Heart Failure

Predictors of Outcome in Patients With Suspected Myocarditis

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Background—The objective of this study was to identify the prognostic indicators in patients with suspected myocarditis who underwent endomyocardial biopsy.

Methods and Results—Between 1994 and 2007, 181 consecutive patients (age, 42±15 years) with clinically suspected viral myocarditis were enrolled and followed up for a mean of 59±42 months. Endomyocardial biopsies were studied for inflammation with histological (Dallas) and immunohistological criteria. Virus genome was detected by polymerase chain reaction. The primary end point was time to cardiac death or heart transplantation. In 38% of the patients (n=69), the Dallas criteria were positive. Immunohistological signs of inflammation were shown in 50% (n=91). Genomes of cardiotropic virus species were detected in 79 patients (44%). During follow-up, 22% of the patients (n=40) reached the primary end point. Three independent predictors were identified for the primary end point, namely New York Heart Association class III or IV at entry (hazard ratio, 3.20; 95% confidence interval, 1.36 to 7.57; P=0.008), immunohistological evidence of inflammatory infiltrates in the myocardium (hazard ratio, 3.46; 95% confidence interval, 1.39 to 8.62; P=0.008), and β-blocker therapy (hazard ratio, 0.43; 95% confidence interval, 0.21 to 0.91; P=0.027). Ejection fraction, left ventricular end-diastolic pressure, and left ventricular end-diastolic dimension index were predictive only in univariate, not in multivariate, analysis. Neither the Dallas criteria nor the detection of viral genome was a predictor of outcome.

Conclusions—For patients with suspected myocarditis, advanced New York Heart Association functional class, immunohistological signs of inflammation, and lack of β-blocker therapy, but not histology (positive Dallas criteria) or viral genome detection, are related to poor outcome. (Circulation. 2008;118:639-648.)

Key Words: biopsy ■ cardiomyopathy ■ immunohistochemistry ■ molecular biology ■ myocarditis

Vir al myocarditis has been identified by sophisticated tools for the analysis of endomyocardial biopsies1-4 as an important causal factor responsible for the progression to dilated cardiomyopathy,1,5-7 currently the most frequent reason for heart transplantation.7 The manifestation of myocarditis or inflammatory cardiomyopathy varies, with a broad spectrum of symptoms ranging from asymptomatic courses over presentations with signs of myocardial infarction to devastating illness with cardiogenic shock.5,6-8 The affected patient may recover or develop dilated cardiomyopathy with heart failure and the need for heart transplantation.6,8-9 The prognostic role of endomyocardial biopsy findings, particularly myocardial inflammation and viral genome detection in concert with clinical and left ventricular functional parameters, has not been studied prospectively in a large cohort. In the present study, patients with clinically suspected myocarditis underwent clinical assessment, echocardiography, and cardiac catheterization, as well as endomyocardial biopsy, with detailed analyses of specimens involving immunohistochemical staining for characterization of inflammation and polymerase chain reaction (PCR)/reverse-transcriptase (RT) PCR for viral genome detection. Our goal was to investigate the independent contribution of these parameters to the risk of cardiac death and the need for heart transplantation. This study is the first to investigate the prognostic impact of left ventricular functional variables, together with clinical data and myocardial biopsy results, in a multivariate fashion.

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Study Subjects
The study enrolled 181 consecutive patients who underwent endomyocardial biopsy as part of an evaluation for clinically suspected myocarditis at our institution between September 1994 and April

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2007. Criteria for inclusion were selected on the basis of previous reports and studies on myocarditis6–11 and were in accordance with the recently published American College of Cardiology, American Heart Association, and European Society of Cardiology recommendations4 to achieve a good comparability to previous works. Patients were included if they experienced an episode of a febrile infection of the bronchial tree, the gut, or the urinary tract within the last 6 months and at least one of the following features not related to myocardial ischemia: impaired global or regional left ventricular systolic function, an increase in serum concentrations of myocardial necrosis markers, pericardial effusion, or sustained or nonsustained ventricular tachycardia or ventricular fibrillation of unknown origin. Coronary artery disease had to be excluded by means of coronary angiography before a patient was eligible to participate. Patients with overt cardiogenic shock requiring vasopressors at initial presentation were not included in the study.

All patients underwent a careful history and physical examination, as well as selected laboratory studies, including thyroid function testing and measurements of antinuclear antibodies. In each patient, left ventricular end-diastolic and end-systolic diameters were measured with 2-dimensionally guided M-mode echocardiography. The study was approved by the appropriate ethics committee. All patients gave written informed consent to include their data in the study.

Study Design and End Points
The study was designed as a prospective longitudinal evaluation with patient follow-up scheduled at 6-month intervals in our heart failure outpatient clinic. All patients with impaired left ventricular function and/or heart failure symptoms received evidence-based medical treatment. Follow-up visits included a physical examination, recording of a 12-lead ECG, and further laboratory studies (eg, echocardiography) at the physician’s discretion. If patients were lost to follow-up, every attempt was made to at least make telephone contact with the patient to determine end-point occurrence.

The primary study end point was the time to cardiac death or heart transplantation. Time to all-cause death or heart transplantation and all-cause mortality were investigated as secondary end points.

Cardiac Catheterization and Endomyocardial Biopsy
Before endomyocardial biopsy, each patient underwent left heart catheterization with coronary angiography to exclude coronary artery disease. Left ventricular end-diastolic pressure was measured with standard fluid-filled catheters. Left ventricular ejection fraction was measured by contrast ventriculography in the 30° right anterior oblique view. If renal failure or excessive end-diastolic pressures did not permit ventriculography, ejection fraction was estimated by echocardiography using the Teichholz method.

The biopsy sample sites (right versus left ventricle, wall segment) were chosen according to the findings of echocardiography or magnetic resonance imaging of the heart with a 1.5-T Magnetom Sonata (Siemens Medical Solutions, Erlangen, Germany) to reduce the sampling error and to maximize the sensitivity and specificity of the method.11 Because most patients had evidence of left ventricular involvement, left ventricular biopsies were taken in 163 patients (90.1%), with additional right ventricular endomyocardial biopsy in 6 patients. Selective right ventricular endomyocardial biopsy was performed in 18 subjects (9.9%). Biopsy specimens were taken with a dedicated biopsyte (H1518.02-A, Endoflex, Voerde, Germany) advanced through various 7F coronary guiding catheters (LA7-JR40/AL10/JL40, Medtronic, Danvers, Mass) to reach prespecified regions of interest in the left and right ventricles. At least 4 biopsy specimens (median, n=5) with a diameter of 1 to 3 mm were harvested immediately and under strictly sterile conditions: 2 to 3 biopsy specimens were fixed in 4% buffered formaldehyde for hematoxylin and eosin, Masson’s trichrome, and Giemsa staining and performance of immunohistochemistry; 2 to 3 cardiac tissue samples were quick-frozen or fixed in RNAlater (Ambion Inc, Foster City, Calif) for PCR detection of viral genomes without a loss of sensitivity.4,12 The study was performed according to clinical practice in most centers.4,10,11 Biopsy specimens were investigated within 24 hours.

Analysis of Endomyocardial Biopsies
Endomyocardial biopsy findings were classified in 3 ways: by histopathological analysis alone, by immunohistochemistry, and by the presence or absence of viral genomes.

Histopathological Analysis
Histopathological examinations were done on 4-μm-thick tissue sections from paraffin-embedded endomyocardial biopsies stained with hematoxylin and eosin, Masson’s trichrome, and Giemsa and were examined by light microscopy. Histological analysis followed the Dallas criteria,13 which have previously been considered the gold standard for the biopsic evaluation of suspected myocarditis.4 According to this classification, acute myocarditis is defined by lymphocytic infiltrates in association with myocyte necrosis. Borderline myocarditis is characterized by the presence of inflammatory infiltrates without microscopic signs of myocyte injury. For statistical analysis, the 2 categories of acute (n=5 patients) and borderline (n=64 patients) myocarditis were combined and judged as a positive biopsy according to the Dallas criteria.

Immunohistochemistry
For immunohistological staining, paraffin-embedded tissue sections were treated with an avidin-biotin-immunoperoxidase method according to the manufacturer’s protocol (Vectastain Elite ABC Kit, Vector, Burlingame, Calif). The following monoclonal antibodies were applied for identification, localization, and characterization of mononuclear cell infiltrates: CD3 for T cells (Novocastra Laboratories, Newcastle on Tyne, UK), PGM1 (CD68) for macrophages and natural killer cells (DAKO, Glostrup, Denmark), and HLA-DR-α (DAKO, Hamburg, Germany) to assess HLA class II expression in professional antigen-presenting immune cells. According to the World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies, endomyocardial biopsies were considered to be inflamed after immunohistochemical detection of focal or diffuse mononuclear infiltrates with >14 leukocytes per 1 mm² (CD3+ T lymphocytes and/or CD68+ macrophages) in the myocardium, in addition to enhanced expression of HLA class II molecules.14–16

Molecular Biological Detection of Viral Genomes
Enterovirus species (comprising coxsackieviruses and echoviruses), parvovirus B19, adenoviruses, Epstein-Barr virus, and human herpesvirus type 6 were evaluated by nested PCR/RT-PCR from deep-frozen or RNAlater-fixed endomyocardial biopsy specimens as described.13 For RT-PCR analyses, RNA was transcribed into cDNA by RT according to the protocol of the manufacturer (AGS, Heidelberg, Germany). The enzymatic amplification of cDNA respectively DNA was performed as nested PCR on a Perkin-Elmer GeneAmp PCR System 9600 (Applied Biosystems, Weiterstadt, Germany) in two 30-cycle programs. As an internal control for successful isolation of nucleic acids, the housekeeping gene GAPDH was detected by PCR.11,17 A biopsy was considered positive for viral infection if viral genome was detected by PCR, and specificity was confirmed by automatic DNA sequencing of viral amplification products.18

Patients were prospectively analyzed for all viruses studied except a subset of 35 patients with retrospective viral genome detection who were enrolled before April 1997.

Statistical Analysis
Cox proportional-hazards regression analysis was performed to assess the association of clinical and hemodynamic variables and endomyocardial biopsy findings with primary and secondary end-point occurrence. After univariate screening, any candidate variable with a value of P<0.10 was forced to enter a multivariate model, which then identified independent predictors of outcome defined by a multivariate value of P<0.05. As a confirmation test, a second multivariate model with stepwise conditional forward logistic regression was applied. Survival curves of patients grouped by prespecified variables were calculated by the Kaplan-Meier method and compared with the log-rank test. For multiple pairwise comparisons of
Table 1. Baseline Characteristics of the Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
<th>n*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>42.4±15.3</td>
<td>181</td>
</tr>
<tr>
<td>Men</td>
<td>122 (67.4)</td>
<td>181</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>39 (21.5)</td>
<td>181</td>
</tr>
<tr>
<td>II</td>
<td>52 (28.7)</td>
<td>181</td>
</tr>
<tr>
<td>III</td>
<td>73 (40.3)</td>
<td>181</td>
</tr>
<tr>
<td>IV</td>
<td>17 (9.4)</td>
<td>181</td>
</tr>
<tr>
<td>LV end-diastolic dimension</td>
<td>36.2±6.90</td>
<td>169</td>
</tr>
<tr>
<td>index, mm/m†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>37.7±18.5</td>
<td>179</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>15.6±7.40</td>
<td>158</td>
</tr>
<tr>
<td>Endomyocardial biopsy results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunohistology and histopathology</td>
<td>181</td>
<td></td>
</tr>
<tr>
<td>Immunohistology positive</td>
<td>91 (50.3)</td>
<td>181</td>
</tr>
<tr>
<td>Immunohistology negative</td>
<td>90 (49.7)</td>
<td>181</td>
</tr>
<tr>
<td>Acute myocarditis‡</td>
<td>5 (2.8)</td>
<td>180</td>
</tr>
<tr>
<td>Borderline myocarditis‡</td>
<td>64 (35.4)</td>
<td>180</td>
</tr>
<tr>
<td>No myocarditis‡</td>
<td>112 (61.9)</td>
<td>180</td>
</tr>
<tr>
<td>Detection of viral genome</td>
<td>79 (43.9)</td>
<td>180</td>
</tr>
<tr>
<td>Medication</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>β-Blockers</td>
<td>103 (56.9)</td>
<td>180</td>
</tr>
<tr>
<td>ACE inhibitors or angiotensin converting enzyme</td>
<td>154 (85.0)</td>
<td>180</td>
</tr>
<tr>
<td>antagonists</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldosterone antagonists</td>
<td>69 (38.1)</td>
<td>180</td>
</tr>
</tbody>
</table>

Values are n (%) or mean±SD when appropriate. LV indicates left ventricular; ACE, angiotensin-converting enzyme.

*Number of patients for whom corresponding variable has been identified.
†Dimension was indexed to body height.
‡Histopathology according to the Dallas criteria.

Results

Patient Population

The baseline data of the 181 study patients and results of the endomyocardial biopsy are given in Table 1. Patients were relatively young (median age, 42.4 years), and approximately two thirds were men. Endomyocardial biopsy was performed because of global left ventricular dysfunction in 137 cases (75.7%). Ventricular enlargement (left ventricular end-diastolic dimension index >35 mm/m) with reduced ejection fraction (<45%) was observed in 82 subjects (45.3%), whereas 55 patients had either a depressed ejection fraction (n=45, 24.9%) or an enlarged left ventricle (n=10, 5.5%). In 19 patients with global left ventricular dysfunction, additional biopsy indications like ventricular tachyarrhythmia (n=10) or pericardial effusion (n=9) of unknown origin were present. Patients were included as a result of regional hypokinesia in 12 cases (6.6%) and elevated myocardial necrosis markers in 8 cases (4.4%). In the latter group, 6 presented without and 2 presented with ST-segment elevation. A total of 12 patients (6.6%) had sustained (n=11) or nonsustained (n=1) ventricular tachyarrhythmia with preserved left ventricular ejection fraction as a primary indication for biopsy. A pericardial effusion of unknown origin not associated with left ventricular dysfunction was detected in 12 patients (6.6%). Although half of the patients (n=91, 50.3%) had no or only modest symptoms of heart failure, 90 patients (49.7%) presented with moderately severe or severe heart failure. Eighty-eight subjects (48.6%) had a left ventricular end-diastolic pressure >12 mm Hg.

Endomyocardial Biopsy

Histopathological examination of endomyocardial biopsies according to the Dallas criteria was positive in 69 subjects (38.1%), with the majority (n=64) of findings indicating borderline myocarditis. Positive findings were more frequent after immunohistochemical staining, which revealed significant inflammatory cellular infiltrates in the specimens of 91 subjects (50.3%). In 25 patients with positive immunohistology (27.5%), examination of myocardial specimens only by biopsy showed no evidence of inflammation. In 3 of 69 subjects (4.3%) with Dallas-positive biopsy results, inflammatory processes were not confirmed by immunohistology. Examples of typical immunohistochemical stainings are illustrated in Figure 1 with regard to detection of T lymphocytes, macrophages, and expression of HLA class II molecules compared with uninfamed myocardium of immunohistochemistry-negative hearts. Viral genome was detected in the myocardium of 79 subjects (43.9%), 14 of whom (7.7%) had double infections. The following virus species were detected: parvovirus B19 (n=52, 28.7%), human herpesvirus type 6 (n=20, 11.0%), enterovirus species (n=11, 6.1%), Epstein-Barr virus (n=6, 3.3%), and adenoviruses (n=4, 2.2%). Among the 14 patients with double infections, codetection of parvovirus B19 prevailed (12 of 14 subjects). The most frequent combination of myocardial coinfection was parvovirus B19 and human herpesvirus type 6 (n=7, 3.9%). In 91 patients with immunohistology-proven myocardial inflammation, viral genome was detected in 42 subjects (46.2%). In 89 patients with negative immunohistochemical biopsy findings, viral genome detection was positive in 37 subjects (41.6%). In 8 patients, a possible blood contamination of endomyocardial specimens could not be excluded because PCR revealed genomes of the same virus species in both the myocardium and blood leukocytes. Exclusion of these patients from end-point analysis did not yield different results with regard to clinical outcome (P=0.266 to 0.850 for univariate end-point analysis).

Six patients experienced complications from heart catheterization and endomyocardial biopsy, giving a complication
rate of 3.3%. Five patients showed a new pericardial effusion after biopsy that required pericardiocentesis in 2 patients. One patient had a transient cerebral ischemic attack during catheterization. All complications resolved without sequelae.

Medical Therapy
The medical therapy of the study population is given in Table 1. \( \beta \)-Blockers and aldosterone antagonists were given to 57% and 38% of study subjects, respectively. Because aldosterone antagonist therapy was highly biased with regard to New York Heart Association (NYHA) class (57% in class III/IV, 20% in class I/II) and because of the low prevalence of patients not treated with angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists (15%), only \( \beta \)-blocker medication was considered for clinical end-point analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95% CI)</th>
<th>P</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>1.00 (0.98–1.03)</td>
<td>0.693</td>
<td>1.00 (0.98–1.03)</td>
<td>0.693</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.91 (0.48–1.75)</td>
<td>0.782</td>
<td>0.91 (0.48–1.75)</td>
<td>0.782</td>
</tr>
<tr>
<td>NYHA functional class III/IV</td>
<td>3.43 (1.73–6.77)</td>
<td>&lt;0.001</td>
<td>3.20 (1.36–7.57)</td>
<td>0.008</td>
</tr>
<tr>
<td>LV end-diastolic dimension index, mm/m</td>
<td>1.05 (1.01–1.10)</td>
<td>0.027</td>
<td>1.05 (0.97–1.15)</td>
<td>0.207</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>0.98 (0.96–1.00)</td>
<td>0.026</td>
<td>0.98 (0.96–1.00)</td>
<td>0.026</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>1.05 (1.00–1.09)</td>
<td>0.030</td>
<td>1.00 (0.95–1.05)</td>
<td>0.983</td>
</tr>
<tr>
<td>Positive immunohistology</td>
<td>4.54 (2.01–10.28)</td>
<td>&lt;0.001</td>
<td>3.46 (1.39–8.62)</td>
<td>0.008</td>
</tr>
<tr>
<td>Dallas-positive histopathology*</td>
<td>1.51 (0.81–2.80)</td>
<td>0.195</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evidence of viral genome</td>
<td>0.96 (0.51–1.80)</td>
<td>0.893</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )-Blocker medication</td>
<td>0.50 (0.26–0.95)</td>
<td>0.034</td>
<td>0.43 (0.21–0.91)</td>
<td>0.027</td>
</tr>
</tbody>
</table>

LV indicates left ventricular. Only variables with a univariate value of \( P < 0.10 \) were allowed to enter the multivariate analysis. The HR for continuous variables is interpreted as follows: For every 1-unit increase in the variable, the ratio of the risk of reaching the point is multiplied by the given HR.

*Acute and borderline myocarditis combined.

Follow-Up
During an average follow-up of 58.9 months (median, 53.2 months), 37 patients (20.4%) died, 26 of them for cardiac reasons (13 suffered sudden cardiac death, 13 died of terminal cardiac pump failure). Noncardiac causes of death were malignant tumors (\( n = 4 \)), sepsis (\( n = 3 \)), accident (\( n = 2 \)), gastric perforation (\( n = 1 \)), and terminal liver failure resulting from cirrhosis (\( n = 1 \)). Fourteen patients (7.7%) underwent heart transplantation. A total of 14 patients (7.7%) were lost to follow-up, defined as >12 months since the last follow-up in subjects without end-point occurrence. The primary end point of cardiac death or heart transplantation was reached in 40 patients (22.1%). The secondary end point of all-cause mortality or heart transplantation occurred in 51 patients (28.2%).

Predictors of Outcome
For the primary end point of cardiac death or heart transplantation (Table 2), NYHA functional classes III and IV, high values of the left ventricular end-diastolic diameter index and pressure, a low ejection fraction, and immunohistological detection of inflammation were shown to be significant predictors of poor outcome in the univariate analysis, whereas the Dallas criteria and viral genome detection were not significantly related to outcome. In addition, \( \beta \)-blocker treatment was associated with a better prognosis. Freedom from primary end-point occurrence in relation to clinical and left ventricular functional parameters (Figure 2A through 2D), myocardial biopsy results (Figure 3A through 3D), and
β-blocker treatment (Figure 4) is illustrated by unadjusted Kaplan-Meier curves. Because survival curve construction required dichotomization of the continuous left ventricular functional parameters of diameter, ejection fraction, and pressure, the log-rank probability values in the survival plots (Figure 2B through 2D) differ from the univariate Cox test probability values (Table 2) for these variables. Because viral genome detection in the presence of myocardial inflammation defines a clinically important subgroup of inflamed hearts compared with the latent viral type of persistence in uninflamed hearts, patients with and without myocardial inflammation were subclassified according to the presence or absence of viral genome. However, viral genome detection did not allow further risk stratification in either inflamed or uninflamed hearts (Figure 3D).

After adjustment for covariates, only NYHA functional class, immunohistological findings, and β-blocker therapy remained significant and independent predictors of the primary outcome (Table 2). A risk stratification approach based on these 3 variables is depicted in Figure 5. According to this triple-parameter approach, NYHA class I/II patients taking β-blockers without myocardial inflammation appear to have...
an excellent prognosis, with 100% survival and no need for heart transplantation. In contrast, NYHA class III/IV patients with positive immunohistology without β-blocker therapy have a 5-year transplantation-free survival rate of only 39%. Patients who do not present with these 3 risk factors have an intermediate prognosis, with a 5-year survival rate of 81%.

Except for left ventricular dimension, univariate predictors of the secondary end point of all-cause death or heart transplantation were the same as in the primary end-point analysis (Table 3). Left ventricular functional parameters lost their prognostic value for the prediction of the second secondary end point of all-cause death (Table 4). It was a consistent finding over all 3 end points that functional status according to NYHA classification, immunohistological results of endomyocardial biopsies, and β-blocker therapy were the only significant and independent parameters retained in the multivariate analysis. The second multivariate approach of stepwise conditional forward regression confirmed the results of the forced inclusion technique shown in Tables 2 through 4, yielding multivariate values of \( P = 0.001 \) to 0.002 for the NYHA class, \( P = 0.002 \) to 0.007 for immunohistology, and \( P = 0.011 \) to 0.019 for β-blocker therapy.

Remarkably, the histopathological classification according to the Dallas criteria did not prove to be a significant predictor for either the primary (Table 2 and Figure 3B) or secondary

Figure 3. Unadjusted survival free from cardiac death and heart transplantation according to the findings of endomyocardial biopsy. A, Immunohistological results. B, Histopathological results according to the Dallas criteria. C, Viral genome detection. D, Combination of immunohistological and PCR findings. HTx indicates heart transplantation.
immunohistology. Using NYHA functional class, the immunohistological results of transplantation according to a triple-parameter risk stratification model Figure 5. Interestingly, viral genome detection alone had no positive and -negative subjects (53.2% versus 48.5%; 0.552). Interestingly, viral genome detection alone had no predictive meaning for any of the 3 end points (Figure 3C and Tables 2 through 4).

(Table 3 and 4) end points. Myocardial inflammation was not found to be significantly correlated with the presence of cardiac viral genome. The frequency of inflammatory infiltrates as indicated by immunohistology was similar in virus-cardiac viral genome. The frequency of inflammatory infiltrate in the myocardium, and absence of myocardial inflammation was respectively of poor outcome. The combination of advanced NYHA criteria alone or viral genome detection per se, were predictive of poor outcome.22

Sympathetic activation has been identified as being involved in the progression of left ventricular dysfunction by stimulating inflammation19,20 and apoptosis21 and is related to poor outcome.22 β-Blockers have been shown to improve morbidity and mortality in heart failure.23 Clinical studies are lacking in myocarditis. However, in viral myocarditis in mice with virus-induced expression of cytokines,24 β-blocker treatment improved outcome and reduced inflammation.25 In the present study, the presence of β-blocker therapy was associated with a beneficial prognosis, whereas a lack of β-blocker treatment, particularly in patients with higher NYHA classes and myocardial inflammatory infiltrates, was adversely associated with outcome. This analysis on β-blocker treatment was

Discussion

The purpose of the study was to investigate predictors of outcome in patients with suspected myocarditis. We observed that among the clinical markers, NYHA functional class and β-blocker treatment were able to predict survival free from cardiac death or heart transplantation. Because all patients underwent endomyocardial biopsy, we were able to show that positive immunohistology for invading immune cells and expression of HLA-DR molecules, but not the Dallas criteria alone or viral genome detection per se, were predictive of poor outcome. The combination of advanced NYHA functional class with positive immunohistology and the absence of β-blocker treatment identified subgroups of patients at extremely high risk.

Sympathetic activation has been identified as being involved in the progression of left ventricular dysfunction by stimulating inflammation19,20 and apoptosis21 and is related to poor outcome.22 β-Blockers have been shown to improve morbidity and mortality in heart failure.23 Clinical studies are lacking in myocarditis. However, in viral myocarditis in mice with virus-induced expression of cytokines,24 β-blocker treatment improved outcome and reduced inflammation.25 In the present study, the presence of β-blocker therapy was associated with a beneficial prognosis, whereas a lack of β-blocker treatment, particularly in patients with higher NYHA classes and myocardial inflammatory infiltrates, was adversely associated with outcome. This analysis on β-blocker treatment was
In this respect, it might be relevant was similar in virus-positive and virus-negative patients clinical outcome. Notably, the frequency of inflammation viral genome detection per se was not associated with poor heart failure.

Before this study, no predictors derived from the histopathological evaluation of endomyocardial biopsies for judging the outcome of acute or chronic myocardial inflammation were identified. In addition, the role of endomyocardial biopsies in diagnosing myocarditis has been challenged by a lack of specificity,1,26 risk of complications,4,27,28 and retrospective but calls for randomized β-blocker trials in patients with myocarditis, particularly those at high risk.

Before this study, no predictors derived from the histopathological evaluation of endomyocardial biopsies for judging the outcome of acute or chronic myocardial inflammation were identified. In addition, the role of endomyocardial biopsies in diagnosing myocarditis has been challenged by a lack of specificity,1,26 risk of complications,4,27,28 and sampling error.29,30 In patients with recent onset of cardiac dysfunction, the histopathological Dallas criteria have been described to detect acute or borderline myocarditis in only ≈ 10% of patients.26,31,32 Using echocardiography and magnetic resonance imaging to guide the biopsy to affected areas, we found 2 to 3 endomyocardial biopsies to be sufficient for assessing acute and borderline myocarditis in 38% of patients by the Dallas criteria. The reason for the higher rate of Dallas-positive biopsies compared with previous studies might be the fact that we took the biopsies from the dysfunctional left ventricle (in 163 patients, 90%), not from the less affected right ventricle.

Complementing the histological features of the Dallas criteria with immunohistochemistry revealed myocarditis more frequently, resulting in 50% positive patients. According to multivariate regression analysis, immunohistological assessment of myocardial inflammation, but not the histological Dallas criteria, was observed to be an independent predictor of poor outcome, a finding that warrants consideration in the clinical management of patients with recent-onset heart failure.

In contrast to immunohistologically proven inflammation, viral genome detection per se was not associated with poor clinical outcome. Notably, the frequency of inflammation was similar in virus-positive and virus-negative patients (52.6% versus 49.0%). In this respect, it might be relevant that detection of viral genomes by PCR/RT-PCR as performed in this study is not capable of differentiating viral latency from active viral replication. However, evidence from well-characterized murine models indicates that viral persistence may sustain myocardial inflammation, whereas virus elimination predicts recovery.12 It is conceivable that viral infection triggers an inflammatory process in the myocardium, eg, by expression of cytokines and adhesions molecules, that outlasts the initial replicative phase.24 Therefore, it is reasonable to suggest that virally triggered inflammation without sustained viral genome detection might cause heart failure and poor prognosis. This notion is supported by the findings presented here that inflammation determining poor outcome can occur without evidence of viral genomes, favoring the concept of postviral autoimmunity. Thus, the detection of viral genomes appears to be of etiopathogenic importance only in the presence of an immunohistology-proven reactive inflammatory infiltrate. In cases without inflammation, the situation is one of latent viral persistence. Using quantitative PCR and assessing replicative viral intermediates should prove to be useful in future studies for unequivocal differentiation of replicative viral persistence from viral latency.

With regard to as-yet unestablished antiviral or immunosuppressive treatment strategies,8,33,34 it is mandatory to differentiate chronic active viral myocarditis, defined as myocardial viral infection with cellular inflammation, from postviral autoimmunity and from harmless latent viral persistence without inflammatory infiltrates26 by using the diagnostic approach as described in this study.

There has been interest in redefining the role of myocardial biopsy in the diagnosis and outcome of myocarditis.3,4,11 The data presented here show that immunohistological signs of inflammatory response, either with or

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Age, y</td>
<td>1.02 (0.99–1.04)</td>
<td>0.227</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.92 (0.47–1.81)</td>
<td>0.811</td>
</tr>
<tr>
<td>NYHA functional class III/IV</td>
<td>2.91 (1.48–5.76)</td>
<td>0.002</td>
</tr>
<tr>
<td>LV end-diastolic dimension index, mm/m</td>
<td>1.02 (0.97–1.08)</td>
<td>0.411</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>0.99 (0.97–1.00)</td>
<td>0.114</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>1.03 (0.98–1.08)</td>
<td>0.208</td>
</tr>
<tr>
<td>Positive immunohistology</td>
<td>4.98 (2.08–11.96)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dallas-positive histopathology*</td>
<td>1.98 (1.03–3.79)</td>
<td>0.040</td>
</tr>
<tr>
<td>Evidence of viral genome</td>
<td>0.66 (0.33–1.32)</td>
<td>0.236</td>
</tr>
<tr>
<td>β-Blocker medication</td>
<td>0.44 (0.22–0.88)</td>
<td>0.020</td>
</tr>
</tbody>
</table>

LV indicates left ventricular. Only variables with a univariate value of P<0.10 were allowed to enter the multivariate analysis. The HR for continuous variables is interpreted as follows: For every 1-unit increase in the variable, the ratio of the risk of reaching the point is multiplied by the given HR.

*Acute and borderline myocarditis combined.
without evidence of viral genre and despite negative histological Dallas criteria, can reliably predict cardiovascular death and the need for transplantation. Because immunohistology yields important information, this finding argues in favor of performing a prognostic myocardial biopsy early after presentation. Our data support the recommendations of the AHA, the American College of Cardiology, the European Society of Cardiology, and Baughman suggesting an interdisciplinary approach to redefine myocarditis involving new possibilities of innovative methodological techniques.

Conclusions

Advanced NYHA functional class, immunohistological features of inflammation in endomyocardial biopsies, and the absence of β-blocker treatment are potent risk factors for identifying patients with myocarditis who are likely to deteriorate. The study adds new information on the role of clinical judgment in patients with recent-onset symptoms of myocarditis and emphasizes the role of endomyocardial biopsy in patients with suspected myocarditis.

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This work was supported by the Deutsche Forschungsgemeinschaft (KFO 196 to Dr Böhm, SFB-TR 19 to Drs Kandolf and Klingel).

Disclosures

None.

References

29. Hauck AJ, Kearney DL, Edwards W. Evaluation of post-mortem endomyocardial biopsy specimens from 38 patients with lymphocytic myo-
Since the introduction of the histopathological Dallas criteria in 1987, the role of endomyocardial biopsy in the management of suspected myocarditis has been controversial. We therefore examined the prognostic role of modern endomyocardial biopsy with detailed analysis of specimens involving immunohistochemical staining for characterization of inflammation and polymerase chain reaction for detection of cardiotropic virus species in 181 young patients with clinically suspected myocarditis. In 181 endomyocardial biopsy procedures with 1018 specimens taken mainly from the left ventricle, we had a low number of 6 complications (3.3% per patient, 0.6% per specimen), all of which resolved without sequelae. Neither the histopathological Dallas criteria alone nor the detection of viral genome was a predictor of outcome. Besides functional class, only 2 independent predictors proved to be significant for future occurrence of the primary end point. Although β-blocker treatment was protective, immunohistological evidence of inflammatory infiltrates in the myocardium was associated with a >3-fold increase in risk of cardiac death or heart transplantation (P=0.008). Patients in New York Heart Association class I/II who took β-blockers without myocardial inflammation appeared to have an excellent prognosis, with 100% 5-year transplantation-free survival, whereas New York Heart Association class III/IV patients with positive immunohistology not on β-blocker therapy had an event-free survival rate of only 39%. Hence, myocardial inflammation, which can be detected with high sensitivity by modern immunohistological staining, confers significant prognostic information. This underlines the role of endomyocardial biopsy as an important cornerstone for risk stratification in patients with clinically suspected myocarditis.
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