Presentation, Patterns of Myocardial Damage, and Clinical Course of Viral Myocarditis

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Background—Enteroviruses and adenoviruses have been considered the most common causes of viral myocarditis, but parvovirus B19 (PVB19) and human herpesvirus 6 (HHV6) are increasingly found in endomyocardial biopsy samples.

Methods and Results—Consequently, our aim was to evaluate the prevalence and clinical presentation of cardiac PVB19 and/or HHV6 infection in a cohort of myocarditis patients and to follow its clinical course. In addition, we sought to demonstrate patterns of myocardial damage and to determine predictors for chronic heart failure. Our study design consisted of a cardiovascular magnetic resonance protocol as well as endomyocardial biopsies in the myocardial region affected as indicated by cardiovascular magnetic resonance. One hundred twenty-eight patients were enrolled by clinical criteria. In the group of myocarditis patients (n=87), PVB19 (n=49), HHV6 (n=16), and combined PVB19/HHV6 infections (n=15) were detected most frequently. The remaining patients were diagnosed with healing myocarditis (n=15) or did not have myocarditis (n=26). Patients with PVB19 presented in a manner similar to that of myocardial infarction; most had typical subepicardial late gadolinium enhancement in the lateral wall and recovered within months. Conversely, patients with HHV6 and especially with HHV6/PVB19 myocarditis presented with new onset of heart failure, had septal late gadolinium enhancement, and frequently progressed toward chronic heart failure.

Conclusions—Our data indicate that PVB19 and HHV6 are the most important causes for viral myocarditis in Germany and that the clinical presentation is related to the type of virus. Furthermore, clinical presentation, type of virus, and pattern of myocardial damage are related to the clinical course. (Circulation. 2006;114:1581-1590.)

Key Words: biopsy ■ heart failure ■ magnetic resonance imaging ■ myocarditis ■ cardiomyopathy

Myocarditis is a common cardiac disease that is identified in up to 9% of routine postmortem examinations. It may progress to chronic dilated cardiomyopathy, and it appears to be a major cause of sudden, unexpected death in adults aged <40 years.

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Although the cause of myocarditis often remains unknown, a large variety of infections, systemic diseases, drugs, and toxins have been associated with this disease. Viruses, bacteria, protozoa, and even worms have been implicated as infectious agents. There is a consensus that viruses are a frequent cause of myocarditis in North America and Europe. Initially, coxsackieviruses were thought to be the most common cause of myocarditis because rising antibody titers could be demonstrated in patients during acute myocarditis and convalescence. Later, other enteroviruses as well as adenoviruses were identified in endomyocardial biopsy specimens from patients with clinically suspected myocarditis and from those with idiopathic dilated cardiomyopathy. Thus, for a long time, enteroviruses and adenoviruses have been considered the most common causes of viral myocarditis with possible transition to dilated cardiomyopathy.

Recently, with the frequent detection of parvovirus B19 (PVB19) as well as human herpesvirus 6 (HHV6) in endomyocardial biopsy samples, 2 new suspects have entered the arena. However, the prevalence of those 2 viruses in patients clinically suspected to have myocarditis, their clinical presentation, and their clinical course are widely unknown.

Consequently, our aim was to evaluate the prevalence and the clinical presentation of cardiac PVB19 and/or HHV6 infection in a cohort of myocarditis patients as well as to follow its clinical course. In addition, we sought to demonstrate patterns of myocardial damage and to determine predictors for recovery as well as for progression toward chronic heart failure. Our study design consisted of a cardiovascular magnetic resonance (CMR) protocol, assessing functional parameters and late gadolinium enhancement (LGE), as well
as endomyocardial biopsies in the myocardial region affected as indicated by CMR.9,11 To allow the evaluation of the clinical course, patients were monitored clinically and by repeat CMR scans.

**Methods**

**Patient Population**

We initially identified 257 patients suspected to have myocarditis on the basis of a variable combination of chest discomfort, dyspnea, and altered ECG in conjunction with a history compatible with inflammatory disease such as cough, diarrhea, fever, malaise, and elevated C-reactive protein. Patients were excluded if they had concomitant coronary artery disease (stenosis >50%) (n=31), had coronary spasm defined by catheterization (n=14), or had refused to undergo catheterization (n=34). One hundred twenty-eight of the remaining 178 patients fulfilled the following criteria: (1) history of respiratory and/or gastrointestinal symptoms within 8 weeks before admission; (2) 1 of the following symptoms: fatigue/malaise, chest pain, dyspnea, or tachycardia; and (3) 1 of the following ECG signs: conduction block, ST abnormalities, supraventricular tachycardia, or sustained or nonsustained ventricular tachycardia. All patients gave written informed consent and were included in our study.

**CMR Protocol**

ECG-gated CMR imaging was performed in breath-hold mode with the use of a 1.5-T Magnetom Sonata (Siemens Medical Solutions, Erlangen, Germany). Both cine and LGE short-axis CMR images were prescribed every 10 mm (slice thickness 6 mm) from base to apex. In-plane resolution was typically 1.2×1.8 mm. Cine CMR was performed with the use of a steady state free precession sequence. LGE images were acquired on average 5 to 10 minutes after contrast administration with the use of a segmented inversion recovery gradient echo technique with constant adjustment of inversion time, as previously described.13 The contrast dose (Omniscan [gadodiamide], Amersham Health Deutschland, Braunschweig, Germany) was 0.1 mmol/kg. In addition, fat-saturated and T2-weighted images were obtained to allow differentiation between subepicardial LGE, epicardial fat, and pericardial effusion because, in comparison to LGE, bright epicardial fat will disappear after fat saturation, and pericardial effusion will only appear bright on T2-weighted images.

**CMR Analysis**

Cine and contrast images were evaluated separately by 2 blinded observers as described elsewhere.9,13 In brief, endocardial and epicardial borders were outlined on the short-axis cine images. Volumes and ejection fraction (EF) were derived by summation of epicardial and endocardial contours. The extent of LGE was planimetered on the short-axis contrast images with the use of an image intensity level ≥2 SD above the mean of remote myocardium to define LGE.14

Regional parameters were assessed by dividing each short axis into 12 circumferential segments. For each segment, the extent of LGE was measured with the use of the NIH Image Analysis software package (National Institutes of Health, Bethesda, Md), and the results were expressed as a percentage of the total segment area as well as a percentage of the area of the outer, middle, and inner terities of each segment.

**Myocardial Biopsy Protocol**

At least 5 endomyocardial biopsies were taken from the region showing LGE, as described elsewhere.9 In patients who did not undergo CMR imaging before cardiac catheterization (eg, because of an initial interpretation of the clinical presentation as myocardial infarction), at least 4 biopsies were taken from the left ventricle (lateral wall) after coronary artery disease was excluded, and usually another 4 biopsies were taken from the right ventricle (septum) to minimize sampling errors.

**Histopathological Analysis**

Endomyocardial biopsies were stained with Masson’s trichrome as well as Giemsa and examined by light microscopy.15 For immunohistology, tissue sections were treated with an avidin-biotin-immunoperoxidase method (Vectastain-Elite ABC Kit, Vector, Burlingame, Calif), with application of the following monoclonal antibodies: CD3 (T cells; Novocastra Laboratories, Newcastle, UK), CD68 (macrophages, natural killer cells; DAKO, Hamburg, Germany), and HLA-DR-α (DAKO, Hamburg, Germany).

The detection of f>14 infiltrating leukocytes/mm² (CD3+ T lymphocytes and/or CD68+ macrophages) 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 implies that the inflammation is less extensive (<14 leukocytes/mm²), whereas healed myocarditis is characterized by multifocal fibrosis or scarring without inflammation (0 to 3 leukocytes/mm², which is identical to normal myocardium).16–19

**Detection of Viral Genomes**

DNA and RNA were extracted simultaneously with the use of proteinase-K digestion followed by extraction with phenol/chloroform. Nested polymerase chain reaction/reverse transcriptase–polymerase chain reaction was performed for the detection of enteroviruses (including Coxackie B viruses [CBV] and echoviruses), PVB19, adenoviruses, human cytomegalovirus, Epstein-Barr virus, and HHV6.20 As a control for successful extraction of DNA and RNA, oligonucleotide sequences were chosen from the glyceraldehyde-3-phosphate-dehydrogenase gene.20 Specificity of all viral amplification products was confirmed by automatic DNA sequencing.21

**Statistical Analysis**

Univariate numerical samples are characterized by their median and range or interquartile range; the latter is given by the difference between the third and first sample quartiles. To compare 2 paired or independent samples of numerical observations, we use the Wilcoxon signed rank test or Wilcoxon rank test, respectively. To compare the distributions of several independent groups, the Kruskal-Wallis test is used. In case of multiple comparisons, we adjusted the raw probability values by the Holm stepwise correction method (denoted by P<adj). Proportions in 2 groups are compared by the Fisher exact test. The multivariable analysis is set up by multiple linear regressions guided by a stepwise model selection procedure with the use of the Akaike information criterion. As usual, hypotheses related to probability values <0.05 are considered significant findings. The computations were performed with the statistical package R (www.r-project.org).

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

**Presentation**

Chest pain was the primary reason to seek medical attention for the majority of patients (n=83), followed by symptoms of congestive heart failure (n=36) and malaise (n=9). Eighty-seven of the 128 patients enrolled were diagnosed with myocarditis by histopathology, and viral genomes were demonstrated in the myocardium in 82. Five patients had myocarditis by histopathology, but no infectious agent could be detected. In 15 patients, histopathology revealed healing myocarditis without presence of viral genomes. Patients’ clinical characteristics are summarized in Table 1. The remaining 26 patients did not have myocarditis by histopathology, and no virus was found. In this subgroup, however, 2 cases of Takotsubo cardiomyopathy, 3 cases of mild...
hypertrophic cardiomyopathy, and 1 case of cardiac amyloidosis were diagnosed, possibly explaining symptoms at initial presentation. Nevertheless, in the remaining individuals without histopathological evidence of myocarditis, no definite diagnosis could be achieved.

PVB19 was found in the majority of myocarditis patients (n=49), followed by HHV6 (n=16) and combined PVB19/HHV6 infection (n=15). Coxsackie B viruses and Epstein-Barr virus were only present in 1 patient each (Table 1). PVB19-infected patients sought medical attention early because of severe chest pain (n=49; Tables 1 and 2), clinically often appearing similar to acute myocardial infarction. At initial presentation, the left ventricular ejection fraction (LVEF) in the PVB19 group was only mildly impaired, and the end-diastolic volume (EDV) was normal in most cases (Tables 3 and 4). In HHV6 myocarditis, clinical symptoms at initial presentation were more variable (Tables 1 and 2) as well as the range of EDV and LVEF (Tables 3 and 4). Symptoms of heart failure were present in half of HHV6 patients. Interestingly, the majority of patients diagnosed with combined PVB19/HHV6 myocarditis presented with subacute onset of heart failure (11 of 15), frequently in combination with bundle branch block (8 of 15) and malaise. LVEF at initial presentation in this patient group was severely impaired, and most ventricles were dilated (Tables 3 and 4).

Comparing the symptoms forcing patients to seek medical attention between the different patient groups revealed that the clinical presentation of PVB19 patients was statistically significantly different from that of patients with HHV6 or combined PVB19/HHV6 myocarditis. In contrast, the presentation of HHV6 myocarditis was not significantly different from that of combined PVB19/HHV6 myocarditis (Table 2). In addition, pairwise comparisons revealed a statistically significant difference in EDV at initial CMR between the group of PVB19 and combined PVB19/HHV6 myocarditis patients (P=0.03/P_adj=0.03), whereas there was no significant difference in EDV between PVB19 and HHV6 myocarditis. Significant differences in LVEF at initial CMR were detected between the PVB19 and HHV6 groups (P=0.03/ P_adj=0.03) as well as between the PVB19 and combined PVB19/HHV6 patients (P=0.0003/P_adj=0.0009).

After inclusion and initial CMR, all patients diagnosed with active or healing myocarditis received treatment for heart failure. After loop diuretics for fluid control in overt heart failure were introduced first, patients usually were started on angiotensin-converting enzyme inhibitors or, if these were not tolerated, angiotensin II receptor blockers. In addition, β-blockers were initiated after patients were stable on angiotensin-converting enzyme inhibitors. All drugs were started at low doses with titration to recommended dosages as tolerated. Antiviral, immune-stimulating, or immune-suppressing treatment was not prescribed.

Patterns of Myocardial Damage
LGE representing myocardial damage was initially present in 83 of all 87 patients diagnosed with active myocarditis (95%). In the healing myocarditis group (n=15), LGE was present in 6 cases (40%). Patients without histopathological evidence of myocarditis did not have LGE except for 1 patient diagnosed with cardiac amyloidosis.

In the group of PVB19 patients (n=49), all had LGE except 3. Median LGE was 3.8% of LV mass, ranging from 0% to 18%. All HHV6 patients (n=16) also had LGE (median, 6%LV; range, 3% to 50%). Patients with combined PVB19/HHV6 myocarditis (n=15) had LGE as well, except 1 case. Median LV affection was 6.9%, ranging from 0% to 25% (Figure 1).

### Table 1. Baseline Patient Characteristics

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>n</th>
<th>Age, y</th>
<th>Sex, M/F</th>
<th>Chest Pain</th>
<th>Dyspnea</th>
<th>Malaise</th>
<th>Edema</th>
<th>CK, µL</th>
<th>Symptoms to CMR, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVB19</td>
<td>49</td>
<td>42</td>
<td>35/14</td>
<td>92</td>
<td>16</td>
<td>12</td>
<td>2</td>
<td>296</td>
<td></td>
</tr>
<tr>
<td>HHV6</td>
<td>16</td>
<td>32</td>
<td>11/5</td>
<td>76</td>
<td>8</td>
<td>11</td>
<td>3</td>
<td>155</td>
<td>0.75</td>
</tr>
<tr>
<td>PVB + HHV</td>
<td>15</td>
<td>45</td>
<td>11/4</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>62</td>
<td>0.10</td>
</tr>
<tr>
<td>CBV</td>
<td>1</td>
<td>47</td>
<td>1/0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>EBV</td>
<td>1</td>
<td>55</td>
<td>1/0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>No virus</td>
<td>5</td>
<td>42</td>
<td>11/5</td>
<td>7</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>366</td>
<td>0.94</td>
</tr>
<tr>
<td>Healing</td>
<td>15</td>
<td>43</td>
<td>13/2</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>81</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Age, creatine kinase (CK), troponin I, and symptoms to CMR are mean values. CBV indicates Coxsackie B virus; EBV, Epstein-Barr virus.

### Table 2. Comparison of Patient Groups by Fisher Exact Test

<table>
<thead>
<tr>
<th>Symptom Forcing Patient to Seek Medical Attention</th>
<th>P, PVB19 vs HHV6</th>
<th>P, PVB19 vs PVB+HHV</th>
<th>P, HHV6 vs PVB+HHV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chest pain</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.23</td>
</tr>
<tr>
<td>Heart failure</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.27</td>
</tr>
<tr>
<td>Other</td>
<td>0.0005</td>
<td>0.002</td>
<td>1</td>
</tr>
</tbody>
</table>

IQR indicates interquartile range. Values are express in milliliters. Kruskal-Wallis rank sum test shows that the location of the distribution of at least 1 of the 4 factors differs from the others (P=0.0137). Pairwise comparisons with the use of Wilcoxon rank sum test detect a significant difference in PVB19 and PVB+HHV (P=0.05) (with P value adjustment according to Holm’s method).
In the setting of PVB19 myocarditis, LGE was located predominantly in the lateral wall of the left ventricle, typically originating from the epicardial quartile (n=49) (Figure 2). This was completely different from patients with HHV6 myocarditis (n=16), in whom LGE was most frequently found in the anteroseptal region, often located intramurally, without any contact with the subepicardial region (Figure 2).

In the setting of combined PVB19/HHV6 myocarditis, the pattern was similar, not only affecting the anteroseptal myocardium but forming a midwall area of damage in the entire septum.

In our evaluations of group differences in LGE patterns, pairwise comparisons reveal a statistically significantly different pattern in PVB19 compared with HHV6 (P=0.001), HHV6 compared with HHV (P=0.0002), and combined PVB19/HHV (P=0.0002) (with P value adjustment according to Holm’s method).

TABLE 4. LVEF at Initial CMR

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Minimum</th>
<th>Median</th>
<th>Maximum</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVB19</td>
<td>49</td>
<td>12.0</td>
<td>55.0</td>
<td>80.0</td>
<td>20.3</td>
</tr>
<tr>
<td>HHV 6</td>
<td>16</td>
<td>10.0</td>
<td>42.0</td>
<td>83.0</td>
<td>26.3</td>
</tr>
<tr>
<td>PVB + HHV</td>
<td>15</td>
<td>11.0</td>
<td>25.0</td>
<td>76.0</td>
<td>18.5</td>
</tr>
<tr>
<td>Healing</td>
<td>15</td>
<td>18.0</td>
<td>46.0</td>
<td>70.0</td>
<td>26.0</td>
</tr>
</tbody>
</table>

IQR indicates interquartile range. Values are percentages. Kruskal-Wallis rank sum test shows that the location of the distribution of at least 1 of the 4 factors differs from the others (P<0.001). Pairwise comparisons with the use of Wilcoxon rank sum test detect a significant difference in PVB19 and PVB+HHV (P=0.0002) (with P value adjustment according to Holm’s method).

After an average follow-up time of 138 days, 71 of the 87 patients initially diagnosed with active myocarditis were available for repeat CMR. LGE remained present in 52 patients (73%), whereas in 19 patients LGE had disappeared. Seventeen of those patients were initially diagnosed with PVB19; 1 was diagnosed with HHV6; and in 1, initially no virus was found. Interestingly, LGE remained present in all cases of combined PVB19/HHV6 myocarditis.

In the PVB19 group, the amount of LGE at follow-up had decreased to a median of 1.4%LV (range, 0% to 9%); the difference between initial and final LGE was significantly different (P<0.001). In the HHV6 group, LGE decreased to 4.6%LV (range, 0% to 16%); the difference between initial and final LGE was also significantly different (P<0.027). In contrast, in patients with combined PVB19/HHV6 myocarditis, the amount of LGE during follow-up remained almost constant (median, 6%LV; range, 0% to 25%), and the difference between initial and final LGE was not significant (P=0.57) (Figure 3). The majority of patients initially diagnosed with healing myocarditis but no detectable viruses were asymptomatic at follow-up and thus refused to undergo repeat CMR scans (11 of 15). In the 4 patients scanned at follow-up, no LGE was present.

Comparing the group of patients in whom LGE has disappeared with the group in whom the amount of LGE remained constant or even increased from initial to follow-up CMR (n=16), we found that the group with resolved LGE...
Table 1. Eight individuals experienced reoccurring symptoms of heart failure as well as malaise or arrhythmias. Overall, in those patients functional as well as clinical parameters improved less than in the PVB19 group (Figure 5).

The majority of patients with combined PVB19/HHV6 myocarditis (11 of 15) presented at follow-up with ongoing symptoms of heart failure. LVEF had improved significantly (≥5%) in only 1 patient, whereas in most other patients it remained constant or even decreased. A relevant decrease of EDV was observed in only 3 cases (Figure 5).

Pairwise comparisons did not reveal statistically significant “between-entire-group” differences for the change in LVEF and EDV over time. When we compared only PVB19 patients presenting with impaired EF (<30%) and/or dilated ventricles (>200 mL), similar to the usual HHV6 or combined PVB19/HHV6 patient presentation, the between-group differences were statistically significant only for the unadjusted probability values (PVB19 versus HHV6 myocarditis, P=0.031; PVB19 versus combined PVB19/HHV6 myocarditis, P=0.009). This may be a spurious finding, however.

Predictors of Ventricular Size and Function at Follow-up
For evaluation of predictors, only cases with PVB19 and/or HHV6 myocarditis were considered. According to univariate analysis, LVEF and EDV in the acute setting as well as PVB19 infection, combined infection, and symptoms of chest pain or heart failure at initial presentation were clinical predictors of left ventricular function and left ventricular size at follow-up (Table 5). The strongest CMR predictor for chronic ventricular dysfunction as well as ventricular dilatation was the presence of LGE in the ventricular septum (Table 5).

According to multivariable analysis, EDV in the acute setting, the presence of LGE in the septum at initial presentation, and the total amount of LGE (%LV) were the strongest independent predictors of impaired ventricular function and ventricular dilatation at follow-up. In addition, a combined PVB19 and HHV6 infection was also an independent predictor for chronic impairment of ventricular function (Tables 6 and 7).

Discussion
This is the first study to evaluate the prevalence of cardiac PVB19 and/or HHV6 infections in a group of patients with biopsy-proven myocarditis, to evaluate their clinical presentation, and to follow their clinical course. Our study is unique in that patients underwent repeat CMR and endomyocardial biopsy was directed toward the area of LGE.

Presentation
Our data suggest 2 distinct forms of clinical presentation. The first group presents with symptoms of acute myocardial infarction in the absence of coronary artery disease. In those patients, chest pain usually is severe and/or reoccurring, forcing them to seek medical attention early after onset. Interestingly, their ventricles are not severely dilated in most cases, and they often have normal LVEF (Tables 3 and 4). This presentation was seen in all PVB19 patients, which is consistent with other reports demonstrating that PVB19 myocarditis may mimic acute myocardial infarction.
Why should PVB19 patients predominantly present with severe chest pain of acute onset? PVB19 is known to infect endothelial cells of myocardial vessels but not myocytes, causing endothelial dysfunction as well as inducing migration of inflammatory cells into the myocardial interstitium with subsequent myocyte damage. This combination of ischemia and inflammation explains the infarct-like ECG and elevated troponin level (Table 1). Importantly, myocardial inflammation and injury may remain focal, explaining why LVEF is not diffusely impaired.

The second distinct form of clinical presentation was subacute new onset of heart failure, often in combination with malaise and bundle branch block. These patients typically did not fully recover from their initial infection and finally sought medical attention because of persisting dyspnea and increasing peripheral edema. Chest pain also occurred but was not severe enough to lead to hospital admission. This form of presentation was observed in half of HHV6 and most of combined PVB19/HHV6 patients. Because of the more insidious onset, those patients sought medical attention later than patients with infarct-like symptoms (Table 1). This presentation corresponds to the usual onset of myocarditis described in the literature, and our study extends these clinical observations by identifying HHV6 and especially combined HHV6/PVB19 myocarditis as a causative agent.

Speculating on potential mechanisms resulting in this presentation raises 2 major questions: First, why should HHV6 myocarditis result in heart failure more frequently? Second, why is there no infarct-like presentation in combined PVB19/HHV6 myocarditis, despite the presence of PVB19? Unfortunately, the mechanism by which HHV6 causes myocardial damage is not completely understood. However, there is evidence that natural killer cell activity and a virus-specific immune response play a key role. One reason for the higher
incidence of heart failure in HHV6 myocarditis may be the fact that HHV6 also targets T cells, thus impairing its own elimination. This could result in a higher and prolonged disease activity and therefore ventricular dysfunction. An altered immune response caused by HHV6-infected T cells may also explain why heart failure occurs even more frequently in combined infections because the elimination of the second virus may also be impaired. This hypothesis can furthermore help us to understand the lack of infarct-like symptoms in combined infections because the effects of PVB19 on endothelial function may be different in the presence of HHV6.

Similar to Kühl et al, who found PVB19 and HHV6 to be the most common pathogens in their series of patients with “idiopathic” left ventricular dysfunction, we could demonstrate that PVB19 and HHV6 were the most common viruses in patients with clinical signs of myocarditis. This, however, is in contrast to reports that adenoviruses and enteroviruses most frequently cause myocarditis and may be explained by a shift of the type of virus causing myocarditis.

Pattern of Myocardial Damage
We found LGE in 95% of patients diagnosed with myocarditis by histopathology. In contrast, patients with healing myocarditis showed LGE in only 40%. This finding may be explained by the fact that resorption clears necrotic cells and that myocarditic micro scars shrink over time beyond the resolution of CMR, as described previously.

Importantly, our data reveal 2 patterns of myocardial damage. The most frequent pattern, which was present in 52 patients, was LGE occurring predominantly in the lateral free wall, originating from the epicardial quartile (Figures 1 and 2), confirming earlier findings. This peculiar pattern of subepicardial inflammatory lesions as seen by CMR also confirms previous postmortem examinations. Thus, CMR may noninvasively provide information about myocardial damage that previously could only be obtained in the dissecting room. Interestingly, this lateral pattern was found in the majority of PVB19 patients (Figures 1 and 2). The second most frequent pattern was LGE in the midwall area of the interventricular septum (Figures 1 and 2). This pattern of myocardial damage was associated with HHV6 myocarditis. Fourteen of 16 HHV6 patients as well as 14 of 15 combined PVB19/HHV6 patients showed this pattern (Figure 2).

Our unique finding of different patterns of myocardial damage associated with different viruses cannot be explained on the basis of current knowledge. However, one may

Figure 5. Time course of LVEF as well as EDV displayed from inclusion to follow-up (FU) for all patients diagnosed with PVB19, HHV6, combined infection, and healing myocarditis. Note that patients with single PVB19 myocarditis were most likely to improve if impaired at initial presentation. Medians are indicated by black arrows.
speculate on a virus-specific pathophysiology. HHV6, for example, primarily infects T cells but also cells of the nervous and the cardiac conduction system. In addition, an important property of HHV6 is its ability to establish a latent state after primary infection. Because the primary infection occurs in early childhood, it is possible that HHV6 myocarditis may develop in adulthood. HHV6, for example, primarily infects T cells but also cells of the nervous and the cardiac conduction system. In addition, an important property of HHV6 is its ability to establish a latent state after primary infection. Because the primary infection occurs in early childhood, it is possible that HHV6 myocarditis may develop in adulthood.

### TABLE 5. Predictors of Clinical Outcome: Simple Linear Models (Intercept Not Shown)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient ± SE</th>
<th>P</th>
<th>Coefficient ± SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF acute</td>
<td>0.68 ± 0.07</td>
<td>&lt;0.0001</td>
<td>-0.012 ± 0.0027</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EDV acute</td>
<td>-0.1 ± 0.02</td>
<td>&lt;0.0001</td>
<td>-0.004 ± 0.0004</td>
<td>&lt;0.0001</td>
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<tr>
<td>Virus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HHV6</td>
<td>-4.1 ± 5.04</td>
<td>0.42</td>
<td>0.074 ± 0.14</td>
<td>0.6</td>
</tr>
<tr>
<td>PVB19</td>
<td>17.4 ± 3.33</td>
<td>&lt;0.0001</td>
<td>-0.32 ± 0.1</td>
<td>0.003</td>
</tr>
<tr>
<td>HHV6+PVB19</td>
<td>-20.5 ± 3.9</td>
<td>&lt;0.0001</td>
<td>0.38 ± 0.12</td>
<td>0.003</td>
</tr>
<tr>
<td>Symptom</td>
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<td></td>
</tr>
<tr>
<td>Chest pain</td>
<td>20.53 ± 3.1</td>
<td>&lt;0.0001</td>
<td>-0.4 ± 0.01</td>
<td>0.0002</td>
</tr>
<tr>
<td>Heart failure</td>
<td>-16.3 ± 3.9</td>
<td>&lt;0.0001</td>
<td>0.41 ± 0.11</td>
<td>0.0005</td>
</tr>
<tr>
<td>Other</td>
<td>-16.7 ± 6.0</td>
<td>0.007</td>
<td>0.14 ± 0.18</td>
<td>0.44</td>
</tr>
<tr>
<td>LGE, %LV</td>
<td>-0.57 ± 0.27</td>
<td>0.037</td>
<td>0.02 ± 0.007</td>
<td>0.01</td>
</tr>
<tr>
<td>LGE septal</td>
<td>-1.46 ± 0.23</td>
<td>&lt;0.0001</td>
<td>0.037 ± 0.007</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LGE lateral</td>
<td>0.48 ± 0.25</td>
<td>0.054</td>
<td>-0.003 ± 0.007</td>
<td>0.671</td>
</tr>
</tbody>
</table>

LGE indicates late gadolinium enhancement.

### TABLE 6. Predictors of Clinical Outcome: Multiple Linear Model for EDV at Follow-Up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>SE</th>
<th>Wald</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-9.3</td>
<td>13.01</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>EDV acute</td>
<td>0.75</td>
<td>0.07</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Virus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVB19</td>
<td>15.37</td>
<td>23.72</td>
<td>0.194</td>
<td></td>
</tr>
<tr>
<td>HHV6+PVB19</td>
<td>-26.81</td>
<td>21.79</td>
<td>0.231</td>
<td></td>
</tr>
<tr>
<td>Symptom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart failure</td>
<td>30.234</td>
<td>30.923</td>
<td>0.174</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>41.624</td>
<td>27.518</td>
<td>0.039</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LGE, %LV</td>
<td>2.02</td>
<td>0.74</td>
<td>0.008</td>
<td>0.004</td>
</tr>
<tr>
<td>LGE septal</td>
<td>2.12</td>
<td>0.91</td>
<td>0.023</td>
<td>0.023</td>
</tr>
</tbody>
</table>

LGE indicates late gadolinium enhancement. Residual SE = 41.2 on 62 df. Multiple R² = 0.7879, adjusted R² = 0.7785. F statistic: 81.53 on 3 and 62 df, P = 2.2e⁻¹⁶.

Why would the focus of myocardial damage caused by PVB19 be located in the subepicardium of the left inferolateral wall? One possibility is that myocardial damage in PVB19 myocarditis develops in regions of distal circulation because of PVB19-mediated endothelial dysfunction and microvascular disease causing impaired perfusion to watershed myocardial territories.

Another explanation may be the fact that cardiotropic viruses, including PVB19, can cause polyserositis and pericarditis after initial viremia. Because the left lateral free wall is in direct contact with the pericardium, it is 1 prime location for per continuitatem propagation of PVB19 and inflammation. This hypothesis is also supported by animal studies showing that viral pericarditis occurs first and subsequently is followed by viral myocarditis as well as the fact that a high concentration of viruses can be found in serous secretions, including pericardial fluid. However, if this is correct, one would expect the right ventricular free wall to be affected as well because it has contact with the pericardium as well. Interestingly, Shirani et al demonstrated this postmortem in vivo. In vivo, however, the LGE is mainly visible in the left ventricle, perhaps because of limited spatial resolution (only 1 to 3 pixels in the right ventricular wall) and chemical shift.

At follow-up, we found that left lateral LGE decreased over time in all patients who were not affected by HHV6 (Figure 4). However, in HHV6-associated myocarditis, septal LGE persisted or even increased in some cases of combined HHV6/PVB19 myocarditis, forming a distinct midwall striate-like pattern within the interventricular septum that recently has also been described in patients with idiopathic dilated cardiomyopathy.

### Clinical Course

We found that the clinical course in PVB19 myocarditis was mostly benign, and despite the sudden onset of infarct-like symptoms, all patients improved significantly or recovered fully. This finding is somewhat surprising because other authors found that PVB19 could not be eliminated from the myocardium in nearly 80% of their patients and was associated with cardiac death or heart failure, especially in...
those patients developing viral persistence.\textsuperscript{20,28,42,44–46} However, other groups found PVB19 in patients with normal LVEF, without ventricular dilatation or adverse events,\textsuperscript{8} which is consistent with our present and previous results. In addition, the pathophysiology of PVB19 myocarditis makes a benign clinical course for immunocompetent patients more likely because PVB19 does not directly infect myocytes but rather causes secondary myocardial damage due to migration of inflammatory cells.

In our group with HHV6-related myocarditis, the clinical course was worse. During follow-up, only about half of the patients improved (Figure 5). In the group with combined HHV6/PVB19 myocarditis, the majority did not improve. Based on the fact that HHV6 establishes a latent state after primary infection,\textsuperscript{34} every infected individual is forever susceptible to subsequent periodic viral reactivation. Thus, HHV6-associated myocarditis may also be subject to reactivation, even if the virus has been eliminated from the mycardium temporarily between reactivations, which is consistent with the recent findings from Kühl et al.\textsuperscript{28,43,44} That viral persistence is associated with progressive cardiac dysfunction.

Predictors of Ventricular Size and Function at Follow-Up

Statistical analysis revealed EDV in the acute setting, the presence of LGE in the septum at initial presentation, and the total amount of LGE (\%LV) as the strongest independent predictors of chronic ventricular dysfunction and dilatation. Ventricular enlargement in the acute setting is most likely caused by myocardial damage due to inflammation. Over time, replacement scar and interstitial fibrosis will appear in those areas, causing permanent structural damage to the ventricle. If a certain threshold is met, dilatation may become irreversible, explaining why the EDV in the acute setting has been found to be a predictor of chronic dysfunction and dilatation. This is supported by the finding that the total amount of LGE is also an independent predictor of ventricular function and remodeling because LGE is known to represent irreversible myocardial injury in a variety of cardiac diseases.\textsuperscript{47} In addition, Rochitte et al.\textsuperscript{31} recently described a similar relation between the amount of LGE and ventricular dysfunction in Chagas myocarditis. This relation was also found in our previous study, in which it almost reached statistical significance ($P = 0.09$). However, the mechanism explaining the prognostic importance of septal LGE compared with other locations remains unclear. Obviously, the septal pattern is related to ventricular dysfunction and dilatation because it can be encountered frequently in patients with idiopathic dilated cardiomyopathy,\textsuperscript{42} but further investigation is necessary.

In addition to functional parameters and LGE, a combined PVB19/HHV6 myocarditis has also been identified as an independent predictor of chronic ventricular dysfunction. This finding is consistent with the results of Kühl et al.\textsuperscript{28} Demonstrating that myocardial virus elimination is less common in patients with combined infection, and thus chronic ventricular dysfunction is more common in those patients.

Conclusion

Our data indicate that PVB19 and HHV6 are the most important causes of biopsy-proven viral myocarditis in Germany and that the clinical presentation is related to the type of virus present in the myocardium. Furthermore, clinical presentation, type of virus, and pattern of myocardial damage are related to the clinical course.

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Disclosures

None.

References

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