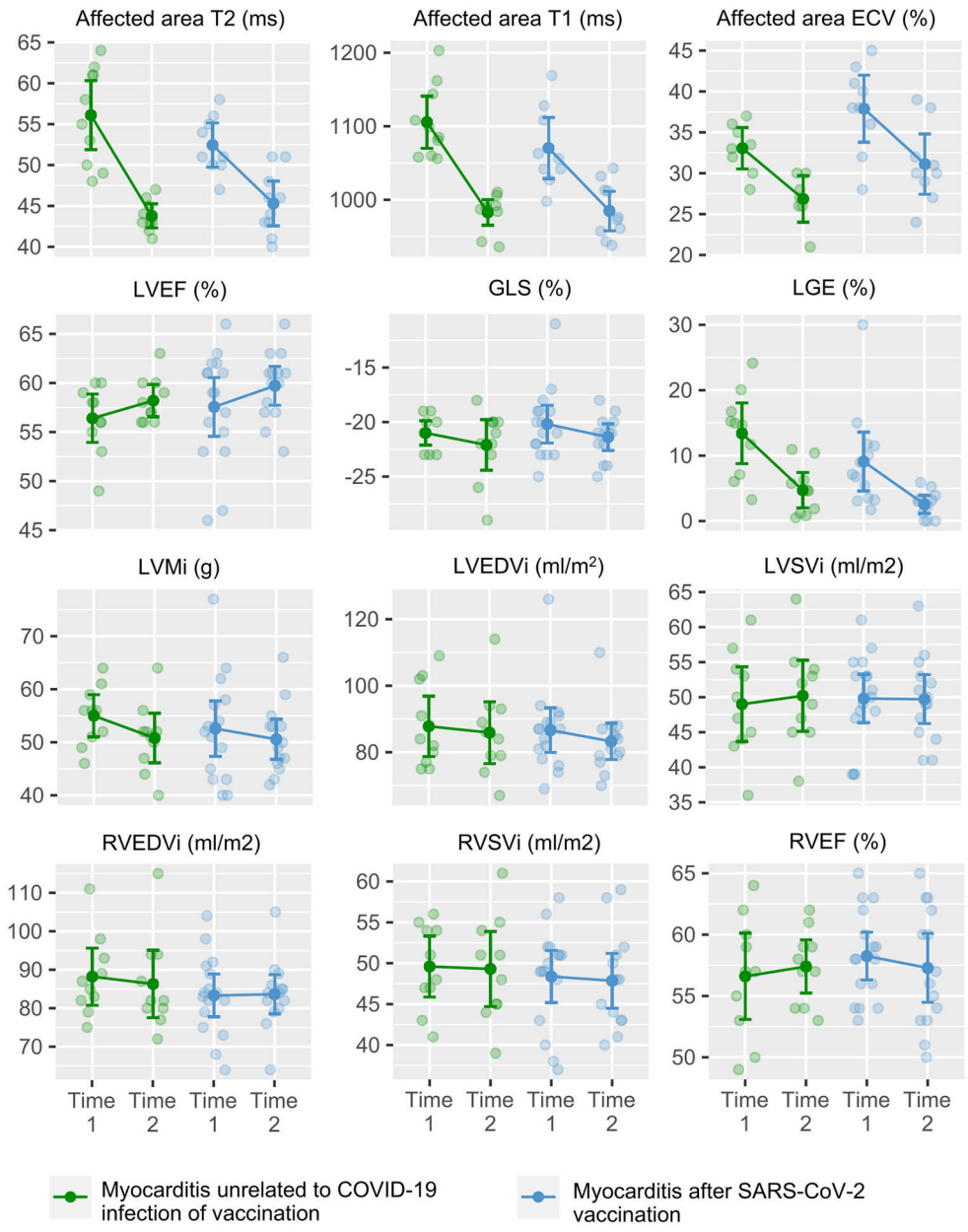

FIGURE 4
CMR metrics of myocarditis patients after SARS-CoV-2 immunization and myocarditis patients unrelated to COVID-19 immunization or infection over the acute phase and follow-up scan. Graphs show the trajectory of CMR metrics between the acute (T1) and follow-up (T2) CMR scans in myocarditis patients after SARS-CoV-2 immunization (in blue) and myocarditis patients unrelated to COVID-19 infection or vaccination (in green). CMR, cardiac magnetic resonance; ECV, extracellular volume; EDVi, left ventricular end diastolic volume index; EF, ejection fraction; ESVi, end systolic volume index; GLS, global longitudinal strain; Mi, mass index; LGE, late gadolinium enhancement; LV, left ventricular; RV, right ventricular; SVi, left ventricular stroke volume index.

system such as VAERS is prone to over- or underreporting based on the knowledge and attention of the reporters (5). Therefore, it should be used as a hypothesis-generating or event detection system (5, 21). Moreover, participants in our study received Gam-COVID-Vac (two doses required) as opposed to the Janssen vaccine (one dose required), which is approved by the Food and Drug Administration for use in the US and is therefore reported in the VAERS.

There are several aspects of the history of our patients that are worth noting. Twenty-five percent of our patients reported immune-mediated diseases. Furthermore, two individuals reported prior acute myocarditis, and one experienced

TABLE 5 Immune response in myocarditis patients after COVID-19 immunization vs. age-, sex- and COVID-19 immunization-matched controls.

	Myocarditis patients after COVID-19 vaccination ($n = 12$)	Age- sex- and immunization-matched controls ($n = 23$)	*P*
Age, years	22 ± 7	22 ± 6	0.924
Sex, male %	12 (100)	23 (100)	NA
Time from the first dose of vaccine to test, days	109 ± 57	108 ± 58	0.983
Time form the second dose of vaccine to test, days	86 ± 60	81 ± 55	0.907
COVID-19 vaccine			
- mRNA vaccine *n* (%)	8 (67%)	18 (78%)	0.814
- vector vaccine *n* (%)	4 (33%)	5 (22%)	
Test after the second dose of COVID-19 vaccine, yes (*n* %)	10 (83%)	18 (86%)	0.432
Previous SARS-CoV-2 infection, yes *n* (%)	3 (25%)	21 (91%)	<0.001
Time from previous SARS-CoV-2 infection, days	224 ± 66	284 ± 73	0.206
Anti-SARS-CoV-2 NCP-IgG (Ratio*) *Cutoff: > 1.1*	0.24 [0.13, 0.49]	0.32 [0.21, 1.23]	0.198
Anti-SARS-CoV-2 NCP-IgM (Ratio*) *Cutoff: > 1.1*	0.31 [0.24, 0.48]	0.33 [0.18, 0.66]	0.715
S1 Ig (U/ml) *Cutoff: ≥ 0.8 U/ml*	10265.5 [2,232, 38327.5]	9,167 [3948.5, 20,050]	0.881
SP1 IgG (RU/ml) *Cutoff: ≥ 11 RU/ml*	1155.5 [284, 1,656]	627 [283, 1537.5]	0.505
SP1 IgA (Ratio*) *Cutoff: ≥ 1.1*	11 [7, 11]	7 [6.5, 10]	0.095
Ag1 – S1 CD4+ (IU/ml) *Cutoff: ≥ 0.15*	1.3 [0.5, 4.5]	0.5 [0.2, 1.0]	0.002
Ag2 – S1 CD4+ CD8+ (IU/ml) *Cutoff: ≥ 0.15*	2.0 [1.0, 4.7]	0.6 [0.2, 1.2]	0.008
Ag3 – S1 CD4+ CD8+, whole genome CD8+ (IU/ml) *Cutoff: ≥ 0.15*	2.4 [1.0, 6.8]	0.8 [0.6, 1.5]	<0.001

Immune response to myocarditis after COVID-19 vaccination vs. age-, sex- and COVID-19 immunization-matched controls. Continuous variables showing a normal distribution are presented as the mean and standard deviations (± SD), and those showing a non-normal distribution are reported as medians and interquartile ranges [IQRs]. Comparisons between participant groups were conducted using independent samples t tests and Mann–Whitney U tests as appropriate.
*Ratio, extinction of the sample/extinction of calibrator; Ag, Antigen; CD, Cluster of differentiation; NA, Not applicable; NCP, Nucleocapsid protein; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SP1, Spike protein 1.

recurrent myocarditis 3 months after vaccination. In the latter case, acute myocarditis was linked to acute gastrointestinal infection; thus, it seems unlikely that this event was associated with vaccination. These findings might suggest a predisposing immune system response, as described previously in the etiology of acute myocarditis unrelated to vaccination (22). We did not find a statistically significant difference between the immune response of participants with predisposing factors and that of those without predisposing factors; however, the limited number of patients in each group precludes meaningful conclusions.

The male predominance of myocarditis after vaccination and myocarditis unrelated to vaccination has been previously described, and the cause is still unknown (23). One leading hypothesis is based on sex hormone disparities. It has been proven that there are differences in sex hormone receptor expression on both immune cells and cardiac tissues (24). The highest free testosterone levels have been described in males aged 12–24 years (25). Moreover, testosterone has a role in interleukin-10 upregulation and interferon-gamma downregulation. However, the direct relationship between testosterone levels and myocarditis has not been conclusively proven. Finally, experimental data demonstrate that Y chromosome-associated genetic factors are also responsible for the higher prevalence of myocarditis among males (26). Vigorous sports activity can trigger the onset of acute myocarditis and should be avoided during ongoing infection (22, 27); this might also be applicable after immunization, especially among young males. Five individuals reported possible acute triggers in our cohort: vigorous physical activity ($n = 4$) and heavy alcohol consumption ($n = 1$) immediately after immunization. In summary, our current findings suggest that the combined effect of genetic predisposition, hormonal factors and acute triggers may contribute to the pathomechanism of myocarditis after COVID-19 vaccination.

Several case reports have provided a visual account of myocarditis after COVID-19 immunization using CMR imaging (28–31), and this is the first study to show the improvement of myocardial injury. Moreover, for context, we provided a control group of myocarditis unrelated to the COVID-19 vaccine or SARS-CoV-2 infection. In our study, the most frequent localization of LGE was the lateral wall of the left ventricle in both myocarditis patients after COVID-19 vaccination and patients with myocarditis unrelated to COVID-19 infection or vaccination. This suggests that based on the CMR image, it

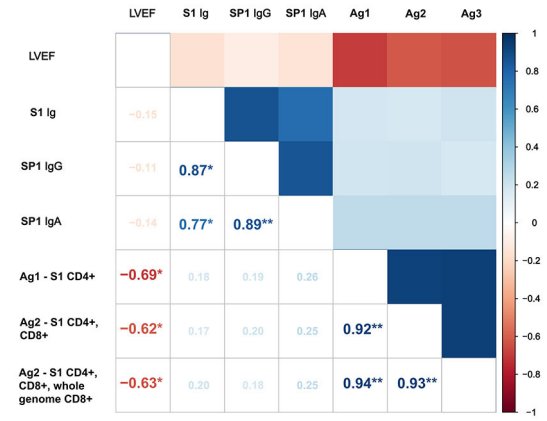

FIGURE 5
Correlation matrix showing the associations between SARS-CoV-2 immune response and LVEF. Positive correlations are shown in blue, and negative correlations are shown in red. Ag, Antigen; CD, Cluster of differentiation; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SP1, Spike protein 1; *$p < 0.05$; **$p < 0.001$.

is impossible to distinguish myocarditis cases post-vaccination from viral myocarditis. Our finding is in line with the recent report from Fronza et al. (32). CMR is a crucial diagnostic tool for myocardial injury. However, clarifying the disease etiology requires a holistic approach, taking into account the patient's history, symptoms and potential predisposing factors.

It has been shown, that acute myocarditis can heal or completely resolve over time (33), and our results support the notion that this is also true for cases potentially linked to the COVID-19 vaccine. We found that T2 mapping returned to the normal range on follow-up for all patients. Moreover, T1 mapping, ECV, and LGE decreased. Data suggest that LGE on the acute CMR scan is not equal to irreversible myocardial damage but the result of myocardial inflammation that can decrease over time and suggests a better prognosis over more extended follow-up periods. Additionally, none of the participants had extensive (>20%) LGE during follow-up, which is also considered a better prognostic marker (27). We found a slight improvement in LVEF during follow-up. Whilst the betterment of GLS values were not significant in our study, as expected based on the literature (34), the overall trend of GLS also suggested a marginal improvement over time when looking at individual data points.

In addition to the production of SARS-CoV-2-specific antibodies, COVID infection also leads to the generation of specific CD4+ and CD8+ cells (35). Increasing evidence supports the essential role of the T-cell-mediated response to SARS-CoV-2 infection; the COVID-specific T-cell response is associated with less severe disease (36, 37). Thereafter, to obtain a comprehensive view regarding the COVID-specific adaptive immune response, it is essential to measure specific antibodies and CD4+ and CD8+ cells from the same individual. Our current data indicate a substantially accelerated COVID-specific T-cell-mediated immune response in the myocarditis group compared to the age-, sex-, and vaccination status-adjusted control population. It is noteworthy that a larger proportion of controls than myocarditis patients had previously had COVID infections.

The rapid onset of symptoms after vaccination is an intriguing phenomenon and might be connected with immune response-mediated pathomechanisms. Reports all over the globe agree that myocarditis starts ∼2–4 days after vaccination. Although data regarding long-term immunity are scarce, it seems that a T-cell response is sustained for several months after infection and appears to be more prolonged than the antibody response. It has also been suggested that the T-cell response to different COVID-19 vaccines differs among age groups (17).

While we believe that acute myocarditis after COVID-19 vaccination is an important cardiovascular adverse effect that may occur after both mRNA and vector vaccines, this should not overshadow the ample evidence that clearly supports the effectiveness of vaccines (38, 39). The question also arose if young patients with COVID-19 are more likely to develop acute myocarditis or other adverse events than individuals after SARS-CoV-2 immunization. The most serious of which is the multisystem inflammatory syndrome in children (MIS-C). Recent evidence from France suggests that COVID-19 vaccination is associated with lower MIS-C incidence among adolescents (40). Moreover, in a new report by Zambrano et al. critically ill MIS-C patients requiring life support, all were unvaccinated, reinforcing the COVID-19 vaccination recommendation for eligible children (41). Therefore, there is an urgent need for an international consensus recommendation regarding an immunization protocol for those who experienced acute myocarditis after their COVID-19 vaccine.

Limitations

The main limitation of our study is the small sample size, which is mainly due to the rare occurrence of myocarditis after COVID-19 vaccination. Although we contacted all Hungarian centers reporting CMR, we could not avoid referral bias to CMR by clinicians. Mapping sequences were available in three institutes out of four. Similarly to other reports of myocarditis after COVID-19 vaccination, we report myocarditis cases of young, male patients. This prevents generalizability of our results to the female or older male population. In the institute where the parametric T2 mapping sequence was not available, oedema was characterized by T2-weighted black blood images alone. Mapping sequences were compared only among those participants who were scanned at the Semmelweis University Heart and Vascular Center (using a Siemens Magnetom Aera

1.5 T scanner) to avoid inter scanner variability. Importantly, our myocarditis control group's history was provided by the referring physician. The control group for the immunological studies did not undergo CMR examination.

Conclusions

In this cohort of myocarditis patients after COVID-19 immunization confirmed by CMR, we found that acute myocarditis can occur after mRNA and vector vaccines, predominantly in individuals with predisposing factors. Upon mid-term follow-up, myocarditis showed improvements in CMR markers, including the LVEF and tissue-specific alterations. The T-cell response was more prominent among myocarditis patients after COVID-19 vaccination than matched controls.

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 National Public Health Center of Hungary. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. 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

HV, LS, GN, BM, RK, and DB contributed to the conception and design of the study. HV, LS, AT, VJ, ZD, GB, ES, ZU, ZS, and GGN contributed to the data acquisition and curation. LS performed the statistical analysis and wrote the first draft of the manuscript. HV refined the manuscript. GN, GGN, ZU, and ZS wrote sections of the manuscript. All authors contributed to manuscript revision, read, 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.2022.961031/full#supplementary-material

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Neutrophil infiltration and myocarditis in patients with severe COVID-19: A post-mortem study

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Aims: To investigate cardiac pathology in critically ill patients with coronavirus disease 2019 (COVID-19) and identify associations between pathological changes and clinical characteristics.

Methods: The present autopsy cohort study included hearts from 26 deceased patients hospitalized in intensive care units due to COVID-19, and was conducted at four sites in Wuhan, China. Cases were divided into a neutrophil infiltration group and a no-neutrophil group based on the presence or absence of histopathologically identified neutrophilic infiltrates.

Results: Among the 26 patients, histopathological examination identified active myocarditis in four patients. All patients with myocarditis exhibited extensive accompanying neutrophil infiltration, and all patients without myocarditis did not. The neutrophil infiltration group exhibited significantly higher rates of detection of interleukin-6 (100 vs. 4.6%) and tumor necrosis factor-alpha (100 vs. 31.8%) than the no-neutrophil group (both $p < 0.05$). On admission, four patients with neutrophil infiltration in myocardium had significantly higher baseline levels of aspartate aminotransferase, D dimer, and high-sensitivity C reactive protein than the other 22 patients (all $p < 0.05$). During hospitalization, patients with neutrophil infiltration had significantly higher maximum creatine kinase-MB (median 280.0 IU/L vs. 38.7 IU/L, $p = 0.04$) and higher troponin I (median 1.112 ng/ml vs. 0.220 ng/ml, $p = 0.56$) than patients without neutrophil infiltration.

Conclusion: Active myocarditis was frequently associated with neutrophil infiltration in the hearts of deceased patients with severe COVID-19. Patients with neutrophil-infiltrated myocarditis had a series of severely abnormal laboratory test results on admission, and high maximum creatine kinase-MB during hospitalization. The role of neutrophils in severe heart injury and systemic conditions in patients with COVID-19 should be emphasized.

KEYWORDS

COVID-19, autopsy, heart, myocarditis, neutrophil infiltration

Introduction

Coronavirus disease 2019 (COVID-19) outbreaks caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) still occur repeatedly and intermittently around the world. Although COVID-19 is mainly characterized by the infection of the lung and respiratory failure, cardiac injury with troponin elevation is evidently associated with mortality (1, 2). In several post-mortem autopsy studies, heart tissue and cardiomyocyte injury including myocardial necrosis were common and non-specific, but the rate of pathology-confirmed myocarditis was low (3, 4). Despite being found in heart tissue, the presence of SARS-CoV-2 as determined via reverse transcription-polymerase chain reaction (RT-PCR) was rarely detected in cardiomyocytes; thus, it was unclear whether direct virus invasion was the primary cause of cardiac injury (5, 6). To date, the precise mechanisms involved in pathological changes in the heart induced by COVID-19 are unclear.

A proportion of patients with COVID-19 progress to critical illness, and are at significantly higher risk of mortality (7, 8). Especially with the current rapid spread of Delta and Omicron variants, an increasing trend of severe cases with worse prognoses has emerged (9). Critically ill patients experience a long stay in the intensive care unit (ICU), and are more prone to developing multiple organ dysfunction syndromes including the heart (10, 11), which may result in substantial histological and immunological changes. Therefore, we conducted a post-mortem pathological study of critically ill patients with COVID-19 to investigate pathological features of hearts and associations between pathological changes and clinical characteristics.

Materials and methods

Study population and specimen disposal

This autopsy cohort study included 26 patients with COVID-19 from Huoshenshan Hospital ($n = 8$), Taikang Tongji Hospital ($n = 5$), Zhongfaxincheng Hospital ($n = 5$), and Wuhan Jinyintan Hospital ($n = 8$), China, who died between 18 February 2020 and 04 April 2020. Patient hospitalization information has been described previously (12). Briefly, all 26 patients had COVID-19 confirmed via nasopharyngeal or pharyngeal PCR analyses of SARS-CoV-2 RNA and were hospitalized in the ICU. Full autopsies were performed with the approval of the relevant ethics committees, and written consent from the patient's relatives in accordance with regulations issued by the National Health Commission of China and the Helsinki Declaration.

Clinical characteristics, laboratory tests, echocardiography results, complications during hospitalization, medications, and invasive procedures undertaken were ascertained from hospitalization records and other sources of information. Time from syndrome onset to hospitalization was also recorded. For laboratory tests including cardiac markers and inflammatory indicators, baseline values on admission and maximum values during hospitalization were recorded. Laboratory tests include creatine kinase (CK), creatine kinase-MB (CK-MB), hypersensitive troponin I (hsTnI), brain natriuretic peptide (BNP), interleukin (IL) 6, hypersensitive C reactive protein (hsCRP), procalcitonin (PCT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured at a core laboratory within each participating site within minutes after blood drainage. Serum samples were then centrifuged, and serum was separated and stored at −80°C for repeated measurements if necessary. To minimize autolysis, decedents were promptly stored at 4°C after death and the range of the post-mortem interval (time of death to time of autopsy) was 4–24 h. For histopathological analysis, autopsy materials were collected, fixed in 4% neutral formaldehyde for at least 24 h, formalin-fixed, and embedded in paraffin.

Pathological analysis

Autopsies of hearts were performed by two experienced pathologists, and ventricle tissues, atrium tissues, and epicardial coronary arteries were collected for further analyses. A median of 25 full-thickness blocks of myocardium was examined histologically (range 11–40 blocks). Pathological changes in hearts were evaluated via hematoxylin and eosin (H&E) staining and immunohistochemical (IHC) staining. H&E staining was performed in accordance with a standard procedure. IHC staining was performed using routine automated diagnostic IHC staining devices (Roche, BenchMark-ultra). Myocarditis was defined as microscopic findings of multiple foci of increased leukocyte infiltration associated with myocyte injury that was not due to another cause (3). The number of myocardium-infiltrating mononuclear cells per mm^2 in a high-power field was counted in each sample with the most inflammation, using IHC staining for CD4 (Zhongshan Jinqiao, #ZM-0418), CD8 (Zhongshan Jinqiao, #ZA-0508), CD20 (Zhongshan Jinqiao, #ZM-0039), and CD68 (Zhongshan Jinqiao, #ZM-0060). Primary antibodies used for IHC staining included IL-6 (Abcam, ab6672, 1:600) and tumor necrosis factor-alpha (TNF-α; Cell Signal Technology, #8184, 1:20). Images were captured using a digital camera (DP73, Olympus) under a light microscope (BX43, Olympus). The diluent without primary antibodies was used as a negative control for IHC staining.

Statistical analysis

Continuous variables are presented as means ± the standard deviation (SD) or medians with ranges for non-parametric data. Categorical data are presented as counts with percentages.

To quantify correlations between pathological findings and clinical characteristics, the Kendall's tau-b index for bivariate correlational analysis was used. Two-sided p-values < 0.05 were considered statistically significant. All statistical analyses were performed via IBM SPSS version 25 and R version 3.6.1.

Results

A total of 26 patients admitted to ICUs due to COVID-19 were included in this pathological study. General patient characteristics and main causes of death have been published previously (12). Briefly, the median age of the study cohort was 68 years (range 53–88 years), and 50% (13 patients) were male subjects. The median duration in the ICU until death was 20 days (range 3–61 days). Twenty patients had at least one comorbidity, including 10 with chronic cardiovascular diseases (three with coronary artery disease, three with cardiac dysfunction, two with valvular heart disease, one with dilated cardiomyopathy, and one with arrhythmia), nine with hypertension, six with chronic pulmonary diseases, and four with diabetes. Most of the 26 patients died of pulmonary injuries related to COVID-19.

Heart failure occurred in 10 (38.5%) patients. Atrial fibrillation was documented in six (23.1%) patients and was the main type of new-onset arrhythmia during hospitalization. Due to their serious illness, various complications emerged during hospitalization in the ICU including respiratory failure, pleural effusion, pneumothorax, anemia, renal dysfunction, and disseminated intravascular coagulation. Eighteen patients received anticoagulation treatment, and two received antiplatelet therapy. Multiple invasive procedures including non–end-stage endotracheal intubation, assisted ventilation, deep vein puncture, bronchoscopy, dialysis, and extracorporeal membrane oxygenation were intermittently or continuously used in critical situations. Treatment information is shown in Supplementary Table S1.

Pathological findings

As described in our previous work, a series of common pathological changes in hearts were found in all 26 patients, including myocardial cell degeneration and scattered necrosis, mild interstitial edema, and infiltration of monocytes and lymphocytes and/or neutrophils (13). Cardiomyocyte hypertrophy, atrophy, and interstitial fibrosis of varying degrees based on underlying diseases were also detected. Morphological analysis of heart tissue blocks identified active myocarditis in only four (15.4%) patients (Figure 3). Neutrophilic infiltrates were detected in all four patients with myocarditis. Diffuse neutrophilic infiltrates were associated with adjacent cardiomyocyte degeneration or necrosis, and involvement of bilateral ventricles and atriums was detected in two of the patients with myocarditis. In one of these patients, there was obvious accompanying myocardial interstitial edema (Figures 1A,B). In the two other patients with active myocarditis, there were multiple small discrete foci of mixed inflammatory cells with visible neutrophils and lymphocytes associated with single-cell necrosis of cardiomyocytes, involving the left ventricle and atrium (Figures 1C,D). All 22 patients without active myocarditis exhibited minor infiltration of scattered mononuclear cells in the myocardial interstitium, rather than neutrophils. To further investigate the severity and properties of inflammation in myocardium, IHC staining was performed to detect the expression of TNF-α and IL-6. Expression of TNF-α and IL-6 in infiltrating inflammatory cells and myocardial interstitial cells was detected in all four patients with neutrophil infiltration (Figures 1E–L), whereas patients without neutrophil infiltration exhibited negative or mild expression of these inflammation-related factors (Figures 1M–P). Patients with neutrophil infiltration were more likely to exhibit TNF-α and IL-6 positivity than those without neutrophil infiltration [TNF-α 100 vs. 31.8% (7/22 cases), $p = 0.022$; IL-6 100% vs. 4.6 (1/22 cases), $p < 0.001$; Table 1].

The immunologic characteristics of myocardium-infiltrating mononuclear cells were analyzed using IHC staining for the helper T cell marker CD4, the cytotoxic T cell marker CD8, the B cell marker CD20, and the monocyte and macrophage marker CD68. All four patients with active myocarditis exhibited very mild infiltration of $CD4^+$, $CD8^+$, and $CD20^+$ lymphocytes, and single or small clusters of $CD68^+$ macrophages, and this pattern was also evident in all 22 patients without active myocarditis (Figure 2). The numbers of each cell type per mm^2 in a high-power field are shown in Table 1. There were no significant differences in $CD4^+$, $CD8^+$, $CD20^+$, or $CD68^+$ cell densities between patients with and without active myocarditis (all $p > 0.05$; Table 1). Kendall's tau-b index indicated no significant correlations between the numbers of each cell type and the levels of TNF-α or IL-6 expression (all $p > 0.05$). Other pathologic findings are shown in Supplementary Figure S1. Two of the four patients with neutrophil infiltration had neutrophil-predominant endocarditis. Dilated cardiomyopathy with tricuspid valve infective endocarditis occurred in one patient without neutrophil infiltration. Epicarditis with focal infiltration of mixed inflammatory cells occurred in three patients with neutrophil infiltration and seven patients without neutrophil infiltration. Mixed thrombi were detected in four patients without neutrophil infiltration, including one in the left atrium, one in the right atrium, and two in the right ventricle. Epicardial coronary arteriosclerosis was detected in nine of the 26 patients, including one with neutrophil infiltration. There was no thrombotic occlusion or endarteritis of the epicardial coronary artery in any of the 26 patients. Intravascular microthrombi in myocardial interstitium were observed via microscopy

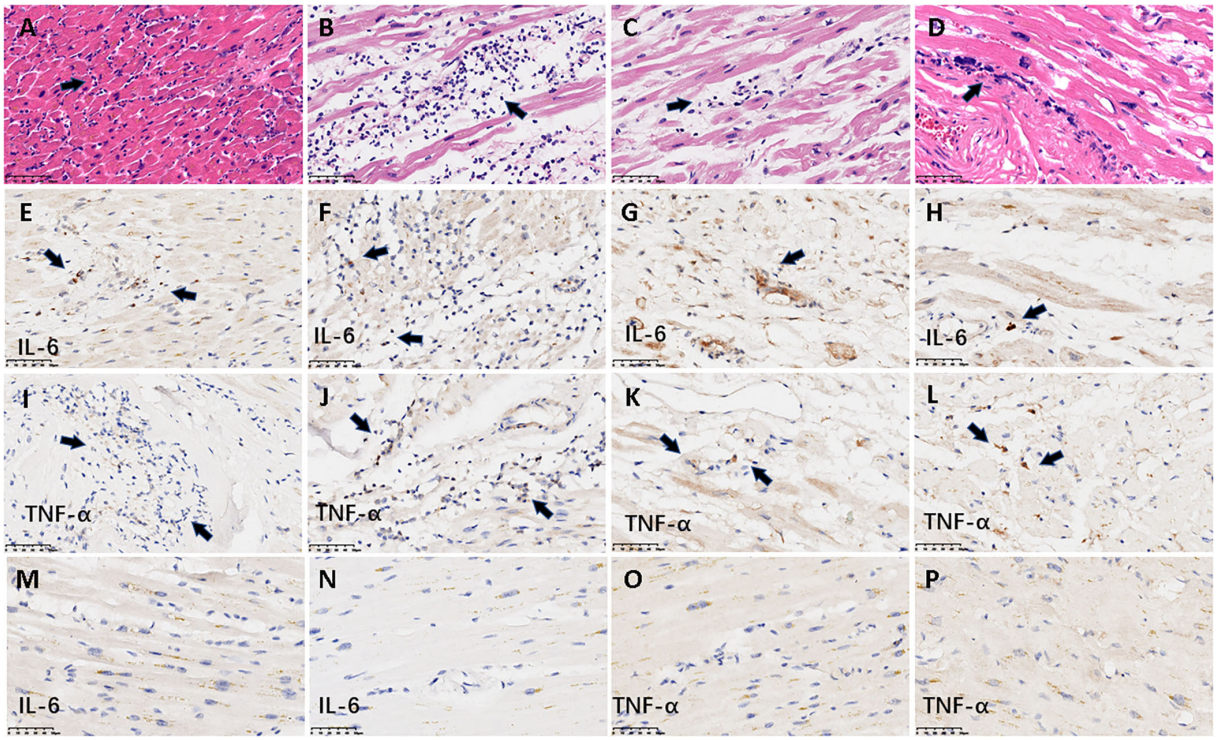

FIGURE 1
Representative histological and IHC findings from hearts. The figure shows the histological and IHC findings from heart tissues. **(A–D)** demonstrate active myocarditis in the four cases. **(A,B)** The histology in the myocardium demonstrated diffuse neutrophilic infiltrates with myocyte injury in a 62-year-old man and a 56-year-old woman, respectively; **(C,D)** multiple small discrete foci of mixed inflammatory cells with visible neutrophils associated with single-cell necrosis of cardiomyocytes in a 63-year-old woman and a 76-year-old man, respectively. The arrows denote the infiltrated neutrophils. **(E–L)** denote the positive IHC staining of IL-6 and TNF-α from the four cases with active myocarditis. **(E–H)** IL-6; **(I–L)** TNF-α. The longitudinal images in the first three rows were derived from the same case (A, E, I from a 62-year-old man; B, F, J from a 56-year-old woman; C, G, K from a 63-year-old woman; D, H, L from a 76-year-old man). Arrows denote the positive signal of IHC staining. **(M–P)** Represent the negative expression of IL-6 and TNF-α from two cases without neutrophil infiltration. **(M,O)** IHC staining from an 81-year-old man; **(N,P)** IHC staining from a 59-year-old woman. Scale bars represent 50μm. IHC: immunohistochemical.

TABLE 1 Pathologic findings of cases with vs. without neutrophil infiltration.

Pathologic findings	Cases with neutrophil infiltration (myocarditis) N = 4	Cases without neutrophil infiltration (myocarditis) N = 22	P value
TNF-α (+), No. (%)	4 (100%)	7 (32%)	0.02
IL-6 (+), No. (%)	4 (100%)	1 (5%)	< 0.001
Number of lymphocytes per mm^2, median (range)	6.5 (range 4–13)	8.5 (range 3–17)	0.45
CD4+ cell			
CD8+ cell	8 (range 5–15)	12 (range 3–50)	0.29
CD20+ cell	2 (range 1–5)	3 (range 1–5)	1.00
CD68+ cell	61 (range 34–89)	50 (range 24–154)	0.83
Microthrombi (+), No. (%)	4 (100%)	8 (36%)	0.02

TNF-α, tumor necrosis factor; IL-6, interleukin-6.

in 12 (46.2%) patients, including all four with neutrophil infiltration and another eight without neutrophil infiltration. The detection rate of cardiac microthrombi in patients with neutrophil infiltration was significantly higher than that in patients without neutrophil infiltration (100% vs. 36.4%, $p = 0.02$; Table 1).

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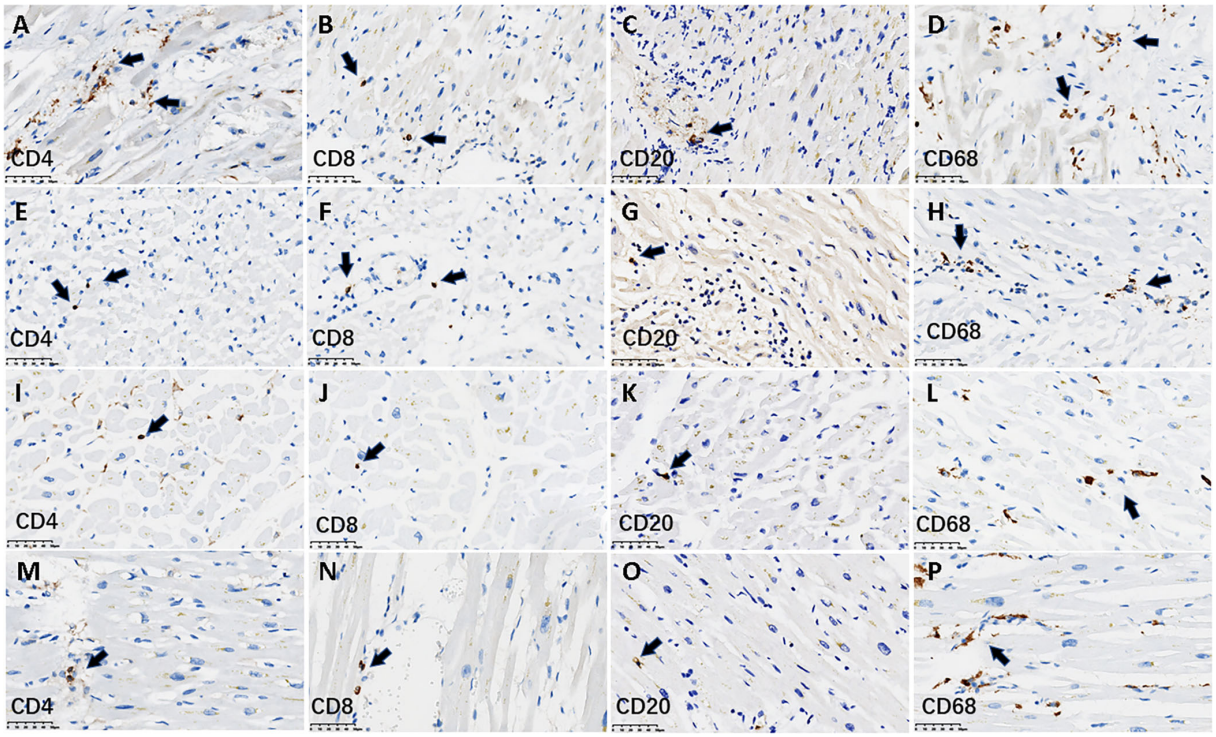

FIGURE 2
Lymphocyte infiltration using IHC staining within myocardium in representative cases. The figure shows the infiltration of lymphocytes and macrophages stratified by $CD4^+$, $CD8^+$, $CD20^+$, and $CD68^+$ cells in the myocardium in four cases. **(A–H)** denote IHC staining for lymphocytes from two cases with neutrophil-infiltrated pathological myocarditis. **(A–D)** 62-year-old man; **(E–H)** a 56-year-old woman. **(I–P)** denote IHC staining for lymphocytes from two cases without pathological myocarditis. **(I–L)** An 81-year-old man; **(M–P)** a 59-year-old woman. The immunostaining of two myocarditis cases showed there was a scattered infiltration of very mild $CD4^+$, $CD8^+$, $CD20^+$ lymphocytes, and single or small clusters of CD68+ macrophages. This pattern was also seen in the other two cases without myocarditis. The arrows denote the lymphocytes or macrophages. Scale bars represent 50 μm. IHC, immunohistochemical.

Clinical characteristics of the neutrophil infiltration and no-neutrophil infiltration groups

To investigate dynamic changes in clinical characteristics in the 26 deceased patients, baseline characteristics at admission, and maximum values of a series of laboratory test parameters reflecting severe medical conditions were analyzed. Baseline characteristics including parameters of cardiac injury, inflammation, coagulation, and liver function at admission are shown in Table 2. The median time from symptom onset to hospital admission in patients with neutrophil infiltration was 20.5 days (range 13–26 days), which was significantly longer than that in patients without neutrophil infiltration (10.0 days, range 1–24 days; $p = 0.02$). Compared to patients without neutrophil infiltration, those with neutrophil infiltration had significantly higher baseline levels of AST, D dimer, and hsCRP (all $p < 0.05$, Table 2, Figure 3). In terms of baseline cardiac markers, median CK was significantly higher in patients with neutrophil infiltration than in those without neutrophil infiltration (277.5 IU/L, range 91.0–486.0 IU/L vs. 36.0 IU/L, range 11.5–547.0 IU/L; $p = 0.03$). CK-MB and BNP were similar in the two groups (both $p > 0.05$; Table 2, Figure 3). Baseline hsTnI was only available for 16 (61.5%) of the 26 patients. In the other 10 (38.5%) patients, hsTnI was not evaluated until they were transferred to the ICU ($n = 6$) or exhibited symptoms indicating heart failure or atrial fibrillation ($n = 4$).

Maximum values of laboratory tests during hospitalization were compared in the two groups (Table 2). Patients with neutrophil infiltration had a significantly higher median peak value of the inflammatory indicator hsCRP than those without neutrophil infiltration (134.0 mg/L, range 98.2–211.0 mg/L vs. 10.0 mg/L, range 9.5–163.5 mg/L; $p = 0.04$). Higher median peak AST was also evident in patients with neutrophil infiltration than in those without neutrophil infiltration, though the difference was not significant (687 IU/L, range 53–1568 IU/L vs. 123 IU/L, range 26–800 IU/L; $p = 0.07$). The median maximum value of CK-MB during hospitalization in neutrophil-infiltrated patients was significantly higher than that in patients without neutrophil infiltration (280.0 IU/L, range 14.0–996.0 IU/L vs. 38.7 IU/L,

TABLE 2 Clinical characteristics of cases with vs. without neutrophil infiltration.

	Neutrophil (–) $N = 22$	Neutrophil (+) $N = 4$	P value	Neutrophil (–) $N = 22$	Neutrophil (+) $N = 4$	P value
Age, years, (median, range)	69 (53–88)	62.5 (56–77)	0.21			
Sex, male, No. (%)	11 (50.00%)	2 (50.00%)	1.00			
Time from symptom onset to hospital admission, days, (median, range)	10 (1–24)	20.5 (10–26)	0.01			
Laboratory test (median, range)	**Baseline characteristics at admission**			**Maximum value during hospitalization**		
CK-MB, IU/L	11.4 (5.5–45.0)	13.5 (7.20–128.0)	1.00	38.7 (5.9–234.7)	280.0 (14.0–996.0)	0.04
CK, IU/L	36.0 (11.5–547.0)	277.5 (91.0–486.0)	0.03	144.9 (36.0–1,997.0)	2,276.5 (91.0–7,491.0)	0.17
hsTnI, ng/ml	NA	NA	NA	0.220 (0.008–8.749)	1.112 (0.008–7.775)	0.56
BNP, pg/ml	55.1 (10.0–1,243.0)	56.7 (10.0–299.1)	0.80	387.0 (89.3–26,000.0)	173.0 (56.0–328.0)	0.19
IL-6, pg/ml	6.89 (16.80–204.10)	16.54 (6.89–26.30)	0.48	63.44 (16.14–5,000.00)	20.28 (11.79–455.00)	0.19
hsCRP, mg/L	10.0 (2.15–160)	96.6 (67.6–211.0)	0.02	10.0 (9.5–163.5)	134.0 (98.2–211.0)	0.04
D-Dimer, mg/L	2.97 (0.36–21.00)	11.92 (4.25–18.19)	0.04	6.45 (0.36–56.63)	14.43 (4.25–50.00)	0.13
Neutrophil, 10×10^9/L	6.34 (3.57–16.66)	12.12 (3.44–20.03)	0.62	17.14 (3.94–50.34)	17.55 (7.71–23.03)	0.67
PCT, ng/ml	0.29 (0.08–1.91)	1.01 (0.05–88.34)	0.89	2.58 (0.14–47.53)	3.37 (0.11–88.34)	0.92
ALT, IU/L	27 (4–110)	52 (34–1,204)	0.20	107 (4–1,000)	128 (40–1,400)	0.46
AST, IU/L	38.4 (9.2–92.6)	55.9 (48.0–1,487.0)	0.04	123 (26–800)	687 (53–1,568)	0.07

CK-MB, creatine kinase-MB; CK, creatine kinase; hsTnI, hypersensitive troponin I; NA, not available; BNP, brain natriuretic peptide; IL-6, interleukin-6; hsCRP, hypersensitive C reaction protein; PCT, procalcitonin; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

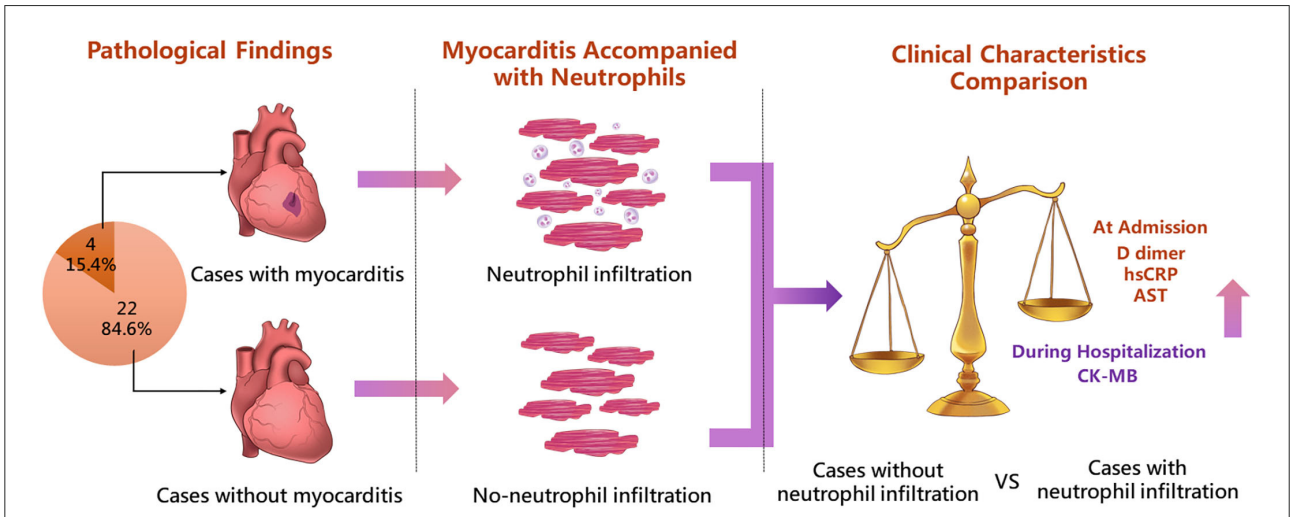

FIGURE 3
Highlight overview. The figure shows the highlight overview of the present autopsy cohort study. Neutrophil infiltration in heart tissue was associated with active pathological myocarditis in COVID-19. Patients with neutrophil infiltration had dramatically elevated levels of both cardiac and systemic laboratory tests, indicating the severe condition of COVID-19. hsCRP, high-sensitivity C reactive protein; AST, aspartate aminotransferase.

range 5.9–234.7 IU/L; $p = 0.04$) (Figure 3). Patients with neutrophil infiltration had higher median peak hsTnI during hospitalization than those without neutrophil infiltration, though the difference was not significant (1.112 ng/ml, range 0.008–7.775 ng/ml vs. 0.220 ng/ml, range 0.008–8.749 ng/ml; $p = 0.56$). Other main laboratory parameters were comparable in the two groups (Table 2). Heart failure occurred in one of the four patients with neutrophil infiltration, and

atrial fibrillation occurred in six of the patients without neutrophil infiltration.

Discussion

Since the global COVID-19 pandemic began, a considerable proportion of patients with COVID-19 have developed critical illnesses, and many have experienced multiple organ failures including the lung, heart, and other organs (14, 15). To investigate specific pathological changes in the heart, we conducted the present autopsy study of hearts of 26 critically ill patients who died of COVID-19 in Wuhan from February 2020 to April 2020. The main findings of the study were that (1) active myocarditis was commonly and specifically accompanied by neutrophil infiltration; (2) the positive IHC detection rates of TNF-α and IL-6 were significantly higher in patients with neutrophil infiltration than in those without neutrophil infiltration, but this was not associated with the extent of lymphocyte or macrophage infiltration; (3) in patients with neutrophil infiltration, the time from syndrome onset to hospitalization was significantly longer than it was in those without neutrophil infiltration, and they exhibited higher baseline levels of CK, AST, hsCRP, and D dimer; (4) patients with neutrophil infiltration had significantly higher levels of CK-MB and non-significantly higher levels of hsTnI than those without neutrophil infiltration.

Role of neutrophil: A new notion of COVID-19-related myocarditis?

Compared to the dramatic pathological changes described in the lung (16, 17), microscopic findings derived from the heart in patients with COVID-19 are less numerous and less specific. Previous post-mortem studies have identified various pathological manifestations, most pertaining to necrosis, myocarditis, inflammatory infiltration, and fibrin microthrombi (3, 4, 18). Compared with other published autopsy studies, the scattered necrotic cardiomyocytes in the current study were more common, probably because all the patients had severe COVID-19 and significantly longer ICU stays, which greatly increased the risks of cardiac injury. Consistent with previous reports, a small proportion of patients had active myocarditis, indicating more severe cardiac injury (19). Surprisingly neutrophil infiltration was detected in all four patients with myocarditis in the present study, but it was rare in patients without myocarditis. A series of trials indicate that neutrophil infiltration into pulmonary tissues causes the deterioration of patients with COVID-19 (20–23). Infiltrating neutrophils may release neutrophil extracellular traps (NETs)—which are extracellular networks of chromatin and microbicidal proteins—in response to SARS-CoV-2 infection, while excessive activation of NETs simultaneously results in lung cell death in critically ill patients (24–27). NETs derived from neutrophils are responsible for multiple pathophysiological changes including microthrombi, angiotensin-converting enzyme 2 activity, and oxidative stress (28, 29). Moreover, NETs are reportedly correlated with cytokine storms. Various cytokines may mediate the migration of neutrophils to injury sites (30). Conversely, the generation of NETs may stimulate the aggravation of cytokine storms including IL-6 via IL-1β (20, 31, 32). In the present post-mortem study, the positive detection rates of IL-6 and TNF-α were significantly higher in the four patients with neutrophil infiltration than in the 22 patients without neutrophil infiltration. Neutrophils, as the first-line regulator of adaptive immunity, are detrimental in cases of cardiac injury (33). Accumulating evidence indicates that autoimmune mechanisms may contribute to the progression of COVID-19 (34, 35). Based on our findings, despite a lack of direct evidence, it is reasonable to speculate that neutrophil infiltration may severely exacerbate cardiac injury in patients with severe COVID-19 by regulating autoimmune responses. Although the identification of NETs and autoimmunity was not part of the present study, results from the study indirectly indicate a strong association between neutrophils and severe cardiac injury.

Of the 26 patients in the current study, SARS-CoV-2 nucleic acids were only found in the heart tissues of five patients via real-time RT-PCR, as reported previously (12). Interestingly, none of these five patients had pathologically diagnosed myocarditis (36). Also, other pathological studies from endomyocardial biopsy (EMB) or autopsy rarely reported direct invasion of SARS-CoV-2 into cardiomyocytes (37). Current evidence still fail to determine the key role of SARS-CoV-2 infection on cardiac injury, while inflammatory infiltration was now regarded as a preliminary cause of heart damage in severe COVID-19. The Dallas Criteria (38) which only depends on histological evidence have already been not fully suitable for diagnosis of myocarditis, and IHC analysis for inflammatory infiltration was particularly advocated (39). A comparison of $CD3^+$ T cells and $CD68^+$ macrophages in patients with COVID-19 and control patients was reported in a recent review (37). There were no significant differences in the total numbers of $CD3^+$ or $CD68^+$ cells in the two groups, whereas $CD68^+$ cell counts were significantly higher in the COVID-19 group than in the control group (37). In the present study, $CD68^+$ macrophages were single or clustered in the myocardium, but there was no significant difference in the numbers of $CD68^+$ cells in patients with and without active myocarditis. Moreover, all patients with myocarditis exhibited neutrophil infiltration accompanied by distinct cytokines, which was not prevalent in patients without myocarditis. Therefore, based on previous investigations of associations between neutrophil infiltration and critical COVID-19, we surmise that neutrophils

and inflammatory infiltration may be a constituent cause of devastating heart damage in cases involving myocarditis. Some researchers have postulated that glucocorticoids may act as an immunomodulator that inhibits cytokine storms and excessive immune responses, improving therapeutic effects in critically ill patients (40, 41). Other specific cytokine inhibitors such as the IL-6 receptor inhibitor tocilizumab are being investigated (42, 43). The role of neutrophil infiltration in severe cardiac injury in patients with COVID-19 warrants further attention.

Cardiac biomarkers: A warning sign of severe COVID-19?

In the present study, patients with cardiac neutrophil infiltration had a significantly longer median ICU stay than patients without neutrophil infiltration, indicating that cardiac neutrophil infiltration may be related to severe COVID-19. Several studies evaluating risk factors for a poor COVID-19 prognosis have identified a series of laboratory predictors of in-hospital mortality, including AST, D dimer, and hsCRP (44, 45). Significant elevation of baseline levels of these three parameters was also found in patients with cardiac neutrophil infiltration in the current study. There may be a link between a relatively severe condition (involving liver function, coagulation, and inflammation) and pathological changes in heart tissues, indicating that COVID-19 can simultaneously cause damage to multiple organs that are not part of the respiratory system. Of the patients in the present study, however, almost half were not tested for cardiac biomarkers until they were transferred to the ICU or exhibited relevant symptoms. This suggests that under an emergent situation, inspection of the heart may be easily neglected by physicians, who mainly focused on treatment strategies based on the respiratory system. Furthermore, patients with neutrophil infiltration exhibited relatively worse conditions both on admission and during their ICU stay. The peak level of CK-MB throughout hospitalization was significantly higher in patients with neutrophil infiltration than in those without neutrophil infiltration. Peak hsTnI was also higher in patients with neutrophil infiltration, although this observation was not statistically significant due to the small sample size. The dramatic elevation of cardiac biomarkers was consistent with severe pathological changes in hearts. In combination with indicators identified in previous studies (1, 2), biomarkers of cardiac injury including CK-MB and troponin may be indicators of heart damage, and predictors of a systemic inflammatory response to COVID-19. A series of observational cohort studies conclusively indicate that CK-MB and other cardiac injury biomarkers are independent predictors of ICU admission and fatality in patients with COVID-19, regardless of the presence or absence of comorbid coronary artery disease (46–48). The current study in combination with previous clinical evidence collectively demonstrates that cardiac biomarkers, as meaningful indicators of critical illness, should be paid particular attention by clinical physicians in patients with COVID-19.

The present study had several limitations. The sample size was low, and findings from 26 patients inevitably entail potential bias, particularly with respect to the association between cardiac injury parameters and neutrophil infiltration. Second, although patients with neutrophil infiltration in hearts had significantly longer ICU stays than patients without, we could not conclude that neutrophil infiltration may prolong the ICU stay because all patients ultimately died, rather than recovering. Further studies may focus on this issue and qualify neutrophil infiltration in hearts as a predictor of prognosis in patients with severe COVID-19. Third, IHC staining for NETs was not performed, though the effects of NETs on COVID-19 have been investigated in previous studies. Lastly, we did not investigate details of heart injury mechanisms due to COVID-19 further, and more research is needed in this regard.

In this autopsy study of heart tissue from critically ill patients who died of COVID-19, active myocarditis was commonly accompanied by neutrophil infiltration. Patients with neutrophil-infiltrated myocarditis had more severe abnormal baseline laboratory test results for AST, D dimer, and hsCRP, and a higher peak value of CK-MB during hospitalization than patients without neutrophil-infiltrated myocarditis. The role of neutrophils in severe heart injury and systemic conditions in COVID-19 should be emphasized.

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 National Health Commission of China and the Helsinki Declaration. The patients/participants provided their written informed consent to participate in this study.

Author contributions

QZ collected the specimens and designed the conduction of study. HZ and XYao were responsible for specimen disposal and pathological analysis of all cases. XYan collected and analyzed the clinical information of recruited patients. SM was in charge

of statistical analysis and manuscript writing. QZ and SM verified the underlying data. YS, YP, MC, CP, SW, ML, and CY provided assistance of staining procedure and figure exhibition. XB, YH, and SZ contributed to the leadership of the whole process of study conduction, and acted as the key role of initiating, designing, conducting, and concluding the study. All authors had full access to all the data in the study and accept responsibility to submit 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.2022.1026866/full#supplementary-material

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Cardiac magnetic resonance follow-up of COVID-19 vaccine associated acute myocarditis

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Background: Mass COVID-19 vaccination campaigns have helped impede the COVID-19 pandemic. In rare cases, some vaccines have led to vaccine associated myocarditis in a specific subset of the population, usually young males. Cardiac magnetic resonance (CMR) can reliably diagnose vaccine associated myocarditis, but follow-up data of CMR proven acute myocarditis is scarce.

Materials and methods: Nine patients with acute vaccine associated myocarditis underwent baseline and follow-up CMR examinations and were compared to baseline parameters at initial presentation and to a group of 20 healthy controls. CMR protocol included functional assessment, T1 and T2 mapping, T2 signal intensity ratio, strain feature tracking, and late gadolinium enhancement (LGE).

Results: Myocarditis patients (n = 9, aged 24 ± 6 years, 8 males) underwent CMR follow-up after an average of 5.8 ± 4.3 months. All patients showed a complete resolution of visual myocardial edema while also demonstrating a reduction in overall LGE extent from baseline to follow-up (4.2 ± 2.1 vs. 0.9 ± 0.8%, $p < 0.001$), although visual LGE was still noted in all patients. Left ventricular ejection fraction was normal at baseline and at follow-up (58 ± 6 vs. 62 ± 4%, $p = 0.10$) as well as compared to a healthy control group (60 ± 4%, $p = 0.24$). T1 (1024 ± 77 vs. 971 ± 34 ms, $p = 0.05$) and T2 relaxations times (57 ± 6 vs. 51 ± 3 ms, $p = 0.03$) normalized at follow-up. Most patients reported a resolution of clinical symptoms, while two (22%) reported new onset of exertional dyspnea.

Conclusion: Patients with COVID-19 vaccine associated acute myocarditis showed a complete, uncomplicated resolution of myocardial inflammation on follow-up CMR, which was associated with a near complete resolution

of symptoms. Minor, residual myocardial scarring was present on follow-up LGE imaging. The long-term implications of the remaining myocardial scar-tissue after vaccine associated myocarditis remain unknown warranting further studies.

KEYWORDS

cardiac magnetic resonance, myocarditis, COVID-19, vaccination, follow-up

Introduction

The start of the coronavirus disease-19 (COVID-19) outbreak caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) at the end of 2019 prompted a world-wide effort to curtail the spread of the pandemic. One possible way to achieve this was the development of safe and effective vaccines. This effort cumulated in a rapid deployment of a few COVID-19 vaccines, most commonly and notably the Ad26.COV2-S [recombinant] (Janssen) vaccine, the mRNA-1273 (Moderna) vaccine, the BNT162b2 (Pfizer/BioNTech) vaccine, and the ChAdOx1-S [recombinant] (AstraZeneca) vaccine. Shortly after mass immunization programs began, reports of vaccine associated adverse reactions, such as fever, deep venous thrombosis, and myocarditis, started to emerge. Myocarditis and pericarditis are rare cardiovascular adverse vaccine reactions with an estimated incidence of approximately 0.48 cases per 100,000 administered vaccines (1). The Vaccine Adverse Event Reporting System (VAERS), set up by the Disease Control and Prevention (CDC) and the U.S. Food and Drug Administration (FDA), currently record 94 cases of reported vaccine associated myocarditis for the Janssen vaccine, 980 for the Moderna vaccine and 1897 for the BioNTech vaccine (2). Typical clinical presentation includes new exertional dyspnea and/or acute chest pain, accompanied by elevated troponin T, hours to days after COVID-19 vaccination. Cardiac magnetic resonance (CMR) studies have shown that the pattern of myocardial involvement in vaccine associated myocarditis was similar to acute viral induced myocarditis (3–5). In accordance with the 2018 modified Lake Louise Criteria for the diagnosis of acute myocarditis, typical findings include generalized or focal edema, prolonged T1 or T2 relaxation times, increased extracellular volume (ECV), and focal necrosis on late gadolinium enhancement (LGE) imaging (6). Initial clinical studies suggest a mild clinical course of disease with rapid resolution of symptoms (7). However, there is currently a scarcity of follow-up studies, making it difficult to determine the possible risks associated with vaccine associated myocarditis. This study reports follow-up CMR findings in patients after initial acute vaccine associated myocarditis.

Materials and methods

This retrospective study was approved by the appropriate institutional ethics committee and performed in concordance with the Declaration of Helsinki and International Conference on Harmonization of Good Clinical Practice. The requirement for written informed consent was waived. Patients who underwent initial CMR with diagnosis of acute vaccine-associated myocarditis and subsequent follow-up CMR at the Department of Diagnostic and Interventional Radiology from September 2021 to August 2022 were retrospectively identified.

Initial symptoms for referral for CMR included exertional dyspnea, chest pain, fever, or palpitations with associated elevated troponin T, within hours or days of receiving a COVID-19 vaccine. All patients received at least one dose of a COVID-19 vaccine approved for use in the European Union. Initial CMR results were positive for myocarditis as defined by the 2018 Lake Louise criteria (6). Reasons for follow-up referrals were standardized CMR follow-up of acute myocarditis according to local guidelines, examination before return to physical activity, or persistent cardiac symptoms under exertion. Clinical patient information was gathered through the local hospital information system.

The control group consisted of healthy subjects without previous myocarditis and no cardiovascular disease history who underwent CMR for study control reasons. Controls were age-matched to the myocarditis cohort and had normal CMR results without structural abnormalities.

Cardiac magnetic resonance protocol

All CMR examinations were performed using clinical whole-body MRI systems (Ingenia 1.5T or 3.0T; Philips Healthcare, Best, The Netherlands). Signal reception was achieved by a 16-channel torso coil using a digital interface. A signal intensity correction algorithm (CLEAR: Constant LEvel AppeaRance; Philips Medical Systems) was utilized to correct for torso-coil related signal inhomogeneities. Short-axis, 2-chamber, 3-chamber, and 4-chamber cine

TABLE 1 Patient characteristics and cardiac magnetic resonance findings.

Parameter	Acute myocarditis baseline ($n = 9$)	Acute myocarditis follow-up ($n = 9$)	Controls ($n = 20$)	*P*-value
Age (years)	24.1 ± 6.4	24.7 ± 6.1	25.9 ± 7.2	0.77
Males (n, %)	8 (89)	8 (89)	18 (89)	0.99
Height (cm)	176 ± 9	176 ± 9	177 ± 8	0.98
Weight (kg)	74 ± 20	78 ± 22	79 ± 11	0.84
LVEF (%)	58 ± 6	62 ± 4	60 ± 4	0.29
LVEDV (ml)	158 ± 32	161 ± 28	157 ± 20	0.95
LVEDVi (ml/m^2)	83 ± 12	82 ± 12	80 ± 8	0.62
IVSD (mm)	8.7 ± 1.8	9.2 ± 1.6	9.3 ± 1.7	0.69
Visual edema (n)[b]	7 (77%)	0 (0%)	0 (0%)	<0.001*
Visual LGE present (n)[b]	9 (100%)	9 (100%)	0 (0%)	<0.001[†]
LGE extent (%)	4.2 ± 2.1	0.9 ± 0.8	0.6 ± 0.2	<0.001*
T2 signal intensity ratio	1.9 ± 0.3	1.7 ± 0.3	1.6 ± 0.3	0.07
T1 relaxation times (ms)[a]	1024 ± 77	971 ± 34	982 ± 62	0.28
T2 relaxation times (ms)[a]	57 ± 6	51 ± 3	51 ± 3	0.07
ECV (%)	24.5 ± 2.1	24.3 ± 1.8	23.3 ± 1.9	0.29
GRS (%)	25.6 ± 6.4	30.2 ± 10.2	22.9 ± 4.8	0.13
GCS (%)	−12.7 ± 1.9	−14.9 ± 2.7	−13.5 ± 2.0	0.17
GLS (%)	−17.0 ± 2.2	−16.4 ± 1.9	−16.2 ± 1.7	0.60
Largest axillary lymph node at injection side (mm)	11.8 ± 2.3	7.8 ± 2.3	6.4 ± 1.3	<0.001*

P-values were derived from ANOVA (with Tukey *post-hoc* tests) unless otherwise noted. [a]Data from one patient was not included due to CMR examination at 3.0 Tesla. [b]$\chi 2$ test. *$p < 0.05$ baseline compared to follow-up and control. [†]$p < 0.05$ control group compared to baseline and follow-up. IQR, interquartile range; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end diastolic volume; LVEDVi, left ventricular end diastolic volume index; IVSD, interventricular septum thickness at diastole; LGE, late gadolinium enhancement; ECV, extra cellular volume; GRS, global radial strain; GLS, global longitudinal strain; GCS, global circumferential strain.

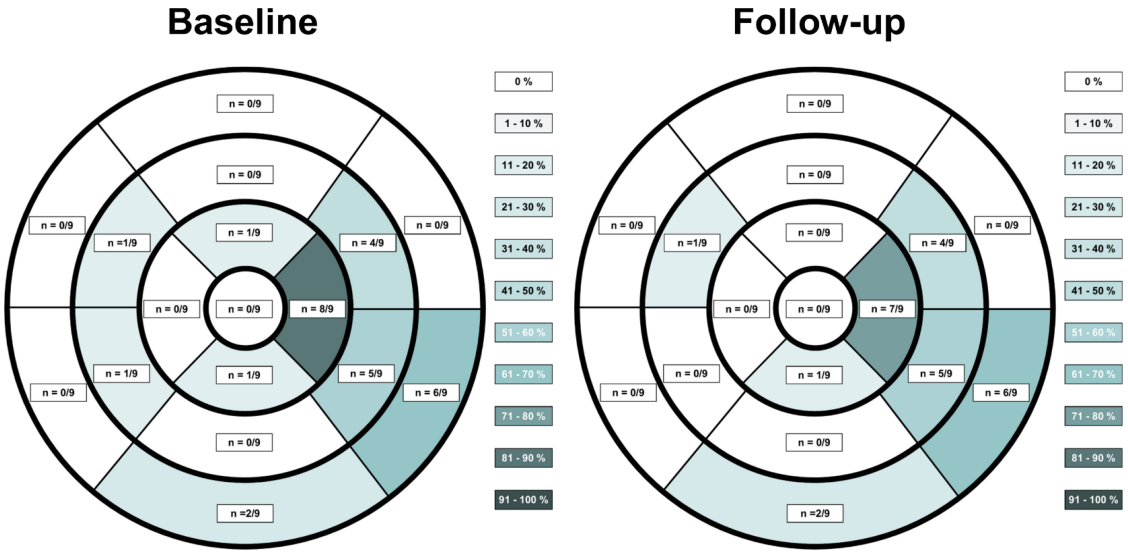

FIGURE 1
Late gadolinium enhancement (LGE) distribution on CMR in patients suffering from vaccine associated myocarditis at acute baseline imaging and at follow-up according to the American Heart Association 17-segment heart model. LGE extent was reduced on follow-up imaging, although LGE findings were still discernable at most previous locations.

views were acquired using electrocardiogram gated, breath-hold steady state free precession sequences for functional analysis. A transversal respiratory-gated fat-suppressed T2-weighted fast spin echo sequence (Philips MultiVane XD, Philips Healthcare, Best, The Netherlands) was acquired for the assessment of axillary and mediastinal

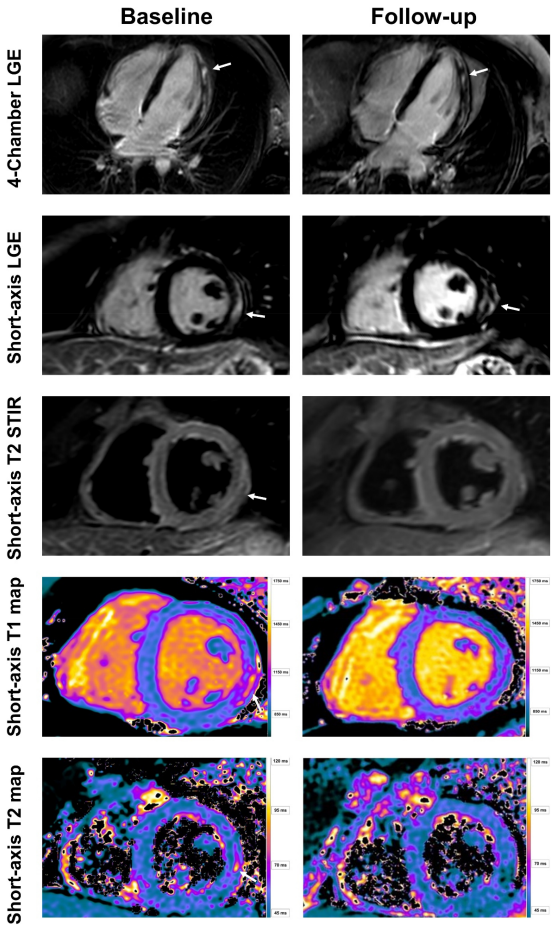

FIGURE 2
Baseline and follow-up cardiac imaging in a 25-year-old male with acute vaccine associated myocarditis after receiving his second vaccine with Pfizer/BioNTech. Short-axis and 4-chamber late gadolinium enhancement (LGE) views demonstrate subepicardial enhancement along the midventricular and apical inferolateral wall (arrows). T2 short-axis short tau inversion recovery (STIR) imaging corresponding to the LGE findings shows a resolution of edema from baseline to follow-up (arrow). Normalization of T1 and T2 relaxation times are also demonstrated over time (arrow). Note, however, the persistent LGE along the inferolateral wall even at follow-up, consistent with scar tissue.

lymphadenopathy, which was also included in the follow-up protocol. Myocardial edema was visualized using T2-weighted short-tau inversion-recovery sequences in short axis and transversal views. T2 STIR images were also used to calculate T2 signal intensity ratio. Myocardial T1 and T2 mapping was performed in end-diastolic short axis views with acquisition of apical, midventricular, and basal sections. A six-echo gradient spin-echo sequence (GraSE) was applied for myocardial T2 mapping (8). Myocardial T1 mapping was achieved using a standard 3(3)3(3)5 modified Look-Locker inversion recovery (MOLLI) acquisition scheme, with post-contrast T1 maps acquired 10 min after the administration of contrast medium (9). For contrast enhancement, a 0.2 mmol/kg of body weight bolus of gadoterate meglumine (Clariscan; GE Healthcare, Chicago, IL, USA) was used. Segmented inversion-recovery gradient-echo sequences for LGE imaging were obtained in short axis, 2-chamber, 4-chamber, and transversal views. The Look-Locker method was utilized to determine the optimal inversion time for LGE image acquisition as previously described (10). Sequence parameters for 1.5 Tesla are summarized in **Supplementary Table 1**.

Image analysis

Image analysis was performed by a board-certified cardiovascular radiologist (J.A.L with 10 years of experience in CMR) and a radiology resident (D.K. with 4 years of experience in CMR) using dedicated software (IntelliSpace Portal, version 12.1.4.; Philips Medical Systems). Papillary muscles were included for the volumetric quantification of the left ventricle. Global systolic radial, longitudinal, and circumferential strain were calculated by using feature tracking strain analysis software (CAAS MR Solutions, version 5.2.1.; Philips Medical Systems) using short-axis, 2-chamber, and 4-chamber balanced steady state free precession cine imaging.

Focal areas of regional high signal intensities in a non-ischemic distribution pattern on T2 short-tau inversion-recovery and on LGE images were visually assessed by consensus agreement of the two readers. Quantitative markers of myocardial edema (T2 signal intensity ratio) and myocardial injury and fibrosis (enhanced areas were defined as those with a signal intensity $\geq$ 3.0 standard deviations above the mean signal intensity of normal myocardium) were calculated as previously reported (11–13). Motion correction was achieved using a software-implemented algorithm (fast elastic image registration, IntelliSpace Portal) for myocardial T1 and T2 relaxation maps, deriving global T1 and T2 relaxation times. Hematocrit-corrected global ECV values were calculated as previously described (12, 14, 15). For scans at 1.5 Tesla, institution specific cutoffs ($\geq$1000 ms for myocardial T1 relaxation times and $\geq$ 55.9 ms for myocardial T2 relaxation times) for the assessment of the 2018 Lake Louise criteria were used as previously described (16). LGE distribution was classified according to the American Heart Association 17 segment heart model (17). LGE localization was classified according to wall involvement (subepicardial, midmyocardial, subendocardial, transmural, or patchy). Axial T2 weighted images were assessed for axillary lymph node enlargement and compared to previous imaging in the injection arm of the vaccine. For the control group, the largest axillary lymph node of either side was used. The largest short axis diameter measured in millimeter was recorded.

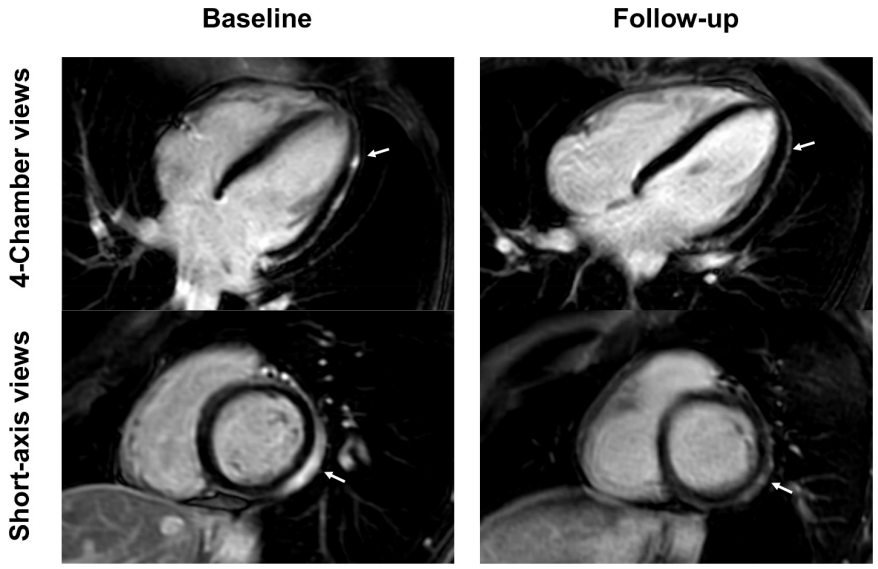

FIGURE 3
Short-axis and 4-chamber late gadolinium enhancement (LGE) views in a 28-year-old, previously healthy male after receiving his second Moderna vaccine. Reduction of subepicardial enhancement along the lateral wall from baseline (arrows) to follow-up 11-months later consistent with myocardial scarring. This patient reported new onset of occasional exertional dyspnea after initial acute vaccine associated myocarditis.

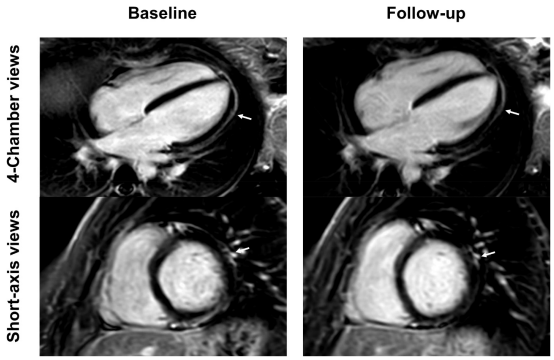

FIGURE 4
Short-axis and 4-chamber late gadolinium enhancement (LGE) views in a 26-year-old, previously healthy female after receiving the first dose of the Pfizer/BioNTech vaccine. Nearly complete resolution of LGE (arrows) at follow-up 2 months later with minor enhancement discernable at the apical lateral segment. Clinical correlation showed a complete resolution of previous symptoms which included chest pain and exertional dyspnea.

Statistical analysis

Prism (version 8.4.1; GraphPad Software) and Jamovi (version 2.2; The Jamovi Project) were used for statistical analysis. Data are given as means ± standard deviation or as percent to absolute frequency. Continuous variables were summarized as median with interquartile range (IQR) or as mean ± standard deviation, as appropriate. Normal distribution was checked using the Shapiro–Wilk test. For comparison of continuous variables and inter-individual variables the Student's *T*-test was used. A paired *T*-Test was used for the comparison of means in variables recorded at baseline and at follow-up. Mann–Whitney-*U* test was used for non-normal distributed data. Dichotomous variables were compared by using the $\chi 2$ test. One-way analysis of variance (ANOVA) followed by Tukey *post-hoc* multiple comparison tests was performed to compare variables in three groups. The level of statistical significance was set to $P < 0.05$.

Results

Patient characteristics

In total, nine patient datasets with baseline and follow-up CMR (8 male [89%], aged 24 ± 6 years) were available for retrospective analysis. A detailed comparison with 20 age-/and gender-matched controls (18 males [89%], aged 26 ± 7 years) is given in **Table 1**. Patients with vaccine associated myocarditis received the following vaccines: Pfizer/BioNTech (n = 7, 78%) of which one was a first dose, four were the second dose, and two were a booster dose (third vaccination); Moderna (n = 1, 11%) of which it was a second dose; Janssen (n = 1, 11%) first dose (only one dose required). Highly sensitive troponin T levels were elevated in all nine patients (median 644 ng/l [IQR: 159–930 ng/l]). All patients were treated with either cardioprotective or anticoagulative medication: 6 out of 9 (67%) were treated with beta-blockers, 4 (44%) with ACE-inhibitors, 2

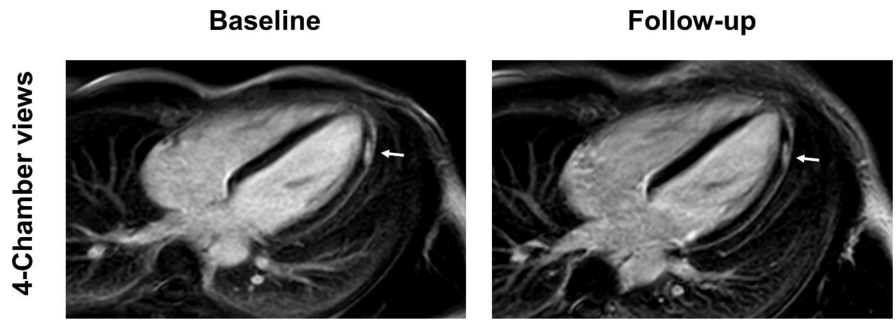

FIGURE 5
4-chamber late gadolinium enhancement (LGE) views of a 36-year-old, previously healthy male after receiving the third dose of the Pfizer/BioNTech vaccine. The LGE lesion at the apical lateral wall (arrow) reduced at follow-up 3 months later, but was still visible, a finding which is consistent with scar tissue. Patient reported complete resolution of clinical symptoms which included chest pain and palpitations.

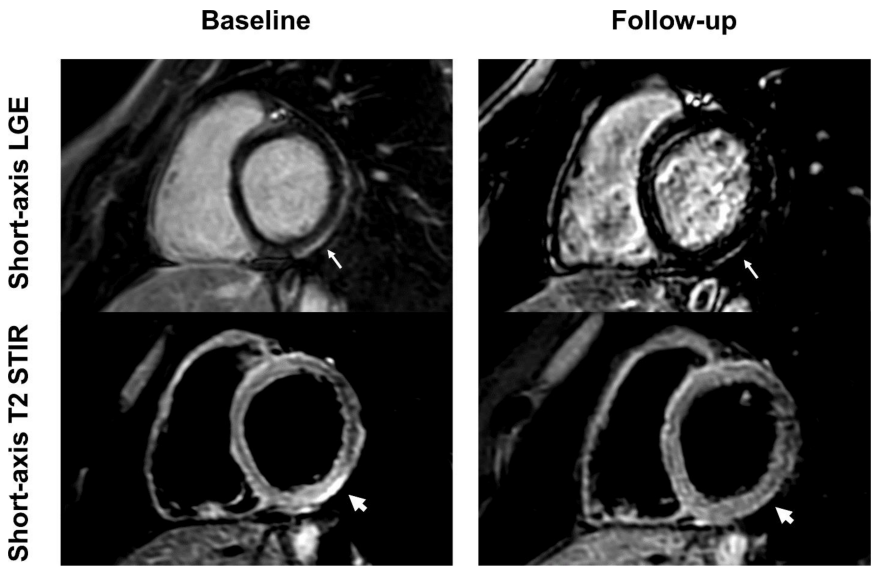

FIGURE 6
Short-axis late gadolinium enhancement (LGE) and short-axis T2 short tau inversion recovery (STIR) views of a 24-year-old, previously healthy male after receiving the second dose of the Pfizer/BioNTech vaccine. LGE at the basal inferolateral wall (thin arrows) shows a marked decrease at 6-month follow-up, with only minimal remaining findings. T2 STIR imaging shows focal myocardial edema corresponding to the location of the LGE (thick arrows) with complete resolution at follow-up. The patient reported complete resolution of previous clinical symptoms but complained of new onset exertional dyspnea.

(22%) with low molecular weight heparin, and one (11%) with a diuretic.

Clinical symptoms

All nine had clinical symptoms of acute myocarditis at initial scan: 8 out of 9 (89%) presented with chest pain, 3 (33%) with exertional dyspnea, and 2 (22%) with occasional fever. At follow up, only 1 out of 9 (11%) patients reported persistent chest pain, 2 patients reported new onset of exertional dyspnea (22%), and none reported fever. Patients did not report signs of infection prior to vaccination. Median number of days to symptom onset after vaccination was 0 days (IQR: 0–1 days; mean 0.6 ± 1.0 days), median time to initial CMR was 6.5 days (IQR: 5.3–12.8 days; mean 8.1 ± 3.9 days), and median time to follow-up was 3.0 months (IQR: 2.0–10.5; mean 5.8 ± 4.3 months).

Cardiac magnetic resonance results

T1 and T2 relaxation times from the same scanner at the same field strength (1.5T) were available for 8 patients while one patient received both, baseline and follow-up scans using a 3.0T MRI scanner. T1 and T2 maps from this patient were

excluded from consecutive analysis. All patients demonstrated positive LGE findings typical for acute myocarditis. The most common LGE distribution pattern was subepicardial (n = 9, 100%), followed by midwall (n = 3, 33%) or diffuse transmural involvement (n = 2, 22%). LGE distribution according to the American Heart Association 17-segment heart model at baseline and at follow-up is depicted in **Figure 1**. Visual myocardial edema was noted in 7 out of 9 patients at baseline (78%) with complete resolution at follow-up in all patients. Direct baseline to follow-up imaging comparisons are shown in **Figures 2–7**. All p-values within the text are derived from a paired t-test analysis between baseline and follow-up data unless otherwise noted. All patients demonstrated a significant reduction of overall LGE extent from baseline to follow-up (4.2 ± 2.1 vs. 0.9 ± 0.8%, p = 0.001), although persistent LGE was noted in all patients at follow-up, consistent with post-inflammatory scar tissue. In two cases (22%) visual LGE on follow-up was barely discernable, and in all cases visual LGE demonstrated a reduction in signal intensity. Septal LGE sparring was noted in most patients and only 2 patients demonstrated septal LGE. T2 relaxation times were noted to be significantly higher at baseline compared to follow-up (57 ± 6 vs. 51 ± 3 ms, p = 0.03). T1 relaxation times were also higher at baseline, although not statistically significant (1024 ± 77 vs. 971 ± 34 ms, p = 0.05).

No significant differences between baseline and follow-up investigation were noted for left ventricular ejection fraction (58 ± 6 vs. 62 ± 4%; p = 0.10), left ventricular end diastolic volume (158 ± 32 vs. 161 ± 28 ml; p = 0.62), or left ventricular end diastolic volume index (83 ± 12 vs. 83 ± 9 ml/m^2; p = 0.87). A significant reduction in axillary lymph node size was noted between baseline and follow-up (11.8 ± 2.3 vs. 7.8 ± 2.3 mm; p = 0.003). A slightly significant improvement in systolic global circumferential strain (GCS) was noted from baseline to follow-up (−12.7 ± 1.9 vs. −14.9 ± 2.7%, p = 0.04). No such statistically significant difference was noted for systolic global radial strain (GRS, 25.6 ± 6.4 vs. 30.2 ± 10.2%, p = 0.12) or global longitudinal strain (GLS, −17.0 ± 2.2 vs. −16.4 ± 1.9%, p = 0.08) between baseline and follow-up. **Table 2** provides an overview of some currently available data regarding vaccine associated myocarditis.

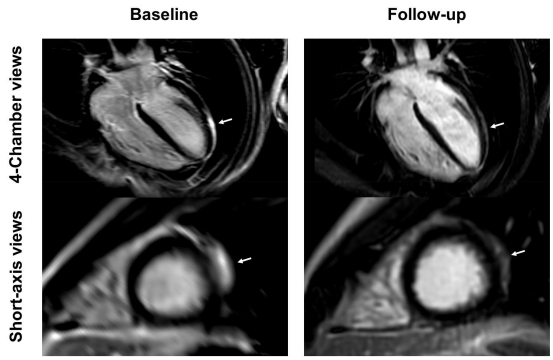

FIGURE 7
4-chamber and short-axis late gadolinium enhancement (LGE) views of a 15-year-old male after receiving the second dose of the Pfizer/BioNTech vaccine. Subepicardial and pericardial LGE at the apical lateral wall (arrows) shows a marked decrease at the 9-month follow-up with residual findings. Resolution of chest pain at follow-up was accompanied by new onset of occasional exertional dyspnea.

Discussion

This retrospective study analyzed clinical and CMR data from nine, non-hospitalized patients with vaccine associated myocarditis regarding parameters such as left ventricular function, LGE extent, and myocardial T1 and T2, at initial imaging and at follow-up as well as to an age and sex matched control group. While vaccine associated myocarditis is a rare possible complication of currently available COVID-19 vaccines, its clinical presentation and CMR characteristics at the acute stage and follow-up should be known to cardiovascular imaging physicians.

Vaccine associated myocarditis is more likely to occur after vaccination with mRNA-based vaccines, although we report of one case after vaccination with a vector vaccine (Janssen). The number of received doses also seems to play a role as vaccine associated myocarditis is less likely to be noted after the first dose, usually causing symptoms after the second or third booster dose, suggesting that prior exposure is necessary for the development of vaccine associated myocarditis. However, the exact pathomechanism of vaccine associated myocarditis remains unknown. Current theories focus on a hypersensitivity reaction or cross reactions of spike proteins with myocardial contractile proteins (33, 34).

Collaborating previously published findings, we found vaccine associated myocarditis to predominantly affect younger males (1, 3, 35). Typically, clinical course of disease is mild with rapid resolution of symptoms within a few months (17, 19, 25, 28, 33), although persistent LGE on CMR indicative of fibrous scar tissue was noted in all our patients. Resolution of other CMR findings (elevated T1 and T2 times, focal or diffuse edema) are to be expected as the extent of the inflammatory process diminishes over time. Other studies have noted a relatively normal left ventricular ejection fraction in vaccine associated myocarditis compared to patients with other causes of myocarditis (4). We found similar findings in our study, as parameters of myocardial function were not noticeably impaired. Cardiac strain has been shown to improve the diagnostic performance of the 2018 revised Lake Louise Criteria and provide prognostic value regarding major adverse cardiovascular events for acute myocarditis (36–39). Strain analysis might be able to detect subtle changes in myocardial

TABLE 2 An overview of current studies concerning COVID-19 vaccine associated myocarditis.

References	Patients (n)	Males (n)	Age (years)	1st/2nd dose (n)	Vaccine P/M/A/J (n)	Symptoms	CMR findings
Ahmed et al. (18)	7	7	25	0/7	5/2/0/0	Chest pain, fatigue, dyspnoea, elevated troponin T	Acute non-severe myocarditis after vaccination
Evertz et al. (19)	10	10	26	2/8	4/6/0/0	Chest pain, dyspnoea	Subepicardial LGE and edema, normal LVEF, normal global longitudinal strain
Fronza et al. (4)	21	17	31	4/17	9/12/0/0	Chest pain	LGE findings in all patients
Kravchenko et al. (3)	9	7	24	2/7	8/1/0/0	Chest pain, elevated troponin T, fatigue	All LLC positive patients demonstrated elevated troponin T and LGE on CMR
Abellan et al. (20)	3	3	29	0/3	0/3/0/0	Chest pain, elevated troponin T	Acute non-severe myocarditis after vaccination
Diaz et al. (21)	20	15	36*	4/16	9/11/0/0	N/A	Acute non-severe myocarditis or perimyocarditis after vaccination
Bautista García et al. (22)	1	1	39	0/1	1/0/0/0	Fever, chest pain	Edema and LGE
Isaak et al. (23)	1	1	15	0/1	1/0/0/0	Fever, myalgia, chest pain, elevated troponin T	Subepicardial LGE. Normal left ventricular function
Jain et al. (24)	63	58	16	1/62	59/4/0/0	Fever, chest pain, fatigue, headache	Mild LVEF dysfunction, edema, LGE
Kim et al. (25)	4	3	38	0/4	2/2/0/0	Fatigue, chest pain	All patients demonstrated subepicardial LGE and elevated T1 and T2 times
Larson et al. (26)	8	8	32	1/7	5/3/0/0	Chest pain	Elevated troponin T in 6 patients. All patients demonstrated LGE findings, most with associated oedema
Marshall et al. (27)	7	7	17*	0/7	7/0/0/0	Chest pain, elevated troponin T	All patients presented with LGE, hyperaemia, and cardiac oedema
Montgomery et al. (28)	23	23	25*	3/20	7/16/0/0	Chest pain, elevated troponin T	CMR was performed in 8 of 23 cases with findings including edema and abnormal LGE
Abu Mouch et al. (29)	6	6	–	1/5	5/0/0/0	Chest pain	Elevated troponin T in 4 out of 6. All patients demonstrated LGE. Uncomplicated resolution
Perez et al. (30)	7	6	50	1/6	3/4/0/0	Chest pain, dyspnoea, fatigue	LGE, pericardial involvement in 50% of the cases
Rosner et al. (31)	7	7	27	2/7	5/1/0/1	Chest pain, elevated troponin T, fever	Cardiac edema in 5 patients. LGE in all patients
Shaw et al. (32)	4	2	24	2/2	3/1/0/0	Chest pain	Edema and LGE
Truong et al. (7)	139	126	16	12/128	131/5/0/1	Chest pain, fever, myalgia	Edema and LGE

P, Pfizer/BioNTech; M, Moderna; A, AstraZeneca; J, Johnson & Johnson; N/A, not available; CMR, cardiac magnetic resonance; LGE, late gadolinium enhancement; LLC, Lake Louise criteria; LVEF, left ventricular ejection fraction. All data are presented as mean unless otherwise noted. *Data reported as median.

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tissue earlier when other traditional prognostic markers such as left ventricular ejection fraction (LVEF) or LGE are normal. Strain encoded MRI has been reported to be able to identify patients with subclinical LVEF dysfunction, potentially at risk for heart failure (40). In ischemic heart disease, strain encoded MRI was able to differentiate between reversible and irreversible myocardial injury (41). Reduced GLS and GCS have also been associated with edema in suspected acute myocarditis (42). Feature tracking strain analysis did not show any statistically significant difference in systolic GRS or GLS between the three groups. A decrease in GLS has been previously described to be a negative prognostic marker for major adverse cardiovascular events (36). A small, but discernable improvement was noted for GCS going from baseline to follow-up, as well as an insignificant improvement in GRS and worsening in GLS. The difference in these values might be attributed to the small patient population of this study. Resolution of symptoms was noted in most patients, although two patients reported new onset of occasional exertional dyspnea. This may be in part due to residual LGE findings on CMR, which has been previously described as a marker of unfavorable prognosis when paired with a resolution of associated edema (43). The full implications of remaining myocardial scar tissue are unknown in such a young patient group. The risk of developing ventricular arrhythmias for such young patients after vaccine-induced acute myocarditis is currently unknown. A preference for lateral ventricular wall involvement for LGE with septal sparring has been observed in the majority of patients, indicating a more favorable prognosis regarding the development of arrhythmias (44–47).

Our study suffers from limitations, including the small patient cohort and variable follow-up times. Most statistical comparisons were not corrected for multiple testing due to the small data set. As of date, there are no published consensus criteria for the diagnosis of vaccine associated myocarditis after COVID-19 vaccination. We therefore defined it as myocarditis like symptoms after COVID-19 vaccination with abnormal CMR findings in previously healthy people accompanied with elevated troponin T levels within a reasonable number of days of COVID-19 vaccination. Not all CMR examinations were performed on the same machine or at the same field strength, diminishing data sets available for statistical analysis. Furthermore, the reference standard for diagnosis of myocarditis, endomyocardial biopsy, was not performed as it is not part of the best standard of care practice at our institution. Further studies comparing CMR findings in vaccine associated myocarditis with other causes of myocarditis akin to data published by Fronza et al. offer an outlook for potential future research (35).

Conclusion

Vaccine associated myocarditis tends to affect younger, predominantly male patients and shows abnormal CMR findings such as focal or diffuse edema, elevated T1 and T2 relaxation times, and LGE. While the overall prognosis seems to be favorable and a rapid resolution of symptoms is observed, reduced, yet persistent LGE findings indicative of myocardial fibrosis in light of complete resolution of edema have been noted. Further studies are needed to examine the long-term effects of the remaining scar-tissue and develop recommendations for patients with a history of vaccine associated myocarditis regarding booster doses.

Data availability statement

The raw data supporting the conclusions of this article can be provided upon reasonable request by the authors, without reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethikkommission der Medizinischen Fakultät Bonn, University Hospital Bonn, Venusberg-Campus 1, 53127, Bonn. Written informed consent from the participants or their legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

JL and DKr contributed to the conception and design of the study and performed the statistical analysis. DKr organized the database and wrote the first draft of the manuscript. JL, AI, DKu, DKr, CP, and SZ wrote sections of the manuscript. All authors contributed to the manuscript revision, read, 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.1049256/full#supplementary-material

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Case report: mRNA-1273 COVID-19 vaccine-associated myopericarditis: Successful treatment and re-exposure with colchicine

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Introduction: Vaccine-induced myocarditis is a rare complication of messenger RNA (mRNA) COVID-19 vaccines.
Case presentation: We report a case of acute myopericarditis in a recipient of allogeneic hematopoietic cells following the first dose of the mRNA-1273 vaccine and the successful administration of a second and third dose while on prophylactic treatment with colchicine to successfully complete the vaccination.
Conclusion: Treatment and prevention of mRNA-vaccine-induced myopericarditis represent a clinical challenge. The use of colchicine is feasible and safe to potentially reduce the risk of this rare but severe complication and allows re-exposure to an mRNA vaccine.

KEYWORDS
mRNA vaccine, myocarditis, colchicine, allogeneic stem cells transplantation, COVID-19, mRNA-1273

Introduction

Vaccine-induced myocarditis is a rare complication of messenger RNA (mRNA) vaccines against SARS-CoV-2. There are no reliable data about this adverse event in patients who received allogeneic hematopoietic cell transplantation (HCT). We report a case about a severe clinical course of mRNA-vaccine-induced myopericarditis, its challenging diagnostic process and plausible pathological background, the therapeutical use of colchicine in the acute phase of the disease, and also as a successful prophylaxis by re-exposure to mRNA vaccine against SARS-CoV-2.

Abbreviations
ECG, electrocardiogram; GvHD, graft versus host disease; HCT, hematopoietic stem cell transplantation; MRI, magnetic resonance imaging; mRNA, messenger RNA; NTproBNP, N-terminal prohormone of brain natriuretic peptide; NSAID, non-steroidal anti-inflammatory drug; P-gp, transport protein p-glycoprotein.

Case presentation

A 70-year-old patient presented to the emergency department complaining of severe back pain radiating to the chest and dyspnea occurring within 12 h following the first dose of the mRNA-1273 vaccine (Spikevax, Moderna). Twenty months earlier, the patient underwent allogeneic hematopoietic cell transplantation for primary myelofibrosis. At the time of presentation, he was in complete remission without signs of active graft versus host disease (GvHD) or infectious disease. Current medication included bisoprolol, ursodeoxycholic acid, budesonide, esomeprazole, insulin, valaciclovir, co-trimoxazole, and gabapentin.

On admission, he was hemodynamically stable (blood pressure 136/79 mmHg, heart rate 79 bpm), afebrile, and adequately oxygenated (oxygen saturation 99% in ambient air). Besides arterial hypertension, no other cardiovascular risk factors were present. An electrocardiogram (ECG) showed concave ST-elevation and PQ-segment depression in several ECG leads and not related to a single coronary artery (**Figure 1A**). Initial laboratory tests indicated a mildly elevated high sensitivity Troponin T of 53 ng/L (reference <14 ng/L), and an N-terminal prohormone of brain natriuretic peptide (NTproBNP) of 655 ng/L (reference <124 ng/L). The remaining laboratory values are summarized in **Table 1**.

Aortic dissection, pulmonary embolism, and type 1 myocardial infarction were ruled out by computed tomography scan and coronary angiography, respectively. A single-vessel coronary artery disease with a non-critical right coronary artery stenosis was identified that was not assumed to be responsible for the acute symptoms. Bedside echocardiography showed a hemodynamically insignificant pericardial effusion with preserved left systolic and right heart function bedside echocardiography. A cardiac magnetic resonance imaging (MRI) performed 2 days after symptom onset confirmed pericardial effusion and showed a circumferential pericardial contrast enhancement compatible with pericarditis; the myocardium showed no late gadolinium enhancement and no edema (**Figures 1B,C**). However, based on the dynamical rise of troponin levels (peak 1,139 ng/L on day 5), a substantial myocardial injury was present. Combined with the ECG changes and cardiac imaging findings and after ruling out alternative causes, acute mRNA vaccine-related probable myopericarditis was diagnosed. No infections or other medical problems in the weeks prior to the presentation could be identified in the patient's history. A nasopharyngeal swab for

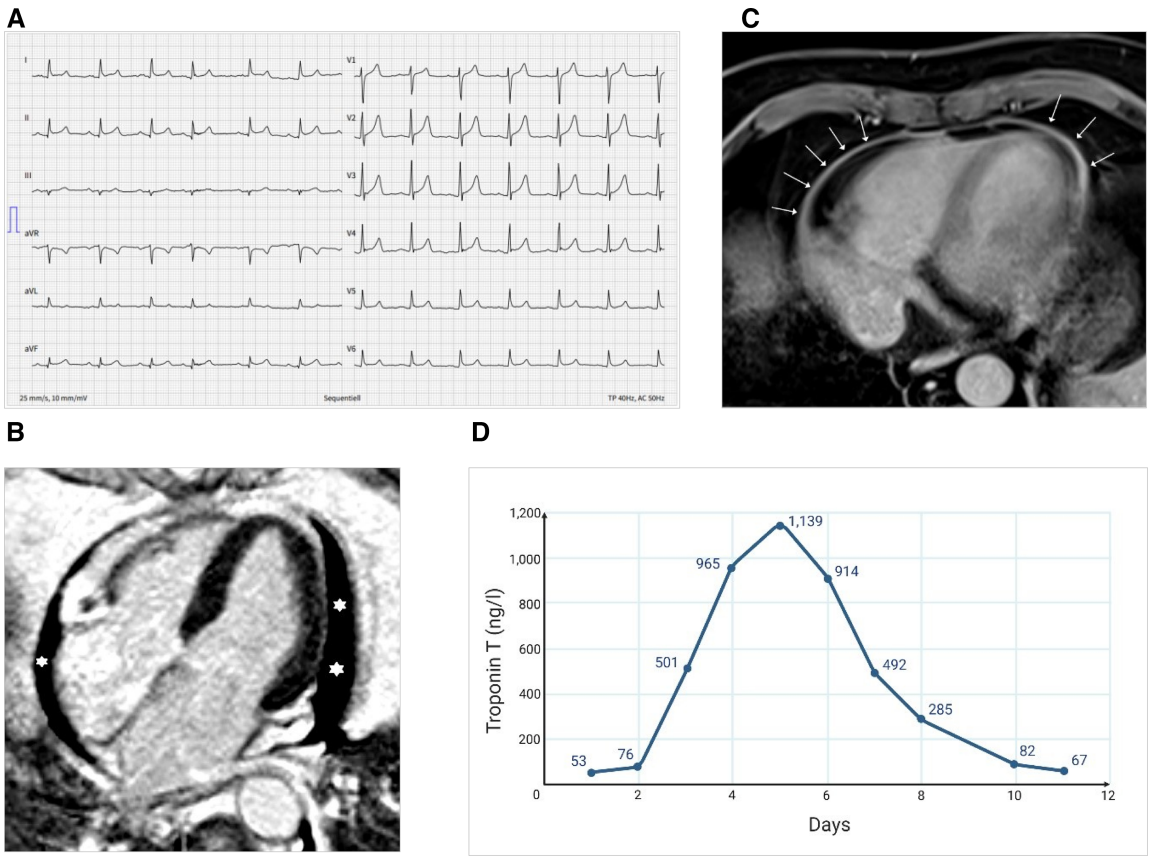

FIGURE 1
Electrocardiographic and imaging findings at admission and course of high-sensitive troponin T. (**A**) ECG showing diffuse concave ST-elevation (II, aVF, V3-6) and PQ-depression (II, aVF). (**B**) MRI showing pericardial effusion (asterisks) in the four-chamber view (late enhancement PSIR). (**C**) MRI showing pericardial enhancement (arrows) (axial T1 DIXON Water only, which is a fat-saturated, non-ECG-triggered). (**D**) Longitudinal evolution of the high-sensitive troponin T levels during the hospitalization (MRI done on day 3; i.e., troponin 501 ng/L).

TABLE 1 Laboratory values at admission.

Laboratory investigation	Results	Reference range
Hemoglobin (g/L)	129	120–160
Leucocytes (G/L)	6.3	3.5–10.0
Neutrophils (G/L)	4.7	1.3–6.7
Lymphocytes (G/L)	0.8	0.9–3.3
Platelets (G/L)	153	150–450
C-reactive protein (mg/L)	18.2	<10.0
Creatine kinase (U/L)	29	38–157
Creatine kinase myocardial band (µg/L)	2.5	<5.0
Troponin T high sensitivity (ng/L)	53	<14
N-terminal prohormone of brain natriuretic peptide (NTproBNP) (ng/L)	655	<125
Alanine transaminase (U/L)	93	8–41
Lactate dehydrogenase (U/L)	223	135–214

respiratory viruses, an autoimmune serology panel, and serological tests for enterovirus and adenovirus antibodies were repeatedly negative. A nasal swab and a negative anti-SARS-CoV-2-Nucleoprotein-IgG/M excluded an acute or previous infection with SARS-CoV-2.

The patient was admitted to the hospital for telemetric monitoring and treatment with a non-steroidal anti-inflammatory drug (NSAID) as well as colchicine 0.5 mg every 12 h without systemic steroids was started.

After 13 days, he was discharged (**Figure 1D**). After 2 months, colchicine was stopped, followed by symptom recurrence after 2 weeks. Troponin was negative then, but C-reactive protein was 16 mg/L (reference <10 mg/L). Echocardiography and a computed tomography scan indicated increased pericardial effusion; thus, a second episode of isolated pericarditis was diagnosed. Therapy with NSAIDs and colchicine was reintroduced and could finally be tapered after 6 weeks without relapse.

Due to the non-availability of alternative (non-mRNA) vaccines in Switzerland at that time and due to the high individual risk for severe COVID in this patient, we discussed a re-exposure with a second dose of an mRNA vaccine to complete the immunization. In order to prevent the re-occurrence of myopericarditis, we initiated a prophylactic therapy with colchicine 0.5 mg every 12 h, starting a week before the second dose. The rationale for using an inflammasome-inhibiting drug as prophylaxis was based on the data suggesting inflammasome-induced inflammation in the pathogenesis of mRNA vaccine-associated myocarditis (1), and data indicating a reduced risk of (inflammasome mediated) mRNA vaccine induced gout flares on colchicine treatment (2). Because the symptoms developed exceptionally rapidly after the first dose, we opted to monitor the patient in the hospital for 72 h. He received the second dose of mRNA-1273 in September 2021, 5 months after the first dose. No symptoms, arrhythmias, or ECG changes occurred. Colchicine was discontinued 4 weeks after the second dose. The clinical course was uneventful. SARS-CoV-2-S-IgG/M antibody levels 5 weeks after second vaccination were >2,500 U/mL. Subsequently, the patient could successfully complete the vaccination course with a third dose of mRNA-1273, again under colchicine prophylaxis for 4 weeks and without evidence of myopericarditis. **Figure 2** shows the timeline of the clinical milestones of the patient.

Discussion and conclusion

We present a case of myopericarditis in temporal association with a first dose of an mRNA COVID vaccine. The patient had classical pericarditis and a substantial dynamic troponin elevation. The troponin level was elevated before an uneventful diagnostic angiography without intervention was performed. The cardiac MRI 2 days following the angiography showed no evidence of ischemia or alternative causes of the troponin elevation. Based on the case definitions for vaccine-associated myocarditis by the Brighton Collaboration, the case fulfilled the criteria for "probable myocarditis" in addition to pericarditis (3).

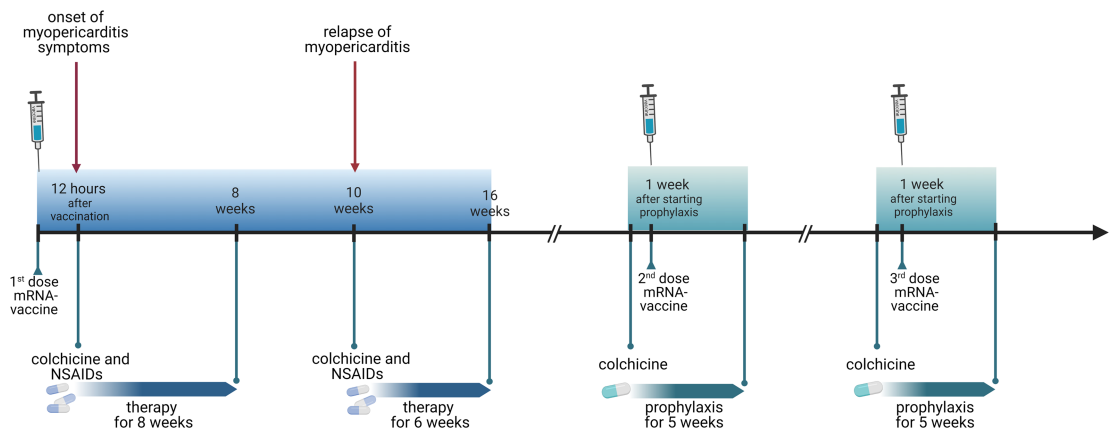

FIGURE 2
Timeline of clinical course (figure created with biorender.com).

Pericarditis following mRNA COVID vaccinations has been reported, although actual incidence rates have not yet been established (4, 5), likely due to a mix between isolated myocarditis and myopericarditis (5). In a case series, Ochs et al. reported five cases of isolated pericarditis occurring within 7 days following COVID vaccination (6). In contrast to our case, these subjects had normal, or borderline elevated, troponin levels. Vaccine-induced myocarditis emerged as a rare but serious adverse event following immunization with mRNA—vaccines against SARS-CoV-2 during the postmarketing surveillance (7, 8). While no cases occurred in the pivotal phase III studies trials (8, 9), epidemiological data estimated the incidence of SARS-CoV-2 vaccine-associated myocarditis between 0.3 and 5.5 per 100 per 100,000 vaccinated persons (7, 10–16). Data from different studies are summarized in **Table 2**. Most frequently, mRNA vaccine-induced myocarditis occurred in young male (<40 years of age) 1–5 days after the second dose of an mRNA vaccine with higher incidence following mRNA-1273 than BNT162b. The clinical course was mostly mild with only a few patients requiring steroids or intensive care treatment while the majority responded well to NSAIDs (14). So far, no further risk factors for the onset of vaccine-induced myocarditis could be identified.

To the best of our knowledge, this is the first case report on mRNA vaccine-induced myopericarditis in a patient who underwent allogeneic HCT. This case shows various specific peculiarities. Our patient was already 70 years old, and the symptoms developed about 12 h after vaccination. Although young men seem to be at the highest risk and the median time to symptom onset is 2–3 days, in real life, the age range of persons affected by mRNA vaccine-associated myocarditis is much broader, and a considerable number of individuals become symptomatic within the first 24 h (14, 17).

The pathogenesis of myopericarditis associated with mRNA vaccination is not entirely understood but may involve a dysregulation of several immunological pathways, including mRNA-induced innate immune stimulation *via* Toll-like receptors, hypersensitivity reaction against vaccine components, molecular mimicry between cardiac self-antigen and the spike protein of SARS-CoV-2, or hormone-dependent alteration of inflammatory pathways (testosterone vs. estrogen) (7, 18–20). Recently, autoantibodies against the interleukin-1 receptor antagonist (IL-1RA) were found in histologically proven myocarditis cases after SARS-CoV-2 vaccination (21). Given the early onset of symptoms after a single dose of the mRNA and in the absence of a previous SARS-CoV-2 infection, it is unlikely that vaccine-induced adaptive immune mechanisms were involved in our case. Previously, an in-depth immunological analysis of a case of mRNA vaccine-associated myocarditis revealed a dysregulation of the innate immune system underlying the pathogenesis of myocarditis (19). The clinical response of our patient to colchicine, an inhibitor of the innate immunity, is well matching this hypothesis. Notably, the transplanted immune system could have interfered with those mechanisms contributing to this side effect presentation. Because this patient underwent allogeneic HCT, we also considered a flare of GvHD in differential diagnosis. Pericarditis as a sign of chronic GvHD is rare, usually manifests as part of a polyserositis and/or other signs of GvHD. Because systemic immunosuppression has already been stopped 7 months prior to the event of myopericarditis and no other clinical signs of GvHD occurred, we consider a GvHD flare highly unlikely.

Our patient presented with a severe clinical course requiring longer in-hospital management because of prolonged precordial pain and delayed troponin normalization. Colchicine is a standard of care for patients with pericarditis (22–25). While less frequently used for the treatment of myopericarditis, a recent study indicated better outcome of myopericarditis if treated with colchicine (26). Based on (i) the observation that the inflammasome pathway, that is targeted by colchicine (**Figure 3**),

TABLE 2 Reported incidence of myocarditis among general population after at least one dose of after SARS-CoV-2 vaccine.

Incidence per 100,000 people	Study population, *n* (median age)	Country	Period	Vaccines	Type of study	References
1.90	38,615,491 (≥12 years)	United Kingdom	December 1, 2020 to August 24, 2021	Comirnaty, Spikevax, Vaxzevria	Self-controlled case series study	Patone, *Nature* 2021 (7)
2.13	2,558,421 (≥16 years)	Israel	December 20, 2020 to May 24, 2021	Comirnaty	Retrospective cohort study	Witberg, *NEJM* 2021 (10)
5.54	5,442,696 (≥16 years)	Israel	December 20, 2020 to May 31, 2021	Comirnaty	Retrospective cohort study	Mevorach, *NEJM* 2021 (11)
1.38	4,931,775 (≥12 years)	Denmark	October 1, 2020 to October 5, 2021	Comirnaty, Spikevax	Population based cohort study	Husby, *BMJ* 2021 (12)
0.29	10,162,227 (≥12 years)	United States of America	December 14, 2020 to June 26, 2021	Comirnaty, Spikevax	Interim analyses of surveillance monitoring of mRNA COVID-19 vaccines	Klein, *JAMA* 2021 (13)
0.85	192,405,448 (≥12 years)	United States of America	December 14, 2020 to June 26, 2021	Comirnaty, Spikevax	Descriptive study of reports of myocarditis to the Vaccine Adverse Event Reporting System (VAERS), national passive reporting system	Oster, *JAMA* 2022 (14)

SARS-CoV-2 vaccine: Comirnaty (BNT162b2, Pfizer/BionTech), Spikevax (mRNA-1273, Moderna), or Vaxzevria (ChAdOx1-S, AstraZeneca).

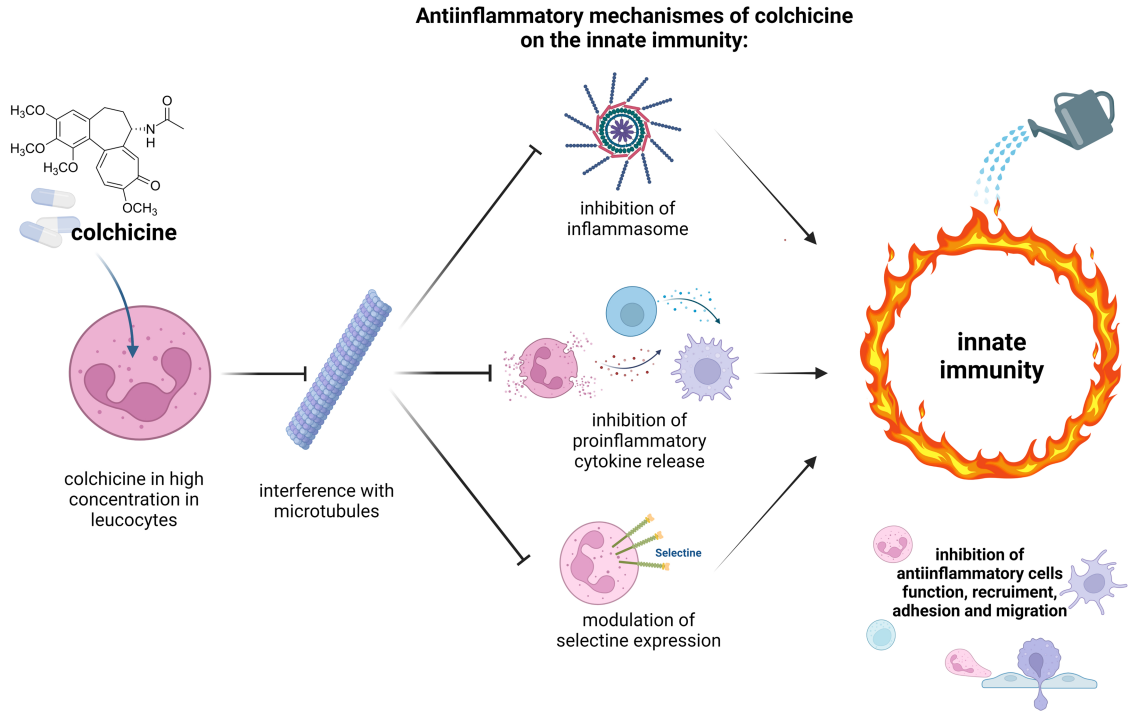

FIGURE 3
Mechanisms of action of colchicine on the innate immunity. High cytoplasmic concentration of colchicine especially in neutrophils are reached because of physiological reduced expression of transport P-glycoprotein (P-gp), which usually excretes drugs out of the cells. Colchicine inhibits assembly and attachment of microtubules. Consequently, several mechanisms of anti-inflammatory cell of the innate immunity are inhibited: inflammasome, a cytosolic multiprotein oligomers complex, which should activate an inflammatory cascade; release of proinflammatory cytokines (such as IL-1-β and IL-18) are downregulated; the expression E- and L-selectine on neutrophil surface, which promote the adhesion and the migration of those cells, are inhibited. This complex and sophisticated model results into inhibition of anti-inflammatory cells functions, recruitment, and motility of the innate immunity (22, 23, 25) (figure created with biorender.com).

is involved in the pathogenesis of mRNA vaccine-associated myocarditis (1), and (ii) the reported reduced risk of gout flares in association with mRNA vaccinations in patients treated with colchicine (2), we speculated that colchicine may reduce the risk for symptom reoccurrence upon re-exposure to an mRNA vaccine. Indeed, colchicine seemed to be effective for both acute treatment and prophylaxis, allowing a safe re-exposure to the mRNA vaccine and thereby the completion of the immunization resulting in an adequate SARS-CoV-2-S-antibody response. Whether colchicine reduces the risk for mRNA vaccine-associated pericarditis and myocarditis should be assessed in larger patient cohorts.

Data on vaccine-re-exposure in patients experiencing a myopericarditis after a previous single dose of vaccination are scare (27). Due to the high individual risk for severe COVID and the ongoing pandemic situation, we decided to re-expose the patient to a second dose with the same vaccine (mRNA-1273 Spikevax; Moderna) but under colchicine prophylaxis. At that time only, mRNA vaccines (mRNA-1273 and BNT162b) were available in Switzerland; therefore, no switching to another class of vaccine was possible. At the time of the second vaccination, no data were available about the differences in the rate of myopericarditis among mRNA vaccines.

This report has some limitations: first, it is a single-case report of an immunocompromised patient after allogeneic HCT, thus precluding generally valid conclusions of our observations. Second, the presentation differed from published cases of mRNA vaccine-induced myocarditis by the absence of typical myocarditis findings, such as late gadolinium enhancement and edema, in the cardiac MRI. However, given the high troponin indicating myocardial injury and the extensive work-up to exclude alternative causes, probable myopericarditis could be diagnosed, according to the proposed case definition of the Brighton Collaboration (3). In line with this, the patient had chest pain over several days, and the troponin levels showed an increase to a peak 5 days following vaccination. Third, we cannot exclude that second dose was well-tolerated independent of colchicine prophylaxis. Finally, we only assessed humoral immunogenicity after the second dose and therefore cannot comment on whether colchicine may have affected the adaptive T-cell response to the vaccination in this patient. However, colchicine is generally not considered to interfere with adaptive immunity (**Figure 3**).

Our observation suggests that, following careful individual risk–benefit assessment, re-exposition can be safe under prophylaxis with colchicine inducing sufficient immunization

thus reinforcing our conviction to continue a uniform and widespread vaccination campaign to protect our fragile patient population.

Data availability statement

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

Ethics statement

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

Author contributions

All authors have contributed substantially to this work. LV and EH collected clinical data on the case; CZ performed the echocardiography; MS performed the MRI; LV reviewed the literature; and LV, JH, and CB wrote the draft of the manuscript. 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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Supplementary material

The Supplementary Material for this article can be found online https://www.frontiersin.org/articles/10.3389/fcvm.2023.1135848/full#supplementary-material.

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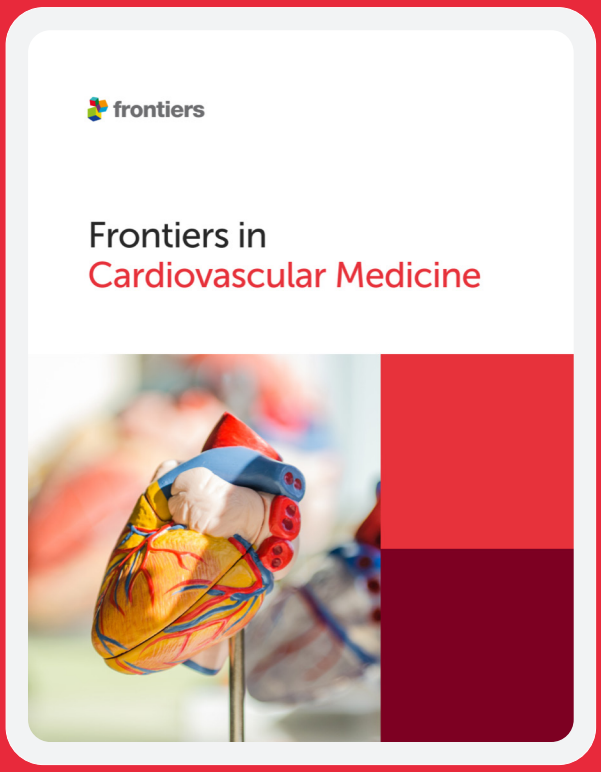

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