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RESEARCH ARTICLE

Intravenous immunoglobulin therapy in adult patients with idiopathic chronic cardiomyopathy and cardiac parvovirus B19 persistence: a prospective, double-blind, randomized, placebo-controlled clinical trial

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Aims Previous uncontrolled studies suggested a possible benefit of intravenous immunoglobulin (IVIg) in parvovirus B19 (B19V)-related dilated cardiomyopathy (DCM). This randomized, double-blind, placebo-controlled, single-centre trial investigated the benefits of IVIg beyond conventional therapy in idiopathic chronic DCM patients with B19V persistence.

Methods and results Fifty patients (39 men; mean age 54 ± 11 years) with idiopathic chronic (>6 months) DCM on optimal medical therapy, left ventricular ejection fraction (LVEF) <45%, and endomyocardial biopsy (EMB) B19V load of >200 copies/μg DNA were blindly randomized to either IVIg ($n = 26$, 2 g/kg over 4 days) or placebo ($n = 24$). The primary outcome was change in LVEF at 6 months after randomization. Secondary outcomes were change in functional capacity assessed by 6-min walk test (6MWT), quality of life [Minnesota Living with Heart Failure Questionnaire (MLHFQ)], left ventricular end-diastolic volume (LVEDV), and EMB B19V load at 6 months after randomization. LVEF significantly improved in both IVIg and placebo groups (absolute mean increase 5 ± 9%, $P = 0.011$ and 6 ± 10%, $P = 0.008$, respectively), without a significant difference between groups ($P = 0.609$). Additionally, change in 6MWT [median (interquartile range) IVIg 36 (13;82) vs. placebo 32 (5;80) m; $P = 0.573$], MLHFQ [IVIg 0 (−7;5) vs. placebo −2 (−6;6), $P = 0.904$] and LVEDV (IVIg −16 ± 49 mL/m^2 vs. placebo −29 ± 40 mL/m^2; $P = 0.334$) did not significantly differ between groups. Moreover, despite increased circulating B19V antibodies upon IVIg administration, reduction in cardiac B19V did not significantly differ between groups.

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Conclusion Intravenous immunoglobulin therapy does not significantly improve cardiac systolic function or functional capacity beyond standard medical therapy in patients with idiopathic chronic DCM and cardiac B19V persistence.
Clinical Trial Registration: ClinicalTrials.gov ID NCT00892112.

Keywords Dilated cardiomyopathy • Heart failure • Intravenous immunoglobulin • Parvovirus B19 • Endomyocardial biopsy

Introduction

Virus persistence has been related to the development and progression of dilated cardiomyopathy (DCM).[1–6] In recent decades, parvovirus B19 (B19V) has become the most frequently found cardiotropic virus in endomyocardial biopsies (EMB), with a reported prevalence of up to 80%.[5–7]

A possible pathogenic effect of B19V is supported by its activation of pro-inflammatory cytokines, reduced endothelial regeneration and induction of apoptosis.[5,8–11] If extensive enough, this might result in endothelial damage, which compromises tissue perfusion and causes cardiac dysfunction.[1,5,8,12] However, in recent times B19V genomes are also frequently found in healthy or diseased hearts of individuals without evidence of myocarditis or DCM, making the clinical significance of B19V within the myocardium still unclear.[5,13,14]

While the causal relationship and pathogenic importance of viral persistence and DCM remain controversial, positive effects on viral load and/or cardiac function of intravenous immunoglobulin (IVIg) have been suggested in retrospective and non-randomized studies.[15–20] Nonetheless, the effect of IVIg therapy in adults with idiopathic chronic DCM and EMB B19V persistence has not yet been prospectively evaluated. We therefore performed a prospective, randomized, double-blind, placebo-controlled trial to evaluate the effect of IVIg on systolic cardiac function and EMB B19V load in adult patients with idiopathic chronic DCM and cardiac B19V persistence.

Methods

Study objectives

The objective of the present single-centre, prospective, randomized, double-blind, placebo-controlled trial (NCT00892112) was to evaluate the incremental value of IVIg therapy beyond conventional heart failure therapy vs. conventional heart failure therapy alone in ambulatory patients with idiopathic chronic (>6 months) DCM and an EMB B19V load of >200 copies/μg DNA.

The primary endpoint was the absolute change of echocardiographically assessed left ventricular ejection fraction (LVEF) from baseline to 6 months. The secondary endpoints included changes in EMB B19V load (copies/μg DNA), cardiac CD45+ inflammatory cells, myocardial collagen volume fraction, 6-min walk test (6MWT) distance, patient quality of life [Minnesota Living with Heart Failure Questionnaire (MLHFQ)], and left ventricular end-diastolic volume (LVEDV) assessed by echocardiography.

The study was performed according to the Declaration of Helsinki and was approved by the institutional Medical Ethics Committee (METC azM/UM). All patients gave written informed consent.

Patient population

Patients that underwent EMB because of idiopathic DCM were screened for eligibility from November 2009 to January 2018. The primary inclusion criteria were: (i) LVEF <45% with a diagnosis of idiopathic chronic (>6 months) DCM on optimal medical therapy; (ii) EMB B19V load of >200 copies/μg DNA, and (iii) age between 18–75 years.

All patients underwent angiography or non-invasive screening to exclude coronary artery disease, a transthoracic echocardiogram to rule out significant valvular disease, and right ventricular EMBs before enrolment.

Patients with significant EMB load (>200 copies μg/DNA) of other cardiotropic viruses (enterovirus, adenovirus, human herpesvirus 6, Epstein–Barr virus), systemic autoimmune disease, renal insufficiency (plasma creatinine >115 μmol/L), or non-idiopathic cardiomyopathy were excluded. The complete list of exclusion criteria is provided in online supplementary *Methods S1*.

Randomization and therapeutic protocol

Patients were randomly and blindly assigned using the minimization randomization method to minimize the imbalance between the number of patients in each treatment group over pre-defined factors (age, gender, LVEF, left ventricular dimensions and EMB B19V load).[21] Patients randomized to the treatment group received a total of 2 g/kg IVIg (Nanogam 50 mg/mL, Sanquin Plasma Products B.V., Amsterdam, The Netherlands) administered as 0.5 g/kg (10 mL/kg) over 6 h on four consecutive days. Placebo consisted of a plasma volume expander (Albuman 40 g/L, Sanquin Plasma Products B.V.) to control for the protein load given by Nanogam, administered as 10 mL/kg over 6 h on four consecutive days. Independent pharmacists prepared the intravenous solutions according to the unique randomization number generated by TEN-ALEA software. All study personnel and participants were blinded to treatment assignment for the duration of the study.

Clinical evaluations

The baseline measurements and final follow-up visit at 6 months included physical examination, transthoracic echocardiogram, laboratory tests, assessment of functional capacity by 6MWT and quality of life using the MLHFQ,[22] and right ventricular EMB to evaluate viral persistence and immunohistological markers of inflammation and fibrosis. Additional follow-up visits with physical examination took place at 2 weeks – including the evaluation of safety and potential side-effects of the study drug and laboratory tests – and 3 months – including additional transthoracic echocardiogram – after baseline. Circulating B19V antibodies – anti-NS1 and anti-VP1/VP2 – were measured at baseline and after the last treatment day, these data were made available after

completion of the study to evaluate whether expected differences in circulating B19V antibodies between treatment arms were reached.

More detailed information is provided in online supplementary *Methods S1*.

Statistical analysis

The sample size estimation was based on our pilot data – patients with a baseline LVEF <45% in the pilot study were used for this power calculation[15] – as well as potential patient withdrawal. Sample size requirements were determined using the following assumptions: (i) expected absolute therapy effect of 10% LVEF improvement with a standard deviation of 10%; (ii) power of 0.90 and alpha of 0.05; (iii) a drop-out of $n = 4$ per group. To ensure enough power for this study, 25 patients per group had to be enrolled.

Normality was assessed visually using histograms and Q-Q plots. Numerical variables are displayed as mean ± standard deviation or median (interquartile range) where appropriate. Categorical variables are displayed as absolute frequencies and percentage values.

Spearman's correlation (r_s) was used to evaluate the correlation between EMB CD45+ cells and EMB B19V load. The changes between 6 months and baseline LVEF (primary outcome), LVEDV, and EMB B19V and the changes between day four and baseline anti-VP1/VP2 concentrations and anti-NS1 were calculated for each subject. Subsequently, the differences between groups were calculated by unpaired Student's *t*-test or Wilcoxon signed-rank test for continuous variables, and Chi-squared or Fisher's exact test for categorical variables as appropriate.

The differences within groups were analysed using paired Student's *t*-test, paired Wilcoxon signed-rank test or McNemar test as appropriate. Additionally, linear mixed-effects modelling – lme4 package in R[23] – was used to assess the difference in change of LVEF over time (baseline, 3 and 6 months) between the treatment groups (IVIg vs. placebo). Time, group and group*time were included as fixed factors, and a random intercept on subject level was included to adjust for the correlation between repeated measurements. Restricted maximum likelihood was used to obtain unbiased estimates of the treatment effects, while maximum likelihood estimation was used for testing fixed effects.

Missing data at follow-up were assumed to be missing at random and were not imputed (likelihood-based approach). In total, three patients ($n = 1$ IVIg; $n = 2$ placebo) withdrew – based on their preference – before the first day of treatment and were therefore not included in the analysis. Additionally, two patients did not show up during echocardiography at 3 months ($n = 2$ IVIg), in three patients LVEDV could not be determined at 6 months ($n = 1$ IVIg; $n = 2$ placebo), and two patients refused EMB at 6 months ($n = 2$ placebo). The former patients have only been excluded from the analysis involving echocardiographic variables at 3 and 6 months, and EMB variables at 6 months, respectively. For the three patients in which LVEDV could not be determined, the Teichholz formula was used to calculate LVEF. The use of the Teichholz formula in all patients did not change the results of this study.

A *P*-value ≤0.05 was considered statistically significant. Statistical analysis was performed using RStudio V1.2.5033.[24]

Results

A total of 526 patients underwent EMB because of unexplained left ventricular dysfunction (LVEF <45%) at our centre from November 2009 to January 2018. A total of 370 (70%) patients had B19V presence, including 148 (40%) patients with an EMB B19V load of >200 copies/µg DNA. Among them, 95 were excluded according to the exclusion criteria (including four patients with cardiac human herpesvirus 6 >200 copies/µg DNA; no patients were excluded due to significant presence of other cardiotropic viruses). Finally, 53 patients (27 IVIg and 26 placebo) agreed to participate in the study. Three of them, however, withdrew before receiving trial medication ($n = 1$ IVIg and $n = 2$ placebo; *Figure 1*) and therefore were not included in the analysis.

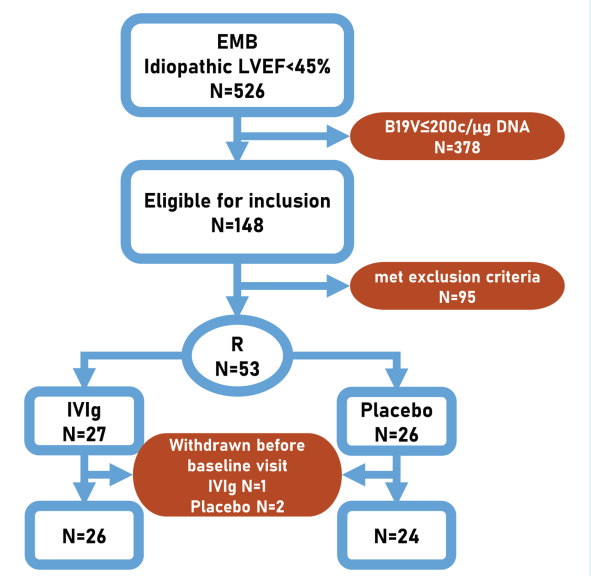

Figure 1 Study flow. B19V, parvovirus B19; c/µg DNA, copies per microgram of DNA; EMB, endomyocardial biopsy; IVIg, intravenous immunoglobulin; LVEF, left ventricular ejection fraction; R, randomized.

Similar clinical characteristics were observed in both treatment groups (*Tables 1* and *2*, online supplementary *Table S1*). Male sex predominated (78%), and mean LVEF was 35 ± 6% for the total population at the time of inclusion.

Effect of therapy on left ventricular function

The primary endpoint, i.e. absolute change in LVEF 6 months after therapy, did not differ significantly between the IVIg and placebo group ($P = 0.609$; *Figure 2A*). The mean LVEF improved significantly in the total patient population (5 ± 9%, $P < 0.001$) from baseline to 6 months. This increase was significant for both the IVIg (5 ± 9%, $P = 0.011$) and placebo group (6 ± 10%, $P = 0.008$). Additionally, the linear mixed-effects model did not show a significant difference in LVEF trajectory over time between groups ($P = 0.923$ for interaction between group and time), indicating that the change in LVEF from baseline to 3 and 6 months was not significantly different between the treatment groups.

The LVEDV decreased significantly in the total patient population from baseline to 6 months (−22 ± 45 mL/m^2, $P = 0.002$). Although

Table 1 Baseline characteristics: placebo vs. intravenous immunoglobulin

Demographics/ presentation	Placebo ($n = 24$)	IVIg ($n = 26$)	*P*-value
Age at diagnosis (years)	53 ± 9	54 ± 13	0.759
Male sex	19 (79%)	20 (77%)	0.848
Weight (kg)	86 ± 14	87 ± 18	0.698
Height (cm)	177 ± 10	178 ± 11	0.918
Heart rate (bpm)	70 ± 8	70 ± 9	0.817
SBP (mmHg)	123 ± 13	123 ± 18	0.857
DBP (mmHg)	77 ± 9	72 ± 10	0.045
Diabetes mellitus	2 (8%)	3 (12%)	0.706
Atrial fibrillation	10 (42%)	5 (19%)	0.084
LBBB	6 (25%)	5 (19%)	0.719
Hypercholesterolaemia	3 (13%)	2 (8%)	0.571
Days from first DCM diagnosis	418 (335;653)	415 (316;801)	0.961
Medical history of acute myocarditis	0 (0%)	3 (12%)	0.236
Medical history of HFH	5 (21%)	4 (15%)	0.721
Family history of DCM	3 (13%)	1 (4%)	0.340
NYHA class III or IV	0 (0%)	1 (4%)	1.000
Lab			
Creatinine (µmol/L)	91 (75;102)	84 (76;99)	0.600
NT-proBNP (pmol/L)	44 (14;98)	33 (8;60)	0.218
CRP	1 (1;3)	2 (1;3)	0.289
Medication[a]			
Beta-blocker (yes/no)	22 (92%)	24 (92%)	1.000
≥50% OMT	12 (50%)	16(62%)	0.592
ACE-inhibitor/ARB (yes/no)	23 (96%)	25 (96%)	1.000
≥50% OMT	21 (88%)	20 (77%)	0.467
Aldosterone antagonist (yes/no)	13 (54%)	8 (31%)	0.165
≥50% OMT	12 (50%)	7 (27%)	0.165
Cardiac devices			
ICD	1 (4%)	3 (12%)	0.611
CRT-D	3 (13%)	1 (4%)	0.340

ACE, angiotensin-converting-enzyme; ARB, angiotensin receptor blocker; CRP, C-reactive protein; CRT-D, cardiac resynchronization therapy with defibrillator; DBP, diastolic blood pressure; DCM, dilated cardiomyopathy; HFH, heart failure hospitalization; ICD, implantable cardioverter-defibrillator; IVIg, intravenous immunoglobulin; LBBB, left bundle branch block; NYHA, New York Heart Association; OMT, optimal medical therapy; SBP, systolic blood pressure.
[a]OMT was calculated based on the 2016 European Society of Cardiology heart failure guidelines.

this change was only significant in the placebo group (placebo −29 ± 40, $P = 0.003$; IVIg −16 ± 49 mL/m^2; $P = 0.116$), the difference between groups was not significant ($P = 0.334$; *Figure 2B*).

No significant treatment effect was observed in a subset of patients with baseline EMB B19V load >500 copies/µg DNA ($n = 10$ placebo; $n = 13$ IVIg) on change of both LVEF (IVIg 4 ± 7% and placebo 9 ± 8%; $P = 0.157$) and LVEDV (IVIg −14 ± 57 mL/m^2 and placebo −42 ± 22 mL/m^2; $P = 0.129$) 6 months after treatment (online supplementary *Tables S3–S6*).

Table 2 Echocardiographic results: placebo vs. intravenous immunoglobulin

	Placebo ($n = 24$)	IVIg ($n = 26$)	*P*-value
Baseline			
LVEDV (mL)	189 ± 52	208 ± 69	0.283
LVESV (mL)	122 ± 38	135 ± 54	0.313
LVEF (%)	35 ± 6	36 ± 7	0.552
3 months[a]			
LVEDV (mL)	178 ± 59	201 ± 72	0.226
LVESV (mL)	112 ± 44	125 ± 53	0.380
LVEF (%)	38 ± 9	39 ± 8	0.703
LVEF absolute change from baseline (%)	3 ± 9	3 ± 6	0.985
6 months			
LVEDV (mL)	164 ± 55	190 ± 60	0.120
LVESV (mL)	100 ± 41	117 ± 46	0.187
LVEF (%)	41 ± 12	41 ± 10	0.921
LVEF absolute change from baseline (%)	6 ± 10	5 ± 9	0.609

IVIg, intravenous immunoglobulin; LVEDV, left lentricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume.
[a]IVIg $n = 24$.

Histological and biochemical changes

Baseline EMB B19V load did not significantly differ between the IVIg and placebo group [481 (334;907) and 354 (287;883) copies/µg DNA, respectively, $P = 0.351$; *Table 3*]. There was a significant reduction in B19V load in the total patient population during the trial [−119 (−338;4) copies/µg DNA, $P = 0.004$],which was comparable between groups ($P = 0.718$; *Figure 3*).

No significant difference in amount of inflammation or collagen volume fraction in EMB was observed between groups at baseline and 6 months (*Table 3*). Changes of CD45+ cells/mm^2 after treatment [placebo −0.9 (−7.2;1.0), $P = 0.098$; IVIg 2.2 (−1.6;5.6), $P = 0.317$] did not significantly differ between the treatment arms ($P = 0.058$). Moreover, B19V and EMB CD45+ cells/mm^2 did not significantly correlate at baseline, neither in the total study population ($r_s = 0.08$, $P = 0.58$), nor in patients ($n = 23$) with an EMB B19V load of >500 copies/µg DNA at baseline ($r_s = -0.18$, $P = 0.42$).

Changes in functional capacity and quality of life

The 6MWT distance increased significantly after 6 months in the total patient population [36 (6;81) m, $P < 0.001$], but did not differ between the IVIg and placebo group [36 (13;82) and 32 (5;80), respectively, $P = 0.573$; *Table 4*].

Quality of life did not differ between the assessment at 6 months and baseline in the total patient population [MLHFQ −2 (−6;6), $P = 0.517$], neither was there a significant difference in change during the trial between the treatment groups [IVIg 0 (−7;5) and placebo −2 (−6;6), $P = 0.904$; *Table 4*). Two patients ($n = 1$ in both

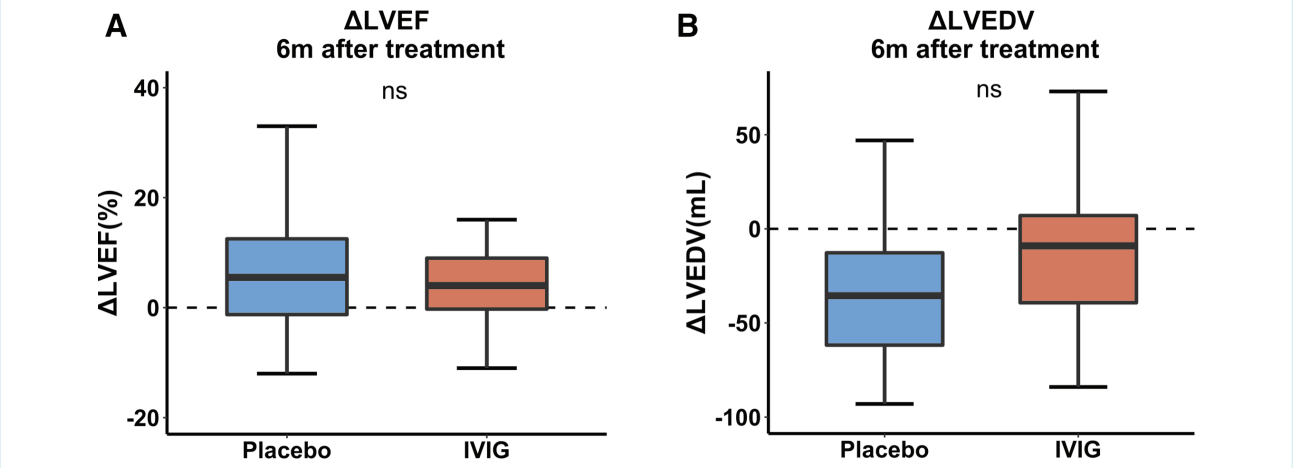

Figure 2 (*A*) Primary endpoint: comparison of change in left ventricular ejection fraction (LVEF) 6 months after therapy. (*B*) Comparison of change in left ventricular end-diastolic volume (LVEDV) 6 months after therapy. IVIG, intravenous immunoglobulin.

Table 3 Endomyocardial biopsy results: placebo vs. intravenous immunoglobulin

	Placebo (n = 24)	IVIg (n = 26)	*P*-value
Baseline			
Cardiac inflammation[a]	6 (25%)	7 (27%)	0.877
CD3 (cells/mm^2)	5.6 (1.8;7.8)	5.1 (2.9;7.0)	0.907
CD45 (cells/mm^2)	6.8 (5.1;12.3)	7.8 (4.7;11.3)	0.808
CD68 (cells/mm^2)	1.3 (0.5;4.0)	2.8 (2.0;4.1)	0.145
Collagen volume fraction (%)	7.2 (4.4;11.0)	7.6 (4.2;13.9)	0.683
B19V (copies/μg DNA)	354 (287;883)	481 (334;907)	0.351
6 months[b]			
Cardiac inflammation[a]	3 (12%)	7 (29%)	0.286
CD3 (cells/mm^2)	5.4 (2.5;6.1)	4.5 (3.1;6.5)	0.645
CD45 (cells/mm^2)	6.4 (5.1;9.1)	8.9 (7.2;11.2)	0.060
CD68 (cells/mm^2)	1.2 (0.7;2.8)	2.1 (1.5;3.6)	0.090
Collagen volume fraction (%)	6.5 (4.8;8.7)	8.4 (4.8;13.9)	0.177
B19V (copies/μg DNA)	245 (200;633)	349 (200;891)	0.298

B19V, parvovirus B19; CD, cluster of differentiation; IVIg, intravenous immunoglobulin.
[a]Defined as ≥14 infiltrating CD45+ cells/mm^2 (including up to four infiltrating CD68+ cells/mm^2) of total myocardial area.
[b]IVIg n = 24.

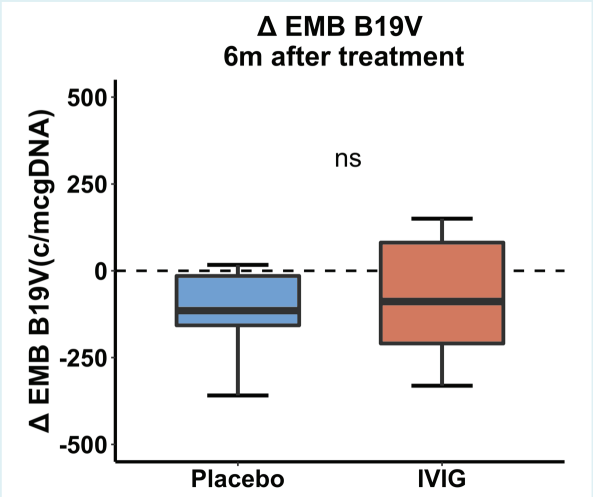

Figure 3 Comparison of change in endomyocardial biopsy (EMB) parvovirus B19 (B19V) load (copies/μg DNA) 6 months after therapy. IVIG, intravenous immunoglobulin.

groups) were classified as New York Heart Association class ≥III after 6 months.

Adverse drug effects and heart failure medication changes

No adverse event led to the interruption or discontinuation of the study treatment, and no serious adverse drug reaction occurred during the study. Headaches were significantly more often reported in the IVIg group compared to placebo (42% and 17%, respectively, P = 0.048). In nine patients, heart failure medication changes occurred during the study period (n = 5 IVIg and n = 4 placebo, respectively; P = 0.99). Medication usage at baseline (*Table 1*) and after 6 months (online supplementary *Table S2*) was not different between the treatment groups. None of the patients underwent cardiac device implantation or upgrade during the study period.

Effect of therapy on circulating parvovirus B19 antibodies

A significant increase in B19V antibodies after 4 days of treatment was observed in patients receiving IVIg as compared to placebo (both $P < 0.001$), reflected by an increase of positive anti-NS1 tests (from 4% to 81%, $P < 0.001$) and increased anti-VP1/VP2 [+869 (761;1045) U/mL, $P < 0.001$]. In contrast, anti-NS1 (from 4% to 9%, P = 0.99) and anti-VP1/VP2 [−50 (−97;-26) U/mL, $P < 0.001$] did not significantly increase in the placebo group.

Table 4 Functional capacity and quality of life: placebo vs. intravenous immunoglobulin

	Placebo ($n = 24$)	IVIg ($n = 26$)	*P*-value
6-min walk test (m)			
Baseline	487 (466;552)	468 (421;542)	0.336
6 months	554 (480;581)	508 (448;613)	0.778
Change	32 (5;80)	36 (13;82)	0.573
Quality of life (MLHFQ)			
Baseline	18 (8;34)	11 (6;36)	0.514
6 months	20 (5;41)	17 (3;29)	0.633
Change	−2 (−6;6)	0 (−7;5)	0.904

IVIg, intravenous immunoglobulin; MLHFQ, Minnesota Living with Heart Failure Questionnaire, with a higher score (range 0–105) indicative of a lower quality of life.

Discussion

This is the first randomized placebo-controlled trial evaluating the therapeutic effects of IVIg in patients with idiopathic chronic DCM and EMB proven B19V persistence, showing that IVIg (2 g/kg) did not result in any improvement of cardiac function, functional capacity or quality of life after 4 days of treatment.

The mode of action of IVIg is versatile with a broad range of activities: IVIg preparations are known to have anti-infectious, anti-inflammatory, and immunomodulating properties.[25,26] The administration of IVIg resulted in a significant increase of anti-B19V antibodies (anti-NS1 and anti-VP1/VP2) in the treated patients, but did not result in a significant reduction of cardiac B19V load or inflammation. IVIg has no beneficial effects in the majority of patients with idiopathic chronic DCM and a cardiac B19V load of >200 copies/μg DNA. After the initiation of this study, several studies revealed that the EMB B19V load of DCM patients was comparable to controls with either diseased or healthy hearts.[5] Also, B19V load is not affected by immunosuppression in inflammatory DCM with significant B19V load, as recently published,[27] also indicating that B19V might be an innocent bystander. Adjudicating IVIg treatment solely based on cardiac function and B19V presence does not seem to be an effective strategy given the likely latent intracellular state of the virus in the majority of patients.[28] Therefore, additional determinants beyond viral load – e.g. active viral replication, location of the virus, co-infection, inflammation and genetic background – might be crucial for B19V to yield a pathogenic potential in DCM. A better understanding of these mechanisms is crucial to select a subgroup of patients that still could benefit from IVIg therapy.[5,14,29–31]

Our negative findings in chronic DCM are in line with the IMAC trial where IVIg on top standard therapy was given in patients with recent-onset DCM and did not improve outcome.[32] DCM is the result of multiple underlying environmental, genetic and immunological insults and therefore likely does not follow a unidirectional treatment strategy.[33] Identifying downstream pathophysiological processes will be essential to develop future targeted treatment strategies for subsets of DCM patients.

The used total dosage (2 g/kg IVIg) within this study is known as a high-dose therapy and is the same as previously studied in peripartum cardiomyopathy,[34] acute myocarditis in children,[35] recent-onset DCM,[32] and our retrospective pilot study in idiopathic cardiomyopathy with EMB B19V persistence.[15] While the present study is unable to assess the possible beneficial effect of prolonged administration of IVIg (beyond 4 days or on a weekly/monthly basis), the lack of any improvement of either cardiac function, viral load, inflammation, or functional capacity makes a clinical relevant beneficial effect unlikely. We included chronic DCM patients and still observed a significant increase in systolic cardiac function in the total patient population, underscoring the fact that functional recovery may take longer than 6 months upon optimal heart failure therapy.[36,37]

Limitations

The number of included patients in our trial is small. Moreover, given the limited information available on this topic before the initiation of this study, we decided to perform the power calculation solely based on the primary endpoint, which theoretically could have resulted in a type 2 error for the secondary endpoints. However, due to the randomized and double-blind nature of the study and corresponding negative findings, we would not favour larger clinical trials with IVIg in B19V-related DCM which are based solely on cardiac B19V load. The inclusion of patients with EMB B19V >200 copies/μg DNA in the current study was based on our previous results in post-mortem samples of non-cardiac diseased subjects.[15] After the initiation of this study, a load of >500 copies/μg DNA was suggested to be a clinically relevant threshold given its association with cardiac inflammation.[38] In a sub-analysis of patients with EMB B19V >500 copies/μg DNA, we did not find a trend for a beneficial effect of IVIg on any of the endpoints. Nonetheless, a small effect cannot be excluded due to the limited number of patients in this sub-analysis and as a consequence, insufficient power. Moreover, a sub-analysis in patients with EMB B19V >500 copies/μg DNA and EMB proven inflammation ($n = 7$) could not be performed due to the lack of power. Lastly, due to the small sample size and over-representation of males (78%) – in which DCM is known to be more prevalent – the effect of gender could not be evaluated within this trial.[39,40]

Conclusion

Intravenous immunoglobulin therapy does not provide additional benefit on cardiac systolic function or functional capacity beyond standard medical therapy in patients with idiopathic chronic DCM and cardiac B19V persistence.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Conflict of interest: none declared.

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