Prevalence of Parvovirus B19 and Human Bocavirus DNA in the Heart of Patients with no Evidence of Dilated Cardiomyopathy or Myocarditis

Friedhelm Kuethe,1,a Juha Lindner,1,b Klaus Matschke,2 Juergen J. Wenzel,1 Päivi Norja,3 Katrin Ploetze,3 Sarah Schaal,1 Virginia Kamvissi,1 Stefan R. Bornstein,1 Uta Schwanebeck,2 and Susanne Modrow4

1Department of Medicine and 2Coordinating Centre for Clinical Trials, University of Dresden, 3Department of Cardiovascular Surgery, Heart Centre Dresden, Dresden, 4Institute of Medical Microbiology and Hygiene, University of Regensburg, Regensburg, Germany; and 5Department of Virology, Haartman Institute, University of Helsinki, Helsinki, Finland

Background. Although the DNA of parvovirus B19 (B19V) is frequently detected in patients with dilated cardiomyopathy or myocarditis, whether the parvovirus causes disease is questionable, since even in healthy individuals the virus persists in various tissues. The same question applies to human bocavirus (HBoV). We have determined the prevalence and quantity of B19V and HBoV DNA in heart tissue of patients who were not experiencing virus-related heart diseases and analyzed whether the seroprevalence corresponded to DNA prevalence in the heart.

Methods. Samples of left-atrium heart tissue and serum were obtained from 100 patients who underwent open-heart surgery. Serum immunoglobulin (Ig) G and IgM against proteins encoded by B19V and HBoV were detected by enzyme-linked immunoabsorption assay and immunoblotting. B19V and HBoV DNA concentrations were determined by quantitative real-time polymerase chain reaction (PCR) in heart tissue and serum samples. Nested PCRs for VP1, K71, and GT3 identified the B19V genotypes.

Results. The prevalences of serum IgG specific for B19V and HBoV were 85% and 96%, respectively. Of all the patients, 85% had B19V DNA detected in heart tissues, and 4% displayed low-level B19V viremia, whereas only 5% of heart tissue samples and none of the serum samples demonstrated HBoV DNA. The sensitivity of B19V serological testing for B19V DNA in heart samples was 0.96 (95% confidence interval, 0.92–1.0). Specificity was 0.8 (95% confidence interval, 0.6–1.0), and the positive predictive value was 0.96 (95% confidence interval, 0.92–1.0). B19V genotypes 1 and 2 were present in 11% and 89% of heart tissue samples, respectively. B19V genotype 3 was not detected in any of the samples.

Conclusions. Our data suggest that B19V but not HBoV demonstrates a lifelong persistence in the heart. The detection of B19V DNA in heart tissue showed no correlation with clinical symptoms. We strongly recommend that serological testing become a standardized procedure for future studies, to obtain representative data concerning the prevalence of B19V in the heart.

Parvovirus B19 (B19V) and human Bocavirus (HBoV) are members of the Paroviridae. B19V causes erythema infectiosum, transient aplastic anemia, and hydrops fetalis [1–3] and is associated with arthritis, hepatitis, and vasculitic syndromes [4–7]. HBoV causes respiratory disease in children and infants [8–10]. More than 90% of the adult German population has been seropositive for both B19V and HBoV [11, 12].

Although no information is available on the cell tropism of HBoV, B19V infects erythroid progenitor cells of the bone marrow via adsorption to the blood group antigen P [13, 14]. The heart is a key focus in the study of B19V-associated diseases, because B19V DNA is found in the hearts of patients with acute myocarditis, dilated cardiomyopathy, and peripartum cardiomyopathy [15–20]. Nevertheless, whether B19V causes heart disease is highly questionable, because B19V DNA is
found in various tissues of healthy adults [21–23] and is also found in the myocardium of patients without cardiomyopathy or myocarditis [17, 24]. Furthermore, a recent study found no differences in the T cell–mediated immune response or in serological parameters of patients with myocarditis or dilated cardiomyopathy with detectable B19V DNA in the myocardium, compared with healthy individuals [25]. Whether the persistence of viral DNA in tissues is a unique feature of B19V or can also be observed with other human paroviruses is not known.

The aim of this study was to analyze the prevalence of paroviral DNA in heart tissue obtained from patients who did not have myocardial diseases that might be associated with viral pathogens. In this regard, the group of patients investigated can be regarded as representing the normal adult population. The data supply further evidence that B19V DNA commonly persists asymptomatically in myocardial tissue.

**METHODS**

**Patient population.** From December 2007 through March 2008, we obtained 3–5 g of tissue from the left atrium of 100 patients who underwent open-heart surgery. The surgical procedures included valve replacement or correction and coronary artery bypass grafting, alone or in combination. Patients with cardiomyopathy were excluded from the study. The tissue was frozen in liquid nitrogen immediately after removal and stored at −80°C until nucleic acid preparation. All patients underwent determination of the ejection fraction via echocardiography in standard manner and views or left ventricular angiography. Basic characteristics and operating procedures are listed in Table 1. The local ethics committee approved the study, and all patients gave written informed consent for the data to be included in the study.

**Detection of B19V- and HBoV-specific antibodies.** B19V-specific immunoglobulin (Ig) G and IgM antibodies were detected in serum samples with use of standardized enzyme-linked immunosorbent assay (ELISA; Biotrin International) and Western Blot assay (recomLine; Mikrogen GmbH) according to the manufacturers’ instructions [26]. HBoV-specific IgG and IgM were detected by ELISA as described elsewhere [27].

**Preparation and detection of B19V and HBoV DNA.** DNA extraction from serum samples and from cardiac tissue of the left atrium was performed with the QIAamp DNA Mini Kit (Qiagen). Briefly, tissue samples were minced and ~50 mg of tissue was transferred to microcentrifuge tubes containing lysis buffer and proteinase K. Tissue lysis was performed at 56°C overnight. Further sample work-up was performed according to the manufacturers’ instructions. All samples were analyzed in duplicate.

B19V and HBoV DNA were quantitated by real-time TaqMan polymerase chain reaction (PCR). Sequences of primers and probes for the B19V PCR for the combinatorial detection of B19V genotypes 1–3 were as described elsewhere [28]. To amplify the HBoV genome sequences, the following primers and probes were used: forward primer, 5′-CCACCTATCGTCTTGCACTGCG-3′ (nucleotides 2586–2606); reverse primer, 5′-TTTTCCCCCGATGTACTCTCCC-3′ (nucleotides 2619–2639) probe, FAM-5′-TCGAAGACCTCAGACCAAGTGATGAAGACG-3′-TAMRA (nucleotides, 2608–2637) (nucleotide positions according to GenBank accession number DQ000496.1). An initial denaturation phase of 10 min at 95°C was followed by 45 cycles at 95°C (15 s) and 60°C (1 min).

Quantitative values for B19V and HBoV DNA were calculated from simultaneous detection of serial dilutions of cloned PCR target fragments, which were used to generate standard curves. The number of cells in the respective tissue samples was calculated with use of quantitative PCR targeting a non-coding chromosomal region upstream of the human pyruvate dehydrogenase ß gene [29].

**Detection of B19V genotypes.** B19V genotype analysis was performed at the Haartman Institute for Virology (Helsinki, Finland). Again, DNA from 50 mg samples of myocardium was isolated using the QIAamp DNA Mini Kit. All samples were analyzed by 3 nested PCRs: VP1 PCR was used to amplify B19V genotypes 1 and 2, K71 PCR was used to amplify genotype 2, and GT3 PCR was used to amplify genotype 3. The sensitivities

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients (n = 100)</th>
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<tbody>
<tr>
<td>Sex, male/female</td>
<td>76/24</td>
</tr>
<tr>
<td>Age, mean years ± SD</td>
<td>68.7 ± 8.7</td>
</tr>
<tr>
<td>BMI, mean kg/m² ± SD</td>
<td>28.9 ± 4.5</td>
</tr>
<tr>
<td>Hypertension</td>
<td>97</td>
</tr>
<tr>
<td>Diabetes</td>
<td>48</td>
</tr>
<tr>
<td>Smoking</td>
<td>32</td>
</tr>
<tr>
<td>NYHA classification, I/II/III/IV</td>
<td>11/51/35/3</td>
</tr>
<tr>
<td>Ejection fraction, mean % ± SD</td>
<td>54.7 ± 14.6</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
</tr>
<tr>
<td>AVR</td>
<td>23</td>
</tr>
<tr>
<td>AVR and AA</td>
<td>3</td>
</tr>
<tr>
<td>AVR and MVR</td>
<td>2</td>
</tr>
<tr>
<td>CABG</td>
<td>52</td>
</tr>
<tr>
<td>CABG and AVR</td>
<td>8</td>
</tr>
<tr>
<td>CABG and MVR</td>
<td>5</td>
</tr>
<tr>
<td>MVR</td>
<td>6</td>
</tr>
<tr>
<td>MVR and TVR</td>
<td>1</td>
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</tbody>
</table>

**NOTE.** Data are no of patients, unless otherwise indicated. AA, aortic aneurysm; AVR, aortic valve replacement; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); CABG, coronary artery bypass grafting; MVR, mitral valve replacement or reconstruction; NYHA, New York Heart Association classification; SD, standard deviation; TVR, tricuspid valve reconstruction.
of VP1 PCR and K71 PCR were measured elsewhere (sensitivity, 5 copies/reaction). The sensitivity of GT3 PCR was measured with a dilution series of plasmid clone V9, resulting in a value of 5 copies/reaction as well. The primers for VP1 and K71 PCR have been described elsewhere [30]. For nested GT3 PCR, the outer primer sequences were as follows: forward primer, 5′-ACCCATTTTCTGTGTTAACTTGT-3′; reverse primer, 5′-CGAGGAC-3′. The forward and reverse internal primer sequences were 5′-CAGTGACAACTAAGTCAAATAA-3′ and 5′-AAGGATTATCTAAAGAAATG-3′, respectively. The PCR products were separated by 1% agarose gel electrophoresis for VP1 and 2% for K71 and GT3, and the products were detected by ethidium bromide staining.

**Statistical analysis.** Discrete variables are expressed as counts (percentage) and continuous variables were expressed as means ± standard deviation (SD), unless stated otherwise. Frequency comparison was done by χ² test. For data that was not normally distributed, the Mann-Whitney U test was used. Sensitivities, specificities, positive predictive values, and negative predictive values with precise 95% confidence intervals (CIs) were calculated for single and composite markers. P values of <.05 were considered to be statistically significant. For statistical analysis, JMP Statistical Software, version 7.01 (SAS Institute), was used.

**RESULTS**

**Study population.** Altogether, 100 white patients underwent cardiac surgery (76 men and 24 women; mean age [± SD], 68.7 ± 8.7 years; range, 46–86 years). Of these, 15 were aged <60 years, 34 patients were aged 60–69 years, 45 patients were aged 70–79 years, and 6 patients were aged ≥80 years. The mean ejection fraction (± SD) was 54.7% ± 14.6%; the ejection fraction was >55% in 58 patients and below 35% in 16 patients. Cardiac insufficiency was symptomatic in 38% of patients; 35 were in New York Heart Association classification stage III, and 3 were in stage IV (Table 1). No patient had cardiomyopathy or myocarditis.

**Serum analysis for viral DNA and virus-specific antibodies.** ELISA and immunoblotting were used to detect B19V-specific antibodies in serum. By ELISA alone, the seroprevalence for all patients was 79%, and of 85 patients positive for B19V DNA in the heart, 76 were IgG positive. The sensitivity of serological testing for B19V DNA in the heart was 0.89 (95% CI, 0.82–0.96), specificity was 0.8 (95% CI, 0.6–1.0), positive predictive value was 0.96 (95% CI, 0.92–1.0), and negative predictive value was 0.57 (95% CI, 0.36–0.78). Combining ELISA data with those from immunoblotting, seroprevalence increased to 85%, and of the 85 patients positive for B19V DNA, 82 were IgG positive. The resultant sensitivity was 0.96 (95% CI, 0.92–1.0), specificity was 0.8 (95% CI, 0.6–1.0), positive predictive value was 0.96 (95% CI, 0.92–1.0), and negative predictive value was 0.8 (95% CI, 0.6–1.0) (Table 2).

Of all B19V-seropositive individuals, 97.6% had antibodies against conformational epitopes of the viral capsid proteins. Antibodies against linear VP2 epitopes were found in 5.9% of serum samples. Antibodies against the viral nonstructural protein NS1 were detected in 2.4% of patients. VP2-specific IgM was detected in 1 patient. In 4% of patients, low levels of B19V DNA (up to 100 genome equivalents [geq]/mL) were observed in serum samples. Because none of the patients were IgM positive, this situation is indicative of persistent low level B19V production and has been observed in individuals with and without symptoms [25, 30].

In 96% of the serum samples, HBoV-specific IgG antibodies were detected, but neither HBoV-specific IgM nor HBoV DNA were observed in any of the serum samples.

**Detection of B19V and HBoV DNA in the heart.** In total, 85% of patients had B19V DNA detected in the heart. The mean number (± SD) of geq per million cells of heart tissue (geq/1 × 10⁶ cells) was 146.6 ± 163.6, and the maximum con-

### Table 2. Analysis of Parvovirus B19 (B19V) and Human Bocavirus (HBoV) DNA and Specific Antibodies

<table>
<thead>
<tr>
<th>Assay</th>
<th>No of patients</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Positive predictive value (95% CI)</th>
<th>Negative predictive value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B19V DNA detection in heart[^a^]</td>
<td>85</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>B19V DNA in serum</td>
<td>4</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>HBoV DNA in heart</td>
<td>5</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>B19V IgM ELISA in serum</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>B19V IgG ELISA in serum</td>
<td>79</td>
<td>0.89 (0.78–0.92)</td>
<td>0.8 (0.6–1.0)</td>
<td>0.96 (0.92–1.0)</td>
<td>0.57 (0.36–0.78)</td>
</tr>
<tr>
<td>B19V IgG ELISA and WBA in serum</td>
<td>85</td>
<td>0.96 (0.92–1.0)</td>
<td>0.8 (0.6–1.0)</td>
<td>0.96 (0.92–1.0)</td>
<td>0.8 (0.6–1.0)</td>
</tr>
<tr>
<td>HBoV IgM ELISA in serum</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>HBoV IgG ELISA in serum</td>
<td>96</td>
<td>...</td>
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</tbody>
</table>

**NOTE.** CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin WBA, Western blot assay.

[^a^]: The mean (± standard deviation) level of DNA detected was 147 ± 164 genome equivalents per 1 × 10⁶ cells.
Figure 1. Distribution of patients with different levels of parvovirus B19 (B19V) genome equivalents per 1 million cells in the heart, measured by quantitative polymerase chain reaction.

centration in our study population was 1025.0 geq/1 × 10^6 cells. Around one-half (51%) of the patients had values <100 geq/1 × 10^6 cells (Figure 1). The amount of B19V DNA was not significantly different in patients with an ejection fraction <35%, compared with patients with an ejection fraction of ≥55% (P = .412), and the same frequency of patients were positive for B19V DNA in both groups (P = .348). The frequency of patients positive for B19V DNA was not significantly different with increasing age (patients aged <60 years, 13 of 15 positive; 60–69 years, 28 of 34 positive; 70–79 years, 38 of 45 positive; and ≥80 years, 6 of 6 positive).

HBoV DNA was found in heart tissue of 5% of patients. The mean age (±SD) of HBoV DNA–positive patients was similar to that of HBoV DNA–negative patients (68.5 ± 8.6 vs 73.6 ± 8.4 years; P = .249). Patients with detectable HBoV DNA in the myocardium had similar mean ejection fraction values (±SD) to patients without detectable HBoV DNA (53.0% ± 6.7% vs 54.8% ± 14.9%; P = .612).

**Detection of B19V genotypes in the heart.** When heart tissue was examined for human B19V DNA, 87 samples were positive by the VP1 PCR. Only 9 of these samples were negative for K71 (which indicated that they contained genotype 1). The other 78 samples had both VP1 and K71; therefore, they contained genotype 2 DNA. All 85 patients who were determined to be positive for B19V DNA by the quantitative PCR specific for genotypes 1–3 in Regensburg (Germany) had positive test results confirmed at the institute in Helsinki. This verification at 2 different laboratories was performed to exclude cross-contamination artifacts. The results included 2 samples with low copy numbers that had given negative results with the combinatorial B19V DNA detection approach used by the group in Germany. Because no sample was positive for GT3, genotype 3 was absent from all tissue samples. B19V DNA–positive patients were analyzed according to age; in the group of patients aged <60 years, 25% were positive for genotype 1 and 75% for genotype 2. Among those aged 60–69 years, 8% were positive for genotype 1 and 92% for genotype 2, among those aged 70–79 years, 11% were positive for genotype 1 and 89% for genotype 2 (Figure 2). All patients aged ≥80 years were positive for genotype 2.

**DISCUSSION**

Viral infections of the heart have become a central issue in studying the pathogenesis of myocarditis and dilated cardiomyopathy. Experimental data, mostly derived from mouse models, have suggested that myocarditis causes dilated cardiomyopathy [31]. It has been thought that dilated cardiomyopathy can occur as a late sequel of viral myocarditis, because of either viral persistence [32] or a chronic immune process initially triggered by a viral infection [33]. Sensitive molecular methods have detected viral nucleic acids in almost 70% of endomyocardial biopsies [34].

In the past, enteroviruses were reported to be the most common cause of myocarditis and dilated cardiomyopathy [31, 35]. Because B19V DNA has repeatedly been detected in up to 65% of endomyocardial biopsies, B19V has become the prime suspect [34, 36]. However B19V DNA has also been found in myocardium from healthy heart donors [37], in the hearts of patients with normal left ventricular function [17], and in the hearts of patients with amyloidosis or lupus erythematosus [24], although the number of patients investigated was small. These findings raise the question as to whether B19V really does cause the underlying heart disease or whether it is only an innocent bystander present in the heart as a consequence of an earlier infection, typically occurring during childhood or
adolescence [11]. The present study aimed to determine the prevalence of B19V DNA in patients who are not experiencing cardiomyopathy or myocarditis. It also raises the question of whether HBoV DNA also persists in the myocardium and whether tissue persistence is a general feature of parvovirus pathobiology [38].

In the present study, 85% of the patients demonstrated B19V DNA in the myocardium. The seroprevalence was also up to 85%, and the sensitivity and positive predictive value of the combined serological approach were 96%. Therefore, almost all seropositive patients had myocardial B19V DNA. Although the study subjects underwent cardiac surgery, their clinical conditions were not associated with viral infections, and thus, the observed B19V DNA prevalence can be regarded as representing the adult German population. In contrast, HBoV DNA was found in only 5% of the patients, although almost all patients had HBoV IgG detected. This suggests that, unlike B19V, HBoV infections do not lead to viral DNA persistence in heart tissue. Because the viral replication cycle, including target cells or cellular receptors, is currently unstudied for HBoV, the reason for this difference remains to be elucidated.

Because the seroprevalence and the myocardial prevalence of B19V DNA are so closely correlated, we may question the relevance of B19V DNA detection in patients with myocarditis or dilated cardiomyopathy. Several previous studies have actually reported a lower frequency of B19V DNA detection in endomyocardial biopsies of patients with inflammatory cardiomyopathy or myocarditis [19, 39, 40], compared with our results. This is not surprising, considering the very small sample size of endomyocardial biopsies, compared with tissue obtained during open-heart surgery. We extracted nucleic acids from 50 mg of tissue. According to the results obtained from quantification of the human pyruvate dehydrogenase ß gene, the samples included an averaged of 2.5 \times 10^6 cells. In view of the low number of B19V geqs per cell, analysis of endomyocardial biopsies obtained from diseased hearts with a high content of fibrotic tissue may entail an enhanced sampling error. Because our results indicate that B19V DNA is present in almost all individuals with previous B19V infection, B19V DNA–negative endomyocardial biopsies of seropositive individuals should be regarded with skepticism. Our data are supported by a recent finding that 96% of left ventricular tissue samples are B19V DNA–positive when study subjects are also seropositive [41]. That study also showed that there is no difference between tissue from the left atrium or left ventricle with regard to viral persistence. In view of our new findings, we strongly recommend serological testing for all future studies, to warrant a standard of quality and to prevent misinterpretation of data.

One may ask whether the presence of B19V DNA in myocardial tissue causes the underlying disease, or whether it is a pathogenetically unrelated viral persistence. Using quantitative PCR to quantitate pathogen-specific nucleic acids might be the first step in resolving this question. As long as data are based exclusively on qualitative DNA analysis, the interpretation is delicate. The results shown here explain the observation that the presence of B19V DNA does not predict the outcomes of patients with suspected myocarditis or dilated cardiomyopathy [24, 42]. Copy numbers of up to 1000 B19V geq/1 \times 10^6 cells, the highest that we detected, should be regarded as a normal diagnostic finding, especially because in acute diseases definitely associated with B19V infections, such as hydrops fetalis, copy numbers are significantly higher [28]. It has been suggested that B19V may induce acute myocarditis that clinically mimics ischemic heart disease [15] and is responsible for vasospasm of coronary arteries [43]. Therefore, it would be very informative to assess B19V DNA concentrations as a measure of virus load in future studies or case presentations. However our data do not definitively exclude the possibility that adult primary exposure to B19V might lead to acute cardiac disease.

A recent publication inferred that genotype 2 of B19V was predominate in patients with dilated cardiomyopathy and was the only genotype found in heart tissue of patients aged >65
years [18]. The patients with genotype 1 detected were younger on average and had poorer left ventricular function than did patients with genotype 2 detected. The authors speculated that genotype 1 might trigger a more severe autoimmune reaction. In our patients, genotype 2 was predominante. However, we did find genotype 1 in older patients. Another study of 523 non-cardiac biopsies also demonstrated genotype 1 in older patients and in all patients born after 1973 [30]. It therefore seems unlikely that different viral subtypes really evoke different immunological responses. More likely, the apparent association of genotype 1 with poor left ventricular function reflected other characteristics of patients with an earlier onset of dilated cardiomyopathy, such as genetic predisposition, causing an age-related bias of the samples. This assumption is supported by data from in vitro neutralization that used serum samples from subjects infected with B19V genotype 1, which also inhibited genotype 2 infection [44]. Furthermore, 100% cross-reactivity of IgG antibodies against either VP1 or VP2 protein can be found between B19V genotypes 1 and 2 [45]. Because data on cellular immune reactions against VP1 and VP2 of genotype 2 have not been published, it is unclear whether T cells may be similarly cross-reactive. However, the 3 B19V genotypes were transcribed with comparable efficiency in various cell types, suggesting a similar tropism [45]. These previous studies and our own findings, which exclude sampling errors, indicate there is no genotype difference in the pathobiology of B19V infections. The patients we investigated are representative of the age-matched German population. We found a high seroprevalence of B19V, which corresponded with the cardiac prevalence of B19V DNA. In contrast, no persistence of HBoV DNA was observed, suggesting that the lifelong DNA persistence in the heart tissue is a characteristic feature of B19V. Our results imply that persistent B19V DNA is not pathogenetic. On this basis, we strongly recommend age-matched serological testing and quantitative PCR for future studies. Correct interpretation of the data will help elucidate any clinical significance of viral DNA detected in cardiac tissue.

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References


