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Myocarditis and inflammatory cardiomyopathies: Diagnosis, treatment and future directions

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Published in
Frontiers in Cardiovascular Medicine
Frontiers in Immunology

ISSN 1664-8714
ISBN 978-2-8325-4021-3
DOI 10.3389/978-2-8325-4021-3

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Myocarditis and inflammatory cardiomyopathies: Diagnosis, treatment and future directions

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Citation

Bobbio, E., Vergaro, G., Gentile, P., Bollano, E., eds. (2023). *Myocarditis and inflammatory cardiomyopathies: Diagnosis, treatment and future directions*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-4021-3

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Editorial
PUBLISHED 14 November 2023
DOI 10.3389/fcvm.2023.1321494

OPEN ACCESS

EDITED AND REVIEWED BY
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RECEIVED 14 October 2023
ACCEPTED 03 November 2023
PUBLISHED 14 November 2023

CITATION
Gentile P, Bollano E, Vergaro G and Bobbio E (2023) Editorial: Myocarditis and inflammatory cardiomyopathies: diagnosis, treatment and future directions.
Front. Cardiovasc. Med. 10:1321494.
doi: 10.3389/fcvm.2023.1321494

Editorial: Myocarditis and inflammatory cardiomyopathies: diagnosis, treatment and future directions

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KEYWORDS

myocarditis acute and fulminant, heart failure, sarcoidosis, systematic review, imaging, cardiomyopathy

Editorial on the Research Topic
Myocarditis and inflammatory cardiomyopathies: diagnosis, treatment and future directions

Myocarditis is defined as an inflammatory condition affecting the myocardium and is typically diagnosed based on the Dallas criteria (1). These criteria encompass histological factors, such as the presence of an inflammatory infiltrate combined with degeneration and necrosis, as well as immunological and immunohistochemical parameters (1). Determining the exact incidence of acute myocarditis (AM) is challenging due to the reliance on endomyocardial biopsies for precise diagnosis. As a rough estimate, AM was believed to impact approximately 22 out of every 100,000 individuals before the beginning of the COVID-19 pandemic in 2020 (2).

The clinical presentation of this condition encompasses a variety of signs and symptoms, such as chest pain, heart failure, ventricular arrhythmias, or shortness of breath (3, 4). Although most patients presents with mild symptoms, myocarditis can also lead to acute heart failure and life-threatening arrhythmias or cardiogenic shock (3, 4).

Based on contemporary guidelines, cardiac magnetic resonance (CMR) plays a pivotal role in the diagnostic work-up of suspected AM, however, endomyocardial biopsy (EMB) is still considered the gold standard for the diagnosis (5, 6). Despite its invasive character and the relatively low sensitivity, the information derived from EMB is fundamental for identifying the mechanisms and deciding therapy and should be used for select patients (4).

To gain insight into myocarditis and inflammatory cardiomyopathies, the present Research Topic, entitled "Myocarditis and Inflammatory Cardiomyopathies: Diagnosis, treatment and future directions" aggregated relevant and original research studies, reviews, clinical cases and meta-analysis that explore this pathophysiological entity.

We identified two original research papers that studied clinical and CMR predictors in patients with myocarditis. Firstly, Cannatà et al. conducted an analysis involving 199 patients with CMR-confirmed AM and found that AM cases presenting with life-threatening arrhythmias were associated with a higher risk of adverse events. In their registry three-

quarters of patients with AM presented with chest pain, which was associated with a benign prognosis. A protective value of a chest pain as presenting manifestation was also observed by Bohbot et al. The Authors studied clinical and CMR predictors in 388 hemodynamically stable patients with AM. They found that the absence of oedema, reduced ejection fraction, and the extent of late gadolinium enhancement were all associated with early adverse outcomes.

Patients with myocarditis following the administration of mRNA SARS-CoV-2—vaccination were studied by Shiyovich et al. and Schroth et al. Shiyovich et al. specifically focused on adolescents aged 12–15 years who experienced myocarditis following the administration of the BNT162b2 mRNA COVID-19 vaccine. Their study revealed that the CMR imaging findings, consistent with the clinical course, resembled those observed in older patients. These findings suggested relatively mild myocarditis in this population, potentially indicating a favourable clinical course and outcomes. On the other hand, Schroth et al. conducted a study involving 59 patients (80% males, mean age 29 years) with CMR-diagnosed mild myocarditis resulting from mRNA SARS-CoV-2 vaccinations. This study aimed to identify predictors of persistent symptoms in individuals with vaccine-related myocarditis. A significant portion of their patients (24%) reported enduring symptoms, including chest pain (67%), dyspnoea (58%), and an increasing occurrence of fatigue (42%) and palpitations (17%). Schroth et al. observed that patients with persistent symptoms were predominantly females and older individuals.

In their review, Garg et al. investigated CMR findings in cases of myocarditis associated with COVID and SARS-CoV-2 vaccination. They observed similarities in myocardial injury patterns among acute disease, post-COVID, and SARSCoV-2 vaccination, suggesting a non-specific underlying pathophysiology. The authors posit that most instances of myocardial inflammation may arise from generic inflammatory injury rather than direct viral damage.

To gain a more comprehensive understanding of myocarditis pathogenesis and provide valuable guidance for drug development and clinical treatment, Xuan et al. conducted a study exploring the intricate interactions among cardiomyocytes (CMs), cardiac fibroblasts (CFs), and endothelial cells (ECs). CFs and ECs are susceptible to pathogen infection and can release immunologically active substances, contributing to the inflammatory response. Additionally, CFs influence the fibrosis process and the long-term prognosis of the heart through changes in the extracellular matrix (ECM). Furthermore, their interactions with CMs may either enhance or hinder the disease progression.

In this research topic focusing on myocarditis and inflammatory cardiomyopathies, we encounter three manuscripts addressing rare forms of these conditions. Firstly, there is a review on cardiac sarcoidosis, and secondly, two clinical cases discuss myocarditis associated with immune checkpoint inhibitors (ICIs) and tuberculous myocarditis.

Sarcoidosis is an inflammatory disease characterized by multisystem non-caseating granulomas, which most commonly affects the lungs (7, 8). The development of sarcoidosis-associated PH (SAPH) significantly increases mortality in individuals with sarcoidosis. Zhang et al. conducted a meta-analysis of 25 studies spanning 12 countries to determine the prevalence of SAPH in general and advanced sarcoidosis populations. Their findings, based on right heart catheterization, revealed a pooled prevalence of SAPH at 6.4% (95% CI: 3.6%–9.1%) in the general sarcoidosis population, with pre-capillary PH at 6.5% (95% CI: 2.9%–10.2%).

A rare form of myocarditis is associated to the use of ICIs. A case of pembrolizumab-induced myocarditis following COVID-19 infection was described by Nishiyama et al. It's worth noting that in patients with acute myocarditis, elevated myocardial markers and electrocardiographic changes may precede clinical symptoms, underscoring the importance of regular myocardial marker measurements and electrocardiographic monitoring during ICI administration (9).

Zhang et al. described a rare case of tuberculous myocarditis (TM), an exceedingly rare manifestation of Mycobacterium tuberculosis (TB) infection. In this patient, a definitive diagnosis of TM was established, and histopathological findings were consistent with sinus node involvement, as determined through autopsy results. Furthermore, the authors provide an insightful overview of the challenges associated with diagnosing myocardial TB.

The objective of this Research Topic was to address significant knowledge gaps in this protean clinical entity. Substantial efforts need to be made to identify the precise underlying causes for individual patients in various situations, enabling the customization of targeted therapies.

Author contributions

PG: Conceptualization, Writing – original draft, Writing – review & editing. EBol: Conceptualization, Writing – review & editing. GV: Writing – review & editing. EBob: Conceptualization, Data curation, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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.

The handling editor MC declared a past co-authorship with the author GV.

Publisher's note

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frontiers in Cardiovascular Medicine

SYSTEMATIC REVIEW
published: 17 January 2022
doi: 10.3389/fcvm.2021.809594

Prevalence of Sarcoidosis-Associated Pulmonary Hypertension: A Systematic Review and Meta-Analysis

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OPEN ACCESS

Edited by:
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Reviewed by:
Junhua Yuan, Qingdao University, China
Joseph Barney, University of Alabama at Birmingham, United States

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

Received: *05 November 2021*
Accepted: *20 December 2021*
Published: *17 January 2022*

Citation:
Zhang S, Tong X, Zhang T, Wang D, Liu S, Wang L and Fan H (2022) Prevalence of Sarcoidosis-Associated Pulmonary Hypertension: A Systematic Review and Meta-Analysis. Front. Cardiovasc. Med. 8:809594. doi: 10.3389/fcvm.2021.809594

Background: Sarcoidosis-associated pulmonary hypertension (SAPH) is associated with poor prognosis, conferring up to a 10-fold increase in mortality in patients with sarcoidosis, but the actual prevalence of SAPH is unknown.

Methods: The PubMed, Embase, and Cochrane Library databases were systematically searched for epidemiological studies reporting the prevalence of SAPH up to July 2021. Two reviewers independently performed the study selection, data extraction, and quality assessment. Studies were pooled using random-effects meta-analysis.

Results: This meta-analysis included 25 high-quality studies from 12 countries, with a pooled sample of 632,368 patients with sarcoidosis. The prevalence of SAPH by transthoracic echocardiography in Europe, the United States and Asia was 18.8% [95% confidence interval (CI): 11.1–26.5%], 13.9% (95% CI: 5.4–22.4%) and 16.2% (95% CI: 7.1–25.4%) separately, and the overall pooled prevalence was 16.4% (95%CI: 12.2–20.5%). By right heart catheterization (RHC), the pooled prevalence of SAPH was 6.4% (95% CI: 3.6–9.1%) in general sarcoidosis population, and subgroup analyses showed that the prevalence of SAPH was 6.7% (95% CI: 2.4–11.0%) in Europe and 8.6% (95% CI: −4.1 to 21.3%) in the United States. Further, the prevalence of pre-capillary PH was 6.5% (95% CI: 2.9–10.2%). For the population with advanced sarcoidosis, the pooled prevalence of SAPH and pre-capillary PH by RHC was as high as 62.3% (95% CI: 46.9–77.6%) and 55.9% (95% CI: 20.1–91.7%), respectively. Finally, the pooled prevalence of SAPH in large databases with documented diagnoses (6.1%, 95% CI: 2.6–9.5%) was similar to that of RHC. Substantial heterogeneity across studies was observed for all analyses ($I^2 > 80\%$, $P < 0.001$).

Conclusions: The sarcoidosis population has a relatively low burden of PH, mainly pre-capillary PH. However, as the disease progresses to advanced sarcoidosis, the prevalence of SAPH increases significantly.

Keywords: sarcoidosis-associated pulmonary hypertension, sarcoidosis, pulmonary hypertension, prevalence, meta-analysis

INTRODUCTION

Sarcoidosis is an inflammatory disease characterized by multisystem non-caseating granulomas with unknown causes, which most commonly affects the lungs and its surrounding lymph nodes but can also involve other organs, including the liver, kidneys, brain, heart, eyes, skin, and sinuses (1). The prevalence of sarcoidosis and its clinical presentation vary greatly according to patient sex, age group, ethnicity, and geographical region (2, 3). Consistently, the incidence of sarcoidosis is the highest among African Americans and lowest among Asians (4). Many patients with sarcoidosis have favorable outcomes, with a significant proportion showing spontaneous remission without systemic therapy. However, a small number of cases progress to advanced sarcoidosis, which is the end-stage of sarcoidosis associated with significant mortality (5, 6). The mortality rate of sarcoidosis is ∼5% (5, 7).

Pulmonary hypertension (PH) is a hemodynamic and pathophysiological state characterized by an increase in the mean pulmonary arterial pressure (≥25 mmHg); a subgroup of pre-capillary PH is defined by an additional criterion of a pulmonary arterial wedge pressure of ≤15 mmHg (8). PH is a well-recognized complication of sarcoidosis, resulting in poor prognosis (9–11). The development of sarcoidosis-associated PH (SAPH) confers up to a 10-fold increase in mortality in patients with sarcoidosis (7, 12). SAPH is also an independent cause of death in patients with advanced pulmonary sarcoidosis (5). Therefore, it is important to know the actual prevalence of PH in patients with sarcoidosis for timely screening and treatment.

To date, the actual prevalence of SAPH is unknown; however, the condition is associated with the stage at which patients are assessed for PH. In recent years, researchers have been interested in the prevalence of SAPH and extensive related studies have been conducted worldwide. However, the results are somewhat different for the limited sample sizes and these studies are individually underpowered to effectively address this issue. Although there are relevant literatures describing and discussing this topic (13, 14), there are no meta-analysis reporting the epidemiology of SAPH globally. Therefore, we performed a systematic review and meta-analysis to comprehensively calculate the prevalence of SAPH in general and advanced sarcoidosis populations.

METHODS

Data Source and Search Strategy

This systematic review and meta-analysis followed the Meta-analysis of Observational Studies in Epidemiology (MOOSE) (**Supplementary Table 1**) (15) and Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines (**Supplementary Table 2**) (16) guidelines. We performed a systematic literature search of the PubMed, Embase, and Cochrane Library databases up to July 6, 2021. The search strategy was as follows: (sarcoidosis) AND [(pulmonary hypertension) OR (pulmonary arterial hypertension) OR (PH) OR (PAH)]. The language of the searched papers was English. All reference lists of the included studies were manually searched for additional studies. Moreover, we conducted a web-based search in Internet search engines (such as Baidu Scholar and Google Scholar). As the current meta-analysis was based on previously published studies, no ethical approval or patient consent was required.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: (1) observational study, including cross-sectional, prospective, and retrospective studies; (2) study involving the prevalence of SAPH (including pre-capillary PH and post-capillary PH); and (3) study providing the total number of sarcoidosis cases to calculate the standard error. The exclusion criteria were as follows: (1) missing essential information; (2) duplicated data used in different studies; and (3) reviews, meta-analyses, *in vitro* studies, and study protocols.

Data Extraction

Two authors (Zhang and Tong) used a pre-designed data extraction form to independently extract data from all eligible studies. A third investigator intervened when there was any disagreement or doubt. We extracted the following information: first author; year of publication; country; sample size; number of SAPH cases identified by transthoracic echocardiography (TTE); number of SAPH cases identified by right heart catheterization (RHC); and patient age, sex, ethnicity, smoking status, and sarcoidosis stage. Notably, in the included studies, if the probability of PH was judged to be high, intermediate, or low (or the PH severity was classified as mild, moderate, and severe), the high and intermediate probabilities (or moderate and severe PH) were defined as the presence of PH. The diagnostic criteria of SAPH in included studies are shown in **Supplementary Table 3**. Advanced disease is mainly for patients who are at risk for death or loss of organ function (5), so in our analysis, advanced sarcoidosis refers mainly to patients with Stage IV sarcoidosis, those for lung transplantation, and those with severe life-threatening symptoms. In the study that only provided medians and ranges for several groups, we transformed the data into mean and standard deviation (SD) according to the validated methods described by Wan et al. (17), and then combined the mean and SD of multiple groups. When necessary, we also requested further information from the corresponding authors of the original studies.

Quality Assessments

For the quality assessments, two authors (Zhang and Tong) independently assessed the risk of bias of the included studies using an adapted risk of bias tool for prevalence studies (**Supplementary Table 4**) (18). Selection, non-response, measurement, and analysis biases were assessed using this tool. The possible answers for every item were "low risk" or "high risk," which were scored as "0" or "1." Scores of 0–3, 4–6, and 7–10 were defined as low, moderate, and high risk of bias, respectively. Any disagreement was clarified and confirmed by a third investigator.

Data Analysis and Statistical Methods

In this study, all analyses were performed using Stata 12.0, with statistical significance set at $P < 0.05$. We applied a random-effects model to obtain a pooled prevalence and a corresponding 95% confidence interval (CI) from various studies. Heterogeneity was tested using $\chi 2$ and I^2 tests. A $P < 0.10$ suggested significant between-study heterogeneity. Thresholds for the interpretation of I^2 were as follows: 0– 40%: might not be important; 30–60%: may represent moderate heterogeneity; 50–90%: may represent substantial heterogeneity; 75–100%: considerable heterogeneity (19). Sensitivity analyses were conducted to explore the sources of heterogeneity of results across studies by sequentially excluding eligible studies. Publication bias was assessed by Egger's and Begg's tests, with $P < 0.05$, indicating potential bias. A trim-and-fill analysis was performed to identify possible asymmetry and assess the robustness of the conclusions. Publication bias was not assessed when fewer than 10 studies were included in our analysis. If there was significant heterogeneity, subgroup analysis was further performed.

RESULTS

Study Characteristics

As shown in **Figure 1**, the initial literature search in the PubMed, Embase, and Cochrane Library databases and other sources identified 1,857 studies. After checking for duplicates, 575 studies were excluded. And 1,202 studies were removed after title and abstract review. Furthermore, three articles were excluded because they were reviews. The remaining 77 studies were further assessed for eligibility through a full-text review. Of these, 49 studies were subsequently excluded for a lack of usable data. Three studies were removed for duplicated data used in another study (13, 20, 21). Finally, the analysis included 25 studies (7, 11, 12, 22–43). The studies were conducted in the United States ($n = 10$), Europe ($n = 9$), and Asia ($n = 6$). Of these, 14 studies reported the prevalence of SAPH by TTE, 11 studies reported the prevalence of SAPH by RHC, and eight studies reported the prevalence of pre-capillary PH by RHC. Furthermore, four other studies were conducted using large databases with only documented records for the diagnosis of sarcoidosis and PH (25, 26, 28, 29). In addition, five studies included patients with advanced sarcoidosis (11, 12, 27, 37, 43); the others included the general sarcoidosis population. The basic characteristics of the included studies are shown in **Table 1**. The quality score of all included studies was 1–3 points, and all studies were deemed to have low risks of selection, non-response, measurement, and analysis biases (**Supplementary Table 5**).

Prevalence of Sarcoidosis-Associated Pulmonary Hypertension by Transthoracic Echocardiography

Fourteen studies (11, 22–24, 30–36, 38, 40, 42) reported the prevalence of SAPH by TTE. Among them, one study targeted a population with advanced sarcoidosis, among which the

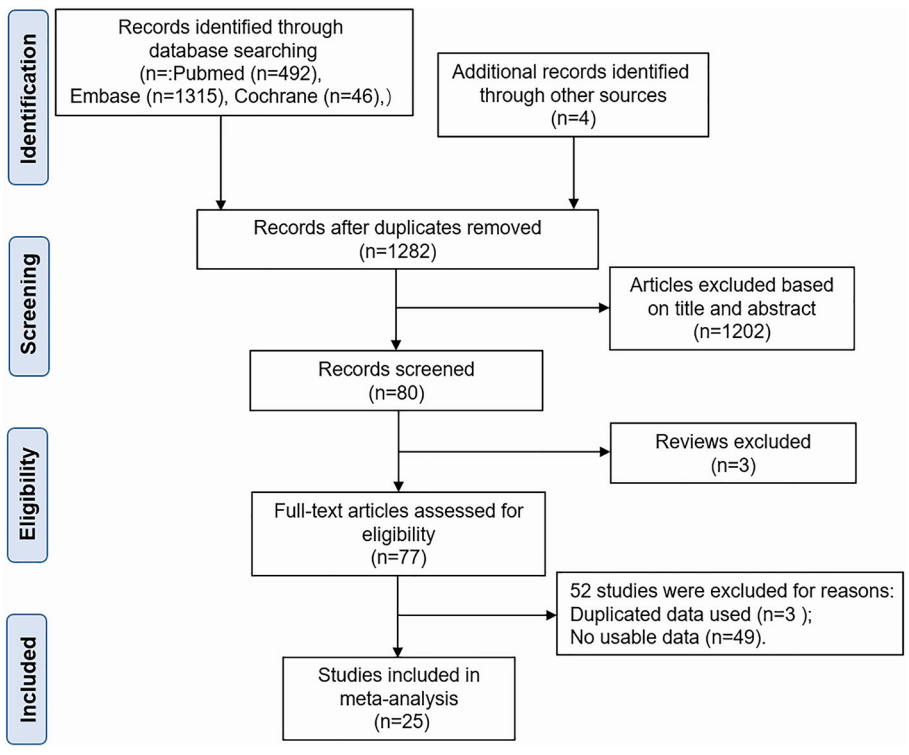

FIGURE 1 | Flow diagram of the selection of studies for inclusion.

TABLE 1 | Characteristics of included studies.

Study	Country	Sarcoidosis					SAPH						
		N	Age	Sex (M/F)	Ethnicity	Smoking (yes/no)	SAPH by TTE (*N*)	SAPH by RHC (*N*)	Pre-capillary PH by RHC (N)	SAPH from database (*N*)	Age*	Sex* (M/F)	Sarcoidosis* stage (0/1/2/3/4)
Pabst et al. (34)	Germany	111	52.2 ± 14.9	65/56	NR	NR	23	5	4	N/A	66.25 ± 7.80	NR	0/0/0/3/1
Shorr et al. (43)	USA	363	46	127/236	71.6% A-A	NR	NR	268	NR	N/A	46.5 ± 7.8	97/171	NR
Huitema et al. (24)	Netherlands	479	NR	NR	NR	NR	42	17	NR	N/A	58.7 ± 12.9	13/4	NR
Rapti et al. (33)	Greece	313	54.08 ± 13.39	121/192	NR	110/203	37	9	NR	N/A	NR	NR	NR
Milman et al. (37)	Demark	24	NR	16/8	Danish	NR	NR	19	18	N/A	46.61 ± 6.87	14/5	0/0/3/0/16
Sulica et al. (42)	USA	354	NR	NR	NR	NR	54	NR	NR	N/A	50.3 ± 1.6	17/37	2/2/7/4/23
Handa et al. (40)	Japan	212	57.67 ± 14.30	55/157	Japanese	46/166	12	NR	NR	N/A	58.9 ± 13.0	7/5	2/3/1/4/2
Maimon et al. (35)	Israel	127	56.70 ± 13.46	37/91	NR	39/88	36	NR	NR	N/A	64.3 ± 11	12/25	1/5/13/17/0
Baughman et al. (12)	USA	130	53.04 ± 11.43	39/91	50.77% white	NR	NR	70	50	N/A	52 (24–76)	17/33	1/7/10/7/25
Gangemi et al. (27)	USA	28	59.23 ± 6.35	13/15	78.57% black	NR	NR	11	NR	N/A	NR	NR	NR
Kirkil et al. (7)	USA	452	50 (25–78)	139/313	68.8% white; 30.1% A-A	NR	NR	NR	29	N/A	NR	NR	NR
Huitema et al. (32)	Netherlands	89	51.69 ± 11.19	60/29	NR	NR	37	25	NR	N/A	55.8 ± 9.0	16/9	0/0/0/0/25
Smedema et al. (30)	Netherlands	87	53.27 ± 10.04	57/30	NR	NR	15	NR	NR	N/A	NR	NR	NR
Baughman et al. (39)	USA	142	51 (26–81)	41/101	61.97% A-A	NR	NR	NR	14	N/A	NR	NR	NR
Nardi et al. (11)	France	111	NR	NR	NR	NR	33	NR	NR	N/A	NR	NR	NR
Mirsaeidi et al. (31)	USA	108	NR	NR	NR	NR	6	NR	NR	N/A	NR	NR	NR
Baughman et al. (41)	USA	1,223	NR	370/853	56.50% white, 43.50% A-A	NR	NR	30	25	N/A	NR	NR	NR
Bourbonnais et al. (38)	USA	162	47 ± 12	38/124	88.3% A-A, 11.7% White	23/139	35	25	22	N/A	NR	NR	NR
Alhamad et al. (36)	Saudi Arabia	96	50.47 ± 13.75	32/64	NR	NR	20	NR	NR	N/A	49.2 ± 14.2	3/17	0/2/6/3/9
Utpat et al. (22)	India	68	42.7	27/41	NR	NR	9	NR	NR	N/A	NR	NR	NR
Özen et al. (23)	Turkish	55	52.7 ± 10.1	10/45	NR	6/49	8	3	0	N/A	64 ± 2.646	0/3	0/1/0/0/2
Tiosano et al. (25)	Israel	3,993	64.2 ± 15.7	1,471/2,522	NR	1,342/2,651	N/A	N/A	N/A	269	NR	NR	NR
Serrano et al. (26)	Spain	5,484	60.62 ± 16.28	2,395/3,089	NR	NR	N/A	N/A	N/A	337	NR	NR	NR
Frank et al. (28)	Germany	9,106	55.4 ± 15.5	4,288/4,818	NR	NR	N/A	N/A	N/A	254	NR	NR	NR
Patel et al. (29)	USA	609,051	55 ± 14	199,769/409,282	43.9% white, 49.5% black	NR	N/A	N/A	N/A	52,442	NR	NR	NR

*NR, not reported; N/A, not applicable; A-A, African-American. *Data were extracted in the priority order of pre-capillary PH by RHC, SAPH by RHC, and SAPH by TTE.*

prevalence of SAPH by TTE was 29.7% (11). In the general sarcoidosis population, the prevalence of SAPH by TTE in Europe, the United States, and Asia were 18.8% (95% CI: 11.1–26.5%), 13.9% (95% CI: 5.4–22.4%), and 16.2% (95% CI: 7.1–25.4%), separately. Overall, 334 of 2,261 sarcoidosis patients had SAPH by TTE and the pooled prevalence of SAPH by TTE was 16.4% (95% CI: 12.2–20.5%) (**Figure 2**). However, the heterogeneity between studies was substantial ($I^2 = 88.3\%$, $P < 0.001$). A sensitivity analysis to explore the effect of each study on the pooled meta-results showed no substantial changes in the pooled prevalence, indicating the stability of our meta-analysis (**Supplementary Figure 1**). In terms of publication bias, Begg's and Egger's tests revealed *P*-values of 0.059 and 0.003, respectively, indicating some publication bias. However, there was no indication of publication bias by trim-and-fill method (no trimming was performed, and the data were unchanged) (**Supplementary Figure 2**).

Prevalence of Sarcoidosis-Associated Pulmonary Hypertension by Right Heart Catheterization

Eleven studies (12, 23, 24, 27, 32–34, 37, 38, 41, 43) described the prevalence of SAPH by RHC. Of these, seven studies focused on the general sarcoidosis population, while four studies focused on the population with advanced sarcoidosis. In 2,432 general sarcoidosis patients, 114 had SAPH by RHC, with an estimated prevalence of SAPH by RHC of 6.4% (95% CI: 3.6–9.1%) (**Figure 3**). Among the population with advanced sarcoidosis, the estimated prevalence of SAPH by RHC was 62.3% (95% CI: 46.9–77.6%) (**Figure 4**). Significant heterogeneity across studies was observed for both the general ($I^2 = 88.0\%$, $P < 0.001$) and advanced sarcoidosis ($I^2 = 89.4\%$, $P < 0.001$) populations. Sensitivity analysis revealed that our meta-results were stable in both groups (**Supplementary Figures 3**, **4**).

Subgroup analyses by geographical region showed persisting heterogeneity. The pooled prevalence of SAPH by RHC was 6.7% (95% CI: 2.4–11.0%) in Europe and 8.6% (95% CI: −4.1 to 21.3%) in the United States for the general sarcoidosis population (**Figure 5**).

Prevalence of Sarcoidosis Associated Pre-capillary Pulmonary Hypertension by Right Heart Catheterization

Eight studies (7, 12, 23, 34, 37–39, 41) showed the prevalence of pre-capillary PH by RHC in patients with sarcoidosis, six of which focused on the general sarcoidosis population. In the general sarcoidosis population, the estimated prevalence of pre-capillary PH by RHC was 6.5% (95% CI: 2.9–10.2%) (**Figure 6**),

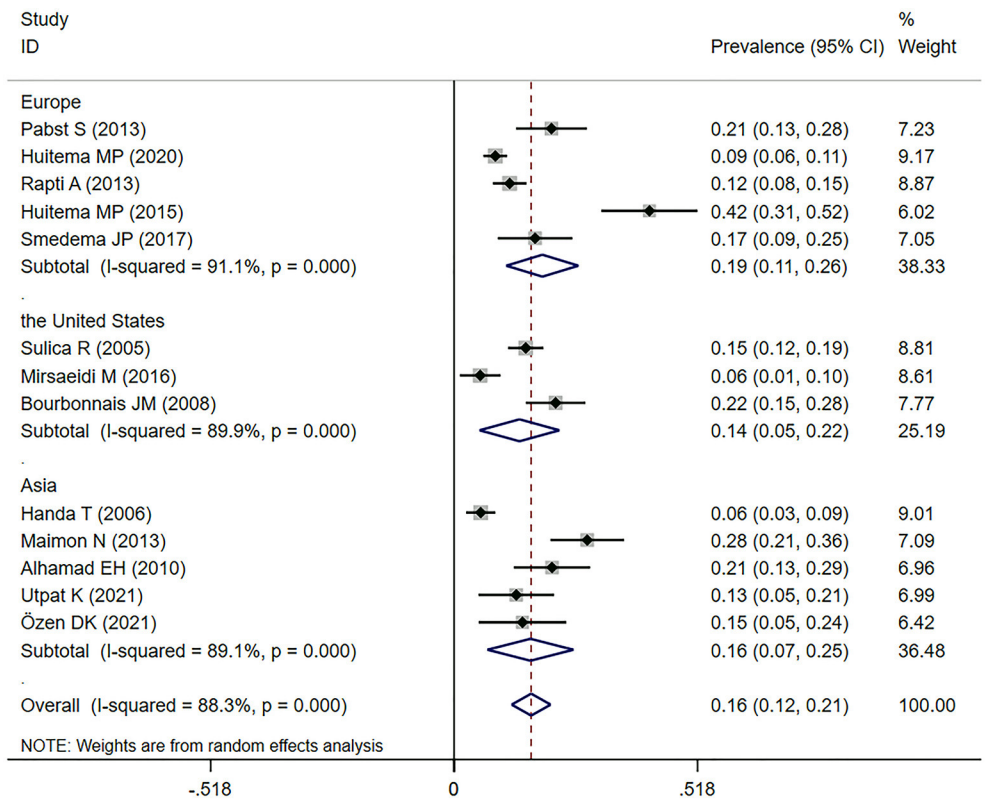

FIGURE 2 | Forest plot of the pooled prevalence of SAPH by TTE in the general sarcoidosis population. SAPH, sarcoidosis-associated pulmonary hypertension; TTE, transthoracic echocardiography.

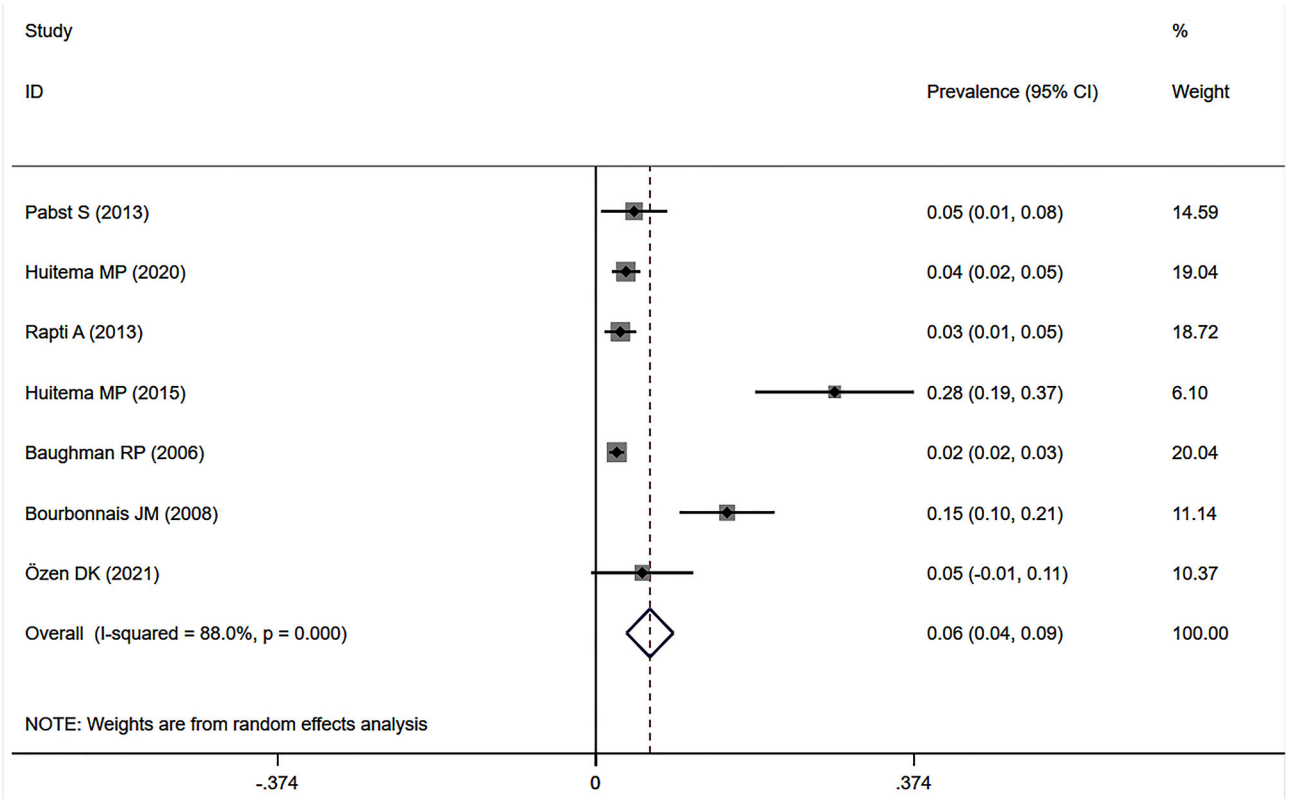

FIGURE 3 | Forest plot of the pooled prevalence of SAPH by RHC in general sarcoidosis population. SAPH, sarcoidosis-associated pulmonary hypertension; RHC, right heart catheterization.

with a high degree of heterogeneity across studies ($I^2 = 89.3\%$, $P < 0.001$). Sensitivity analysis revealed that our meta-results were stable (**Supplementary Figure 5**). In addition, two studies reported the prevalence of advanced sarcoidosis, with a pooled prevalence of pre-capillary PH by RHC of 55.9% (95% CI: 20.1–91.7%) (**Figure 7**).

Prevalence of Sarcoidosis-Associated Pulmonary Hypertension in Large Databases With Documented Records for Diagnoses

Four studies (25, 26, 28, 29) used data on the prevalence of SAPH in large databases with documented records for diagnoses of sarcoidosis and PH (i.e., the chronic disease registry of Clalit Health Services in Israel, the Spanish National Hospital Discharge Database, the patient-individual health insurance claims data in Germany, and the National Inpatient Sample database in the United States). Among 627,634 sarcoidosis records, 53,302 had PH. The pooled prevalence of SAPH was 6.1% (95% CI: 2.6–9.5%) (**Figure 8**), with a non-negligible heterogeneity ($I^2 = 99.7\%$, $P < 0.001$). Sensitivity analysis revealed the stability of our meta-analysis results (**Supplementary Figure 6**).

DISCUSSION

Sarcoidosis can affect multiple organs, including the lungs, liver, kidneys, brain, heart, eyes, skin, and sinuses, and can cause a variety of complications. Among them, cardiovascular complications, such as conduction abnormalities, arrhythmias, heart failure, and PH, are associated with higher mortality (14, 44, 45). SAPH is closely associated with poor prognosis, conferring up to a 10-fold increase in mortality in patients with sarcoidosis. Therefore, estimating the prevalence of SAPH is critical to the treatment and prognosis of sarcoidosis and essential for adequate health services planning and organization.

This is the first comprehensive meta-analysis to estimate the prevalence of SAPH in patients with sarcoidosis. Twenty-five high-quality studies from 12 countries met the inclusion criteria, with a pooled sample of 632,368 patients with sarcoidosis. In the general sarcoidosis population, the pooled prevalence of SAPH was 16.4% by TTE and 6.4% by RHC but was as high as 62.3% by RHC in the population with advanced sarcoidosis. Furthermore, the estimated prevalence of pre-capillary PH by RHC was 6.5% in the general sarcoidosis population and 55.9% in the population with advanced sarcoidosis. The prevalence of SAPH in large databases with documented diagnoses was 6.1%.

The mechanisms of PH in sarcoidosis patients are unclear and multifactorial. The possible triggers include

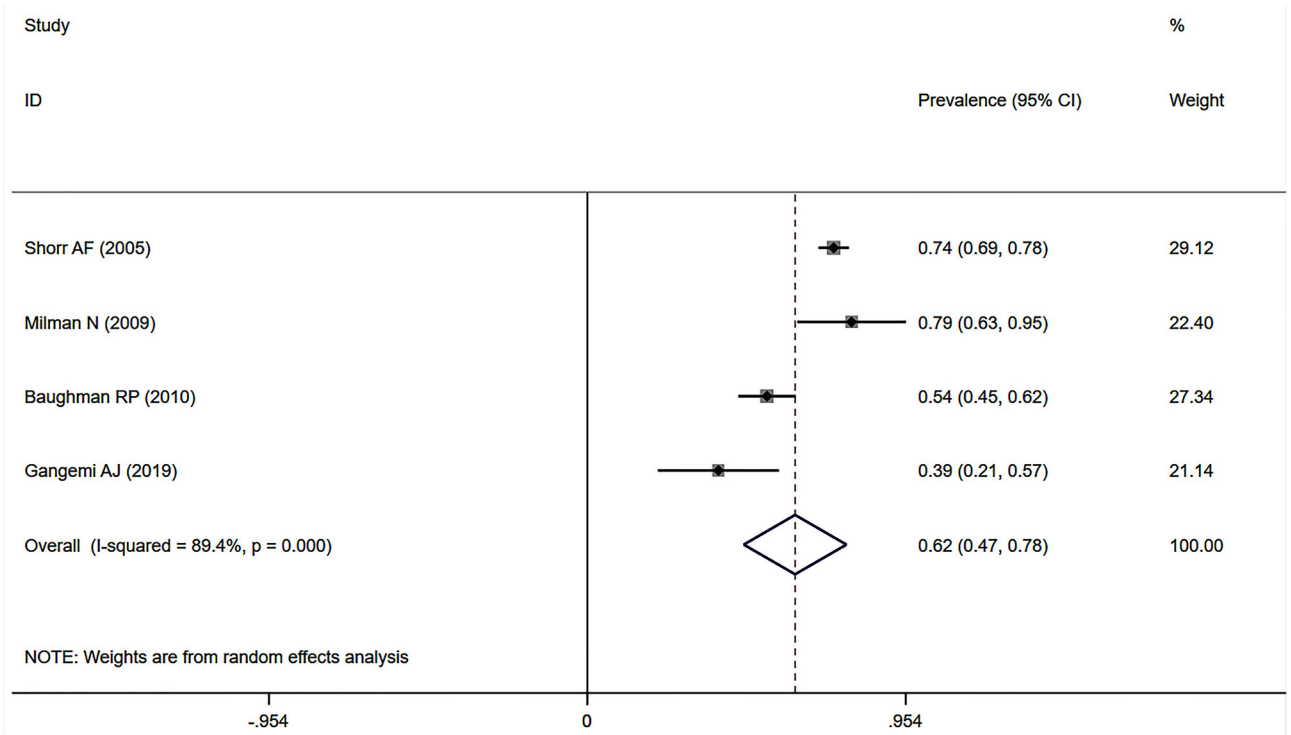

FIGURE 4 | Forest plot of the pooled prevalence of SAPH by RHC in advanced sarcoidosis population. SAPH, sarcoidosis-associated pulmonary hypertension; RHC, right heart catheterization.

progressive pulmonary fibrosis and hypoxic vasoconstriction (46), small vessel inflammatory vasculopathy (46, 47), diffuse alveolar-capillary multiplication (48, 49), pulmonary veno-occlusive disease (50), chronic thromboembolism and non-thromboembolic pulmonary embolism (51–53), extrinsic compression of pulmonary vessels caused by lymphadenopathy (54), myocardial involvement (55), sleep-disordered breathing (56), and liver dysfunction causing portopulmonary hypertension and anemia (14). Many confounders also affect the prevalence of SAPH, particularly the stage at which sarcoidosis patients are assessed (14). In our study, SAPH occurred much more frequently in patients with advanced sarcoidosis.

Surprisingly, our meta-analysis showed that the prevalence of pre-capillary PH by RHC (6.5%) was similar to that for SAPH by RHC (6.4%), or even slightly higher in general sarcoidosis patients, mainly because the studies included in each analysis were not completely consistent. To address this problem, we performed a meta-analysis of four studies (23, 34, 38, 41) that reported both the prevalence of PH and pre-capillary PH by RHC for general sarcoidosis patients, the results of which showed that the prevalence of SAPH by RHC (6.5%) (**Supplementary Figure 7**) was higher than that of pre-capillary PH (5.8%) (**Supplementary Figure 8**). In all included studies, only two studies (31, 33) excluded patients with cardiac dysfunction. Thus, our results showed that SAPH was mostly pre-capillary PH to some extent, with few parts related to cardiovascular conditions (mainly left heart disease) (57).

We also estimated the prevalence of SAPH in large databases with documented records for diagnoses. Surprisingly, the prevalence of SAPH in the large databases (6.1%) was similar to that by RHC (6.4%). These results indicated that the clinical diagnosis of SAPH mainly depends on RHC rather than TTE. The higher prevalence of PH by TTE than that by RHC is mainly based on the peak tricuspid regurgitation velocity or an estimated systolic pulmonary arterial pressure, which can only assign the echocardiographic probability of PH. Finally, the gold standard for the diagnosis of PH is RHC (8). When interpreted in a clinical context, TTE should always be performed initially in cases of suspected PH because TTE is non-invasive and cheaper, and if there are indications of PH, RHC should be considered to confirm the diagnosis of PH. In addition, TTE can help detect the cause of PH.

The presence of SAPH is an independent risk factor for poor prognosis in patients with sarcoidosis (46, 58, 59). As this meta-analysis aimed to calculate the prevalence of SAPH to inform clinic settings, attention should be paid to therapy after the confirmation of SAPH. The pathophysiology of SAPH is relevant to individual treatment. Currently, the approved medical therapies for PH are mainly for patients with group 1 pulmonary arterial hypertension (PAH); however, SAPH may be somewhat similar to PAH (54). Studies on PAH-directed therapies in the treatment of SAPH have

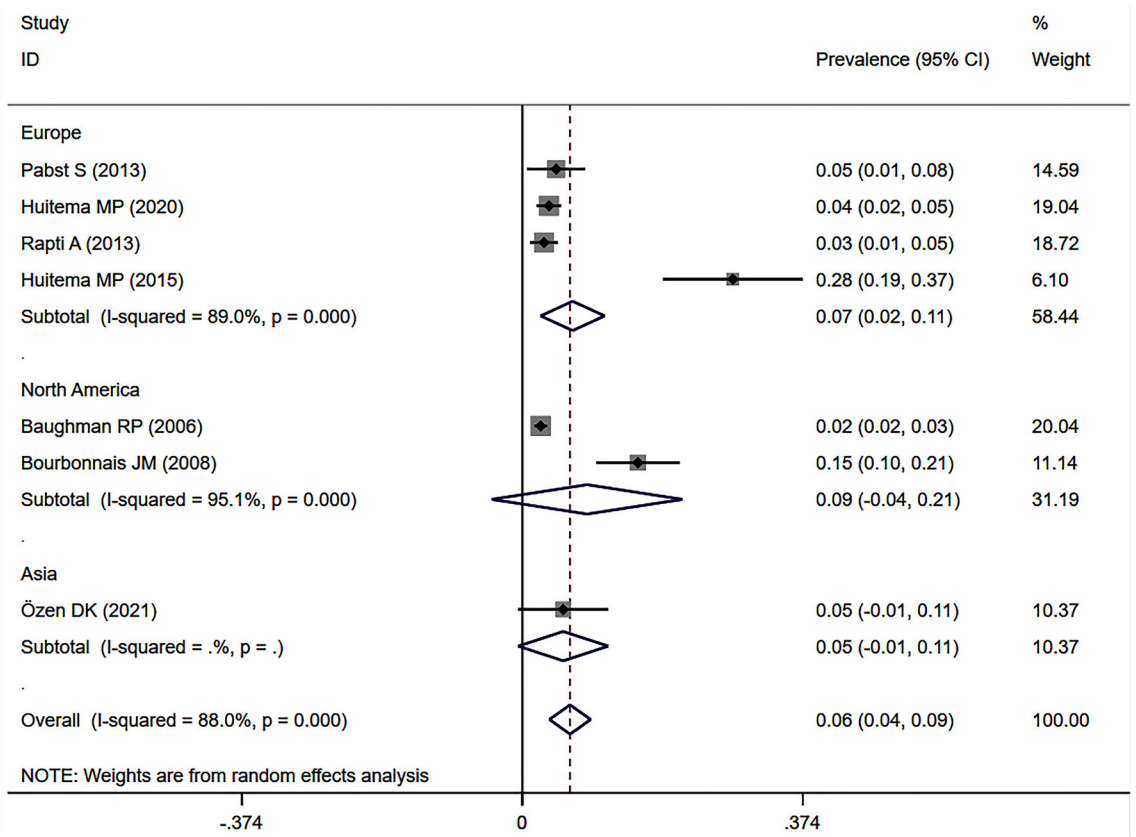

FIGURE 5 | Subgroup analysis of the prevalence of SAPH by RHC in the general sarcoidosis population by geographical regions. SAPH, sarcoidosis-associated pulmonary hypertension; RHC, right heart catheterization.

demonstrated that pulmonary vasodilators (mainly referred to as endothelin receptor antagonists, phosphodiesterase inhibitors, and prostacyclins) can improve hemodynamics and functional status (21, 60–64). Additional treatments for SAPH include combination therapy with pulmonary vasodilators and immunomodulation, diuretics for volume optimization, stenting for mechanical vascular obstruction, pulmonary endarterectomy, balloon pulmonary angioplasty, and even lung transplantation (14, 54).

In our meta-analysis, the heterogeneity between studies was substantial, with many analyses showing $I^2 > 80\%$. Previous meta-analyses on prevalence studies have reported similar results (65, 66). The subgroup analyses failed to determine the reasons for this significant heterogeneity. There was insufficient information or quantity of studies to conduct subgroups according to sex, smoking, and the stage of sarcoidosis. The incidence of sarcoidosis in different races is different and evaluating the impact of ethnicity on SAPH is of significance. However, few included studies reported information on ethnicity and detailed data on the prevalence of SAPH in different races were not available. Thus, this study could not conduct a subgroup analysis by ethnicity. SAPH is also related to the severity of lung disease and chronic hypoxemia, but there was insufficient information to analyze the severity of PH based on current published literature. In addition, the inconsistent use of TTE and RHC to confirm the diagnosis of PH in all included studies contributed to the heterogeneity.

This study has several strengths. First, this was the first meta-analysis to estimate the prevalence of SAPH. Second, the 25 high-quality included studies from 12 countries provided a sufficient sample size with a pooled sample of 632,368 patients with sarcoidosis, which is powered to effectively address this issue. Third, our study assessed the prevalence of SAPH from multiple perspectives, including TTE, RHC, and data from a large database with documented records for diagnoses, as well as the prevalence of pre-capillary PH (a subgroup of PH) in patients with sarcoidosis. Nevertheless, this study also has some limitations arising from the included studies. First, because the prevalence of SAPH was not the main focus but was rather integrated into some studies, the reporting was often suboptimal for the purpose of this meta-analysis. Second, our meta-analysis identified significant publication bias, particularly regarding the prevalence of SAPH by TTE, which might be associated with the small sample sizes in most of the included studies and the English language restriction during study screening. Third, the included studies were concentrated in the United States ($n = 10$), Europe ($n = 9$), and Asia ($n = 6$), which might affect the generalizability of

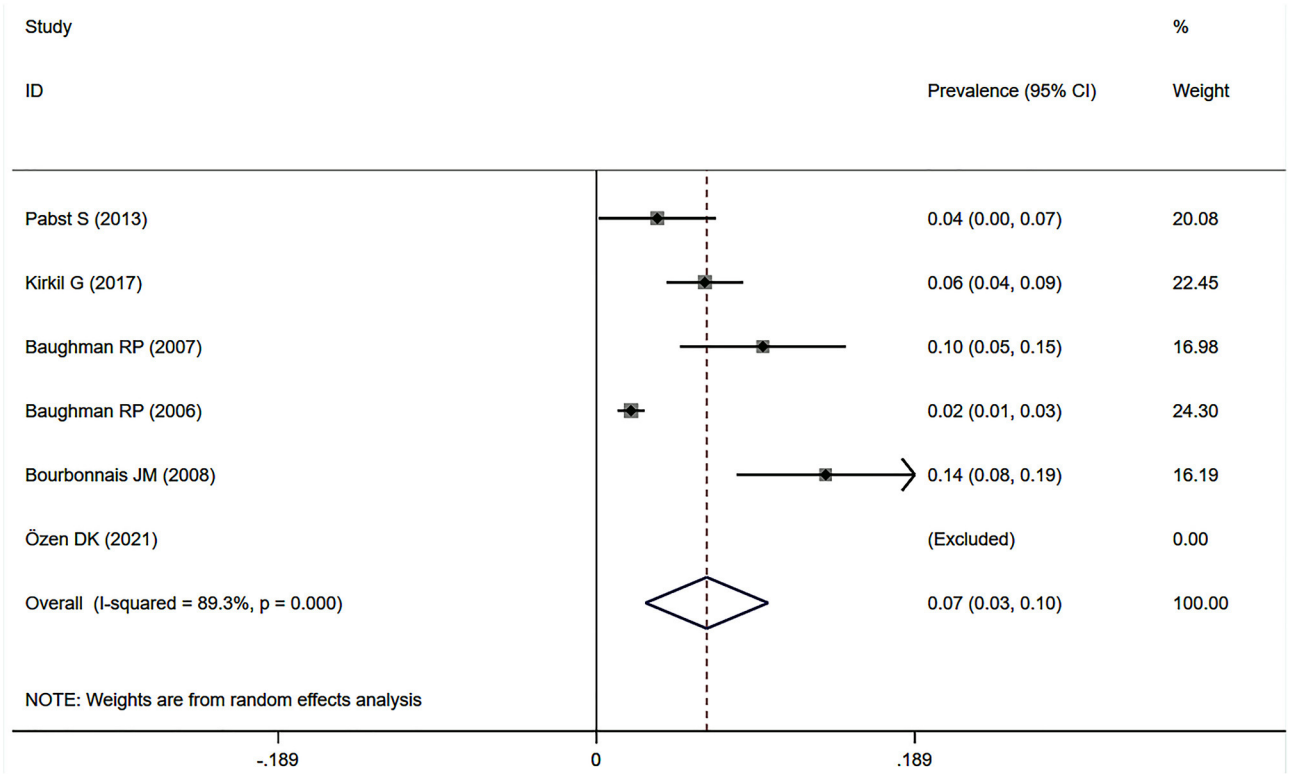

FIGURE 6 | Forest plot of the pooled prevalence of pre-capillary PH by RHC in general sarcoidosis population. PH, pulmonary hypertension; RHC, right heart catheterization.

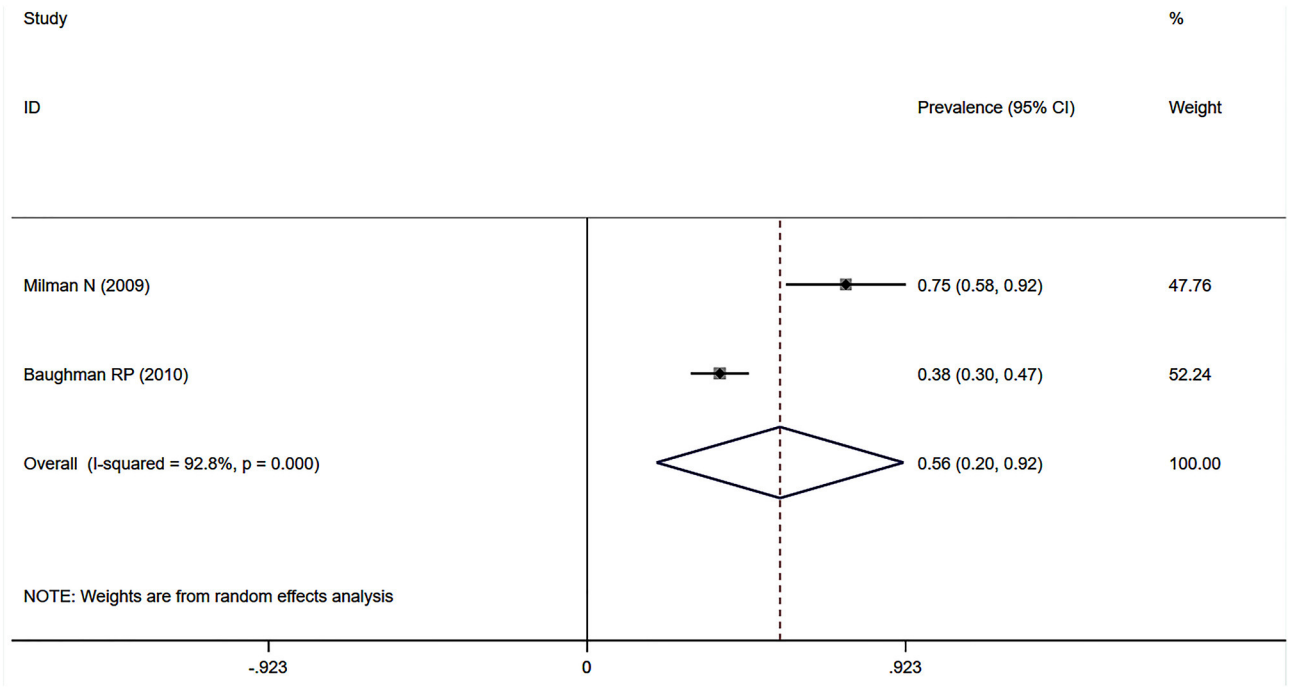

FIGURE 7 | Forest plot of the pooled prevalence of pre-capillary PH by RHC in the population with advanced sarcoidosis. PH, pulmonary hypertension; RHC, right heart catheterization.

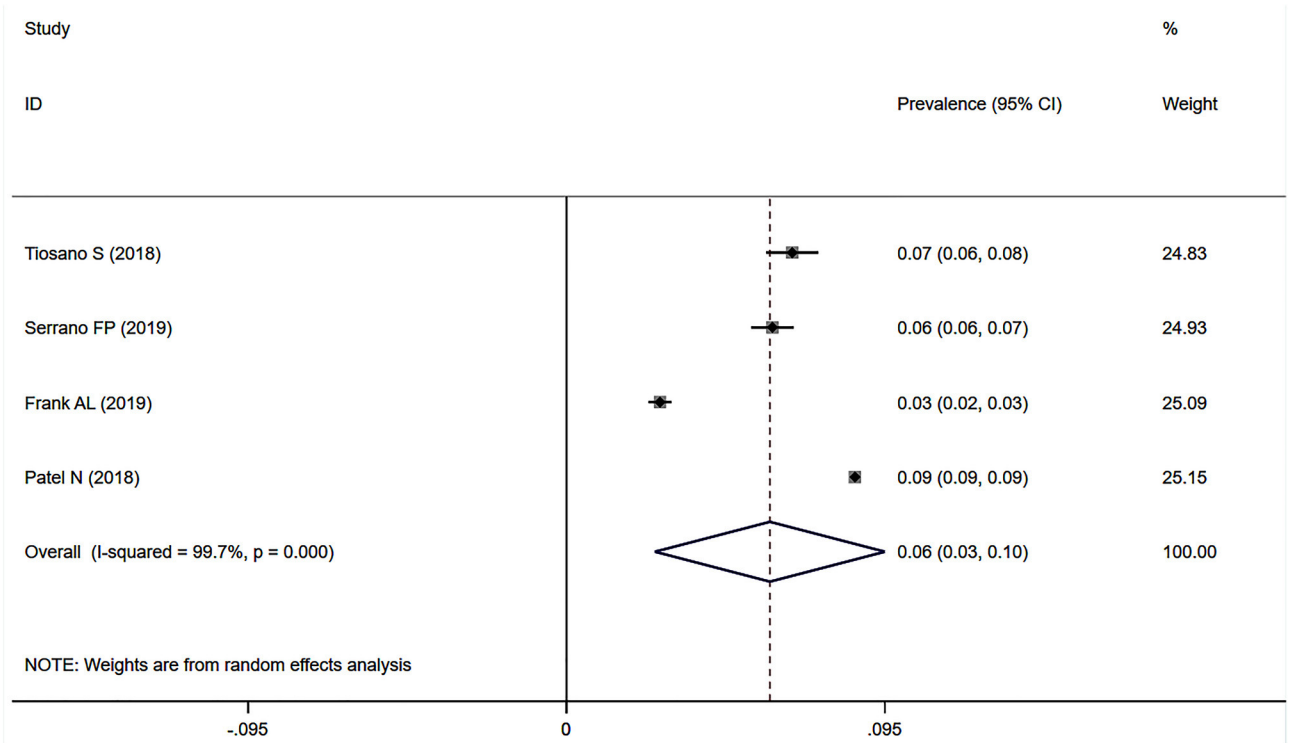

FIGURE 8 | Forest plot of the pooled prevalence of SAPH in large databases with documented diagnoses. SAPH, sarcoidosis-associated pulmonary hypertension.

the findings. Finally, some analyses included small numbers of studies, resulting in limited statistical confidence. Despite these limitations, we were able to minimize bias throughout the entire analysis process.

CONCLUSIONS

Estimating the prevalence of SAPH is essential for adequate planning and organizing health services. Based on the available literature, the pooled prevalence of SAPH was 16.4% by TTE and 6.4% by RHC in the general sarcoidosis population but was as high as 62.3% by RHC in the population with advanced sarcoidosis. The estimated prevalence of pre-capillary PH by RHC was 6.5% in the general sarcoidosis population and 55.9% in the population with advanced sarcoidosis. To yield more accurate prevalence estimates, more high-quality cohort studies are warranted and procedures and prevalence studies should be standardized.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

HF and XT conceived of the study. SZ, XT, and TZ performed the literature search and selection, data extraction, and assessment of risk of bias. SZ, TZ, DW, SL, and LW carried out the data analysis. SZ drafted the manuscript. All authors critically revised the article and approved the final draft for publication.

FUNDING

This study was supported by China Postdoctoral Science Foundation (2020M673259), Post-Doctor Research Project, West China Hospital, Sichuan University (2020HXBH013), and 1•3•5 project for disciplines of excellence–Clinical Research Incubation Project, West China Hospital, Sichuan University (2019HXFH008).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.809594/full#supplementary-material

Supplementary Figure 1 | Results of the sensitivity analysis of the prevalence of SAPH by TTE. SAPH, sarcoidosis-associated pulmonary hypertension; TTE, transthoracic echocardiography.

Supplementary Figure 2 | Results of the trim-and-fill analysis of SAPH by TTE in the general sarcoidosis population. SAPH, sarcoidosis-associated pulmonary hypertension; TTE, transthoracic echocardiography.

Supplementary Figure 3 | Results of the sensitivity analysis of the prevalence of SAPH by RHC in the general sarcoidosis population. SAPH, sarcoidosis-associated pulmonary hypertension; RHC, right heart catheterization.

Supplementary Figure 4 | Results of the sensitivity analysis of the prevalence of SAPH by RHC in the population with advanced sarcoidosis. SAPH, sarcoidosis-associated pulmonary hypertension; RHC, right heart catheterization.

Supplementary Figure 5 | Results of the sensitivity analysis of the prevalence of pre-capillary PH by RHC in the general sarcoidosis population. PH, pulmonary hypertension; RHC, right heart catheterization.

Supplementary Figure 6 | Results of sensitivity analysis of the prevalence of SAPH based on documented diagnoses in large databases. SAPH, sarcoidosis-associated pulmonary hypertension.

Supplementary Figure 7 | Forest plot of the pooled prevalence of SAPH for studies reporting both PH and pre-capillary PH. SAPH, sarcoidosis-associated pulmonary hypertension; PH, pulmonary hypertension.

Supplementary Figure 8 | Forest plot of the pooled prevalence of pre-capillary PH in sarcoidosis patients for studies reporting both PH and pre-capillary PH. PH, pulmonary hypertension.

Supplementary Table 1 | MOOSE checklist (15).

Supplementary Table 2 | PRISMA checklist.

Supplementary Table 3 | Diagnostic criteria of pulmonary hypertension in included studies.

Supplementary Table 4 | Risk of bias quality assessment tool.

Supplementary Table 5 | Quality assessment of included studies.

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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.

frontiers | Frontiers in Cardiovascular Medicine

REVIEW
published: 07 April 2022
doi: 10.3389/fcvm.2022.882027

The Roles of Cardiac Fibroblasts and Endothelial Cells in Myocarditis

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In myocarditis caused by various etiologies, activated immune cells and the immune regulatory factors released by them play important roles. But in this complex microenvironment, non-immune cells and non-cardiomyocytes in the heart, such as cardiomyocytes (CMs), cardiac fibroblasts (CFs) and endothelial cells (ECs), play the role of "sentinel", amplify inflammation, and interact with the cardiomyocytes. The complex interactions between them are rarely paid attention to. This review will re-examine the functions of CFs and ECs in the pathological conditions of myocarditis and their direct and indirect interactions with CMs, in order to have a more comprehensive understanding of the pathogenesis of myocarditis and better guide the drug development and clinical treatment of myocarditis.

Keywords: myocarditis, cardiac fibroblasts, endothelial cells, cross-talk, extracellular vesicles

OPEN ACCESS

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

Received: *24 February 2022*
Accepted: *16 March 2022*
Published: *07 April 2022*

Citation:
Xuan Y, Chen C, Wen Z and Wang DW (2022) The Roles of Cardiac Fibroblasts and Endothelial Cells in Myocarditis. Front. Cardiovasc. Med. 9:882027. doi: 10.3389/fcvm.2022.882027

INTRODUCTION

The pathological standard of myocarditis refers to the infiltration of inflammatory cells, with or without myocardial necrosis in the staining of heart tissue sections according to the Dallas definition (1). A recent study estimates that the global prevalence of myocarditis is ∼22 out of 100,000 patients per year (2). A variety of infectious pathogens, systemic diseases, drugs, and toxins can cause the disease (3). Myocarditis can appear with a variety of different characteristics, from mild symptoms of chest pain to ventricular arrhythmia or fatal cardiogenic shock (3). Inflammatory cells and inflammatory factors in innate immunity and adaptive immunity play important roles in the occurrence and development of myocarditis, and there has been review (4) clarifying the roles of various types of immune cells. However, the vast majority of the heart is non-inflammatory cell. As our understanding of the human immune response continues to increase, many cell types, such as CFs, ECs that were initially thought to be just bystanders have been proven to play vital roles in the inflammatory process and even determine the direction and intensity of the immune response. However, there is little research on the role of non-inflammatory cells in the process of myocarditis. Thus, in this review, we will briefly delineate the role of non-inflammatory cells, non-CMs including CFs and ECs, and focus on the everlasting cross-talk between CFs, ECs and CMs in the progression of cardiovascular diseases.

CFs

Fibroblasts in the adult mouse heart make up about 15% of the non-myocytes in the heart (5). The basic function of cardiac CFs is to synthesize a collagen-rich ECM network to provide structural integrity and biomechanical signals (6). They are dynamic participants in ventricular physiology and pathophysiology. CFs can be activated either by their infection or invasion

(7, 8) or by heart stress. Activated CFs will secret inflammatory factors, chemokines, or other immunomodulatory substances and produce large amounts of ECM. In myocarditis, virus infection or cytokines induces fibroblast activation state and then CFs function through virus transmission, cell damage, chemokines, and inflammatory factors production, making heart inflammation to keep getting worse. At the same time, the extracellular matrix produced by CFs promotes myocarditis from acute inflammatory infiltration to the chronic phase (**Figure 1**).

Pathogenic Infection of CFs

CFs can directly serve as the host of the virus. Lindner et al. demonstrated that when both CMs and CFs are infected with coxsackie virus B3 (CVB3), the viral replication within CFs increases by 10-folds, indicating that they play a key role in promoting viral load in myocarditis (9). Within 9 h after infection, the CMs network loses its ability to contract spontaneously, and then decomposes and is replaced by overgrown CFs that survive from the infection (10).

Infected CFs can continue to damage the heart function at different stages of myocarditis. In the acute phase of viral myocarditis, after CFs are attacked and die, the virus in the cell continues to attack various types of cells, thereby making it a storage and transmission reservoir for the virus in the infection (11). While in the chronic phase, the virus that resides in CFs may be an important cause of persistent inflammation, heart dilation, and even heart failure. In addition, viral infection can cause damage to CFs and their release of damage-associated molecular patterns (DAMPs), such as HGMB1 (12).

Inflammation Reaction Caused by CFs

Although CFs are not a component of the immune system, they play an important role in the occurrence and development of inflammation. The expression of chemokines has been found in a variety of disease processes related to tissue damage and leukocyte recruitment.

Eosinophils are multifunctional granulocytes that help trigger and regulate inflammation. It is reported that eosinophils aggravate the pathological severity and mortality of eosinophilic myocarditis (13). In general, the transport of eosinophils to the heart in eosinophilic myocarditis depends on the expression of the chemokine receptor CCR3 (14) and its ligands CCL11, CCL24, and CCL26 in the mouse model and the heart of patients with eosinophilic myocarditis. However, CFs produce CCL11 under the regulation of two cytokines IL-4 and IL-13 ADDIN EN.CITE during myocarditis (15).

IL-17 is involved in the pathogenesis of autoimmune myocarditis, and it has been shown that neutralizing IL-17 can reduce the severity of myocarditis (16). *In vitro*, the stimulation with TNF-α, IL-1β, and IL-17 can induce CFs to produce CCL20. CCL20 promotes adhesion of Th17 on endothelium and induces Th17 cell migration (17), thereby producing more IL-17 to form a vicious circle. What's more, IL-17A acts on the differentiation of Ly6C (low) monocytes into macrophages through CFs-derived GM-CSF *in vitro*, indicating that CFs promote monocyte differentiation and proliferation, and regulate monocytes and monocyte-derived macrophages phenotype and function (18).

The continuous expression of cytokines was observed in the mouse model of dilated cardiomyopathy (DCM) after myocarditis. Compared with other cytokines, the expression of the IL-1β gene in the chronic phase was relatively higher, and it was related to the ratio of heart weight/body weight and the degree of fibrotic lesions (19). IL-1β secreted by CFs initiates the inflammatory process and attracts proinflammatory immune cells. In addition, CFs-secreted IL-1β leads to the maintenance of inflammation in the later stage of wound healing (20).

Overall, in myocarditis caused by a variety of factors, CFs can participate in the entire inflammatory reaction process and even the chronic phase by secreting chemokines or inflammatory factors. Most of the inflammation involved in CFs is harmful, but there are also beneficial parts, so it is particularly important to find the reason for these differential effects and carry out corresponding interventions.

The Change of ECM Leads to the Formation of Fibrosis

The ECM components in the interstitium of the heart form a complex network structure, which provides structural support for several different cell types, and integrates extracellular signals and cellular responses (21). The ECM is a highly dynamic structure that exists in all tissues and undergoes continuous controlled remodeling. In the body, CFs are considered to be a key regulator of ECM structure. CFs residing in the heart are not only the main producer of ECM components but also the main source of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs). In inflammatory heart disease, cytokines, growth factors can change ECM components and the production of MMPs and TIMPs by stimulating the migration and proliferation of CFs or mediating the interaction between CFs and other cell types (22).

For example, TNFα and IL-1β lead to increased expression of MMP-2, MMP-9, and MMP-13 in CFs (23) which cause ECM remodeling. On the one hand, prolonged activation time of CFs and progressive fibrosis can lead to further deformation of the tissue structure and deterioration of heart function. ECM remodeling during myocarditis can cause arrhythmia and even heart failure. On the other hand, ECM components may adsorb parasite antigens (24) and cytokines (25) that may contribute to the establishment and continuation of inflammation.

The regulation of matrix metalloproteinases (MMPs) in the acute and chronic phases of viral myocarditis and its role in myocardial interstitial remodeling have been reviewed (26) in detail. Therefore, adjusting the function of CFs to regulate cardiac inflammation and cardiac remodeling is gradually turning into a better solution.

ECs

ECs are arranged in the circulatory system and are essential for maintaining and regulating vascular homeostasis (27) by protecting material transport, controlling vascular permeability, and regulating vascular tension (28). They are the largest cell population in non-cardiomyocytes of the adult mouse

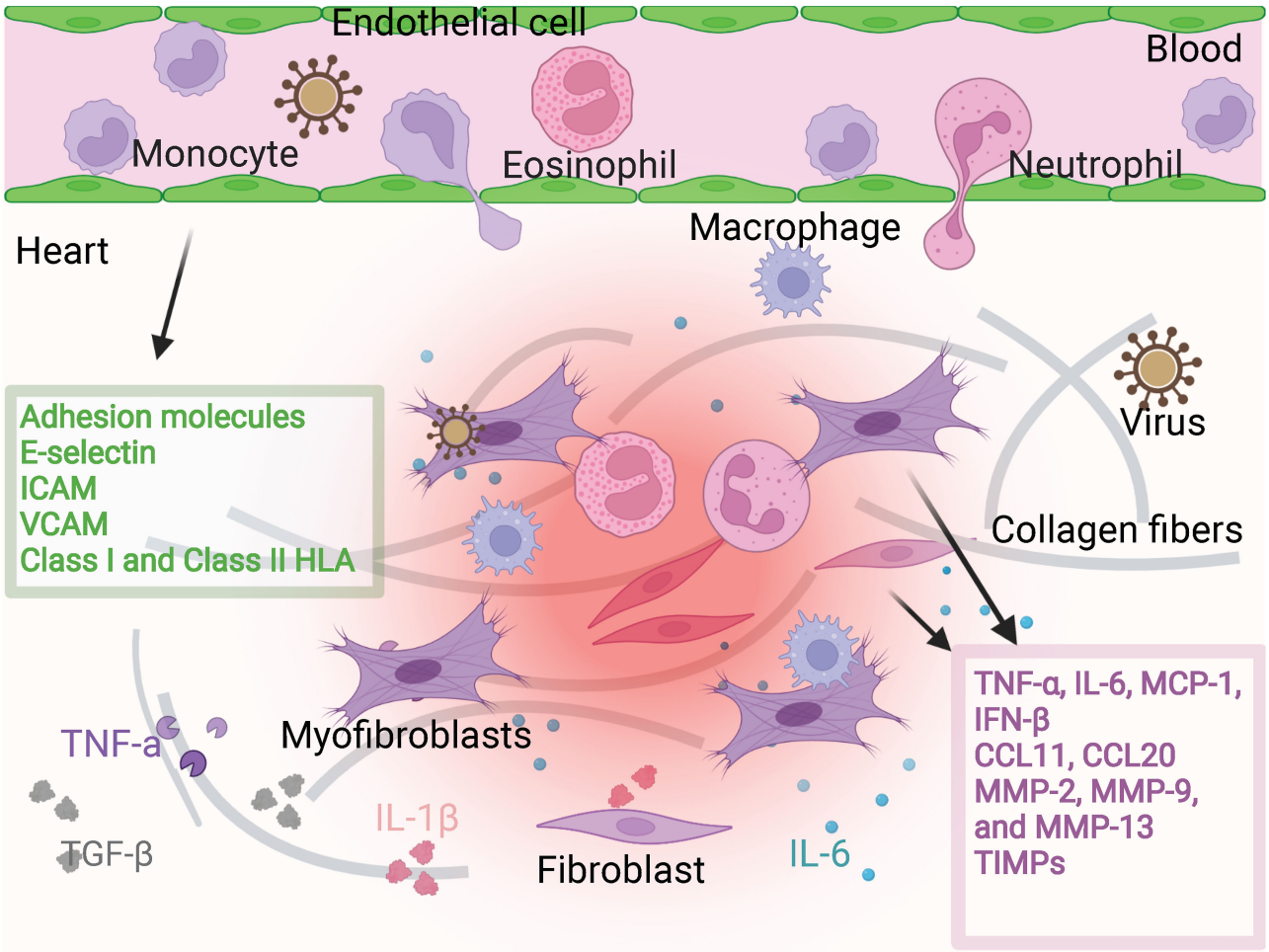

FIGURE 1 | Fibroblast and endothelial cell activation. Fibroblasts are activated in viral infection or in the environment containing cytokines. Then they produce pro-inflammatory factors and chemokines such as TNF-α, IL-6, MCP-1, IFN-β, CCL11, CCL12. They can also produce MMP family proteins to regulate cardiac interstitial fibrosis. Under the stimulation of these microenvironments, endothelial cells produce adhesion molecules to regulate the adhesion and infiltration of inflammatory cells. The participation of fibroblasts and endothelial cells promotes more severe cardiac inflammatory infiltration and more severe fibrosis.

heart, accounting for about 60% (5). Resting ECs isolate leukocyte-interacting proteins in specialized secretory vesicles (29), so they cannot interact with white blood cells. In addition, resting ECs also inhibit the transcription of other adhesion molecules, such as E-selectin, vascular cell adhesion molecule 1 (VCAM1), and to a large extent inhibit the transcription of intercellular adhesion molecule 1 (ICAM1) and pro-inflammatory cytokines (30).

However, under the influence of certain factors, ECs can be transformed into an activated state. In DCM developed from autoimmune myocardial inflammation, it is shown that endothelial activation in organ-specific diseases affects the function of blood vessels throughout the body. Vallbracht et al. proved this and found that patients with myocardial inflammation showed impaired endothelial function of radial artery, and these patients have ruled out the risk factors of classic arterial damage, such as coronary artery disease, severe left ventricular failure, diabetes or atherosclerosis (31). For myocarditis, myocardial inflammatory infiltration, endothelial activation, direct viral toxicity, and circulating cytokines cause endothelial dysfunction in patients with persistent myocardial viruses (32). The persistence of the myocardial virus can independently cause endothelial dysfunction. For patients with myocardial leukocyte infiltration, endothelial dysfunction is more obvious (32). Nearly half of patients with DCM have increased T lymphocyte density and increased immune activation of ECs and mesenchymal cells in cardiac biopsies (33).

ECs not only act as a transport device for immune cells in circulation, forming a mechanical barrier to resist invaders, but also produce chemokines, interleukins, interferons, and growth factors through paracrine after they are activated. In addition, they can induce the expression of adhesion molecules such as E-selectin, P-selectin, ICAM, or VCAM to attract and transfer immune cells to inflammatory sites (34) (**Figure 1**).

Pathogenic Infection of ECs

Many pathogenic microorganisms not only infect CMs and CFs, but also the ECs. Among *T. cruzi* and more than 20 viruses related to myocarditis, they are known to infect human and/or animal ECs (35). Parvovirus B19 is considered the pathogen in most cases of chronic myocarditis (36). And PVB19-related inflammatory cardiomyopathy is characterized by infection of the ECs of the heart in small arteries and veins (37).

Bültmann et al. reported (38) a patient with severe myocarditis, whose radioactive *in situ* hybridization detected the viral genome in ECs, but did not detect the viral genome in CMs or other tissue components. What's more, humans or mice infected with PVB19 show more particles related to endothelial cell damage in the blood circulation (39). Due to the high viral load in cardiac ECs, PVB19 infection of ECs is sufficient to induce coronary microcirculation damage and secondary CMs necrosis (40).

However, ECs, like a barrier that the virus needs to cross, can to a certain extent provide a buffer for the time when the heart damage occurs so that the immune system can have a longer time to exert a stronger immune effect before the virus invades the CMs (41).

Inflammation Reaction Caused by ECs

Adhesion molecules (42) are a class of membrane receptors with various functions, including cell migration, cell-cell interaction, and cell-to-extracellular matrix adhesion. They are involved in various processes such as growth, differentiation, migration, and apoptosis. The adhesion molecules VCAM-1, ICAM-1, and E-selectin expressed on the vascular endothelium act as ligands for the counter-receptors of circulating inflammatory cells (43).

The expression of endothelial cell surface markers is dynamic and is affected by circulating cytokines and other stimuli (44). The inflammation state of endomyocardial biopsy in patients with dilated cardiomyopathy was evaluated by immunohistochemistry. It was found that 45.8% of patients had endothelial inflammation activation, which was manifested by increased expression of at least three adhesion molecules (Class I and Class II HLA, ICAM-1, VCAM-1) (45).

The increased expression of endothelial adhesion molecules in the myocardium of patients with infectious myocarditis may be related to pro-inflammatory cytokines. TNFα and IL-1β increase the expression of adhesion molecules ICAM-1, VCAM-1 and E-selectin on the ECs of the human coronary artery and cardiac microvascular system (46). Cytokines and chemokines driven by *T. cruzi* can also regulate adhesion molecules like VCAM-1 and ICAM-1 on the ECs of target tissues, and play a key role in cell recruitment (24).

CROSS-TALK AMONG CFs, ECs, AND CMs: DIRECT AND INDIRECT INTERACTION

The functions of various cells are not independent, they share the characteristics of repetitiveness and mutual reinforcement or containment. CMs are the most important cell type for the heart to perform biological functions. So we focus on the interaction between CMs and CFs, and CMs and ECs (**Figure 2**). On the one hand, these interactions promote the heart's adaptive response to internal and external stimuli and compensate or overcome these pressures. On the other hand, these interactions can also cause pathological remodeling related to heart disease, speed up the disease process, and lead to heart failure and cardiogenic death. Therefore, the study of the interaction between cells will help us to employ a synergistic protective effect between cells and avoid the expansion of malignant events.

Direct Cell-to-Cell Interaction

There are mechanical coupling and electrical coupling (47) between CMs, CFs, and ECs. This kind of spatial connection enables the direct exchange of information between each other. Research shows blocking contact between CMs and CFs using antibodies against cardiac fibroblast plasma membrane protein or connexin will inhibit cardiomyocyte adhesion and reduce IL-6 production, but will not reduce TNFα production (48). However, the direct interaction is very dependent on the tight connection in space. Compared with the indirect action, its range of action is smaller. Therefore, we mainly discuss the indirect interaction between cells.

Indirect Cell-to-Cell Interaction: Paracrine Effects of Released Cytokines/Chemokines or Others

CF-CM and CM-CF

The interaction between CFs and CMs leads to paracrine of regulatory factors, which in turn affects cardiac conductivity, and hypertrophy, contraction (49), and apoptosis of CMs. While CMs change the proliferation, migration, differentiation, and matrix production capacity of CFs through paracrine (**Table 1**).

For example, TGF-β1 is the prototype of a large family of structurally related secreted dimeric proteins. It is pleiotropic and plays an important role in intercellular signal transduction (64). Disturbance of TGF-β action can lead to pathological conditions, including cardiovascular disease (65), fibrotic disease, and cancer (66). When subjected to mechanical stretching, TGF-β secreted by CMs and CFs induces the growth response of CMs and CFs in an autocrine/paracrine manner. In addition, the inflammatory factors released by immune cells will also promote the release of TGF-β from CFs. The increase of TGF-β will promote the protein synthesis rate of static CMs (67) and change the function of CMs.

In cardiac remodeling, myofibroblasts can induce CMs hypertrophy through cross-talk between CFs and CMs (68). IL-6 signaling in CMs co-cultured with CFs is activated to promote CMs hypertrophy (69). In addition, when CMs are co-cultured with cardiac CFs, the production of tumor necrosis factor (TNF)-α is also upregulated (48).

Similarly, CMs also have a paracrine effect on CFs. For example, the pro-inflammatory cytokine hypoxia-induced mitotic factor (HIMF) was overexpressed in CMs *in vitro*, and the cell supernatant was used as a conditioned medium and co-cultured with CFs. The results (52) show that CMs induce

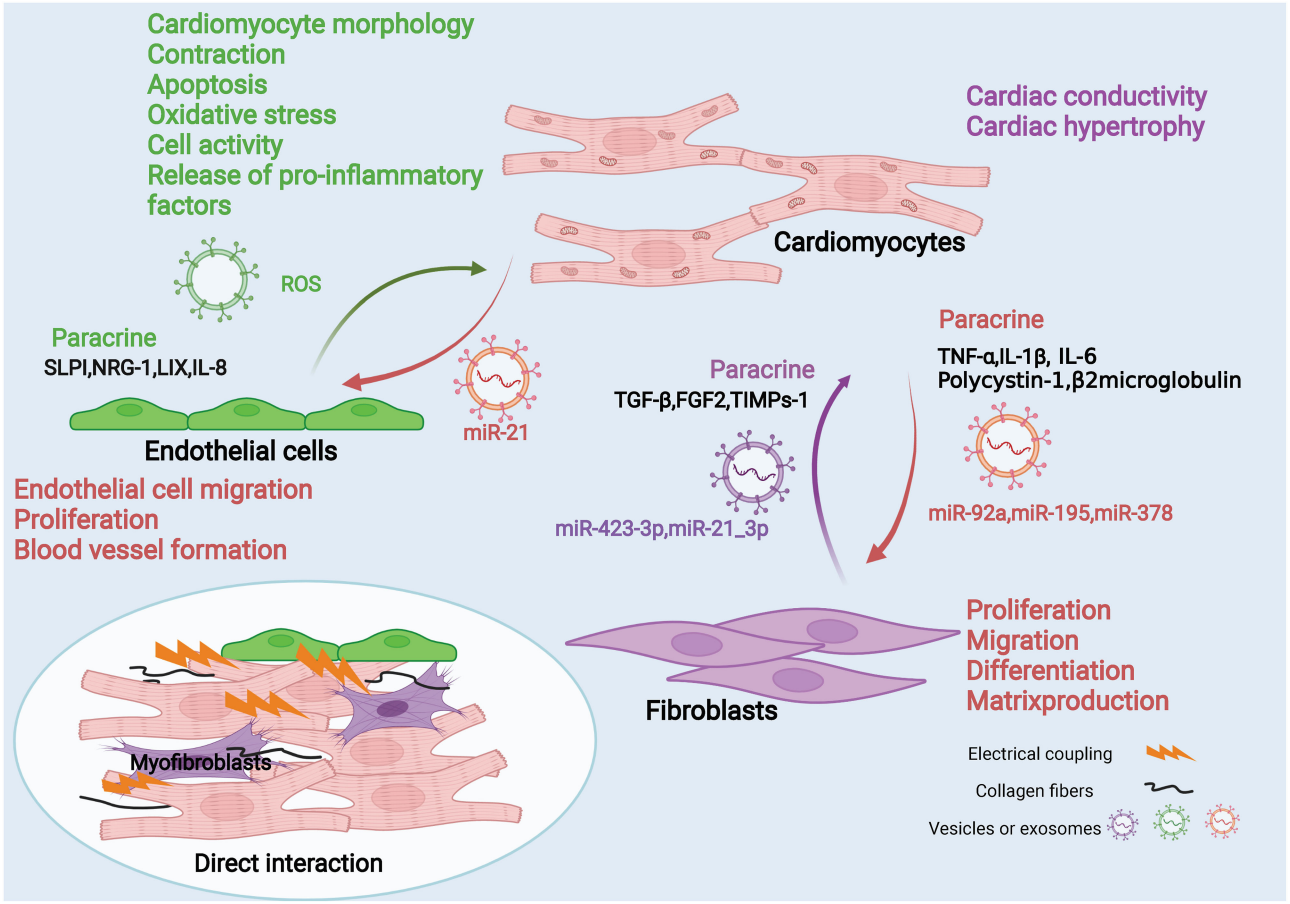

FIGURE 2 | Fibroblasts, endothelial cells and cardiomyocytes interaction diagram. Fibroblasts, endothelial cells can interact with cardiomyocytes through mechanical coupling and electrical coupling. They also act through paracrine effects and/or cell-secreted vesicles or exosomes. Cardiomyocytes secrete pro-inflammatory factors such as TNF-α, IL-1β, and IL-6 through paracrine effects to act on fibroblasts, and they can also deliver intracellular miRNAs through exosomes, such as miR92a, miR195, and miR378. These mediators can affect fibroblast proliferation, migration, differentiation and matrix production. Fibroblasts can also secrete factors such as TGF-β through paracrine action or deliver miRNAs into cardiomyocytes through exosomes, which affect cardiomyocyte contraction and cardiac hypertrophy. Similarly, endothelial cells can secrete effector molecules such as IL-8 through paracrine effects, and can also carry ROS through exosomes, thereby affecting various functions of cardiomyocytes. In contrast, cardiomyocytes can affect endothelial cell migration, proliferation, and angiogenesis by secreting exosomes carrying molecules such as miR-21.

CFs migration, proliferation, and myofibroblast differentiation by paracrine IL-6.

EC-CM and CM-EC

Since ECs and CMs are relatively close in space, they can conduct direct cell signal transduction and two-way communication through paracrine. The interaction between CMs and ECs is essential for heart development, postnatal function, and heart repair (70). ECs influence cardiomyocyte morphology, contractility, apoptosis, oxidative stress, cell activity, and release of pro-inflammatory factors in a paracrine manner. At the same time, CMs can promote endothelial cell migration, proliferation, and blood vessel formation in the same way (**Table 2**).

For example, Endothelin-1 (ET-1) is described as a 21 amino acid peptide produced by ECs and is the most effective known endogenous vasoconstrictor. CMs mainly express ETA receptors. Under normal physiological conditions, ET-1 promotes the contraction of CMs. However, under pathological conditions, increased ET-1 can damage the contractile function of CMs through ETA receptors (81).

In addition to the direct effect of substances secreted by one type of cell on another type of cell, the substances secreted by cells can affect them through synergistic or antagonistic effects with other cells. Pummerer et al. showed that normal hearts are not easily affected by T cells that react with myosin (82). In myocarditis, the cardiac mesenchymal cells expressing MHC class II are significantly increased and the expression of endothelial ICAM-1 is strongly upregulated, making the heart vulnerable to passive metastasis and myosin responds to T cell attack.

In general, ECs and CMs can communicate extensively through secreted substances or "permissible action", which is essential for the overall functioning of the heart.

TABLE 1 | Table of CF-CM and CM-CF interactions in the cardiovascular field.

Disease or treatment	Cross-talk	Mode of action	Acting molecule	Cell effects	References
Heart development	CF-CM	Paracrine	–	CMs are specialized as ventricular conduction system-like cells, CNMs maturation	(50, 51)
Heart failure caused by TAC	CM-CF	Paracrine	IL-6	CFs migration, proliferation, and myofibroblast differentiation	(52)
	CM-CF	Extracellular vesicles	miR-378	CFs proliferation, fibrosis	(53)
	CM-CF	Paracrine	β2 microglobulin	CFs activation	(54)
MI	CM-CF	Extracellular vesicle	miR-92a	Myofibroblast activation	(55)
–	CM-CF	Exosomes	miR-195	Myofibroblast activation	(56)
	CF-CM	Paracrine	TGF-β	CMs hypertrophy	(57)
DOX-induced cardiotoxicity	CF-CM	Paracrine	FGF2	CMs damage	(58)
Early hypoxia	CM-CF	Paracrine	TNF-α, IL-1β	CFs migration	(59)
Ang II-induced cardiac hypertrophy	CF-CM	Exosomes	miR-21-3p	CMs hypertrophy	(60)
H/R	CF-CM	Paracrine	TIMPs-1	CMs viability	(61)
	CF-CM	Exosomes	miR-423-3p	CMs viability and apoptosis	(62, 63)
	CM-CF	Paracrine	Polycystin-1	CFs differentiate, profibrotic factors expression	(54)

–, No specific information is given in the original text.
TAC, transverse aortic constriction; MI, myocardial infarction; DOX, Doxorubicin; FGF2, fibroblast growth factor 2; H/R, Hypoxia–reoxygenation; Ang II, angiotensin II; Gal-3, Galectin-3; sFRP2, Secreted frizzled-related protein 2.

TABLE 2 | Table of EC-CM and CM-EC interactions in the cardiovascular field.

Disease or treatment	Cross-talk	Mode of action	Acting molecule	Cell effects	References
ET-1 pre-stimulated ECs	EC-CM	Paracrine	–	CMs hypertrophy	(71)
Hypoxic ECs	EC-CM	Paracrine	TGFβ1	CMs apoptosis	(72)
H/R	EC-CM	Paracrine	SLPI	CMs damage	(73)
	EC-CM	Microvesicles (MVs)	ROS	CMs apoptosis and oxidative stress	(74)
OGD	CM-EC	Exosomes	miR-21	Angiogenic activity	(75)
Ischemic preconditioning	EC-CM	Exosomes	–	CMs activity	(76)
–	hiPSC-CM -EC	Exosomes	miR-21-3p	Angiogenesis, ECs migration and proliferation	(77)
LPS	EC-CM	Exosomes	–	CMs damage	(78)
DCM	EC-CM	Paracrine	NRG-1	CMs contractility	(79)
Hypercholesterolemia	EC-CM	Paracrine	Lipopolysaccharide-induced chemokine (LIX) and IL-8	CMs apoptosis, Expression of the proinflammatory cytokines	(80)

–, No specific information is given in the original text.
ET-1, endothelin-1; SLPI, Secretory leukocyte protease inhibitor; ROS, Reactive Oxygen Species; OGD, Oxygen–glucose-deprivation; hiPSC-CM, Human induced pluripotent stem cell-derived cardiomyocytes; LPS, Lipopolysaccharide; NRG-1, Neuregulin-1.

Indirect Cell-to-Cell Interaction: Extracellular Vesicles-A New Pathway of Communication

The above-mentioned paracrine substances can play a corresponding role through the binding of receptors on the corresponding target cells. These substances are of fewer types and have a limited range of action. However, the production of EVs, as a new pathway of communication, enriches the medium of communication and expands the distance of interaction between cells.

EVs are cell-derived membrane structures that are secreted or shed from the plasma membrane after fusion of the endosome with the plasma membrane. Exosomes (83), as the current hottest research type in EVs, are membrane vesicles that carry biologically active molecules such as proteins, lipids, messenger RNA (mRNA), and micro RNA (miRNA) which

mediate intercellular communication between different cell types within the body, thereby affecting normal and pathological states. And they have become a key medium for intercellular communication. Exosomes can be induced by many cell events including cell death, hypoxia, stress, oncogene expression, differentiation, and viral infection (84). They deliver downstream signals in the following three ways: cell internalization, direct fusion with the cell membrane, and receptor-ligand interaction (85). All major heart cell types including CMs, ECs, and CFs release exosomes to regulate cell function. What is special is that the protein content of cardiac exosomes is significantly different from other types of exosomes in the literature, which contain cytosolic, sarcomeric, and mitochondrial proteins (86).

For example, CFs-derived exosomes mediate crosstalk between CFs and CMs and lead to cardiomyocyte hypertrophy (60) via internal miRNA. Exosomes isolated from hypoxic CMs promote CFs apoptosis and inhibit their proliferation, migration, and invasion (87) via internal lncRNA.

In addition, exosomes can transfer genetic material between cells. CVB can cause a variety of life-threatening inflammatory diseases. It is a member of the picornaviridae family and is a non-enveloped single-stranded RNA virus related to human diseases including myocarditis (88) and pancreatitis. Although it is well-known that CVB spreads through cytolysis, recent reports reveal a second route in which CVB can be encapsulated in host membrane components and escape the cell in the form of exosome-like particles (89). Recent evidence suggests that CVB and other picornaviruses hijack the host membrane and obtain the envelope. Obtaining the envelope may provide unique benefits for CVB virus particles, such as resistance to neutralizing antibodies and effective non-lytic virus transmission (90). While EVs participate in the transmission of HSV-1, in which microvesicles (MVs) containing virus particles released by infected cells are endocytosed by immature cells, leading to productive infection (91).

TARGETING FIBROBLASTS OR ENDOTHELIAL CELLS IN THE TREATMENT OF MYOCARDITIS

As mentioned above, fibroblasts are activated under the influence of viruses or cytokines. Therefore, reducing the invasion of viruses in fibroblasts and reducing the stimulation of fibroblasts by other inflammatory factors are important means to prevent their activation. The study by Heim et al. showed that Ribavirin, the antiviral drug, has high cell-specific activity in fibroblasts, and it can reduce the viral load in fibroblasts (92). Moreover, Kania et al. demonstrated TGF-β-mediated myofibroblast differentiation and progression of myocardial fibrosis in human and mouse myocarditis (93). Anti-TGF-β treatment prevented myocardial fibrosis in mice with autoimmune myocarditis. In addition, IL-1 can prompt uninfected fibroblasts to enhance the induction of inflammation-related genes in the presence of infected fibroblasts. Blockade of IL-1 receptor signaling with an IL-1 receptor antagonist and knockdown of IL-1 receptor using siRNA abolished cytokines in human fibroblasts during CVB3 infection (94).

Fibroblasts can produce a variety of inflammatory factors and chemokines after activation. Among them, CCL20 is a chemokine that can effectively recruit Th17 cells. The neutralization of CCL20 can effectively reduce cardiac inflammation (17). In addition, blocking HMGB1 secreted by fibroblasts can effectively improve cardiac fibrosis in EAM mice (12). Studies have shown that fibroblasts in the heart are a heterogeneous cell population, in which Sca-1+ cardiac fibroblasts are not only efficient GM-CSF producers, but also have certain plasticity (95). They have a certain degree of plasticity to alter their own cytokine production under the influence of local microenvironment. Therefore, exploring the characteristics of different subpopulations of fibroblasts in myocarditis and finding targeted interventions based on their characteristics can better inform the treatment of patients with myocarditis.

After endothelial cells are infected by virus, the expressions of cell adhesion molecules such as ICAM-1, integrin αvβ3, P- and E-selectin are increased and the release of coagulation factor-von Willebrand factor (VWF) is also increased (96). The increase of adhesion molecules makes the circulating inflammatory cells more likely to adhere to blood vessels, while the increase of coagulation factors aggravates the retention of inflammatory cells and affects the execution of vascular function through the coagulation pathway. Telbivudine, an antiviral drug clinically used to treat hepatitis B, exerts endothelial protection in HPV B19 infected endothelial cells and can improve HPV B19-associated inflammatory cardiomyopathy (97). In addition, CVB3 virus can also infect endothelial cells through Coxsackie and adenovirus receptor (CAR). Bosentan and valsartan are angiotensin II receptor antagonists for the treatment of hypertension. In CVB3-induced myocarditis, bosentan and valsartan treatment can dose-dependently downregulate the levels of CAR mRNA and protein which is required for viral entry into cells. Reduction of CAR significantly reduced viral load in CVB3-infected Human Umbilical Vein Endothelial Cells (HUVECs) (98, 99). What's more, House Dust Mite Specific Antibodies, which can bind to cardiac vascular endothelial cells to weaken their barrier function, have been detected in the serum of patients with myocarditis. Since barrier dysfunction may lead to local tissue inflammation, removal of this antibody may be effective in relieving myocarditis (100). Moreover, compared with healthy rats, rats with autoimmune myocarditis exhibited marked mobilization of Endothelial Progenitor Cells (EPCs) from the bone marrow to the periphery, and their ability to adhere to fibronectin, mature endothelial cells, and cultured cardiomyocytes was significantly reduced. Metastasis of splenocyte-derived EPCs partially attenuates myocardial injury induced by experimental myocarditis. Of these, the transformation of EPCs into resident mature endothelial cells appears to be the most important reason (101). Therefore, augmentation of damaged endothelial cells by cell therapy could also serve as a promising therapeutic approach.

Among endothelial cell-derived proteins with altered secretion or expression in myocarditis, depletion of functional selectin ligand expression reduces the migration of pathogenic

$CD8^+$ T cells to the heart, which in turn reduces cardiac inflammatory infiltration and cardiomyocyte damage in myocarditis. In addition, the expression of MHC class II in myocarditis endothelial cells with dilated cardiomyopathy is increased (102). Studies have shown that loss of MHC class II expression on endothelial cells protects mice from experimental autoimmune myocarditis (103). Cyclosporine and statins reduce endothelial MHC class II levels and may prevent diffuse endothelial dysfunction in myocarditis (98). What's more, the antisense ICAM-1 inhibitor alicaforsen has been studied in four Phase 2 studies in ulcerative colitis (UC), and analysis suggests that alicaforsen enema may provide an effective and potential lasting response (104). However, it still needs more evidence to confirm whether these drugs can affect the expression of ICAM-1 in endothelial cells in myocarditis and exert their therapeutic effect on myocarditis.

In conclusion, the factors that cause the activation of fibroblasts and endothelial cells or the active substances produced by fibroblasts or endothelial cells that affect the occurrence and development of myocarditis can be used as potential targets for clinical treatment of myocarditis.

CONCLUSIONS

CFs and ECs can be infected by pathogens and release immunologically active substances to participate in the inflammatory response. In addition, CFs affect the fibrosis process and long-term prognosis of the heart through ECM changes. At the same time, through the interaction with the CMs, they may play a synergistic or antagonistic effect during the disease process.

However, there are still many problems. First of all, many of the interactions between cells are experiments conducted *in vitro*, such as the use of conditioned media or co-cultivation. It's difficult to perform *in vivo* experiments due to technical problems. However, the *in vitro* environment is quite different from the *in vivo* environment which has a more abundant neurohumoral regulation process.

In addition, a problem faced by cell experiments under *in vitro* conditions is that the conditioned medium may not be able to reproduce the observed effect. The composition and content of the conditioned medium will vary with the intervention environment, such as cell content, cell viability, cell intervention time, and the selection of cell lines, etc. That will make the results unstable.

Finally, the body is in a complex internal environment. The virus infection or the release of inflammatory factors both have beneficial and harmful aspects. This is closely related to the time and space relationship between cells. How to balance the pros and cons, and how to determine the correct intervention intensity, intervention time, and intervention methods is an important issue currently facing.

In general, a correct understanding of the function and mode of action of various cells, especially the non-inflammatory cells of the heart, such as CFs and ECs, will be more conducive to our understanding of diseases. And it contributes to the development of new myocarditis pathogenesis and corresponding treatment strategies.

AUTHOR CONTRIBUTIONS

DWW conceived this review, organized, and critically revised the manuscript. YX did major work of writing the manuscript. CC made contributions to editing the manuscript. ZW gave final approval of the version to be published. All authors were fully aware of and approved the submission of this manuscript.

FUNDING

This work was supported by grants from the National Natural Science Foundation of China (81630010 and 81790624 to DWW, 82070395 to ZW), the Natural Science Foundation of Hubei Province (2020CFA016 to CC), and the Tongji Hospital Clinical Research Flagship Program (2019CR207 to DWW). No funding bodies had any role in the program design, preparation of the manuscript, or decision to publish.

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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.

frontiers | Frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 29 April 2022
doi: 10.3389/fcvm.2022.886607

Clinical and Cardiovascular Magnetic Resonance Predictors of Early and Long-Term Clinical Outcome in Acute Myocarditis

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

Received: 28 February 2022
Accepted: 12 April 2022
Published: 29 April 2022

Citation:
Bohbot Y, Garot J, Hovasse T, Unterseeh T, Di Lena C, Boukefoussa W, Tawa C, Renard C, Limouzineau I, Duhamel S, Garot P, Tribouilloy C and Sanguineti F (2022) Clinical and Cardiovascular Magnetic Resonance Predictors of Early and Long-Term Clinical Outcome in Acute Myocarditis. Front. Cardiovasc. Med. 9:886607. doi: 10.3389/fcvm.2022.886607

Introduction: The natural history of acute myocarditis (AM) remains partially unknown and predictors of outcome are debated. We sought to assess the impact of various cardiac magnetic resonance (CMR) parameters on early and long-term prognosis in a population of patients with AM.

Materials and Methods: In a two-center longitudinal study, we included consecutive patients with diagnosis of AM based on CMR and without hemodynamic compromise. The primary endpoint was the occurrence of an event in the acute phase (≤15 days). Secondary endpoints were the occurrence of major adverse cardiac events (MACE) and recurrence of AM during follow-up.

Results: Three hundred and eighty-eight patients were included [mean age 38.5 years, 77.3% male, mean ejection fraction (EF):56%] of which 82% (317) presented with chest pain. CMR was performed 4 ± 2 days after index presentation. Overall, 38 patients (9.8%) had an event at the acute phase, 41 (10.6%) presented at least one MACE during follow-up (median 7.5 years, 6.6–8.9) and 30 (7.7%) experienced a recurrence of AM. By multivariate analysis, the independent predictors of initial complications were absence of chest pain (OR [95%CI] = 0.35 [0.15–0.82]), presence of syncope/pre-syncope (OR [95%CI] = 3.56 [1.26–10.02]), lower EF (OR [95%CI] = 0.94 [0.91–0.98] per%), myocardial extent of late gadolinium enhancement (LGE) (OR [95%CI] = 1.05 [1.002–1.100] per%) and absence of edema (OR [95%CI] = 0.44 [0.19–0.97]). Only age (HR [95%CI] = 1.021 [1.001–1.041] per year) and an initial alteration of EF (HR [95%CI] = 0.94 [0.91–0.97] per%) were associated with MACE during follow-up. Factors independently associated with AM recurrence were myocarditis prior to the index

episodes (HR [95%CI] = 5.74 [1.72–19.22]) and viral syndrome at the index episode (HR [95%CI] = 4.21 [1.91–9.28]).

Conclusion: In routine consecutive hemodynamically stable patients with diagnosis of AM based on CMR, absence of edema, reduced EF, and extent of LGE were associated with early adverse outcome. Only age and EF were associated with long-term events.

Keywords: cardiovascular magnetic resonance, outcome, late gadolinium enhancement, left ventricular ejection fraction, myocarditis

INTRODUCTION

Acute myocarditis (AM) has an underlying viral etiology in most cases and remains difficult to diagnose because of heterogeneous clinical presentations (1). Although the vast majority of patients have no hemodynamic compromise at the acute stage, AM may have a fulminant presentation. It may also lead to potentially life-threatening complications and subsequently to dilated cardiomyopathy and heart failure (2). Endomyocardial biopsy (EMB) is the current gold standard to confirm the diagnosis of AM, but it is invasive and may lack sensitivity due to the focal nature of the disease (3–5). For these reasons, its use is currently limited to severe forms of AM. Indeed, its main interest is to identify particular etiologies that could benefit from specific treatments (3, 5). Cardiovascular magnetic resonance (CMR) has been recognized as the most appropriate non-invasive diagnostic method for AM diagnosis (6, 7). Besides the evaluation of left ventricular (LV) volumes and function, it carries unprecedented diagnostic value for the accurate depiction of myocardial inflammatory lesions and irreversible myocardial damage (6–13). Some CMR parameters have been identified as potential predictors of outcome, although their value remains controversial (14). Previous studies showed that the presence, extent and location of late gadolinium enhancement (LGE) at the acute phase were predictive of poor prognosis (15–17), whereas others did not (18, 19). Those apparent discrepancies may be due to relatively short clinical follow-up in most studies, and especially to the fact that the patient populations are heterogeneous. The study sought to assess the impact of various CMR parameters on early and long-term prognosis in a population of patients with AM diagnosed by CMR.

MATERIALS AND METHODS

Study Protocol

This retrospective study was conducted in two French tertiary centers [Institut Cardiovasculaire Paris Sud (ICPS) and Amiens University hospital] and included consecutive patients with a CMR-based diagnostic of AM. Patients from ICPS (n = 203) were included between October 2008 and December 2011 as previously described (18) and patients from Amiens University hospital (n = 185) were included between 2010 and 2015. Patients who underwent CMR at the time of acute presentation with at least 3 of the following criteria: (1) chest pain, (2) a recent episode (< 1 month) of acute viral infection, (3) repolarization abnormalities on electrocardiogram, (4) elevated troponin, were eligible for this study. A coronary angiography was performed in case of significant ST segment elevation on the ECG or at the discretion of the physicians when deemed appropriate. The diagnosis of AM was based on the presence of intramyocardial and/or subepicardial LGE indicative of myocardial damage, and at least 1 of the following criteria: (1) presence of myocardial edema identified by the presence of spontaneous subepicardial and/or intramyocardial increased signal intensity on T2-weighted spin echo images, (2) subepicardial and/or intramyocardial early gadolinium enhancement indicative of hyperemia, and (3) absence of microvascular obstruction and subendocardial LGE. Exclusion criteria were: (1) Age < 18 years, (2) the presence of severe life-threatening non-cardiac disease, (3) presence of other cardiac disease, and (4) severe hemodynamic compromise that precluded the CMR study. When AM was diagnosed by CMR, medical treatment usually included β-blockers and angiotensin-converting enzyme inhibitors for at least 6 weeks and aspirin if there was an associated pericarditis. In case of acute or subacute heart failure or rhythm disorders, specific treatments were given when appropriate. The study was approved by both institutions local Ethic Committees and conducted in compliance with institutional policies, national legal requirements and the revised principles of the Declaration of Helsinki. The data underlying this article will be shared on reasonable request to the corresponding author.

Cardiovascular Magnetic Resonance Acquisitions

CMR was performed between within 7 days of first symptoms using a Siemens Magnetom Espree® 1.5 T scanner (Erlangen, Germany) and a General Electric Optima MR450 W, 1.5 T (Milwaukee, Wisconsin, United States) using an 8-element phased-array coil. Cine-MR images in the long axis (2, 3, and 4 chambers) and in the short axis, covering the left ventricle from the base to the apex, were obtained using a fast-imaging Steady State Free Precession (SSFP) sequence. Myocardial edema was studied in matched locations using a triple inversion-recovery T2-weighted turbo spin echo sequence with fat and blood suppression inversion pulses (T2 STIR). Next, a bolus of gadolinium contrast (0.1 mmol/kg) was injected with an injector at a dose of 4 ml/s. Presence of LGE was assessed 10 min after the administration of the contrast in matched locations through the use of inversion-recovery 2D fast spoiled gradient echo sequence with an inversion time (TI) set to null normal myocardial signal (determined by TI scout sequence).

Cardiovascular Magnetic Resonance Analysis

All CMR images were independently reviewed by two experienced physicians and, in case of discrepancies, by a third observer to reach a consensus. The myocardium was divided into 17 segments according to the North American Society of Myocardial Imaging consensus (20). LV volumes and ejection fraction were derived by summation of epicardial and endocardial contours. Myocardial edema was defined as a spontaneous hypersignal on black-blood T2-STIR images. LGE extent was evaluated using a semi-quantitative analysis. Each of the 17 myocardial segments of the left ventricle was divided into 3 layers (outer, middle and inner). Myocardial lesions were evaluated in the 3 layers of each segment of the myocardium (18). Myocardial lesions were finally evaluated by planimetry with the use of an automated cut-off greater than 5 *SD* above the mean signal intensity of the myocardium and expressed as a percentage of the total LV myocardial area.

Follow-Up and Endpoints

The primary clinical endpoint was the occurrence of an event in the acute phase of AM (within 15 days of diagnosis). This criterion was composite and included cardiac death, development or worsening of heart failure (determined by clinicians in each center on the basis of clinical symptoms, signs, laboratory results, and chest X-rays according to the Framingham criteria), sustained ventricular arrhythmia or complete atrioventricular block. Patients were followed over time [median follow-up: 7.5(IQR:6.6–8.9) years] and the secondary clinical endpoints were the occurrence of major adverse cardiac events (MACE) and the recurrence of AM. Events were ascertained by direct patient interview and clinical examination and/or repeated telephone calls to physicians, patients, and (if necessary) next of kin. The adjudication of clinical event was based on a consensus reached by 2 senior cardiologists. MACEs were defined by the occurrence of at least one of the following events: cardiac death, cardiac transplantation, documented sustained ventricular arrythmia, hospitalization for heart failure and hospitalization for cardiac causes. Recurrence of AM was defined as a new episode of clinically suspected AM, at least 6 months after the index episode, with the presence of edema in T2 STIR and LGE.

Statistical Analysis

Baseline continuous variables are expressed as mean ± standard deviation or median [interquartile range], and categorical variables are expressed as numbers and percentages. The normality of data was assessed using the Skewness and Kurtosis normality test. The differences between the groups were assessed with the chi-square test or Fisher exact test for categorical variables and Student's *t*-test for continuous variables. Factors associated with the occurrence of events in the acute phase were investigated using multivariate logistic regression analyses including all significant variables in univariate analysis with a p-value < 0.1 to ensure that we do not drop any potentially useful variables from the multivariate analysis. As the presence of a LGE was mandatory to retain the diagnosis of AM in this study, we could not test its impact on the prognosis Factors associated with the occurrence of MACE during follow-up were identified using a Cox multivariate analysis that included all variables with a p-value < 0.10 in univariate analysis. Colinearity was considered in the multivariate analysis if the Pearson coefficient of correlation was > 0.6 or if a covariate's standard error was > 5.0. Collinear variables were removed from the multivariate analysis. The limit of statistical significance was $p < 0.05$ and all tests were two-tailed. Data were analyzed using SPSS 27.0 (SPSS Inc. Chicago, IL).

RESULTS

Baseline Clinical Characteristics

Of the 441 patients who were eligible for this study, 388 were included (**Figure 1**). Baseline clinical characteristics are reported in **Table 1**. The mean age was 38.5 years and 77.3% of patients were male. Men were younger with more dilated LV than women (**Table 1**). Most patients (N = 317.82%) presented with chest pain, ECG showing ST segment or T wave abnormalities and a moderate troponin increase. Of these 317 infarct-like patients, 165 (52%) had a rapid coronary angiography for an elevated ST segment in at least 2 contiguous leads, showing angiographically normal arteries. Coronary angiography was waived in the remaining 152 patients (48%) due to the absence of ST elevation and a very low cardiovascular risk profile (mean age 33 years, absence of cardiovascular risk factors). The other symptoms at initial presentation included flu-like syndrome, palpitations, dyspnea and syncope/pre-syncope. Patients with an infarct-like presentation had lower LV EF (p = 0.025), more frequent edema (p = 0.004) and LGE more frequently involving the subepicardial layer of the myocardium (**Table 2**). Out of the 388 patients enrolled, 32 (8.2%) were lost to follow-up and were censored at the date of the last clinical contact.

Cardiovascular Magnetic Resonance Data

The mean time between symptoms onset and CMR performance was 4 ± 2 days. Mean LV EF was 56% and mean LV end-diastolic volume was 73 ml/m2. Initial LV dilatation (LV end-diastolic volume > 100 ml/m^2) was found in 37 patients (9%). Regional wall motion abnormalities were found in 123 patients (31.7%). Increased signal intensity on T2-STIR images was detected in 245 patients (63.1%). LGE was present in all patients and involved predominantly the lateral wall (84.8%) and subepicardial layer of the LV myocardium (91.0%) (**Figure 2**). The mean LGE extent was 10.9 ± 7.4% of LV myocardial area. CMR revealed pericardial effusion in 92 patients (23.6%) (**Table 1**).

Outcome at the Acute Phase of Acute Myocarditis

Thirty-eight patients (9.8%) experienced an event within 15 days of the AM diagnosis: 1 cardiac death, 22 development or worsening of heart failure (16 were onset and 6 were worsening with mild dyspnea at the time of initial presentation but

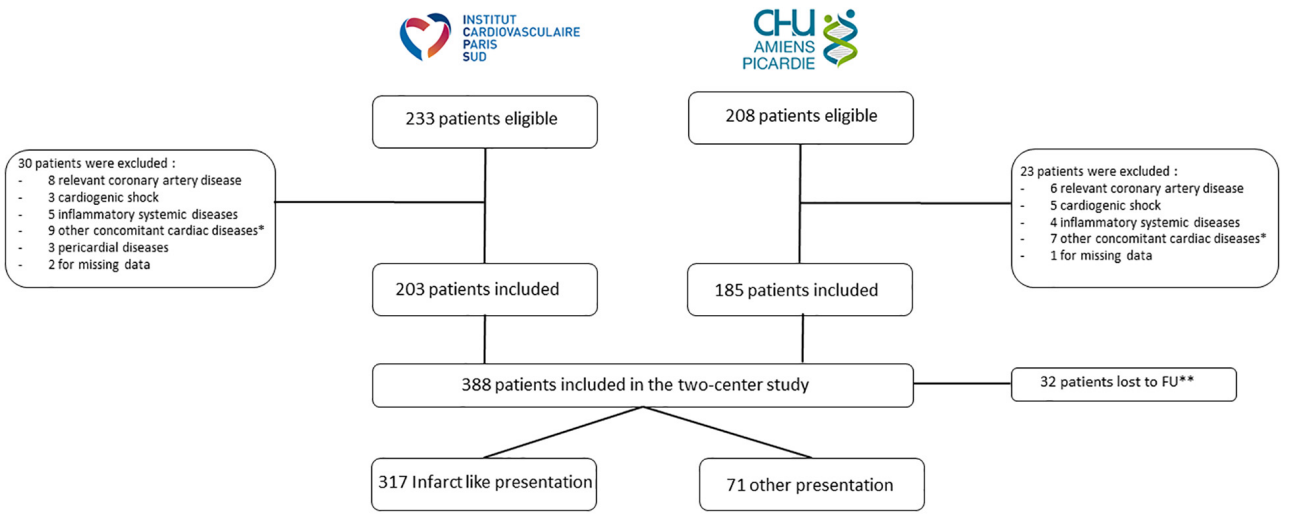

FIGURE 1 | Flow chart of the study. *Other cardiac diseases were chemotherapy-induced cardiomyopathy, valve disease, arrhythmogenic right ventricular dysplasia, hypertrophic cardiomyopathy. **Censored at the date of the last clinical contact. FU, follow-up.

TABLE 1 | Baseline characteristics of study patients according to gender.

Variables	Total (388)	Men (*n* = 300)	Women (*n* = 88)	*p*-value
Age (years)	38.5 ± 17	35 ± 15	49 ± 18	**<0.001**
History of cardiovascular disease (*N*,%)	6 (1.5)	4 (1.3)	2 (2.3)	0.622
Prior history of myocarditis (*N*,%)	7 (1.8)	7 (2.3)	0 (0.0)	0.358
Recent acute viral infection (*N*,%)	115 (29.7)	96 (32.0)	19 (21.6)	0.064
Chest pain (*N*,%)	317 (81.7)	254 (84.8)	63 (71.6)	0.007
Syncope/pre-syncope (*N*,%)	27 (6.9)	20 (6.7)	7 (8.0)	0.639
Time between symptoms onset and CMR (days)	4 ± 2	4 ± 2	5 ± 4	0.971
Initial LV ejection fraction (%)	56 ± 8	56 ± 8	56 ± 9	0.765
Presence of areas of hypokinesia (*N*,%)	123 (31.7)	91 (30.3)	32 (36.4)	0.299
Initial end-diastolic LV volume (ml/m^2)	73 ± 19	75 ± 19	67 ± 18	**0.001**
LV dilatation (*N*,%)	37 (9.5)	30 (10.0)	7 (8.0)	0.682
Pericardial effusion (*N*,%)	92 (23.7)	66 (22.0)	26 (29.5)	0.155
Wall distribution of LGE				0.206
Subepicardial (*N*,%)	353 (91.0)	276 (92.0)	77 (87.5)	
Midwall (*N*,%)	35 (9.0)	24 (9.0)	11 (12.5)	
Lateral wall involved (*N*,%)	329 (84.8)	256 (85.3)	73 (83.0)	0.613
Presence of T2-hypersignal (*N*,%)	245 (63.1)	197 (65.7)	48 (54.5)	0.061
Myocardial extent of LGE (% myocardial surface area)	10.9 ± 7.4	11.1 ± 7.7	10.6 ± 6.3	0.595

Continuous variables are expressed as mean ± 1 standard deviation and categorical variables as percentages and counts.
CMR, cardiac magnetic resonance; LGE, late gadolinium enhancement; LV, left ventricular.
Bold value reffered to p ≤ 0.05.

frank clinical worsening during hospitalization) 13 sustained ventricular arrythmias, and 2 complete atrioventricular blocks. Patients with an early event were older (49 vs. 37 years, $p < 0.001$), had less chest pain but more syncope or pre-syncope at the time of diagnosis than those with no event (both $p < 0.001$) (**Table 3**). Regarding CMR results, Patients with an early event had lower LV EF ($p < 0.001$), less edema (39.5% vs. 65.7%, $p = 0.002$) but a more extensive LGE ($p = 0.042$) (**Table 3**). By multivariate logistic regression analysis, the absence of chest pain (OR [95%CI] = 0.35 [0.15–0.82]; $p = 0.016$), the presence of syncope or pre-syncope (OR [95%CI] = 3.56 [1.27–10.02]; $p = 0.016$), lower LV EF (OR [95%CI] = 0.94 [0.91–0.98] per% decrease; $p = 0.006$), myocardial extent of LGE (OR [95%CI] = 1.05 [1.002–1.100] per% increase; $p = 0.049$) and absence of edema (OR [95%CI] = 0.44 [0.19–0.97];$p = 0.041$) were independently associated with occurrence of events in the acute phase of AM.

Major Adverse Cardiac Events During Follow-Up

Forty-one patients (10.5%) developed at least one MACE during follow-up [median: 7.5(IQR:6.6–8.9) years] including cardiac

TABLE 2 | CMR characteristics according to initial presentation.

Variables	Infarct-like presentation (*n* = 317)	Other presentations (*n* = 71)	*p*-value
CMR variables			
Initial LV ejection fraction (%)	56 ± 7	54 ± 1	**0.025**
LV ejection fraction ≤ 45% (*N*,%)	31 (9.8)	12 (16.9)	0.095
Initial end-diastolic LV volume (ml/m^2)	74 ± 19	71 ± 18	0.186
LV dilatation (*N*,%)	33 (10.4)	4 (5.6)	0.268
Pericardial effusion (*N*,%)	77 (24.3)	15 (21.1)	0.645
Wall distribution of LGE			**0.019**
Subepicardial (*N*,%)	294 (92.2)	59 (83.1)	
Midwall (*N*,%)	23 (7.3)	12 (16.9)	
Lateral wall involved (*N*,%)	267 (84.2)	62 (87.3)	0.587
Septal wall involved (*N*,%)	85 (26.8)	22 (31.0)	0.467
Anterior wall involved (*N*,%)	54 (17.0)	8 (11.3)	0.284
Septal + lateral (*N*,%)	73 (23.0)	21 (29.6)	0.283
Presence of areas of hypokinesia (*N*,%)	102 (32.2)	21 (29.6)	0.778
Presence of LGE in 2 opposed walls (*N*,%)	98 (30.9)	29 (40.8)	0.124
Myocardial extent of LGE (% myocardial surface area)	10.8 ± 7.5	11.7 ± 7.1	0.540
Number of segments with LGE (*N*,%)	4.1 ± 2.4	4.2 ± 2.5	0.696
Presence of T2-hypersignal (*N*,%)	211 (66.6)	34 (47.9)	**0.004**
Number of segments with T2-hypersignal (*N*,%)	2.3 ± 2.3	2.3 ± 1.7	0.968

Continuous variables are expressed as mean ± 1 standard deviation and categorical variables as percentages and counts.
CMR, cardiac magnetic resonance; LGE, late gadolinium enhancement; LV, left ventricular.
Bold value reffered to p ≤ 0.05.

death/heart transplantation in 6 patients, documented sustained ventricular tachycardia in 6 patients, hospitalization for cardiac causes in 15 patients and hospitalization for heart failure in 14 patients. Patients who developed MACE during follow-up were older (46 vs. 38 years, $p = 0.001$) and had experienced more syncope/pre-syncope during the acute episode ($p = 0.016$) (**Table 4**). Patients with MACEs had lower LV EF ($p < 0.001$), a more extensive LGE ($p = 0.036$) which involved more frequently the septal wall ($p = 0.017$), and was more often localized in the midwall layer of the LV ($p = 0.002$) compared to patients without MACEs. By multivariate Cox analysis, only age (HR [95%CI] = 1.021 [1.001–1.041] per year; $p = 0.035$), and lower LV EF (HR [95%CI] = 0.94 [0.91–0.97] per% decrease; $p < 0.001$), remained independently associated with MACE occurrence (**Figure 3**). Only lower LV EF (HR [95%CI] = 0.90 [0.87–0.94] per% decrease; $p < 0.001$), remained independently associated with the occurrence of MACE in patients presenting with edema and LGE ($n = 245$).

Estimated 7-year event free survival was 94 ± 1% for patients with LV EF > 45% and 81 ± 6% for patients with LV EF ≤ 45% (Log Rank $p < 0.001$) (**Figure 4A**). Estimated 7-year event free survival was 94 ± 2% for patients with LGE extent < 10% of myocardial mass and 91 ± 2% for patients with LGE extent ≥ 10% of myocardial mass (Log Rank $p = 0.736$) (**Figure 4B**).

Recurrence of Acute Myocarditis

During follow-up, 30 patients (7.7%) experienced a recurrence of AM with a mean delay of 4.9 ± 3.1 years after the initial episode. Patients with recurrent myocarditis reported more viral syndrome at the time of the index episode (50% vs. 27.9%; $p = 0.020$) and had more often a prior history of myocarditis before this index episode ($p = 0.012$) (**Table 5**). By multivariate Cox analysis, these two variables remained independently associated with an AM recurrence during follow-up (HR [95%CI] = 5.74 [1.72–19.22]; $p = 0.005$ for prior myocarditis and HR [95%CI] = 4.21 [1.91–9.28]; $p < 0.001$ for viral syndrome).

DISCUSSION

In this two-center study of 388 consecutive patients with a diagnosis of AM based on CMR with a mild presentation, the absence of edema, reduced LV EF, and extent of LGE were associate with early outcome while the only independent CMR predictor of an adverse long-term outcome was an initial impairment of LV EF. The presence and extent of acute focal myocardial edema and the extent of myocardial tissue damage on LGE-CMR were not independently related to clinical outcome over a long median follow-up of 7.5(6.6–8.9) years (**Figure 5**).

Interestingly, the presence of edema was associated with a better early outcome, suggesting a potential protective role of inflammatory response in the acute phase of AM. McLellan et al. showed consistent results in patients presenting with acute cardiomyopathy, demonstrating that the extent of myocardial inflammation identified by high global relative enhancement was predictive of left ventricular function recovery (21). Vermes et al. reported in a population of 37 AM, that the presence of global and/or regional edema on admission was the sole independent predictor of a subsequent recovery of LV EF, likely reflecting a recovery of reversibly injured myocardium

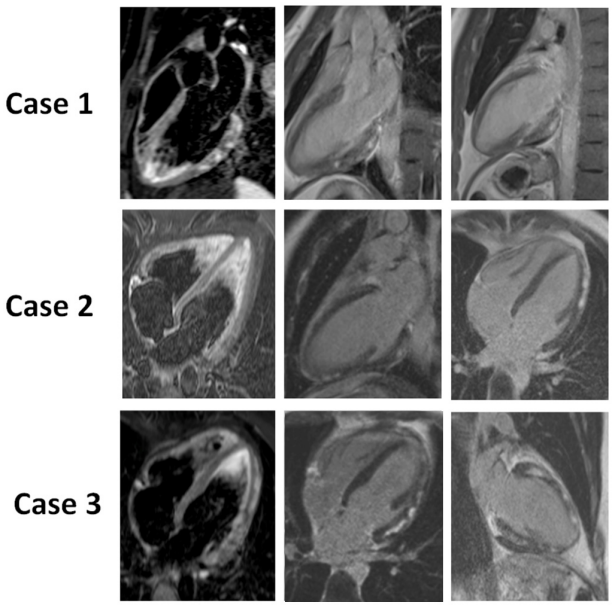

FIGURE 2 | CMR of 3 patients with acute myocarditis: Case 1: a 21-year-old male patient with chest pain and ST elevation. CMR shows a marked focal edema (T2 STIR) and an intramyocardial nodular LGE of the inferolateral wall. Case 2: a 28-year-old male patient with chest pain, dyspnea, ST depression and elevated troponin. CMR shows no focal edema (T2 STIR) and a subepicardial nodular and linear LGE of the latero-apical and infero-apical walls. Case 3: a 33-year-old female patient with chest pain, palpitations, negative T waves and elevated troponin. CMR shows a moderate focal edema (T2 STIR) of the latero-apical wall and a diffuse intramyocardial and subepicardial, nodular and linear LGE. Images in the left panel of the figure are obtained in Black blood T2-weighted short-inversion-time inversion-recovery (STIR) sequence and allow the identification of edema. Images of the mid and right panel are based on inversion-recovery prepared T1-weighted sequences, performed 10 min after intravenous administration of gadolinium chelate and allow the identification of LGE.

(22). Our data do not explain the mechanism by which edema appears protective. However, as in AM, interstitial space could be increased by the presence of edema and not just by focal fibrosis, we can postulate, that in patients with both LGE and edema, there is active inflammation with the possibility of future healing, whereas in those without edema, LGE would correspond to fibrosis that would be more likely to persist over time. However, it is noteworthy that identification of edema is not always easy with T2 STIR sequences because of several limitations. Indeed, the high signal from stagnant blood in the LV cavity, the low signal-to-noise ratio, and the high sensitivity to myocardial motion complicates its identification. More recent sequences with quantitative T1 and T2 mapping techniques might be well suited to detect myocardial edema or other myocardial tissue alterations in AM with higher sensitivity, but they were not available at the time of our study (23). Nevertheless, these sequences also present some limitations. Firstly, the values can vary significantly between CMR machines and from one manufacturer to another. Secondly, thresholds for the diagnosis of AM are currently missing and comparative data with histopathology is lacking. It also seems difficult in clinical practice to make the diagnosis of AM using only T1/T2 mapping in patients without focal edema and without LGE, with the risk of over diagnosing this condition.

In agreement with previous studies of patients with AM diagnosed either by EMB or CMR (4, 18, 24–26), the initial LV EF determined by CMR was an independent predictor of early and long-term outcome. Patients with AM and LGE are more likely to have greater myocardial damage than patients with AM but no LGE as it may favor progressive left ventricular remodeling and dysfunction and eventually leads to heart failure (14, 26). Furthermore, LGE may play a key role in the genesis of ventricular arrhythmias by promoting reentrant circuits (27). According to a recent meta-analysis, the presence of LGE on baseline CMR in AM is an important independent prognostic marker that portends an increased risk of major adverse cardiac events (14). However, studies evaluated the impact of LGE presence in AM are very heterogeneous. Grün et al. reported that the presence of LGE was independently predictive of long-term all-cause and cardiac mortality in patients with myocarditis proven by biopsy (15). It is important to note that our study population was very different from the latter study where a substantial number of patients had severe heart failure and LV dysfunction (45% in NYHA III-IV, mean LVEF: 45%). Viral genomes are frequently detected in the EMBs of patients with left ventricular systolic dysfunction and the persistence of these viruses, often in the form of recurrent infections, may be involved in the transition from myocarditis to dilated cardiomyopathy (28), or may precipitate the development of heart failure (29). In our study, the presence of LGE was mandatory to establish the diagnosis of AM and therefore, we could not assess the impact of its presence on the prognosis.

The prognostic impact of LGE extent in AM is debated. Gräni et al. found that a 10% increase in the extent of LGE led to a 79% increase in the risk of MACE, after adjustment for LVEF (17). However, their population was different from ours. Indeed, unlike in the current study, only 44% of patients had LGE but almost 50% of patients had heart failure and/or left ventricular dysfunction (mean LVEF 49% vs. 56%) which may partly explain the conflicting results (17). White et al. have recently reported in a series of 100 patients with AM diagnosed by CMR, that neither the presence nor the extent of LGE was associated with improvement in LV EF or reduction in LV end-diastolic volume index at 12 months (19). A pilot study by Aquaro et al. involving patients with AM, preserved LV EF and NYHA class I, suggested that LGE in this acute setting is not synonymous with definitive fibrosis and that the persistence of LGE on repeat CMR at 6 months is associated with poor outcome (30). In our study, the extent of LGE was independently associated with early outcome but not with long-term MACE after adjustment to LV EF, which remained the strongest prognostic marker. It is therefore possible that the extent of LGE at initial presentation reflects the severity of the disease in the early phase, but not at long term, as its course is not predictable and only its persistence or worse its progression may be associated with long term events.

A recent study suggested a relationship between anteroseptal localization of LGE and worse prognosis (16). whereas in the current study, septal localization of LGE was associated with

TABLE 3 | Patients with/without clinical event at the acute phase of AM.

Variables	Event in the acute phase (*n* = 38)	Absence of event in the acute phase (*n* = 350)	*p*-value
Clinical variables			
Age (years)	49 ± 19	37 ± 16	**<0.001**
Male sex (*N*,%)	25 (65.8)	275 (78.6)	0.100
Prior history of myocarditis (*N*,%)	0 (0.0)	7 (2.0)	1.00
Recent acute viral infection (*N*,%)	7 (18.4)	108 (30.9)	0.135
Chest pain (*N*,%)	18 (47.4)	299 (85.4)	**<0.001**
Syncope/pre-syncope (*N*,%)	11 (28.9)	16 (4.6)	**<0.001**
CMR variables			
Initial LV ejection fraction (%)	52 ± 13	56 ± 8	**<0.001**
LV ejection fraction ≤ 45% (*N*,%)	13 (34.2)	30 (8.6)	**<0.001**
Initial end-diastolic LV volume (ml/m^2)	78 ± 21	73 ± 18	0.146
LV dilatation (*N*,%)	6 (15.8)	31 (8.9)	0.237
Pericardial effusion (*N*,%)	10 (26.3)	82 (23.4)	0.690
Wall distribution of LGE			0.065
Subepicardial (*N*,%)	31 (81.6)	322 (92.0)	
Midwall (*N*,%)	7 (18.4)	28 (8.0)	
Lateral wall involved (*N*,%)	35 (92.1)	294 (84.0)	0.318
Septal wall involved (*N*,%)	13 (34.2)	94 (26.9)	0.343
Anterior wall involved (*N*,%)	5 (5.3)	60 (17.1)	0.063
Septal + lateral (*N*,%)	12 (31.6)	82 (23.4)	0.318
Presence of areas of hypokinesia (*N*,%)	17 (44.7)	106 (30.3)	0.097
Presence of LGE in 2 opposed walls (*N*,%)	15 (39.5)	112 (32.0)	0.366
Myocardial extent of LGE (% myocardial surface area)	13.4 ± 8.6	10.6 ± 7.0	**0.042**
Number of segments with LGE (*N*,%)	4.5 ± 2.8	4.0 ± 2.4	0.311
Presence of T2-hypersignal (*N*,%)	15 (39.5)	230 (65.7)	**0.002**
Number of segments with T2-hypersignal (*N*,%)	2.6 ± 2.5	2.3 ± 2.2	0.184

Continuous variables are expressed as mean ± 1 standard deviation and categorical variables as percentages and counts.
AM, acute myocarditis; CMR, cardiac magnetic resonance; LGE, late gadolinium enhancement, LV, left ventricular.
Bold value reffered to p ≤ 0.05.

TABLE 4 | Patients with/without MACE at follow-up.

Variables	MACE at follow-up (*n* = 41)	Absence of MACE at follow-up (*n* = 347)	*p*-value
Clinical variables			
Age (years)	46 ± 17	38 ± 16	**0.001**
Male sex (*N*,%)	30 (73.2)	270 (77.8)	0.554
Prior history of myocarditis (*N*,%)	1 (2.4)	6 (1.7)	0.545
Recent acute viral infection (*N*,%)	11 (26.8)	104 (30.0)	0.599
Chest pain (*N*,%)	29 (70.7)	288 (83.0)	0.084
Syncope/pre-syncope (*N*,%)	7 (17.1)	20 (5.8)	**0.016**
CMR variables			
Initial LV ejection fraction (%)	50 ± 11	57 ± 8	**<0.001**
LV ejection fraction ≤ 45% (*N*,%)	14 (34.1)	29 (8.4)	**<0.001**
Initial end-diastolic LV volume (ml/m^2)	76 ± 24	73 ± 18	0.241
LV dilatation (*N*,%)	7 (15.9)	30 (8.7)	0.092
Pericardial effusion (*N*,%)	9 (22.0)	83 (23.9)	0.849
Wall distribution of LGE			**0.002**
Subepicardial (*N*,%)	31 (75.6)	322 (92.8)	
Midwall (*N*,%)	10 (24.4)	25 (7.2)	
Lateral wall involved (*N*,%)	36 (87.8)	293 (84.4)	0.818
Septal wall involved (*N*,%)	18 (43.9)	89 (25.6)	**0.017**
Anterior wall involved (*N*,%)	5 (12.2)	57 (16.4)	0.653
Septal + lateral (*N*,%)	16 (39.0)	78 (22.5)	**0.032**
Presence of areas of hypokinesia (*N*,%)	18 (43.9)	105 (30.3)	0.110
Presence of LGE in 2 opposed walls (*N*,%)	18 (43.9)	109 (31.4)	0.115
Myocardial extent of LGE (% myocardial surface area)	14.1 ± 7.7	10.3 ± 7.4	**0.036**
Number of segments with LGE (*N*,%)	5.1 ± 2.8	3.9 ± 2.3	**0.004**
Presence of T2-hypersignal (*N*,%)	21 (51.2)	224 (64.6)	0.123
Number of segments with T2-hypersignal (*N*,%)	2.8 ± 2.7	2.2 ± 2.1	0.100

Continuous variables are expressed as mean ± 1 standard deviation and categorical variables as percentages and counts.
CMR, cardiac magnetic resonance; LGE, late gadolinium enhancement; LV, left ventricular; MACE, major cardiac events.
Bold value reffered to p ≤ 0.05.

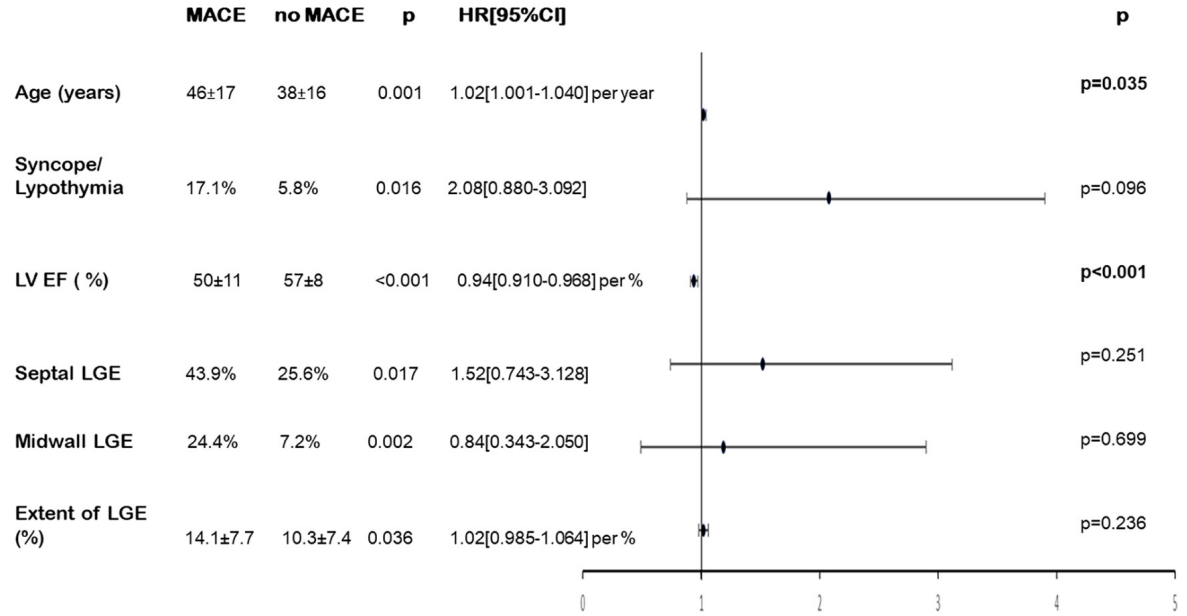

FIGURE 3 | Univariate and multivariate analysis of variables associated with MACE during follow-up. Forrest plot: Hazard ratio and 95% confidence interval for risk of MACE during follow-up. LGE, late gadolinium enhancement; LV EF, left ventricular ejection fraction; MACE, major cardiac events.

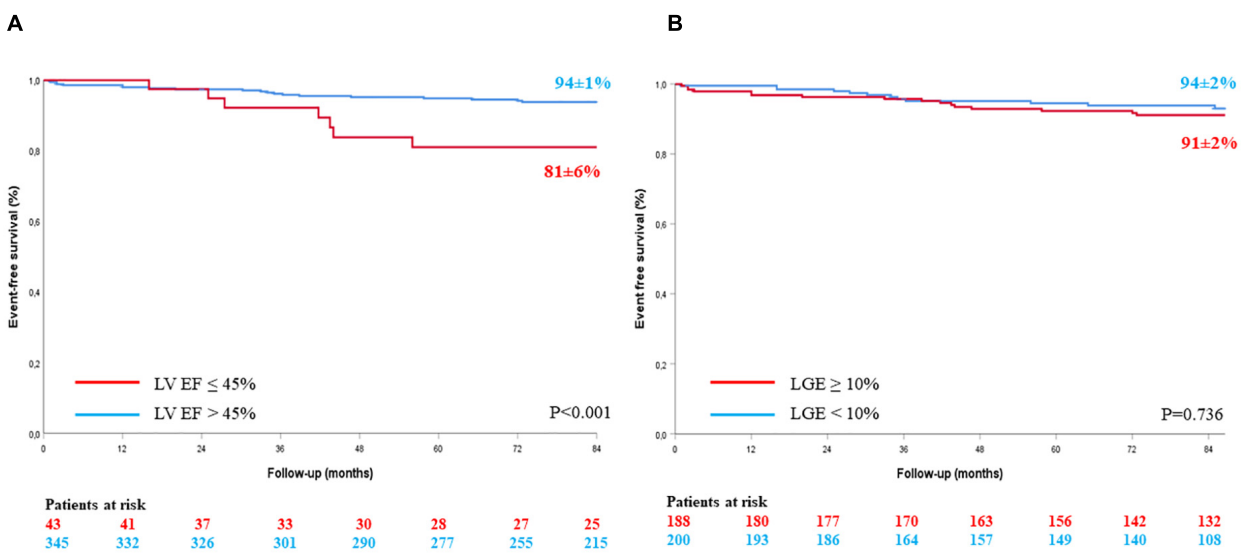

FIGURE 4 | Kaplan Meier event-free survival curves according to LV EF **(A)** and LGE extension **(B)**. LGE, late gadolinium enhancement; LV EF, left ventricular ejection fraction.

MACE in univariate analysis but not in multivariate analysis. However, the prevalence of anteroseptal involvement was lower in our study (39% of patients with LGE vs. 27% in our study), which may explain this discrepancy. In concordance with our data, Aquaro et al. reported that the extent of LGE was not associated with MACE occurrence (16).

It is important to note that the current findings are only valid in patients with a diagnosis of AM based on CMR with a predominantly infarct-like presentation and without hemodynamic compromise. However, this is the most common presentation of the disease in clinical practice by far. The diagnosis of AM was based on a combination of clinical, electrocardiographic and/or biological arguments, as well as CMR, which showed intramyocardial and/or subepicardial lesions strongly indicative of acute non-ischemic tissue damage. The CMR diagnostic criteria were slightly different from the

TABLE 5 | Patients with/without recurrence of AM during follow-up.

Variables	Recurrence of AM during follow-up (n = 30)	No recurrence of AM during follow-up (n = 358)	p-value
Clinical variables			
Age (years)	46 ± 17	38 ± 16	0.202
Male sex (*N*,%)	24 (80.0)	276 (77.1)	0.824
Prior history of myocarditis (*N*,%)	3 (10.0)	4 (1.1)	**0.012**
Recent acute viral infection (*N*,%)	15 (50.0)	100 (27.9)	**0.020**
Chest pain (*N*,%)	26 (86.7)	291 (81.3)	0.625
Syncope/pre-syncope (*N*,%)	2 (6.7)	25 (7.0)	1.00
CMR variables			
Initial LV ejection fraction (%)	50 ± 11	57 ± 8	0.477
LV ejection fraction ≤ 45% (*N*,%)	4 (13.3)	29 (8.4)	0.760
Initial end-diastolic LV volume (ml/m^2)	76 ± 24	73 ± 18	0.461
LV dilatation (*N*,%)	4 (13.3)	33 (9.2)	0.512
Pericardial effusion (*N*,%)	6 (20.0)	86 (24.0)	0.823
Wall distribution of LGE			0.744
Subepicardial (*N*,%)	27 (90.0)	326 (91.1)	
Midwall (*N*,%)	3 (10.0)	31 (8.9)	
Lateral wall involved (*N*,%)	24 (80.0)	305 (85.2)	0.430
Septal wall involved (*N*,%)	10 (33.3)	97 (27.1)	0.824
Anterior wall involved (*N*,%)	8 (26.7)	54 (15.1)	0.117
Septal + lateral (*N*,%)	8 (26.7)	86 (24.0)	0.824
Presence of areas of hypokinesia (*N*,%)	13 (43.3)	110 (30.7)	0.158
Presence of LGE in 2 opposed walls (*N*,%)	9 (30.0)	118 (33.0)	0.841
Myocardial extent of LGE (% myocardial surface area)	14.1 ± 7.7	10.3 ± 7.4	0.609
Number of segments with LGE (*N*,%)	5.1 ± 2.8	3.9 ± 2.3	0.303
Presence of T2-hypersignal (*N*,%)	21 (70.0)	224 (62.6)	0.555
Number of segments with T2-hypersignal (*N*,%)	2.8 ± 2.7	2.2 ± 2.1	0.100

Recurrence of AM was defined as a new episode of clinically suspected AM, at least 6 months after the index episode, with the presence of edema in T2 STIR and LGE. AM, acute myocarditis; CMR, cardiac magnetic resonance; LGE, late gadolinium enhancement; LV, left ventricular.
Bold value reffered to $p \leq 0.05$.

original Lake-Louise criteria (in effect at the time of the study) according to which a diagnosis of AM is made when at least 2 parameters among T2 hypersignal, early enhancement and late enhancement are met (12). In our study, the presence of subepicardial and/or intramyocardial LGE lesions was required for the diagnosis as this parameter is more specific and limits the possibility of including patients with myocardial edema due to another cause. Similarly, we did not include clinical suspicions of AM with a negative CMR, because we believe that the diagnosis may be questionable in those situations. Finally, to our knowledge, we report the largest study of patients with acute myocarditis confirmed by the presence of non-ischemic typical myocarditis LGE patterns on CMR with the longest follow-up (median 7.5 years).

Limitations

Our study presents the limitations inherent to retrospective analyses. Coronary artery disease has not been ruled out by specific examination in all patients. However, none of the patients had an LGE pattern consistent with myocardial infarction, and coronary angiography was performed when deemed necessary to rule out an acute coronary syndrome. EMB was not performed to confirm the diagnosis of AM. This study did not provide comparisons between CMR lesions and biomarkers to predict clinical outcomes because these markers were not available for all patients. Also, the empirical treatment based on β-blockers and angiotensin-converting enzyme inhibitors might have influence the outcome of patients. T1 and T2 mapping techniques were not available at the time of our study and we cannot rule out the possibility that patients without focal edema might have another diagnosis, even if all patients had a clinical presentation suggestive of AM. CMR does not allow to distinguish the most common lymphocytic-dominant myocarditis from more severe forms of AM such as giant cell, eosinophilic and sarcoid myocarditis, which might be associated with poor outcomes. However, our population consisted only of hemodynamically stable patients with AM of presumed viral origin. Recent data suggest that patients with inherited cardiomyopathies can present with acute myocarditis and that they tend to have worse prognosis compared to "pure" myocarditis (31-33). Unfortunately, this interesting information was not known during the inclusion period of the study and therefore data on family history of cardiomyopathy or AM and genetic testing were not available in our database. Although follow-up is long, it is possible that some events occurred later and that LGE extent could be associated with those later events.

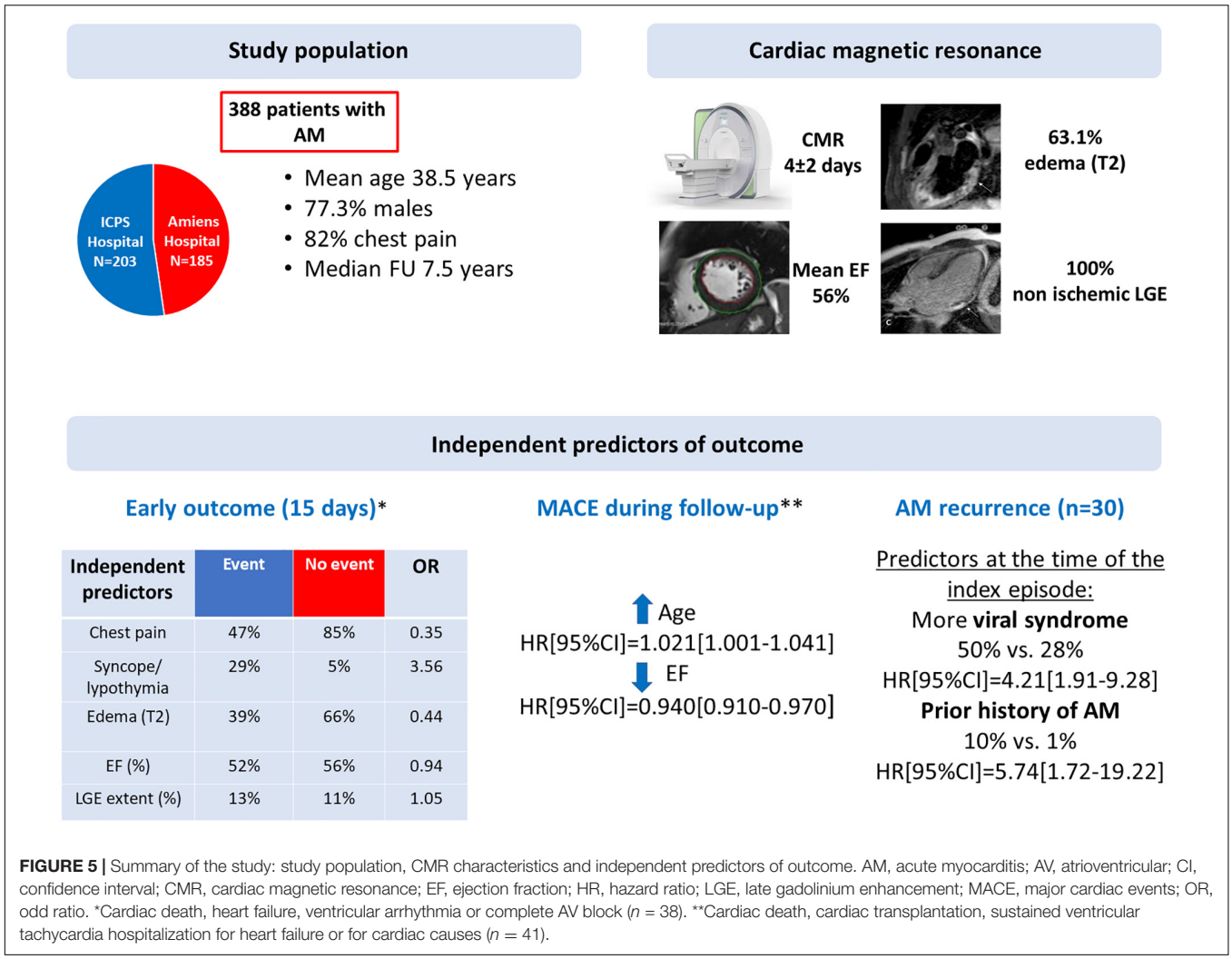

FIGURE 5 | Summary of the study: study population, CMR characteristics and independent predictors of outcome. AM, acute myocarditis; AV, atrioventricular; CI, confidence interval; CMR, cardiac magnetic resonance; EF, ejection fraction; HR, hazard ratio; LGE, late gadolinium enhancement; MACE, major cardiac events; OR, odd ratio. *Cardiac death, heart failure, ventricular arrhythmia or complete AV block (n = 38). **Cardiac death, cardiac transplantation, sustained ventricular tachycardia hospitalization for heart failure or for cardiac causes (n = 41).

Finally, all patients included in this study had early CMR, which represents a selection bias because it excluded the most severe patients.

CONCLUSION

In consecutive routine patients diagnosed with acute myocarditis by CMR with mild presentation and without severe hemodynamic compromise, the absence of edema, reduced LV EF, and extent of LGE were associated with early outcome. In contrast, only age and initial LV EF impairment were predictors of long-term adverse outcome, whereas the presence of myocardial edema and the extent of LGE were not.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics committee of ICPS and Amiens University Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JG, FS, and YB: design of the study. FS, CTa, CD, WB, IL, and YB: data acquisition. YB and FS: statistical analysis. JG, YB, and FS: analysis and interpretation of the data, drafting of the manuscript. All authors substantially contributed to the manuscript and reviewed the manuscript.

ACKNOWLEDGMENTS

We thank the administrative and technical staff of the ICPS and Amiens University Hospital.

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Brief Research Report
PUBLISHED 05 October 2022
DOI 10.3389/fcvm.2022.978592

OPEN ACCESS

EDITED BY
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SPECIALTY SECTION
This article was submitted to
Cardiovascular Imaging,
a section of the journal
Frontiers in Cardiovascular Medicine

RECEIVED 26 June 2022
ACCEPTED 18 August 2022
PUBLISHED 05 October 2022

CITATION
Shiyovich A, Plakht Y, Witberg G,
Rotstein A, Aviv Y, Wiessman M,
Kornowski R and Hamdan A (2022)
Myocarditis following COVID-19
vaccination in adolescents: Cardiac
magnetic resonance imaging study.
Front. Cardiovasc. Med. 9:978592.
doi: 10.3389/fcvm.2022.978592

Myocarditis following COVID-19 vaccination in adolescents: Cardiac magnetic resonance imaging study

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Introduction: Vaccination-associated myocarditis was reported following COVID-19 vaccine initially among persons aged 16 or older and recently among adolescents aged 12–15.

Objectives: To describe the clinical and cardiac magnetic resonance (CMR) characteristics of adolescents aged 12–15 with myocarditis following the administration of the BNT162b2 mRNA COVID-19 vaccine.

Methods: CMR of adolescents (age 12–15) with a clinical diagnosis of myocarditis within 42 days following the first COVID-19 vaccine were analyzed.

Results: A total of 182,605 adolescent were vaccinated, out of which 9 were diagnosed with clinically adjudicated myocarditis while CMR was performed in 5/9 patients (56%). Median age was 15 years (range 13–15), 4/5 (80%) males. All the patients we previously healthy. The ECG upon presentation was abnormal in 3/5 (60%) of patients. All cases were classified as clinically mild and no patient required inotropes or mechanical circulatory support treatment. The median follow-up time, for the 5-included patients, was 206 (IQR 192–229, range 179–233) days. During the follow-up, no re-admissions, deaths, or any other cardiac events have occurred.

The median time between the diagnosis to the CMR was 104 days (range 27–149). The median left ventricular ejection fraction was within normal range 65% (range 62–69). Native T1 was available in four patients, the local T1 value was increased in three of them. T2 values were available in two patients and were all within normal range. The median late gadolinium enhancement (LGE) was 2% (range 0–6%) with inferolateral wall being the most common location (3/5). The patterns of the LGE were as following: (i) mid-wall in 3 patients; (ii) epicardial in 1-patient. LGE in the pericardium was present in 2/5 patients with pericardial effusion present in 4/5 patients with a median diameter of 4 mm (range 3–5 mm) at end-systole.

Conclusions: CMR findings and clinical course of adolescents with COVID-19 vaccination associated myocarditis, are similar to those of older patients, being relatively mild and potentially implying favorable outcomes.

KEYWORDS

MRI, COVID-19, vaccine, myocarditis, adolescents

Introduction

Pfizer-BioNTech BNT162b2 messenger-RNA (mRNA) vaccines have demonstrated exceptional safety and real-world effectiveness in preventing severe disease and death from COVID-19. Concerns about vaccination-related myocarditis in young men were initially raised in Israel (1, 2). The Incidence of such myocarditis was highest among males aged 16–29 years, with a relatively favorable clinical course and mild cardiac magnetic resonance (CMR) imaging findings in initial reports (3, 4). Subsequently, mRNA COVID-19 vaccine was approved for adolescents aged 12–15 years and initial evidence of vaccine-associated myocarditis in this age group were published as well (5, 6). Our objective in the current report was to describe the clinical course and CMR imaging characteristics of adolescents aged 12–15 with myocarditis following the administration of BNT162b2 mRNA COVID-19 vaccine.

Methods

Study population

Data regarding 12–15 year old adolescents vaccinated between June 2nd, 2021 and November 30th, 2021 were obtained from Clalit Health Services (CHS) database. Potential myocarditis cases were identified throughout the period of 42 days after the administration of the first vaccine dose by ICD-9 codes. Subsequently, the diagnosis was adjudicated by cardiologists who carefully reviewed each patients' full-medical record. Follow-up data from the computerized medical record was performed until February 26th, 2022, as previously described (1).

This study was approved by the institutional review board and performed consistently with the Helsinki declaration.

CMR imaging

CMR imaging was performed using either 1.5 T scanner (Ingenia; Philips Medical System) or 3 T scanner (Magnetom Vida; Siemens Healthineers), implementing standardized imaging protocols. CMR protocol comprised of multiplanar cine imaging for acquisition of cardiac function, volumes, and mass, and late gadolinium (LGE) for scar imaging. At 1.5 T scanner Balanced steady state free precession, single breath-hold modified inversion recovery Look-Locker (MOLLI) was used for T1 mapping and a navigator gated black blood prepared gradient spin-echo sequence was used for T2 mapping. At 3 T scanner, myocardial T1 mapping was performed using MOLLI sequence. Native T1 and T2 mapping, and postcontrast T1 mapping were acquired in a 3 short-axis slices (apical, mid-ventricular and basal).

Analysis of the CMR was performed as previously reported (3, 4). For 1.5T scanner abnormal native T1 and T2 values were defined as greater than 1,060 ms and greater than 57 ms; respectively (7) and for 3T scanner abnormal native T1 values were defined as greater than 1,105 ms (8).

Statistical analysis

Descriptive statistical methods were applied in the current study. Baseline characteristics of the patients are presented as counts (%) for categorical variables and median (range) or mean (±standard deviation) for continuous variables, as appropriate.

Results

A total of 182,605 adolescent were vaccinated during the study period, out of which 23 had an ICD-9 code of myocarditis. Following adjudication 9 adolescents were clinically confirmed to have a diagnosis of myocarditis. Throughout the study period CMR was performed in 5/9 patients (56%) who were included in the current study. Patient characteristics are presented in Table 1. Median age was 15 years (range 13–15), 4/5 (80% males). One patient presented following the first vaccine (3 days) and 4 after the second vaccine (median of 3 days). All the patients we previously healthy. The median temperature upon presentation was 37.8 (range: 36.5–38.6)°C. All patients presented with acute chest pain. The ECG upon presentation was abnormal in 3/5 (60%) of patients. The median value of the peak Troponin T level was 730 ng/L (IQR = 146–1,647) and the median C-reactive protein was 8.5 mg/dL (IQR = 4.2–14.2). The median left-ventricular ejection fraction, as

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

Age	Sex	past medical history	Symptoms	ECG	Peak Troponin (ng/L)	Time from vaccine and symptoms (days)	Time from vaccine and CMR (days)	WMA	LVEDV (ml)	LESVD (ml)	LVEF (%)	T1 local (ms)	T1 Global (ms)	T2 local (ms)	T2 global (ms)	LGE localization	LGE %	LGE pattern	Pericardial enhance-ment	Pericardial effusion	Pericardial diameter
15	M	None	Chest pain	Normal	146	3	149	N	133	42	69			51	49	–	0	-	N	N	-
15	M	None	Chest pain	Normal	730	5	107	N	144	50	65	1,042	1,044			Infero-lateral (medial)	2	Mid-wall	Y	Y	
15	M	None	Chest pain	Diffuse STE	1,190	3	104	N	132	51	62	1,076	1,075	53	48	Inferior, infero-lateral (basal/medial) and lateral (apical)	3	Mid-wall	N	Y	3
14*	F	None	Chest pain	Diffuse STE	350	2	95	N	138	49	68	1,313	1,251	NA	NA	Infero-lateral, inferoseptal (basal/medial)	2	Mid-wall	N	Y	4
13	M	None	Chest pain	Inferior STE	1,647	3	27	N	137.8	52.9	62	1,067	1,037	NA	NA	Inferior, infero-lateral (basal/medial) and lateral (apical)	6	Epicardial	Y	Y	5

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

* CMR was performed at 3T scanner, while the rest at 1.5T scanner.

10.3389/fcvm.2022.978592

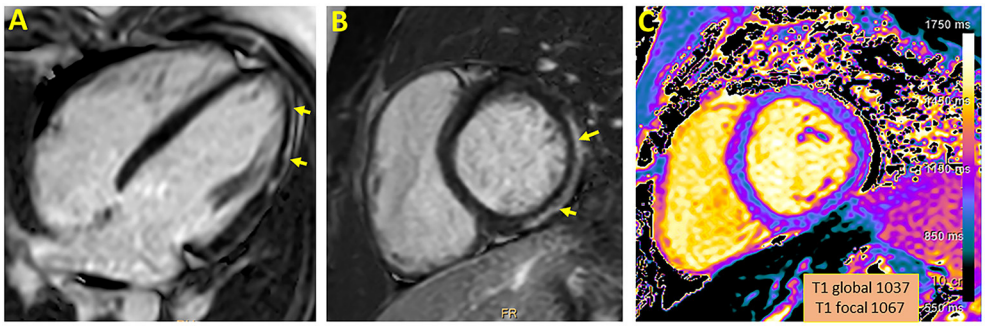

FIGURE 1
Representative CMR imaging in 4-chamber **(A)** and short axis **(B)** view demonstrating late gadolinium enhancement involving epicardial wall of the inferior and inferolateral segment. A corresponding myocardial injury in native T1 mapping is presented **(C)**.

evaluated by echocardiography at diagnosis, was 60% (range: 55–65%). All cases were classified as clinically mild and no patient required inotropes or mechanical circulatory support treatment. The median time of in-hospital stay was 4 days (range 2–6 days). The median follow-up time, for the five-included patients, was 206 (IQR 192–229, range 179–233) days. During the follow-up, no re-admissions, deaths, or any other cardiac events have occurred.

As presented in Table 1, the median time between the diagnosis to the CMR was 104 days (range 27–149). The median left ventricular ejection fraction, based on CMR, was within normal range 65% (range 62–69%). There were no regional wall motion abnormalities detected in all patients. Native T1 was available in 4 patients, the local native T1 value was increased in three of them. T2 values were available in 2 patients and were all within normal range. The median LGE was 2% (range 0–6%) with inferolateral wall being the most common location (3/5). The patterns of the LGE were as following: (i) mid-wall in 3 patients; (ii) epicardial in 1-patient. LGE in the pericardium was present in 2/5 patients with pericardial effusion present in 4/5 patients with a median diameter of 4 mm (range 3–5 mm) at end-systole. A representative CMR imaging finding is displayed in Figure 1.

Discussion

The current study reports and elaborate CMR imaging findings, clinical presentation and clinical outcomes of 12–15 year-old adolescents with a clinical diagnosis of myocarditis following the first and second Pfizer BNT162b2 mRNA COVID-19 vaccination in adolescence. This seems to be a rare adverse event (9 out of 182,605 adolescents, 0.0049%) in this group of patients with a male predominance, a mild clinical course and favorable outcomes, which are all consistent with previous initial reports for this and other age groups (1, 2). The CMR imaging findings observed here-in further support the mild and overall benign myocarditis following COVID-19 vaccination, consistent with the CMR findings among older patients (3, 4). In addition, these findings of relatively low LGE%, and its predominant pattern (inferolateral) and normal/near-normal LVEF are known to be strong predictors of favorable long-term outcomes both when the CMR performed at presentation and at follow-up (9), yet this remains to be proven in this particular group of patients.

Limitations of the current study include a relatively small number of patients, lack of confirmation by a myocardial biopsy, CMRs were not performed systematically in all patients and with a non-identical, often delayed interval from diagnosis.

Conclusions

In conclusion, the CMR imaging findings, consistently with the clinical course, of 12–15 year-old adolescents with vaccine-associated myocarditis following the Pfizer BNT162b2 mRNA COVID-19 vaccination, are similar to those of older patients, being relatively mild and potentially implying favorable clinical course and outcomes of these patients. However, the long-term consequences of this vaccine-associated cardiac myocarditis are not yet fully defined and should be studied.

Data availability statement

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

Ethics statement

This study was reviewed and approved by Clalit Health Services institutional review board. Written informed consent was not required for this study in accordance with the local legislation and institutional requirements.

Author contributions

AS, AH, and RK conceived and planned the study. AS, AH, and AR reviewed the CMR tests. AS and AH contributed to

the interpretation of the results and drafted the manuscript. AS, AH, YA, MW, YP, and GW obtained patient related clinical data and contributed to sample preparation and data analysis. All authors provided critical feedback and helped shape the research, analysis, and manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 13 October 2022
DOI 10.3389/fcvm.2022.1037837

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Heart Failure and Transplantation, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 06 September 2022
ACCEPTED 28 September 2022
PUBLISHED 13 October 2022

CITATION
Cannata A, Bhatti P, Roy R, Al-Agil M, Daniel A, Ferone E, Jordan A, Cassimon B, Bradwell S, Khawaja A, Sadler M, Shamsi A, Huntington J, Birkinshaw A, Rind I, Rosmini S, Piper S, Sado D, Giacca M, Shah AM, McDonagh T, Scott PA and Bromage DI (2022) Prognostic relevance of demographic factors in cardiac magnetic resonance-proven acute myocarditis: A cohort study.
Front. Cardiovasc. Med. 9:1037837.
doi: 10.3389/fcvm.2022.1037837

Prognostic relevance of demographic factors in cardiac magnetic resonance-proven acute myocarditis: A cohort study

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Aim: Acute myocarditis (AM) is a heterogeneous condition with variable estimates of survival. Contemporary criteria for the diagnosis of clinically suspected AM enable non-invasive assessment, resulting in greater sensitivity and more representative cohorts. We aimed to describe the demographic characteristics and long-term outcomes of patients with AM diagnosed using non-invasive criteria.

Methods and results: A total of 199 patients with cardiac magnetic resonance (CMR)-confirmed AM were included. The majority (n = 130, 65%) were male, and the average age was 39 ± 16 years. Half of the patients were White (n = 99, 52%), with the remainder from Black and Minority Ethnic (BAME) groups. The most common clinical presentation was chest pain (n = 156, 78%), with smaller numbers presenting with breathlessness (n = 25, 13%) and arrhythmias (n = 18, 9%). Patients admitted with breathlessness were sicker and more often required inotropes, steroids, and renal replacement therapy ($p < 0.001$, $p < 0.001$, and $p = 0.01$, respectively). Over a median follow-up of 53 (IQR 34–76) months, 11 patients (6%) experienced an adverse outcome, defined as a composite of all-cause mortality, resuscitated cardiac arrest, and appropriate implantable cardioverter defibrillator (ICD) therapy. Patients in the arrhythmia group had a worse prognosis, with a nearly sevenfold risk of adverse events [hazard ratio (HR) 6.97; 95% confidence interval (CI) 1.87–26.00, $p = 0.004$]. Sex and ethnicity were not significantly associated with the outcome.

Conclusion: AM is highly heterogeneous with an overall favourable prognosis. Three-quarters of patients with AM present with chest pain, which is associated with a benign prognosis. AM presenting with life-threatening arrhythmias is associated with a higher risk of adverse events.

KEYWORDS

myocarditis, presentation, sex, ethnicity, outcomes, cardiac magnetic resonance (CMR)

Introduction

Acute myocarditis (AM) is an inflammatory disease of the myocardium occurring most commonly after viral infection, autoimmune disease, or exposure to toxins (1–3). It is characterised by a heterogeneous clinical presentation, ranging from subclinical or minimally symptomatic forms to a life-threatening fulminant presentation with cardiogenic shock or cardiac arrest (4–6).

Early studies of AM mostly included patients with endomyocardial biopsy (EMB)-confirmed AM and suggested a relatively high mortality rate (∼25% at 5 years) (7–11). However, EMB is invasive and therefore reserved for more severe cases, and these studies are likely to have selected more high-risk patients. Recent studies, using cardiovascular magnetic resonance (CMR) in place of EMB to confirm the diagnosis of AM (12, 13), have suggested a more benign clinical course and that clinical presentation can predict prognosis (14–20).

Many of these studies were either small or included small numbers of cases from contributing hospitals, with a consequent risk of selection bias. Furthermore, very few studies report patient ethnicity, and a vast majority of patients in studies where it is reported are White, reflecting local demographics (8, 15, 18). Finally, the impact of sex in a robust, CMR-proven cohort has not been investigated. The objective of this study was to describe the demographic characteristics and outcomes of a large, unselected and ethnically diverse population of patients with AM admitted to a large tertiary Centre in the UK.

Materials and methods

Study design

This was a retrospective cohort study. We included all consecutive patients aged ≥18 years admitted to two hospitals within one hospital Trust (King's College Hospital, London, UK and Princess Royal University Hospital, Orpington, UK), which has a dedicated myocarditis service, between 12th February 2009 and 4th October 2021 with a diagnosis of AM.

The study was conducted under London South-East Research Ethics Committee approval (reference 18/LO/2048) granted to the King's Electronic Records Research Interface (KERRI) (21). We used an open-source retrieval system for unstructured clinical data (CogStack) to identify patients. The CogStack engine, developed at King's College London (22), was used with Elasticsearch to process structured and unstructured textual clinical data from various hospital databases. Additional data cleansing was performed using Python in JupyterLab and returned as CSV spreadsheets.

Further unique patients were identified from our local Hospital Episode Statistics (HES) data if they had a discharge diagnosis of AM in the first diagnostic position according to appropriate ICD codes (B33.2 viral carditis, I01.2 acute rheumatic myocarditis, I09.0 rheumatic myocarditis, I40 AM, I41 myocarditis in diseases classified elsewhere, I51.4 myocarditis, unspecified). We also searched the hospital and Intensive Care Unit (ICU) discharge summaries for inpatients discharged alive containing the keywords "myocarditis" or "myopericarditis." Finally, we searched for patients that died during the study period where the keywords "myocarditis" or "myopericarditis" were given as a cause of death on the death notification. Patients and public were not involved in the design of this study.

Diagnostic criteria for AM were defined according to 2013 ESC-position statement on myocarditis and 2018 Lake Louise Criteria for AM (4, 11, 23, 24), which were independently applied to retrieved data by a minimum of two authors. Patients were included if they presented to the hospital with a consistent clinical presentation and one or more diagnostic criteria based on ECG, cardiac enzymes or cardiac imaging structure and function, in the absence of significant coronary artery disease (CAD) on invasive or non-invasive coronary imaging or low likelihood of CAD (patients <40 years with low clinical suspicion). In addition, all patients had CMR confirmation of AM according to consensus recommendations (24). CMR was considered acceptable if it included cine imaging, late-gadolinium enhancement imaging, and parametric mapping of myocardial T1 and T2. Patients with suspected/confirmed COVID-19 or vaccine-related AM were excluded. Patients with cardiac sarcoidosis were also excluded. All patients underwent

a history and physical examination. Laboratory parameters, echocardiography and CMR findings were recorded.

Clinical presentation, demographic characteristics, and outcomes

We categorised patients into three groups based on their main clinical presentation: (1) chest pain; (2) breathlessness; and (3) arrhythmia. Chest pain presentations were defined as acute chest pain (either ischaemic or pericarditic-sounding) in the absence of CAD and significant left ventricular systolic dysfunction (LVSD). Breathlessness presentations were defined as new onset or progressive HF syndrome. Life-threatening arrhythmias were defined as advanced atrioventricular block, sustained ventricular arrhythmias, or aborted sudden cardiac death. We also categorised patents according to their self-reported ethnicity, and sex. Main clinical presentation was defined by interrogation of the medical record by two authors (DB and AC). Disagreements were resolved by a third author (PS). For each patient, data on baseline co-morbidities and cardiovascular risk factors were obtained using a mix of CogStack retrieval, which was manually validated, and manual searches of the electronic patient record. The primary outcome was a composite of all-cause mortality, resuscitated cardiac arrest, and appropriate implantable cardioverter defibrillator (ICD) therapy following hospital discharge. Appropriate ICD therapy was defined as an appropriate shock for life-threatening arrhythmias.

Statistical analysis

The results were reported in line with the "Strengthening the Reporting of Observational Studies in Epidemiology" (STROBE) guidelines (**Supplementary Table 1**) (25). Continuous variables were expressed as mean and standard deviation, or median and interquartile range (IQR), where appropriate. Categorical variables were expressed as counts and percentage. Comparisons between groups were made by the analysis of variance (ANOVA) test on continuous variables or the Student's *t*-test, or by the non-parametric Mann–Whitney test when appropriate. The Chi-square test or the Fisher's exact tests were calculated for discrete variables.

Survival curves for the primary outcome were estimated and compared between groups by means of the log-rank test. Univariable Cox regression models were performed to obtain hazard ratios for adverse events in the study population. Given the low number of events in the population, multivariable analysis was not performed to avoid overfitting. $p < 0.05$ was considered significant. All analyses were performed with IBM SPSS Statistics Version 28.0 (IBM Corp., Armonk, NY, USA) and R (R Foundation for Statistical Computing, Vienna, Austria).

Results

Derivation of the cohort

Screening hospital and ICU discharge summaries for keywords "myocarditis" or "myopericarditis" revealed 683 unique patients. Three additional patients were identified by screening death notifications for the same keywords. A further 152 unique patients were identified by searching for admissions with ICD-10 codes corresponding to myocarditis. Discharge summaries and patient records were screened for eligibility and 207 cases were excluded due to not being a discharge diagnosis or death notification of AM. A further eight patients were excluded as they were younger than 18 years old at time of admission. Next, data on diagnostic criteria were extracted. 196 patients had insufficient evidence of AM, CAD was not sufficiently excluded in 18, and an alternative diagnosis was evident in 161. A further 49 patients had clinically suspected AM that was not proven by CMR or EMB. Finally, 199 patients with CMR-proven AM were included in our cohort. Only one patient underwent EMB.

Baseline characteristics

A total of 199 patients with CMR-proven AM were included in the study (**Table 1**). The majority (n = 130, 65%) were male and the average age was 39 ± 16 years. The study population was ethnically diverse. While most patients were White (n = 99, 52%), Black, African, Caribbean, or Black British (n = 61, 32%), Asian (n = 8, 4%), and "other" (n = 24, 12%) ethnicities were also represented. The most common clinical presentation was with chest pain (n = 149, 75%), with smaller numbers presenting with breathlessness (n = 32, 16%) and arrhythmias (n = 18, 9%). Fewer than half of patients (n = 80, 41%) experienced prodromal symptoms, which included flu-like symptoms in 26% (n = 51). Only a small number of patients had a pre-existing autoimmune disorder (n = 24, 12%) and the most common cardiac co-morbidity was hypertension (n = 26, 13%). Among those with arrhythmic presentation, 14 patients had sustained ventricular tachycardia or ventricular fibrillation.

At admission, all patients underwent ECG, blood testing and CMR as a minimum. Most patients had an abnormal ECG at presentation (n = 149, 74%), predominantly consisting of repolarisation abnormalities (n = 72, 39%) with a smaller proportion demonstrating ST elevation (n = 48, 26%). Overall, renal function was mildly impaired (eGFR 77 ± 22 ml/min/m^2) with elevated inflammatory markers (CRP 65 ± 85 U/L). Peak troponin was elevated at 771 times the upper limit of normal (×ULN). Of patients that had an echo at baseline (n = 173, 87%), two thirds presented with normal left ventricular ejection fraction (LVEF).

TABLE 1 Characteristics of study patients.

Baseline characteristics		
	n patients	199
	Male sex, *n* (%)	130 (65%)
	Age at admission, years	39.2 ± 16.3
Ethnicity	White, *n* (%)	99 (52%)
	Black, African, Caribbean, or Black British, *n* (%)	61 (32%)
	Asian, *n* (%)	8 (4.2%)
	Other, *n* (%)	24 (12%)
History	Any prodromal symptoms, *n* (%)	80 (41%)
	Flu-like symptoms, *n* (%)	51 (26%)
Clinical presentation	Chest pain	149 (75%)
	Breathlessness	32 (16%)
	Arrhythmia	18 (9.0%)
Co-morbidities	Autoimmune disorders, *n* (%)	24 (12%)
	Previous myocarditis, *n* (%)	7 (3.6%)
	Hypertension, *n* (%)	26 (13%)
	Dyslipidaemia, *n* (%)	11 (5.6%)
	Diabetes, *n* (%)	12 (6.1%)
	CKD, *n* (%)	8 (4.1%)
	PAD, *n* (%)	1 (0.5%)
	Previous MI, *n* (%)	1 (0.5%)
	Alcohol, *n* (%)	4 (2.0%)
Baseline medication	RAASi, *n* (%)	14 (7.3%)
	Beta blocker, *n* (%)	15 (7.8%)
	MRA, *n* (%)	0 (0%)
	Diuretics, *n* (%)	5 (2.6%)
	Immunosuppressant, *n* (%)	19 (9.9%)
	Statin, *n* (%)	19 (9.9%)
	Aspirin, *n* (%)	17 (8.9%)
Admission observations	Temperature, °C	37.04 ± 0.80
	Fever > 37.5, *n* (%)	36 (23%)
	Systolic blood pressure, mmHg	121 ± 19
	Heart rate, bpm	83 ± 23
Presenting ECG	Normal, *n* (%)	50 (26%)
	Sinus rhythm, *n* (%)	173 (92%)
	ST elevation, *n* (%)	48 (26%)
	Other repolarisation abnormalities, *n* (%)	72 (39%)
	LBBB, *n* (%)	9 (5%)
	QRS duration, ms	95 ± 18
	QT_c	396 ± 53
	Q waves	12 (6.9%)
Bloods	Creatinine, mg/dl	98 ± 76
	eGFR, ml/min/m^2	77 ± 22
	Urea, mmol/L	6.3 ± 6.0
	Sodium, mEq/L	137.8 ± 4.2
	Potassium, mEq/L	4.32 ± 0.60
	Haemoglobin, g/dl	135 ± 21
	CRP, U/L	65 ± 85
	White cell count, 10^9/L	148 (79%)
	Neutrophils, 10^9/L	9.8 ± 4.3
	Lymphocytes, 10^9/L	7.3 ± 4.2
	Monocytes, 10^9/L	1.73 ± 0.85
	Basophils, 10^9/L	0.61 ± 0.30
	Eosinophils, 10^9/L	0.04 ± 0.05
	ESR, mm/h	0.12 ± 0.18
	TSH, mIU/L	37 ± 38
	Free T4, pmol/L	2.28 ± 2.48
	Peak troponin, ×ULN	771 ± 1,570
	NT-proBNP, pg/ml	1,272 ± 5,734
Echo	LVEDD, mm	48.5 ± 6.4
	LVEDV, ml	112 ± 35
	IVSDd, mm	10.01 ± 2.44
	No LVSD, *n* (%)	108 (63%)
	Mild LVSD, *n* (%)	20 (12%)
	Moderate LVSD, *n* (%)	23 (13%)
	Severe LVSD, *n* (%)	20 (12%)
	LVEF, %	51 ± 11
CMR	LVEDV indexed, ml/m^2	86 ± 17
	LV mass indexed, g/m^2	57 ± 15
	LVEF, %	57 ± 10
	RVEDV indexed, ml/m^2	88 ± 27
	RVEF, %	57 ± 7
In-hospital management		
Place of care	Cardiology, *n* (%)	132 (68%)
	General medicine, *n* (%)	46 (24%)
	Other wards, *n* (%)	16 (8.2%)
Advanced therapies	Inotropes, *n* (%)	18 (9.4%)
	Intravenous immunoglobulin, *n* (%)	2 (1.0%)
	Steroids, *n* (%)	14 (7.3%)
	Renal replacement therapy, *n* (%)	12 (6.2%)
Discharge medication	Aspirin, *n* (%)	72 (37%)
	Colchicine, *n* (%)	41 (21%)
	RAASi, *n* (%)	94 (48%)
	Beta blocker, *n* (%)	98 (51%)
	MRA, *n* (%)	11 (5.7%)
	Diuretics, *n* (%)	21 (11%)
	Statin	31 (16%)
	Amiodarone, *n* (%)	3 (1.5%)
	Immunosuppressant, *n* (%)	28 (14%)

CKD, chronic kidney disease; PAD, peripheral arterial disease; MI, myocardial infarction; RAASi, renin angiotensin aldosterone system inhibitors; MRA, mineralocorticoid receptor antagonist; LBBB, left bundle branch block; eGFR, estimate glomerular filtration rate; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TSH, thyroid stimulating hormone; NT-proBNP, N-terminal pro-brain natriuretic peptide; LVEDD, left ventricular end-diastolic dimension; LVEDV, left ventricular end diastolic volume; IVSDd, Interventricular septal diameter in diastole; LVSD, left ventricular systolic dysfunction; LVEF, left ventricular ejection fraction; LV, left ventricle; RVEDV, right ventricular end diastolic volume; RVEF, right ventricular ejection fraction.

Overall, most patients (n = 132, 68%) were managed in a cardiology ward. Only a small minority were treated with advanced therapies, including inotropes (n = 18, 9%), and renal replacement therapy (RRT) (n = 12, 6%). Immunosuppression was started in 16 patients (8%). Specifically, 2 patients (1%) received intravenous immunoglobulin (IVIG) and 14 patients received steroids (7%). At discharge, approximately half of patients were on renin-angiotensin-aldosterone system (RAAS) inhibitors. Cardiac devices were present in 21 patients (8%).

Clinical presentation

Baseline characteristics according to clinical presentation are summarised in **Table 2**. Patients presenting with chest pain were younger and more frequently male compared to other presentations, while ethnicity was equally distributed. Co-morbidities and baseline medications were broadly similar between patients with different presentations, although fewer patients with a chest pain presentation had hypertension (p = 0.018) and more patients in the breathlessness group were on immunosuppression (p = 0.002). Admission observations were similar between groups, though patients with a breathlessness presentation had a significantly higher heart rate than chest pain or arrhythmia presentations ($p < 0.001$).

Patients with a chest pain presentation were more likely to have ST elevation than other presentations (31% compared to 14% with breathlessness and none with arrhythmia, p = 0.004), while patients in the arrhythmia group were more likely to have LBBB (p = 0.006). Patients with breathlessness had worse renal function, lower Hb and higher inflammatory markers than other presentations ($p < 0.001$ for all). Peak troponin was highest in the chest pain group and lowest in those presenting with breathlessness (p = 0.02). Mean LVEF was higher in the chest pain group compared to breathlessness and arrhythmia patients (53 ± 9 vs. 40 ± 13 vs. 45 ± 12%, respectively, $p < 0.001$). A total of 78% of patients presenting with breathlessness had LVSD, and more often had moderate or severe LVSD compared to those with chest pain or arrhythmias at presentation (65 vs. 17 vs. 38%, respectively, $p < 0.001$).

In-hospital management was significantly different between groups. Less than one fifth of patients admitted with breathlessness were managed on a cardiology ward compared to three quarters for other presentations (17% for breathlessness vs. 76% for chest pain vs. 71% for arrhythmia, $p < 0.001$). With the exception of IVIG, advanced therapies were more commonly used in breathlessness presentations (inotropes $p < 0.001$, steroids $p < 0.001$, RRT p = 0.01). Furthermore, patients with a breathlessness presentation were more likely to be treated with RAAS inhibitors (p = 0.042) as well as mineralocorticoid receptor antagonists (MRA, $p < 0.001$), diuretics ($p < 0.001$) and/or immunosuppression ($p < 0.001$). Arrhythmia patients were more likely to be treated with beta blockers ($p < 0.001$) or statins (p = 0.003), while patients presenting with chest pain were more likely to be treated with colchicine ($p < 0.001$).

Ethnicity differences in acute myocarditis

Ethnic differences among patients presenting with AM are presented in **Table 3**. No differences in age, sex, clinical presentation, comorbidities, or baseline treatment were evident. Compared to White patients, Black and Minority Ethnic (BAME) patients had more repolarisation abnormalities on ECG (47 vs. 32%, p = 0.04) and a more pronounced leucocytosis (neutrophils 10.3 ± 4.2 vs. $9.2 \pm 4.1 \times 10^9$/L, p = 0.025; lymphocytes 7.8 ± 4.1 vs. $6.7 \pm 4.0 \times 10^9$/L, p = 0.018). BAME and White patients had similar rates of moderate or severe left ventricular (LV) systolic dysfunction, measured with echo (23 vs. 27%, p = 0.47), and no differences on CMR. Inpatient and discharge medications were also indistinct between groups.

Sex differences in acute myocarditis

Baseline characteristics according to sex are summarised in **Table 4**. Compared to men, women were older (44.5 ± 18.1 vs. 36.4 ± 14.6, p = 0.003), while ethnicity was equally distributed. Women were also more likely to have a prior autoimmune disorder ($p < 0.001$), hypertension (p = 0.002) and renal insufficiency (eGFR < 60 mL/min/m^2, p = 0.023). More women were on aspirin and/or immunosuppression at the time of presentation (p = 0.035 and <0.001, respectively).

Women were more likely to present with breathlessness compared to men (p = 0.001), and were also more tachycardic at admission (89 ± 24 vs. 80 ± 22 bpm, p = 0.003). Men were more likely to have a chest pain presentation along with ST elevation ($p < 0.001$ for both).

Women with AM had worse renal function ($p < 0.001$ for eGFR), lower Hb ($p < 0.001$) and higher NT-proBNP (p = 0.004) than men. However, men had significantly higher peak troponin ($p < 0.001$). Inflammatory markers were similar between the groups. Mean LVEF was similar between the groups using both echo and CMR, but both modalities indicated more LV dilatation in men ($p < 0.001$ for echo).

In-hospital management was significantly different between groups with higher use of steroids, inotropes and RRT in women ($p < 0.001$ for all). Immunosuppression was also more commonly prescribed for women at discharge ($p < 0.001$), but other treatments were similar.

TABLE 2 Characteristics of study patients according to clinical presentation.

Baseline characteristics		Chest pain	Breathlessness	Arrhythmia	*P*-value
	n patients	156 (78%)	25 (13%)	18 (9%)	–
	Male sex, *n* (%)	114 (73%)	5 (20%)	11 (61%)	<0.001
	Age at admission, years	35.8 ± 14.5	51.0 ± 18.8	52.6 ± 14.0	<0.001
Ethnicity	White, *n* (%)	99 (52%)	76 (50%)	14 (58%)	0.983
	Black, African, Caribbean, or Black British, *n* (%)	61 (32%)	49 (32%)	7 (29%)	
	Asian, *n* (%)	8 (4.2%)	6 (4.0%)	1 (4.2%)	
	Other, *n* (%)	24 (12%)	20 (13%)	2 (8.3%)	
History	Any prodromal symptoms, *n* (%)	68 (44%)	8 (32%)	4 (22%)	0.129
	Flu-like symptoms, *n* (%)	45 (29%)	3 (12%)	3 (17%)	0.136
Co-morbidities	Autoimmune disorders, *n* (%)	15 (9.7%)	6 (24%)	3 (17%)	0.092
	Previous myocarditis, *n* (%)	5 (3.2%)	1 (4.0%)	1 (5.6%)	0.622
	Hypertension, *n* (%)	15 (9.7%)	7 (28%)	4 (22%)	0.018
	Dyslipidaemia, *n* (%)	7 (4.5%)	1 (4.0%)	3 (17%)	0.120
	Diabetes, *n* (%)	7 (4.5%)	3 (12%)	2 (11%)	0.130
	CKD, *n* (%)	3 (1.9%)	4 (16%)	1 (5.6%)	0.007
	PAD, *n* (%)	1 (0.6%)	0 (0%)	0 (0%)	>0.999
	Previous MI, *n* (%)	1 (0.6%)	0 (0%)	0 (0%)	>0.999
	Alcohol, *n* (%)	1 (0.6%)	0 (0%)	3 (17%)	0.004
Baseline medication	RAASi, *n* (%)	11 (7.4%)	1 (4.0%)	2 (11%)	0.777
	Beta blocker, *n* (%)	9 (6.0%)	3 (12%)	3 (17%)	0.129
	MRA, *n* (%)	0 (0%)	0 (0%)	0 (0%)	–
	Diuretics, *n* (%)	2 (1.3%)	2 (8.0%)	1 (5.6%)	0.075
	Immunosuppressant, *n* (%)	9 (6.0%)	7 (28%)	3 (17%)	0.002
	Statin, *n* (%)	11 (7.4%)	2 (8.0%)	6 (33%)	0.007
	Aspirin, *n* (%)	11 (7.4%)	2 (8.0%)	4 (22%)	0.118
Admission observations	Temperature, °C	37.04 ± 0.82	37.13 ± 0.86	36.90 ± 0.45	0.850
	Fever > 37.5, *n* (%)	28 (23%)	7 (37%)	1 (7.1%)	0.152
	Systolic blood pressure, mmHg	121 ± 16	124 ± 31	117 ± 18	0.576
	Heart rate, bpm	80 ± 17	100 ± 26	84 ± 44	<0.001
Presenting ECG	Normal, *n* (%)	39 (26%)	5 (23%)	6 (33%)	0.763
	Sinus rhythm, *n* (%)	141 (95%)	20 (91%)	12 (67%)	0.001
	ST elevation, *n* (%)	45 (31%)	3 (14%)	0 (0%)	0.004
	Other repolarisation abnormalities, *n* (%)	54 (37%)	10 (45%)	8 (44%)	0.65
	LBBB, *n* (%)	4 (3%)	1 (5%)	4 (25%)	0.006
	QRS duration, ms	94 ± 15	91 ± 19	113 ± 32	0.017
	QT_c	389 ± 47	413 ± 71	432 ± 57	0.005
	Q waves	7 (5.1%)	4 (18%)	1 (7.1%)	0.070
Bloods	Creatinine, umol/L	90 ± 68	127 ± 101	121 ± 91	0.084
	eGFR, ml/min/m^2	81 ± 18	60 ± 30	64 ± 25	<0.001
	Urea, mmol/L	5.3 ± 3.8	11.8 ± 12.1	7.4 ± 4.5	<0.001
	Sodium, mEq/L	138.2 ± 2.8	136.1 ± 5.9	137.3 ± 9.0	0.029
	Potassium, mEq/L	4.23 ± 0.48	4.70 ± 0.82	4.58 ± 0.86	0.006
	Haemoglobin, g/dl	138 ± 17	117 ± 27	136 ± 29	<0.001
	CRP, U/L	60 ± 81	114 ± 100	41 ± 82	<0.001
	White cell count, 10^9/L	119 (82%)	21 (88%)	8 (44%)	0.002
	Neutrophils, 10^9/L	9.2 ± 3.6	12.4 ± 6.4	11.8 ± 4.9	0.007
	Lymphocytes, 10^9/L	6.7 ± 3.4	10.2 ± 6.3	8.7 ± 4.9	0.014
	Monocytes, 10^9/L	1.72 ± 0.68	1.35 ± 0.97	2.32 ± 1.49	0.006

(Continued)

TABLE 2 (Continued)

Baseline characteristics		Chest pain	Breathlessness	Arrhythmia	*P*-value
	Basophils, 10^9/L	0.61 ± 0.29	0.56 ± 0.35	0.65 ± 0.30	0.568
	Eosinophils, 10^9/L	0.04 ± 0.04	0.05 ± 0.07	0.04 ± 0.04	0.501
	ESR, mm/h	0.12 ± 0.12	0.16 ± 0.38	0.09 ± 0.14	0.042
	TSH, mIU/L	36 ± 37	36 ± 32	51 ± 57	0.789
	Free T4, pmol/L	2.22 ± 2.62	2.76 ± 2.91	1.99 ± 1.24	0.820
	Peak troponin, ×ULN	858 ± 1,702	301 ± 491	694 ± 1,280	0.020
	NT-proBNP, pg/ml	471 ± 2,936	3,897 ± 10,726	3,162 ± 9,000	0.277
Echo	LVEDD, mm	47.8 ± 5.7	50.0 ± 8.3	52.7 ± 6.9	0.020
	LVEDV, ml	108 ± 30	119 ± 52	130 ± 36	0.074
	IVSDd, mm	10.10 ± 2.40	9.91 ± 2.71	9.39 ± 2.44	0.449
	No LVSD, *n* (%)	95 (72%)	5 (22%)	8 (50%)	<0.001
	Mild LVSD, *n* (%)	15 (11%)	3 (13%)	2 (12%)	
	Moderate LVSD, *n* (%)	14 (11%)	6 (26%)	3 (19%)	
	Severe LVSD, *n* (%)	8 (6.1%)	9 (39%)	3 (19%)	
	LVEF, %	53 ± 9	40 ± 13	45 ± 12	<0.001
CMR	LVEDV indexed, ml/m^2	86 ± 15	80 ± 24	98 ± 31	0.423
	LV mass indexed, g/m^2	57 ± 15	59 ± 13	70 ± 20	0.166
	LVEF, %	58 ± 9	51 ± 15	55 ± 9	0.017
	RVEDV indexed, ml/m^2	88 ± 18	70 ± 26	143 ± 100	0.124
	RVEF, %	56 ± 8	61 ± 8	55 ± 4	0.213
In-hospital management					
Place of care	Cardiology, *n* (%)	116 (76%)	4 (17%)	12 (71%)	<0.001
	General medicine, *n* (%)	33 (22%)	10 (42%)	3 (18%)	
	Other wards, *n* (%)	4 (2.6%)	10 (42%)	2 (12%)	
Advanced therapies	Inotropes, *n* (%)	8 (5.3%)	8 (33%)	2 (11%)	<0.001
	Intravenous immunoglobulin, *n* (%)	2 (1.3%)	0 (0%)	0 (0%)	>0.999
	Steroids, *n* (%)	6 (4.0%)	7 (29%)	1 (5.6%)	<0.001
	Renal replacement therapy, *n* (%)	6 (4.0%)	5 (21%)	1 (5.6%)	0.010
Discharge medication	Aspirin, *n* (%)	58 (38%)	8 (32%)	6 (33%)	0.779
	Colchicine, *n* (%)	40 (26%)	1 (4.0%)	0 (0%)	<0.001
	RAASi, *n* (%)	66 (44%)	17 (68%)	11 (61%)	0.042
	Beta blocker, *n* (%)	65 (43%)	18 (72%)	15 (83%)	<0.001
	MRA, *n* (%)	4 (2.7%)	6 (24%)	1 (5.6%)	<0.001
	Diuretics, *n* (%)	10 (6.6%)	9 (36%)	2 (11%)	<0.001
	Statin	18 (12%)	5 (21%)	8 (44%)	0.003
	Amiodarone, *n* (%)	0 (0%)	2 (8.0%)	1 (5.6%)	0.010
	Immunosuppressant, *n* (%)	14 (9.3%)	11 (44%)	3 (17%)	<0.001

CKD, chronic kidney disease; PAD, peripheral arterial disease; MI, myocardial infarction; RAASi, renin angiotensin aldosterone system inhibitors; MRA, mineralocorticoid receptor antagonist; LBBB, left bundle branch block; eGFR, estimate glomerular filtration rate; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TSH, thyroid stimulating hormone; ULN, upper limit of normal; NT-proBNP, N-terminal pro-brain natriuretic peptide; LVEDD, left ventricular end-diastolic dimension; LVEDV, left ventricular end diastolic volume; IVSDd, Interventricular septal diameter in diastole; LVSD, left ventricular systolic dysfunction; LVEF, left ventricular ejection fraction; LV, left ventricle; RVEDV, right ventricular end diastolic volume; RVEF, right ventricular ejection fraction.

Outcomes

Over a median follow-up of 53 (IQR 34–76) months, 11 patients (6%) experienced the primary outcome. Of those, 10 died (5 in the chest pain group, 2 in the breathlessness group and 3 in the arrhythmia group) and 1 patient experienced a successful appropriate ICD shock for monomorphic VT. No patients had resuscitated cardiac arrest.

Overall, 3-year, event-free survival was 96%. Patients in the chest pain group had a more favourable prognosis compared to those presenting with breathlessness or arrhythmia (**Figure 1**, 3-year event-free survival 98% in the chest pain group, 96% in the breathlessness group, and 89% in the arrhythmia group,

TABLE 3 Characteristics of study patients according to ethnicity.

Baseline characteristics		BAME	White	*P*-value
	n patients	93	99	–
	Male sex, *n* (%)	65 (70%)	59 (60%)	0.136
	Age at admission, years	38.7 ± 14.6	39.2 ± 17.8	0.742
History	Any prodromal symptoms, *n* (%)	43 (47%)	34 (35%)	0.091
	Flu-like symptoms, *n* (%)	26 (28%)	23 (23%)	0.451
Clinical presentation	Chest pain	66 (71%)	78 (79%)	0.293
	Breathlessness	19 (20%)	12 (12%)	
	Arrhythmia	8 (8.6%)	9 (9.1%)	
Co-morbidities	Autoimmune disorders, *n* (%)	10 (11%)	14 (14%)	0.479
	Previous myocarditis, *n* (%)	4 (4.3%)	3 (3.1%)	0.714
	Hypertension, *n* (%)	11 (12%)	13 (13%)	0.786
	Dyslipidaemia, *n* (%)	7 (7.6%)	4 (4.1%)	0.298
	Diabetes, *n* (%)	6 (6.5%)	6 (6.1%)	0.910
	CKD, *n* (%)	4 (4.3%)	3 (3.1%)	0.714
	PAD, *n* (%)	1 (1.1%)	0 (0%)	0.484
	Previous MI, *n* (%)	1 (1.1%)	0 (0%)	0.484
	Alcohol, *n* (%)	2 (2.2%)	2 (2.0%)	>0.999
Baseline medication	RAASi, *n* (%)	6 (6.7%)	7 (7.3%)	0.867
	Beta blocker, *n* (%)	7 (7.8%)	8 (8.3%)	0.889
	MRA, *n* (%)	0 (0%)	0 (0%)	–
	Diuretics, *n* (%)	0 (0%)	5 (5.2%)	0.060
	Immunosuppressant, *n* (%)	8 (8.9%)	11 (11%)	0.563
	Statin, *n* (%)	9 (10%)	9 (9.4%)	0.885
	Aspirin, *n* (%)	8 (8.9%)	9 (9.4%)	0.908
Admission observations	Temperature, °C	37.16 ± 0.87	36.91 ± 0.69	0.067
	Fever > 37.5, *n* (%)	22 (27%)	12 (17%)	0.130
	Systolic blood pressure, mmHg	120 ± 20	122 ± 18	0.225
	Heart rate, bpm	86 ± 26	80 ± 19	0.071
Presenting ECG	Normal, *n* (%)	22 (24%)	25 (27%)	0.706
	Sinus rhythm, *n* (%)	83 (93%)	84 (90%)	0.472
	ST elevation, *n* (%)	21 (24%)	26 (28%)	0.474
	Other repolarisation abnormalities, *n* (%)	42 (47%)	29 (32%)	0.04
	LBBB, *n* (%)	3 (4%)	6 (7%)	0.49
	QRS duration, ms	94 ± 17	96 ± 20	0.764
	QT_c	392 ± 54	399 ± 52	0.450
	Q waves	7 (8.8%)	5 (5.7%)	0.453
Bloods	Creatinine, mg/dl	107 ± 93	89 ± 56	0.059
	eGFR, ml/min/m^2	76 ± 22	78 ± 21	0.072
	Urea, mmol/L	5.9 ± 6.2	6.8 ± 5.9	0.004
	Sodium, mEq/L	137.9 ± 3.6	137.8 ± 4.8	0.834
	Potassium, mEq/L	4.29 ± 0.56	4.35 ± 0.59	0.565
	Haemoglobin, g/dl	133 ± 20	138 ± 22	0.053
	CRP, U/L	67 ± 87	57 ± 72	>0.999
	White cell count, 10^9/L	66 (73%)	76 (84%)	0.051
	Neutrophils, 10^9/L	9.2 ± 4.1	10.3 ± 4.2	0.025
	Lymphocytes, 10^9/L	6.7 ± 4.0	7.8 ± 4.1	0.018
	Monocytes, 10^9/L	1.80 ± 0.86	1.72 ± 0.83	0.427
	Basophils, 10^9/L	0.57 ± 0.33	0.65 ± 0.25	0.001

(Continued)

TABLE 3 (Continued)

Baseline characteristics		BAME	White	*P*-value
	Eosinophils, 10^9/L	0.04 ± 0.05	0.05 ± 0.04	0.189
	ESR, mm/h	0.13 ± 0.13	0.12 ± 0.22	0.140
	TSH, mIU/L	41 ± 39	34 ± 38	0.390
	Free T4, pmol/L	2.12 ± 1.88	2.42 ± 3.00	0.820
	Peak troponin, ×ULN	916 ± 2,087	643 ± 898	0.351
	NT-proBNP, pg/ml	863 ± 4,471	1,691 ± 6,780	0.263
Echo	LVEDD, mm	47.2 ± 6.4	49.7 ± 6.2	0.018
	LVEDV, ml	107 ± 33	116 ± 37	0.231
	IVSDd, mm	10.46 ± 2.62	9.53 ± 2.17	0.016
	No LVSD, *n* (%)	52 (62%)	53 (66%)	0.348
	Mild LVSD, *n* (%)	9 (11%)	9 (11%)	
	Moderate LVSD, *n* (%)	15 (18%)	7 (8.8%)	
	Severe LVSD, *n* (%)	8 (9.5%)	11 (14%)	
	LVEF, %	51 ± 10	51 ± 12	0.707
CMR	LVEDV indexed, ml/m^2	84 ± 18	88 ± 17	0.132
	LV mass indexed, g/m^2	60 ± 18	56 ± 12	0.557
	LVEF, %	58 ± 9	57 ± 11	0.341
	RVEDV indexed, ml/m^2	86 ± 20	90 ± 32	0.468
	RVEF, %	57 ± 9	56 ± 6	0.546
In-hospital management				
Place of care	Cardiology, *n* (%)	60 (67%)	68 (70%)	0.256
	General medicine, *n* (%)	25 (28%)	19 (20%)	
	Other wards, *n* (%)	5 (5.6%)	10 (10%)	
Advanced therapies	Inotropes, *n* (%)	9 (9.9%)	9 (9.5%)	0.924
	Intravenous immunoglobulin, *n* (%)	1 (1.1%)	1 (1.1%)	>0.999
	Steroids, *n* (%)	4 (4.4%)	9 (9.5%)	0.175
	Renal replacement therapy, *n* (%)	5 (5.5%)	7 (7.4%)	0.603
Discharge medication	Aspirin, *n* (%)	32 (35%)	38 (39%)	0.570
	Colchicine, *n* (%)	21 (23%)	20 (21%)	0.683
	RAASi, *n* (%)	42 (46%)	49 (51%)	0.550
	Beta blocker, *n* (%)	45 (49%)	49 (51%)	0.884
	MRA, *n* (%)	4 (4.4%)	7 (7.2%)	0.421
	Diuretics, *n* (%)	10 (11%)	11 (11%)	0.939
	Statin	12 (13%)	17 (18%)	0.393
	Amiodarone, *n* (%)	0 (0%)	3 (3.1%)	0.247
	Immunosuppressant, *n* (%)	12 (13%)	16 (16%)	0.524

BAME, Black and Minority Ethnic group; CKD, chronic kidney disease; PAD, peripheral arterial disease; MI, myocardial infarction; RAASi, renin angiotensin aldosterone system inhibitors; MRA, mineralocorticoid receptor antagonist; LBBB, left bundle branch block; eGFR, estimate glomerular filtration rate; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TSH, thyroid stimulating hormone; ULN, upper limit of normal; NT-proBNP, N-terminal pro-brain natriuretic peptide; LVEDD, left ventricular end-diastolic dimension; LVEDV, left ventricular end diastolic volume; IVSDd, Interventricular septal diameter in diastole; LVSD, left ventricular systolic dysfunction; LVEF, left ventricular ejection fraction; LV, left ventricle; RVEDV, right ventricular end diastolic volume; RVEF, right ventricular ejection fraction.

$p = 0.003$). Outcomes were similar between ethnicities (**Figure 2**, 3-year event-free survival 97% in White patients vs. 96% in BAME patients, $p = 0.6$) and sexes (**Figure 3**, 3-year event-free survival 96% in females vs. 97% in males, $p = 0.82$).

In univariable analyses, the variables most strongly associated with the primary outcome were increasing age ($p = 0.003$), arrhythmia clinical presentations ($p = 0.004$), comorbidities including hypertension, dyslipidaemia and diabetes (all $p < 0.001$), sodium ($p = 0.003$), and LV septal wall thickness ($p = 0.045$) (**Table 5**). Compared to chest pain presentations, arrhythmia presentations were associated with a nearly sevenfold risk of the primary outcome [hazard ratio (HR) 6.97; 95% confidence interval (CI) 1.87–26.00, $p = 0.004$], whereas breathlessness presentations were not significantly associated with outcome. Sex and ethnicity were not associated with the outcome.

TABLE 4 Characteristics of study patients according to sex.

Baseline characteristics		Female	Male	*P*-value
	n patients	69	130	–
	Age at admission, years	44.5 ± 18.1	36.4 ± 14.6	0.003
Ethnicity	White, *n* (%)	40 (59%)	59 (48%)	0.276
	Black, African, Caribbean, or Black British, *n* (%)	17 (25%)	44 (35%)	
	Asian, *n* (%)	4 (5.9%)	4 (3.2%)	
	Other, *n* (%)	7 (10%)	17 (14%)	
History	Any prodromal symptoms, *n* (%)	23 (33%)	57 (45%)	0.127
	Flu-like symptoms, *n* (%)	14 (20%)	37 (29%)	0.188
Clinical presentation	Chest pain	43 (62%)	106 (82%)	0.001
	Breathlessness	20 (29%)	12 (9.2%)	
	Arrhythmia	6 (8.7%)	12 (9.2%)	
Co-morbidities	Autoimmune disorders, *n* (%)	17 (25%)	7 (5.5%)	<0.001
	Previous myocarditis, *n* (%)	2 (2.9%)	5 (3.9%)	>0.999
	Hypertension, *n* (%)	16 (23%)	10 (7.8%)	0.002
	Dyslipidaemia, *n* (%)	5 (7.2%)	6 (4.7%)	0.521
	Diabetes, *n* (%)	7 (10%)	5 (3.9%)	0.116
	CKD, *n* (%)	6 (8.7%)	2 (1.6%)	0.023
	PAD, *n* (%)	1 (1.4%)	0 (0%)	0.350
	Previous MI, *n* (%)	0 (0%)	1 (0.8%)	>0.999
	Alcohol, *n* (%)	0 (0%)	4 (3.1%)	0.300
Baseline medication	RAASi, *n* (%)	8 (12%)	6 (4.8%)	0.088
	Beta blocker, *n* (%)	9 (13%)	6 (4.8%)	0.038
	MRA, *n* (%)	0 (0%)	0 (0%)	–
	Diuretics, *n* (%)	3 (4.4%)	2 (1.6%)	0.348
	Immunosuppressant, *n* (%)	14 (21%)	5 (4.0%)	<0.001
	Statin, *n* (%)	9 (13%)	10 (8.1%)	0.251
	Aspirin, *n* (%)	10 (15%)	7 (5.6%)	0.035
Admission observations	Temperature, °C	37.16 ± 0.84	36.97 ± 0.76	0.132
	Fever > 37.5, *n* (%)	16 (28%)	20 (20%)	0.247
	Systolic blood pressure, mmHg	124 ± 25	119 ± 14	0.376
	Heart rate, bpm	89 ± 24	80 ± 22	0.003
Presenting ECG	Normal, *n* (%)	19 (30%)	31 (25%)	0.471
	Sinus rhythm, *n* (%)	62 (97%)	111 (90%)	0.078
	ST elevation, *n* (%)	5 (7.8%)	43 (35%)	<0.001
	Other repolarisation abnormalities, *n* (%)	29 (46%)	43 (35%)	0.14
	LBBB, *n* (%)	5 (8%)	4 (4%)	0.28
	QRS duration, ms	95 ± 22	96 ± 16	0.067
	QT_c	409 ± 62	388 ± 45	0.006
	Q waves	7 (11%)	5 (4.5%)	0.120
Bloods	Creatinine, mg/dl	106 ± 96	93 ± 62	0.019
	eGFR, ml/min/m^2	69 ± 27	81 ± 17	<0.001
	Urea, mmol/L	8.0 ± 8.6	5.4 ± 3.7	0.023
	Sodium, mEq/L	137.2 ± 4.8	138.2 ± 3.8	0.008
	Potassium, mEq/L	4.34 ± 0.65	4.31 ± 0.57	0.756
	Haemoglobin, g/dl	123 ± 22	142 ± 18	<0.001
	CRP, U/L	70 ± 92	63 ± 82	0.508
	White cell count, 10^9/L	10.8 ± 4.8	9.3 ± 3.9	0.017
	Neutrophils, 10^9/L	8.5 ± 4.7	6.7 ± 3.7	0.008

(Continued)

TABLE 4 (Continued)

Baseline characteristics		Female	Male	*P*-value
	Lymphocytes, 10^9/L	1.63 ± 0.82	1.78 ± 0.86	0.207
	Monocytes, 10^9/L	0.56 ± 0.28	0.63 ± 0.30	0.075
	Basophils, 10^9/L	0.04 ± 0.05	0.04 ± 0.04	0.519
	Eosinophils, 10^9/L	0.11 ± 0.24	0.12 ± 0.12	0.7
	ESR, mm/h	43 ± 39	34 ± 38	0.180
	TSH, mIU/L	2.77 ± 3.33	1.93 ± 1.59	0.414
	Free T4, pmol/L	14.70 ± 3.29	15.12 ± 2.89	0.530
	Peak troponin, ×ULN	334 ± 632	1,013 ± 1,860	<0.001
	NT-proBNP, pg/ml	3,019 ± 9,003	190 ± 758	0.004
Echo	LVEDD, mm	46.8 ± 5.7	49.5 ± 6.5	0.003
	LVEDV, ml	100 ± 29	119 ± 36	<0.001
	IVSDd, mm	9.58 ± 2.54	10.25 ± 2.36	0.029
	No LVSD, *n* (%)	37 (61%)	71 (65%)	0.392
	Mild LVSD, *n* (%)	5 (8.2%)	15 (14%)	
	Moderate LVSD, *n* (%)	9 (15%)	14 (13%)	
	Severe LVSD, *n* (%)	10 (16%)	10 (9.1%)	
	LVEF, %	49 ± 12	51 ± 10	0.574
CMR	LVEDV indexed, ml/m^2	79 ± 17	90 ± 16	0.005
	LV mass indexed, g/m^2	52 ± 16	61 ± 14	<0.001
	LVEF, %	57 ± 12	57 ± 9	0.913
	RVEDV indexed, ml/m^2	74 ± 18	96 ± 27	<0.001
	RVEF, %	58 ± 9	56 ± 6	0.083
In-hospital management				
Place of care	Cardiology, *n* (%)	32 (47%)	100 (79%)	<0.001
	General Medicine, *n* (%)	22 (32%)	24 (19%)	
	Other wards, *n* (%)	14 (21%)	2 (1.6%)	
Advanced therapies	Inotropes, *n* (%)	14 (21%)	4 (3.2%)	<0.001
	Intravenous immunoglobulin, *n* (%)	1 (1.5%)	1 (0.8%)	>0.999
	Steroids, *n* (%)	12 (18%)	2 (1.6%)	<0.001
	Renal replacement therapy, *n* (%)	9 (13%)	3 (2.3%)	<0.001
Discharge medication	Aspirin, *n* (%)	19 (28%)	53 (42%)	0.052
	Colchicine, *n* (%)	14 (21%)	27 (21%)	0.891
	RAASi, *n* (%)	30 (44%)	64 (51%)	0.375
	Beta blocker, *n* (%)	36 (53%)	62 (49%)	0.620
	MRA, *n* (%)	6 (8.8%)	5 (4.0%)	0.200
	Diuretics, *n* (%)	11 (16%)	10 (7.9%)	0.078
	Statin	12 (18%)	19 (15%)	0.658
	Amiodarone, *n* (%)	2 (2.9%)	1 (0.8%)	0.281
	Immunosuppressant, *n* (%)	20 (29%)	8 (6.3%)	<0.001

BAME, Black and Minority Ethnic group; CKD, chronic kidney disease; PAD, peripheral arterial disease; MI, myocardial infarction; RAASi, renin angiotensin aldosterone system inhibitors; MRA, mineralocorticoid receptor antagonist; LBBB, left bundle branch block; eGFR, estimate glomerular filtration rate; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TSH, thyroid stimulating hormone; ULN, upper limit of normal; NT-proBNP, N-terminal pro-brain natriuretic peptide; LVEDD, left ventricular end-diastolic dimension; LVEDV, left ventricular end diastolic volume; IVSDd, Interventricular septal diameter in diastole; LVSD, left ventricular systolic dysfunction; LVEF, left ventricular ejection fraction; LV, left ventricle; RVEDV, right ventricular end diastolic volume; RVEF, right ventricular ejection fraction.

Discussion

We report one of the largest, single-centre observational analyses of patients hospitalised with AM. We used the ESC position statement on myocarditis and 2018 Lake Louise Criteria to identify nearly 200 patients with CMR-confirmed AM. Importantly, this is the first study of AM in an ethnically diverse population and the first to examine, in detail, sex differences in CMR-proven AM. There are three main findings from our analysis. First, the overall prognosis of

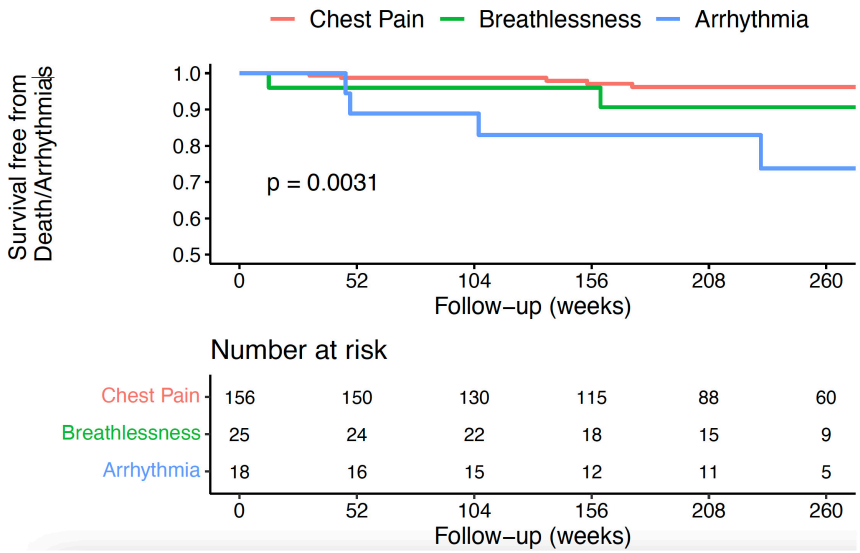

FIGURE 1
Kaplan–Meier curve for all-cause mortality, resuscitated cardiac arrest, and appropriate implantable cardioverter defibrillator (ICD) therapy following hospital discharge in patients with AM, according to clinical presentation.

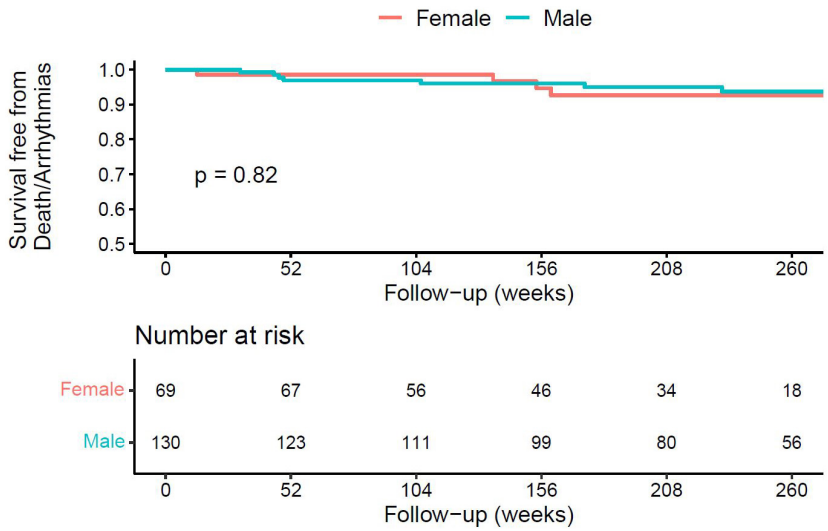

FIGURE 2
Kaplan–Meier curve for all-cause mortality, resuscitated cardiac arrest, and appropriate implantable cardioverter defibrillator (ICD) therapy following hospital discharge in patients with AM, according to ethnicity.

CMR-proven AM is benign, with a 3-year event-free survival of 96%. Furthermore, adverse outcomes were virtually exclusively confined to patients with arrhythmia presentations (11% at 3 years of follow-up). Second, there are no major differences in baseline characteristics between patients with different ethnicities. Third, while outcomes are similar for men and women with myocarditis, women have a distinct phenotype, including prior autoimmune disease, more comorbidities, and more breathlessness presentations.

The presentation of AM is highly heterogeneous. Consequently, its epidemiology is incompletely understood (5, 6, 23, 26). Historically, the diagnosis of AM relied on histology obtained from EMB. However, EMB is invasive and not universally available, significantly limiting the widespread applicability of this technique and likely underestimating the real prevalence of the disease (23). Furthermore, most data on the natural history of AM comes from highly selected, EMB-confirmed cases. With increasing availability of CMR, the

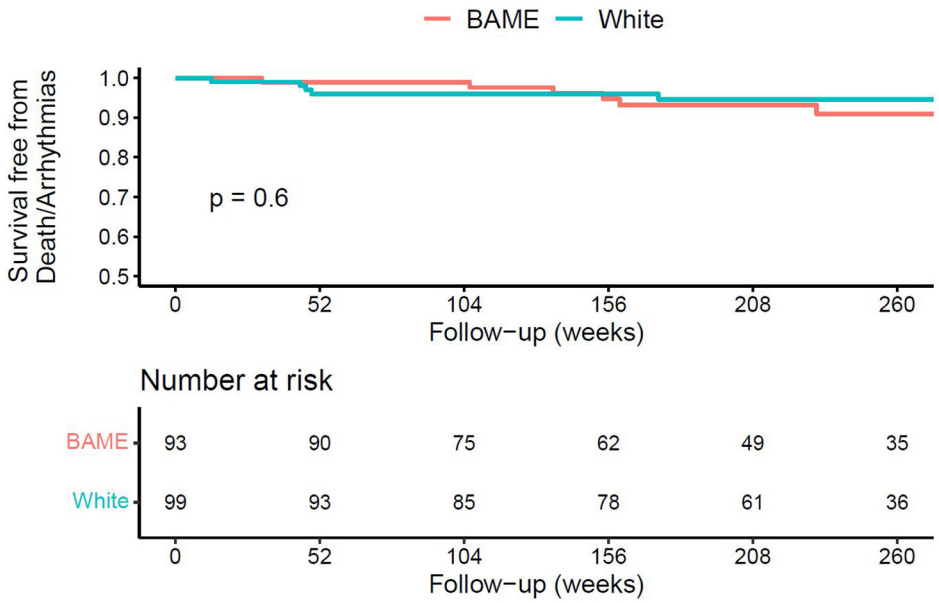

FIGURE 3
Kaplan–Meier curve for all-cause mortality, resuscitated cardiac arrest, and appropriate implantable cardioverter defibrillator (ICD) therapy following hospital discharge in patients with AM, according to sex.

diagnosis of AM has become more accessible (5, 24, 26, 27). As a result, there is increasing research interest in the use of CMR, rather than EMB, to confirm AM cases. The increased diagnostic yield achieved with CMR has allowed a more detailed and accurate characterisation of patients across the spectrum of AM presentations.

In our cohort, the most common presentation was with chest pain, accounting for three-quarters of cases, with smaller numbers presenting with breathlessness or malignant arrhythmias. These findings are very similar to those of Ammirati et al., who described the clinical course of 443 patients with AM in Italy (15). In their cohort, 73% of patients presented with uncomplicated AM, defined as AM with preserved LVEF and no significant arrhythmia, of whom 97% presented with chest pain. Furthermore, the 5-year incidence of heart transplantation or death in their study was low (5.2%) (15). They found that adverse events were virtually completely confined to patients with a breathlessness or arrhythmia presentations, and that outcomes in patients presenting with chest pain without significant LVSD were good (5-year incidence of death or heart transplantation of 18 vs. 0.3%, respectively). Our study supports these findings in a different healthcare system, time period, and diverse patient population.

Interestingly, we observed a worse prognosis in the arrhythmia compared to breathlessness group. This is contrary to a previous study that described worse clinical outcomes in patients with heart failure presentations than observed in our cohort (11). However, this study used heart transplantation in EMB-proven AM as an endpoint, which is likely to select for sicker patients and may partially account for the different findings. Furthermore, our breathlessness cohort was not limited to patients with LVSD. Our analysis provides a more heterogeneous and contemporary population, and therefore reflects the current prognosis across the spectrum of AM. Arrhythmia patients were older (53 ± 14 years) and, interestingly, less pro-inflammatory than breathless patients. Arrhythmia patients also had larger LV dimensions at presentation, which might suggest AM is a "second hit" in these patients (28, 29).

A higher incidence of AM among men is consistent across studies (4). Previous epidemiological studies have found women with AM to be older, less frequently present with ST elevation, and have no differences in LVEF or all-cause mortality (30, 31). This is consistent with our findings. Mirna et al. also observed lower presenting CRP in women. Animal models of autoimmune myocarditis have suggested attenuation of the immune response in female rats (32). In particular, female rats display preserved LVEF, higher anti-inflammatory $Arg1^{+}$ macrophages, and a lack of increase in pro-inflammatory modulators (such as TLR4, IL6, IL1β, and iNOS). However, we did not observe differences in CRP, instead finding more neutrophils despite significantly lower troponin. We also observed more autoimmune disorders and immunosuppression in women, suggesting complex interactions between AM, sex and immunity. Finally, this is the first study to specifically examine the role of ethnicity in AM. Ethnic variation has been implicated in several cardiovascular conditions, including heart failure, and response to treatment (33–35). We did

TABLE 5 Cox proportional univariable analyses evaluating the association of baseline characteristics with all-cause mortality, resuscitated cardiac arrest, and appropriate implantable cardioverter defibrillator (ICD) therapy following hospital discharge.

Variable		HR	95% CI		*P*-value
	Age at admission (per 10 years)	1.06	1.03	1.1	<0.001
	Male sex	0.87	0.25	2.96	0.82
Ethnicity	White vs. BAME	0.73	0.22	2.39	0.6
History	Any prodromal symptoms	0.14	0.02	1.11	0.06
Clinical presentation	Chest pain	Ref			
	Breathlessness	2.02	0.39	10.4	0.4
	Arrhythmia	6.97	1.87	26	0.004
Co-morbidities	Autoimmune disorders	1.57	0.34	7.25	0.57
	Hypertension	7.91	2.41	25.9	<0.001
	Dyslipidaemia	18.9	5.74	62.1	<0.001
	Diabetes	11.4	3.32	39.2	<0.001
	CKD	2.72	0.35	21.3	0.34
Admission observations	Systolic blood pressure	1	0.97	1.04	0.82
	Heart rate	1	0.98	1.03	0.67
Presenting ECG	Normal	0.28	0.04	2.16	0.22
	Sinus rhythm	0.26	0.07	0.98	0.047
	ST elevation	0.58	0.13	2.71	0.49
	LBBB	1.84	0.72	4.66	0.2
	QT_c	1	0.99	1.01	0.97
Bloods	Creatinine	1	1	1.01	0.12
	eGFR	0.98	0.96	1	0.11
	Urea	0.98	0.85	1.12	0.74
	Sodium	0.88	0.81	0.96	0.003
	Potassium	1.61	0.72	3.57	0.24
	Haemoglobin	0.98	0.96	1.01	0.16
	CRP	1	0.99	1.01	0.53
	White cell count	1	0.88	1.15	0.96
	Neutrophils	1	0.87	1.15	0.97
	TSH	0.87	0.41	1.82	0.77
	Free T4	1	0.63	1.61	0.99
	Peak troponin	1	1	1	0.63
	NT-proBNP	0.99	0.98	1.01	0.62
Echo	LVEDD	0.99	0.89	1.1	0.85
	LVEDV	1	0.98	1.02	0.89
	IVSDd	1.21	1	1.45	0.045
	No LVSD	Ref	–	–	–
	Mild LVSD	1.23	0.14	11	0.9
	Moderate LVSD	3.99	0.89	17.9	0.07
	Severe LVSD	1.37	0.15	12.3	0.8
	LVEF	0.97	0.92	1.02	0.27
CMR	LVEDV indexed	1.02	0.96	1.08	0.58
	LV mass indexed	1	0.92	1.08	0.92
	LVEF	1.07	1	1.15	0.056
	RVEDV indexed	0.99	0.93	1.05	0.72
	RVEF	1.11	0.92	1.34	0.29

BAME, Black and Minority Ethnic group; CKD, chronic kidney disease; PAD, peripheral arterial disease; MI, myocardial infarction; renin angiotensin aldosterone system inhibitors; MRA, mineralocorticoid receptor antagonist; LBBB, left bundle branch block; eGFR, estimate glomerular filtration rate; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TSH, thyroid stimulating hormone; ULN, upper limit of normal; NT-proBNP, N-terminal pro-brain natriuretic peptide; LVEDD, left ventricular end-diastolic dimension; LVEDV, left ventricular end diastolic volume; IVSDd, Interventricular septal diameter in diastole; LVSD, left ventricular systolic dysfunction; LVEF, left ventricular ejection fraction; LV, left ventricle; RVEDV, right ventricular end diastolic volume; RVEF, right ventricular ejection fraction.

not observe any major difference between White and BAME patients, and ethnicity was not associated with outcome in univariable analyses.

The use of advanced or immunosuppressive therapy in hospital was low, and mostly confined to patients presenting with breathlessness. In the total population there was limited use of inotropes (9%), IVIG (1%), steroids (7%), and/or RRT (6%). Use of these therapies was slightly lower than described elsewhere, likely reflecting differences in local practices but also reflecting the benign nature of most AM cases diagnosed using CMR. In our study, a lower proportion of patients with breathlessness were managed on cardiology wards. This was not associated with worse outcomes in our study, despite being well known that patients with acute HF have worse outcomes when managed on general medical wards (36–38).

The adverse event rate in our study is significantly lower than that seen in many previous studies. Most of these studies were performed prior to the widespread availability of CMR and used EMB to confirm AM. It is therefore likely that this predominantly reflects differences in study design and patient selection, leading to lower estimates of mortality. Most studies with ≥90% of cases confirmed by CMR indicate 100% survival with follow-up up to 5 years (14–16, 19, 20, 39, 40), with the most conservative estimate being 95.7% survival at 4.7 years of follow-up (17). This is significantly higher than EMB-selected cohorts, that have reported mortality as high as 56% at 4.3 years (41). The variables with the strongest association with adverse events were clinical presentation, and markers of organ damage or compromised haemodynamics. Most of these variables are available at initial presentation and may help identify which patients might need more advanced support or observation.

Limitations

As a retrospective study of prospectively collected data we recognise several important limitations. We attempted to capture all patients admitted with AM by performing keyword searches of electronic health records and death certificates. However, some patients with AM may have been missed, potentially leading to selection bias. We included patients admitted to two hospitals with AM. The patient populations served by these hospitals may not reflect the general population in the UK or other countries. Furthermore, clinical practice may differ from other national and international centres, which may limit the generalisability of our results. Although all cardiac follow-up that occurred in our institution was captured, it was possible that some endpoints were missed. Patients that moved abroad and subsequently died would have been missed, as would patients that had ICDs implanted at another centre. Finally, unstable patients who could not undergo CMR may have been missed. EMB was not routinely performed and post-mortem analysis was not available, so we were unable to assess aetiology. Despite these limitations, our findings are very similar to those of a comparable multicentre study in terms of clinical presentation and outcomes (15). Finally, although ours is a relatively large study by the standards of AM, our cohort was small with a low number of events. We could therefore not perform multivariable analysis and our study power was limited in identifying variables with a less strong association with adverse events. The use of surrogate endpoints in future AM studies (such as change in EF) may provide sufficient power to identify factors that are independently associated with outcome, especially in male and female subgroups. In addition, studies that examine inflammation in patients with AM would be welcomed.

Conclusion

Acute myocarditis has highly heterogeneous clinical presentations. In our analysis, two thirds of patients with myocarditis present with chest pain and we identified several differences between male and female patients. Overall, AM is associated with a benign prognosis, especially for chest pain presentations. Those presenting with life-threatening arrhythmias are at higher risk of adverse events.

Data availability statement

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

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

AC, TM, PS, and DB: design of the study. AC, PB, RR, MA-A, AD, EF, AJ, AS, JH, BC, SB, AK, MS, SR, AB, IR, SP, DS, PS, and DB: data collection. AC, PS, and DB: data analysis and writing the manuscript. MG, AMS, TM, PS, and DB: supervision. All authors critically revised the manuscript.

Funding

AC was supported by the British Heart Foundation (FS/CRTF/21/24175). AMS was supported by the British Heart

Foundation (CH/1999001/11735). DB was supported by the King's BHF Centre of Research Excellence (RE/18/2/34213). The funders played no role in study design, collection, analysis, interpretation of data, writing of the report, or in the decision to submit the manuscript for publication. They accept no responsibility for the contents.

Acknowledgments

We wish to acknowledge the CogStack team at King's College Hospital NHS Foundation NHS Trust for their support with data retrieval.

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.2022.1037837/full#supplementary-material

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 25 January 2023
DOI 10.3389/fcvm.2023.1115389

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Pediatric Cardiology, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 03 December 2022
ACCEPTED 09 January 2023
PUBLISHED 25 January 2023

CITATION
Chochkova-Bukova LA, Funken D, Bukova M, Genova KZ, Ali S, Stoencheva S, Paskaleva IN, Halil Z, Neicheva I, Shishmanova A, Kelly KS and Ivanov IS (2023) Cardiac MRI with late gadolinium enhancement shows cardiac involvement 3–6 months after severe acute COVID-19 similar to or worse than PIMS. *Front. Cardiovasc. Med.* 10:1115389. doi: 10.3389/fcvm.2023.1115389

Cardiac MRI with late gadolinium enhancement shows cardiac involvement 3–6 months after severe acute COVID-19 similar to or worse than PIMS

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Background: Coronavirus disease 2019 (COVID-19) in children is rarely severe. However, severe courses occur, especially in the presence of risk factors. A minority of children develop pediatric inflammatory multisystem syndrome (PIMS) with substantial morbidity. While the importance of cardiac involvement after PIMS is well established, its role after severe acute COVID-19 remains unclear. We aim to compare cardiac sequelae of children after severe acute COVID-19 using cardiac MRI and compare them with patients after PIMS.

Methods: For this prospective cohort study, we recruited patients with acute COVID or PIMS in a single center. Clinical follow-up, lab work, ECG, and echocardiography were done within 2 days after disease onset and 3–6 months after discharge. At the last visit 3–6 months later, cardiac MRI (CMR) with late gadolinium enhancement (LGE) was performed to evaluate cardiac sequelae and compare both groups.

Results: Data were obtained from $n = 14$ patients with PIMS and $n = 7$ patients with severe acute COVID-19. At the start of the respective disease, left ventricular (LV) ejection fraction was reduced in seven patients with PIMS but none in the acute COVID-19 group. Transient mitral valve insufficiency was present in 38% of patients, of whom PIMS accounted for 7/8 cases. Eight patients (38%) with PIMS presented coronary artery abnormalities, with normalization in 7/8 patients. A significant decrease in LV mass index 3–6 months after disease onset was observed in both groups. MRI follow-up revealed non-ischemic myocardial pattern of LGE in 12/21 patients- in all (6/6) after severe acute COVID-19 and in less than half (6/14) after PIMS. Normal body weight-adjusted stroke volumes and end-diastolic volumes were found in 20/21 patients.

Conclusions: We show that children suffering from severe acute COVID-19 have a similar, or worse, cardiac risk profile as patients with PIMS. Both patient groups should therefore receive close pediatric cardiac follow-up examinations. Cardiac MRI is the technique of choice, as most patients presented with delayed LGE as a sign of persistent cardiac injury despite normalization of laboratory and echocardiographic findings.

KEYWORDS

cardiac imaging, echocardiography, SARS-CoV2, heart involvement, Multisystem Inflammatory Syndrome in Children (MIS-C)

1. Introduction

Coronavirus disease 2019 (COVID-19) usually has mild or no symptoms in children and adolescents. However, severe courses may occur, especially in patients with pre-existing risk factors such as chronic underlying disease or obesity (1). Pediatric Inflammatory Multisystem Syndrome (PIMS), also referred to as Multisystem Inflammatory Syndrome in children (MIS-C), is a severe acute inflammatory disease affecting various organs. PIMS usually manifests 2–8 weeks after coronavirus infection in children. The condition is rare but severe and its pathophysiology is still not well understood. However, numerous reports have led to a complete characterization of its clinical features, but a misdirected and overshooting immune response is suspected as a predisposition. Data on secondary damage to the cardiovascular system varies depending on the utilized techniques, parameters, and follow-up period. Results range from complete remission to 36% measurable long-term damage (2–5).

Despite significant clinical overlap between severe acute COVID-19 and PIMS, some peculiarities in presentation patterns and organ involvement may help their differentiation. Severe respiratory symptoms are more frequently seen in severe acute COVID-19, whereas severe cardiac involvement is more common in PIMS (6). Data on secondary damage to the cardiovascular system varies depending on the utilized techniques, parameters, and follow-up period. Results range from complete remission to 36% measurable long-term damage (2, 10).

Although SARS-CoV2 infection usually has a benign course, the presence of risk factors such as pre-existing chronic disease and adiposity may imply a severe disease in pediatric patients. In the United States, up to one-third of children with COVID-19-related hospitalization were reported to have severe disease (7–9). While the importance of cardiac involvement after PIMS is well established, its magnitude after severe acute COVID-19 remains unclear. A cardiac MRI screening study in adult patients who recovered from COVID-19 suggested that up to 60% of COVID-19 survivors had developed myocarditis, regardless of disease severity (10). This is especially concerning from a pediatric point of view, as most pediatric SARS-CoV2 cases are mild or asymptomatic. However, there is increasing evidence that myocarditis may also develop after pediatric COVID-19 infection. These children are at risk of significant but unnoticed cardiac sequelae (10–12). Cardiac MRI is the reference standard for the evaluation of myocardial structure and function in the follow-up of myocarditis (13). During the acute or subacute phase, areas of LGE have been established as a sign of necrosis and fibrosis of the myocardium. Myocardial edema is usually identified and qualitatively assessed on TIRM images. The presence of a prolonged T2 relaxation time is an additional sign of increased myocardial water content. The present prospective study aims to compare children's cardiac sequelae after severe acute COVID-19 and after PIMS.

2. Materials and methods

This is a monocentric study conducted prospectively from September 2021 until February 2022 at the Department of Pediatrics and Medical Genetics, Medical University Plovdiv. Legal consent was obtained from the patient's guardians and patients older than 16 years. Analyses were conducted in accordance with the local institutional review board of Plovdiv Medical University Scientific Ethics Committee (P-2501/2022_RKNE_F6EAA81C7D).

The inclusion criteria for the study were severe acute COVID-19 infection or PIMS according to the Center for Disease Control (CDC, US) criteria for pediatric patients published in May 2020 (14). Patients with pre-existing heart conditions, sepsis, or biological treatment were excluded from this study.

Polymerase chain reaction (PCR) from nose and throat swabs and/or serology for SARS-CoV-2 was performed on all cases suspected of acute COVID-19 or PIMS. Positive cases were screened to fulfill the criteria for severe acute SARS-CoV2 infection. Children presenting with fever, clinical and biochemical inflammation features, and single or multiorgan dysfunction were screened for possible PIMS.

The PIMS group was further evaluated for fulfilling complete or incomplete Kawasaki disease (KD) criteria and for hemodynamic stability. Based on those criteria, patients were divided into 4 groups-KD/KD-like or no-KD with or without hemodynamic shock. Disease severity was assessed for all patients using the PEdiatric Logistic Organ Dysfunction score 2 (PELOD2) (15). Chest radiographs, CT scans, abdominal ultrasounds, and other investigations were performed as clinically indicated. Patients were monitored continuously and received daily ECG and echocardiography checks. After clinical stabilization, the frequency of examinations was reduced to as clinically required. Clinical laboratory tests were performed regularly. Results after admission and 3–6 months later were analyzed for this study.

2.1. Cardiac monitoring

2.1.1. ECG and echocardiography

ECGs were performed routinely. Echocardiograms included 2-dimensional, Doppler, and M-mode modalities. For this study, we compared an initial examination within the first 2 days of hospitalization with a follow-up examination 3–6 months after discharge. Global left ventricular (LV) systolic function was assessed with a 2D method (16). Left ventricular ejection fraction (LVEF) was categorized based on the Teichholtz and Simpson EF methods. Normal values for EF in children were between 56 and 78%. EF was classified as normal (EF $\geq$ 55%), slightly reduced (EF 41–55%), moderately reduced (EF 31–40%), and markedly reduced (EF $\leq$ 30%) (11). Mitral regurgitation was assessed qualitatively and semiquantitatively according to the mitral jet and its components in color Doppler mode. Mitral regurgitation was graded as mild, moderate, or severe based on the size and extent of the color-flow Doppler signal into the left atrium, left atrial and left ventricular size, respectively. Coronary artery measurements were from inner to inner edge. *Z*-scores were calculated for left ventricular end-diastolic diameter (LVEDD), interventricular septal thickness (IVS), left ventricular posterior wall thickness in diastole (LVPW) (16), coronary artery (CA) diameter (17), and left ventricular mass (18). Left ventricular mass was estimated using the Devereux equation. Left ventricular mass index (LVMI) was calculated with body surface area.

2.1.2. Cardiac MRI

MRI was performed between 3 and 6 months after disease onset using a 1,5 Tesla whole-body scanner (Siemens Magnetom Aera,

Erlangen, Germany) according to a standardized protocol. This included modules for the evaluation of cardiac morphology, function, contractility, and tissue characteristics of changes in the myocardium using T1 and T2 mapping for quantification.

Areas of myocardial edema were assessed on T2/turbo inversion recovery magnitude (TIRM) images. A signal intensity ratio of myocardium/skeletal muscle of ≥2.0 was classified as abnormal, indicating myocardial edema. The type of late gadolinium enhancement (LGE) was classified according to its location in endocardial, intramural, epicardial, or transmural type. LGE images were evaluated by phase-sensitive inversion recovery (PSIR). Values of T1 and T2 relaxation times were measured in areas with edema and/or LGE and in the unaffected myocardium. The threshold for abnormal relaxation time was >1,100 ms for T1 and >49 ms for T2 images, respectively. Normal values were determined on 20 healthy age-matched volunteers with no history of cardiovascular disease who received chest MRIs in the same center before the beginning of this study in September 2021.

The volumes and function of both ventricles were calculated using the ARGUS software (Siemens, Erlangen, Germany). Semi-automatic delineation of endocardial and epicardial contours of the myocardium of both ventricles was manually corrected for each image in end-systole and end-diastole. All volumes and masses were normalized to the body surface area of the respective patient.

2.2. Statistical analysis

Statistical analysis and visual representation of data were performed using the statistical programming language R Version 1.3.1093 (https://www.r-project.org/). Non-parametrical Mann–Whitney test was used for comparison of non-dependent groups, Wilcoxon signed-rank test was used for dependent comparisons. Statistical significance was defined at a p-value of <0.05.

3. Results

3.1. Cohort characterization

During the 6-month study period, 21 patients were recruited. The median age was of 12.0 years (5.0–18.0 years). Fourteen out of 21 patients (67%) were male. All patients were of Caucasian descent.

One-third (7/21) of the patients suffered from severe acute COVID-19, and 14 met the criteria for PIMS. Among the 14 patients classified as PIMS, 11 showed complete or incomplete criteria of Kawasaki's disease, and the other three suffered from no-KD PIMS with cardiovascular shock as major presentation. No patients with severe acute COVID-19 developed PIMS during f/u, and no patients with PIMS had a history of prior symptomatic severe SARS-CoV2 infection.

All 21 patients presented with pyrexia with a median fever duration of 8 days. The 5 other most common symptoms were respiratory (81%), gastrointestinal (62%), conjunctivitis (52%), sore throat (52%), and hepatosplenomegaly (48%) (Table 1). Five patients with PIMS had acute kidney injury.

All except one of our patients showed hypoxemia and received oxygen therapy. Two patients suffering from acute COVID-19 needed respiratory support- one on invasive ventilation and one on high-flow. Most children (18/21) received 10 L/min oxygen *via* facemask with oxygen reservoir bag. The PaO_2/FiO_2 (P/F) ratio ranged from 60 to 448, with only four patients with PIMS over the ARDS threshold of 300.

Ten patients with PIMS (8 with KD-like presentation) had cardiovascular shock and received inotropes and vasopressors. No patients with severe acute COVID-19 needed catecholamines. No

TABLE 1 Cohort characterization.

		Severe acute COVID-19	PIMS
Number of cases		$n = 7$	$n = 14$
Sex	Male	6 (86%)	8 (57%)
	Female	1 (14%)	6 (43%)
Age [median]	Years	12	12
Risk factors	Adipositas (BMI > 30)	4 (57%)	1 (7%)
	Pre-existing medical condition	1 (14%)	2 (14%)
Echocardiography	Normal	7 (100%)*	14 (100%)
	Pathologic (EF, coro, effu, MI)	0	0
Cardiac MRI	Nomal	0	7 (50%)
	Pathologic	6 (100)**	7 (50%)
ECG	Normal	6 (86%)	13 (93%)
	Pathologic	1 (14%)	1 (7%)
Clinical symptoms	Persistent fever [median duration]	100% [8 days]	100% [8 days]
	GI symptoms	1 (14%)	12 (86%)
	Rash	0	9 (64%)
	Conjunctivitis	0	11 (79%)
	Neurolog. symptoms	2 (28%)	4 (29%)
	Respiratory symptoms	7 (100%)	10 (71%)
	Sore throat	3 (43%)	8 (57%)
	Myalgia	0	4 (29)
	Swollen hands/feet	0	8 (57%)
	Lymphadenopathy	0	8 (57%)
	Kawasaki criteria complete	na	5 (36%)
	HSM	0	10 (71%)

Characteristics of 21 pediatric patients with severe acute COVID-19 or pediatric inflammatory multisystem syndrome (PIMS). BMI, body mass index. Pre-existing medical conditions were Sturge-Weber-syndrome in one patient with acute COVID-19 and two patients in the PIMS group suffering from thalassemia and asthma or B cell leukemia, respectively. Pathologic echocardiography was defined as at least one of the four criteria (1) pericardial effusion, (2) reduced ejection fraction, (3) coronary anomaly, or (4) mitral valve regurgitation. *In four patients, due to adiposity, the body surface area was >2 m^2 resulting in an inapplicability of pediatric z-scores. All values were normal according to adult criteria and therefore considered normal. MRI, magnetic resonance imaging. **No MRI data available in one patient with acute COVID-19. ECG, electrocardiogram; GI, gastrointestinal; Neurolog., neurological, consisting of headache, altered consciousness, meningoencephalitis, and encephalopathy; HSM, hepatosplenomegaly.

patients needed extracorporeal life support, and all patients were discharged alive and in an improved condition.

All patients with severe acute COVID-19 received a low PELOD 2 score of 0–1 points except one patient with 4 points. Scores in the PIMS groups were also mild, ranging from 0 to 5 points. There was no correlation between severity score and cardiac involvement (Supplementary material).

3.2. Clinical laboratory results

All patients showed elevated inflammatory markers (leukocytosis, elevated C-reactive protein, and ferritin). As expected, elevation of cardiac disease markers (troponin T, pro-BNP) was more profound in the PIMS group but normalized in all patients after 3 months (Figure 2). Laboratory results did not correlate with MRI findings or disease severity (Supplementary Figures 1, 2).

3.3. Imaging

3.3.1. Electrocardiography and echocardiography

ECG abnormalities were present in 5 (24%) and normalized in all patients after 3–6 months.

Left ventricular ejection fraction (LVEF) was reduced in seven patients with PIMS but none in the acute COVID-19 group. The reduction of LVEF resolved in all patients. Interestingly, only one patient with acute COVID-19 showed reduced LVEF during follow-up examination at 3–6 months.

The left ventricular mass index did not differ in patients with COVID-19 or PIMS at the time of disease onset. Both groups showed a significant reduction in LVMI in the follow-up examination (Supplementary Figure 3). Transient mitral valve insufficiency (MI) was present in 8 (38%) of patients, 7 of them with PIMS. While most patients with PIMS recovered, we found newly developed mild MI during follow-up in 2 patients, one in each group.

Eight patients (38%) with PIMS presented coronary artery dilatation, with normalization in 7/8 patients. One patient with PIMS showed persistent coronary dilatation after 6 months of follow-up.

3.3.2. Cardiac MRI

Cardiac MRI was obtained in all but one severe acute COVID-19 patient who aborted the examination due to a panic attack. A non-ischemic myocardial pattern of late gadolinium enhancement (LGE) was seen in 12 of 20 patients (Figure 1). All severe acute COVID-19 patients (6/6) were affected compared to only 43% (6/14) of PIMS patients (Figure 2A). There was no difference in the type of myocardial involvement between both groups (Figure 1). The left ventricle free wall was most involved with a distribution pattern starting at the epicardial surface and progressing to intramural zone. All areas (basal, mid, and apical) were affected with varying locations and grades.

Additionally, in 6 patients, limited LGE in the intraventricular septum was seen in basal and midseptum areas. This was observed in four children after severe COVID-19 and two with PIMS without significant differences between the two groups. The overall amount of affected areas in all patients was <25% of the total myocardium (Figure 3). The distribution of LGE zones and, correspondingly, of prolonged T1 relaxation times revealed a non-ischemic inflammatory damage pattern. T1 relaxation times tended to be longer in patients with acute COVID-19 but did not differ significantly from patients with PIMS (Figure 2C). LGE could not be attributed to a certain subgroup of patients with PIMS as LGE was found in 2/3 patients with no KD-like PIMS and in 4/11 of KD-like PIMS (Figure 2B). The same was true for the 8 patients with coronary dilatation, where 4/8 showed LGE, data not shown.

The MRI data showed no differences in body weight-adjusted stroke volumes. End-diastolic volumes were all normal. Decreased cardiac contractility and EF were seen in only one severe acute COVID-19 patient. A decreased ejection fraction was found in one PIMS patient. Residual non-extensive edema was observed in one patient, accompanied by prolonged T2 relaxation time.

3.4. Therapy

All patients suffering from acute COVID-19 received corticosteroids [dexamethasone, mean dose 0.4 mg/kg/days (0.2–0.8 mg/kg/days)] or methylprednisolone [mean dose 1 mg/kg/days (0.9–2.0 mg/kg/days)], for a mean of 15 days, low molecular weight heparin (dose 1 mg/kg/days), and an antiplatelet agent. The patient who was put on mechanical ventilation also received treatment with remdesevir. All patients with PIMS were treated additionally with intravenous immunoglobulin 1–2 g/kg over 24 h after an average of 3 days after disease manifestation. In 2 patients, intravenous immunoglobulin was administered over 2–3 days due to severe circulatory shock.

4. Discussion

The evaluation of cardiac sequelae after severe acute COVID-19 in childhood and comparison with the better-characterized PIMS patients will help assess these patients' risk better and may improve recommendations for follow-up and overall outcome.

4.1. Late gadolinium enhancement persists in all patients after severe acute COVID-19 infection

In this study, we assessed the cardiac disease severity in patients with acute severe COVID-19 or PIMS to identify their risk profile and compare cardiac sequelae. Through a combination of cardiac MRI with readily available point-of-care examinations, such as ECG and echocardiogram, we found a significant but underreported risk for secondary cardiac damage in pediatric patients after acute severe SARS-CoV2 infection. This effect was mostly evident in MRI examination in the form of a non-ischemic pattern of LGE. This type of myocardial injury was reported in 30% of 47 adult patients with COVID-19 (19).

In most of our patients, a patchy mid-wall and sometimes subepicardial LGE was found. This distribution pattern is typically seen during or after myocarditis (20). In COVID-19 patients with MISC or isolated heart involvement, an identical MRI image with this myocarditis type is seen in all degrees of myocardial involvement (21).

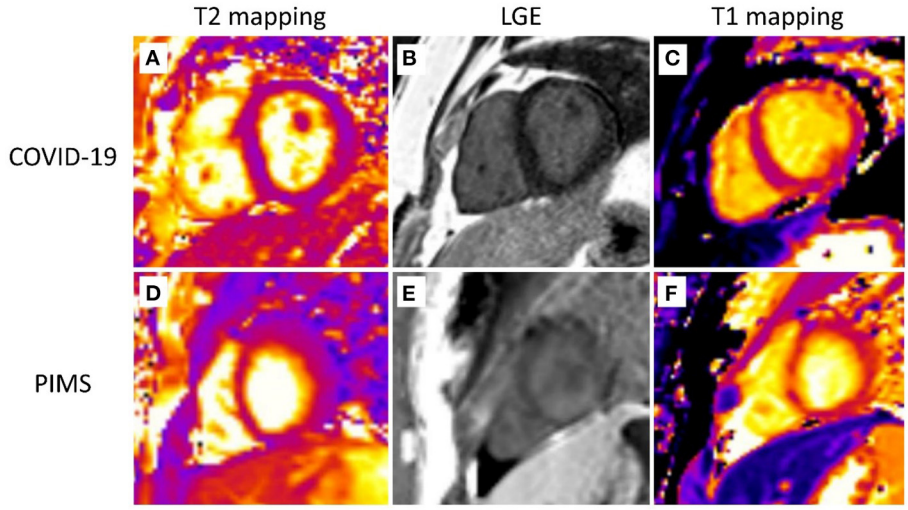

FIGURE 1
Cardiovascular magnetic resonance. Representative cardiovascular magnetic resonance images are shown. The first row depicts a 15-year-old patient after severe COVID-19 infection. T2 mapping **(A)**, LGE **(B)**, and T1 mapping **(C)** images display subepicardial and intramural LGE and prolonged T1 relaxation (up to 1,200 ms) along the anterior and anterolateral free wall of the LV with no signs of myocardial edema (normal T2 relaxation time-—45–47 ms). The second row shows images of an 11-year-old patient with pediatric multiorgan inflammatory syndrome (PIMS). T2 mapping **(D)**, LGE **(E)**, and T1 mapping **(F)** images display similar changes (subepicardial and intramural LGE and prolonged T1 relaxation up to 1,180 ms with normal T2 relaxation time) with a slightly different distribution—along the lateral and posterolateral free wall of the LV.

Edema in the myocardium can be observed in myocarditis up to 6 months after the disease, depending on the severity of myocardial involvement (22).

Noteworthy, all patients (6/6) after severe acute COVID-19 were affected by LGE compared to only 35.7% (5/14) of patients with PIMS diagnosis. These data support previous studies showing that myocarditis may persist or even worsen despite normalized cardiac enzymes and inflammatory parameters (13, 23). Delayed LGE has been shown to be a good parameter for identifying this persistent disease activity despite normalizing biomarkers (23).

We also observed that at the time of pathological MRI findings, the previously elevated troponin T, pro-BNP and markers of inflammation had already normalized completely. Moreover, clinical laboratory results did not correlate with disease severity or occurrence of LGE. Two recent studies in younger adults with acute myocarditis but otherwise limited cardiovascular risk profile highlight the importance of this finding and its clinical implications (24, 25). Myocardial scarring detected by LGE persisted in 54% of the patients after 1 year (24). Moreover, it was shown that patients with mid-wall anteroseptal myocardial LGE were associated with a worse prognosis than other LGE patterns despite preserved LVEF (25). This matches our observations as all patients with acute COVID-19 showed normal LVEF but presented with LGE.

Our findings are in contrast with a recent report showing normal cardiac MRI in a cohort of 17 patients 2 months after severe acute COVID-19 or PIMS (26). In our view, these contradictory results can mainly be attributed to the use of LGE. The extent of cardiac involvement is primarily reflected through LGE changes in our data. In contrast, parameters such as ejection fraction and contractility are affected rarely and show no difference between PIMS and severe COVID-19. Our MRI study showed no difference in ventricular systolic stroke and diastolic volume. This observation exemplifies the superiority of cardiac MRI with LGE over native MRI or echocardiography for the follow-up for ongoing myocardial disease activity in COVID-19 patients (27).

4.2. Pathophysiology of myocardial disease

Most data on cardiac MRI currently available are from the subacute or convalescent phase and report high recovery rates of ventricular function (28, 38, 40) or mostly mild continuous cardiac dysfunction (29–32). Two recent studies report myocardial edema in 30–50% of patients (33); LGE was seen in 14% of patients (34). The frequently seen combination of myocardial edema and hyperemia without focal myocardial necrosis or fibrosis (36) indicates indirect myocardial injury due to severe acute inflammation rather than direct cardiomyocyte damage (37). Recent studies describe a myocarditis-type of PIMS (38, 39) that differs from the first described Kawasaki-type coronary artery disease PIMS (35, 41). Our results support these findings, as we saw a myocarditis-like distribution pattern in all patients with LGE. The decrease in LV mass in all patients further supports a long-lasting inflammatory component of cardiac involvement in SARS-CoV-2.

Interestingly, myocarditis after mRNA vaccines or PIMS demonstrates a similar pattern of myocardial injury (42, 43). This suggests that the general immune response to SARS-CoV2 may lead to a myocarditis-type inflammation, as seen in all our patients after severe acute COVID-19.

Although several studies using MRI for cardiac follow-up are currently conducted, many long-term recommendations for PIMS are being extrapolated from follow-up studies of children with

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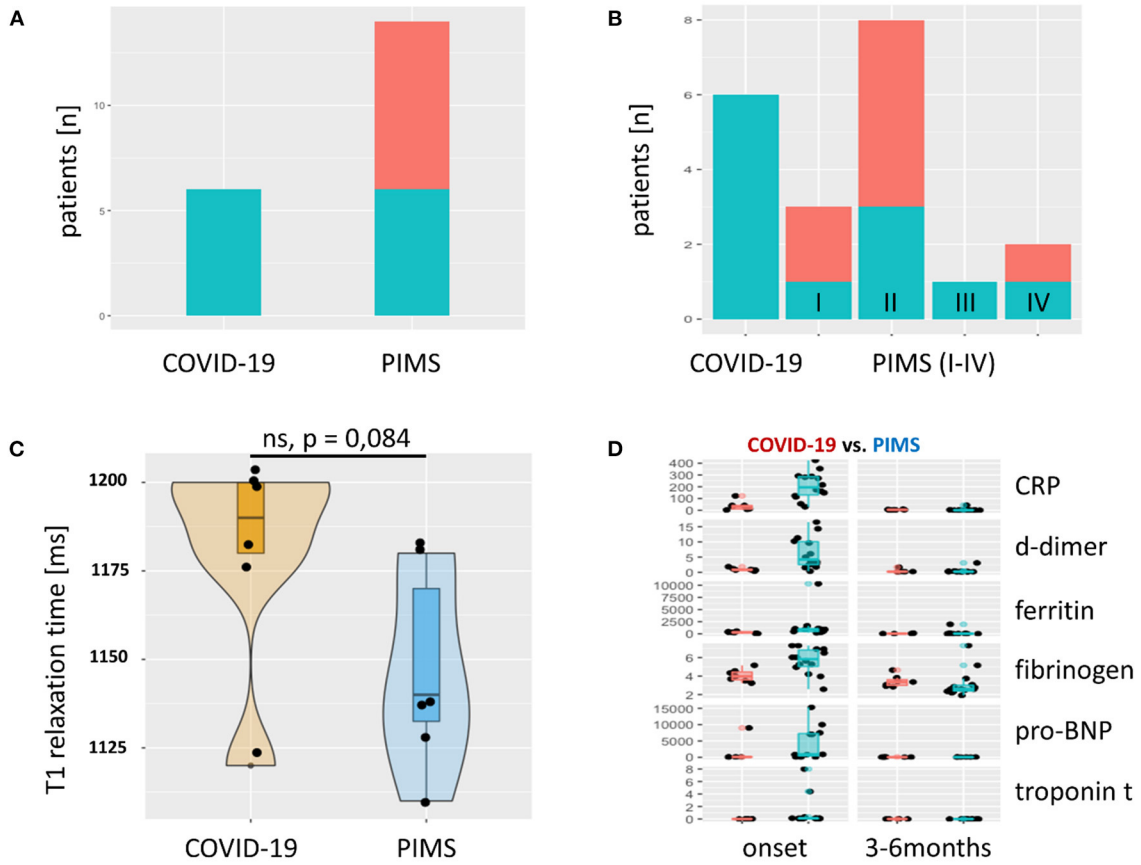

FIGURE 2
Late Gadolinium Enhancement (LGE) and T1 relaxation times and laboratory results. **(A)** The absolute frequency of LGE as bar graph. LGE (green) was found in every severe acute COVID-19 patient. In the group of patients with PIMS, we observed LGE in 6/14 (43%). Red = unaffected patients. In **(B)**, the PIMS group was divided into four subgroups: (I) Kawasaki-like without shock symptoms, (II) Kawasaki-like with shock, (III) PIMS without shock, and (IV) PIMS with shock. LGE could not be attributed to one of these four subgroups, although a trend toward group II Kawasaki with shock symptoms was seen. T1 relaxation time in areas of the myocardium with LGE is shown in **(C)** as violin plots with superimposed scatter plots. This shows a prolongation of relaxation time above the norm (<1,100 ms, determined in 20 healthy subjects) in both groups. Patients with severe acute COVID-19 showed an even more pronounced prolongation of T1 relaxation time than patients with PIMS. There was no significant difference between the two groups ($p = 0.084$). Solid line = median. Markers of inflammation and myocardial damage of patients with COVID-19 (red) and PIMS (blue) at the time of disease onset and last visit are compared in **(D)**. All markers are more elevated in PIMS group but normalize completely until the last visit after 3–6 months. CRP, C-reactive protein [mg/L]; d-dimer [mg/L fibrinogen equivalent units], ferritin [ng/L], fibrinogen [g/L], pro-BNP, pro-brain natriuretic peptide [pg/mL], troponin t [ng/mL].

Kawasaki disease and viral myocarditis. We recommend a close follow-up of all patients with PIMS or severe COVID-19. As cardiac involvement might occur despite normalized laboratory results and echocardiography, we recommend cardiac MRI after 3–6 months. In the case of abnormal findings, the examination should be repeated 9–12 months after disease onset.

4.3. Echocardiography, clinical findings, and implications for cardiac outcome

We attempted to find echocardiographic parameters that correlate with the MRI findings and thus be suitable and affordable in follow-up. In doing so, we hypothesized that myocardial edema is present at disease onset, increasing the LVMI. However, both groups showed an average left ventricular mass (score < 2). At follow-up, we observed a significant decrease in left ventricular mass and the resulting LVMI in both groups. We chose LVMI because both the pediatric *z*-scores for LV mass and the LV mass/height centiles are not validated for obese patients, as in our cohort. Normal *z*-scores for LV mass between +2 and −2 at disease onset indicate a normal LV mass without extensive edema. However, as MRI was not performed at disease onset, we cannot rule out the presence of subtle edema at this time.

According to the literature, a more pronounced cardiac involvement was seen in the patients diagnosed with PIMS (6, 41). Reduced cardiac function and mitral valve regurgitation both normalized in most patients within 6 months. We found more patients with relevant pathologies in the follow-up echocardiography than during the acute disease. This observation fits with the MRI data showing the persistence of myocardial involvement in all patients after acute COVID-19.

Echocardiographic findings after COVID or PIMS mostly consist of depressed LV function, coronary artery dilation or aneurysm, mitral regurgitation, and pericardial effusion (34, 35, 44, 45). Studies reporting a broad range of disease severity from mild infection to PIMS arrive at an overall rate of 30–40 percent for depressed

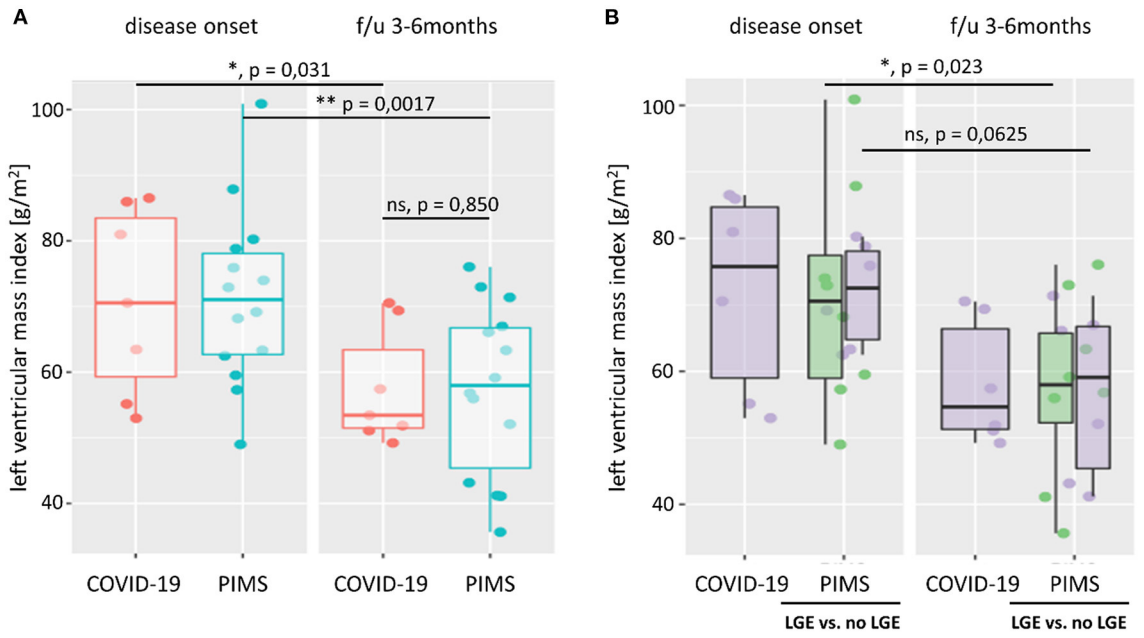

FIGURE 3
Left ventricular mass index. Left ventricular mass index (LVMI) was calculated from body surface area, and left ventricular mass was estimated using the Devereux equation. In **(A)**, we compare LVMI of patients with severe acute COVID-19 (red) or pediatric inflammatory multiorgan syndrome (PIMS, blue) at the time of disease onset and last follow-up (f/u) visit 3–6 months later. At both time points, there was no significant difference in LVMI between the two diagnoses ($p = 0.971$ at disease onset; $p = 0.850$ at f/u). However, both groups saw a significant reduction in LV mass (not shown) and LVMI at f/u. As shown in **(B)**, there was also no significant difference in LVMI between patients with PIMS with or without (green) late gadolinium enhancement (LGE) in cardiac MRI at the time of disease onset ($p = 0.660$) or f/u ($p = 1$). The difference n LVMI between non-LGE PIMS was more pronounced ($p = 0.023$) than in PIMS with LGE ($p = 0.0625$). See also Supplementary Figure 3 for LV z scores and connected dot plots.

LV function and 8–24 percent for CA abnormalities (6, 34, 41, 46, 47). Case series of only severely affected patients reported significantly higher rates of impaired LV function (~50–60%) and CA abnormalities (~20–50%) (4, 6, 47, 48). Our data show less acute functional involvement of the heart. We saw reduced LV function only in patients with PIMS. The same is true for transient MI, which also occurred in 7/8 cases of patients after PIMS.

A recent study that included 503 patients reports a favorable outcome for both conditions: LV function normalized within 30 days in 91% of patients, and nearly all patients with available data had normal LV EF at 90-day follow-up. In more than 75% of patients with CA aneurysms, they regressed to normal (Z-score < 2.5) within 30 days and in all patients up to 90 days (6).

Another study describing echocardiographic findings in 286 children with PIMS reported similar numbers: 34% of patients had depressed LV EF, 42% had mitral regurgitation, and 28% had pericardial effusions. Interestingly, of the 42 patients in whom cardiac MRI was performed in 42 patients, 34% of patients showed evidence of myocardial edema, but LGE was found in only 14% of patients (34). Several studies have reported abnormal strain patterns in patients with LV dysfunction (33, 35). Although strain analysis was not utilized in this study, we report a significant decrease in LV mass after both PIMS and COVID-19 despite normalized LV function (Supplementary material).

For the return to sports and physical activity, we follow the recommendations of the American Academy of Pediatrics of 2022, which recommend a sports break of 3–6 months after severe acute COVID-19 or PIMS. For patients who showed LGE on cardiac MRI after this period, we recommend resumption of light exercise in case of unremarkable physical examination, ECG, and no new onset of symptoms such as syncope, shortness of breath, chest pain, or palpitations.

4.4. Risk stratification of patients with severe acute COVID-19 or PIMS

The pattern of clinical presentation helps distinguish between acute COVID-19 and PIMS. Feldstein et al. (6) report that laboratory findings for cardiac and inflammatory markers correlate with the severity of cardiac involvement. However, identifying severe acute COVID-19 in pediatric patients is rather difficult due to the lack of a consensus definition for severe disease.

To minimize selection bias and identify possible subgroups at risk, we applied the already established PELOD2 score, which was developed to assess the severity of multiple organ dysfunction syndrome in the PICU on a continuous scale (15). However, we found the disease severity relatively underrepresented in the setting of severe acute COVID-19 and PIMS—partly by not considering ARDS in case of high flow oxygen therapy. A recent large multi-center suggests that early identification of children likely to progress to severe disease may be achieved close to admission using readily available parameters (49). It will be exciting to see if this method's predictive value might help identify children at high risk for cardiac involvement.

4.5. Limitations of this study and future directions

One of this study's limitations is the availability of MRI data only as a follow-up examination. Due to the recruitment of severe cases and the risk of anesthesia, cardiac imaging was not feasible in the acute phase of the respective disease. The comparison of only severely ill patients is another limitation of our study. To exclude the resulting selection bias, repeat studies should also include mild courses and compare patients of all severity levels. The comparatively small group size also hinders robust conclusions. More extensive studies are needed to validate our results and make well-founded recommendations. Another essential task for subsequent studies is to evaluate the clinical significance of ongoing myocardial disease activity and to report long-term follow-ups of these patients.

5. Conclusion

In summary, this study shows that children suffering from severe acute COVID-19 infection have a similar cardiac risk profile to patients with PIMS, despite differences in clinical presentation. Both groups showed persistent cardiac involvement and should therefore receive routine cardiological follow-up. Cardiac MRI should be performed during the follow-up of severe acute COVID-19 or PIMS patients, as most of them had delayed late enhancement, even after laboratory and echocardiographic findings had normalized.

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 Plovdiv Medical University Scientific Ethics Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

LC-B, MB, and KG conceived and designed the analysis, collected the data, contributed data or analysis tools, performed the analysis, and wrote the paper. DF conceived and designed the analysis, performed the analysis, and wrote the paper. SA, SS, IP, ZH, IN, AS, and KK collected the data and wrote the paper. II conceived and designed the analysis, contributed data or analysis tools, performed the analysis, and wrote the paper. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Medical University Plovdiv, Project No. P-2394/COV6/2021; N12;N8.

Acknowledgments

We are grateful to the Medical University Plovdiv for funding this project. We wish to thank all the patients, family members, and staff from all the units that participated in the study.

Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2023.1115389/full#supplementary-material

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TYPE Case Report
PUBLISHED 16 February 2023
DOI 10.3389/fimmu.2023.1078838

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to
Cancer Immunity
and Immunotherapy,
a section of the journal
Frontiers in Immunology

RECEIVED 24 October 2022
ACCEPTED 06 February 2023
PUBLISHED 16 February 2023

CITATION
Nishiyama K, Morikawa K, Shinozaki Y, Ueno J, Tanaka S, Tsuruoka H, Azagami S, Ishida A, Yanagisawa N, Akashi YJ and Mineshita M (2023) Case report: Electrocardiographic changes in pembrolizumab-induced fatal myocarditis. *Front. Immunol.* 14:1078838. doi: 10.3389/fimmu.2023.1078838

Case report: Electrocardiographic changes in pembrolizumab-induced fatal myocarditis

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Immune checkpoint inhibitor (ICI)-induced myocarditis is rare but fatal. Because of the rapid course of ICI-induced myocarditis, understanding of clinical course is only possible through information from case reports. We report a case of pembrolizumab-induced myocarditis in which we were able to document the course of electrocardiographic changes from onset to death. A 58-year-old woman with stage IV lung adenocarcinoma, who had completed her first cycle of pembrolizumab, carboplatin, and pemetrexed, was admitted with pericardial effusion. She underwent pericardiocentesis after admission. A second cycle of chemotherapy was administered 3 weeks after the first cycle. Twenty-two days after admission, she developed a mild sore throat and tested positive for SARS-CoV-2 antigen. She was diagnosed with mild coronavirus disease 2019 (COVID-19), isolated, and treated with sotrovimab. Thirty-two days after admission, an electrocardiogram showed monomorphic ventricular tachycardia (VT). Suspecting myocarditis caused by pembrolizumab, the patient was started on daily methylprednisolone after coronary angiography and endocardial biopsy. Eight days after the start of methylprednisolone administration, she was considered to have passed the acute stage. However, four days later, R-on-T phenomenon triggered polymorphic VT and she died. The impact of viral infections such as COVID-19 on patients be treated with immune checkpoint inhibitors is still unknown and we need to be careful with systemic management after viral infections.

KEYWORDS

irAE, lung cancer, myocarditis, pembrolizumab, COVID-19

Introduction

The advent of immune checkpoint inhibitors (ICIs) has revolutionized cancer treatment. ICIs sustain T-cell activation and exert the anti-tumor effects by blocking immunosuppressive signaling from antigen-presenting cells and tumor cells (1). Currently, seven ICIs are approved for the treatment of cancer. Specifically, they are pembrolizumab, nivolumab (PD-1 inhibitors), atezolizumab, durvalumab, avelumab (PD-L1 inhibitors), ipilimumab, and tremelimumab (CTLA-4 inhibitors). ICIs have shown efficacy in the treatment of lung cancer, but they also cause various immune-related adverse events (irAEs). Among them, myocarditis is rare but has the highest mortality rate among all irAEs (2). In cancer therapy, the incidence of myocarditis has been reported to be 1.14% for all ICIs, 0.5% for PD-1 inhibitors, 2.4% for PD-L1 inhibitors, and 3.3% for CTLA-4 inhibitors (3). In the KEYNOTE-189 trial, which evaluated the efficacy and safety of platinum doublet and pembrolizumab combination chemotherapy in patients with non-squamous non-small cell lung cancer, myocarditis was reported in only one case (0.2%) (4).

Recently, the coronavirus disease 2019 (COVID-19) pandemic has had a major impact on healthcare. COVID-19 is an acute respiratory illness caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is known that some COVID-19 patients develop cytokine release syndrome (CRS), in which inflammation-inducing cytokines are increased and the immune system is activated (5-7). Theoretically, COVID-19 infection could further activate the immune system of cancer patients being treated with ICI, resulting in severe irAEs. We report a case of pembrolizumab-induced myocarditis that developed after COVID-19 infection, in which we were able to document the course of electrocardiographic changes from onset to death.

Case presentation

A 58-year-old female with a smoking history of at least 35 pack years had no medical history of dyslipidemia, diabetes mellitus, hypertension or other medical conditions, and no family history of coronary artery disease. She received her second COVID-19 vaccination 6 manths ago and no other vaccinations. She was diagnosed with left lower lobular adenocarcinoma of the lung that had metastasized to the left hilar and right mediastinal lymph nodes, invading the pericardium. The tumor was negative for epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), with a programmed death ligand 1 (PD-L1) tumor proportion score (TPS) of 25%. She visited her previous physician complaining of dyspnea after completing her first cycle of pembrolizumab, carboplatin, and pemetrexed 9 days earlier. Subsequently, she was referred to our hospital due to a worsening pericardial effusion on computed tomography (CT) scan (Figure 1).

On initial examination, she was afebrile with a blood pressure of 125/78 mm Hg, a heart rate of 124 beats/min, and an oxygen saturation of 97% on 3 liters per minute of oxygen administration. Blood tests showed no elevation of creatine kinase (CK), creatine kinase–myocardial band (CK-MB) or troponin T. Electrocardiogram showed sinus tachycardia and low-voltage QRS complexes (Figure 2A), while transthoracic echocardiography (TTE) revealed pericardial effusion. We diagnosed her with cardiac tamponade, and she underwent pericardiocentesis, removing 500 ml of bloody fluid by drainage tube. Subsequently, her symptoms and tachycardia improved, and her oxygen saturation was 96% without oxygen administration. Cytology from the pericardial fluid revealed class V and neoplastic cells consistent with metastatic lung adenocarcinoma but no genetic mutation was detected by highly sensitive next-generation sequencing gene panel assay. The pericardial fluid drainage tube was removed 6 days later since there was no re-accumulation of pericardial fluid.

A second cycle of chemotherapy was administered 3 weeks after the first cycle since her blood tests revealed declining tumor markers, and no regrowth of the primary tumor on CT scan. Twenty-two days after admission, she developed a mild sore throat and tested positive for SARS-CoV-2 antigen. She was diagnosed with mild COVID-19, isolated, and treated with sotrovimab. Twenty-seven days after admission, blood tests showed elevated CK, CK-MB and troponin T, and a negative T wave appeared on electrocardiogram (Figure 2B).

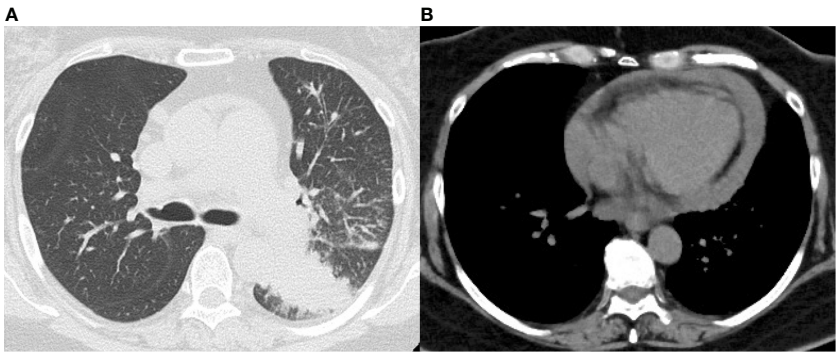

FIGURE 1
Chest computed tomography on admission. **(A)** Lung window shows a mass shadow in the lower lobe of the left lung. **(B)** Mediastinal window shows pericardial effusion.

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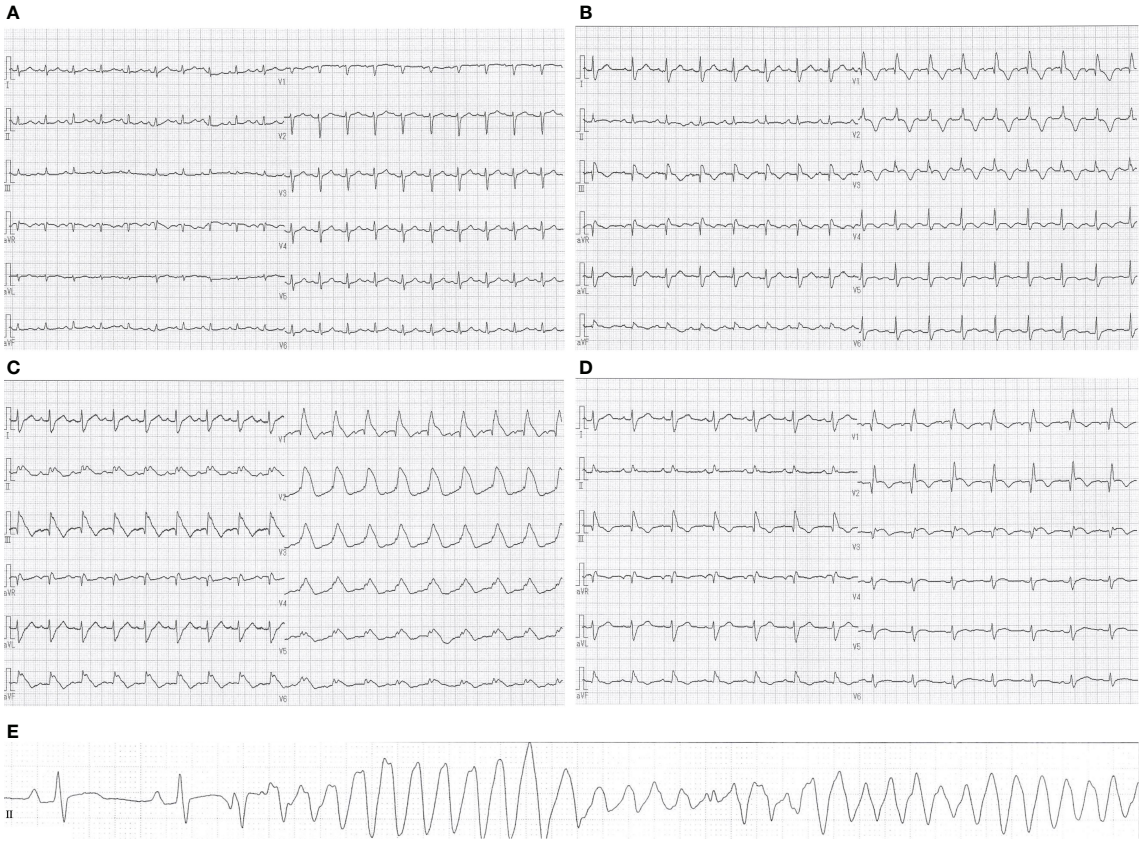

FIGURE 2
Changes in electrocardiographic waveforms during hospitalization. The admission electrocardiogram showed **(A)** sinus tachycardia and low-voltage QRS complexes. **(B)** Negative T waves appeared on electrocardiogram 27 days after admission, and **(C)** monomorphic VT appeared 5 days later. Eight days after the start of methylprednisolone administration, **(D)** negative T waves remained but ST-segment elevation was no longer present. Pre-death electrocardiogram showed **(E)** R-on-T phenomenon triggered polymorphic VT. QT/QTc intervals: **(A)** 313/443 ms, **(B)** 395/507 ms, **(C)** 433/572 ms, **(D)** 408/482 ms, **(E)** 400/408 ms.

She did not complain of palpitations or chest pain. TTE showed a left ventricular ejection fraction of about 70%, with no re-accumulation of pericardial fluid, ventricular wall thickening, or ventricular hypokinesis. However, CK and CK-MB continued to rise on blood tests, and palpitations appeared 5 days later. She was afebrile with a blood pressure of 118/82 mm Hg, a heart rate of 111 beats/min, and an oxygen saturation of 93% on 3 liters per minute of oxygen administration. Differential diagnoses were considered, with myocarditis most concerning, followed by acute coronary syndrome, takotsubo cardiomyopathy, pericardial effusion, and pulmonary embolism. An electrocardiogram showed monomorphic VT (Figure 2C). Blood tests revealed CK 5906 U/l, CK-MB 141.7 ng/ml, troponin T 0.721 ng/ml, and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) 1368 pg/ml. Pulmonary embolism was subsequently ruled out with computed tomography–angiography of the chest. Her hemodynamics had been stable, and she was started on continuous intravenous amiodarone. The next day, coronary angiography and endomyocardial biopsy (EMB) were performed. Her coronary arteries were found to be normal. Cardiac magnetic resonance (CMR) was not performed due to infection control.

We suspected pembrolizumab-induced myocarditis and initiated daily methylprednisolone (1 mg/kg/day) immediately after EMB. The following day, CK and CK-MB decreased on blood test (Figure 3) and ventricular tachycardia disappeared on electrocardiogram. Eight days after the start of methylprednisolone administration, negative T waves remained on electrocardiogram, but ST-segment elevation was no longer present (Figure 2D); therefore, she was considered to have passed the acute stage and amiodarone administration was terminated. Nine days after EMB, she was diagnosed histologically as having acute lymphocytic myocarditis. Myocardial tissue collected at the EMB showed an infiltrate of inflammatory cells predominantly composed of lymphocytes (Figure 4A) and granulation fibrosis of the stroma (Figure 4B). Immunostaining of the tissue showed an inflammatory cell infiltrate predominantly composed of CD8-positive T lymphocytes (Figure 4C). A viral genome study of the tissue was not available at our institution. Twelve days after the start of methylprednisolone administration, R-on-T phenomenon triggered polymorphic VT (Figure 2E). It immediately degenerated into ventricular fibrillation and cardiopulmonary resuscitation was attempted, but she died.

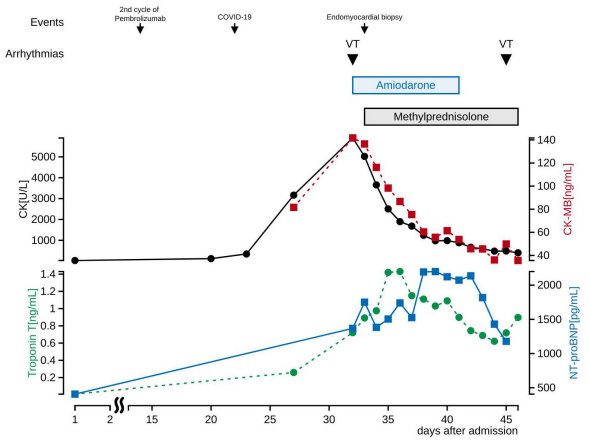

FIGURE 3
Cardiac biomarkers and electrolytes in blood tests after admission. CK and CK-MB decreased initially after methylprednisolone administration, and troponin T and NT-proBNP decreased later. Electrolytes values at the first occurrence of VT were K 4.1 mEq/L, Ca 9.1 mg/dL, Mg 1.8 mg/dL, and at the second occurrence of VT were K 4.7 mEq/L, Ca 8.9 mg/dL, Mg 2.0 mg/dL. Drugs that induce VT are not used.

Discussion

This is the first report of pembrolizumab-induced myocarditis after COVID-19 infection and is also a valuable case in which electrocardiographic changes of myocarditis could be recorded in detail. Although the patient died as a result of arrhythmia, we were able to confirm that corticosteroids are markedly effective in the acute phase of pembrolizumab-induced myocarditis.

Myocarditis is an inflammatory disease of the myocardium caused by viral infection, autoimmunity, or drugs (8). The definitive diagnosis of myocarditis is made by EMB. Myocarditis is classified as eosinophilic, lymphocytic, giant cell, granulomatous or pleomorphic based on the type of cells infiltrating the myocardium. In recent years, ICI-induced myocarditis has been reported with the spread of ICI, and COVID-19-associated myocarditis with the COVID-19 pandemic.

ICI-induced myocarditis occurs when ICIs maintain T lymphocyte activity, T lymphocytes infiltrate the myocardium, and the immune response is excessive (9). The median time of onset was reported to be 34 days after the first ICI administration (3). Histological findings of EMB have been reported to show myocardial infiltration of CD4- positive lymphocytes, CD8-positive lymphocytes, and CD68-positive macrophages (10–12). Corticosteroids are often used in the initial treatment of ICI-induced myocarditis, and other immunosuppressive agents are also considered in corticosteroid-resistant patients (11, 12, 13). Guidelines published in 2018 by the American Society of Clinical Oncology and the National Comprehensive Cancer Center Network recommend treatment with 1 to 2 mg/kg of prednisone for ICI-induced myocarditis (15).

On the other hand, COVID-19-associated myocarditis is thought to result from direct damage to the myocardium by the virus and myocardial damage by the host's immune response (16). The exact incidence of COVID-19-associated myocarditis is unknown because of diagnostic difficulties; some reports indicate that 5.0% of COVID-19 patients developed new onset myocarditis (17). Fulminant myocarditis caused by COVID-19 has been reported to produce ventricular dysfunction and heart failure within 2 to 3 weeks after infection with SARS-CoV-2 (18, 19). Histological findings of EMB shows infiltration of CD4- and CD8-positive lymphocytes in myocardial tissue, as well as CD68-positive macrophages in patients with severe clinical symptoms, such as fulminant myocarditis (20, 21). There are reports that the SARS-CoV-2 genome was detected in myocardial tissue from some COVID-19 patients (22–25). However, there have been reports of virus-negative COVID-19-associated myocarditis, and the authenticity of the SARS-CoV-2 genome remains uncertain (26). Although the treatment of COVID-19-associated myocarditis has not yet been established, corticosteroids are not recommended in viral myocarditis (27).

This patient developed myocarditis 41 days after the first dose of pembrolizumab and 11 days after SARS-CoV-2 infection. Myocardial tissue showed histological findings of acute lymphocytic myocarditis. The timing of onset and histological findings of myocarditis were consistent with both pembrolizumab-induced myocarditis and COVID-19-associated myocarditis. We considered pembrolizumab-induced myocarditis most likely since that myocarditis improved markedly after corticosteroid administration. However, COVID-19-associated myocarditis also causes myocardial damage due to the immune response, so it cannot be completely ruled out. COVID-19 infection has been reported to increase the risk of serious irAEs and may have triggered the development of pembrolizumab-induced myocarditis in this case (28).

An electrocardiogram is a simple test that records the heart's electrical signals and is often used to detect arrhythmias and myocardial disorders. In this case, symptoms of myocarditis, such as palpitations and chest pain, were not present at first, and it was difficult to suspect myocarditis from the symptoms alone. However, we were able to suspect myocarditis at an early stage based on elevated CK and electrocardiographic changes. In addition, frequent ECG testing after the onset of myocarditis made it possible to document ECG changes during the course of treatment for myocarditis. Poor prognostic factors in electrocardiograms of acute myocarditis have been reported as pathological Q wave, wide QRS complex, QRS/T angle ≥ 100°, prolonged QT interval, high-degree atrioventricular block and malignant ventricular tachyarrhythmia (29–32). There are also reports of a high incidence of heart block, such as complete atrioventricular block and right bundle branch block, in electrocardiograms of patients with ICI-induced myocarditis (33). Her ECG showed no heart block, but a wide QRS complex, QRS/T angle ≥100°, prolonged QT interval and malignant ventricular tachyarrhythmia, which predicted a poor prognosis.

There have been several case reports of successful treatment of pembrolizumab-induced myocarditis (34–36). However, in this case, she survived the acute stage of myocarditis without the use of an extracorporeal circulatory device, but the resulting arrhythmia

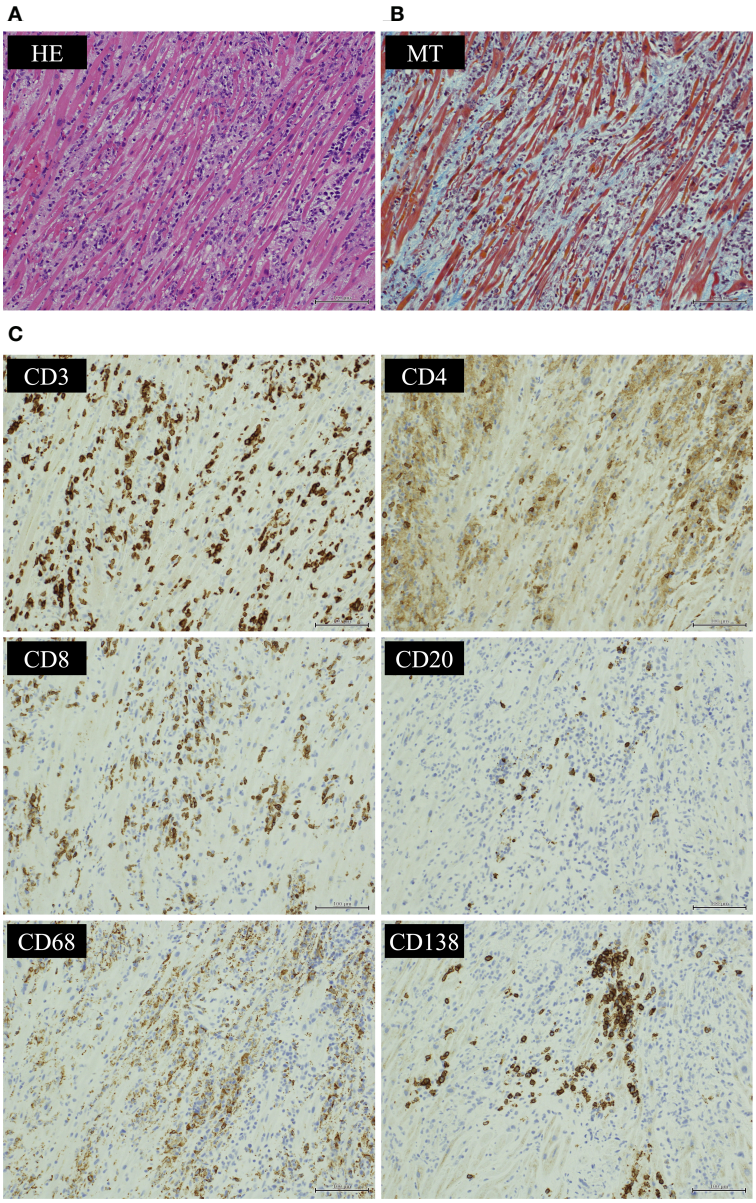

FIGURE 4
Histological findings of endocardial biopsy. Histological findings of the myocardium showed **(A)** an infiltrate of inflammatory cells predominantly composed of lymphocytes by hematoxylin-eosin (HE) staining and **(B)** granulation fibrosis of the stroma by Masson's trichrome (MT) staining. Immunostaining of the tissue showed **(C)** CD3-positive T cells infiltrated more than CD20-positive B cells. CD8-positive T lymphocytes infiltrated more than CD4-positive T lymphocytes. Infiltration of CD68-positive macrophages and CD138-positive plasma cells was also observed. (HE ×200, MT ×200 and CD3/CD4/CD8/CD20/CD68/CD138 immunostaining ×200).

in the post-acute stage resulted in her death. It is suggested that the arrhythmia was caused by severe myocardial damage due to acute myocarditis. The reason for the severe myocardial damage may be related to COVID-19 infection and pericardial invasion of lung cancer. This patient had been infected with COVID-19 prior to the onset of myocarditis, so infection control measures were necessary. This limited the types of tests that could be performed and delayed the diagnosis of myocarditis. It also took longer to respond to emergencies, making it difficult to deal with fatal arrhythmias. In addition, the possibility of COVID-19-associated myocarditis was considered at the pre-treatment stage, which caused a delay in the initiation of corticosteroid administration. There is a report of pembrolizumab-induced myocarditis in a patient with pericardial infiltration of lung cancer (37). Thus, the administration of ICI to patients with pericardial infiltration of tumor may have resulted in excessive lymphocyte infiltration into the myocardium.

There are several limitations in the present case report. First, it was difficult to perform an CMR on COVID-19-infected patients at our institution, and second, we were unable to perform a viral genome study of myocardial tissue. A viral genome study of myocardial tissue might have brought us closer to identifying the cause of myocarditis.

Conclusion

We report a case of pembrolizumab-induced myocarditis that developed after COVID-19 infection, in which we were able to document the course of electrocardiographic changes from onset to death. In myocarditis, elevated myocardial markers and electrocardiographic changes may precede clinical symptoms, so regular myocardial marker measurements and electrocardiographic testing are important. In addition, ECG examination is useful even after the start of treatment, since the prognosis may be inferred from ECG changes. Early diagnosis of pembrolizumab-induced myocarditis is important because early administration of corticosteroids may improve the prognosis. The impact of viral infections such as COVID-19 on patients with ICIs is unknown, and the appearance of irAEs after infection should be noted.

Data availability statement

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

Author contributions

The manuscript was drafted by KN and KM. KN, KM, YS, JU, ST, HT, SA, AI, and MM examined and treated the patient. NY performed the histopathological assessment. YA gave clinical advice. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

Publisher's note

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Case Report
PUBLISHED 15 June 2023
DOI 10.3389/fcvm.2023.1159292

OPEN ACCESS

EDITED BY
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RECEIVED 05 February 2023
ACCEPTED 30 May 2023
PUBLISHED 15 June 2023

CITATION
Zhang L, Yan H, Wang Y and Huang F (2023) Case report: Sudden unexpected death due to tuberculous myocarditis involving sinus node at autopsy.
Front. Cardiovasc. Med. 10:1159292.
doi: 10.3389/fcvm.2023.1159292

Case report: Sudden unexpected death due to tuberculous myocarditis involving sinus node at autopsy

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Tuberculous myocarditis (TM) is an extremely rare manifestation of *Mycobacterium tuberculosis* (TB) infection. Although TM is a critical cause of sudden cardiac death, only a few cases have been reported. We report the case of an older patient with pulmonary TB with a history of fever, chest tightness, paroxysmal palpitations, and electrocardiographic evidence of sinus node conduction abnormalities on admission. Although emergency physicians observed these unusual clinical manifestations, no timely differential diagnosis was made nor interventions were performed. A definitive diagnosis of TM and histopathological findings compatible with sinus node involvement were made based on autopsy outcomes. Herein, we describe the clinical presentation and pathological features of a rare form of *Mycobacterium TB*. In addition, we provide an overview of issues related to the diagnosis of myocardial TB.

KEYWORDS

tuberculous myocarditis, sinus node, *Mycobacterium tuberculosis*, pulmonary tuberculosis, myocardial tuberculosis

Introduction

Although tuberculosis (TB) is a curable disease, it is one of the top 10 causes of death and the leading cause of mortality from a single infectious disease globally (ranking above human immunodeficiency virus and malaria) (1). Therefore, tubercle bacilli infection remains a significant global public health issue (2). The World Health Organization estimates that approximately 10 million new cases of TB and 1.3 million TB-related deaths occur annually (3). More than 95% of deaths due to TB occur in developing and less developed countries (4). In addition, the global risk of TB-associated infection and mortality has increased significantly in recent years owing to the substantial destruction of the public health system due to the recent COVID-19 pandemic (5, 6).

TB is a multisystemic disease that can infect any body organ; however, specific body sites, such as the heart, thyroid, and skeletal muscles, are rarely affected (7). Tuberculous myocarditis (TM) is a rare type of extrapulmonary TB. Only 1%–2% of all patients with TB have heart involvement, and <0.3% die due to the disease (8, 9). Nonetheless, the literature on myocardial TB is limited. The first case of TM was reported in 1664, and sudden death due to myocardial TB was first described in 1977 (7, 10). The clinical symptoms of myocardial TB are diverse and lack specificity, and TB infection often coexists in other organs. To this end, it can be challenging to obtain a timely and

accurate judgment in the early diagnosis of TB in clinical practice; TB is usually diagnosed by postmortem examination (11).

Due to the lack of large-scale clinical samples and data relating to myocardial TB, the reporting and dissemination of such cases is very significant. Here, we describe a unique case of sudden unexpected death due to TM in a patient with the indication of sinus node conduction abnormalities as the initial presentation. A definitive diagnosis was established based on postmortem autopsy findings and pathological examinations. This case report describes in detail the clinical manifestations, pathological features, physical examinations, and laboratory tests outcomes of the patient to raise awareness about TM.

Case description

A 55-year-old male patient was admitted to the hospital with complaints of fever, cough, chest tightness, and paroxysmal palpitations for 1 week. He had a history of TB infection of the lungs 2 years ago. He received routine first-line anti-TB therapy for over 8 months (including isoniazid, rifampin, ethambutol, and pyrazinamide) and was discharged.

At admission, the patient has discontinued anti-TB treatment and was not taking any medication, including hormone preparations, to control other chronic diseases. Additionally, he denied a history of diabetes, hypertension, or tumors. Notably, he reported several sudden chest pain episodes lasting for a few seconds, accompanied by dizziness and amaurosis during the past week. He underwent a general clinical examination at a local outpatient community hospital setting. Previous medical records showed temporary sinus bradycardia with premature atrial beats on electrocardiogram (ECG) (**Figure 1A**), which spontaneously recovered to a normal rhythm after rest.

Based on physical examination at admission, he had a height of 169 cm, body mass index of 18.13 kg/m^2, temperature of 36.9°C, respiration rate of 18 breaths/min, blood pressure of 109/64 mmHg, and mild malnutrition. No anemia or palpable superficial node enlargement were detected. ECG showed a type II second-degree sinoatrial block (**Figure 1B**). However, the patient had no specific symptoms other than mild palpitations and chest tightness; therefore, an atropine challenge test was not

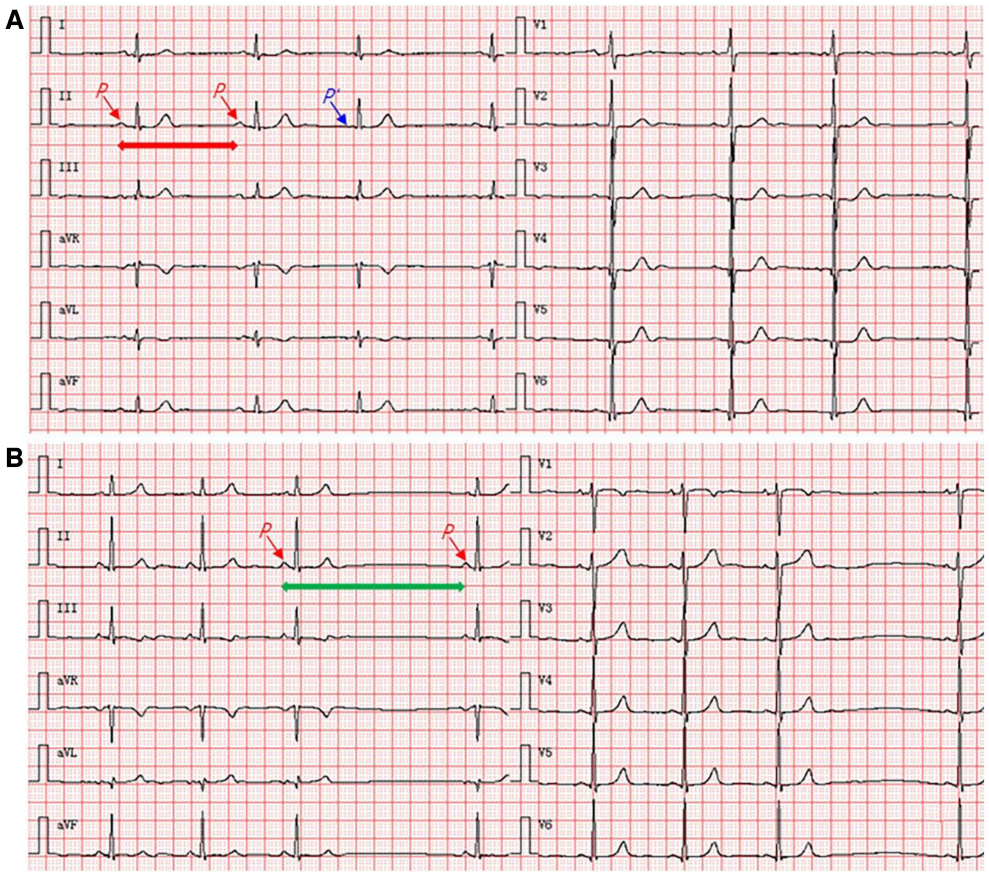

FIGURE 1
Twelve-lead synchronous ECG shows sinus node conduction abnormalities. (A) An increase in the *P*–*P* intervals and the rhythm of <60 beats/min represent sinus bradycardia (take longer horizontal red arrow blue in the lead II as the example); the third *P*-wave (*P'*) in all 12 leads that occurred earlier indicates premature atrial beats (take shorter horizontal blue arrow in the lead II as the example). (B) A single missed beat occurred between the third and fourth *P*-waves, but the increase in *P*–*P* interval is twice as long as the previous *P*–*P* intervals in all 12 leads (take longer horizontal green arrow in the lead II as the example), indicating the occurrence of type II second-degree sinoatrial block. ECG, electrocardiogram.

performed. Lung auscultation revealed scattered pulmonary rhonchi and mild rales in both lungs. Chest radiography revealed fibrotic tuberculous lesions without acute processes. Blood biochemical tests revealed a white blood cell count of 8.06×10^9/L, polymorphonuclear neutrophils level of 96.2%, and high-sensitivity C-reactive protein level of 106.0 mg/L, indicating the presence of infection or inflammation. Other biochemical tests revealed a creatine kinase (CK) level of 109 IU/L, CK-MB level of 16 IU/L, and cardiac troponin I level of 0.02 ng/ml. In addition, serological tests for Hepatitis B, syphilis, and HIV were negative.

Given that the condition of the patient was stable, the emergency physician initially diagnosed the patient with recurrent TB or cardiovascular diseases and prescribed over-the-counter remedies, including dextromethorphan hydrobromide and traditional cough syrup, to relieve cough and reduce sputum and recommended that the patient visits the Specialty Hospital of Infectious Diseases the following day for a further comprehensive examination of the lungs and heart. Unfortunately, after returning home, the patient suddenly experienced severe chest pain, dyspnea, and cardiac arrest at midnight and died after unsuccessful resuscitation attempts. Following the request of the patient's family and the law of China, the body was delivered for a forensic autopsy to examine the cause of the unexpected death.

The autopsy revealed that the epicardium of the right auricle and right atrium were studded with slightly off-white tubercles (**Figure 2A**). The cut surface of the myocardium showed irregular gray-white lesions on the ventricular wall (**Figure 2B**). The main trunk of the left coronary artery showed the presence of atheromatous plaques with lumen stenosis (<25%). All other branches of the coronary artery were normal, and there was no evidence of pleural or pericardial involvement. Multiple white nodules, 1–2 mm in diameter, were observed with partial calcification in cut sections of the lungs. The hilar lymph nodes were grayish-white in color and enlarged. No significant abnormalities were observed in any other organ. Histological examination of the sinus node region revealed granulomas, focal fibrosis, and fatty infiltration (**Figure 2C**). The myocardium also showed a broad context of fibrosis with infiltrated lymphocytes, many Langhans giant cells, and caseating epithelioid granulomas (**Figure 2D**). Although acid-fast bacillus was not detected in the myocardium, tubercle bacillus deoxyribonucleic acid (DNA) was confirmed by real-time polymerase chain reaction (PCR) on formalin-fixed lung tissue. Tubercle bacilli were detected in the granulomatous areas of the lung tissue using Ziehl–Neelsen (ZN) staining (**Figure 3**).

Discussion

TM is a rare but recognized the cause of sudden death. Currently, there are no reliable statistics regarding its incidence (12), although previous studies have reported that the frequency

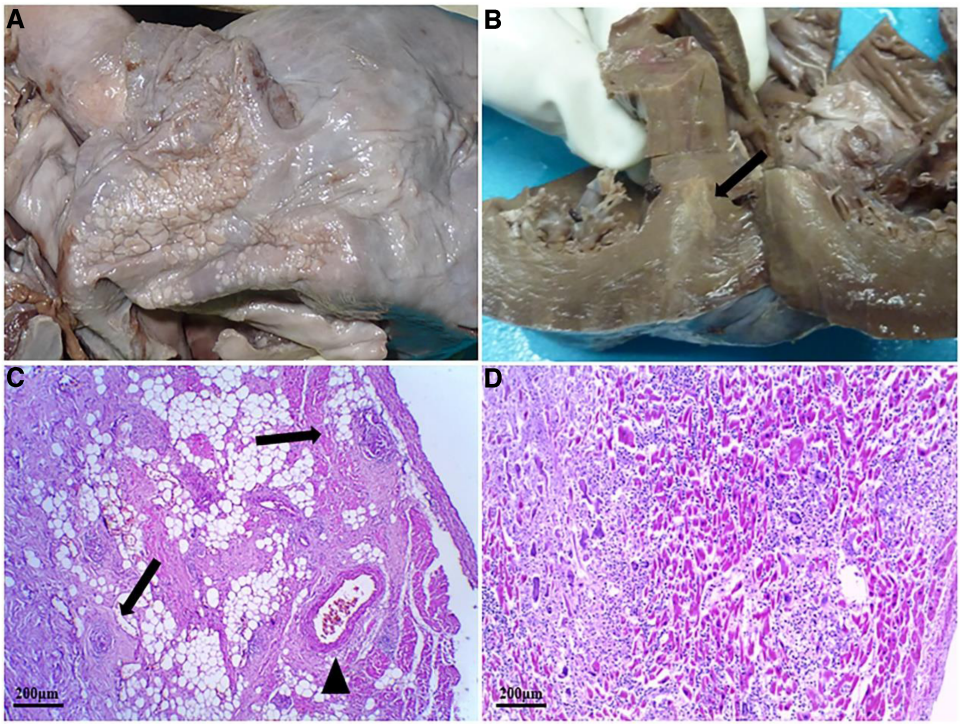

FIGURE 2
Morphological and histological examination of the heart. (A) Small off-white tubercles dotted the epicardium of the right auricle and right atrium. (B) The cut section of the myocardium of the interventricular septum and the anterior wall of the left ventricle shows whitish lesions (black arrow). (C) Several caseating epithelioid granulomas infiltration (black arrow) around the sinoatrial node artery (black triangle) using HE staining. (D) Numerous caseating necrotizing epithelioid cell granulomas in the myocardium of the left ventricle using HE staining. HE, hematoxylin–eosin staining.

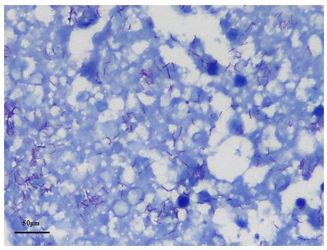

FIGURE 3
Positive Ziehl–Neelsen staining for tubercle bacilli in both lungs (oil immersion).

of myocardial TB is approximately 0.14% at autopsy, and most TB involvement is localized to the pericardium (10, 13). The relative resistance to TB infection may be related to the protective effect of myocardial motion and lactic acid production (14). Cardiac involvement is generally secondary to TB foci in other organs and is caused by hematogenous seeding, lymphatic spread, and contiguously from the pericardium (15). In the present case, TB lesions existed primarily in the lungs and myocardium but did not affect mediastinal lymph nodes or the pericardium. To our knowledge, this is the first reported case of pathologically proven TM, potentially with sinus node involvement, making this case very unusual. Histologically, TM is classified into three distinct types: miliary (due to systemic hematogenous dissemination), caseating nodular, and uncommon diffuse infiltration with lymphocytes and macrophages (16). Based on pathological examination, the present case belongs to the third category.

The clinical presentation of TM varies but is generally associated with the disease severity and affected site, ranging from asymptomatic, pericarditis, arrhythmias, valve dysfunction, ventricular outflow tract obstruction, and impaired myocardial contractility to heart failure (17). Prior to death from TM, most patients have no relevant medical history and are asymptomatic. In some cases, the initial clinical symptoms of TM are similar to those of myocardial ischemia (18). In the current case, the patient presented with myocardial involvement, including chest pain, dizziness, and amaurosis, several weeks before admission, suggesting possible myocardial insufficiency. However, there was no increase in sensitive biomarkers of myocardial injury after admission, except for an abnormal ECG of sinus node conduction. Schnitzer reported a case of miliary TM with palpitations and ventricular tachycardia as the initial manifestations, and the patient eventually died of ventricular arrhythmia (19). In contrast, Dı´az-Peromingo et al. described a cured patient with myocardial TB who was clinically characterized by long QT syndrome (20). Furthermore, Darwish et al. reported a patient with a TB infection of the whole heart who presented with atrial fibrillation and a 1:1 atrioventricular block, and sinus rhythm was restored after anti-TB treatments (21). These case studies illustrate that the presentation of patients with myocardial TB is varied and complex, and early diagnosis and treatment may prevent fatal outcomes. In the current case, in the absence of heart failure and other cardiac pathologies (including coronary and valvular diseases), the presentation of sudden dyspnea and cardiac arrest before death suggests the occurrence of severe arrhythmia, which may be directly related to the involvement of the sinus node. Symptoms of impaired cardiac function, granulomatous lesions of the sinus node, and electrocardiographic evidence of sinus node conduction abnormalities support this hypothesis of sudden death.

Diagnosis of TM remains challenging because consensus-based diagnostic criteria are lacking (18, 22). Histopathological evaluation remains the standard method for determining the presence of TB lesions. Other confirmatory examinations, including acid-fast staining and PCR, have widely been used to detect TB infection. However, *Mycobacterium TB* has been reported to yield negative results using these methods in several cases. In this study, we analyzed myocardial TB-related sudden deaths since 1970 (**Table 1**) and found that, of the 12 cases identified, 4 were positive for acid-fast bacilli staining, 6 were negative, and the remaining were not tested using ZN staining of myocardial tissue. Studies have shown that the PCR method has 96%–100% specificity for detecting *Mycobacterium TB* in pericardial fluid; however, some reports have shown that it has only 15%–20% sensitivity (17). du Toit-Prinsloo and Saayman described TM confirmed by histomorphology despite PCR results being negative (7). Researchers have speculated whether the reliability of PCR depends on the specificity of the primer and DNA concentration and may be related to endogenous inhibitors or fixation procedures (7, 29). Hence, a negative PCR result cannot rule out TB infection (30). In this present case, no tubercle

TABLE 1 Cases of sudden death due to myocardial TB since 1970.

Authors	Year	Sex	Age	Diagnostic method	Extra- cardiac involvement
Behr et al. (23)	1977	M	21, 35	HE, ZN (–)	MLN
Wallis et al. (24)	1984	M	31	HE, ZN (–)	Lung, liver, kidney, MLN
Chan and Dickens et al. (13)	1992	M	71	HE, ZN	Lung, liver, spleen, kidney, bone marrow
Dada et al. (11)	2000	M	25	HE, PCR, ZN (–)	Pericarditis
Biedrzycki and Baithun (14)	2006	F	20	HE, ZN	Lung, kidney, liver
Silingardi et al. (9)	2006	F	33	HE, PCR, ZN (–)	Lung, MLN, liver, spleen
Amonkar et al. (25)	2009	F	65	HE, ZN (–)	Liver
Kanchan et al. (26)	2010	M	58	HE	None
du Toit-Prinsloo and Saayman (7)	2016	M	35	HE, ZN (–), PCR (–)	Lung, spleen
Kumar et al. (8)	2018	M	40	HE, ZN	Not examination
Chan (27)	2018	M	56	HE, ZN, Culture	Lung, diaphragm, spleen
Paliwal et al. (28)	2021	F	17	HE	Kidney, paratracheal, MLN

TB, tuberculosis; HE, hematoxylin–eosin staining; ZN, Ziehl–Neelsen staining; MLN, mediastinal lymph nodes; M, male; F, female; PCR, polymerase chain reaction; (–), negative.

bacilli were detected using ZN staining; however, the presence of tubercle bacillus DNA was identified using real-time PCR in formalin-fixed tissue.

A definitive diagnosis of myocardial TB can only be made from an endomyocardial biopsy (EMB) sample in clinical settings (31); however, no previous studies have examined the application of EMB sampling to cardiac TB. Recently, several studies have identified that cardiac magnetic resonance (CMR) imaging can differentially identify TM in patients with normal troponin levels and ECG findings (1). More so, positron emission tomography-computed tomography can distinguish between infiltrative myocardial TB and cardiac sarcoidosis, which could help delineate the extent of TB involvement and guide the biopsy procedure (32).

Conclusively, TM is a rare and fatal disease that is usually diagnosed postmortem due to its typically asymptomatic presentation. Without consensus on diagnostic criteria for myocardial TB, histopathological assessment remains the dominant method for identifying TB lesions. In this case, we have highlighted the co-application of acid-fast staining and PCR techniques to definitively diagnose a case of suspected TB. In future suspected cases of TB, physicians should consider the possibly enhanced risk of TM in patients with a history of TB, signs of myocardial involvement, and abnormal ECG findings.

Data availability statement

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

Ethics statement

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

Author contributions

LZ and HY wrote the original draft. YW and FH reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgments

The authors appreciate the patient's legal guardian for their consent to this research project, and thank Prof. F. J. Huang of West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University, for referring the case. In addition, we would like to thank Editage (www.editage.cn) for English language editing.

Conflict of interest

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

Publisher's note

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Opinion
PUBLISHED 15 June 2023
DOI 10.3389/fcvm.2023.1186556

OPEN ACCESS

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RECEIVED 14 March 2023
ACCEPTED 16 May 2023
PUBLISHED 15 June 2023

CITATION
Garg R, Hussain M and Friedrich MG (2023) Phenotyping myocardial injury related to COVID and SARS-CoV-2 vaccination: insights from cardiovascular magnetic resonance. Front. Cardiovasc. Med. 10:1186556. doi: 10.3389/fcvm.2023.1186556

Phenotyping myocardial injury related to COVID and SARS-CoV-2 vaccination: insights from cardiovascular magnetic resonance

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KEYWORDS

COVID-19, COVID vaccine, CMR (cardiovascular magnetic resonance), myocardial injury, phenotype

Introduction

Myocardial injury has been reported in patients with acute or previous COVID-19 and in relation to the SARS-CoV-2 vaccine (1). While infrequent, cardiac involvement has been associated with an impaired outcome (2). Several different mechanisms have been proposed in the literature for each entity. As the prime non-invasive diagnostic procedure for myocardial inflammation, cardiovascular magnetic resonance imaging (CMR) has played an essential role during the pandemic in diagnosing associated myocardial inflammation, having a similar diagnostic accuracy compared to invasive endomyocardial biopsy (3). There has been an extensive body of literature on CMR findings in all these syndromes that showed different patterns of myocardial pathology such as global myocardial edema, irreversible myocardial injury such as necrosis and scarring, as well as pericardial effusion. Several such studies have reported similarities of such patterns to those found in myocardial inflammation triggered by other viruses (4, 5), albeit with some difference in some studies (6, 7).

We aim to summarize the CMR findings and discuss areas of similarity.

Myocardial injury in acute COVID-19

Prevalence

Though the most virulent manifestation of COVID-19 is acute respiratory distress syndrome, cardiac injury reflected through elevated troponin concentrations has been increasingly reported (8, 9). Various studies have reported an overall prevalence of acute myocardial injury ranging 5%–38% (10). One study described myocardial injury prevalence of 36% with significant association with death and a higher troponin-I associated with a higher risk of death (11).

Clinical presentation

Myocardial injury is typically characterized by chest pain, dyspnea and palpitations, with or without elevated cardiac biomarkers such as high-sensitivity cardiac troponin (hsTn) and/

or creatinine kinase MB (12). Notably, electrocardiographic and echocardiographic findings in patients with COVID-19-related myocardial injury can be normal (13).

Proposed mechanisms

Several etiologies have been proposed to cause myocardial injury in COVID-19, including direct viral injury, pre-existing chronic injury, supply-demand imbalance, multi-organ failure (14–16), stress-induced cardiomyopathy Takotsubo, or plaque rupture with ischemic events (16–18).

Infection with SARS-CoV-2 impairs endothelial function and hemostatic balance, increases thrombin activity, reduces plasminogen activator inhibitor-1 activity and accelerates the production of fibrin degradation products (19). Endothelial inflammation with vascular edema and disseminated intravascular coagulation may lead to microvascular dysfunction. These factors may exacerbate myocardial oxygen supply/demand imbalance due to hypoxia and tachycardia (20).

Diagnostic utility of CMR

CMR is unique in its capability to non-invasively characterize myocardial inflammation and injury. It is considered the gold standard imaging modality in diagnosing myocarditis (21). The CMR criteria for assessing myocardial inflammation ("Lake Louise Criteria") include a high myocardial signal intensity in T2-weighted images indicating myocardial edema, increased early uptake of gadolinium with a high signal intensity in T1-weighted images (early gadolinium enhancement) as an indicator for hyperemia and capillary leakage, and a high signal intensity in late gadolinium enhancement (LGE) demonstrating necrosis or scar. Based on these criteria, CMR can identify myocardial damage with a sensitivity of 78% and specificity of 88% [AUC 0.83 (0.79–0.86)] (22). In 2018, T1 mapping and T2 mapping were included (3), leading to a similar or higher accuracy (75%–91.8%) as the original criteria (23, 24). The Lake Louise Criteria have significant value in guiding patient management (25) and are also part of the recommendations for using CMR in COVID-19 (26).

Pertinent CMR findings

Myocardial necrosis and scar assessed by LGE imaging are associated with an impaired outcome, while LGE-negative patients have an excellent prognosis, regardless of symptoms (27, 28). Myocarditis-induced injury is typically localized sub-epicardial and/or intramurally, frequently in the basal to mid-inferolateral segments (29). Myocardial edema as an invariable component of active inflammation can be visualized by native T2 mapping. This technique can verify active inflammation with a sensitivity of 89%. Native T1 mapping is also sensitive to myocardial injury and edema and thus can be used to visualize tissue inflammation (30).

Myocardial injury in post-COVID syndrome

Prevalence

While most patients with mild-moderate disease recover within 2 weeks, there is a percentage of the population which do not return to baseline even after 14–21 days (31). The prevalence of post-COVID-19 myocardial injury remains uncertain, with reported data ranging from 0.5% to 20% (32–35). Recent data indicate that as much as 7% of COVID-19-related mortalities may be attributable to myocarditis (36).

Clinical presentation

The Royal College of General Practitioners in the UK has divided COVID-19 infection into: (1) Acute COVID-19 (within 4 weeks after disease onset), (2) Ongoing Symptomatic COVID-19 (persisting for 4–12 weeks), and (3) Post-COVID-19 Syndrome (or Long COVID), if persistent after 12 weeks (32). Persistent chest pain has been reported in up to 20% of COVID-19 survivors over a 2-month follow-up and recurrent palpitations in up to 9% after 6 months, with shortness of breath reported in up to 8% (37).

Proposed mechanisms for post-COVID-19 syndromes

The underlying pathophysiology is poorly understood, including a systemic inflammatory response with cytokine storm, counter-balanced by a compensatory anti-inflammatory response syndrome to prevent widespread multiorgan dysfunction (38) as well as virus persistence and latent virus reactivation of SARS-COV-2. For long COVID, adrenal insufficiency and cerebral dysregulation have been discussed.

Diagnostic utility of CMR

CMR in COVID-19-related cardiac injury is highly mandated and rapidly growing, due to its ability not only to diagnose acute and chronic sequelae of myocardial inflammation but also to provide a more detailed understanding of the pathophysiological phenomenon behind cardiac involvement and differentiate them from other various pathological etiologies (39).

In patients that have recovered from COVID-19, the reported incidence of myocardial inflammation varies greatly, ranging from 2.4% to 30% (2), in one controversial paper even 78%. Non-ischemic scar patterns among participants suggest a non-ischemic cause of cardiac injury (40, 41). One study reported lower left ventricular ejection fraction, higher left ventricular volumes, higher native T1 values consistent with myocardial edema or interstitial fibrosis (42), and high T2 values suggesting myocardial edema (43) when compared with healthy control subjects and risk factor–matched control subjects. These findings correlated with higher

levels of hsTn and active lymphocytic inflammation on endomyocardial biopsy specimens (41). Native T1 and T2 mapping provided the best discriminatory ability to detect COVID-19-associated myocardial disease (41).

CMR may play an important role not only during pandemics but also afterwards, as it can detect persistent scar tissue as well as right and left ventricular remodeling (44–46).

SARS-CoV-2 vaccine-related myocarditis

Prevalence and clinical presentation

There is significant epidemiological research and evidence on the reported adverse effects of myocarditis and pericarditis from these vaccines (47–53). The Center of Disease Control (CDC) in the US reported that cases of myocarditis were highest following the second dose of mRNA vaccination in young males (54). Patients usually present with chest pain, shortness of breath, fatigue, or palpitations, in order of prevalence (55, 56), with a temporal relationship to vaccine administration (52, 55, 57). The incidence remains low as seen in multiple studies and vaccine safety reports (28, 59), currently reported as between 0.5 and 2 per 100,000 people (54, 60).

Given the very high absolute number of vaccinations, however, this is and will remain a significant clinical problem.

Proposed mechanisms

Among several proposed mechanisms for this injury are a dysregulated immune response (61), and activation of the complement system via immune complex formation involving anti-spike protein antibodies (62). Another potentially important mechanism proposed is a direct effect of vaccine nanoparticles on the myocardium, with a subsequent complement activation (63).

Diagnostic utility of CMR

Most studies used the updated Lake Louise Criteria to diagnose myocarditis (3). CMR findings when summarized from multiple case series and original research articles report the severity of myocardial injury as mild (6, 52, 53, 57, 64–69). In several CMR studies on cardiac involvement in COVID-19, myocarditis was more prevalent than pericarditis. LGE was predominantly located in the inferior and inferolateral regions, subepicardial pattern and with co-located edema. Fronza et al. (70) and Groschel et al. (6), among others, described the regional distribution pattern of CMR findings, suggesting a basal/lateral predilection for irreversible injury. Fewer articles reported or mentioned pericarditis, which reported a lower prevalence of pericardial involvement with isolated pericarditis or co-located with myocardial LGE.

Discussion

CMR as the non-invasive diagnostic standard in patients with myocardial involvement in systemic disease has revealed evidence for myocardial inflammation and inflammatory injury in patients with acute COVID-19 or thereafter. This is of clinical importance as such injury may occur in less severe cases and still cause persisting symptoms and impair prognosis. In fact, patients with acute, chronic, or post-vaccination disease all appear to present with similar symptoms, most often non-typical chest pain, shortness of breath, palpitations, and fatigue. Of note, most studies have not found a significant relationship between symptoms and CMR findings (6, 71, 72).

In many post-COVID patients, regional or global myocardial edema can be found (32, 73–75). The typical distribution is a non-ischemic pattern with subepicardial scarring inferior and inferolateral, mostly basal and mid-ventricular. A few studies reported intra-myocardial injury in vaccine-related myocarditis compared to the other 2 groups with a more subepicardial pattern (6). Pericardial effusion and pericarditis were less common. In our experience, pericardial effusion, however, is often localized (mostly lateral-basal) and thus may have been missed in previous studies.

Groschel et al. (6) recently compared CMR findings between the three syndromes and found a higher global T1 in the post-COVID group compared to controls, and a higher basal T1 in the post-COVID and COVID vaccination group. The group with myocardial involvement after vaccination also had a higher segmental involvement rate. No statistical difference was found in myocardial T2 and ECV between the groups, but global T2 values between post-COVID and controls were significant. The most common regions were basal and midventricular, lateral, inferior, or inferolateral, with a similar distribution frequency among the groups. The authors speculated that the difference in higher T1 and lower T2 times could be confounded by age, BMI, or weight (76, 77).

Summarizing the literature, there is conflicting data from studies about inflammation and results for myocardial T1 and T2, likely because the duration between illness and CMR was variable, representing different stages of the disease. Furthermore, the composition of the studied patient populations was variable, some with in-patients or ICU admissions, others with out-patient settings. Moreover, symptom burden and proximity of CMR to the presence of symptoms were also variable. Finally, patient demographics and co-morbidities varied widely. The similarity in the scar pattern amongst the three groups (6, 67) as also seen in this case series at our center (67), with co-located edema and LGE, however, suggests a common pathophysiology (**Figure 1**).

As explained in the subsections above, the underlying pathophysiology for cardiac injury in the three COVID-related conditions may reflect endotheliitis as direct injury and a systemic cytokine storm as indirect injury in the acute-COVID group, a compensatory anti-inflammatory response in post-COVID, and a dysregulated immune response in the COVID-vaccine group.

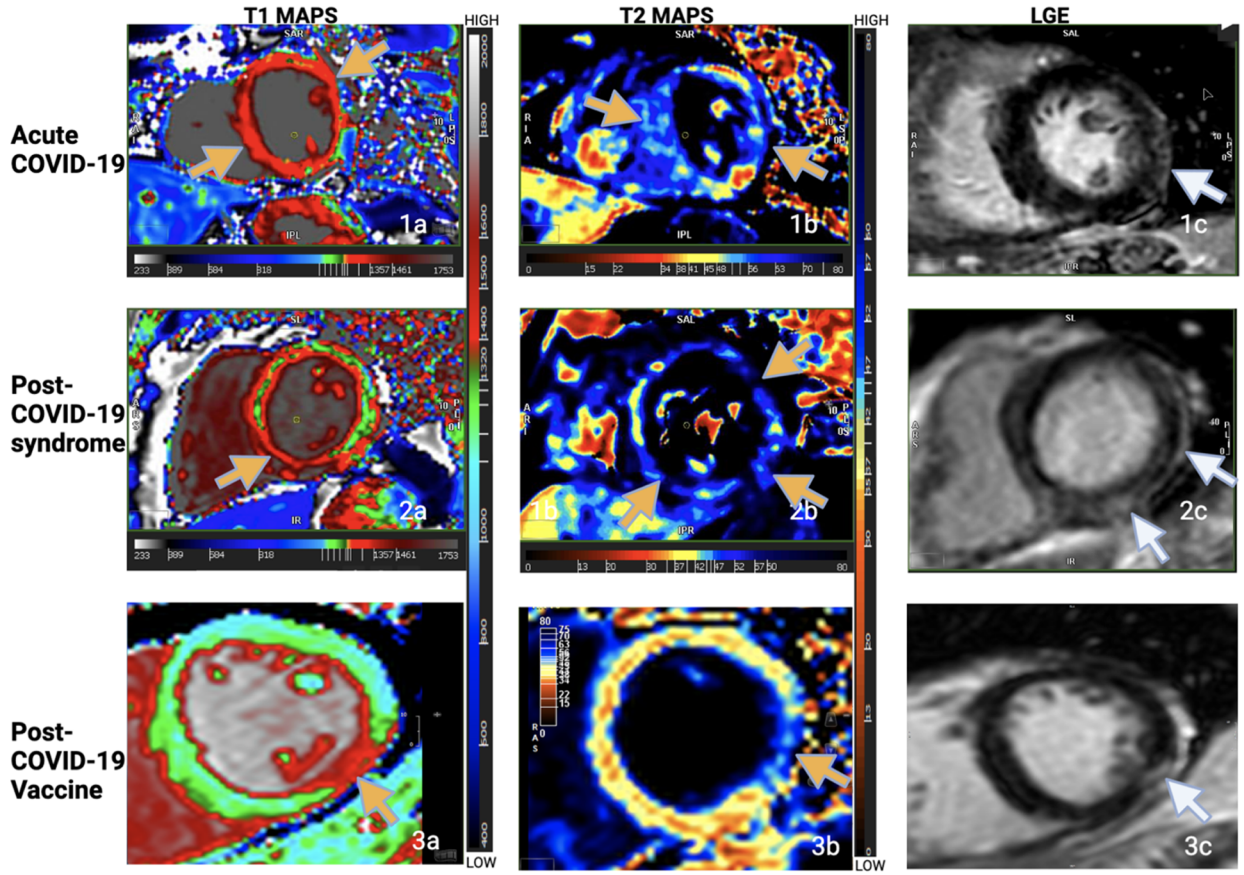

FIGURE 1
T1 maps, T2 maps, LGE imaging from one patient from each of the groups. Increased T1 and T2 times in the maps as shown by the yellow arrows. Subepicardial lateral or inferolateral scar in the basal or mid slices as shown by the white arrows. Note the pattern and location of myocardial injury in all three groups. (Figure adapted from the abstract poster by Garg et al. "Comparison of CMR Findings in Symptomatic Patients with Different COVID-Related Syndromes", SCMR 25th annual scientific sessions, 2022. I am an author of this article; the conference grants authors the right to reuse their own figures without permission in future work.).

Endotheliitis, caused by either direct viral entry or activation of complement cascade may be a potential explanation. This endothelial injury may lead to microvascular dysfunction explaining the symptoms and CMR findings in these patients.

Several studies have studied microvascular dysfunction in acute COVID-19 (78, 79), post COVID (80–82), and post-COVID-vaccine (83) groups. This possibly common pathophysiology needs further exploration with methods such as stress CMR or novel non-invasive techniques like oxygenation-sensitive CMR (84).

The published evidence is still incomplete and subject to several limitations.

In most studies, only symptomatic patients were studied. Biopsy data are scarce, and while CMR is the de facto modality of choice and widely used for diagnosing myocarditis, endomyocardial biopsy (EMB) is still by many considered the gold standard. Most of the time, CMR findings are not corroborated with EMB because of the invasive nature of the latter and current guidelines that restrict it to specific, more severe cases. T1 and T2 values may vary between scanners and are therefore not generalizable between different centers or scanners. Furthermore, as mentioned above, reading CMR images requires expertise and are subject to inter-observer variability, likely explaining the varying prevalence of myocarditis between published cohorts. In athletes, regional fibrosis at the insertion points of the right ventricle, a non-specific finding, may be misinterpreted as inflammatory and lead to an overestimation of the incidence of myocardial involvement. Finally, abnormal LGE represents irreversible injury regardless of its stage. It could be acute, but also reflect such an insult years ago. Therefore, studies confined to LGE or T1 mapping lack information on acute or active inflammation (84).

In summary, CMR studies indicate a similarity of myocardial injury patterns between acute disease, post-COVID, or SARS-CoV-2 vaccination, suggesting a non-specific pathophysiology. It is therefore plausible that most instances of myocardial inflammation stem from generic inflammatory injury rather than direct viral injury. From a clinical standpoint, the exact underlying mechanism, however, is only partly significant. Barring rare cases of viral persistence (which is improbable in COVID), CMR-confirmed myocardial inflammatory injury (necrosis in a non-ischemic regional distribution) accompanied

by edema as an active inflammation marker suffices for informing clinical decision-making.

Alas, therapeutic options for acute myocardial inflammation are limited. To combat COVID-related myocardial injury, we must develop better, personalized immune modulation strategies. The ball in the game against COVID-related myocardial injury is in the therapy court.

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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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 21 June 2023
DOI 10.3389/fcvm.2023.1204232

OPEN ACCESS

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RECEIVED 11 April 2023
ACCEPTED 06 June 2023
PUBLISHED 21 June 2023

CITATION
Schroth D, Garg R, Bocova X, Hansmann J, Haass M, Yan A, Fernando C, Chacko B, Oikonomou A, White J, Alhussein MM, Giusca S, Ochs A, Korosoglou G, André F, Friedrich MG and Ochs M (2023) Predictors of persistent symptoms after mRNA SARS-CoV-2 vaccine-related myocarditis (myovacc registry). Front. Cardiovasc. Med. 10:1204232. doi: 10.3389/fcvm.2023.1204232

Predictors of persistent symptoms after mRNA SARS-CoV-2 vaccine-related myocarditis (myovacc registry)

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Aims: Epidemiological surveillance has raised safety concerns for mRNA SARS-CoV-2-vaccination-related myocarditis. We aimed to analyze epidemiological, clinical and imaging findings associated with clinical outcomes in these patients in an international multi-center registry (NCT05268458).

Methods and results: Patients with clinical and CMR diagnosis of acute myocarditis within 30 days after mRNA SARS-CoV-2—vaccination were included from five centers in Canada and Germany between 05/21 and 01/22. Clinical follow-up on persistent symptoms was collected. We enrolled 59 patients (80% males, mean age 29 years) with CMR-derived mild myocarditis (hs-Troponin-T 552 [249–1,193] ng/L, CRP 28 [13–51] mg/L; LVEF 57 ± 7%, LGE 3 [2–5] segments). Most common symptoms at baseline were chest pain (92%) and dyspnea (37%). Follow-up data from 50 patients showed overall symptomatic burden improvement. However, 12/50 patients (24%, 75% females, mean age 37 years) reported persisting symptoms (median interval 228 days) of chest pain (n = 8/12, 67%), dyspnea (n = 7/12, 58%), with increasing occurrence of fatigue (n = 5/12, 42%) and palpitations (n = 2/12, 17%). These patients had initial lower CRP, lower cardiac involvement in CMR, and fewer ECG changes. Significant predictors of persisting symptoms were female sex and dyspnea at initial presentation. Initial severity of myocarditis was not associated with persisting complaints.

Conclusion: A relevant proportion of patients with mRNA SARS-CoV-2-vaccination-related myocarditis report persisting complaints. While young males are usually affected, patients with persisting symptoms were predominantly females and older. The severity of the initial cardiac involvement not predicting these symptoms may suggest an extracardiac origin.

KEYWORDS

covid vaccination, myocarditis, persistent symptoms, predictors, outcome

Introduction

The corona virus disease of 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), led to the quick development and approval of multiple vaccines against the disease, some of which are based on the emerging messenger RNA (mRNA) technology.

Currently, a total of 6 vaccines in Canada and 5 in Germany are authorized for prevention of COVID-19, including 2 mRNA-based vaccines: Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273). These vaccines were the first to receive emergency use authorization by the United States Food and Drug Administration (USFDA) (1) to reduce the risk and severity of COVID-19. As of May 21st, 2022, 67% of the world population has received at least 1 dose of an approved COVID-19 vaccine. A total of more than 30 million (85% of total population) people in Canada have received at least one dose of an approved COVID-19 vaccine as of May 13, 2022 (2); this number being more than 63 million (76% of total population) people in Germany as of May 26, 2022 (3).

Numerous studies, including case reports and epidemiological research, have suggested the development of mRNA vaccine-related myocarditis and pericarditis, particularly in younger men (4). Notably, however, the clinical trials for both mRNA vaccines have not recorded nor reported myocarditis, potentially missing this adverse event (5–8).

The Centers for Disease Control and Prevention's (CDC's) review of vaccine safety data in the Vaccine Adverse Event Reporting System (VAERS) reporting system (9), from December 2020 to August 2021 showed that out of the over 350 million vaccinated: "rates of myocarditis were highest following the second dose of an mRNA vaccine among males in the age group 12–24 years."

These data not only support previous observations but also confirm their low incidence.

CDC vaccine safety datalink identified that in the 0–7 days post-vaccination, especially after the second dose, both vaccines were associated with an increased risk of myocarditis and pericarditis in the 18–39-year-old age group; estimated to be 22.4 excess cases per million second doses after Pfizer vaccine and 31.2 excess cases per million second doses after Moderna vaccine, suggesting higher incidence after Moderna compared to Pfizer (9).

These registries however should be interpreted with caution because they are only a passive safety signal detection system accompanied by reporting bias. This alone cannot be used to derive causality or any definite conclusions (10, 11).

A study to evaluate the safety of BNT162b2 mRNA vaccine in Israel, with approximately 900,000 vaccinated participants and unvaccinated controls, concluded that the risk of myocarditis after COVID-19 infection (risk ratio = 18.28) surpasses the risk of myocarditis after BNT162b2 vaccine (risk ratio = 2.43) (12), which was reported in the CDC's morbidity and mortality weekly report (MMWR) published on July 9th, 2021 (13). CDC also recommends that the second dose of COVID-19 vaccines should be deferred in patients who had myocarditis or pericarditis after receiving the first dose, with certain exceptions, until additional data is available (14). A consensus document supported by working groups of the European Society of Cardiology has recently been published and emphasizes the rare occurrence of post-vaccine myocarditis compared to myocarditis associated with COVID-19 (15).

In recent years, cardiac MRI (CMR) has become the primary modality for the non-invasive diagnosis of myocarditis (16). A recent recommendations paper suggested that CMR is useful in patients with suspected myocarditis or myopericarditis whenever there is uncertainty in making a diagnosis or to determine the extent of injury and inflammation (17).

We aim to analyze the risk factors associated with clinical outcomes in patients with mRNA COVID-vaccine related myocarditis, diagnosed using CMR. To our knowledge, no study has analyzed the outcomes in terms of symptom severity in these patients and correlated them with CMR findings from a multi-center cohort. Our findings will give the clinician a better understanding of the clinical course, CMR imaging findings and predictors for the outcome of mRNA-vaccine related myocarditis.

Methods

Study design and study population

This multicenter register study (NCT05268458) includes 59 cases of SARS-CoV-2—mRNA-vaccine related myocarditis from 3 hospitals in Canada and 2 hospitals in Germany between 05/2021 and 01/2022 (7 patients from McGill University Health Centre in Montreal, Canada; 26 patients from Foothills Medical Center in Calgary, Canada; 6 patients from Sunnybrook Health Sciences Centre in Toronto, Canada; 9 patients from University Hospital Heidelberg, Germany; and 11 patients from Theresienkrankenhaus in Mannheim, Germany). The study was approved by the ethics committee of the Landesärztekammer Baden-Württemberg, Germany (F-2021-126) as well as the institutional review boards of all participating Canadian sites. All research was performed in accordance with the relevant guidelines and regulations. Informed consent was obtained from all patients or, in the case of minors, from their parent or legal guardian.

Vaccine-related myocarditis was defined as cases of clinically suspected myocarditis with positive CMR findings within 30 days after SARS-CoV-2—mRNA—vaccination without any other plausible etiology. Data on demographics, previous medical history, previous and current symptoms as well as clinical course were gathered from the patients' records. Laboratory results including NT-proBNP, high sensitivity troponin T, GFR, leucocytes and CRP were collected. Every patient received one follow-up phone call at least 4 weeks after hospital discharge. Follow-up data included information on persisting symptoms and New York Heart Association (NYHA) functional classification.

Image acquisition

Every patient received a CMR as part of their routine clinical work-up in the participating study site, with the site-specific clinical MRI protocol for myocarditis. MRI studies were performed on commercial 1.5 T or 3 T scanners with a standard cardiac surface or body coil (McGill University Health Centre: Signa Premier, General Electric, USA; Foothills Medical Center: Magnetom Prisma and Skyra, Siemens Healthineers, USA; Sunnybrook Health Sciences Centre: Magnetom Vida and Sola, Siemens Healthineers, USA; University Hospital Heidelberg: Ingenia and Ingenia CX, Philips Healthcare, Best, The Netherlands; Theresienkrankenhaus Mannheim: Signa Architect, General Electric, USA and Magnetom Avanto, Siemens Healthineers, Germany). All MRI protocols included standard long-axis and short-axis stack cine sequences (steady state free precession) and Late Gadolinium Enhanced (LGE) images (phase sensitive inversion recovery) acquired 10 min after administration of intravenous contrast (0.1–0.2 mmol/kg body weight). For edema sensitive sequences either standard long-axis and short-axis stack short tau inversion recovery images (STIR) and/or native T2 maps were acquired. Imaging protocols included native T1 maps (modified Look-Locker inversion recovery) in all but 12 patients. Native T2 maps were acquired for all but 10 patients. Mapping sequences were acquired as short-axis stack of at least 3 slices. T1 and T2 maps were either automatically generated on the scanner or images were transferred to a workstation for further analysis.

Image analysis

Image analysis was performed directly at each participating study center by an experienced reader blinded to follow-up with at least 3–5 years of experience in Cardiac MRI using certified software (cvi42, Circle Cardiovascular Imaging, Calgary, Canada). Functional data including ventricular volumes, mass and bi-ventricular ejection fraction were measured and calculated after manual contour definition. The presence of LGE was evaluated visually as well as semi-quantitatively using the 5-standard deviation method for each segment of the AHA model (18). In addition, the predominant LGE pattern was identified (subendocardial, mid-wall or subepicardial). If available, T1 and T2 maps were segmented, and average relaxation times were calculated globally and for each segment according to the AHA model.

Statistical analysis

Statistical analysis of all provided data by the participating study centers was carried out at the main study center (Theresienkrankenhaus Mannheim, Germany) using IBM SPSS Statistics for Windows (Version 21.0. Armonk, NY: IBM Corp.). Categorical variables are presented as counts (percentages) and continuous variables as means (standard deviation) or medians [interquartile ranges (IQRs)] depending on data distribution. The Shapiro-Wilk test was used to test for normal distribution. To aggregate mapping data from multiple MRI scanners, T1 and T2 relaxation times were converted to *Z*-scores (19) using the provided local reference ranges for each scanner. *Z*-scores are multiples of standard deviations from the mean of a normally distributed population. Clinical, laboratory and imaging parameters were compared between the subgroups with and without persisting symptoms using Student's *t*-test, Mann-Whitney *U*-test, Chi-Square or Fisher's exact test where applicable. Association of clinical symptoms and extent of myocardial involvement was tested using Spearman's correlation. The relationship of clinical, laboratory and imaging parameters with symptom persistence was assessed using logistic regression analysis. Due to the retrospective and multicentric character of our study, not all data endpoints were available for every patient. For univariate and multivariate regression analysis, the median value of the respective group was used to fill in missing variables. All statistical tests were two-tailed. A p-value < 0.05 was considered statistically significant.

Results

Baseline data

Patient characteristics

From the five participating study centers, 59 patients were included in the study. An overview of baseline data is given in Table 1. Forty-seven patients (80%) were male (male/female ratio 3.92). Mean (±SD) age was 29 ± 13 years. Fifteen patients (25%) had a history of cardiac or pulmonary disease, i.e., asthma in 8 (14%), coronary artery disease in 5 (8%), arterial hypertension in 2 (3%), and previous myocarditis in 2 (3%) patients. In 10 patients (17%), myocarditis occurred after the first vaccination dose, of which 2 (3%) received mRNA-1273 (Moderna) and 8 (14%) received BNT162b2 (Pfizer-BioNTech). In 49 patients (83%), myocarditis occurred after the second vaccination dose, of which 15 (25%) received two doses of mRNA-1273 (Moderna) and 24 (41%) received two doses of BNT162b2 (Pfizer-BioNTech). Six patients (10%) were vaccinated with BNT162b2 (Pfizer-BioNTech) as a first dose and mRNA-1273 (Moderna) as a second dose. Four patients (7%) received other vaccine combinations.

Clinical findings

None of the patients had heart failure symptoms prior to the vaccination. The medical history of one patient included a perimyocarditis more than 10 years prior. Two patients reported a previous infection with SARS-CoV-2 without remaining symptoms prior to vaccination. Median [IQR] onset of symptoms was 3 [2–5] days after vaccination. An overview of baseline and follow up clinical symptoms is given in Figure 1. The most common symptom was chest pain, occurring in 54 (92%) of cases. Other symptoms were dyspnea in 22 (37%), fatigue in 4 (7%), nausea in 4 (7%) and palpitations in 4 (7%) of patients. 8 (14%) patients presented with dyspnea NYHA class II, 5 (9%) with NYHA class III, and 8 (14%) with NYHA class IV. 51 (86%) patients were admitted to the hospital with a median

10.3389/fcvm.2023.1204232

TABLE 1 General patient characteristics.

	All patients ($n = 59$)	With pers. Symptoms ($n = 12$)	Without pers. Symptoms ($n = 38$)	p value*
Age, years	29 ± 13	37 ± 16	28 ± 12	0.04
Male (%)	47 (80)	3 (25)	35 (92)	<0.01
Height, cm	177 ± 9	171 ± 11	178 ± 8	0.03
Weight, kg	80 ± 18	73 ± 15	82 ± 18	0.09
BMI, kg/m^2 [IQR]	25 [22–28]	24 [22–27]	25 [22–28]	0.38
Prev. medical history (%)				
Asthma	8 (14)	1 (8)	5 (13)	1.00
Coronary artery disease	5 (8)	0 (0)	3 (8)	1.00
Hypertension	2 (3)	0 (0)	2 (5)	1.00
NYHA before symptom onset				
NYHA I (%)	59 (100)	12 (100)	38 (100)	-
Vaccination before symptom onset				
One dose mRNA-1273 (%)	2 (3)	0 (0)	2 (5)	1.00
One dose BNT162b2 (%)	8 (14)	0 (0)	7 (18)	0.17
First and second dose mRNA-1273 (%)	15 (25)	4 (33)	11 (29)	1.00
First and second dose BNT162b2 (%)	24 (41)	7 (58)	10 (26)	0.08
First dose BNT162b2, Second dose mRNA-1273 (%)	6 (10)	0 (0)	6 (16)	0.31
Other Combinations (%)	4 (7)	1 (8)	2 (5)	1.00
Clinical presentation				
Days after vaccine [IQR]	3 [2–5]	4 [2–6]	3 [2–4]	0.31
Chest pain (%)	54 (92)	10 (83)	35 (92)	0.58
Dyspnea (%)	22 (37)	8 (67)	11 (29)	0.04
Fatigue (%)	4 (7)	2 (17)	1 (3)	0.14
Nausea (%)	4 (7)	0 (0)	4 (11)	0.56
Palpitations (%)	4 (7)	0 (0)	3 (8)	1.00
Peak NYHA[a]				
NYHA I (%)	36 (63)	4 (33)	26 (70)	0.04
NYHA II (%)	8 (14)	3 (25)	5 (14)	0.39
NYHA III (%)	5 (9)	4 (33)	1 (3)	0.01
NYHA IV (%)	8 (14)	1 (8)	5 (14)	1.00
Admission to hospital (%)	51 (86)	6 (50)	36 (95)	<0.01
Duration of hospitalization, median days [IQR]	3 [1–4]	1 [0–2]	3 [2–4]	0.01
Admission to ICU (%)	17 (29)	1 (8)	11 (29)	0.25
Duration on ICU, median days [IQR]	0 [0–1]	0 [0–0]	0 [0–1]	0.32
Laboratory tests				
Peak NT-proBNP[b], pg/ml	258.8 [141.8–430.8]	61 [61–61]	233 [154.3–434]	0.15
Peak hs-troponin-T[c], ng/L	552 [249–1,193]	317.5 [82.8–664.8]	371 [244.4–1,052.8]	0.42
Peak CRP[d], mg/L	28 [12.5–50.6]	7.9 [5.4–14.2]	42.2 [15.6–58.8]	0.01
Peak Leucocytes[e], n/nl	7.8 [6.7–9.5]	7.6 [7–8.5]	7.8 [6.8–9.2]	0.76
Discharge hs-troponint-T[f], ng/l	197 [19.5–619]	174 [73.1–459.3]	191 [14.8–532]	0.68
Discharge CRP[g], mg/L	7.9 [3.7–24.5]	8 [5.9–8.7]	8.7 [3.6–36.1]	0.41
Discharge Leucocytes[h], n/nl	6.5 [5.4–7.7]	6.4 [5.7–7.2]	6.4 [5.1–7.6]	0.83
ECG findings				
ST segment changes (%)	22 (37)	1 (8)	16 (42)	0.04

Numbers are presented as number (percentage), mean ± standard deviation or median [inter quartile range].
*p value for group comparison between patients with and without persisting symptoms using Student's-t, Mann-Whitney-U, Chi-Square or Fisher's exact test depending on data type and distribution.
[a]Available in 57 baseline and 49 follow up cases.
[b]Available in 22 baseline and 18 follow up cases.
[c]Available in 49 baseline and 40 follow up cases.
[d]Available in 48 baseline and 39 follow up cases.
[e]Available in 49 baseline and 40 follow up cases.
[f]Available in 49 baseline and 40 follow up cases.
[g]Available in 35 baseline and 26 follow up cases.
[h]Available in 47 baseline and 38 follow up cases.

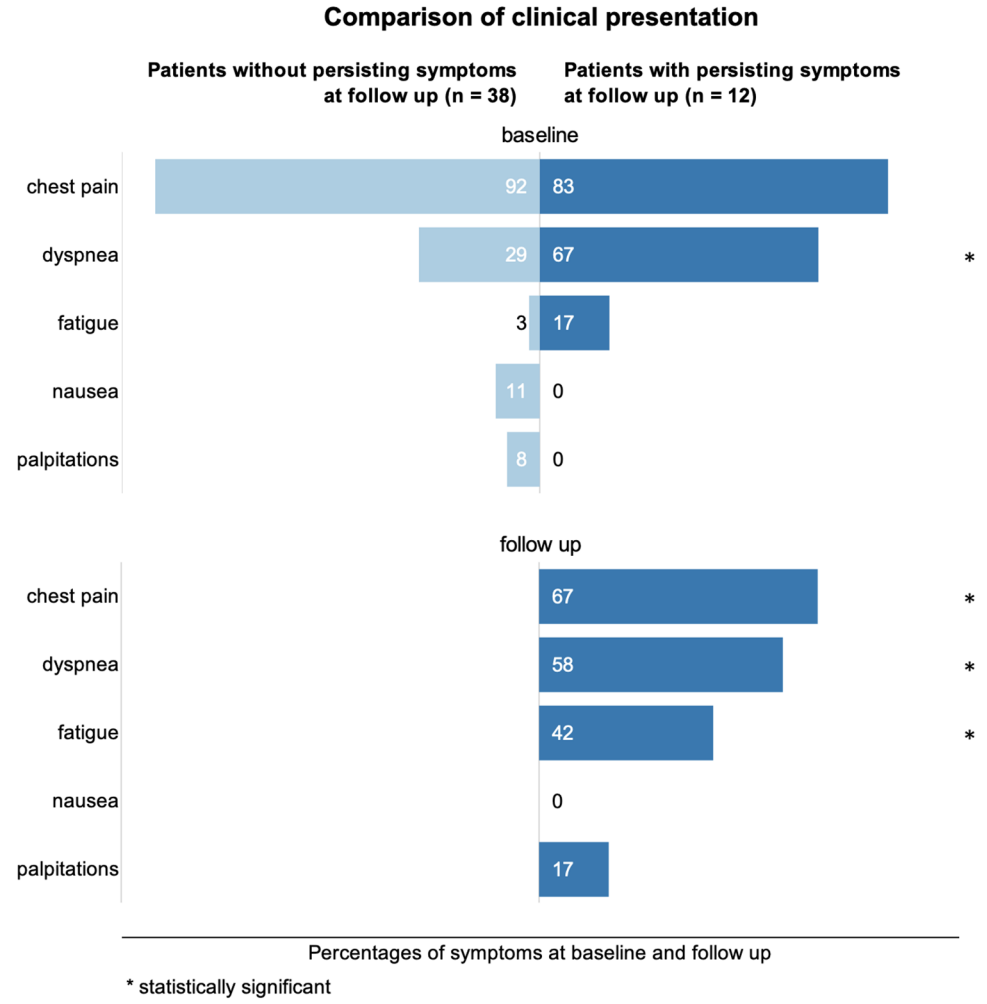

FIGURE 1
Comparison of clinical symptom occurrences in percent at baseline and follow up. Statistically significant differences between the groups with and without persisting symptoms are marked with an asterisk.

[IQR] hospitalization duration of 3 [1–4] days. 17 (29%) patients were admitted to an ICU. Twenty-two patients (37%) showed ST segment changes in the electrocardiogram (ECG). In routine laboratory workup, elevated levels of hs-troponin-T (available in 49 patients; elevated in all, 100%) and CRP (available in 48 patients; elevated in 42, 87.5%) were found. Median [IQR] peak value of hs-troponin-T was 552 [249–1,193] ng/L (reference range <14ng/L). Median [IQR] peak CRP was 28 [12.5–50.6] mg/L (reference range <5mg/L). White cell count was normal in most patients. NT-proBNP was only available in a minority of cases. All abnormal parameters improved over the course of hospital stay.

Imaging findings

A summary of CMR findings is provided in Table 2. The CMR exam was performed after a median [IQR] of 3 [2–8] days after symptom onset. Most patients had a normal LV and RV systolic function with a mildly reduced left ventricular ejection fraction (LVEF <50%) in 9 (15%) cases. The dominant LGE pattern was subepicardial in 52 (91%) cases. Midwall LGE was found in 6 (11%) cases. The typical distribution of LGE as well as of T1 and T2 *Z*-scores is summarized in Figure 2, showing a predilection for the inferior and inferolateral segments at basal and midventricular planes in LGE, and a more heterogenous distribution of segments with a *Z*-score >2 in T1 and T2 mapping. However, the average segmental T2 relaxation time was relatively increased in the inferior and inferolateral basal segments and in the apex, thus, being more in line with LGE distribution. Pericardial effusion was noted in 13 (22%) and pericarditis in 7 (12%) patients.

Follow up

Follow-up data were available for 50 (85%) patients (9 lost to follow-up, 15%). The median [IQR] follow-up interval was 228 [110–307] days after initial diagnosis. At follow-up, 38 (76%) patients were asymptomatic while 12 (24%) patients had persisting symptoms. In the group with persisting symptoms, chest pain was reported by 8 (63%), dyspnea by 7 (58%), fatigue by 5 (42%) and palpitations by 2 (17%) patients, marking no

TABLE 2 Baseline CMR results.

	All patients ($n = 59$)	With pers. Symptoms ($n = 12$)	Without pers. Symptoms ($n = 38$)	p value *
Left ventricle				
Ejection fraction, %	57 ± 7	62 ± 4	57 ± 7	0.03
End diastolic volume, ml	160 ± 38	128 ± 38	168 ± 37	<0.01
End systolic volume, ml	67 [55–81]	48 [43–56]	73 [62–83]	<0.01
Ejection fraction <50%, *n* (%)	9 (15)	0 (0)	7 (18)	0.17
GLS[a], %	−15 [−17 to −13]	−14 [−16 to −13]	−15 [−17 to −14]	0.32
GCS[a], %	−17 [−18 to −16]	−17 [−18 to −16]	−17 [−18 to −15]	0.82
GRS[a], %	27 [25–29]	27 [26–30]	26 [23–29]	0.82
Right ventricle[b]				
Ejection fraction, %	54 ± 6	58 ± 7	53 ± 6	0.06
End diastolic volume, ml	167 [138–186]	122 [99–144]	172 [153–191]	<0.01
End systolic volume, ml	82 [57–93]	48 [42–59]	82 [62–98]	<0.01
Area of left atrium[c], cm^2	20 ± 4	17 ± 4	22 ± 4	<0.01
Area of right atrium[c], cm^2	21 ± 4	20 ± 5	20 ± 4	0.99
Pericardium				
Pericardial effusion, *n* (%)	13 (22)	0 (0)	11 (29)	0.046
Signs of pericarditis, *n* (%)	7 (12)	3 (25)	2 (5)	0.082
Late gadolinium enhancement[d]				
Subendocardial LGE, *n* (%)	0 (0)	0 (0)	0 (0)	–
Midwall LGE, *n* (%)	6 (11)	0 (0)	5 (14)	0.58
Subepicardial LGE, *n* (%)	52 (91)	7 (64)	36 (97)	0.01
Maximum segmental LGE burden[e], %	20 [8–41]	4 [0–14]	22 [9–39]	0.01
Number of visually affected segments, median [IQR]	3 [2–5]	2 [0–4]	3 [2–5]	0.046
Native T1-mapping[f]				
Global *Z*-score, median [IQR]	1.68 [1.16–3.09]	1.2 [1.15–1.55]	2.49 [1.18–3.33]	0.04
Maximum segmental *Z*-score, median [IQR]	4.59 [3.07–6.24]	3.46 [3.17–4.03]	4.69 [3.09–6.29]	0.14
Number of affected segments (*Z*-score >2), median [IQR]	7 [2.5–11]	4 [3–5]	9 [3–13]	0.06
Myocardial edema, median [IQR] affected segments[g]	1 [0–3.5]	0.5 [0–2.5]	1 [0–2]	0.83
T2-mapping[h]				
Global *Z*-score, median [IQR]	−0.3 [−0.7 to 1]	0 [−1 to 1]	−0.3 [−0.6 to 0.5]	0.65
Maximum segmental *Z*-score, median [IQR]	2.3 [1–4]	2.4 [1–3.3]	2.1 [0.9–4.3]	0.94
Number of affected segments (*Z*-score >2), median [IQR]	1 [0–4]	1 [0–2]	1 [0–4.5]	0.80

Numbers are presented as number (percentage), mean ± standard deviation or median [inter quartile range].
*p value for group comparison between patients with persisting symptoms and without using Student's-*t*, Mann-Whitney-*U*, Chi-Square or Fisher's exact test depending on data type and distribution.
[a]Available in 39 baseline and 34 follow up cases.
[b]Available in 39 baseline and 33 follow up cases.
[c]Available in 58 baseline and 49 follow up cases.
[d]Available in 57 baseline and 48 follow up cases.
[e]Available in 56 baseline and 47 follow up cases.
[f]Available in 47 baseline and 38 follow up cases.
[g]Using STIR as fallback and T2-mapping if available.
[h]Available in 49 baseline and 40 follow up cases.

significant reduction of chest pain and dyspnea when compared to initial presentation and even increasing rates of fatigue and palpitations. These findings are summarized in Figure 3.

Differences in patient characteristics and clinical presentation

As shown in Table 1, patients without persisting symptoms were predominantly male (35 men, 92%) and were 28 ± 12 years old. Patients with persisting symptoms were predominantly female (9 women, 75%) and 9 years older on average. Patients who received two doses of BNT162b2 (Pfizer-BioNTech) were significantly more likely to have persisting symptoms, while no significant difference was found for all other vaccine combinations. As can be seen in Figure 1, the spectrum of initial symptoms was different between both groups. While chest pain was the most reported initial symptom by patients with and without persisting symptoms, patients in the former group presented more often with dyspnea (8 patients, 67%) and fatigue (2 patients, 17%) as additional symptoms. In the baseline ECG, ST-segment changes occurred significantly less often in the group with persisting symptoms (1 patient, 8%). Laboratory workup revealed no difference in hs-troponin-T between both groups. However, peak CRP was significantly lower in the group of patients with persisting symptoms, showing only mildly elevated values with a median [IQR] of 7.9 [5.4–14.2] mg/L. Thirty-six patients without symptoms (95%) were admitted to the hospital

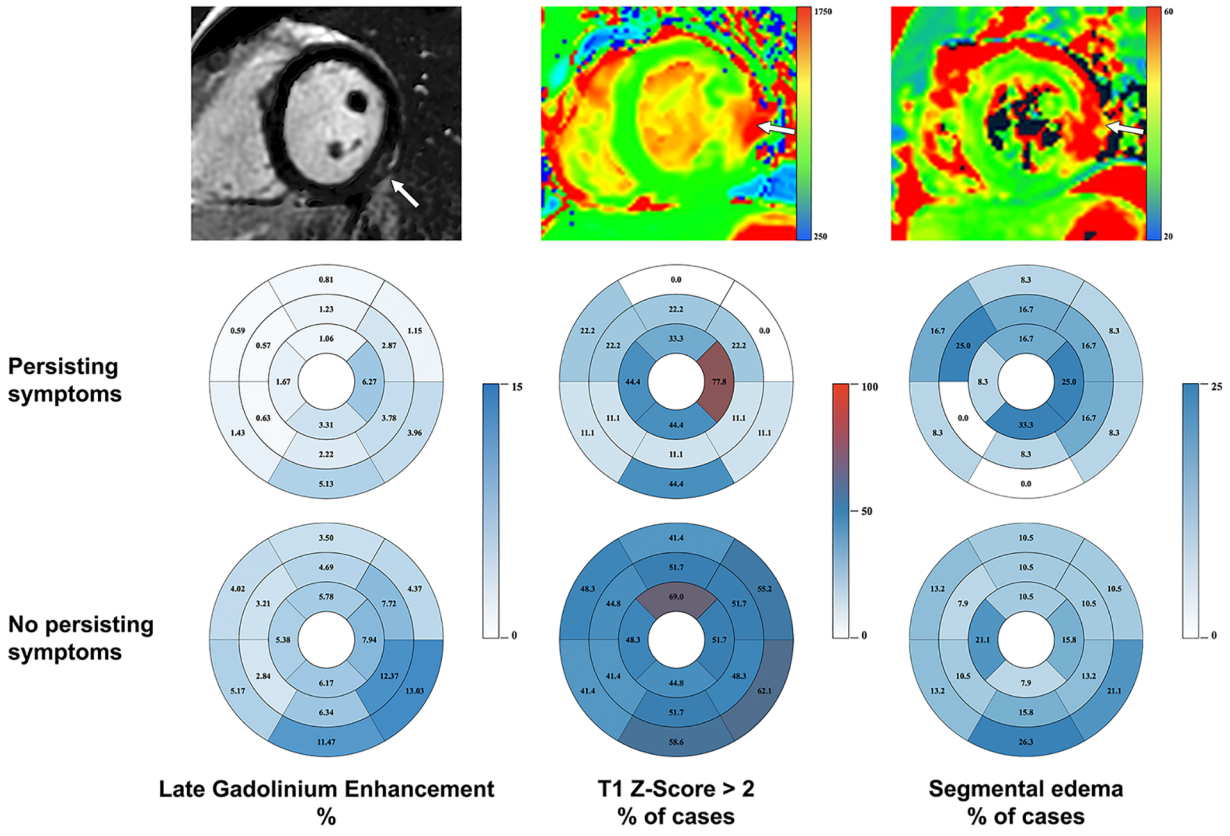

FIGURE 2
Distribution of late gadolinium enhancement, T1-mapping and edema (using STIR as fallback and T2 mapping if available) at baseline CMR. First row: typical imaging presentation of post-vaccination myocarditis in LGE, T1-mapping and fluid-sensitive sequences. A predilection for basal inferior and inferolateral segments is demonstrated (pathology marked by arrow). Second and third row: average LGE burden by segment, segmental involvement in T1-mapping and segments affected by edema for patients with and without persisting symptoms projected on the AHA model.

at initial presentation, while only 6 patients with persisting symptoms (50%) were hospitalized.

Imaging findings

CMR revealed a smaller number of affected segments in LGE and native T1 mapping in the group with persisting symptoms (Table 2, Figure 2). We found no statistically significant difference between both groups in T2 mapping. The baseline left ventricular ejection fraction (LVEF) was significantly higher in patients with persisting symptoms. None of these patients displayed pericardial effusion vs. 11 (patients 29%) without persisting symptoms. However, pericardial enhancement suggestive of pericarditis was more common in the former group.

Predictors of outcome and extent of cardiac involvement

We used logistic regression to analyze the relationship between clinical, laboratory and imaging parameters and persisting symptoms at follow up. Table 3 summarizes the results of the univariate and multivariate regression models. Univariate analysis showed increasing odds for persisting symptoms by age, female sex, and dyspnea at initial presentation, while duration of hospitalization, peak CRP and various CMR parameters representing the severity of myocardial involvement decreased the odds. A three-parameter multivariate logistic regression model consisting of sex (female), duration of hospitalization, and peak CRP predicted persisting symptoms with an accuracy of 96% and a high level of correlation.

Correlation of symptoms at initial presentation with markers for the extent of myocardial involvement was calculated using Spearman's Rho (Table 4). A weak inverse association of dyspnea with hs-troponin-T and number of affected segments in LGE was shown. Conversely, dyspnea was positively associated with the number of affected segments in T2 mapping. Chest pain showed a weak association with number of affected segments in LGE.

Discussion

The aim of this multi-center study was to characterize the clinical course and outcome of vaccine-associated myocarditis after SARS-CoV-2 vaccination, evaluate the typical findings in laboratory and CMR workup as well as identify predictors for an unfavorable outcome. To our knowledge, no study has correlated clinical, laboratory and imaging parameters with patient symptoms at baseline and follow-up.

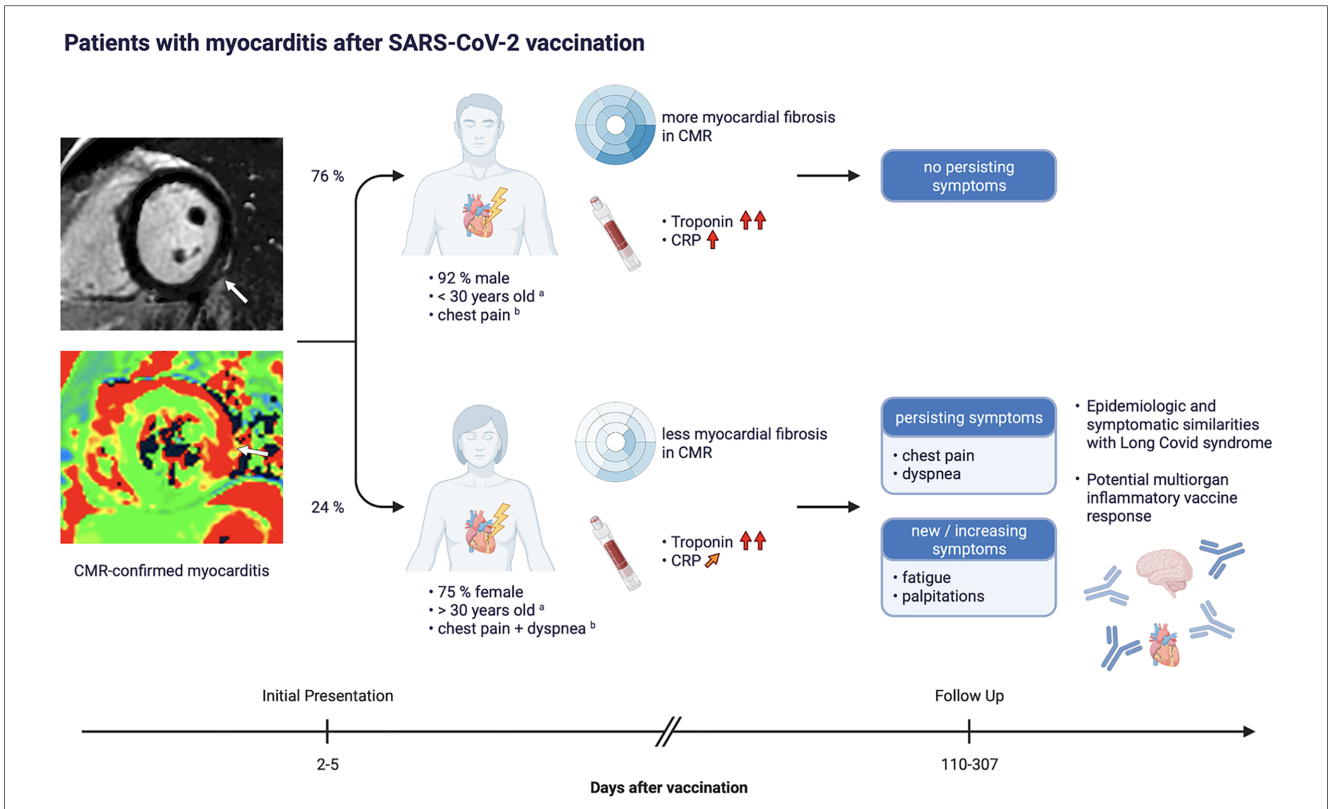

FIGURE 3
Timeline and overview of occurrence of persisting symptoms in the study population. [a]On average, patients with persisting symptoms were females older than 30, patients without persisting symptoms were males younger than 30. [b]Symptoms occurring in the majority of cases.

Summarizing the results from our analysis, we found that patients experiencing vaccination associated myocarditis were predominantly young males (male/female ratio 3.92; mean age 29 years). Most myocarditis cases occurred after the second dose of either mRNA vaccination (83%). Most of these cases were after BNT162b2 (Pfizer-BioNTech) (56%), likely because most administered vaccine doses in Canada (20) and Germany (21) are Pfizer-BioNTech by far. The most common symptoms at baseline were chest pain (92%) and dyspnea (37%). Laboratory work up revealed elevated troponin levels in all patients and elevated CRP in most of them (87.5%). CMR at baseline showed signs of myocarditis in accordance with revised Lake Louise Criteria (16). At follow up (median follow up interval 228 days) symptoms had resolved in most cases (76%), which is an encouraging finding. However, 24% of patients reported persisting complaints. The most common symptoms at follow up were again chest pain (67%) and dyspnea (58%), followed by notably increasing rates of fatigue (42% vs. 17% at baseline) and palpitations (17%, up from 0%) within this group. Patients with persisting symptoms were predominantly females (75%) and older. Interestingly, patients with persisting symptoms at follow-up had a significantly lower peak CRP, a lower rate of ST-segment changes in ECG, and a less myocardial involvement on CMR at initial presentation. While 95% of patients without persisting symptoms at follow-up were initially admitted to the hospital, only 50% of patients with persisting symptoms had been initially admitted to the hospital.

The new mRNA-based vaccines against SARS-CoV-2 have been developed at an unprecedented pace marking a turning point in vaccine development and a testament to the role of mRNA technology. These vaccines are generally considered safe with the most frequent side effects reported by the CDC being pain, swelling and redness at the site of injection, all of which being more pronounced after the second dose (22). Involvement of the myocardium was not shown in the COVID-19 mRNA vaccine trials which could be due to the rare incidence or the faster pre-authorization with lower number of participants, requiring the need for post-marketing surveillance (5, 6) using passive and active surveillance systems like VAERS and BEST (Biologics Effectiveness and Safety, Sentinel Initiative).

Shortly after the start of the global vaccination campaign, first reports began to appear which suggested an association between vaccination and myocardial injury. By now, the epidemiology of this vaccine-related myocarditis has been investigated by multiple large scale epidemiologic trials and its incidence is estimated to be 0.34–2.13 per 100.000 administered doses, occurring more often in younger males (23–28). The estimated catchment area for our study was about 6.5 million people. However, given the existence of multiple medical facilities in the same region as our participating sites, and our suspicion of a considerable volume of unreported cases owing to the predominantly mild course of disease, the incidence of post-vaccination myocarditis cannot be estimated with confidence for this study. The general male-dominant demographic of vaccine-related myocarditis is well

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TABLE 3 Regression analysis for persisting symptoms in univariate and multivariate models.

Univariate analysis	Odds ratio	95% CI	*p* value
Age	1.05	1.001–1.096	0.047
Sex (Female)	35	6.022–203.433	<0.01
Dyspnea at initial presentation	5	1.223–19.709	0.03
Duration of hospitalization	0.54	0.32–0.9	0.02
Peak CRP	0.88	0.796–0.97	0.01
ST segment changes in ECG	0.13	0.015–1.069	0.06
CMR parameters			
LV Ejection fraction	1.13	1.002–1.268	0.05
LV End diastolic volume	0.97	0.943–0.99	0.01
LV End systolic volume	0.92	0.872–0.973	<0.01
RV Ejection fraction	1.12	1.003–1.247	0.04
RV End diastolic volume	0.96	0.937–0.986	<0.01
RV End systolic volume	0.91	0.862–0.968	<0.01
Area of left atrium	0.71	0.569–0.885	<0.01
Signs of pericarditis	6.00	0.869–41.443	0.07
Subepicardial LGE	0.08	0.012–0.484	0.01
Maximum segmental LGE burden	0.93	0.875–0.985	0.01
Number of affected segments in LGE	0.71	0.497–1.01	0.06
Global T1 *Z*-score	0.43	0.22–0.838	0.01
Multivariate model	Accuracy	R^2	*p* value
	0.96	0.80	<0.01
	Odds ratio	95% CI	*p* value
Sex (Female)	67.9	2.3–1,990.5	0.01
Duration of hospitalization	0.44	0.16–1.21	0.11
Peak CRP	0.87	0.767–0.997	0.04

Values calculated using binomial logistic regression analysis for the development of persisting symptoms as dependent variable.

represented by our baseline patient collective. Interestingly, most patients with persisting symptoms at follow-up did not fall into that demographic group by being older on average (median >30 years old) and predominantly female.

CMR findings in vaccine-related myocarditis and pericarditis have been characterized in multiple studies, case reports and case series (29–36). In summary, myocardial affection has usually been described as mild and occurring predominantly in inferior and inferolateral segments with a subepicardial distribution of LGE and edema. Similarly, in our study, CMR at baseline showed that most patients had normal LV and RV function, with LGE having a predominantly subepicardial distribution (91%), in basal and midventricular, inferior and inferolateral segments. Patients who had persisting symptoms at follow-up showed a smaller extent of myocardial involvement in CMR. LGE imaging revealed a smaller amount of myocardial fibrosis which is also reflected by significantly lower global and segmental affection in native T1 mapping. T2 mapping was not statistically different between the two groups. However, pericarditis was the only CMR abnormality that was more common in patients with persisting symptoms. The extent of irreversible injury in CMR was generally small, and the LVEF was normal (>50%) in most patients. Only a minority of patients showed a mildly reduced LVEF. Differences in EF, EDV and ESV between both groups can be explained by the fact that the group with persisting symptoms was mostly females while most patients without persisting symptoms were males, considering that the median values of the mentioned parameters were within the sex-specific normal range (37).

Similar baseline troponin levels in patients with and without persisting symptoms suggest no differences in myocardial injury (38). However, patients with a mild localized myocarditis as in our study and without a significant reduction of LVEF would generally not be expected to present with prolonged dyspnea or fatigue. When considering the smaller extent of myocardial involvement in CMR and lower inflammatory laboratory markers for patients with persisting symptoms one can draw the

TABLE 4 Correlation of symptoms and cardiac involvement using spearman's rho.

Correlation of clinical symptoms and markers for cardiac involvement					
Peak hs-Troponin-T	Spearman's Rho	*p* value	Peak CRP	Spearman's Rho	*p* value
Chest pain	0.08	0.59	Chest pain	0.06	0.69
Dyspnea	−0.36	0.01	Dyspnea	0.00	0.97
Fatigue	0.04	0.77	Fatigue	−0.26	0.08
Nausea	−0.23	0.11	Nausea	0.03	0.85
Palpitations	−0.06	0.67	Palpitations	−0.07	0.66
LGE[a] n of path. Segments	Spearman's Rho	*p* value	T1-Mapping[b] n of path. segments	Spearman's Rho	*p* value
Chest Pain	0.31	0.02	Chest Pain	0.10	0.49
Dyspnea	−0.30	0.02	Dyspnea	0.05	0.74
Fatigue	−0.17	0.20	Fatigue	−0.14	0.35
Nausea	0.13	0.32	Nausea	−0.09	0.55
Palpitations	0.02	0.89	Palpitations	0.06	0.67
T2-Mapping[b] n of path. segments	Spearman's Rho	*p* value			
Chest Pain	−0.07	0.65			
Dyspnea	0.44	0.00			
Fatigue	−0.06	0.67			
Nausea	0.03	0.83			
Palpitations	0.10	0.50			

[a]Path. segment defined as visual presence of LGE.
[b]Path. segment defined as segmental *Z*-score >2.

conclusion that myocardial affection alone does not sufficiently explain these persisting symptoms. As this is a retrospective study collecting already acquired clinical data, follow-up imaging or laboratory data was not available, and a prolonged course of acute myocarditis cannot be excluded with certainty. However, a prolonged acute myocardial inflammation in patients with persisting symptoms at follow up seems implausible given the limited extent of initial myocardial involvement.

The spectrum of reported persisting symptoms in this study—especially the increasing occurrence of fatigue and palpitations—shows similarities with Long-COVID-19 syndrome (39), which is associated with female sex and age as well (40). Similar to our group with persisting symptoms only a minority of patients with post-COVID-19 sequelae show an elevation of CRP (41). Several mechanisms have been proposed regarding long-COVID-19. Multiple studies have attributed it to a dysregulated immune response (39–42). Differences in immune system reaction between men and women could explain the female predilection for persisting symptoms we observed in our study. It has been shown that men and women have different gene expression patterns in immune cells leading to differences in pathogen response (43). Additionally, the X chromosome itself has been described to be an important factor in determining the intensity of the immune response (44, 45). In a study on humoral immune reaction to a trivalent inactivated influenza vaccine, women produced a stronger response with higher antibody levels (46). A stronger immune reaction in females in response to initial myocardial impairment after vaccination, which is suspected to be immune-mediated due to similarities between the spike protein and cardiac self-antigens like α-myosin, might more often lead to a systemic dysregulation causing persisting symptoms by affecting multiple self-antigens in different organ systems (47).

Microvascular dysfunction is another mechanism which has been proposed for cardiovascular injury in acute COVID-19 and long-COVID-19 patients (48–51). Like in other studies on microvascular dysfunction in post-COVID-19 sequelae, chest-pain has been the most common symptom at follow up in our study (51–54). Whether this presumed microvascular impairment is a direct consequence of the spike protein binding to the angiotensin-converting enzyme 2 receptor or whether this triggered a local immune response in our patients remains unclear at this time. Additionally, no immunological laboratory markers or parameters regarding microvascular impairment have been collected in our study. Future immunologic and pathophysiologic studies are needed to gather evidence whether microvascular dysfunction and/or a systemic immune reaction similar to long-COVID-19 syndrome are indeed responsible for the persisting symptoms we reported.

Limitations

Limitations of the study include the absence of a control group, lack of follow-up imaging data and the presumptive nature of the association given no biopsy or serology were routinely obtained. Due to the retrospective nature of this study, there are some inherent limitations due to differences in technical parameters and CMR protocol for image acquisition. Furthermore, the heterogeneity of available laboratory data for our patients precluded feasible statistical comparison for NT-proBNP, a parameter not measured in most patients exhibiting mild symptoms. There is also the possibility of recall bias due to the recent media attention on this condition. Since no routine SARS-CoV-2—tests were performed between hospital discharge and gathering of follow-up data, a SARS-CoV-2 infection during this time interval cannot be ruled out for all patients. As the number and vaccine distribution of the base population of all participating hospitals is not known, no epidemiological conclusions on the vaccine associated myocarditis risk should be drawn from this study. Even in this multi-center study, the patient number is relatively low due to the rare occurrence of post-vaccination myocarditis. In this study, 9 out of 59 patients were lost to follow-up, which could potentially be attributed to their mild course of disease leading to diminished motivation for participation in the follow-up process. Additionally, there is no long-term follow-up for these patients yet available, so we cannot comment on the prognosis and long-term implications.

Conclusion

In our observational study a relevant proportion of patients with confirmed vaccine-related myocarditis reported persisting complaints. While mRNA vaccine-related myocarditis usually affects young males, these patients with persisting symptoms were predominantly females and older. There are clinical similarities between persisting symptoms after mRNA vaccine-related myocarditis and long-COVID. Since the severity of the initial cardiac involvement was not a predictor of persisting symptoms, cardiac impairment does not sufficiently explain these symptoms. Future studies will show whether immune-triggered microvascular dysfunction or a systemic inflammatory syndrome could be a potential pathogenetic mechanism.

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 all individual centers ethics committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

DS and RG responsible for conception of study design, data acquisition and analysis, statistical assessment, and drafting of the

manuscript. XB involved in data acquisition and analysis. JH involved in statistical assessment and revision of the manuscript. MH involved in revision of the manuscript. AY involved in statistical assessment and revision of the manuscript. CF involved in data acquisition and revision of the manuscript. BC involved in data acquisition and revision of the manuscript. AO involved in data acquisition and revision of the manuscript. JW involved in revision of the manuscript. MA involved in data acquisition and revision of the manuscript. SG involved in revision of the manuscript. AO involved in revision of the manuscript. GK involved in statistical assessment and revision of the manuscript. FA involved in data acquisition. MF involved in statistical assessment and revision of the manuscript. MO involved in conception of study design, statistical assessment, and revision of the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

Publisher's note

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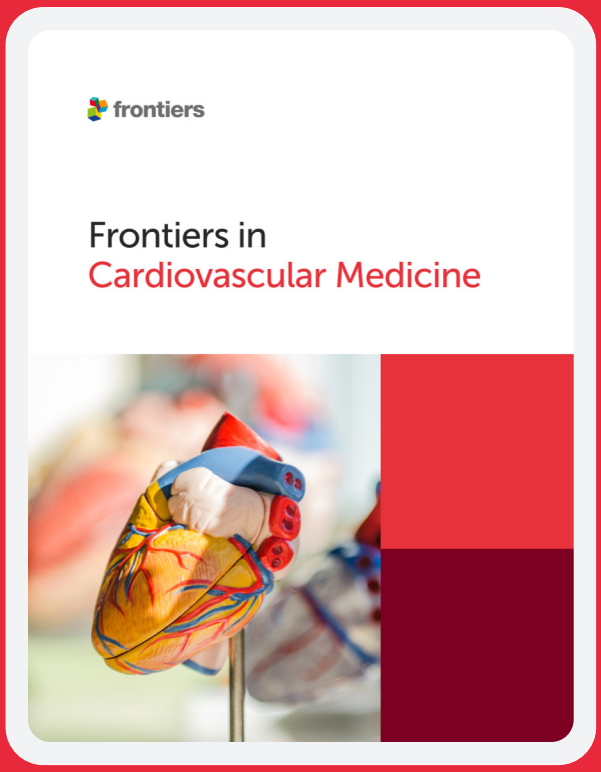

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