

# Sex differences in natural history of cardiovascular magnetic resonance- and biopsy-proven lymphocytic myocarditis

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## Abstract

**Aims** The role of sex in determining the profile and the outcomes of patients with myocarditis is largely unexplored. We evaluated the impact of sex as a modifier factor in the clinical characterization and natural history of patients with definite diagnosis of myocarditis.

**Methods and results** We retrospectively analysed a single-centre cohort of consecutive patients with definite diagnosis of myocarditis (i.e. endomyocardial biopsy or cardiac magnetic resonance proven). Specific sub-analyses were performed in cohorts of patients with chest pain, ventricular arrhythmias, and heart failure as different main symptoms at presentation. The primary outcome measure was a composite of all-cause mortality or heart transplantation (HTx). We included 312 patients, of which 211, 68% of the whole population, were males. Despite no clinically relevant differences found at baseline presentation, males had a higher indexed left ventricular end-diastolic volume ( $62 \pm 23$  mL/m<sup>2</sup> vs.  $52 \pm 20$  mL/m<sup>2</sup>,  $P = 0.011$  in males vs. females, respectively) at follow-up evaluation. At a median follow-up of 72 months, 36 (17%) males vs. 8 (8%) females experienced death or HTx ( $P = 0.033$ ). Male sex emerged as predictors of all-cause mortality or HTx in every combination of covariates (HR 2.600; 1.163–5.809;  $P = 0.020$ ). Results were agreeable regardless of the main symptom of presentation.

**Conclusions** In a large cohort of patients with definite diagnosis of myocarditis, females experienced a more favourable long-term prognosis than males, despite a similar clinical profile at presentation.

**Keywords** Sex differences; Myocarditis; Epidemiology; Prognosis

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## Introduction

The presence of sex-related differences in the epidemiology and pathophysiology of cardiovascular diseases has become evident over time, with women experiencing more favourable natural history in several cardiovascular conditions compared with men.<sup>1</sup> The specific adverse cardiac remodelling and the observed unfavourable cardiac response to stress might be the putative mechanism underlying the greater susceptibility

to the development of heart failure (HF) or dilated cardiomyopathy (DCM) in males,<sup>2</sup> but conclusive evidence is currently lacking.

Myocarditis is an inflammatory disease of the myocardium characterized by great heterogeneity of clinical presentation and evolution.<sup>3,4</sup> In this condition, a key role seems to be played by a maladaptive response of the immune system to specific triggers,<sup>5</sup> and the presence of specific individual genetic background has been postulated.<sup>6</sup> However, the role of

sex in clinical presentation and evolution of myocarditis has been largely unexplored, except for data from animal studies showing more severe disease in males.<sup>7</sup> Recent studies report a female-to-male ratio between 1:1.5 and 1:1.7 in series of patients with myocarditis.<sup>8</sup> This finding was further confirmed in populations of non-ischaemic DCM, where males were more susceptible to develop disease characterized by poor outcome, regardless of the response to therapy.<sup>2,9</sup> Conversely, women frequently exhibit stronger innate and adaptive immune responses than men, and this could theoretically contribute to their increased susceptibility to inflammatory and autoimmune diseases.<sup>10</sup>

The aim of the present study, therefore, was to investigate the potential impact of sex in the clinical presentation and long-term follow-up in a large cohort of unselected patients with a definite diagnosis of myocarditis.

## Methods

This was a single-centre, retrospective, observational cohort study conducted at the Cardiothoracovascular Department of the Trieste University Hospital, Italy. Informed consent was obtained from all subjects, and the study was conducted under the local Regional Institutional Review Board approved the study (identifier 43\_2009).

### Study design, inclusion, and exclusion criteria

We retrospectively analysed data of consecutive patients who received a circumstantiated diagnosis of myocarditis between 2005 and 2019.<sup>11</sup>

The diagnosis of myocarditis was confirmed by endomyocardial biopsy (EMB) or by cardiac magnetic resonance (CMR), according to the recommendation of the position statement on the diagnosis and management of myocarditis from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases.<sup>12</sup> In detail, EMB was reserved to patients with high-risk or intermediate-risk syndromes, as previously reported.<sup>3</sup> The diagnosis was made in accordance with Dallas Criteria<sup>13</sup> and immunohistochemistry analysis<sup>11</sup>; cases with borderline myocarditis were excluded from the present study. In low-risk patients [i.e. patients with chest pain, normal left ventricular ejection fraction (LVEF), and electrically and haemodynamically stable], the diagnosis was confirmed by CMR according to Lake Louise Criteria.<sup>3,14,15</sup> Only CMR exams performed within 14 days from index admission were included in this analysis. In the acute phase, patients older than 35 years and/or  $\geq 1$  risk factor for coronary artery disease in the presence of troponin elevation consistent with myocardial infarction underwent coronary angiography ( $n = 162$ ) or computed tomography ( $n = 9$ ) to exclude coronary artery disease. Finally, patients with

sarcoidosis and eosinophilic and giant cell myocarditis were excluded from this analysis.

Pre-specified criteria for final inclusion were centrally revised by two cardiologists (M.C., M.M.).

Clinical and echocardiographic data were systematically evaluated at diagnosis and at last available clinical evaluation. All the echocardiographic and CMR images were independently revised by operators (M.C., G.G., C.B., and A.P.), blinded to the outcome of the patients.

According to the primary symptom at clinical presentation, patients were categorized in three groups: HF, arrhythmic (i.e. hypokinetic or hyperkinetic life-threatening ventricular arrhythmias), and chest pain. Patient with fulminant myocarditis were mostly in the HF group. When two or more symptoms were present, the predominant clinical presentation was chosen in order to classify the patient.

### Echocardiography

Echocardiographic analysis was performed by expert operators, blinded to patients' data. Left ventricle (LV) quantitative analysis was performed based on 2015 and 2016 ASE/EACVI recommendations, and LVEF was calculated according to the modified Simpson rule in apical four-chamber and two-chamber views.<sup>16,17</sup>

### CMR assessment

CMR was performed using 1.5-T scanners with dedicated cardiac software, a phased-array surface receiver coil, and vectorcardiogram triggering. CMR images were acquired according to the protocols recommended by the Society for Cardiovascular Magnetic Resonance.<sup>18</sup> In detail, we acquired cine steady-state free precession (cine-SSFP) images during apnoea, T2-weighted short-tau inversion recovery (STIR) imaging and/or T2 mapping for myocardial oedema, and late gadolinium enhancement (LGE) in T1-weighted inversion recovery sequences at 5–10 min after gadolinium injection (0.1 mmol/kg) in the short-axis (9–14 images covering the entire LV), two-chamber, three-chamber, and four-chamber planes.

Ventricular volumes and morphology were quantified from the cine images and the presence of LGE was visually assessed. Oedema was evaluated using the signal intensity ratio of the myocardium versus skeletal muscle on T2-weighted images,<sup>19</sup> and regional enhancement was evaluated on an 18-segment model of the LV.

### Outcome measures

The primary study outcome was a composite of all-cause death and heart transplantation (HTx). Information concern-

ing the study endpoint and the causes of death were obtained directly from the patient during the follow-up examinations or by telephone contact with patients, their relatives, or general practitioners. The end of follow-up was established as 30 June 2021. These events were collected at scheduled follow-up evaluations, from electronic health record system, and, if needed, through telephone contacts with patients' general practitioners and/or relatives. CV events were independently assessed by two cardiologists (M.C., A.P.) blinded to patients' baseline characteristics.

## Statistical analysis

Categorical data are presented as percentages and numbers, normally distributed continuous data as mean  $\pm$  standard deviation, and non-normally distributed variables as median and interquartile range (IQR). Unpaired and paired Student's *t*-test was used, when appropriate, for comparison of normally distributed data, whereas Mann–Whitney test was used for abnormally distributed data. The  $\chi^2$  test or the Fisher test was used, when appropriate, to compare categorical variables expressed as proportion.

The Kaplan–Meier method was used to estimate the global survival and the composite end point curve, and the log-rank test was used to compare the curves.

Univariable Cox proportional hazards models were applied to find predictors of the endpoint. Considering the limited number of the events, we evaluated the adjusted sex effect, estimating a set of different multivariable Cox models, each time changing the combination of the other covariates and considering only variables with  $P < 0.1$  at the univariate analysis. We defined a  $P$  value  $< 0.05$  as statistically significant.

All analyses were performed using IBM SPSS Statistics 24 package (New York, NY) package (New York, NY) statistical software and the software R (R Foundation for Statistical Computing, Vienna, Austria; <https://www.r-project.org>), packages 'rms', 'survminer'.

## Results

### Clinical characterization

The overall population included 406 patients. In 86 patients, CMR and EMB were negative and were excluded from the cohort. Of the 320 patients remaining, eight were excluded due to diagnosis of sarcoidosis and eosinophilic or giant cell myocarditis. Thus, the study population included 312 patients (68% males) with histologically proven [37.5%,  $n = 117$ , of which 78 patients (67%) were male and 39 patients (33%) female] or CMR-confirmed myocarditis (62.5%,  $n = 195$ ) (Figure S1). Of them, 60% ( $n = 187$ ) presented with chest pain, 34% ( $n = 106$ ) with HF, and 6% ( $n = 19$ ) with life-

threatening ventricular arrhythmias. In each of these subgroups, the proportion of males was consistently higher than females: 73% in chest pain subgroup, 59% in HF subgroup, and, 63% in arrhythmia subgroup (global  $P$  value = 0.045).

Baseline, last clinical, and echocardiographic data characteristics of the study population comparing males and females were available for 255 patients and are summarized in Table 1. The time from baseline to the last available clinical or imaging data was a median of 30 [IQR 10–77] months. At baseline, males and females had similar age, LVEF and LV dimensions. Compared to females, males showed a higher rate of LGE at CMR (40 vs. 23% in males and females, respectively,  $P < 0.001$ ). This was particularly evident in the chest pain subgroup (49 vs. 29% in males and females, respectively;  $P < 0.001$ ). LGE pattern was non-ischaemic in all patients. Interestingly, at last available follow-up, males had significantly dilated LV compared with females [left ventricle end-diastolic volume indexed (LVEDVi) and left ventricle end-systolic volume index (LVESVi)  $62 \pm 23$  mL/m<sup>2</sup> vs.  $52 \pm 20$  mL/m<sup>2</sup>,  $P = 0.011$ , and  $30 \pm 21$  mL/m<sup>2</sup> vs.  $25 \pm 14$  mL/m<sup>2</sup>,  $P = 0.046$ , in males and females, respectively].

During follow-up, ICD was implanted in 36 patients (11%), 14 females and 22 males. The majority (i.e. 70%) was implanted in primary prevention for persistent severe left ventricular systolic dysfunction. The other patients (i.e. 30%) were implanted in secondary prevention after a life-threatening ventricular arrhythmia at presentation.

Characteristics of sex-specific patient profiles in the three subgroups according to the pattern of disease onset are described in Table S1. In the subgroup of HF patients, despite no differences in LVEF, LVEDVi, and LVESVi at baseline, males showed higher LVEDVi and LVESVi values ( $77 \pm 30$  vs.  $63 \pm 25$  mL/m<sup>2</sup>,  $P = 0.048$  and  $48 \pm 28$  vs.  $33 \pm 16$  mL/m<sup>2</sup>,  $P = 0.034$  in males and females, respectively) and significantly lower median LVEF values at last available follow-up (42 vs. 50%,  $P = 0.004$  in males and females, respectively). Consistently, in the chest pain and arrhythmic subgroups, males showed a more pronounced adverse LV remodelling in terms of LV volumes at last clinical available evaluation.

### Outcomes

The outcomes data were available for all the patients. During a median follow-up of 72 (IQR 43–122) months, 44 (14%) patients met the primary endpoint of all-cause mortality or HTx. As shown in the Kaplan–Meier curves, male sex was associated with an increased risk of experiencing the primary endpoint compared to the female sex [36 (17%) vs. 8 (8%),  $P = 0.033$ ] (Figure 1).

Similar results were seen in the subgroup of patients with HF at the onset. Of note, no female patients experienced the primary outcome measure in the chest pain group (Figure

**Table 1** Baseline and follow-up characteristics of the whole population according to sex

Data at baseline			
Population N = 312	F = 101 (32%)	M = 211 (68%)	P value
Age (years)	42 ± 19	36 ± 17	0.678
NYHA class, n (%)			0.323
I	45 (55%)	112 (64%)	
II	13 (16%)	24 (14%)	
III	12 (15%)	22 (13%)	
IV	12 (15%)	17 (10%)	
Diabetes mellitus, n (%)	3 (3%)	8 (4%)	0.765
Hypertension, n (%)	16 (16%)	26 (12%)	0.403
Family history for DCM, n (%)	4(4)	8(4)	0.953
<b>QRS length (ms)</b>	<b>93 ± 10</b>	<b>101 ± 16</b>	<b>&lt;0.001</b>
LVEF baseline (%)	50 (30–62)	56 (36–61)	0.282
LVEF baseline < 40%, n (%)	33 (33)	52 (25)	0.244
LVEDVi baseline (mL/m <sup>2</sup> )	58 ± 30	60 ± 22	0.542
MR moderate/severe, n (%)	11 (11%)	19 (9%)	0.769
RV systolic dysfunction, no. (%)	10 (10%)	16 (8%)	0.868
Restrictive pattern, n (%)	23 (23)	40 (27)	0.447
<b>Non-ischaeamic LGE, n (%)</b>	<b>23 (23%)</b>	<b>83 (40%)</b>	<b>&lt;0.001**</b>
<b>Beta-blocker, n (%)</b>	<b>48 (49%)</b>	<b>73 (34%)</b>	<b>0.031</b>
ACE-i/ARB, n (%)	26 (26%)	36 (17%)	0.067
MRA, n (%)	14 (14%)	17 (10%)	0.129
Diuretics, no. (%)	26 (26%)	35 (16%)	0.701
Data at last available follow-up			
Population N = 255	F = 78 (31%)	M = 177 (69%)	P value
Beta-blocker, n (%)	37 (61)	56 (49)	0.145
ACE-i/ARB, n (%)	11 (18)	19 (17)	0.499
MRA, n (%)	29 (48)	37 (32)	0.130
ICD at follow-up, n (%)	14 (18%)	22 (13%)	0.415
LVEF at follow-up (%)	55 (49–64)	57 (49–62)	0.577
<b>LVEDVi at follow-up, (mL/m<sup>2</sup>)</b>	<b>52 ± 20</b>	<b>62 ± 23</b>	<b>0.011</b>
<b>LVESVi at follow-up, (mL/m<sup>2</sup>)</b>	<b>25 ± 14</b>	<b>30 ± 21</b>	<b>0.046</b>

ACE-i, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BNP, brain natriuretic peptide; BMI, body mass index; COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; HR, heart rate; ICD, implantable cardiac defibrillator; LGE, late gadolinium enhancement; LVEF, left ventricular ejection fraction; LVEDVi, left ventricular end-diastolic volume indexed; LVESVi, left ventricular end-systolic volume indexed; MRA mineral corticoid antagonists; MR, mitral regurgitation; NYHA, New York Heart Association; RV, right ventricle; SBP systolic blood pressure.

In bold *P* value < 0.05\*\*. CMR was performed in 91, 58, and 51% of patients with chest pain, arrhythmias, and heart failure at the onset, respectively.

52). In total, 32 patients (26 males and 6 females) died, and 31 patients (26 males and 5 females) underwent heart transplant. Of the 31 transplanted patients, 12 are still living, and 19 have passed away. *Table S2* shows the incidences of the main components of endpoints during follow-up in the overall population and in the three subgroups. Males had higher rates of major events both in HF and chest pain groups (*Figure 2*). Arrhythmic subgroup was too small in numbers to derive solid statistical conclusions. *Table S3* shows the baseline and follow-up characteristics dividing the population according to the primary outcome. The male sex was more prevalent in the event group showing that the prognostic role of sex was maintained among these subgroups.

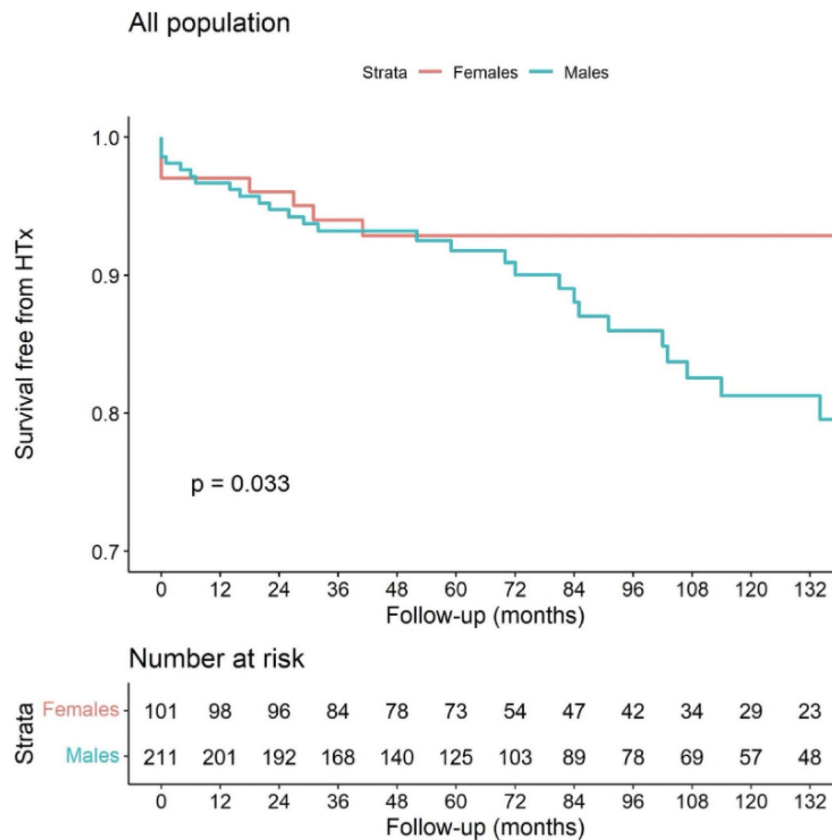
Additional analysis showing the role of sex in patients underwent only EMB or CMR was performed. For both groups, sex remained independently associated with outcome: (I) HR 4.129 (1.245–13.828; *P* = 0.021) among the 117 patients with biopsy-proven myocarditis and (II) HR

8.229 (1.001–67.775; *P* = 0.045) among the 195 patients with CMR confirmed myocarditis.

Univariable and multivariable analyses showed male sex emerged as strongly associated to all-cause mortality or HTx in every combination of covariates (*Table S4*). In the multivariable model with the highest chi-square value, independent predictors of death-HTx, other than sex (HR 2.600; 1.163–5.809; *P* = 0.020), were LVEF <50% (HR 11.496; 1.964–67.277; *P* = 0.007), and restrictive filling pattern (HR 2.682; 1.243–5.788; *P* = 0.012) (*Table 2*).

## Discussion

The present study provides deeper insight into the sex-related impact on clinical presentation and long-term cardiovascular outcomes of myocarditis. To the best of our

**Figure 1** Long-term HTx-free survival according to the sex in the overall population.

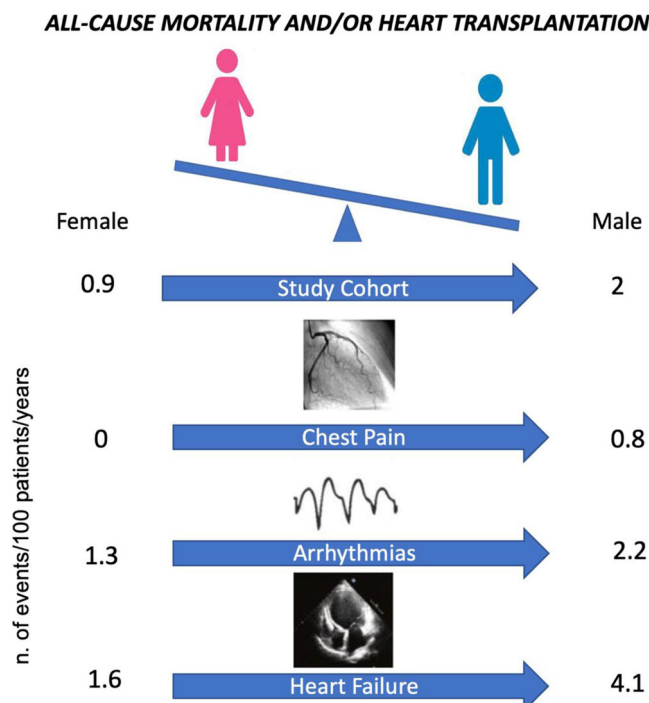
knowledge, this population represents a large cohort of well-characterized patients with a substantial available follow-up (median of 6 years) in which the possible prognostic role of sex was investigated across a wide clinical spectrum of myocarditis.

The main findings can be summarized as follows: (I) In the overall population and in each subgroup of clinical presentation, male sex was predominant. (II) Male and female patients with myocarditis have similar clinical and echocardiographic characteristics at baseline, except for a higher presence of LGE at CMR in males. (III) Male sex, systolic and diastolic dysfunction at baseline were associated with an increased risk of all-cause mortality or HTx during follow-up. This may have been driven by particularly adverse cardiac remodelling in males. (IV) The sex-related difference in prognosis was particularly evident in the subgroup of HF patients, but was consistent in the chest pain subgroup, which are traditionally considered at low risk in light of a normal LVEF at presentation. Finally, the awareness of sex differences and the awareness of the ‘protective’ effect of female sex on the natural history of the disease might have major implications for clinical evaluation and modern management of patients with myocarditis.

### The influence of sex in the pathophysiology of myocarditis

Women constitute a largely under-represented and under-investigated subgroup of patients, accounting for only 30% of participants in clinical trials.<sup>20</sup> Therefore, data from retrospective analyses on large cohorts with long-term follow-up are fundamental to understand the role of sex differences in specific settings of cardiovascular disease. From this perspective, the present study provides important information on sex-related differences for the clinical management of patients with myocarditis. In fact, we found an association between sex and long-term cardiovascular outcome. Our results are relevant considering that female sex is associated with a higher susceptibility to autoimmune diseases and are expected to have more severe consequences due to the stronger innate and adaptive immune responses.<sup>10</sup>

Available studies exploring the mechanisms explaining our results are not conclusive and the ‘sex-tailored approach’ is still a grey area under investigation. In the experimental myocarditis murine model, development of DCM was associated with sex-related differences in the inflammatory response, partially influenced by differences in sex hormones.<sup>21</sup>

**Figure 2** Incidence rates of all cause of mortality and/or HTx. Incidence rates are measured as number of events per 100 patients/years.**Table 2** Univariable analyses and main multivariable analysis

	Univariable		Multivariable <sup>a</sup>	
	HR (95% CI)	P value	HR (95% CI)	P value
Male sex, n (%)	<b>3.319 (1.026–4.801)</b>	<b>0.043</b>	<b>2.600 (1.163–5.809)</b>	<b>0.020</b>
Age (years)	0.999 (0.982–1.016)	0.911		
BMI	0.985 (0.914–1.062)	0.698		
Diabetes mellitus	0.909 (1.128–0.145)	0.875		
Family history for DCM	2.298 (0.298–17.709)	0.425		
Onset with arrhythmia	1.283 (0.455–3.618)	0.637		
Onset with HF	<b>3.807 (1.965–7.373)</b>	<b>&lt;0.001</b>	0.970 (0.234–4.026)	0.996
Onset with chest pain	<b>0.197 (0.09–0.428)</b>	<b>&lt;0.001</b>		
Restrictive pattern	3.103 (1.571–6.128)	0.001	<b>2.682 (1.243–5.788)</b>	<b>0.012</b>
LVEF <50% at baseline	<b>14.004 (4.254–46.102)</b>	<b>&lt;0.001</b>	<b>11.496 (1.964–67.277)</b>	<b>0.007</b>
LVEDVi at baseline	<b>1.019 (1.010–1.027)</b>	<b>&lt;0.001</b>		
LBBB	1.730 (0.607–4.928)	0.305		
Beta-blockers	0.796 (0.311–2.041)	0.635		

BMI, body mass index; DBP, diastolic blood pressure; HF, heart failure; HR, heart rate; LBBB, left bundle branch block; LVEDVi, left ventricular end-diastolic volume index; LVESVi, left ventricular end-systolic volume index; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; SBP, systolic blood pressure.

In bold P value < 0.05.

<sup>a</sup>We report the multivariable model showing the highest chi-square value among the ones estimated.

In human myocarditis, male sex has been reported as a potential risk factor associated with a higher incidence of cardiac fibrosis and more intense cardiac inflammation, leading to the development of DCM.<sup>22,23</sup> Some initial insights are derived from the Intervention in Myocarditis and Acute Cardiomyopathy (IMAC)-2 Study, which only included patients with HF and LVEF <40% due to idiopathic DCM, myo-

carditis, or peripartum cardiomyopathy and reported greater rates of myocardial recovery and longer HTx-free survival in women compared with men.<sup>22</sup> In a recent study, the long-term outcome of women affected by DCM was more favourable compared with those of men, and sex emerged as an important independent factor, particularly for cardiovascular outcome.<sup>24</sup> Our study confirms and expands this

knowledge to a population including all spectrums of clinical onset of myocarditis.

Our knowledge on the role of sex in outcome prediction compared to other parameters remains incomplete. Although some studies investigated the role of sex in patients with myocarditis, the vast majority included selected cohorts, such as fulminant or arrhythmic myocarditis, or did not include the sex in univariable or multivariable analyses.<sup>25–29</sup>

Current understanding of how sex-related differences influence the prevalence and evolution of cardiovascular diseases is still poor and further research on large populations is needed.

## The prognostic role of sex

In the present study, clinical and echocardiographic characteristics of patients at presentation were similar between men and women, but men showed worse outcomes. This data adds an important piece of information to the understanding of sex differences in this clinical context. Men are known to have larger hearts than women,<sup>30</sup> even after adjusting for body surface area, whereas women have increased LVEF values compared with men.<sup>31</sup> In our cohort, ventricular dimensions (even if indexed for body surface area) were similar between males and female at clinical presentation, but men had more dilated LV at follow-up assessment, suggesting a possible sex difference in the evolution of the disease. Specifically, this finding suggests the intriguing possibility that males were less likely to develop LV reverse remodelling compared with females. However, focused studies on this topic are needed in the future. Interestingly, our results further highlight the role of CMR as important tool for prognostic stratification in myocarditis: Men did show higher rates of LGE, which has been previously demonstrated to predict a low probability of LV reverse remodelling.<sup>32</sup>

Moreover, it might be possible to speculate how the association between sex and outcomes may be due to the influence of sex in determining not only the presence of LGE but also its extent. In fact, the extent of LGE might be the best variable to consider for a multivariable Cox analysis in future investigations. So far, standardization of LGE quantification is lacking and represents an important gap of knowledge, mostly in non-ischaemic cardiomyopathies. According to these findings, female patients may be less prone to produce a fibrotic scar in the myocardium in response to an inflammatory cardiac injury, but more research is required in this field. Moreover, our results underline the need to assess myocardial derangement through evaluation of systolic and diastolic dysfunction, which were confirmed to be associated with an adverse outcome, as previously demonstrated in DCM.<sup>33</sup> Further studies are needed to explore the best techniques and parameters to detect and quantify the magnitude of ventricular derangement. Furthermore, a study evaluating

this topic in the era of Lake Louise Criteria is not present yet and definitely needed.

Although the influence of sex on outcome was evident in the overall study population, subgroup analyses showed its prominent role in patients presenting with HF, highlighting the suggested genetic overlap between myocarditis and DCM,<sup>34</sup> in which it is known the detrimental role of male sex.<sup>24</sup> However, our results question the overall benign natural history of myocarditis presenting with chest pain and normal LVEF, suggesting that sex-related differences are important in this setting. In fact, only males experienced the primary endpoint, although we did not observe hard events in the females in this specific subgroup.

Taking all things into consideration, these findings could indicate that men with myocarditis might require closer cardiological follow-up, particularly if they present without LV systolic dysfunction, in light of a higher tendency towards unfavourable ventricular remodelling in the long-term follow-up, thereby affecting their overall survival. According to our results, a careful characterization of the presence and extent of ventricular derangement by means of a multiparametric evaluation including EKG, echocardiography, and CMR (even in myocarditis with low-risk presentation<sup>11</sup>) emerges as a critical tool in the contemporary management of myocarditis.

Finally, our study findings might suggest an important intimate connection between myocarditis and DCM. This connection is tangible and could be attributed to the combination of genetic and immunologic factors, with myocarditis being the hidden bridge, between these two uncharted territories. Therefore, in clinical practice, genetics and family history should be systematically evaluated, not only in cardiomyopathies but also in myocarditis. Conversely, the immunologic point of view might be the missing key in future diagnostic approach and management, not only of myocarditis but also of cardiomyopathies and HF, as suggested by recent clinical trials.<sup>35</sup>

## Limitations

There are several limitations that should be acknowledged. This is a single-centre, retrospective analysis that was conducted on patients with myocarditis followed in a tertiary referral centre for the diagnosis and treatment of cardiomyopathies. Therefore, despite consistency in the entire clinical spectrum of myocarditis, these results may not be generalized to all patients presenting with the disease. Furthermore, the number of women in our cohort was relatively small, possibly reflecting underdiagnosis of women with myocarditis in clinical practice, as known for many other cardiovascular diseases. Additionally, the majority of the overall population was Caucasian, so we were not able to explore the presence of ethnicity differences in our study.

The long enrolment period, although providing the possibility for a reliable statistical analysis, can be considered a known limit of studies analysing rare diseases with low event rate. Evaluation of LGE presence and extent, as well as its prognostic value, could not be investigated, as CMR data were not available for all patients. Given its widely recognized prognostic role in myocarditis, it is reasonable to hypothesize that LGE, and especially the extent of LGE, would have been associated with outcome. Finally, it is unclear how pharmacological therapies, such as beta-blockers and angiotensin-converting enzyme inhibitors, have influenced the occurrence of the study outcome in our cohort. Further research is necessary to confirm findings from this study in larger multicentric populations.

## Conclusion

In a large cohort of well-characterized patients with confirmed myocarditis, our results suggest that male sex is associated with a higher risk of all-cause mortality and HTx in long-term follow-up. Sex emerged as a factor affecting the natural history of myocarditis in terms of adverse ventricular remodelling and poor global outcome, particularly in male patients presenting with systolic and diastolic dysfunction.

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## Conflict of interest

All authors declare no conflict of interest.

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## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Baseline and follow-up characteristics according to sex in the subgroups of patients presenting with heart failure, chest pain and arrhythmias at the onset.

**Table S2.** Incidence rates of events as number of events per 100 patients/years.

**Table S3.** Baseline and follow-up characteristics according to the primary outcome of death for all cause/heart transplant.

**Table S4.** Different multivariable Cox models, each time maintaining male sex and changing the combination of the other covariates.

**Figure S1.** Consort flow diagram of the population.

**Figure S2.** Long-term HTx-free survival according to the sex in the subgroups of patients presenting with chest pain (panel A), arrhythmias (panel B), heart failure (panel C). HTx indicates heart transplantation. Interestingly, no female experienced the outcome measure in chest pain group vs. 9 (6%) males.  $P = 0.1$ . The subgroup of patients with HF at the onset experienced more adverse events than the other groups.  $P = 0.019$ .

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