Viral Persistence in the Myocardium Is Associated With Progressive Cardiac Dysfunction

Uwe Kühl, PhD; Matthias Pauschinger, MD; Bettina Seeberg, MD; Dirk Lassner, PhD; Michel Noutsias, MD; Wolfgang Poller, MD; Heinz-Peter Schultheiss, MD

Background—Cardiotropic viral infections have been suspected as one possible cause of myocarditis and dilated cardiomyopathy. Although adverse outcomes in dilated cardiomyopathy patients have been documented, the natural course of heart diseases caused by cardiotropic viruses is unknown.

Methods and Results—Consecutive patients (n=172) with biopsy-proven viral infection in endomyocardial biopsies (EMBs) were followed up by reanalysis of EMBs and hemodynamic measurements after a median period of 6.8 months (range, 5.4 to 11.9). Nested polymerase chain reaction (PCR) and reverse transcription–PCR were performed to analyze the genomic sequences. Myocardial inflammation was assessed by histology and immunohistology. At baseline, 32.6% of EMBs in the study group contained enteroviral (EV) RNA, 8.1% adenovirus (ADV) DNA, 36.6% parvovirus B19 (PVB19) DNA, and 10.5% human herpesvirus type 6 (HHV6) DNA. In 12.2% of the samples, dual infection with PVB19 and HHV6 was present. Follow-up analysis of EMBs by PCR documented spontaneous clearance of viral genomes in 36.2% (55/151) of all patients with single infections. Virus-specific clearance rates were 50% for EV, 35.7% for ADV, 22.2% for PVB19, and 44.4% for HHV6. In patients with dual infection with PVB19 and HHV6, HHV6 was cleared in 42.8% (9/21), whereas PVB19 persisted in all 21 patients. Clearance of viral genomes was associated with a significant improvement in left ventricular ejection fraction (LVEF), improving from 50.2±19.1% to 58.1±15.9% (P<0.001). In contrast, LV function decreased in patients with persisting viral genomes (LVEF, 54.3±16.1% versus 51.4±16.1%, P<0.01).

Conclusions—In this first biopsy-based analysis of the course of viral heart disease, we show that EV, ADV, PVB19, and HHV6 persistence detected in the myocardium of patients with LV dysfunction was associated with a progressive impairment of LVEF, whereas spontaneous viral elimination was associated with a significant improvement in LV function. (Circulation. 2005;112:1965-1970.)

Key Words: heart diseases ■ myocarditis ■ cardiomyopathy ■ polymerase chain reaction ■ immunohistochemistry

Cardiotropic viral infections are important causative factors in dilated cardiomyopathy (DCM), which appears to occur as a late sequela of acute viral myocarditis.1–4 In the past, mostly enteroviruses (EVs) have been identified5–7 and are associated with unfavorable clinical and hemodynamic outcomes.3,8,9 Recently, other viral genomes have been detected in endomyocardial biopsies (EMB) from adults who presented with the clinical phenotype of acute or chronic myocarditis and DCM. Among identified viral genomes that have been reported in EMBs of ≈67% of patients with this clinical setting, parvovirus B19 (PVB19) and human herpesvirus type 6 (HHV6) are the most frequently encountered pathogens.3,4,10 The natural course and possible relevance of persistent viral infection for improvement, persistence, or progression of myocardial dysfunction are currently unknown. The present study was a biopsy-based analysis of the spontaneous course of cardiac infections with various viruses in follow-up biopsies of patients with regionally or globally impaired myocardial function.

Methods

Patients

Between July 2001 and September 2004, 841 patients were admitted to our institution for EMB to further elucidate a possible inflammatory and/or infectious cause of their disease because the clinical presentation had suggested myocarditis in the past or DCM. In this study, we enrolled 172 consecutive patients in whom polymerase chain reaction (PCR) analysis had detected viral genomes in the biopsy sample at the initial clinical presentation. Patients clinically presenting with acute myocarditis of recent onset with signs of myocardial injury (eg, mimicking acute myocardial infarction) were not included. The majority of enrolled patients (89%) complained of symptoms of moderate heart failure with fatigue, reduced physical capacity, or dyspnea on exertion. Most patients were in New York Heart Association classes II and III (II, 68%; III, 30%; and IV, 2%).

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TABLE 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>No.</th>
<th>Median age (range), y</th>
<th>46.5 (55.6–34.4)</th>
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<tbody>
<tr>
<td>Male</td>
<td>90 (54.6)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>172 (100)</td>
<td></td>
</tr>
<tr>
<td>Median time from onset of symptoms to EMB (range), mo</td>
<td>5.1 (1.5–24.1)</td>
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<tr>
<td>Preceding infection</td>
<td>88 (51.2)</td>
<td></td>
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<tr>
<td>Interval between infection and symptoms, wk</td>
<td>5.5 (1.9, 20.7)</td>
<td></td>
</tr>
<tr>
<td>Pericardial effusion, %</td>
<td>12 (7.0)</td>
<td></td>
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<tr>
<td>Median systolic BP, mm Hg</td>
<td>120 (110, 130)</td>
<td></td>
</tr>
<tr>
<td>Median diastolic BP, mm Hg</td>
<td>75.5 (70, 80)</td>
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</tbody>
</table>

Medication use
- Gliclazide: 73 (42.4)
- ACE inhibitors or ARBs: 140 (81.4)
- β-Blockers: 94 (54.7)
- Diuretics: 96 (55.8)
- Spironolactone: 70 (40.7)
- Angiotensin receptor blocker: 21 (12.2)
- ICD/pacemaker: 12 (7.0)/8 (4.6)
- RV/LV bundle block: 13 (7.5)/23 (13.4)

Cardiac function parameters
- Median LVEDP, mm Hg: 9 (6, 14)
- Median EF, %: 52.5 (37.2, 66.0)
- Median cardiac index, L·min⁻¹·m⁻² BSA: 3.3 (2.7, 4.0)
- Median stroke volume index, mL/min: 45 (36, 55)
- Median PC, mm Hg: 8 (6, 11)
- Median PAP, mm Hg: 14 (11, 18)
- Echocardiography: Median left atrial dimension, mm: 38 (34, 43)
- Median LVEDD, mm: 57 (51, 64)
- Median LVESD, mm: 39 (31, 53)
- Median fractional shortening, %: 29 (19, 38)
- Median ES, mm: 9 (3, 17)
- Global wall-motion abnormality: 112 (65.1)
- Regional wall-motion abnormality: 60 (34.9)

BP indicates blood pressure; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; ICD, implantable cardioverter/defibrillator; BSA, body surface area; EDP, end-diastolic pressure; PC, pulmonary capillary wedge pressure; PAP, pulmonary artery pressure; and ES, mitral valve E-point to septal separation. Data are presented as the median value (range), median value (25th, 75th percentiles), or No. (%) of subjects.

The demographic and clinical characteristics of patients are shown in Tables 1 and 2.

Coronary artery disease and other possible causes of myocardial dysfunction had been excluded by angiography before biopsy in all patients. At the baseline biopsy, the patients presented with either regional (35%) or global (65%) wall motion abnormalities. To determine the course of the viral infection, all patients underwent follow-up biopsy. Ejection fraction (EF) was determined angiographically. Additionally, standard 2-dimensional and M-mode echocardiography was performed for all patients in our echocardiographic department 1 day before biopsy (median interval, 6.8 months; range, 5.4 to 11.9). For each echocardiogram, left ventricular (LV) end-diastolic (LVEDD) and end-systolic (LVESD) diameters were measured by M-mode echocardiography in the parasternal long-axis view according to the leading-edge method. Percentage fractional shortening was calculated in a standardized manner. Medication use, including angiotensin-converting enzyme inhibitors, β-blockers, diuretics, cardiac glycosides, and warfarin, was stable throughout the follow-up period without significant differences between patients who developed virus genome persistence and those who did not.

EMB and Right-Heart Catheterization
Eight EMBs were obtained from the right side of the ventricular septum of each patient with use of a flexible bioptome (Westmed) via the femoral vein approach. There were no biopsy-related adverse events. Two EMBs were used for histological evaluation according to the Dallas criteria11 and immunohistochemistry,6 whereas the remaining 4 EMBs were subjected to DNA and RNA extraction for amplification of the viral genomes. After the EMBs were obtained, the patients underwent right heart catheterization. Right atrial, right ventricular, pulmonary arterial, and pulmonary capillary wedge pressures (all in mm Hg) and cardiac index (L · min⁻¹ · m⁻² body surface area) were recorded. The protocol was approved by the Human Research Committee of the Charite, Campus Benjamin Franklin, Berlin, and all patients gave written, informed consent before treatment.

Etiologic Investigations

Detection of Viral Genomes by Nested PCR
Detection of viral genomes by nested PCR was carried out as published recently.3,4,12 In brief, nested PCR/reverse transcription–PCR was performed on RNA extracted from EMBs for EVs (including coxsackieviruses and echoviruses) and in DNA for adenovirus (ADV), PV, and HHV6. As a control for successful extraction of DNA and RNA from heart muscle tissue, oligonucleotide sequences were chosen from the DNA sequences of the glyceraldehyde 3-phosphate dehydrogenase gene. Specificity of all amplification products was confirmed by automatic DNA sequencing.12–14

Histological and Immunohistological Evaluation
Myocardial inflammation was defined by the detection of infiltrating lymphocytes (median cell count >7.0 cells/mm²) in association with enhanced expression of cellular adhesion molecules (HLA-III or CD54) expressed on interstitial or endothelial cells.15,16 Samples containing low numbers of infiltrating lymphocytes (median cell count <7.0 cells/mm²), especially those without enhanced cellular
adhesion molecule expression, were defined as having no significant myocardial inflammation.

**Statistical Analysis**

Statistical analysis was performed with JMP Statistical Discovery Software, version 3.1.6 (SAS Institute, Inc). All results are presented as median value (25th, 75th percentile), except when stated otherwise. Follow-up data were analyzed with a paired t test. Qualitative data were compared by the $\chi^2$ test. A probability value (2 sided) <0.05 was considered statistically significant.

**Results**

**Biopsy Findings**

At baseline, 63 (36.6%) of the 172 patients' EMBs were positive for PVB19, 56 (32.6%) for EV, 18 (10.5%) for HHV6, and 14 (8.1%) for ADV. Dual infection with PVB19 and HHV6 was present in 21 (12.2%) biopsy specimens (Table 3). The spontaneous course of viral infections in these 172 patients was followed up for a median of 6.8 months (range, 5.4 to 11.9). At the time of the follow-up biopsy, spontaneous clearance of viral genomes was found in 55 of 151 (36.4%) patients with single infections (EV n=28 [50.0%], ADV n=5 [35.7%], PVB19 n=14 [22.2%], and HHV6 n=8 [44.4%]) (Figure 1). In patients with PVB19 and HHV6 dual infections, the HHV6 infection had been cleared in 42.8% (n=9), whereas PVB19 genomes persisted in all 21 cases.

Histological analysis did not detect active or borderline myocarditis in any of the analyzed samples at baseline or follow-up. On immunohistological staining, significant CD3$^+$ T-lymphocytic infiltrates with a median number of 11.6 (8.4 to 17.2) CD3$^+$-positive lymphocytes/mm$^2$ in association with enhanced cellular adhesion molecule expression were detected in 67 patients' (38.9%) baseline biopsy samples (versus 2.8 cells/mm$^2$ [1.8 to 5.3] in the remaining 105 patients). At follow-up, enhanced myocardial inflammation was present in EMBs of 34/172 (19.8%) patients (CD3$^+$, 10.9 cells/mm$^2$ [8.4 to 13.5] versus 2.8 cells/mm$^2$ [1.8 to 4.6]), and increased numbers of inflammatory lymphocytes were detected more frequently in patients who developed virus persistence (26/108 [24.1%] versus 8/64 [12.5%], $P<0.05$). Enhanced HLA-I/DR and CD54 expression was significantly correlated with infiltrating inflammatory cells, but it was independent of the course of viral infection (data not shown).

**Hemodynamic Course**

Regardless of the virus involved, complete clearance of viral genomes (n=64) was associated with a significant improvement in LVEF, improving from 50.2±19.1% to 58.1±15.9% ($P<0.001$, Figure 1). An increase in systolic LV function was found to be independent of the infectious agent. In contrast to the favorable hemodynamic course of patients who eliminated the viral genomes, virus persistence was associated with a lack of hemodynamic improvement. Between baseline and follow-up, LVEF significantly decreased from 54.3±16.1% to 51.4±16.1% ($P<0.01$) in these patients (n=108), despite the relative short follow-up period of 6.8 months. Hemodynamic changes after spontaneous HHV6 clearance but PVB19 persistence were not significant ($P=0.49$) in patients with PVB19/HHV6 dual infection, whereas persistence of both viruses was associated with a mild progression of LV dysfunction ($P=0.06$). Hemodynamic improvement occurred in patients with both mild and severe LV dysfunction. The improvement was more pronounced in patients with an EF <45% (n=51) compared with patients with an EF ≥45% (Figure 2). In this subgroup, EF improved from 29.6±7.8% to 44.0±13.6% ($P<0.001$, n=24) in association with virus elimination, whereas EF did not change in patients who developed viral persistence (32.4±8.4% versus 33.9±15.8%, $P=0.57$). In patients with an EF >45% (n=121), EF improved from 62.6±11.5% to 66.6±10.1% ($P<0.01$, n=40) or deteriorated from 61.6±10.4% to 57.2±11.2% ($P<0.001$, n=81). The aforementioned hemodynamic changes were independent of the patients' medication regimen, which did not differ significantly between the virus-positive and virus-negative cohorts and that had been kept constant during the follow-up period. In contrast to the viral course, changes in myocardial inflammatory cells were not additional predictors of the hemodynamic course.

**Discussion**

**Relation Between Clinical and Virological Course**

A broad spectrum of viral genomes has been detected in EMBs from patients with clinically suspected myocarditis in the past and DCM. So far, EVs have been linked to the development of myocarditis and its progression to DCM. Recently, we and others have detected other frequent viral genomes (eg, ADV, PVB19, and HHV6) in the myocardium of patients presenting with acute heart failure caused by myocarditis, with a sudden onset of cardiac symptoms mimicking acute myocardial infarction and with chronic LV dysfunction diagnosed as “idiopathic” DCM. The natural course of these viral infections and the prevalence of viral persistence in these groups have not been investigated yet. To address this issue and to elucidate the relevance of virus persistence with respect to LV function, we conducted follow-up EMBs in 172 consecutive, virus-positive patients with persistent LV dysfunction.

During follow-up of patients with clinically suspected myocarditis in the past or with heart failure of unknown origin, one may observe either “spontaneous” improvement or progression of ventricular dysfunction despite constant...
heart failure medication. Our results suggest that these “spontaneous” changes in cardiac function may actually reflect the dynamic course of an underlying cardiotropic viral infection. As shown here, virus clearance was associated with a spontaneous improvement in LVEF, regardless of the type of virus involved. It appears that patients with a lower EF (<45%) improve more than do those with milder EF dysfunction. This is reminiscent of a similar phenomenon in a prior study of interferon-β–induced virus elimination.\textsuperscript{12} LVEF did not improve or even deteriorated in patients with viral persistence. Taking into consideration the slow but continuous development of LV dysfunction in DCM, the even mild deterioration of LVEF observed during the short follow-up period of 6.8 months may progress to substantial myocardial dysfunction over years.

**Molecular Pathomechanisms of Viral Heart Disease**

Even small amounts of persistent viral genomes may cause further progression of the disease, by direct cytopathic effects of virus-encoded proteins via virus-associated signaling pathways resulting in the release of cytokines,\textsuperscript{18–25} by alterations of the extracellular cardiac matrix or the cytoskeleton,\textsuperscript{26–28} or by chronic myocardial inflammation.\textsuperscript{15,16,29,30} So far, the causes of the highly variable natural courses of virus-associated heart disease are unknown but may include changes in cardiac virus load, as well as the host’s primary immune responses to the virus.
If the viruses were cleared spontaneously and thus, no pathogenic agents were detected in the myocardium, diagnostic procedures should result in resolved myocarditis or “idiopathic” DCM. In patients with “resolved” myocarditis, ventricular function may recover completely if the initial myocardial damage was minor. In other patients, persistence or further progression of ventricular dysfunction may result from myocardial remodeling after the initial virus-induced injury of cardiac tissues.

Myocardial inflammation was detected in 40% of the virus-positive patients during baseline EMB. This inflammation was significantly reduced at follow-up but still primarily seen in virus-positive patients (23.9% versus 12.5%, \( P<0.05 \), respectively). Further follow-up of virus-negative patients with inflammation would be required to distinguish an inflammatory process resolving after virus clearance from virus-induced persistent inflammation, often referred to as and indistinguishable from (auto)immune myocarditis or chronic inflammatory cardiomyopathy.

Conclusions

A broad spectrum of viral genomes has been detected in patients with de novo wall motion abnormalities or persistent LV dysfunction, clinically often referred to as past myocarditis or DCM. The influence of this chronic viral infection on myocardial function is unknown, because biopsy-based follow-up data on patients infected with these viruses have never been obtained. By following up a large cohort of patients with different virus infections, we could show that spontaneously occurring virus clearance is associated with spontaneous hemodynamic improvement. In contrast, LV function deteriorates in patients with virus persistence, even within a short follow-up period of 6.8 months and despite constant heart failure medication. These data indicate that persisting cardiac viral infections may constitute a major cause of progressing LV dysfunction in patients with clinically suspected past myocarditis or DCM. The data furthermore indicate that only an EMB-derived virus analysis allows accurate diagnosis in patients with clinically suspected myocarditis or DCM, which is mandatory for effective antiviral immunomodulatory treatment of these patients.

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References


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