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A prospective study of biopsy-proven myocarditis: prognostic relevance of clinical and aetiopathogenetic features at diagnosis

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Aims Myocarditis may be idiopathic, viral, and/or immune; frequency of these forms and prognosis are ill-defined. We aimed at identifying aetiopathogenetic and prognostic markers in myocarditis, including viral genome on endomyocardial biopsy (EMB) by polymerase chain reaction (PCR) and serum anti-heart autoantibodies (AHA).
Methods and results We studied 174 patients, 110 males, aged 36 ± 18 years, median follow-up 23.5 months, range 10–54; 85 patients had active myocarditis and 89 borderline myocarditis (no diffuse or severe inflammation) (Dallas criteria). Serum AHA were detected by indirect immunofluorescence. PCR was used to detect virus. Six-year actuarial survival was 73%. AHA were found in 56% of patients and positive PCR in 26%. Univariate predictors of death/transplantation were young age, longer symptom duration, giant cell myocarditis, NYHA II–IV, positive PCR, presentation with LV dysfunction, clinical signs/symptoms of heart failure, and echocardiographic and haemodynamic indexes of cardiac dysfunction. By Cox univariate analysis, highest risk was conferred by clinical signs/symptoms of left (HR = 4.3, CI 1.7–10.8, $P = 0.002$) and right heart failure (HR 3.4, CI 1.5–7.3, $P = 0.002$).
Conclusion In myocarditis, biventricular dysfunction at diagnosis was the main predictor of death/transplantation. AHA identified immune-mediated myocarditis in the majority of cases. Viral genome was a univariate predictor of adverse prognosis. Our approach of using AHA and positive PCR as aetiopathogenetic markers should help patient selection and recruitment in future studies on aetiological therapy.

Introduction

In a patient subset, myocarditis and dilated cardiomyopathy (DCM) represent the acute and chronic stages of an inflammatory myocardial disease, which may be idiopathic, viral, and/or autoimmune.[1,2] Diagnosis of myocarditis is based on endomyocardial biopsy (EMB)[1,2]; prognostic significance of features at presentation is ill-defined and management is hampered by difficulties in establishing aetiopathogenesis.[2–9]

Polymerase chain reaction (PCR) on EMB tissue has become the gold standard for the diagnosis of viral myocarditis or cardiomyopathy.[10–19] Some, but not all, studies suggested that positive PCR for virus may be an unfavourable predictor, and it has been proposed that additional prospective data are needed.[7] Using indirect immunofluorescence (IFL), circulating organ- and disease-specific anti-heart autoantibodies (AHA) are detected, which represent non-invasive autoimmune markers in myocarditis and DCM.[9,20–25] Prospective data in myocarditis patients characterized by viral PCR and AHA to identify distinct aetiopathogenetic subsets are lacking. This would be clinically relevant, as some studies suggest a favourable effect of aetiology-directed therapies.[12,17,18,26]

In this prospective study, we aimed at assessing aetiopathogenesis and prognostic relevance of features at diagnosis in myocarditis, including a positive PCR for virus and serum AHA as viral and autoimmune markers, respectively.

Methods

Patients

Study subjects were 174 consecutive patients (110 males, mean age 36 ± 18 years), admitted to our institution, a tertiary referral centre for arrhythmias and heart transplantation, from January 1992 to

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May 2005, with cardiac symptoms and clinical suspicion of myocarditis, in the absence of known non-inflammatory causes, including coronary artery disease. Patients were excluded if they did not give written informed consent to EMB; none refused to give consent. All patients underwent transthoracic Doppler echocardiography (TTE), complete heart catheterization, right ventricular (RV) EMB, and selective coronary angiography. TTE RV ejection fraction (RVEF) was calculated as described[27]; severe RV dilation was defined as RV end-diastolic volume $>100\,mL/m^2$ and severe RV dysfunction as RVEF $<35\%$. The local Ethics Committee approved the study design and written informed consent was obtained for all patients.

Histology, immunohistology, and molecular analysis on endomyocardial biopsy

Three to five EMB samples from each patient were obtained and processed.[2] Histological diagnosis was based on the Dallas criteria.[28] Immunohistochemistry was used for the characterization of inflammatory infiltrates; cutoff for positive immunohistochemistry was that of an inflammatory infiltrate count ≥ 14 common leucocyte antigen positive cells/mm^2.[2] One or two frozen EMB specimens per patient were used for PCR and reverse transcriptase PCR analysis and for detection of cardiotropic viruses' genome.[2,12,14–16] Patients were prospectively analysed for all viruses studied, except for Parvovirus B19 that started on year 2000. To exclude passive blood contamination, blood samples were collected for each patient at the same time of EMB and tested by PCR for the same virus in the presence of a positive result on EMB. The frequency of positive PCR in myocarditis was compared with that observed in RV EMB obtained during life, for diagnostic purposes or peri-operatively at the time of heart transplantation, in our established control groups of histopathologically confirmed non-inflammatory heart disease ($n = 13$, 11 males, age range 4–71 years, of whom five with restrictive cardiomyopathy, two with valvular heart disease, four with congenital heart disease, and two with amyloidosis), ischaemic heart disease ($n = 17$, 15 males, age range 44–56 years), and normal heart donors ($n = 8$, seven males, age range 33–56 years).[14–16]

Anti-heart autoantibodies testing by standard indirect immunofluorescence

For the detection of AHA, sera, available in 130 patients, were tested by standard IFL at one-tenth dilution on 4 μm thick unfixed fresh frozen cryostat sections of blood group O normal human atrium and skeletal muscle.[9,20,21] The frequency of AHA in myocarditis was compared with that of our control groups of histopathologically confirmed non-inflammatory heart disease ($n = 160$, 80 males, aged 37 ± 17 years, of whom $n = 55$ rheumatic heart disease, $n = 67$ hypertrophic cardiomyopathy, and $n = 38$ congenital defects), ischaemic heart disease ($n = 141$, 131 males, aged 44 ± 14 years), and normal subjects ($n = 270$, 123 males, age 35 ± 11).[9,20,21] Forty-one of the 141 ischaemic patients, aged 47 ± 12 years, 28 males, 31 in NYHA III and 10 in NYHA IV, had suffered a myocardial infarct 6 months to 10 years (median 2 years) previously; ejection fraction ranged from 16 to 44% (mean 30 ± 7).[9,20,21]

Follow-up

All patients were invited for follow-up at 3–6-month intervals at a dedicated outpatient clinic. Assessment included physical examination, 12-lead electrocardiogram, and TTE on each visit. Treatment did not include immunosuppressant, anti-viral, or immunomodulatory treatments.

Statistics

Results for quantitative features are given as mean $\pm$ SD or as median (interquartile range) for variables deviating from the normal distribution. Student's *t*-test, one-way analysis of variance, χ^2 test, Fisher's exact test, or Kolmorogov–Smirnov test were used as appropriate. Endpoints/outcomes of interest during the follow-up period were death or heart transplantation. The Kaplan–Meier method was used to construct life tables of the likelihood of survival free from heart transplantation or death. Differences between actuarial curves were analysed by the Mantel–Haenszel log-rank test. Cox univariate analysis was used to assess associations between clinical and diagnostic features and survival to death or heart transplantation; results are expressed with the hazard ratios (HRs) and their associated 95% confidence intervals (CIs). All *P*-values were two-tailed; *P*-values less than 0.05 were considered to indicate statistical significance. All statistical analyses were performed using the SPSS software version 12.0 (SPSS, Inc., Chicago, IL, USA; 1998).

Results

Clinical features at presentation

Baseline features at presentation are detailed in *Table 1*. History of non-cardiac autoimmune disease was present in 9% of patients and allergy in 17%. Clinical presentation was

Table 1 Baseline features of the 174 myocarditis patients

Family history of autoimmune disease (%)	20 (11)
Family history of non-ischaemic heart disease (%)	60 (34)
Acute viral infection in the last 6 months (%)	62 (36)
History of myocarditis (clinical/biopsy-proven) (%)	28 (16)/10 (6)
Presentation (groups I/II/III) (%)	33 (19)/ 94 (54)/ 47 (27)
NYHA (I/II/III/IV) (%)	80 (46)/27 (15)/ 56 (32)/11 (6)
Afib/other non-sinus rhythm (%)	10 (6)/12 (7)
Bundle branch block (%)	27 (15)
Atrioventricular block (%)	17 (10)
TTE LV end-diastolic volume (mL/m^2)[a]	83 (63–120)
TTE LVEF (%)	43 ± 15
Severe RV dilation by TTE (%)	10 (6)
Severe RV systolic dysfunction by TTE (%)	7 (4)
Angiographic LV end-diastolic volume (mL/m^2)	111 ± 45
Angiographic LVEF (%)	47 ± 18
Mean aortic pressure (mmHg)	82 ± 14
LV systolic pressure (LVSP) (mmHg)[a]	118 (100–130)
LV end-diastolic pressure (mmHg)[a]	12 (8–20)
Mean capillary wedge pressure (PCW) (mmHg)[a]	11 (7–18)
Mean right atrial pressure (mRA) (mmHg)[a]	4 (2–7)
Pulmonary artery systolic pressure (mmHg)[a]	30 (25–38)
Pulmonary artery diastolic pressure (PAD) (mmHg)[a]	10 (6–15)
Mean pulmonary artery pressure (mmHg)[a]	16 (12–22)
RV systolic pressure (mmHg)[a]	30 (25–38)
RV end-diastolic pressure (RVEDP) (mmHg)[a]	5 (2–8)
Cardiac output ($L/min/m^2$)[a]	3.1 (2.7–3.7)

Afib, atrial fibrillation.
[a]Median (25–75th).

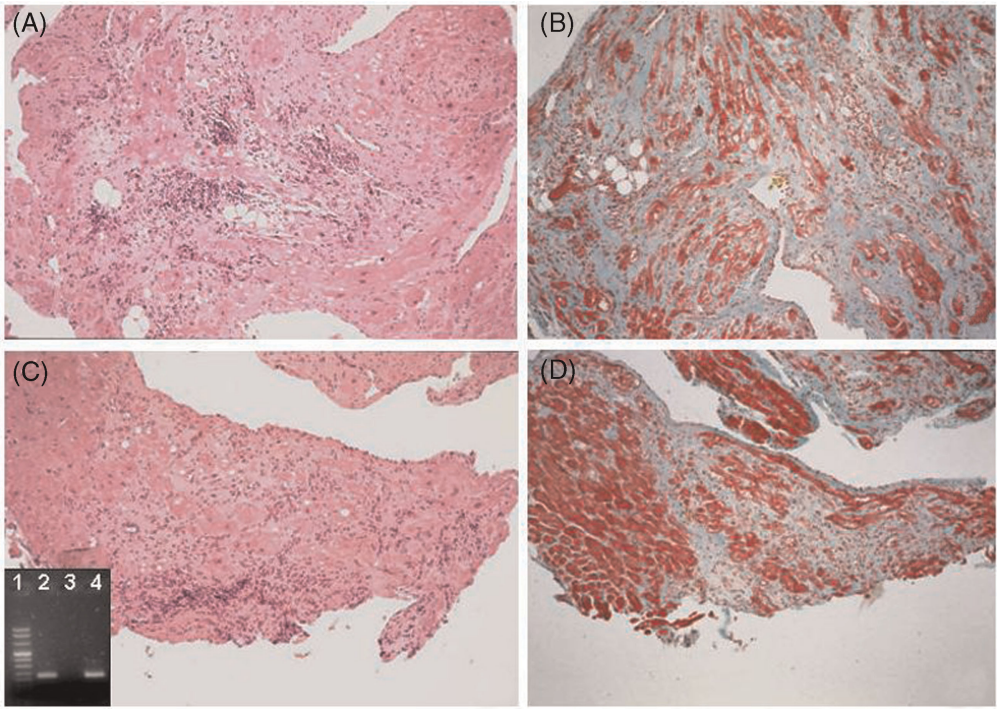

Figure 1 Active myocarditis: extensive inflammatory infiltrates, myocyte necrosis and fibrosis on endomyocardial biopsy (*A* and *C*: haematoxylin–eosin and *B* and *D*: Masson trichrome stain). Original magnification: ×10. Insert: polymerase chain reaction analysis for parvovirus B19: line 1: DNA marker; line 2: endomyocardial biopsy positive for parvovirus B19; line 3: negative control (reagents without template); and line 4: parvovirus positive control.

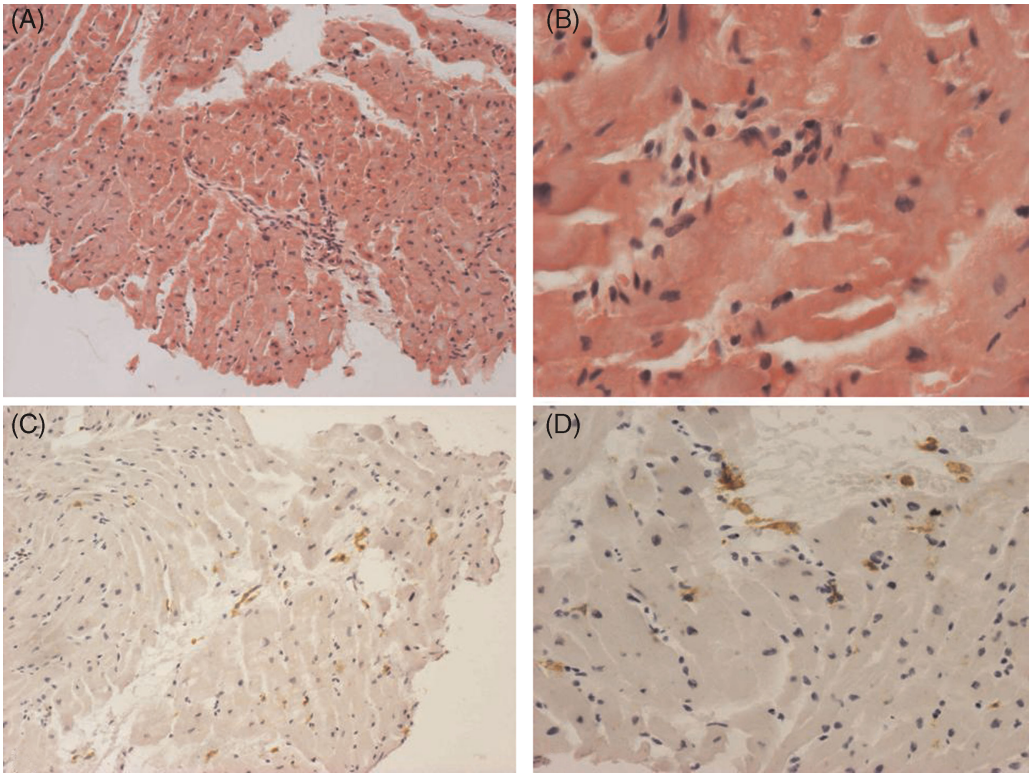

Figure 2 Borderline myocarditis: sparse lymphocytes without myocyte necrosis on endomyocardial biopsy (*A* and *B*: haematoxylin–eosin, magnification: ×10 and ×40, respectively). (*C*) and (*D*): immunohistochemistry for CD3. Magnification ×10 and ×30. PCR analysis was negative for viruses (data not shown).

with arrhythmia and/or syncope (19%, group I), symptomatic or asymptomatic LV and/or RV dysfunction (54%, group II), and chest pain at rest with abnormal cardiac enzymes (median troponin I levels of 8 ng/mL, interquartile range 1.5–20.6) and normal coronary arteries (27%, group III). Cardiac symptoms preceding hospital admission (median duration 0.5 months, interquartile range 0–3) were often reported; 86 patients were in NYHA class I, 51 in II, 30 in III,

and seven in IV. NYHA distribution at diagnosis, shown in *Table 1*, was worse when compared with that prior to hospital admission ($P = 0.001$). Two patients presented with heart failure in the peri-partum. In addition to the ECG findings shown in *Table 1*, ST-T abnormalities in the absence of bundle branch block or LV hypertrophy were found in 69 (40%) patients: negative T waves in 42, of whom 11 with associated ST-elevation of non-ischaemic type, ST-elevation of non-ischaemic type only in 25, and ST-depression in two. On EMB, 85 patients had active and 89 borderline myocarditis in the absence of diffuse or severe inflammation (lymphocytic in 162, giant cell in five, and others in seven) (*Figures 1* and *2*).

Anti-heart autoantibodies and viral genome by polymerase chain reaction: frequency and clinical correlates by univariate analysis

AHA of IgG class were detected in 73 (56%) of the study patients: 54 (41%) of the organ-specific type and 19 (15%) of the partially organ-specific type (*Figure 3*). The frequency of organ-specific AHA was higher ($P < 0.0001$) in myocarditis (41%) than in non-inflammatory heart disease (1%), ischaemic heart disease (1%), or normal blood donors (2.5%). Similarly, the frequency of the partially organ-specific AHA was higher ($P < 0.0001$) in myocarditis (15%) than in non-inflammatory heart disease (4%), ischaemic heart disease (1%), or normal subjects (3%). The finding of positive

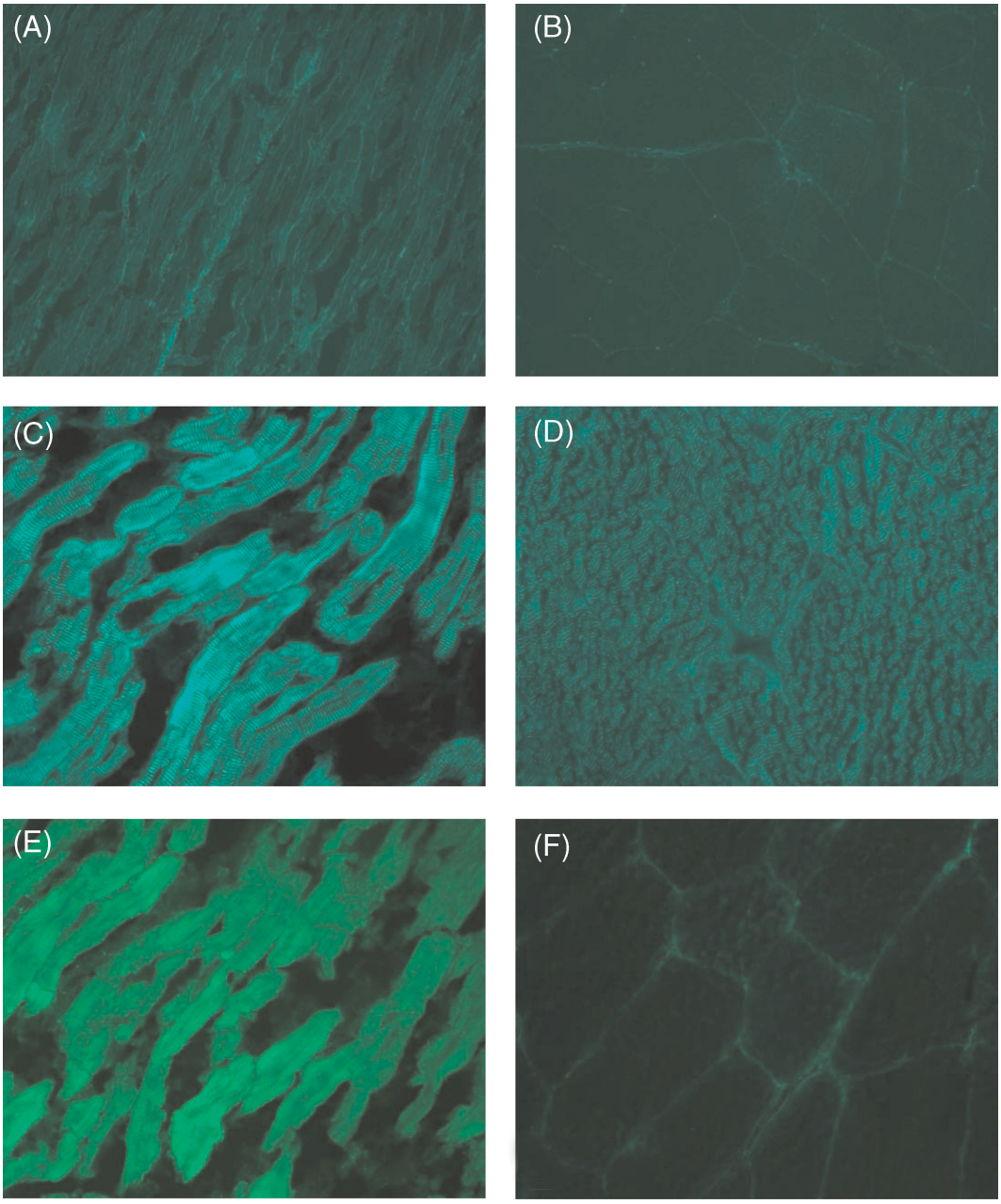

Figure 3 Blood group O normal human atrium (left panels) and skeletal muscle (right panels) stained with (*A* and *B*) an anti-heart autoantibodies-negative control serum from a normal subject. No myocyte or muscle staining is present. (*C* and *D*): a serum from a myocarditis patient, containing partially organ-specific anti-heart autoantibodies. A fine striational indirect immunofluorescence is visible on atrial tissue; skeletal muscle is weakly positive. (*E* and *F*): a serum from a myocarditis patient, containing organ-specific anti-heart autoantibodies. A diffuse cytoplasmic indirect immunofluorescence is visible on atrial myocytes, not on skeletal muscle. Magnification ×400.

Table 2 Associations with anti-heart autoantibodies and polymerase chain reaction status in myocarditis patients

	AHA positive ($n = 73$)	AHA negative ($n = 57$)	*P*-value
Family history of heart disease (%)	33 (45)	15 (26)	0.03
AFib or non-sinus rhythm (%)	13 (18)	2 (3.5)	0.04
Troponin I (ng/mL)[a]	7.4 (0.66–20)	13 (7.4–43.6)	0.09
Symptom duration (months)[a]	0.25 (0–5.5)	0.5 (0–2)	0.40
RVEDP (mmHg)[a]	5 (1–6)	5 (2–10)	0.80
PCR positive/ negative for virus[b]	12/47	9/30	0.74
	PCR positive ($n = 31$)	PCR negative ($n = 89$)	*P*-value
Clinical LV failure (%)	21 (68)	34 (38)	0.005
Clinical RV failure (%)	12 (39)	15 (17)	0.01
Symptom duration (months)[a]	1.5 (0–12)	0.3 (0–2.5)	0.20
TTE LVEF (%)	38 ± 14	45 ± 14	0.04
mRA (mmHg)[a]	5 (3.5–10.5)	4 (2–6.5)	0.40
Cardiac output (L/ min/m^2)[a]	2.9 (2.5–3.1)	3.2 (2.7–3.9)	0.20
LV stroke volume (mL/min/m^2)[a]	39 (30–54)	50 (36–62)	0.08
AHA positive/ negative[b]	12/9	47/30	0.74

Abbreviation as in *Table 1*.
[a]Median (25–75th).
[b]PCR available in 98 of the 130 patients tested for AHA.

Table 3 Frequency of virus-positive patients by polymerase chain reaction

Virus	PCR positive[a], *n* (%)
Mumps	3 (2.5)
Adenovirus	6 (5)
Herpes simplex virus	1 (1)
Epstein–Barr virus	5 (4)
Cytomegalovirus	3 (2.5)
Enterovirus	15 (12.5)
Influenzavirus A	0 (0)
Influenzavirus B	0 (0)
Parvovirus B19	3 (2.5)
Hepatitis virus C	2 (2)

[a]Five of the 120 patients were positive for more than one virus.

family history of non-ischaemic heart disease (defined as the presence of one or more alive or dead family members with cardiomyopathy in the absence of documented coronary artery disease) was more frequent among AHA-positive myocarditis patients; the proportion of those with atrial fibrillation or non-sinus rhythm was higher and peak troponin I levels were non-significantly lower (*Table 2*).

The frequencies of viral genomes by PCR are detailed in *Table 3*. Overall, 31 (26%) of the 120 tested were virus-positive; five patients were positive for more than one virus. The frequency of PCR-positive samples was higher ($P = 0.007$) in myocarditis (26%) than in disease or normal control samples (0%). LV or RV failure was more frequent among PCR-positive patients; TTE–LV ejection fraction (LVEF) was lower and the LV stroke volume was non-significantly lower (*Table 2*).

Of the 98 patients in whom combined PCR and AHA was available, myocarditis was classified as autoimmune (positive AHA and virus-negative PCR) in 48% of patients, viral (virus-positive PCR and negative AHA) in 9%, viral and immune (virus-positive PCR and positive AHA) in 12%, and idiopathic and/or cell-mediated (virus-negative PCR and negative AHA) in 31%.

Predictors of death or transplantation and survival curves

At follow-up (median duration 23.5 months, interquartile range 10–54), 124 patients were alive without being transplanted, 26 dead or transplanted, and 24 (14%) lost; 121 patients were in NYHA I/II and three in III, with LVEF of 56 ± 13%. Actuarial survival was 87% at 2 years, 80% at 3 years, and 73% at 6 years, respectively (*Figure 4*, left panel); actuarial survival was lower in group II patients ($P = 0.0005$) (*Figure 4*, right panel). Probability or survival was also lower in giant cell myocarditis ($P = 0.004$) (*Figure 5*, top panel) and among patients with a positive PCR ($P = 0.02$) (*Figure 5*, bottom panel). A positive PCR was more common among dead/transplanted (7/15, 47%) than alive without being transplanted patients (17/93, 18%, $P = 0.01$).

Associations between clinical and diagnostic features and survival to death or heart transplantation by the Cox univariate analysis are detailed in *Table 4*. Predictors were young age, clinical signs/symptoms of left and right heart failure, presentation with LV dysfunction, NYHA II–IV, longer symptom duration, and echocardiographic and haemodynamic indexes of left and right heart dysfunction. Highest risk was conferred by clinical signs/symptoms of left (HR = 4.3, CI 1.7–10.8, $P = 0.002$) and right heart failure (HR 3.4, CI 1.5–7.3, $P = 0.002$).

Discussion

Prognostic relevance of clinical and diagnostic features at presentation

This prospective study provides evidence for the unfavourable prognostic value of left and right heart dysfunction at presentation in biopsy-proven myocarditis. RV dysfunction at diagnosis may reflect a greater severity of myocarditis in the RV, and/or its higher susceptibility to viral and/or immune-mediated damage.[29] RV dysfunction is also a powerful predictor of adverse prognosis in advanced heart failure secondary to ischaemic or non-ischaemic DCM.[30]

In our study, as in other reports,[4,31] the proportion (27%) of dead or transplanted patients at 6 years is high.[4,31] Thus, biopsy-proven myocarditis should be regarded as a potentially ominous disease, particularly in the young presenting with LV and/or RV failure, and should lead to closer follow-up and prompt diagnosis by EMB.[2,12,27,32] We observed no EMB-related complications. The adjunct of immunohistochemistry to the histological Dallas criteria, as

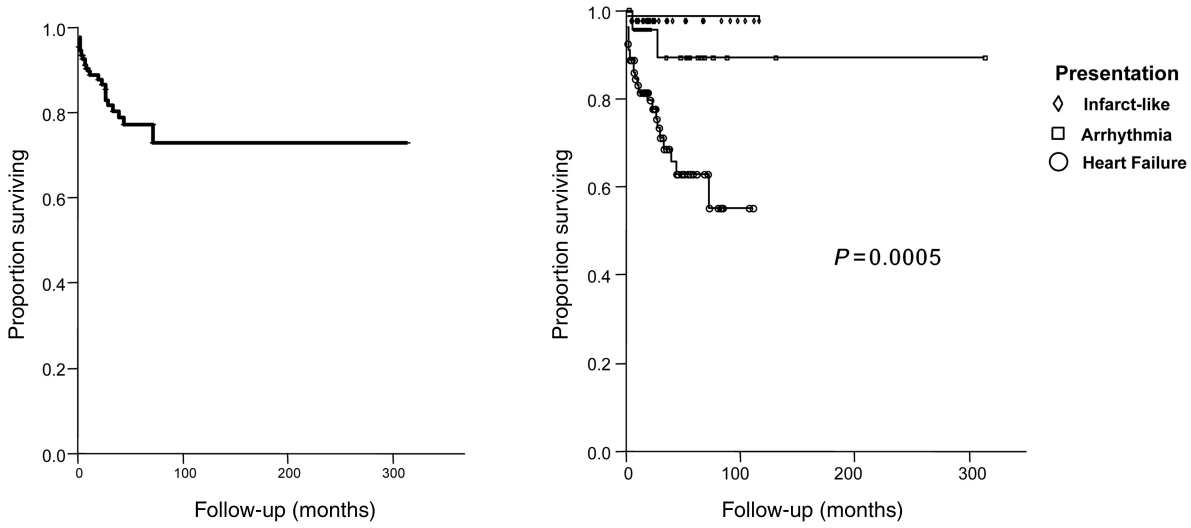

Figure 4 Left panel: probability for myocarditis patients of remaining free from death or transplantation. Right panel: probability for myocarditis patients of remaining free from death or transplantation according to clinical presentation (log-rank test). Patients with heart failure presentation had a worse outcome when compared with those with infarct-like or arrhythmic onset.

confirmed here, enhances the sensitivity of EMB; more than half of our patients, with a Dallas diagnosis of borderline myocarditis, would have not been unequivocally diagnosed in the absence of immunohistochemistry. All of them fulfilled the recognized immunohistochemical cutoff of $\geq$14 common leucocyte antigen positive cells/mm^2.[2] Only four of our 174 patients fell into the previous definition of 'fulminant' myocarditis,[4] and of these, three had giant cell and one peri-partum myocarditis. McCarthy *et al.*[4] excluded both forms from their analysis. This may be misleading because, as reported[3] and confirmed here, giant cell myocarditis has a worse outcome when compared with other histological types.

Viral genomes at presentation and prognosis

In our study, a positive PCR for virus was a univariate predictor of adverse prognosis. Our results are among the first prospective data in adult myocarditis diagnosed according to the World Health Organization definition (e.g. the Dallas histological criteria with the adjunct of immunological and immunohistochemical criteria).[1,28]

In keeping with others,[10–13,18,19] we found that the frequency of positive PCR in myocarditis was higher (26%) than in the controls (0%), supporting a causative association in adults. As far as the frequencies of the individual viruses are concerned, these have been highly variable.[7,10–14,18,19,33] Many factors may influence these figures: age (paediatric vs. adult), ethnicity, geographic and temporal variations in the epidemiology of viral infections, duration of symptoms in relation to the timing of EMB, number of EMB samples tested by PCR, and the use of autopsy myocardium rather than frozen EMB.[7] This prevents us from drawing any epidemiological conclusion. The number of samples tested by PCR was lower in our study than in some,[32,34,35] but not all, previous studies.[10,18,19] Recent cross-sectional studies, reporting a much higher frequency of positive PCR for one or more viruses, were in patients with idiopathic LV dysfunction[32] or LV diastolic dysfunction in the absence of myocarditis,[34] thus their findings are not comparable with our study.

Anti-heart autoantibodies status at presentation and prognosis

We found AHA in a high proportion (56%) of myocarditis patients; the low frequency in controls and the association of AHA with family history for cardiomyopathy are in keeping with what is observed in other autoimmune diseases.[36,37] AHA are more often detected in the acute phase of myocardial inflammation than in the chronic stage, e.g. DCM.[9,20,22,23] AHA in DCM are associated with 'early' disease,[20] and their titres become reduced with disease progression[22]; further, AHA are present in asymptomatic relatives, years before any echocardiographic abnormality.[21,36] Thus, present evidence suggests that AHA represent early markers. Autoimmune myocarditis, identified by positive AHA and negative PCR, was the most common form (48%), suggesting that a majority of patients may benefit of immunosuppression. No aetiopathogenetic markers were used in the Myocarditis Treatment trial, producing negative results in relation to the role of immunosuppression.[8] Conversely, recent studies suggest their beneficial effects in immune-mediated myocardial disease, identified by HLA up-regulation on EMB or serum AHA,[12,26] in keeping with its efficacy in non-cardiac autoimmune disease.[37] A prospective randomized trial, recruiting AHA positive and PCR negative patients for immunosuppression, is warranted.

In this study, 31% of cases were negative for both AHA and PCR. These might be classified as 'idiopathic myocarditis' and could reflect viral myocarditis, owing to yet unknown pathogens, or, most likely, a cell-mediated autoimmune form[37] that might also benefit of immunosuppression. AHA occurred in association with positive PCR for virus in 12% of patients. These patients might be candidates for anti-viral and, after virus clearance, immunosuppression or combined anti-viral and immunosuppressive therapy.

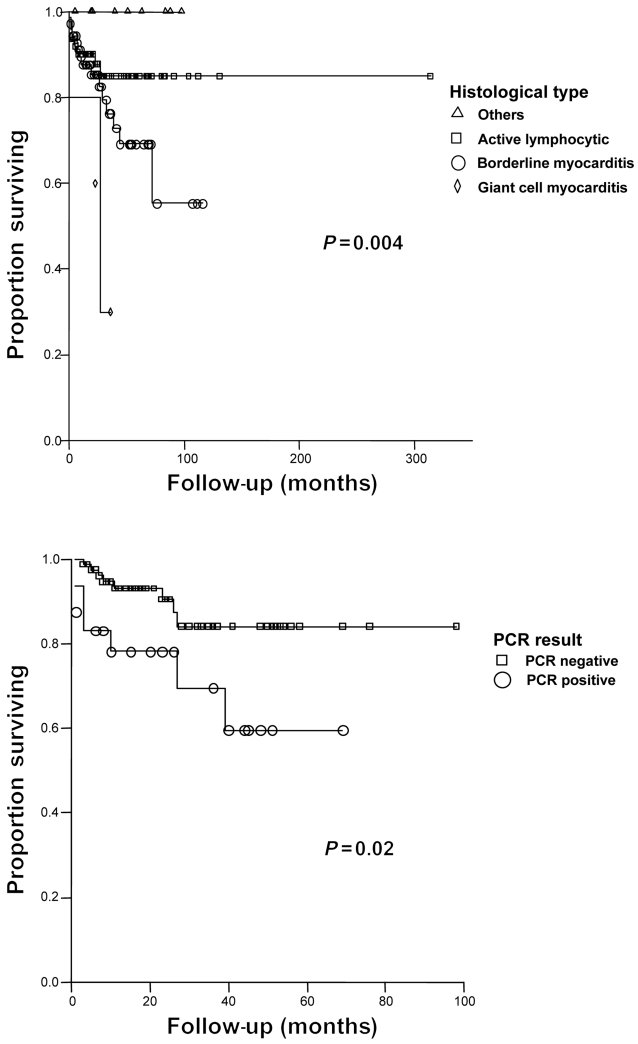

Figure 5 Top panel: probability for myocarditis patients of remaining free from death or transplantation according to histological type (log-rank test); patients with giant cell myocarditis had a lower probability of survival than those with active lymphocytic, borderline myocarditis, or other histological types (sarcoid, polymorphic, and oeosinophilic). Bottom panel: probability for myocarditis patients of remaining free from death or transplantation according to polymerase chain reaction result for virus (log-rank test); patients with positive polymerase chain reaction had a lower probability of survival than those with negative polymerase chain reaction.

Conclusions

In biopsy-proven myocarditis, biventricular dysfunction at diagnosis was the main unfavourable predictor. AHA identified immune-mediated myocarditis in the majority of cases. Viral genome was a univariate predictor of adverse prognosis. Our approach of using AHA in conjunction with PCR to identify aetiopathogenesis in the individual subject should set the basis for patient characterization and recruitment in future studies on aetiological therapy.

Acknowledgements

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Table 4 Associations between clinical and diagnostic features and survival to death or heart transplantation in myocarditis patients by the Cox univariate analysis

	HR	CI	*P*-value
Young age (years)	1.03	1.01–1.05	0.03
Clinical LV failure (%)	4.3	1.7–10.8	0.002
Clinical RV failure (%)	3.4	1.5–7.3	0.002
Presentation (groups I/II/III)	2.7	1.4–4.9	0.001
NYHA (II, III, or IV) (%)	1.6	1.1–2.3	0.01
Bundle branch block (%)	1.5	0.9–2.4	0.09
Longer symptom duration (months)	1.05	1.02–1.08	0.0001
Lower TTE LVEF (%)	1.09	1.04–1.15	0.001
Higher TTE LVEDV (mL/m^2)	1.009	1–1.017	0.05
Lower angiographic LVEF (%)	1.07	1.03–1.13	0.001
Lower LVSP (mmHg)	1.03	1.01–1.05	0.001
Higher PCW (mmHg)	1.06	1.004–1.12	0.03
Higher mRA (mmHg)	1.2	1.1–1.3	0.0001
Higher PAD (mmHg)	1.06	1.01–1.1	0.01
Higher RVEDP (mmHg)	1.07	1.04–1.1	0.0001
Severe TTE RV dilation (%)	1.2	0.8–1.8	0.20
Severe TTE RVEF depression (%)	1.6	1.07–2.4	0.02
CO (L/min/m^2)	1.9	1.08–4.1	0.08

Abbreviations as in text and *Table 1*.

Conflict of interest: none declared.

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