Brief Report

Parvovirus B19 in Endomyocardial Biopsy of Patients With Idiopathic Dilated Cardiomyopathy: Foe or Bystander?

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ABSTRACT

Background: Parvovirus B19 (PVB19) has emerged as one of the viruses possibly inducing chronic myocarditis and subsequent idiopathic dilated cardiomyopathy (IDCM). The aim of this work was to investigate the presence and long-term consequences of PVB19-DNA within myocardial biopsies from patients with IDCM and to compare the findings with those from donor hearts (control group).

Methods and Results: Forty hospitalized IDCM patients (age 47 ± 12 y) with mean left ventricular ejection fraction 27 ± 12% were included. The presence of PVB19-DNA in myocardial biopsies and of IgG and IgM antibodies in patient sera was analyzed. The control group consisted of 20 donor hearts. The follow-up time was 112 ± 57 months. PVB19-DNA was found in myocardial biopsies of both patients (73%) and control samples (55%; $P = .25$).

Three deaths and 8 heart transplantations occurred in the IDCM group, and 6 deaths in the control group (ie, the recipients of the control hearts). No difference in transplantation-free survival between the PVB19-DNA positive/negative IDCM patients or transplant recipients was found.

Conclusions: PVB19-DNA is a common finding in both patients with IDCM and in healthy donor hearts, not affecting prognosis. These findings support the view that PVB19 is an innocent bystander, frequently found in myocardium with low DNA copies, and not a plausible cause of IDCM. (*J Cardiac Fail 2019;25:60–63*)

Key Words: Dilated cardiomyopathy, parvovirus B19, transplantation.

Idiopathic dilated cardiomyopathy (IDCM) is regarded as a heterogeneous disease of multifactorial etiopathogenesis. During the past few decades it has been generally accepted that serum antibodies directed against common viruses, signifying acute infection, are rarely found in patients with IDCM. In 1996, the human parvovirus B19 (PVB19) was detected in the myocardium of patients with acute myocarditis.[1] Chronic myocarditis was later proposed as a causative mechanism of IDCM,[2] and since then, several studies on this topic have been published.[3–5] Data on the prevalence of PVB19 in explanted and in donor hearts were published 2004,[6] showing an extremely low prevalence (7%–9%).

In 2016, in one of the few studies focusing on prevalence of a viral genome in the myocardium of IDCM patients and prognosis, it was suggested that PVB19 does not influence long-term outcome.[7] Patients with acute myocarditis were not excluded in that study. The pathogenic significance of myocardial PVB19 in the development of IDCM remains unclear.[8]

The aim of the present study was to investigate the presence of PVB19-DNA in myocardium from IDCM patients and from donor hearts and to assess the effect of viral load on prognosis of IDCM patients and recipients of PVB19-positive donor hearts. The latter has not been described before.

Methods

In this prospective analysis, myocardial biopsies from 40 patients with IDCM were compared with those from control samples (20 donor hearts obtained during transplantations

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[HTs]). After ruling out other possible etiologies with the use of routine diagnostic work-up, the diagnosis of IDCM was confirmed. Death and HT were registered during follow-up. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. The Ethics Committee of Gothenburg University approved the study.

Laboratory Investigations

Endomyocardial biopsies were fixed in paraformaldehyde and embedded in paraffin. Sections were stained with the use of routine histochemical techniques. The presence of antibodies against PVB19 was analyzed in patient sera with the use of an IgG and IgM enzyme immunoassay (Biotrin).

For the analysis of PVB19-DNA, fresh biopsy specimens were homogenized in a Magnalyser instrument (Roche) in a volume of 220 μL, and DNA in the homogenized material was extracted in a Magnacompact instrument (Roche) with the use of 100 μL and 200 μL output volumes. Details regarding the method have been published previously.[9,10]

Statistical Methods

Comparison between groups was done by means of Mann-Whitney test or Wilcoxon signed-rank test. Proportions were compared with the use of the chi-square test. Survival curves were constructed with the use of the Kaplan-Meier method. The influence of prognostic variables on survival was analyzed by using a Cox regression hazard model. Only variables significant in the univariate analysis were used in the multivariate analysis. A P value of $<.05$ was considered to be significant.

Results

Patients

Baseline demographic and clinical characteristics of the study group are presented in Table 1. The median time from debut of heart failure symptoms to diagnostic biopsy was 5 months. The follow-up time after diagnosis was 112 ± 57 months. PVB19 was found in myocardial biopsies in 29 patients (73%) and 11 control samples (55%; $P = .25$). Follow-up echocardiography was performed in all patients but the elapsed time between baseline and follow-up assessments was not standardized. At follow-up, left ventricular ejection fraction (LVEF) had improved (mean 45% ± 14) in 21 patients (53%) and was unchanged or worse (mean 23% ± 6) in the remaining patients ($P < .001$). Among the patients who improved, 62% had PVB19-positive biopsies, whereas the proportion was 79% among patients with unchanged or worsened LVEF ($P = .46$).

Laboratory Findings

There were no signs of acute myocarditis or storage disease in the analyzed biopsies. The mean Ct value for PVB19 in the IDCM biopsies was 38.8 ± 2.40, and the mean Ct value for betaglobin was 31.3 ± 1.57. From the latter Ct value it can be approximated that the analyzed biopsy tissue contained ~50,000 cells. The control myocardial samples (donor hearts) were larger and therefore the mean Ct value for betaglobin was lower, 27.5 ± 0.89, suggesting that the analyzed tissue contained ~500,000 cells. Accordingly, the parvovirus Ct values in the controls were lower: 35.5 ± 2.50. However, the estimated PVB19 copy numbers per cell were similar in patients (3.13 copies/1000 cells, 505 copies/μg cellular DNA) and control samples (3.25 copies/1000 cells, 524 copies/μg cellular DNA). The real-time polymerase chain reaction (PCR) findings of parvovirus were confirmed by means of sequencing of a subset of samples.

From individuals who presented a positive myocardial PCR signal, whole-blood samples were tested with the same PCR method, and all were negative. No IgM antibodies against PVB19 were detected. In 27 out of 29 PVB19-DNA positive patients, and in one of the PVB19-DNA−-negative patients, IgG antibodies were detected.

There was no difference in Ct values in relation to time from symptom debut to diagnosis: mean Ct value was 38.4, corresponding to 2.30 $\log_{10}$/mL blood, in patients with a median time to diagnosis ≤5 months, and mean Ct value was 38.7, corresponding to 2.21 $\log_{10}$/mL blood, in patients with a median time to diagnosis >5 months ($P = .7$).

Table 1. Baseline Clinical Characteristics and Right Heart Catheterization Hemodynamic Parameters of Patients With Idiopathic Dilated Cardiomyopathy (n = 40)

Characteristic	PVB19-Positive (n = 29)	PVB19-Negative (n = 11)	P Value
Age, y	48 ± 11	46 ± 13	.88
Duration of heart failure, mo	31 ± 55	7 ± 8	.17
Infection at debut of symptoms	11 (38%)	4 (36%)	1.00
Female	11 (38%)	2 (18%)	.29
Familial disease	7 (24%)	2 (18%)	.25
Ejection fraction, %	27 ± 13	26 ± 12	.79
NYHA functional class (I/II/III/IV)	3/9/10/7	0/4/5/2	.66
Heart rate, beats/min	76 ± 25	76 ± 16	.99
RAP, mm Hg	5 ± 4.6	6 ± 5.5	.49
PCWP, mm Hg	14 ± 6.9	14 ± 8.6	.76
Mean arterial pressure, mm Hg	86 ± 15	84 ± 15	.65
Cardiac index, $L \cdot min^{-1} \cdot m^{-2}$	2.3 ± 0.86	2.0 ± 0.49	.36
PVR, $dyne \cdot s \cdot cm^{-5}$	99 ± 90	70 ± 78	.39
SVR, $dyne \cdot s \cdot cm^{-5}$	1626 ± 679	1405 ± 440	.37

P values are according to Pearson χ^2 test (for numbers) and Mann-Whitney U test (for continuous numeric variables). NYHA, New York Heart Association; RAP, right atrial pressure; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; SVR, systemic vascular resistance.

Survival

During the follow-up there were 3 deaths and 8 HTs in the IDCM group and 6 deaths in the control group (ie, the recipients of the control hearts).

Table 2. Univariate Analysis and Cox Proportional Hazard Regression Evaluating the Risk of End Point (Death or Heart Transplantation) in 40 Patients With Idiopathic Dilated Cardiomyopathy

Factor	Univariate *P* Value	Multivariate HR (CI)	*P* Value
Familiar disease	.71		
Heart failure duration	.74		
Gender	.76		
Age	.01	1.00 (0.96−1.05)	.92
PVB19-DNA positive	.45		
Ejection fraction	.04	0.91 (0.84−0.98)	.02
Heart rate	.74		
Cardiac index	.24		
PCW pressure	.19		
Mean arterial pressure	.17		

HR, hazard ratio; CI, confidence interval.

Age ($P = .01$) and LVEF ($P = .04$) were associated with poor outcomes in a univariate analysis, and in the multivariable model only LVEF was an independent predictor of transplantation or death ($P = .02$; Table 2).

No difference in transplantation-free survival was noted between the 2 groups (Fig. 1).

The 6 deaths in the control group occurred during a mean follow-up of 133 ± 65 months. Five of them were cardiac deaths (2 were related to graft failure, 1 to heart failure in relation to pneumonia, 1 to bad compliance, and 1 to sudden cardiac death). The mortality rate was 33% in the PVB19-DNA−positive group and 27% in the PVB19-DNA−negative group ($P = 1.0$).

Discussion

PVB19 was highly prevalent in both healthy and diseased myocardium, and its presence did not affect long-term outcomes of patients with IDCM. Moreover, PVB19-positive donor hearts were not related to worse recipient survival.

The high prevalence of PVB19 IgG antibodies indicates that most of the patients had an earlier PVB19 infection. Schenk et al detected PVB19-DNA in heart samples taken during autopsies of patients without cardiac disease.[11]

The transplantation-free survival of IDCM patients and control recipients was not affected by the presence of PVB19 or by viral load. Our findings are in agreement with those of Lotze et al,[2,4] who showed that PVB19-positive IDCM patients had a rather favorable prognosis.

As pointed out by Verdoschont et al,[8] few studies[5,12] have focused on longitudinal follow-up of IDCM patients who underwent endomyocardial biopsies. We found low PVB19 viral load and no differences in viral load between IDCM patients and control samples, supporting the hypothesis that our findings reflect latent infections with little or no contribution to IDCM pathogenesis.

Thus, this study does not support PVB19 being a main contributing pathogenic factor in IDCM.

Our work has some limitations: the number of patients included was low and only 17 end points (cardiac death or HT) were registered; we did not investigate virus replication (PVB19 -mRNA) or the prevalence of coinfection with other cardiotropic viruses.

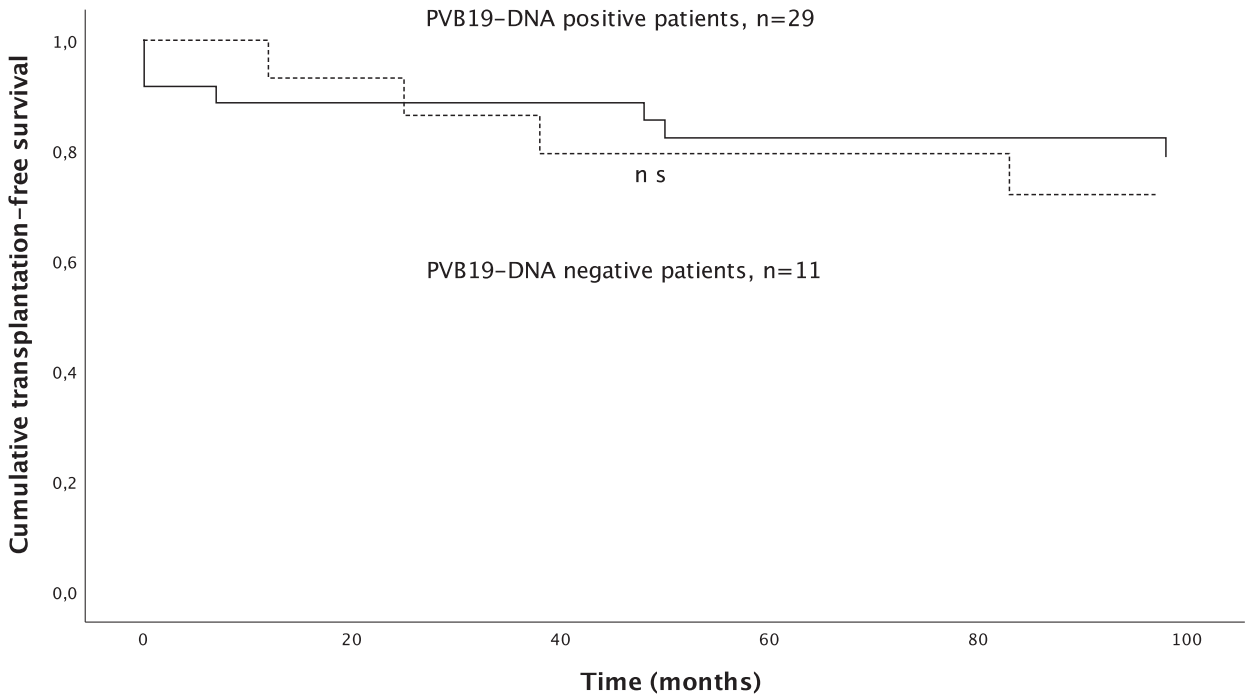

Fig. 1. Risk of end point (death or heart transplantation) in 40 patients with idiopathic dilated cardiomyopathy, according to the presence of parvovirus B19 DNA in myocardial biopsies.

One strength of this work is the long follow-up period with no patients lost to follow-up. Also, this is the first study to investigate survival of recipients of PVB19 positive donor hearts.

In conclusion, our findings support the view that PVB19 is an innocent bystander, frequently found in cardiac tissue with low DNA copies, and not a plausible cause of IDCM. Furthermore, the presence of PVB19-DNA does not, by itself, appear to affect survival or need for HT. Whether coinfection with other cardiotropic viruses, active replication, or patient susceptibility could play a role in certain cases remains to be determined.

Disclosures

None.

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