Original article

Intravenous immunoglobulin therapy for patients with idiopathic cardiomyopathy and endomyocardial biopsy-proven high PVB19 viral load

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Background: Parvovirus B19 (PVB19) persistence in the heart has been associated with progressive cardiac dysfunction and evolution to dilated cardiomyopathy. In the present study, we investigated whether immunomodulation with intravenous immunoglobulin (IVIg) in addition to conventional heart failure therapy is safe and achieves virus reduction. Such therapy might improve cardiac function in patients with chronic dilated cardiomyopathy (DCM) and a significant PVB19 viral load in the heart.
Methods: PVB19 viral load was studied in 25 post-mortem cardiac samples of patients with a normal heart. Then, 17 consecutive patients (mean age 53 ±3 years) with DCM and symptomatic heart failure for >1 year with a PVB19 viral load in endomyocardial biopsies of >250 copies/μg DNA were treated with a high dose of IVIg (2 g/kg).
Results: The post-mortem cardiac samples revealed a PVB19 presence in 80% with a mean load of 131 ±40 copies/μg DNA. In the treated patients, IVIg resulted in a significant decrease of PVB19 viral load from 1,420 ±216 to 619 ±200 copies/μg DNA (*P*=0.004) and significantly improved the ejection fraction from 33 ±3% 6 months before treatment and 34 ±3% at baseline to 41 ±3% 6 months (*P*=0.001) after IVIg therapy. The New York Heart Association classification significantly improved from 2.5 ±0.1 at baseline to 2.1 ±0.1 at follow-up (*P*=0.004). No therapy-related complications were noted.
Conclusion: The present pilot study demonstrates that IVIg significantly reduces viral load and improves cardiac function in patients with DCM related to increased PVB19 viral load in the heart.

Introduction

The link between myocarditis and dilated cardiomyopathy (DCM) is increasingly recognized [1]. Myocarditis is an inflammatory myocardial disease with a variable natural history, ranging from spontaneous recovery, subclinical course with evolution to DCM or early death from multisystem failure [2,3]. The progression to DCM can result from both direct viral-mediated damage or secondary autoimmune-mediated myocardial injury [4–8]. This secondary autoimmunity is induced by molecular mimicry, inducing cross-reaction between antiviral antibodies with autoantigen-binding properties [9]. Viral persistence has been associated with progressive cardiac dysfunction [10], whereas spontaneous virus elimination results in a significant improvement in left ventricle (LV) function [10,11]. Recent studies using viral PCR in endomyocardial biopsies point towards a shift from enterovirus (EV) and adenovirus (ADV) to human herpesvirus 6 (HHV6) and especially parvovirus B19 (PVB19) as the most frequent viruses found in patients with acute myocarditis [12–16]. However, both human PVB19 and HHV6 can persist lifelong in the heart and in other tissues, such as the bone marrow, skeletal muscle, skin and liver [17–19]. To address virus presence and viral load in normal hearts, we studied the occurrence of these viruses in post-mortem myocardial samples of individuals with normal hearts.

Standard heart failure treatment, such as β-blockade or angiotensin inhibition, does not target the immune system, which is needed for elimination of the virus. Hence, a specific treatment that hampers the viral load and improves the immune response related to these cardiotropic viruses might lead to an improvement in cardiac function. It was shown that intravenous immunoglobulin (IVIg) from pooled human plasma donors contains high titres of neutralizing antibodies to PVB19 [20,21]. IVIg has been successfully used for autoimmune disorders and, in particular, in PVB19-induced diseases, such as fetal hydrops, pure red-cell aplasia, polyarteritis nodosa, immune thrombocytopenic purpura and PVB19 infection during pregnancy [22–28]. However, to our knowledge, IVIg has not yet been used in the treatment of PVB19-associated cardiomyopathy. We therefore performed a feasibility study to investigate whether IVIg in conjunction with conventional therapy in a subgroup of patients with DCM and significant PVB19 viral load could reduce PVB19 in the heart and improve cardiac function and clinical symptoms.

Methods

Autopsy samples

A total of 25 endomyocardial biopsy (EMB) samples from normal hearts were obtained from routine autopsies in our hospital. Biopsies were taken from the right ventricular septal wall in accordance with the location of biopsies obtained from transcatheter sampling. A comprehensive post-mortem morphological examination of the patients was performed to find the underlying cause of death. Patients with a prior cardiac history, cardiac-associated death or signs of cardiac disease at autopsy or histological analysis were excluded. The protocol was approved by the Human Research Committee of the University Hospital Maastricht (Maastricht, the Netherlands).

Patients treated with IVIg

Between March 2006 and April 2008, 47 patients were known with an impaired LV function for at least 1 year underwent EMBs to further elucidate a possible origin of their heart disease at the Maastricht University Hospital. All patients underwent a coronary angiography and transthoracic echocardiogram to exclude significant coronary artery disease (>70% stenosis) or valvular disease. Patients did not suffer from hypertension, ischaemia, valvular disease or ethyl abuses. Also systemic diseases, such as sarcoidosis, haemochromatosis, other systemic autoimmune diseases or giant cell myocarditis, were excluded. All patients (n=17) with high myocardial PVB19 titres (>250 copies/μg DNA) were selected and treated with IVIg, excluding patients with a low viral load where lifelong persistence does not relate to cardiac disease. The selection of the cutoff value was based on our findings obtained from the mean of normal hearts, as described below. Serum serology was performed, excluding patients with anti-PVB19 immunoglobulin M antibodies as marker of a recent primary PVB19 infection, prior to treatment. Patients received standard medical treatment for heart failure at least 6 months before IVIg treatment. A conventional cardiovascular treatment regimen was maintained during the study. Echocardiographic measurements were performed 6 months before treatment, just before treatment and 6 months after treatment. Follow-up EMB were taken at 6 months. The Human Research Committee of the University Hospital Maastricht reviewed and approved the protocol.

Therapy

All patients received stable standard heart failure medication including diuretics, β-blockers, aldactone, angiotensin-converting enzyme inhibitors and/or angiotensin II receptor blockers for at least 6 months before inclusion. IVIg (Sanquin, Amsterdam, the Netherlands) was administered during hospitalisation, with a total dose of 2 g/kg of immunoglobulin administered as 0.5 g/kg intravenously over a period of 6 h on each of 4 consecutive days [29]. All patients had continuous cardiac monitoring during therapy.

Non-invasive evaluation

Echocardiographic measurements were performed in the standard parasternal, apical and subxiphoidal views according to the recommendations of the American Society of Echocardiography [30] (Sonos 5500; Philips Medical Systems, Best, the Netherlands). Several dimensions were measured: left ventricular end-diastolic and end-systolic diameter (LVEDD and ESD) and the end-diastolic thickness of the septum and LV posterior wall. Left ventricular end-diastolic and end-systolic volume were obtained from the apical four- and two-chamber views by the modified Simpson's method. Left ventricular ejection fraction (LVEF) was calculated in a standard manner and was used to assess global left ventricular systolic function. Echocardiograms were analysed blindly – without knowing the patients name or date of exam – by one experienced investigator (SS).

Endomyocardial biopsy

Right ventricular EMBs were obtained using a transcatheter bioptome (Cordis, Miami, FL, USA). A total of six endomyocardial septal biopsies were taken from the right ventricle. Two specimens were used for immunohistological analysis and four for the detection of viral genomes.

Immunohistological analysis

For immunohistological analysis of myocardial inflammation, formalin-fixed, paraffin-embedded EMBs were

analysed according to the Dallas classification [31] and infiltrating inflammatory cells were quantified as defined by the task force of the World Heart Federation's Council on Cardiomyopathies [13–16]. Tissue sections of 4 μm thickness were subjected to immunohistochemical staining. Slides were stained using Sirius red for collagen and $CD3^+$, $CD4^+$, $CD8^+$, $CD20^+$, $CD45^+$ and $CD68^+$ antibodies (DAKO, Glostrup, Denmark) for the different inflammatory cells. The total tissue area of the myocardial biopsies on the histological slide was determined by morphometrical analysis (morphometrical software: Leica Qwin, version 3, Cambridge, UK) and the number of staining inflammatory cells were counted and expressed per mm^2, as described previously [32]. Collagen volume fraction was quantified as percentage Sirius red stained area per total myocardial tissue area, excluding perivascular and endocardial fibrosis. The analysis was performed by one experienced investigator (RvS) blinded to patient details.

Detection of viral genomes

DNA of PVB19, ADV, HHV6 and Epstein–Barr virus (EBV) was extracted from two pooled endomyocardial biopsies using a QIAamp DNA blood mini kit (Qiagen, Venlo, the Netherlands). Extractions were performed according to the manufacturer's instructions. DNA concentrations were determined using a nanodrop instrument (Thermo Fischer Scientific, Wilmington, DE, USA). RNA for enterovirus detection was isolated using TRIZOL reagents (Invitrogen, Paisley, Scotland, UK). Before extraction, all samples were spiked with murine cytomegalovirus (CMV) DNA or RNA, which was used as an extraction and amplification control. Reverse transcription was performed using Taqman reverse transcriptase reagents (Applied Biosystems, Foster City, CA, USA). Primers and probe sequences were obtained from the literature [33–35]. The PCR mix consisted of 20 μl isolated DNA, final concentrations of 600 nM of each primer and 200 nM of the probe and 1× absolute QPCR mix (Abgene, Epsom, UK). All probes were labelled with FAM™ as a reporter dye and TAMRA™ as a quencher dye. All real-time PCR reactions were performed using an ABI prism 7000 (Applied Biosystems). The PCR assay used has a linear quantitative range from 1.0×10^2–1.0×10^8 copies with a detection probability above 95%. Below this range semiquantitative detection is performed by extrapolation of the standard curve. We evaluated the variation for each DNA concentration in the standard curve over a period of 3 months. Our results showed that standard deviation was ≤0.15 $\log_{10}$ for all concentrations >500 copies/ml. Only for the two lowest concentrations (500 and 200 copies/ml) standard deviations up to a maximum of 0.38 $\log_{10}$ were measured. The quality of the assays was assured by positive and negative controls as well as a test on amplification inhibition in each sample by an external amplification control. For quantification of viral loads, standard curves were included in each run.

Statistical analysis

Statistical analysis was made using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) software. The central tendency and spread of the data are reported as mean ±SEM. The statistical analysis of patients before and after IVIg therapy was performed using the paired *t*-test. Correlation calculation was performed using Pearson's correlation coefficient. Qualitative data between subgroups were analysed by the χ^2 test and continuous data were compared by the *t*-test. Statistical significance was accepted at the 95% confidence interval ($P<0.05$).

Results

Viral load in control hearts

Myocardial samples were obtained from 25 autopsy patients aged 64 ±2 years. Viral genome could be amplified in 80% (20/25) of the biopsy specimens of normal hearts obtained from autopsy. PVB19 and HHV6 were the most prevalent viruses, present in 76% and 32%, respectively. EBV was detected in 12% of the specimens, whereas no other cardiotropic viruses (ADV or EV) could be detected. Viral loads detected for PBV19, HHV6 and EBV were 131 ±40, 33 ±13 and 4 ±11 copies/μg DNA, respectively. On the basis of these findings, a cutoff of 250 copies/μg DNA was selected based on the sum of three standard errors above the mean of normal population.

Intravenous immunoglobulin reduces viral load in diseased hearts

A total of 17 consecutive patients, mean age 54 ±3 years, with chronic DCM and a PVB19 viral load of >250 copies/μg DNA were enrolled and treated with 2 g/kg IVIg at the Maastricht University Hospital. Serum serology performed in all patients revealed immunoglobulin G seropositivity for PVB19 in all cases. None of the serum samples was positive for PVB19 immunoglobulin M. Mean duration of heart failure symptoms at the time of treatment was 3 ±1 years. Patients were on stable standard heart failure treatment for at least 6 months. Baseline patient characteristics are listed in Table 1. IVIg administration was well-tolerated. No serious adverse events or side effects were noted.

Mean viral load was 1,460 ±216 copies/μg DNA for PVB19 (131 ±40 copies/μg DNA in controls; $P<0.001$) and 75 ±35 copies/μg DNA for HHV6 (33 ±13 copies/μg DNA in controls; $P=0.23$). All except one patient underwent a second EMB 6 months following IVIg treatment. Follow-up EMBs showed a significant decrease in PVB19 load from 1,460 ±216 to 619 ±200

copies/μg DNA (P=0.004; Table 2, Figure 1). Whereas 15 out of 17 patients had a significant decrease in PVB19 viral load, two patients (numbers 9 and 14) showed an increase in PVB19 viral load accompanied by an increase in lymphocyte inflammation. Five patients had a coinfection of PVB19 and HHV6 in their hearts at baseline. Other cardiotropic viruses (EV, ADV or EBV) could not be detected (Table 2). IVIg treatment eliminated HHV6

Table 1. Patient baseline characteristics

Patient	Age, years	Gender	Duration of heart failure, years	Medication		
				ACE inhibitors or AT2 antagonist	β-Blockers	Diuretics
1	45	Male	2	Yes	Yes	No
2	65	Male	3	Yes	Yes	Yes
3	56	Female	1	No	Yes	No
4	58	Male	3	Yes	Yes	Yes
5	32	Male	10	Yes	Yes	Yes
6	73	Male	1	Yes	Yes	No
7	39	Male	1	Yes	No	Yes
8	56	Male	2	Yes	Yes	No
9	57	Female	1	No	Yes	No
10	52	Male	2	Yes	Yes	Yes
11	58	Female	1	Yes	Yes	Yes
12	57	Female	2	Yes	Yes	Yes
13	51	Female	3	Yes	Yes	Yes
14	51	Male	2	Yes	Yes	Yes
15	55	Male	4	Yes	Yes	Yes
16	37	Male	9	Yes	Yes	No
17	57	Male	1	Yes	Yes	Yes
Mean	53	–	3	–	–	–
SEM	3	–	1	–	–	–

ACE, angiotensin-converting enzyme; AT2, angiotensin II receptor.

Table 2. Clinical, echocardiographic and virological data of all patients over time by treatment

Patient	6 Months pre-baseline			Baseline							6-Month follow-up						
	EF	EDD	ESD	NYHA	EF	EDD	ESD	PVB19[a]	HHV6[b]	CD45	NYHA	EF	EDD	ESD	PVB19[a]	HHV6[b]	CD45
1	35	52	41	III	34	55	44	1,363	94	5.9	IIa	57	48	25	345	0	5.7
2	30	62	54	IIb	27	55	48	1,174	409	23	I	50	54	40	35	0	16.9
3	50	50	40	IIa	49	49	37	988	98	6.8	I	45	53	31	13	85	9.6
4	25	54	41	III	20	54	41	3,572	0	10.2	IIa	28	60	48	369	0	9.7
5	29	60	55	III	28	62	51	2,041	0	16	IIa	40	50	25	ND	ND	ND
6	23	60	50	III	18	67	56	409	0	ND	III	20	52	57	76	0	2.6
7	54	48	38	IIb	54	48	40	1,375	0	22.7	IIb	53	64	36	108	0	3.3
8	32	55	45	IIa	33	55	46	1,696	445	3.7	IIa	40	51	42	852	0	3.7
9	42	52	41	III	47	54	41	1,788	196	13	III	52	53	26	2,736	4	17.4
10	50	48	34	IIa	63	44	29	558	0	42.5	IIa	55	46	33	100	0	26.8
11	40	54	35	IIb	45	45	35	2,185	0	1.2	IIa	48	47	35	985	0	8.7
12	18	71	65	IIa	18	71	61	2,830	0	4.4	IIa	20	66	60	1,623	0	6.2
13	27	57	50	IIb	26	58	51	927	0	11.7	IIb	35	54	45	100	0	ND
14	42	58	50	IIa	42	57	49	1,558	0	12	IIa	42	51	42	1,858	0	15.7
15	15	79	73	IIb	17	77	71	1,262	0	6.2	IIb	17	74	68	263	0	5.6
16	30	58	51	IIb	35	58	48	259	0	12.7	IIa	46	54	40	189	0	8.5
17	21	65	59	III	25	63	58	324	0	22.8	IIb	40	56	45	251	0	4.7
Mean	33	57	48	2.5	34	57	48	1,430	73	13.4	2.1[c]	41[c]	55	41[d]	619[c]	6	9.7
SEM	3	2	3	0.1	3	2	3	216	35	2.6	0.1	3	2	3	200	5	1.7

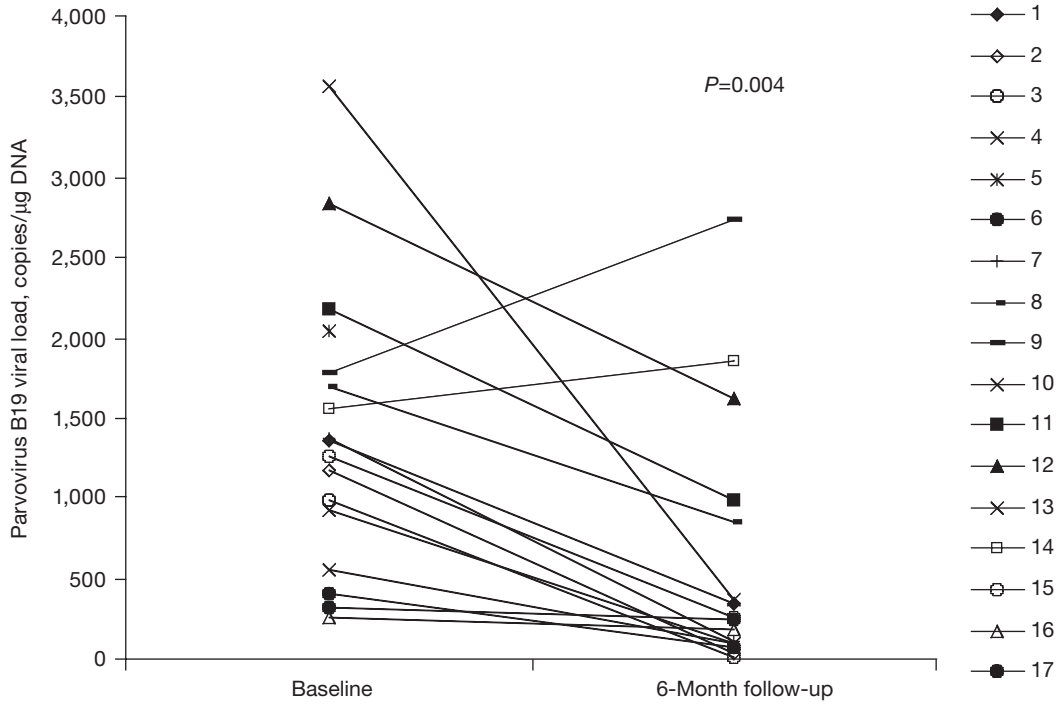
[a]PVB19, copies parvovirus B19 (genome/μg DNA). [b]HHV6, copies human herpesvirus 6 (genome/μg DNA). [c]P<0.005 compared with baseline. [d]P=0.006 compared with baseline. CD45, CD45$^+$-staining lymphocytes/mm^2; EDD, end-diastolic diameter (mm); EF, ejection fraction (%); ESD, end-systolic diameter (mm); ND, not determined; NYHA, New York Heart Association classification.

in four out of five patients (Table 2). According to the histological Dallas criteria, none of the specimens was graded as active or borderline myocarditis, either at baseline or follow-up. Immunohistochemical analyses revealed collagen volume fraction, $CD3^+$, $CD4^+$, $CD8^+$, $CD20^+$, $CD45^+$ and $CD68^+$-staining inflammatory cells at varying intensities in both the baseline and follow-up samples (Table 3). Eight patients (47%) had increased lymphocytic inflammation (defined as >12 $CD45^+$-staining lymphocytes/mm^2) at baseline and four (24%) at follow-up assessment. We observed a moderate increase in $CD4^+$- and $CD8^+$-staining lymphocytes, which was significant for the latter. There was no correlation between reduction in viral load and immunohistochemical inflammation, indicated by the moderate increase in inflammation despite good antiviral response to IVIg therapy in some patients and *vice versa*.

Immunoglobulin treatment improves cardiac function

LVEDD and LVEF did not significantly differ at baseline compared with 6 months before treatment (Table 2). LVEF significantly improved from 34 ±3% at baseline

Figure 1. Changes in left ventricular ejection fraction over time by treatment



Numbers relate to the patient number.

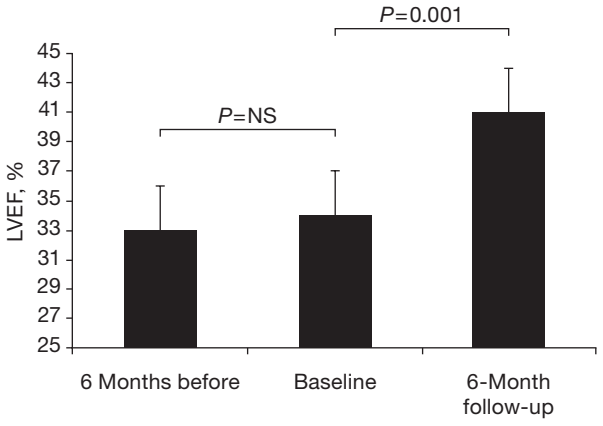
Table 3. Immunohistochemical analyses[a]

Cell type[b]	Baseline	6-Month follow-up	*P*-value
$CD3^+$, cells/mm^2	2.6 ±0.7	5.7 ±2.2	NS
$CD4^+$, cells/mm^2	0.9 ±0.4	1.5 ±0.7	NS
$CD8^+$, cells/mm^2	1.8 ±1.0	3.7 ±1.5	0.03
$CD20^+$, cells/mm^2	0.2 ±0.1	0.2 ±0.1	NS
$CD45^+$, cells/mm^2	13.4 ±2.6	9.7 ±1.7	NS
$CD68^+$, cells/mm^2	3.8 ±1.4	4.2 ±1.2	NS
CVF, %	5.9 ±1.7	6.2 ±1.0	NS

[a]All values are mean ±SEM. [b]$CD3^+$-, $CD4^+$-, $CD8^+$-, $CD20^+$- and $CD45^+$-staining lymphocytes/mm^2; $CD68^+$-staining macrophages/mm^2. CVF, collagen volume fraction; NS, not significant.

to 41 ±3% 6 months after IVIg treatment (P=0.001; Table 2, Figure 2). IVIg also affected LV diastolic and systolic dimensions: both LVEDD and left ventricular end systolic diameter (LVESD) progressively decreased from baseline to 6 months, with a significant reduction in LVESD (Table 2, Figure 2). Treatment resulted in a significant decrease of clinical complaints of dyspnea and/or fatigue, as revealed by an improvement in New York Heart Association (NYHA) classification from 2.5 ±0.1 at baseline to 2.1 ±0.2 at 6 months (P=0.004); the non-treated DCM patients with low PVB19 titres maintained an impaired cardiac function with an LVEF of 32 ±2 at baseline and 34 ±2 at a mean follow-up of 9 months (P=0.07). LVEDD and LVESD also remained stable. The two patients (numbers 9 and 14) with an increase in PVB19 load and associated inflammation still demonstrated improvement in cardiac function. No correlation was found between cardiac improvement and reduction in viral load or inflammation.

Figure 2. Changes in PVB19 viral load over time by treatment



Left ventricular ejection fraction (LVEF) was assessed by quantitative echocardiography. NS, not significant; PVB19, parvovirus B19.

Discussion

This is the first pilot study to demonstrate that IVIg in addition to standard heart failure treatment is a safe and effective therapy to reduce myocardial PVB19 and HHV6, and to improve cardiac function in patients with chronic DCM and high PVB19 viral load.

Infections with parvoviruses are characterized firstly by direct infection of target organs, including erythrocyte aplasia, or secondly as a result of the host response to infection. The immune response is the fifth children's disease, which is also called erythema infectiosum, and the polyarthropathy caused by human PVB19 infection. In addition, myocarditis can be another presentation of parvovirus disease, not only in humans through infection with human PVB19 but also in animals through infection with related parvoviruses (for example, feline/canine parvovirus, duck parvovirus and goose parvovirus), with widespread degeneration of striated cardiac and smooth muscles [36].

We studied PVB19 viral load in normal hearts and revealed a high 80% prevalence, but a low, presumably clinically irrelevant, viral load. Recent reports indicated that PVB19 might persist lifelong in the heart [17–19], as described in other organs including the bone marrow, skeletal muscle and the skin [17–19], but the load in the heart was not specified. The sole observation of lifelong PVB19 presence does not exclude a link between PVB19 presence and cardiac disease. Importantly, persistent infection of PVB19 in the bone marrow or skin in certain patients can cause severe cytopenia or erythema infectiosum [37]. Concordantly, as for other herpesviruses, HHV6 might also reactivate and cause cardiac injury [15]. As such, a dilated heart might be regarded as an immunocompromised environment where a persistent PVB19 or HHV6 might increase its load and presumably cause additional cardiac injury [15]. Whether this cardiac injury is a direct consequence of active viral infection with viral genome replication, synthesis of viral proteins and cell damage or an indirect consequence of immunological humoral and/or cellular processes is still a matter of debate. Previously, despite proven presence of PVB19 DNA in biopted endomyocardial tissue, the presence of PVB19 proteins in the myocardium has not been addressed. However, Escher *et al.* [38] were recently able to confirm the presence of PVB19 capsid proteins in endomyocardial biopsies from DCM patients using immunohistological detection. Within endomymocardial tissue positive for PVB19 DNA, PVB19 capsid proteins were also present; these proteins were most prominent in interstitial cells and additionally in cardiomyocytes and endothelial cells.

In the second part of our study we treated DCM patients with a high PVB19 viral load, excluding patients with a low and possibly clinically insignificant virus load. Treatment with IVIg significantly decreased PVB19 viral load in the dilated heart and improved cardiac function at 6-month follow-up; the untreated patients with low PVB19 titres remained stable. We also observed a significant increase in $CD8^+$-staining lymphocytes, which might reflect a change in the immune response following IVIg treatment.

IVIg is a safe and well-tolerated therapy for the treatment of a number of autoimmune and systemic inflammatory diseases [23,24,39,40]. The mode of action of IVIg is complex, owing to its broad range of activities: IVIg preparations are known to have anti-

infectious, anti-inflammatory and immunomodulating properties. Firstly, the anti-infectious properties of IVIg are based on its ability to neutralize viruses and microbial toxins. Secondly, IVIg in high doses has a well-established anti-inflammatory action, which is a property of the Fc fragment and its associated glycan – more specifically, the terminal sialic acid [41]. Lastly, the immunomodulating properties of IVIg result from its ability to both dampen inappropriate immune activation and to enhance microbial specific immunity [42]. Inflammatory processes and autoimmune mechanisms serve as important mediators in cardiovascular diseases [43]. Specifically, in our subgroup of patients with heart failure and related PVB19 persistence, the anti-infectious properties combined with the immunomodulatory and anti-inflammatory effects of IVIg seem likely to be beneficial [44].

A recent study by Kindermann *et al.* [45] indicated that immunohistochemical signs of inflammation are associated with an adverse outcome in a cohort of patients with recent onset heart failure suspected for myocarditis. Our group of patients had a long-lasting cardiomyopathy and studies judging the value of immunohistological inflammation in this population of patients are lacking. Most of our patients did not have an increased infiltration of inflammatory cells, concordant with previous studies, indicating that virus persistence might evolve as a chronic low-grade, but difficult to detect, inflammation responsible for matrix degradation, cardiomyocyte damage and secondary reparative fibrosis [45–48].

Both PVB19 and HHV6 are increasingly recognized as important cardiotrophic viruses inducing cardiac injury and dysfunction [10,12]. In some of our patients with a high PVB19 viral load, a coinfection with HHV6 was noted. It has been demonstrated that spontaneous elimination of these viruses results in improvement of cardiac function, whereas their persistence triggers progressive deterioration of left ventricular function [22–25]. IVIg therapy resulted in a significant decrease of PVB19 and elimination of HHV6, suggesting that the immunomodulation together with the anti-infectious properties of IVIg contribute to the virus reduction/elimination and the improvement of cardiac function. That reduction of PVB19 viral load might improve organ function is in line with previous studies indicating that IVIg is an effective treatment to reduce inflammation and improve clinical outcome in other PVB19-related diseases, such as fetal hydrops, pure red-cell aplasia, polyarteritis nodosa, immune thrombocytopenic purpura and PVB19 infection during pregnancy [22–28]. Similarly, chronic pain and/or fatigue syndromes reported in subgroups of patients are associated with PVB19 and these patients might benefit from treatment with IVIg [49–51].

A beneficial effect of IVIg for heart failure was first reported in an uncontrolled study of 40 patients with both ischaemic and non-ischaemic DCM [52,53]. However, a randomized trial in 62 patients with recent onset cardiomyopathy or myocarditis did not confirm a beneficial effect of IVIg compared with placebo: both IVIg and placebo treatment improved cardiac function to a similar extent [53]. Whereas spontaneous evolution of heart failure and effective standard antiheart failure treatment might in part explain the results, the lack of EMBs needed for viral diagnosis might also obscure the possible beneficial effects of IVIg in patients most in need of it (that is, those with PVB19 persistence). Cardiac biopsies should be part of the routine diagnosis in patients with idiopathic cardiomyopathy, not only to demonstrate the presence of virus, but also to look for increased inflammation using specific immunostaining techniques to detect (subtypes of) infiltration of different inflammatory cells.

Thus, our approach differed considerably from previous studies by the careful selection of patients eligible for IVIg. Firstly, we only treated patients with a stable history of heart failure with documented unaltered cardiac function and on standard heart failure treatment, making spontaneous recovery as a mechanism less likely. Secondly, we only included patients who had a significant viral load of PVB19, for whom IVIg therapy is most rational. Although we consider our uncontrolled pilot study to be a proof-of-concept because we treated patients during stable disease, it can be anticipated that earlier treatment – before irreversible myocardial damage due to reparative fibrosis occurs – might result in a better outcome. Previous reports indicated that viral DNA can be pseudo-reduced owing to a decrease in inflammatory cells carrying viral DNA [54]. In the present study, there was no significant decrease in inflammatory cells in the heart; therefore, the reduction in PVB19 viral DNA cannot be attributed to a reduction in inflammatory cells.

Despite the limitations of an uncontrolled pilot study, we are the first to demonstrate that IVIg is a safe and possibly effective therapy for reducing PVB19 and HHV6 in the heart. Although a relatively small number of patients with DCM and EMB-proven high PVB19 viral load was treated, this promising novel therapy might improve overall cardiac function and clinical variables in addition to optimal conventional heart failure treatment regimens. The potential effectiveness on other cardiotrophic viruses, a more prolonged dosing schedule and the ideal dosage of IVIg still need to be studied in randomized placebo-controlled trials to verify the promising results reported here.

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Disclosure statement

The authors declare no competing interests.

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