Myocardial Parvovirus B19 Persistence: Lack of Association with Clinicopathologic Phenotype in Adults with Heart Failure

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Abstract

**Background**—Multiple viruses have been isolated from the heart, but their significance remains controversial. We sought to determine the prevalence of cardiotropic viruses in endomyocardial biopsy (EMB) samples from adult heart failure (HF) patients and to define the clinicopathologic profile of patients exhibiting viral positivity.

**Methods and Results**—EMB from 100 patients (median EF 30%, IQR 20-45%) presenting for cardiomyopathy evaluation (median symptom duration 5 months, IQR 1-13 months) were analyzed by polymerase chain reaction for adenovirus, cytomegalovirus, enteroviruses, Epstein-Barr virus, and parvovirus B19. Each isolate was sequenced and viral load was determined. Parvovirus B19 was the only virus detected in EMB samples (12% of subjects). No subject had anti-parvovirus IgM antibodies, but all had IgG antibodies, suggesting viral persistence. The clinical presentation of parvovirus-positive patients was markedly heterogeneous, with both acute and chronic HF, variable ventricular function, and ischemic cardiomyopathy. No subject met Dallas histopathological criteria for active or borderline myocarditis. Two patients with a positive cardiac MRI and presumed “parvomyocarditis” had similar viral loads as autopsy controls without heart disease. The oldest parvovirus-positive subjects were positive for genotype 2, suggesting lifelong persistence in heart tissue.

**Conclusions**—Parvovirus B19 was the only virus isolated from EMB samples in this series of adult HF patients from the United States. Positivity was associated with a wide array of clinical presentations and heart failure phenotypes. Our studies do not support a causative role for parvovirus B19 persistence in HF and therefore advocate against the use of antiviral therapy for these patients.

**Key Words:** heart failure, viruses, biopsy, cardiomyopathy, myocarditis
Despite comprehensive evaluation including heart biopsy, approximately half of patients with initially unexplained heart failure are labeled as having idiopathic cardiomyopathy.\textsuperscript{1} The high prevalence of these poorly understood cardiomyopathies has led to ongoing interest about a potential role for viral infection leading to heart failure. Multiple cardiotropic viruses have been implicated in the development of myocarditis, dilated cardiomyopathy and isolated left ventricular diastolic dysfunction.\textsuperscript{2-4} The enteroviruses (most commonly coxsackievirus) were the first to be described, followed by adenovirus, and more recently the human erythrovirus known as parvovirus B19.\textsuperscript{5,6} Given the variable detection rates, the clinical significance of viral recovery from heart tissue remains controversial, with some studies claiming viruses are a ubiquitous cause of cardiomyopathy\textsuperscript{7-12} and others arguing that these same viruses may be a normal, non-pathologic element of the host microbiome.\textsuperscript{13-15}

The prevalence of viral recovery in patients presenting for a heart failure evaluation remains uncertain, as does the interaction between cardiotropic viral persistence and clinical phenotype as defined by presenting symptoms, cardiac imaging and histopathology. To date, the majority of studies on have been performed in Europe. We set out to prospectively determine the prevalence of cardiotropic viruses and their associated clinicopathological profiles in adult heart failure patients in the United States undergoing endomyocardial biopsy (EMB).
Methods

Patient Population

Subjects were prospectively enrolled at Brigham and Women’s Hospital (Boston, MA) between June 2004 and May 2008. The institutional review board approved the study protocol and each participant provided written informed consent. Eligible subjects were adult males or non-pregnant females (age >18yrs) undergoing diagnostic endomyocardial biopsy as part of evaluation for new onset cardiomyopathy or refractory heart failure.16 Patients were ineligible for participation if they had an uncorrected coagulopathy (INR>1.3), platelet count <75,000/mm³, right ventricular systolic pressure >60mmHg, body mass index (BMI) >40 kg/m², or significant thoracic deformity, which could increase biopsy risk.

Baseline demographic and clinical data was collected including age, sex, self-identified race, BMI, duration of heart failure symptoms, and presence of viral prodrome. Patients were further characterized according to their dominant clinical feature, which could include one or more of the following: congestive heart failure, ventricular arrhythmia, or chest pain syndrome. Physical examination at enrollment included assessment of resting heart rate, blood pressure, and the presence of an S3 gallop. Patients underwent right heart catheterization using a balloon-tipped, flow-directed catheter. Cardiac output was determined by the Fick method. Coronary angiography was also performed in patients with a history suggestive of ischemic heart disease or with at least two coronary risk factors. Surface echocardiography was performed in all patients to determine cardiac size, function and the presence of a pericardial effusion. With the more widespread use of cardiac magnetic resonance imaging (MRI) imaging towards the
end of the study period, ten patients underwent off-protocol cardiac MRI with gadolinium contrast during their index hospital stay. A final clinicopathological diagnosis was assigned as previously described. Patients with an acute presentation with positive biomarkers and MRI with late-gadolinium enhancement and T2 signal consistent with edema could receive a diagnosis of myocarditis even in the absence of cellular infiltrate on biopsy.

**Endomyocardial biopsy**

Biopsy of the right ventricular septum was performed via the right internal jugular vein with the use of a disposable bioptome. Each histologic specimen was stained with hematoxylin-eosin and examined at a minimum of 4 levels. The median number of biopsies taken per patient was 5 (range 3-8). Special stains, such as Congo red for amyloid or Prussian blue for iron deposition, were performed when appropriate. Histopathologic analysis allowed classification of formalin-fixed biopsies for evidence of myocarditis according to the Dallas criteria. Biopsies were reviewed by two pathologists blinded to both the clinical scenario and results of molecular testing. Each biopsy was scored for myocyte hypertrophy (0-3), perivascular and interstitial fibrosis (0-3), evidence of chronic ischemic injury (sub-endocardial myocyte vacuolization, 0-3) or infarction, along with other findings such as non-cellular infiltration.

In all, there were 110 eligible study subjects who provided informed consent but seven did not complete EMB and three subjects had insufficient sample for viral testing. Of the seven patients not biopsied, three had significant coronary disease on angiography, obviating the need for biopsy to uncover heart failure etiology; one developed transient
complete heart block during right heart catheterization requiring temporary pacemaker; one subject had a right ventricular perforation leading to a small, hemodynamically insignificant pericardial effusion and cessation of further biopsy attempts; one had significant pulmonary hypertension secondary to pulmonary fibrosis precluding biopsy; and one had a recovery in ejection fraction on ventriculography. The remaining 100 subjects participated in the primary analysis. To serve as controls without heart failure, endomyocardial tissue samples were obtained from four autopsied hearts from patients whose causes of death was deemed to be non-cardiac.

**Viral Recovery**

A single biopsy sample was fixed preserved in RNA*later* solution (Ambion, Applied Biosystems, Austin, TX) and viral screening was performed at the John Welsh Cardiovascular Diagnostic Core Laboratory at Baylor College of Medicine, as previously described.\(^{20, 21}\) In brief, total RNA and DNA were isolated simultaneously from EMB samples with the integrity of the human DNA and RNA in the samples confirmed by PCR or reverse-transcriptase (RT)-PCR, respectively. Nested PCR or RT-PCR were performed for adenovirus types 1, 2, 5, 6, and 41, cytomegalovirus, Epstein-Barr virus, enterovirus (coxsackievirus B1-B6, echovirus types 9 and 11, and enterovirus type 71) and parvovirus B19. Positive and negative controls were run for each analysis. All samples were de-identified and analyzed without knowledge of the clinical data. Positive PCR results were confirmed both by repeating the analysis in duplicate (nucleic acid extraction and PCR) and by DNA sequencing.\(^{20, 21}\) For subjects with parvovirus B19
recovery from EMB, serologic testing was performed for parvovirus IgM and IgG antibodies (ARUP National Reference Laboratory, Salt Lake City, Utah).

**Parvovirus Viral Load Detection**

Parvovirus B19 copy number and the amount of endogenous myocardial DNA were determined by a multiplex assay in single reactions using oligonucleotides and TaqMan probes detecting all three parvovirus B19 genotypes (1, 2, and 3), consisting of PF2 5’-ACCACCCCCCATGCCTTA-3’, PR2 5’-TATACCTAAAGTCATGAATCCTTGCA-3’, and PTaq 5’-6-FAM-AAGACTTACACAAGCCT-MGB/NFQ-3’ (Applied Biosystems, Foster City, CA), and myocardial DNA was quantified by measuring human GAD65 gene copy number using the oligonucleotides: GAD65F 5’-TGGAGAAAGGCCCACTTTG-3’, GAD65R 5’-GGAAATCAATCACTTTGGTTGA-3’, and TaqMan probe GAD65Taq 5’-NED-TTCTGCAAGATGTATGGA-MGB/NFQ-3’ (Applied Biosystems, Foster City, CA). The parvovirus 19 qPCR assay was multiplexed with the GAD65 qPCR assay. Plasmids containing parvovirus 19 genotype 1 (pB19-N8) (courtesy of Neal Young, Hematology Branch, NHLBI, Bethesda, MD) or genotype 2 (pLali) (courtesy of Klaus Hedman and Maria Söderlund-Venermo, University of Helsinki, Finland) were used to test the sensitivity and specificity of this parvovirus 19 qPCR assay. Parvovirus assays were performed in triplicate and validated by serial dilution of positive control plasmids. For further details, see Supplemental Information.

**Statistical Analysis**
Clinical and histopathologic features were compared between patients with and without viral genomic recovery using the student’s t-test for normally distributed continuous variables and the Wilcoxon rank sum test for non-normally distributed continuous variables, along with Fisher’s exact test for categorical variables. Indexed viral load was compared between heart failure subjects and controls using the Wilcoxon rank sum test. The authors had complete access to the data and take sole responsibility for the statistical analysis, which was performed using SAS (Cary, NC, version 9.1).
Results

Viral Recovery Rate

Parvovirus B19 was the only virus recovered from failing hearts and was found in 12 of 100 subjects (12.0%; 95% confidence interval 6.4-20.0%) undergoing biopsy for evaluation of cardiomyopathy. No subject had amplification of adenovirus, cytomegalovirus, Epstein-Barr virus or enterovirus. All initial testing was performed in a certified national reference virologic core laboratory. Each parvovirus B19-positive patient had circulating anti-parvovirus B19 IgG but not anti-parvovirus IgM in serum, consistent with remote infection. Sequencing of each isolate revealed parvovirus B19 genotype 1 in 10 of 12 cases; the two remaining subjects had genotype 2 (Supplemental Figure 1). No subject had the more recently discovered genotype 3 of parvovirus B19. Older age was associated with recovery of parvovirus B19 genotype 2 with positivity in the two oldest patients in this series (age 55 and 61 years), consistent with its presence in subjects born before 1973.15

Baseline history, physical examination, echocardiography and hemodynamic assessment are reported in Table 1. Overall this is a middle aged (mean age 50 years) cohort of heart failure patients with mainly subacute or chronic disease (median symptom duration 5 months, IQR 1-13 months). Most patients had systolic dysfunction (median EF 30%, IQR 20-45%), though one-third of subjects biopsied had a preserved ejection fraction. There were few aggregate differences between subjects with and without parvovirus B19. Patients with parvovirus B19 were younger (42 vs. 51 years old, p=0.02) and had lower systolic and diastolic blood pressures compared to their parvovirus B19 negative counterparts. Only two parvovirus B19-positive patients (17%)
recalled a viral prodrome before the onset of heart failure symptoms, a rate similar to those without viral recovery on biopsy (17/88 or 19%, p=0.82 compared to parvovirus B19 positive). The majority of patients biopsied presented with symptoms of heart failure, rather than ventricular arrhythmia or chest pain. More patients with parvovirus had a chest pain syndrome as part of their presentation for biopsy (25% vs. 7% without parvovirus), but this difference did not reach statistical significance (p=0.07).

Echocardiographic and hemodynamic measurements were similar with or without parvovirus B19. Light microscopy of the EMB sample was unable to distinguish parvovirus positive and negative subjects. There was no increase in the degree of fibrosis (p=0.42) or sub-endocardial myocyte vacuolization (p=0.45), both histological markers of chronic ischemic injury. No parvovirus-positive patient had evidence of any cellular infiltrate that met Dallas criteria for myocarditis, even borderline myocarditis. In contrast, 10 subjects (11%) of the patients without viral recovery had a cellular infiltrate meeting Dallas criteria for myocarditis.

A final clinicopathological diagnosis was assigned to each subject, integrating features of the clinical presentation, cardiac imaging and heart biopsy. Idiopathic cardiomyopathy, either dilated or non-dilated, was the most common clinicopathologic diagnosis of patients with both parvovirus B19-positive (75%) and parvovirus B19-negative biopsies (56%). There was no difference in the prevalence of idiopathic dilated cardiomyopathy in the parvovirus positive vs. negative patients (50% vs. 30%, p=0.19). Myocarditis was clinically diagnosed in 2 of the 12 (17%) parvovirus-positive patients based on late gadolinium enhancement on MRI along with positive serum cardiac troponin-I in the absence of Dallas criteria or coronary artery disease. The
frequency of myocarditis was not significantly different in patients biopsied who had no parvovirus B19 recovered (8%, p=0.33).

**Clinical Profiles of Parvovirus B19 in Heart Failure**

Patient specific data about clinical presentation, echocardiography, histopathology, and viral genotype for parvovirus B19 positive subjects are summarized in Table 3. There was marked heterogeneity in clinical presentation among those patients with parvovirus B19. Parvovirus B19-positive subjects with heart failure ranged in age from 21 to 61 years old and were from multiple different ethnic groups (10 Caucasian, 1 Hispanic, 1 African-American).

Cases with parvovirus B19 presented with both recent onset heart failure (<2 weeks) and with chronic, refractory congestive symptoms (nearly 6 years). Subjects with parvovirus B19 had both preserved (4 of 12) and reduced ejection fractions (8 of 12). The degree of left-ventricular remodeling as assessed by end-diastolic dimension was poorly correlated with the duration of symptoms. There was a varying degree of myocyte hypertrophy and fibrosis, with more severe changes consistent with an element of chronic injury found in patients with greater remodeling, as measured by a larger left ventricular end-diastolic dimension and longer symptom duration. None of the 12 subjects with parvovirus B19 amplified by PCR met Dallas criteria for active or borderline myocarditis, despite two subjects having late gadolinium enhancement on cardiac MRI.

Brief clinical vignettes for each parvovirus B19 positive subject are included in the Supplemental Table to further put the clinical presentation, imaging, histopathology and viral studies in context.
**Parvovirus Viral Load**

Viral load was determined in parvovirus B19-positive samples using a multiplex PCR assay that enabled the determination of parvovirus B19 viral load and the amount of endogenous human myocardial DNA in the same reaction. (Figure 1A) Due to the limited amount of sample remaining after previous viral PCR assays and sequencing, only 6 of 12 subjects had sufficient residual DNA for viral load determination. Parvovirus B19 viral load in EMB in these patients ranged widely from ~$10^1$ to $10^5$ copies/µg myocardial DNA. (Figure 1B) The highest viral copy numbers were found in patients with dilated cardiomyopathy. Lower viral copy numbers were found in the two patients with myocarditis compared to those with idiopathic dilated cardiomyopathy or ischemic cardiomyopathy (p=0.31). PCR analysis of cardiac samples taken at autopsy after non-cardiac death revealed parvovirus B19 in two of the four specimens; neither of these patients (one of whom died from sepsis and the other from leukemia) had pathologic evidence of heart disease on autopsy. Parvovirus viral load on autopsy controls was no different than clinical myocarditis in these limited cases (p=0.53).
Discussion

This is the first report defining the prevalence of cardiotropic viruses and their associated clinicopathological profiles in adult heart failure patients in the United States. Among patients routine EMB for evaluation of unexplained cardiomyopathy or refractory heart failure, parvovirus B19 was the sole cardiotropic virus isolated, in marked contrast to the recent literature. Parvovirus B19 genomic was identified by PCR amplification in only 12% of subjects, a frequency of viral recovery significantly lower than recent studies in Europe in similarly selected patient populations.

The clinical profiles of patients with parvovirus B19 were heterogenous and included patients with heart failure of variable duration, ejection fraction and underlying etiology. Parvovirus copy number was low in patients with myocarditis and no different from autopsy controls, suggesting that even in the setting of myocarditis, parvovirus genome positivity alone does not confer causality. Indeed, our findings indicate that parvovirus B19 may be a normal element of the cardiac microbiome, as has been shown for parvovirus persistence in other human tissues.

Prevalence of Parvovirus in Heart Tissue

The temporal trend in viral recovery from cardiac tissue has changed with each decade—coxsackievirus in the 1980s, adenovirus in the 1990s and parvovirus B19 in the 2000s. Overall the reported prevalence of parvovirus B19 in cardiac tissue has varied widely, from 1-85%, depending on subject selection criteria and viral detection methodology. In a recent report from Germany, parvovirus B19 was isolated in over half of 264 consecutive adults presenting with left ventricular dysfunction (34.7%)
with parvovirus alone and 15.4% with another virus co-isolated). Another study from Germany evaluated left atrial tissue in patients undergoing cardiac surgery and found that 85% had evidence of parvovirus B19 by nested PCR. In marked contrast, no subject in the present study had more than one cardiotropic virus isolated and the only virus recovered was parvovirus B19 in only 12% of patients.

Several explanations could account for variable recovery rates in heart failure patients. Both Kuhl report and the present study enrolled patients with a similar mean ejection fraction and duration of symptoms. However, the former only reported patients with idiopathic LV dysfunction, excluding those with alternative heart failure etiologies, such as ischemic cardiomyopathy. Even if we restrict our cohort to those patients with idiopathic heart failure, only 14% had parvovirus recovery. Another explanation is the lack of standardization in PCR assays and amplification thresholds. Our study only included PCR on a single biopsy sample, whereas other studies have used between 4-6 biopsy samples. Increased sampling has the potential to increase the frequency of genuine viral recovery along with the number of different viruses identified in a given patient. Repeated sampling and multiple PCR cycles, however, could contribute to an increase the aggregate false positive rate. In a follow-up series of patients undergoing serial EMB with PCR, 22% of subjects who were initially parvovirus B19 positive had subsequently negative biopsies (4 samples amplified), which is unexpected given the biology of viral persistence.

The seropositive rate of parvovirus B19 IgG in children under 15 years is 30-50% and increases to >90% in the elderly. Given the high background seropositive prevalence worldwide, it is unlikely that infection rates of parvovirus in the United States
and Europe are different. We cannot comment on the background seropositive prevalence of parvovirus in our study suggesting remote infection. These data are consistent with a recent report from an autopsy series showing 46 of 48 patients with parvovirus recovery if seropositive, while all 21 patients that were seronegative had negative cardiac PCR.\(^\text{14}\) This has led to the hypothesis that parvovirus may persist in cardiac tissue after acute infection.

**Parvovirus Infection and Cardiac Persistence**

Viral persistence may represent a state after acute infection and prior to immunologic clearance. Viral persistence has been postulated to be one marker of failure to respond to immunotherapy for idiopathic cardiomyopathy.\(^\text{27, 28}\) Persistence as defined by recovery of virus on serial biopsies has also been associated with deteriorating ejection fraction.\(^\text{10}\) Data from several recent reports have demonstrated parvovirus B19 persistence in asymptomatic individuals without myocarditis or dilated cardiomyopathy.\(^\text{13, 14, 25}\) In our case series, sequencing of parvovirus B19 isolated revealed the two oldest subjects (aged 55 and 61 years) had recovery of genotype 2. Since parvovirus genotype 2 has not been in circulation since at least 1973, this is consistent with life-long myocardial persistence rather than acute infection.\(^\text{15}\) These data suggest that if cardiac tissue is a lifelong reservoir for parvovirus B19, then detection by PCR alone may be insufficient to declare a pathologic effect.

**Clinical Heterogeneity and Viral Load**
Parvovirus was recovered from EMB in patients with a wide variety of putative heart failure etiologies and clinical presentations in this study. Patients had both a preserved ejection fraction (33%) and reduced systolic function (67%). Interestingly, the single subject presenting with acute heart symptoms (<2 weeks) had an ischemic cardiomyopathy that improved with revascularization, yet tested positive for parvovirus on biopsy. The ischemic burden in this patient was recognized only after biopsy had already been performed and revascularization improved cardiac function. Parvovirus has been previously linked to endothelial dysfunction, which may lead to myocardial injury or a presentation mimicking acute myocardial infarction. However, in the current study, parvovirus was not associated with ischemic fibrosis or sub-endocardial vacuolization on histopathology, both markers of chronic ischemic injury.

Parvovirus has previously been recovered from both postmortem and antemortem biopsies in adult and pediatric patients with myocarditis. In the present case series, the frequency of myocarditis defined by cellular infiltrate or cardiac MRI and appropriate clinical syndrome was no different between parvovirus positive and negative subjects. These data confirm the absence of correlation between Dallas criteria infiltrate. Thus, parvovirus B19 recovery alone cannot be claimed to represent the cause of chronic myocarditis. Yet, two parvovirus-positive subjects displayed clinical evidence of myopericarditis with recurrent chest pain, troponin elevation, normal coronary angiography, and a cardiac MRI consistent with myocarditis. This presentation has been described as “parvomyocarditis” and has been shown to mimic acute myocardial infarction.
Although we hypothesized that the two subjects with presumed parvomyocarditis would harbor higher myocardial parvovirus B19 loads than the subjects who were unexpectedly found to be positive for parvovirus B19, the highest parvovirus copy numbers were found in patients with idiopathic cardiomyopathy. In turn, the two patients with presumed parvomyocarditis had similar viral loads as autopsy controls without heart disease. These viral loads, however, are consistent with those previously reported in patients with both systolic and diastolic dysfunction. While we cannot comment on the variation of viral load over time, or the effect of patient age on copy number, these data further argue against a primary pathogenic role for parvovirus B19 in adults with heart failure. More extensive interpretation of these findings is impossible given the limited sample size but may be a target for future studies.

Several small case series have suggested that interferon ß-1b may be useful as a treatment for subjects with dilated cardiomyopathy in whom viral genome is detected on EMB. However, a more recent, longer term follow-up trial (36 months) could not confirm the promising results from pilot studies and showed no significant clinical benefit conferred by interferon therapy in HF patients. Of interest, the lack of therapeutic effect in this recent trial was postulated to be due to the presence of parvovirus B19 in many of the virus-positive subjects. While antiviral therapy may be of benefit for parvovirus B19-associated myocarditis in children in whom primary (acute) infection is more common, our results support recent studies and argue against the use of antiviral therapy in adult HF patients with parvovirus persistence.

Study Limitations
PCR has been subject to assay variability, which we attempted to minimize by performing assays in a certified diagnostic laboratory. There may be selection bias in who was referred for EMB at our center, though the indications for EMB conformed to published indications. Since this data is derived from a single biopsy sample, we are unable to comment on natural history of parvovirus B19 positive patients or its prognostic importance. The sensitivity of viral detection may be low in a single biopsy rather than several. The background prevalence of parvovirus B19 is unknown for this cohort since assays were only obtained on parvovirus B19 positive subjects to confirm chronic rather than acute infection. Since viral loads were determined from samples remaining after primary PCR analyses, not all samples contained sufficient DNA for multiplex qPCR amplification. Although the mere presence of parvovirus B19 genome in myocardium was not found to be clinically significant, a possible role through mRNA or protein production cannot be excluded. Since our PCR assays cannot provide localization of viral elements, it is unclear whether the microvasculature or cardiac myocytes themselves are harboring parvovirus, which could provide further insight into a potential pathomechanism.

Conclusions

Parvovirus B19 was the only virus recovered using PCR of biopsies from adult heart failure patients with new or unexplained cardiomyopathy in this prospective, single-center study in the United States. These data confirm that parvovirus may persist lifelong in the myocardium after acute infection, as has been described for other human tissues. There was marked heterogeneity of clinical presentation associated with parvovirus
recovery and no association with myocardial inflammation. Our studies do not suggest a causative role for parvovirus persistence in HF and advocate against the use of antiviral therapy in these patients.
Acknowledgments

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Disclosures

None.
References


biopsies from patients with suspected myocarditis or idiopathic left ventricular dysfunction. *Zeitschrift fur Kardiologie.* 2004;93:300-309.


Table 1. Baseline Clinical Characteristics

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<tr>
<th></th>
<th>All Subjects (n=100)</th>
<th>Parvovirus Positive (n=12)</th>
<th>Parvovirus Negative (n=88)</th>
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<tr>
<td>Age (yrs)</td>
<td>50 (15)</td>
<td>42 (12)</td>
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<td>Male</td>
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<td>White</td>
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<td>28 (5)</td>
<td>28 (4)</td>
<td>28 (5)</td>
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<td>Duration symptoms (months)</td>
<td>5 (1-13)</td>
<td>4 (1-13)</td>
<td>6 (1-13)</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td>81 (18)</td>
<td>81 (17)</td>
<td>81 (18)</td>
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<td>104 (14)</td>
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<td>Diastolic blood pressure (mmHg)</td>
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<td>S3 gallop</td>
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<td>19</td>
<td>17</td>
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Clinical Presentation

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<td>Heart Failure</td>
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<td>Arrhythmia</td>
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<td>Chest Pain</td>
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<td>Ejection fraction (%)</td>
<td>30 (20-45)</td>
<td>41 (24-51)</td>
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<td>Reduced EF (&lt;50%)</td>
<td>70</td>
<td>67</td>
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<td>LVEDD (cm)</td>
<td>5.7 (1.1)</td>
<td>6.0 (1.6)</td>
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Right heart cath

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<td>RA pressure (mmHg)</td>
<td>8 (6)</td>
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<td>PA systolic pressure (mmHg)</td>
<td>36 (13)</td>
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<td>36 (12)</td>
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<td>PA mean pressure (mmHg)</td>
<td>25 (9)</td>
<td>23 (10)</td>
<td>25 (9)</td>
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<td>Pulmonary wedge pressure (mmHg)</td>
<td>16 (9)</td>
<td>15 (8)</td>
<td>16 (9)</td>
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<td>Cardiac output (L/min)</td>
<td>5.0 (1.6)</td>
<td>4.9 (1.5)</td>
<td>5.0 (1.6)</td>
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<td>2.5 (0.7)</td>
<td>2.5 (0.7)</td>
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<td>SVR (dynes*sec/cm5)</td>
<td>1392 (478)</td>
<td>1381 (436)</td>
<td>1394 (481)</td>
<td>0.93</td>
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<td>PVR (dynes*sec/cm5)</td>
<td>161 (151)</td>
<td>182 (326)</td>
<td>158 (99)</td>
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BMI, body mass index; EF, ejection fraction; LVEDD, left ventricular end-diastolic dimension; RA, right atrium; PA, pulmonary artery; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance

*Categorical variables expressed as percentages and continuous variables as mean (sd), except for symptom duration and EF which are expressed median (quartile 1 - quartile 3).*
Table 2. Final Clinicopathologic Diagnosis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Overall (n=100)</th>
<th>Parvovirus Positive (n=12)</th>
<th>Parvovirus Negative (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic cardiomyopathy</td>
<td>58</td>
<td>9</td>
<td>49</td>
</tr>
<tr>
<td>Dilated</td>
<td>32</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>Non-dilated</td>
<td>26</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>Coronary artery disease/Ischemia</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>10</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Valvular heart disease</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Drug-induced/Toxic</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Alcohol abuse</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Cocaine</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Connective Tissue Disease</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Pheochromocytoma</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Constrictive pericarditis</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Idiopathic restrictive cardiomyopathy</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Primary pulmonary process</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 3. Profiles of Patients with Parvovirus Amplified from Cardiac Biopsy

<table>
<thead>
<tr>
<th>CASE</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Symptom Duration</th>
<th>Viral Prodrome</th>
<th>LVEF (%)</th>
<th>LVEDD (cm)</th>
<th>Baseline Clinical Features</th>
<th>Histopathology and Viral Studies</th>
<th>Final Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>H15</td>
<td>50</td>
<td>M</td>
<td>6 days</td>
<td>No</td>
<td>50</td>
<td>4.2</td>
<td></td>
<td>1+</td>
<td>Negative</td>
</tr>
<tr>
<td>H16</td>
<td>36</td>
<td>F</td>
<td>2 years</td>
<td>No</td>
<td>35</td>
<td>6.1</td>
<td></td>
<td>1+ 1+</td>
<td>1</td>
</tr>
<tr>
<td>H17</td>
<td>61</td>
<td>F</td>
<td>3 months</td>
<td>No</td>
<td>15</td>
<td>7.8</td>
<td></td>
<td>2+ 0</td>
<td>2</td>
</tr>
<tr>
<td>H18</td>
<td>50</td>
<td>M</td>
<td>2 weeks</td>
<td>Yes</td>
<td>15</td>
<td>8.4</td>
<td></td>
<td>2+ 0</td>
<td>Negative</td>
</tr>
<tr>
<td>H19</td>
<td>47</td>
<td>M</td>
<td>2 months</td>
<td>No</td>
<td>40</td>
<td>4.2</td>
<td></td>
<td>0 0 0 0</td>
<td>1</td>
</tr>
<tr>
<td>H27</td>
<td>32</td>
<td>M</td>
<td>13 months</td>
<td>No</td>
<td>50</td>
<td>3.4</td>
<td></td>
<td>2+ 0 0 0</td>
<td>1</td>
</tr>
<tr>
<td>H55</td>
<td>37</td>
<td>F</td>
<td>2 months</td>
<td>No</td>
<td>25</td>
<td>6.8</td>
<td></td>
<td>1+ 1+ 0 0</td>
<td>1</td>
</tr>
<tr>
<td>H64</td>
<td>55</td>
<td>M</td>
<td>8 months</td>
<td>No</td>
<td>40</td>
<td>7.0</td>
<td></td>
<td>1+ 1+ 1+ 0</td>
<td>2</td>
</tr>
<tr>
<td>H77</td>
<td>49</td>
<td>M</td>
<td>2 weeks</td>
<td>No</td>
<td>20</td>
<td>5.9</td>
<td></td>
<td>2+ 0 2+ 0</td>
<td>1</td>
</tr>
<tr>
<td>H92</td>
<td>40</td>
<td>M</td>
<td>4 months</td>
<td>No</td>
<td>15</td>
<td>7.6</td>
<td></td>
<td>1+ 1+ 0 0</td>
<td>1</td>
</tr>
<tr>
<td>H100</td>
<td>25</td>
<td>M</td>
<td>6 years</td>
<td>No</td>
<td>55</td>
<td>5.1</td>
<td></td>
<td>1+ 1+ 1+ 0</td>
<td>Negative</td>
</tr>
<tr>
<td>H102</td>
<td>21</td>
<td>M</td>
<td>6 months</td>
<td>Yes</td>
<td>55</td>
<td>4.6</td>
<td></td>
<td>0 0 0 0</td>
<td>Negative</td>
</tr>
</tbody>
</table>

LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic dimension; CM, cardiomyopathy; DCM, dilated cardiomyopathy; Myocyte hypertrophy, fibrosis, subendocardial vacuolization and infarction were graded on a scale of 0 to 3+. 

Negative 1  Idiopathic Non-dilated CM

Negative 1  Idiopathic DCM

Negative 2  Idiopathic DCM

Negative 1  Idiopathic Non-dilated CM

Negative 1  Idiopathic Non-dilated CM

Negative 1  Idiopathic DCM

Negative 1  Idiopathic DCM

Negative 1  Idiopathic DCM

Negative 1  Myocarditis

Negative 1  Myocarditis
Figure Legends

Figure 1

Panel A. I, Amplification curve of endogenous myocardial DNA from patients H55 and H64 by GAD65 TaqMan assay. II, Amplification curves from samples that were negative for parvovirus B19. III and IV, triplicate amplification curves from samples H55, genotype 1 and sample H64, genotype 2. Inset are triplicate samples resolved by agarose gel electrophoresis stained with ethidium bromide.

Panel B. Parvovirus B19 viral copies per μg myocardial DNA from endomyocardial biopsy. Subjects H16 and H55, were classified as idiopathic dilated cardiomyopathy (DCM), subject H77 had ischemic cardiomyopathy (ICM), and subjects H100 and H102 had myocarditis. A1 and A2 are myocardial autopsy samples from subjects who had no clinical or pathological evidence of heart disease. Viral load was determined by quantitative PCR. Inset is the standard curve analysis of diluted parvovirus 19 plasmid used to extrapolate parvovirus 19 viral copies in the quantitative PCR.
A. 

I. GAD65: H55, H64

II. Negative samples

III. H55 Genotype 1

IV. H64 Genotype 2

B. 

Viral copies/µg myocardial DNA

Heart Failure Etiology

DCM  ICM  Myocarditis  Autopsy

H16  H55  H64  H77  H100  H102  A1  A2
Myocardial Parvovirus B19 Persistence: Lack of Association with Clinicopathologic Phenotype in Adults with Heart Failure
Garrick C. Stewart, Javier Lopez-Molina, Raju V. Gottumukkala, Gregg F. Rosner, Mary S. Anello, Jonathan L. Hecht, Gayle L. Winters, Robert F. Padera, Kenneth L. Baughman and Myra A. Lipes

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