Myocarditis as a precipitating factor for heart failure: evaluation and 1-year follow-up using cardiovascular magnetic resonance and endomyocardial biopsy

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Aims

The aim of this study was to evaluate myocarditis as a precipitating factor for heart failure using cardiovascular magnetic resonance (CMR) and endomyocardial biopsy

Methods and results

Eighty-five patients with suspected myocarditis and 20 controls were evaluated. Seventy-one patients with positive CMR were referred for endomyocardial biopsy and re-evaluation after 1 year. Cardiovascular magnetic resonance was performed using STIR T2-weighted (T2W), early T1-weighted (EGE), and late gadolinium-enhanced (LGE) images. Immunohistological and polymerase chain reaction (PCR) analysis of myocardial specimens was employed.

In patients with myocarditis, T2 and EGE were increased compared with controls (2.6 ± 0.9 vs. 1.57 ± 0.13, P < 0.001 and 7.9 ± 5.5 vs. 3.59 ± 0.08, P < 0.001, respectively). Late gadolinium enhancement was found in all myocarditis patients. Endomyocardial biopsy performed in 50 of 71 patients with positive CMR showed positive immunohistology in 48% and presence of infectious genomes in 80% (mainly Chlamydia, Herpes, and Parvovirus B19). Left ventricular ejection fraction (LVEF) was significantly decreased compared with controls (47.7 ± 19.2 vs. 64 ± 0.2, P < 0.001). After 1 year, CMR showed normalization of T2 and EGE, and decreased LGE. Left ventricular ejection fraction increased in 36.5% of patients, remained stable in 56.5% and decreased in 7% of patients, in whom biopsy showed persistence of the initial infective agents. A negative correlation was identified between EGE, LGE, and LVEF. Patients with positive biopsies had lower LVEFs.

Conclusion

In a Greek population with myocarditis, Chlamydia with viruses was a common finding. Cardiovascular magnetic resonance and PCR proved useful for the detection of myocarditis; EGE and LGE had the best correlation for the development of heart failure. Persistence of the initially detected infective agents was identified in patients who deteriorated further.

Keywords

Myocarditis • Viruses • Chlamydia • Biopsy • Polymerase chain reaction • Coronary artery disease • Magnetic resonance imaging • Heart failure

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Introduction

Myocarditis is inflammation of the heart due to infectious, toxic, or autoimmune processes. The clinical presentation of the disease ranges from non-specific systemic symptoms (fever, myalgia, palpitations, or exertional dyspnoea) to fulminant haemodynamic collapse and sudden death. Myocarditis can also mimic acute myocardial infarction, with sudden onset of chest pain, electrocardiogram (ECG) abnormalities, troponin elevation, arrhythmias, and haemodynamic instability. According to recent data, the prevalence of myocarditis was found to be up to 42% for unexplained deaths in people aged 35 years or younger and was documented as a cause of dilated cardiomyopathy in 5–10% of patients in a large prospective series. The diagnosis of myocarditis is still a challenge, due to the non-specific pattern of clinical presentation and the lack of a standardized diagnostic process. Furthermore, the evolution from myocarditis to heart failure is not fully understood.

Cardiovascular magnetic resonance is a promising tool for diagnosing myocarditis, it has been shown that contrast enhancement evolves from a focal to a disseminated process during the first weeks after the onset of symptoms. According to recent reports from the EuroCMR registry, myocarditis is the most common indication for CMR. However, CMR findings need further validation against immunohistology of myocardial specimens. The only known myocardial biopsy data were obtained in a German population and to our knowledge, except for some case reports, there is minimal data available about the presence of viral genomes in other European countries.

Methods

Patient population

Patients with a clinical suspicion of acute myocarditis were eligible to be included in this prospective study. The inclusion criteria were based on a combination of chest pain, dyspnoea or altered ECG, increase in troponin I and/or NT-proBNP, with or without a history of flu-like symptoms or gastroenteritis, and elevated C-reactive protein within 4–12 (median 8) weeks before admission, and with no evidence of coronary artery disease or spasm defined by catheterization. In addition, 20 matched healthy volunteers with no known history of myocardial inflammation or infarction were also included in the study. The CMR study was performed 3–7 days after patient admission to the hospital. Patients who did not fulfill all of the clinical criteria for myocarditis and had a negative CMR evaluation for myocarditis were excluded from the study. Patients with a positive CMR evaluation for myocarditis were referred for an endomyocardial biopsy (EMB) and then re-evaluated by CMR 1 year later.

Image analysis

In T2W, the signal ratio was measured from the region of interest covering the left ventricular myocardium as well as a skeletal muscle in the same slice. Cardiovascular magnetic resonance functional study

For each subject, localizing scans were obtained to define the long (two-chamber) axis of the left ventricle. A mid-ventricular short-axis view was prescribed, and used to plan a four-chamber view. The short-axis orientation was then defined accurately, perpendicular to both the two- and four-chamber views. To cover the entire left ventricle, 10 contiguous (gap = 0 mm) short-axis slices were acquired in each study. The imaging sequence was a 2D, multi-phase (16 cardiac phases were acquired per cardiac cycle resulting in a temporal resolution of 47 ms for a heart rate of 80 b.p.m.), steady-state free-precession sequence (TE = 1.5 ms, TR = 3.1 ms, flip angle = 70°, slice thickness = 8 mm, acquired in-plane spatial resolution = 1.8 mm × 2.0 mm) characterized by the application of balanced gradients in all directions.

Cardiovascular magnetic resonance evaluation of inflammation

The CMR examination was performed in a 1.5 T Philips Intera system, using STIR T2W, T1W before and early after contrast media injection (EGE), and LGE images. Electrocardiogram-triggered, STIR T2W multislice spin-echo sequencing was performed in axial orientation and the signal ratio measured from the region of interest covering the myocardium of the left ventricle as well as a skeletal muscle in the same slice. Cardiovascular magnetic resonance-triggered T1W multislice spin-echo images were also obtained in axial orientation with identical parameters, before and after an intravenous bolus of 0.1 mmol/kg Gd-DTPA. Measurements after Gd-DTPA were started within 1 min of injection (EGE) in the same area as for T2W. Immediately after the second set of T1W images, 0.1 mmol/kg Gd-DTPA was given again and LGE images were taken 15 min later, using a 3D–T1-TFE sequence, pre-conditioned with a 180° inversion pulse (flip angle = 15°, TE = 1.4 ms, TR = 5.5 ms, TI 225–275 ms as individually optimized to null myocardial signal, matrix 256X192 and slice thickness = 5 mm). Images were analysed according to previously described protocols.

Cardiovascular magnetic resonance functional study

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Image analysis

In T2W, the signal ratio was measured from the region of interest covering the left ventricular myocardium as well as a skeletal muscle in the same slice.

In T1W early enhancement (EGE), which reflects hyperaemia and capillary leakage as a marker of inflammation, the early myocardial enhancement was measured from the region of interest covering the left ventricular myocardium as well as a skeletal muscle in the same slice. To assess the contrast-enhanced images (LGE), all short-axis slices from base to apex were inspected visually to identify areas of normal (completely nulled) myocardium. Mean signal intensity and standard deviation (SD) was derived and a threshold of > 6 SD exceeding the mean was used to define areas of LGE. Summing the planimetered areas of LGE in all short-axis slices yielded the total volume, which was also expressed as a proportion of total LV myocardium (% LGE). The LGE analysis was performed by one experienced
reader and reviewed and confirmed by a second expert reader with both of the independent readers blinded to patient’s identity and clinical profile. Any discrepancies in the analysis between the two readers was then adjudicated by a senior reader with >10 years of CMR experience, also blinded to patient’s identity and clinical profile.

Cine images were used for the evaluation of left ventricular ejection fraction (LVEF). Left ventricular endocardial borders were outlined on the end-diastolic and end-systolic short-axis view images covering the entire LV. Papillary muscles were considered as myocardium. Left ventricular ejection fraction was calculated as follows: LVEF = [(volume at end-diastole – volume at end-systole)/volume at end-diastole]. The MRI-MASS, Medis, Leiden, the Netherlands software was used and the readers were blinded to the clinical data.

Endomyocardial biopsy

Patients who were CMR positive for myocarditis, who had either reduced LVEF and/or a recent increase in troponin and a normal coronary angiogram, underwent an EMB in accordance with current guidelines. Eight EMB specimens were obtained from the right side of the ventricular septum of each patient with clinically suspected myocarditis, using a flexible biopomate (Westmed, Germany) via the femoral vein approach. Four specimens were used for the histological evaluation, and the remaining four specimens were subjected to DNA and RNA extraction, using commercially prepared protocols, to detect viral or microbial genomes. Post-biopsy pericarditis without significant haemodynamic deterioration occurred in 2 of the 50 patients who underwent EMB.

Histopathological analysis

Endomyocardial biopsies were stained with Masson’s trichrome as well as Giemsa stain and examined by light microscopy. For immunohistological identification of cardiac immune cells, tissue sections were treated with an avidin–biotin–immunoperoxidase method according to the manufacturer’s protocol ( Vectastain Elite ABC Kit, Vector), applying the following monoclonal antibodies: CD3 (T cells, Novocastra Laboratories) and PGM1 (macrophages, Dako).

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The detection of >14 infiltrating leucocytes/mm² in the presence of myocyte damage and/or fibrosis, in addition to enhanced HLA class II expression in professional antigen-presenting immune cells and endothelium was used for the diagnosis of active myocarditis. Healing myocarditis was considered if the inflammation was less extensive (<14 leucocytes/mm²), whereas healed myocarditis was characterized by multifocal fibrosis or scarring without inflammation (0–3 leucocytes/mm²), which is identical to normal myocardium.

Detection of viral genomes

DNA and RNA were extracted simultaneously from frozen heart muscle tissue probes. Polymerase chain reaction (PCR)/reverse transcriptase (RT)–PCR was performed for the detection of enteroviruses (EVs, including Coxackieviruses and Echoviruses), adenoviruses (ADVs), Parvovirus B19 (PVB19), Human Cytomegalovirus, Epstein-Barr-virus, Herpes virus 1–6, Chlamydia pneumoniae, and trachomatis.23–25 The quality (purity) and quantity of the extracted nucleic acids (DNA, RNA) was evaluated on a Nanodrop 2000c spectrophotometer (ThermoScientific, Mannheim, Germany). As a control for the successful extraction of RNAs, as well as for the presence of PCR inhibitors a reverse transcription real time PCR assay was designed with primer/probe sequences from the G-6PDH (glucose-6-phosphate dehydrogenase) or b2-microglobulin or human acidic ribosomal protein cDNAs (data not shown). For the verification of the DNA extraction, a conventional PCR assay was designed with specific primers for the human b-globin gene [intraventricular septum (IVS)-2, data not shown]. During the initial application of the experiment, the specificity of the assay was confirmed by checking the primer/probe sequences for possible homologies to all of the gene bank published sequences, by sequence comparison analysis.

Statistical analysis

All measurements are expressed as mean ± SD. Statistical significance of the differences was investigated using unpaired Student’s t-test. Correlation between variables was sought with Pearson’s correlation coefficient. For non-parametric data, the Mann–Whitney test and Spearman’s correlation coefficient were used, respectively. Statistical significance was considered for P < 0.05.

Results

A total of 85 patients with suspected myocarditis (mean age 42 ± 16 years) were included. Chest pain was the main reason for seeking medical attention in the majority of patients (n = 60), followed by symptoms of congestive heart failure (n = 25), arrhythmias in the form of non-sustained ventricular tachycardia, bigeminy or couplets (n = 20), and malaise (n = 5). Increased troponin I levels were found in 20 of 85 patients (23.5%), with range 0.9–20 μg/L (normal values <0.1 μg/L). Increased NT-proBNP levels were found in 15 of 85 patients (17.65%), with range 300–700 ng/L (normal values <125 ng/L). Increased C-reactive protein levels were found in 15 of 85 patients (17.65%) with range 30–60 mg/L and in 10 of 85 patients (11.76%) with range 60–80 mg/L (normal values <5 mg/L).

T2 and EGE values were considered as increased if T2 > 2 and EGE > 4. According to this definition and the lack of adequate clinical symptoms/signs of myocarditis, 14 patients were excluded from the study. In the remaining 71 patients, T2 values were significantly increased compared with controls (2.6 ± 0.9 vs. 1.57 ± 0.13, P < 0.001). The presence of high T2 values was the main characteristic of patients with a recent presentation of the disease, as also described in other studies.26,27 The EGE values were also significantly increased compared with controls (7.9 ± 5.5 vs. 3.59 ± 0.08, P < 0.001). Epicardial and/or intramyocardial LGE was found in all myocarditis patients, but none of the controls. Late gadolinium enhancement was located in the posterolateral wall (PS) of the left ventricle in 55 of 71 patients, in the IVS in 6 of 71 and in both IVS and PS in 10 of 71 patients. The LGE extent was 7.8 ± 6.3% of myocardial mass. Left ventricular end-diastolic (LVEDV) and left ventricular end-systolic (LVEDV) volumes were significantly increased and LVEF significantly decreased in patients with myocarditis compared with controls (187.8 ± 64.6 vs. 123. ± 15.72 mL, 107.8 ± 71 vs. 44.6 ± 6.16 mL and 47.7 ± 19.2 vs. 64 ± 0.2, P < 0.001, respectively).

Endomyocardial biopsy was performed in 50 of the 71 patients. Twenty-four of these patients (48%) were diagnosed with myocarditis by immunohistology (acute 11 of 24 and borderline 13 of 24), while viral or microbial genomes in the myocardium were demonstrated in 40 of 50 (80%). Chlamydia trachomatis was documented in 23 of 50, Herpes 1, 2 in 9 of 50, Parvo B19 in 8 of 50, CMV in 4 of 50, Coxackie in 5 of 50, and EBV in 1 of 50. A combination of Chlamydia with Herpes was found in 8 of 50 and of Chlamydia...
with Parvo B19 in 3 of 50. The combination of Chlamydia with EBV or CMV or Coxsackie was documented in three more patients (each patient with one combination). Combination of Herpes with Parvo B19 was identified in 2 of 50 patients. In 10 patients with Chlamydia in the myocardium, Chlamydia was also identified by PCR in their urogenital system and these patients had all been treated with antibiotics for urogenital infection due to Chlamydia in the past 8–15 months. Chlamydia and Parvo B19 provoked typical LGE lesions in the PS, while the combination of more than two infective factors had a more complicated pattern including LGE in both the IVS and PS. Cardiovascular magnetic resonance images from a patient with myocarditis due to C. trachomatis and Parvo-B19, positive histology and positive PCR are presented in Figures 1–3.

No deaths or heart transplantations were documented after 1 year and the repeat CMR evaluation showed that T2 and EGE were significantly lower compared with values at the first evaluation (1.4 ± 0.1 vs. 2.6 ± 0.9 and 4.1 ± 1.4 vs. 7.9 ± 5.5, respectively, P < 0.05). The LGE extent was significantly decreased compared with the first examination (3.7 ± 4 vs. 7.8 ± 6.3%, P < 0.05) and in 13 of 71 patients was completely resolved. The mean LVEDV, LVESV, and LVEF were 186.1 ± 61.1 mL, 100.1 ± 61.8 mL, and 50.9 ± 16.8%, respectively. Left ventricular ejection fraction increased in 36.5% of patients, remained stable in 56.5% and decreased in 7% of patients who presented with NYHA class II–III symptoms of heart failure. Changes in LVEF of >5% are presented graphically in Figure 4.

Of the 50 biopsied patients, 6 had a further reduction in their LVEF after 1 year. In the initial biopsies, 4 of 6 had a combination of Herpes and Chlamydia and 2 of 6 a combination of Parvo-B19 and Chlamydia. A second EMB in these patients demonstrated the persistence of these infectious agents in their myocardium.

No correlation was identified between T2, at either the initial or after 1 year evaluation, and left ventricular systolic indices. At the initial evaluation, EGE correlated negatively with LVEF (r = −0.30, P < 0.001) (Figure 5) and positively with LVESV (r = 0.23, P < 0.05). Initial EGE also correlated negatively with LVEF after 1 year (r = −0.27, P < 0.05). Late gadolinium enhancement at the 1 year evaluation correlated negatively with LVEF (r = −0.25, P < 0.05).

In addition, patients with a positive histology had a significantly lower LVEF both at the initial (P < 0.001) and 1 year evaluation (P < 0.01) compared with those with negative histology. Patients with positive PCR results had a significantly lower LVEF only during the initial evaluation (P < 0.05) compared with those with negative PCR (Table 1).

**Discussion**

In this study, a CMR protocol including T2, EGE, and LGE was applied in a group of Greek patients with clinically suspected myocarditis. The results of CMR were compared with those for immunohistology and PCR of the myocardium. The best agreement for the detection of myocarditis was between the CMR findings and PCR. Epicardial LGE, located mainly in the PS of the LV, was found in all of our myocarditis patients. Chlamydia trachomatis, Herpes and Parvo B19 or a combination of them, were the most common findings detected by PCR. After 1-year follow-up, LVEF was further decreased in six patients who had positive CMR and coexistence of Chlamydia and viruses on the initial myocardial biopsy. A second EMB in these patients revealed the persistence of the initially identified infectious agents. The EGE during the...
initial evaluation had the best correlation with the initial and 1-year follow-up LVEF values. Patients with either positive immunohistochemistry and/or positive PCR had the lowest LVEF values. The area of LGE after 1 year had a negative correlation with LVEF at that time.

Cardiovascular magnetic resonance is inherently very attractive for the detection of myocarditis, because it is sensitive to tissue changes taking place during inflammation. T2 is increased in inflammation or necrosis due to the development of edema. However, data comparing T2 values with myocardial biopsy and LVEF are missing in the literature. In our patients, higher T2 values were found in patients with the most recent presentation of the disease. However, the increase in T2 values did not correlate with the myocardial biopsy results or with LVEF values and had no correlation with the development of heart failure.

Higher levels of EGE are most likely due to increased cell membrane permeability. This process is an important contributor, because inflammation damages cell membranes through T-cell perforin and B-cell antibody complement-mediated mechanisms. Data comparing EGE values with myocardial biopsy and LVEF are also missing in the literature. In our patients, EGE had a negative

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**Figure 3** Positive polymerase chain reaction analysis indicative of Chlamydia (top) and Parvovirus-B19 (bottom).
correlation with LVEF at both the initial and 1-year follow-up evaluation. This finding further emphasizes its role as an inflammatory parameter that can contribute to LV dysfunction.

The exact pathophysiology of LGE in myocarditis is still under investigation, but it seems that myocardial necrosis in the acute phase plays a major role. In our patients, LGE was located mainly in the posterolateral part of the LV, which is in agreement with previous studies. The fact that LGE was documented in all of our patients seems rather surprising (it has previously been observed in between 30 and 88% of patients according to different studies). However, this may be due to the combination of different viral and microbial agents identified in our patients. Although the currently used CMR protocol could not identify the interstitial and moderately extensive fibrosis revealed at histology, it is important to note that LGE has been shown to have a good correlation with histopathology for detection of fibrosis \( r = 0.70, P < 0.001 \) in both hypertrophic cardiomyopathy and severe aortic stenosis. In addition, the negative correlation between LGE after 1 year and LVEF measured at that time is in agreement with previous studies and emphasizes the well-known role of fibrosis in the development of heart failure.

We also documented that EMB, using immunohistological criteria, was positive in only 48% of the examined patients. Our data are in agreement with previous studies, which have shown that the sensitivity of EMB, even after the application of immunohistological techniques, remains low. According to some studies the histological evaluation of myocardial specimens yields a diagnosis in only 10–20% of cases. Although biopsy provides crucial information regarding the type of inflammatory infiltrates (lymphocytic, neutrophilic, eosinophilic, granulomatous, or giant cell), sampling error remains a serious limitation. It is also important to note that only 4–6 biopsy samples are usually taken, however, a careful post-mortem analysis of proven myocarditis cases demonstrated that >17 samples were necessary to correctly diagnose myocarditis in >80% of cases. Thus, the lack of sensitivity of myocardial biopsy is apparent. Furthermore, EMB is an invasive technique and cannot be easily used for patient follow-up. However, the recent application of cardioscope-guided biopsy has been successfully used to increase the accuracy of histological diagnosis. In addition, there is no doubt that myocardial biopsy is of great value in identifying the inflammatory pathway in different types of myocarditis and/or cardiomyopathies before starting specific anti-inflammatory treatment.

The addition of PCR techniques provides very important information. However, a positive PCR by itself does not constitute proof of myocarditis, because evidence of viral genomes without positive histology has been found in otherwise healthy people. Different infectious agents can be innocent bystanders in the myocardium and only under special conditions, such as immune defects, contribute to clinically overt myocarditis. The combination

**Figure 4** Percentage of patients at 1-year follow-up with a >5% decrease in the left ventricular ejection fraction, stable left ventricular ejection fraction or a >5% increase in the left ventricular ejection fraction.

**Figure 5** At initial evaluation EGE correlated negatively with the left ventricular ejection fraction \( r = -0.30, P < 0.001 \).
of a CMR approach and PCR evaluation of myocardial specimens seems to be more sensitive for the diagnosis of myocarditis than the currently used gold-standard of myocardial histology.26

Viruses, bacteria, fungi, parasites, toxins, and immunological syndromes can cause myocarditis. The presence of Herpes, ParvoB19, or a combination of them has previously been identified in other studies.7 In our population, Chlamydia was the commonest microbial genome and often coexisted with different viruses. The high frequency of Chlamydia in the myocardium is a new finding and its clinical significance should be evaluated carefully. Although the heart is not the primary target for Chlamydia, once the microbes have been carried to the host’s heart, they promote inflammation.36 Myocarditis occurs when the host fails to control the infection. Under these circumstances, the real prevalence of Chlamydia-induced myocarditis may be higher than previously documented.37 It leads to the production of heart muscle-specific epitopes autoantibodies and Chlamydia-mediated heart disease.38

Although we do not know the exact incidence of Chlamydia as a causative factor for myocarditis, the high incidence of Chlamydia in the myocardium of our patients is a rather unusual finding according to data in the literature. However, the fact that 10 of 23 patients with positive myocardial PCR for Chlamydia had a previously well-documented Chlamydia infection may explain the possible role of Chlamydia in the pathogenesis of myocarditis in this population. The combination of Chlamydia with different viruses seems to have a detrimental effect on the development of heart failure. Since Chlamydia can be treated with antibiotics, its aetiological association with myocarditis could have possible therapeutic implications. However, the development of heart failure in patients already treated with antibiotics against Chlamydia shows that the evolution to heart failure is a more complicated phenomenon, due to a combination of both infectious and immunological process.

A broad spectrum of viral genomes has been detected in patients with LV dysfunction, often referred to as ‘past myocarditis’. The influence of this chronic viral infection on myocardial function is unknown, because biopsy-based follow-up data have never been obtained. The 1-year follow-up of our patients revealed that LV function had deteriorated in those patients with a combination of viral and microbial infection, despite the early initiation of intensive heart failure medication. A second EMB in those patients with heart failure, proved the persistence of the initially detected infectious agents. In addition, the finding that patients with either positive histology and/or positive PCR had a lower LVEF both at the initial and 1-year follow-up evaluation emphasizes the importance of positive biopsy findings in the development of heart failure. Our data are in agreement with other studies indicating that the persistence of infectious agents in the heart constitutes a major cause of LV dysfunction.39,40

The current study has several limitations due to (i) the lack of targeted biopsy in the area of LGE, due to higher number of complications28 (ii) the lack of biopsy data in normals (ethical reasons) (iii) the lack of biopsy data in all patients after 1-year follow-up (iv) the inability of the currently used CMR protocol to identify endocardial thrombi <3 mm, LV, and RV microaneurysms detected at angiography and the interstitial and moderately extensive fibrosis revealed at histology, and (v) the lack of long-term evaluation.

In conclusion, after evaluation of a Greek population with myocarditis, Chlamydia with viruses was the most common finding. Cardiovascular magnetic resonance and PCR proved useful for the detection of myocarditis; EGE and LGE had the best correlation for the development of heart failure. Persistence of the initially detected infectious agents was identified in patients who deteriorated further.

Conflict of interest: none declared.

References


Table I Comparison of left ventricular ejection fraction between patients with positive (+) and negative (−) biopsy and polymerase chain reaction analysis results at first evaluation (LVEF(1)) and after 1-year follow-up (LVEF(2))

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