Viral genome changes and the impact of viral genome persistence in myocardium of patients with inflammatory cardiomyopathy

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Abstract

Introduction: Viral infections are considered the most frequent cause of myocarditis and dilated cardiomyopathy (DCM).

Material and methods: We investigated the changes in viral presence and the impact of viral genome persistence in the myocardium on echocardiographic parameters, functional status and some laboratory parameters in a 6-month follow-up. Fifty-four patients with recent onset DCM, left ventricular ejection fraction < 40% and biopsy-proven myocarditis (> 14 mononuclear leukocytes/mm^2 and/or > 7 T-lymphocytes/mm^2) were enrolled. Polymerase chain reaction (PCR) was performed to detect pathogens in the myocardium. Patients were divided according to the administered therapy: standard heart failure medication (46 patients) and immunosuppressive therapy (8 patients).

Results: In the standard heart failure medication group viral clearance was observed in 13 patients and viral persistence in 24 patients in the follow-up period. Comparing both groups, there was no statistically significant difference – LVEF improvement of 12.0 ±11.4% vs. 18.3 ±12.6%, decrease in NYHA class of 0.7 ±0.7 vs. 1.0 ±0.7, decline in NT-proBNP of 1335 ±1933 ng/l vs. 1942 ±3242 ng/l and decrease in infiltrating leukocytes of 11.1 ±15.8 vs. 6.7 ±23.0 cells/mm^2 and T-lymphocytes of 5.8 ±15.1 vs. 1.8 ±10.9 cells/mm^2 (all *p* = NS). A decrease in PCR positive patients from 37 to 29 was observed. The number of PVB19 positive PCR findings decreased from 5 to 4 in patients with immunosuppressive therapy.

Conclusions: A decrease in the number of positive PCR findings in control endomyocardial biopsy was observed. Viral genome persistence was not associated with worse outcome in short-term follow-up.

Key words: polymerase chain reaction, myocarditis, dilated cardiomyopathy, endomyocardial biopsy, inflammatory cardiomyopathy.

Introduction

Dilated cardiomyopathy (DCM) is one of the leading causes of systolic heart failure, particularly in younger patients [1]. Dilated cardiomyopathy remains the diagnosis leading to more than half of all heart transplantations [2]. It has been reported previously that significant inflammatory

AMS

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infiltration (i.e. myocarditis) is present in the myocardium of about one half of patients with DCM [3–5]. In such cases, the condition should be called inflammatory cardiomyopathy (ICM). Myocarditis and ICM can be caused by a variety of infectious and non-infectious conditions [6, 7]. In developed countries, viral infections are considered to be the main etiological factor. Results of trials focused on bioptic diagnostics have shown that viral nucleic acid can be detected in 44–67% of patients with DCM [4, 5, 8]. Recently, parvovirus B19 (PVB19) and also herpes virus type 6 (HHV-6) have been the most commonly detected pathogens in the myocardium [4, 5, 9, 10].

Current understanding of the pathophysiology of viral myocarditis is derived from murine models of enteroviral myocarditis and consists of three distinct phases [11–13]. The acute phase is characterized by direct viral cytotoxicity and the innate immune response. The second subacute phase is associated with a specific immune response, which could have autoimmune features based on the exposure of intracellular antigens and immune cross-reactivity (molecular mimicry). The third phase could be healing when left ventricle (LV) function recovers (in 50–70% of cases), or evolution in noninflammatory DCM. It is questionable whether this course of myocarditis is the same with other viruses, especially with those that do not primarily affect the cardiomyocytes (e.g. PVB19 causes inflammation in endothelial cells) [14].

Another very interesting but at the same time rather confusing fact is that the presence of viral agents is not limited to patients with LV dysfunction but it is often also found in patients with normal ejection fraction who undergo cardiothoracic surgery [10, 15]. Currently published data concerning the impact of viral genome presence in the myocardium are based mainly on follow-up data after initial single diagnostic biopsy. According to some studies, the viral presence is related to poor prognosis [16, 17] but other trials have not proved this association [4, 8]. Besides that, the importance of viral persistence (thus not only of simple presence) in the myocardium is less convincing. There are very few studies addressing this issue that indicate that viral persistence is linked with worse prognosis [17, 18]. All considered, it is still uncertain how close the relation between viral persistence in the myocardium and progression of the disease to DCM is.

The aim of this study was to evaluate the presence of the viral nucleic acid and its changes in patients with ICM in a 6-month follow-up. The evaluation of these changes was performed in a group of patients with standard heart failure therapy and in patients with immunosuppressive medica-

tion added to the standard treatment. We focus mainly on the group with standard heart failure treatment only, and we assessed the persistence of the viral genome and its relation to the changes in the echocardiographic and laboratory (especially natriuretic peptides) parameters and functional outcome, as well as on the change of the number of inflammatory cells in the myocardium.

Material and methods

Patients

Between February 2010 and February 2015, a total of 191 patients with recent-onset DCM were admitted to our institution for initial evaluation. This also included endomyocardial biopsy (EMB) to rule out inflammatory etiology of LV dysfunction. We enrolled 54 patients (41 males and 13 females) with biopsy-proven myocarditis and LV dysfunction confirmed by echocardiography (LVEF < 40%). All of them had to have a history of heart failure symptoms shorter than 6 months and a completed 6-month follow-up. Patients were divided into two groups according to the administered medication. The first one was receiving standard heart failure medication only according to current guidelines [19, 20]. Patients in the second group received immunosuppressive therapy (combination of azathioprine 2 mg/kg/day and prednisone in initial dose 1 mg/kg/day with step decrease; immunosuppression was administered for 3 or 6 months) on top of standard medication (these patients were included in the randomized clinical trial with immunosuppressive therapy CZECH-ICIT (ClinicalTrials.gov Identifier: NCT01877746) [21]). All patients signed informed consent and the study protocol was approved by the local ethics committee.

Patients with coronary artery disease, significant primary valve disease, excessive alcohol intake, administration of cardiotoxic chemotherapy, tachycardia-induced cardiomyopathy or endocrine disorders possibly associated with cardiac disease were excluded.

Methods

At the baseline visit, all patients were clinically examined, their functional status was assessed according to the NYHA classification and routine laboratory tests including natriuretic peptides in serum were done. EMB was performed via the jugular vein under local anesthesia, so the samples were obtained from the right ventricle only. Four samples were obtained for histological and immunohistochemical analysis, and another six samples were tested using real-time polymerase chain reaction (PCR) for detection of potential pathogens. The average number of T-lymphocytes

(CD3+ cells) and mononuclear leukocytes (LCA+ cells) per mm^2 was assessed. Myocarditis was defined as the presence of > 7 CD3+ cells and/or > 14 LCA+ cells per mm^2 in the baseline EMB [18].

The PCR was performed to detect the genomic sequences of parvovirus B19 (PVB19), cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus type 1 and type 2 (HSV-1, 2), human herpesvirus 6 (HHV-6), adenovirus (ADV), *Borrelia burgdorferi* (sensu lato) and reverse transcription-PCR for enterovirus (EV). In PVB19 positive samples, the viral load was expressed as the number of genomic DNA copies per µg of total extracted nucleic acids.

Echocardiography was performed using the Vivid E9 (GE, Milwaukee, WI, USA) machine and M5S probe according to current guidelines [22, 23].

The follow-up admission for performing physical examination with evaluation of functional status, endomyocardial biopsy, echocardiography and laboratory studies (natriuretic peptides in serum) was planned in 6 months ± 14 days. Follow-up echocardiographic examination was performed by the same physician as the initial evaluation.

Statistical analysis

Monitored parameters were described using descriptive analysis and initial values were compared with the values observed after 6 months. Results are presented as an average value with standard deviation and as a median value (25th, 75th percentile). Because most of the monitored parameters do not show a normal distribution (Shapiro-Wilk test), non-parametric tests were performed. The change in each parameter after 6 months from the beginning was evaluated using the paired Wilcoxon test. The Mann-Whitney test was used for comparison of parameters between groups of patients. All analyses were performed at the 5% significance level (i.e. $p < 0.05$ were considered statistically significant).

Results

The demographic and other characteristics of the patients are shown in Table I. Out of 54 enrolled patients, 46 (34 males and 12 females) were treated with standard heart failure medication only, 8 patients (7 males and 1 female) with immunosuppressive therapy added to the standard one.

In the group of patients receiving only standard heart failure medication, viral genome was detected in 37 of 46 patients at the baseline biopsy (i.e. 80%), and follow-up biopsy showed viral genome presence in 29 (63%) patients. According to the 6-month follow-up EMB results, in 24 of 37 initially positive patients (65%) viral genome persisted while in 13 of these patients (35%) no virus was found. Initial characteristics of these two groups did not differ significantly.

In the group with viral clearance, LVEF improved from 26.8 ±8.6% to 38.8 ±12.2% in the 6-month follow-up ($p < 0.01$). NYHA classification grade decreased from 2.3 ±0.7 to 1.6 ±0.5 ($p < 0.01$). The levels of NT-proBNP in serum decreased from 1910 ±1940 ng/l to 575 ±689 ng/l ($p < 0.001$). A decrease of the number of LCA+ cells from 22.5 ±14.7 to 11.3 ±5.6 cells/mm^2 ($p < 0.01$) and in the number of infiltrating CD3+ cells from 8.8 ±15.2 to 3.1 ±2.6 cells/mm^2 ($p < 0.05$) was observed.

The group of patients with viral persistence showed improvement in LVEF from 26.5 ±7.4% to 44.9 ±11.2% ($p < 0.0001$). NYHA class classi-

Table I. Baseline characteristics

Parameter	Standard heart failure therapy	Standard heart failure therapy + immunosuppression
Number of patients	46	8
Age, mean ± SD	42.63 ±12.59	48.75 ±12.79
Sex, men/women	34/12	7/1
NYHA classification	2.46 ±0.71	2.63 ±0.78
Ejection fraction, mean ± SD (%)	25.3 ±7.05	21.38 ±4.09
Duration of symptoms, mean ± SD [months]	2.32 ±2.39	3.5 ±2.55
ACE inhibitors or ARBs	45 (97.8%)	8 (100%)
β-Blockers	44 (96.6%)	7 (87.5%)
Spironolactone	36 (88.3%)	8 (100%)
Diuretics	40 (87%)	8 (100%)
Digoxin	10 (22.7%)	3 (37.5%)

ACE – angiotensin-converting enzyme, ARB – angiotensin receptor blocker.

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fication decreased from 2.5 ±0.5 to 1.5 ±0.5 ($p <$ 0.0001). The level of NT-proBNP decreased from 2733 ±2798 to 820 ±2318 ng/l ($p < 0.001$). A decrease of the number of infiltrating LCA+ cells from 22.5 ±10.5 to 15.8 ±18.6 cells/mm^2 ($p < 0.001$) and in the number of infiltrating CD3+ cells from 7.2 ±4.6 to 5.3 ±9.7 cells/mm^2 ($p < 0.01$) was observed.

Comparing the results in the group with viral genome clearance and the group with viral persistence, there was no statistically significant difference – i.e. improvement in LVEF of 12.0 ±11.4% vs. 18.3 ±12.6%, decrease in NYHA class of 0.7 ±0.7 vs. 1.0 ±0.7, decline in NT-proBNP of 1335 ±1933 ng/l vs. 1942 ±3242 ng/l, decrease in number of infiltrating LCA+ cells of 11.1 ±15.8 vs. 6.7 ±23.0 and CD3+ cells of 5.8 ±15.1 vs. 1.8 ±10.9 (all p = NS). All results are shown in Table II.

The most frequent virus in EMBs was PVB19: at the baseline it was present (isolated or in combination with other viruses) in 33 of all patients (72%), and the number of PVB19 positive patients decreased significantly to 23 (50%) at the time of the follow-up biopsy ($p < 0.05$). At the baseline, PVB19 load was 9.4 ±10.7 copies/μg DNA (range: 0.1–28.2); at the time of the follow-up biopsy, the PVB19 load increased to 43.0 ±89.4 copies/μg DNA (range: 0.1–386). There were no statistically significant changes in the presence of other detected viruses in the follow-up period. The viral genome distribution at the baseline and in the 6-month follow-up biopsy is shown in Figure 1.

In the group of patients treated with immunosuppressive therapy added to the standard one, viral genomes were detected at the baseline in 5 (63%) patients and in the follow-up biopsy in 4 (50%) patients. In one of them, we found the clearance of viral genome, while in 4 patients the virus persisted. The only detected virus was PVB19. PVB19 load was 12.0 ±8.2 copies/μg DNA (range: 1–20.4) at the baseline, and in the follow-up biopsy PVB19 load was 10.5 ±10.0 copies/μg DNA (range: 5–27.6) (Figure 2).

Discussion

Despite significant progress in the development of non-invasive methods such as nuclear magnetic resonance [24–27], the endomyocardial biopsy is still considered as the gold standard in diagnostics of myocarditis [28]. In addition to histological and immunohistochemical evaluation, PCR analysis is an integral part of bioptic samples' evaluation. In the past, enterovirus and adenovirus were considered as the most frequent etiology of viral myocarditis. Recently, there has been described a shift in viral spectrum [29]. Studies focused on bioptic diagnostics in patients with DCM revealed that PVB19 and HHV-6 have become the

most common viral pathogens found in myocardium [4, 5, 9, 10, 30]. These data are consistent with our previous study where PVB19 was present in 56% of all patients, and in 91% of all PCR positive patients [4].

According to our opinion, the significance of this study lies in the evaluation of biopsy samples not only at the baseline but also in a follow-up biopsy performed 6 months after the initial examination. Our previous study showed that a decrease in inflammatory infiltration in the myocardium is related to improvement in LVEF and NYHA class classification and a decrease in NT-proBNP levels [31]. In this study we focused on the evaluation of the change in viral presence and the potential impact of viral persistence on echocardiographic and laboratory parameters, and on the changes in the intensity of myocardial inflammation and functional status of patients. Recently published data showed the association between enterovirus persistence and poor long-term prognosis [17]. Similar results were presented in another study with a broader spectrum of viral pathogens, and here as well viral persistence was associated with worse left ventricle function [18]. Our study did not confirm these results. We found a significant reduction in inflammatory infiltration, improvement in LVEF and functional status in the 6-month follow-up – both in the group with viral genome clearance and the group with persistence of virus in myocardium. There were no statistically significant differences comparing these two groups. In this context, it is important to emphasize that as opposed to the previously mentioned studies [17, 18, 32], enterovirus was not identified in our group while PVB19 in low viral load was the dominating pathogen. Interestingly, an increase of viral load occurred in the group treated with the standard therapy of heart failure despite the decrease in the number of positive findings, which is probably caused by the small sample size. Another important fact is that at the time of previous studies [18] patients were not treated with the whole spectrum of currently available pharmacotherapy of heart failure as is commonly used today. In all the patients in our study, maximum effort to bring them to optimal heart failure treatment according to current guidelines was made [19, 20]. Our results showed that the viral persistence (importantly, without enterovirus presence), at least in short-term follow-up, is not associated with worsening of echocardiographic parameters and functional status.

Our investigation also involved a few patients on immunosuppression added to standard therapy of heart failure as part of a randomized clinical trial with immunosuppressive therapy in patients with inflammatory cardiomyopathy [21]. Results of two randomized clinical trials have shown the

Table II. Comparison of groups of patients according to viral genome changes in myocardium (treated with standard heart failure therapy only)

Parameter	Viral clearance after 6 months (n = 13)			Viral persistence after 6 months (n = 24)			P-value**
	Mean (SD)	Median (25th, 75th percentile)	P-value*	Mean (SD)	Median (25th, 75th percentile)	P-value*	
DD [mm]:							
Initial value	62.85 (6.466)	62.00 (59.00–65.00)		60.54 (7.813)	60.00 (57.50–64.00)		0.4531
After 6 months	57.08 (7.994)	56.00 (51.00–64.00)		55.13 (8.456)	54.50 (48.50–61.00)		0.5556
Difference	−5.77 (8.043)	−4.00 (−9.00 − −1.00)	0.0190	−5.42 (6.467)	−4.50 (−11.00 − −0.50)	0.0003	0.9619
DS [mm]:							
Initial value	54.62 (5.738)	54.00 (50.00–58.00)		52.63 (8.112)	53.00 (48.50–57.00)		0.4931
After 6 months	45.77 (8.278)	45.00 (39.00–51.00)		43.54 (9.632)	42.00 (36.50–50.00)		0.4351
Difference	−8.85 (7.809)	−8.00 (−13.00 − −7.00)	0.0015	−9.08 (7.840)	−10.00 (−16.50 − −1.50)	< 0.0001	0.7499
E/e´:							
Initial value	15.11 (10.039)	12.00 (10.25–15.50)		12.33 (6.105)	10.10 (8.12–16.00)		0.4103
After 6 months	9.90 (4.469)	8.33 (6.19–12.33)		8.65 (3.708)	7.84 (7.19–8.92)		0.4545
Difference	−5.21 (7.855)	−4.74 (−7.17 − 0.33)	0.0266	−3.67 (7.308)	−2.28 (−7.17 − 0.46)	0.0325	0.6685
e´ [cm/s]:							
Initial value	6.35 (1.962)	6.00 (5.00–8.00)		6.98 (2.428)	6.50 (5.00–8.50)		0.4976
After 6 months	7.15 (2.384)	6.00 (5.50–9.00)		7.29 (2.349)	7.00 (6.50–8.50)		0.7374
Difference	0.81 (2.594)	1.00 (0.50–2.00)	0.1384	0.30 (2.675)	1.00 (−2.00 − 2.00)	0.6359	0.7660
EDV [ml]:							
Initial value	198.92 (58.379)	194.00 (168.00–214.00)		187.42 (52.944)	178.50 (162.00–207.00)		0.5995
After 6 months	162.08 (54.758)	152.00 (124.00–205.00)		158.04 (54.209)	146.00 (116.00–190.50)		0.8237
Difference	−36.85 (55.754)	−33.00 (−43.00 − −9.00)	0.0225	−29.38 (40.493)	−31.00 (−57.50 − −4.00)	0.0011	0.8486
ESV [ml]:							
Initial value	143.15 (40.562)	142.00 (107.00–168.00)		140.04 (44.747)	144.50 (114.00–163.00)		0.9113
After 6 months	97.08 (47.019)	91.00 (65.00–121.00)		93.71 (46.844)	80.50 (58.00–120.50)		0.6558
Difference	−46.08 (43.904)	−50.00 (−57.00 − −36.00)	0.0034	−46.33 (41.418)	−49.00 (−79.00 − −11.00)	< 0.0001	0.8736
LVEF (%):							
Initial value	26.85 (8.640)	25.00 (20.00–30.00)		26.54 (7.401)	27.50 (20.00–33.00)		1.0000
After 6 months	38.85 (12.219)	35.00 (30.00–45.00)		44.88 (11.211)	47.00 (39.00–52.50)		0.0972
Difference	12.00 (11.453)	12.00 (5.00–20.00)	0.0039	18.33 (12.617)	20.00 (12.50–26.00)	< 0.0001	0.0822

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Table II. Cont.

Parameter	Viral clearance after 6 months (n = 13)			Viral persistence after 6 months (n = 24)			P-value**
	Mean (SD)	Median (25th, 75th percentile)	P-value*	Mean (SD)	Median (25th, 75th percentile)	P-value*	
RV [mm]:							
Initial value	33.69 (6.395)	34.00 (30.00–35.00)		31.96 (6.721)	33.00 (25.50–38.00)		0.6437
After 6 months	31.23 (3.539)	30.00 (29.00–34.00)		32.13 (5.169)	32.00 (28.00–37.00)		0.6316
Difference	−2.46 (5.532)	−1.00 (−4.00 − 0.00)	0.0938	0.17 (6.329)	1.00 (−4.00 − 3.50)	1.0000	0.2849
s´ [cm/s]:							
Initial value	5.96 (2.145)	6.00 (4.50–7.00)		5.52 (1.951)	5.00 (4.00–7.00)		0.5847
After 6 months	6.77 (2.315)	6.50 (5.00–7.50)		6.98 (2.046)	6.50 (6.00–8.00)		0.5303
Difference	0.81 (1.953)	0.00 (−0.50 − 2.00)	0.2842	1.52 (2.456)	1.50 (−0.50 − 3.50)	0.0072	0.2831
s'tri [cm/s]:							
Initial value	10.54 (2.757)	10.00 (8.00–11.00)		9.74 (1.888)	9.00 (9.00–11.00)		0.6030
After 6 months	12.31 (4.070)	14.00 (9.00–14.00)		12.17 (3.017)	12.00 (10.00–13.00)		0.8726
Difference	1.77 (3.609)	1.00 (0.00–4.00)	0.0898	2.48 (4.077)	2.00 (0.00–4.00)	0.0061	0.5392
TAPSE [mm]:							
Initial value	18.08 (4.078)	17.00 (16.00–19.00)		18.14 (4.443)	17.50 (15.00–21.00)		0.7995
After 6 months	20.23 (4.166)	22.00 (18.00–23.00)		21.63 (3.160)	21.50 (19.00–24.00)		0.5429
Difference	1.92 (5.017)	1.50 (−3.00 − 6.00)	0.1602	3.27 (5.633)	3.00 (−1.00 − 6.00)	0.0173	0.6001
NTproBNP [ng/l]:							
Initial value	1909.85 (1939.007)	1364.00 (640.00–2635.00)		2732.67 (2797.830)	1503.50 (799.00–4345.50)		0.5777
After 6 months	575.15 (689.309)	290.00 (178.00–630.00)		820.35 (2317.808)	161.00 (92.00–318.00)		0.0838
Difference	−1334.69 (1933.186)	−547.00 (−1549.00 − −383.00)	0.0002	−1942.04 (3242.159)	−1120.00 (−3709.00 − −365.00)	0.0004	0.5982
NYHA:							
Initial value	2.31 (0.663)	2.50 (2.00–2.50)		2.48 (0.500)	2.50 (2.00–3.00)		0.3997
After 6 months	1.62 (0.506)	1.50 (1.50–2.00)		1.52 (0.521)	1.50 (1.00–2.00)		0.5704
Difference	−0.69 (0.723)	−0.50 (−1.00 − −0.50)	0.0068	−0.96 (0.706)	−1.00 (−1.50 − −0.50)	< 0.0001	0.2504
LCA+ [cells/mm²]:							
Initial value	22.46 (14.700)	18.00 (16.00–21.00)		22.50 (10.467)	18.00 (16.00–24.50)		0.5851
After 6 months	11.31 (5.633)	10.00 (8.00–14.00)		15.83 (18.619)	12.00 (8.00–15.00)		0.4064
Difference	−11.15 (15.768)	−8.00 (−11.00 − −6.00)	0.0015	−6.67 (23.049)	−7.50 (−14.50 − −2.00)	0.0006	0.8735

Table II. Cont.

Parameter	Viral clearance after 6 months (n = 13)			Viral persistence after 6 months (n = 24)			P-value**
	Mean (SD)	Median (25th, 75th percentile)	P-value*	Mean (SD)	Median (25th, 75th percentile)	P-value*	
CD3+ [cells/mm²]:							
Initial value	8.85 (15.181)	4.00 (2.00–8.00)		7.17 (4.594)	7.00 (4.50–9.00)		0.2776
After 6 months	3.08 (2.565)	2.00 (1.00–4.00)		5.33 (9.734)	3.00 (2.00–4.00)		0.3172
Difference	−5.77 (15.117)	−2.00 (−3.00 − −1.00)	0.0435	−1.83 (10.901)	−3.00 (−7.00 − −0.50)	0.0013	0.3452

Difference = difference of value after 6 months and initial value. * P-value of Wilcoxon pair test for comparison of initial and 6-month parameters. **P-value Mann-Whitney test for comparison parameters between two groups of patients (viral clearance after 6 months vs. viral persistence after 6 months). DD – diastolic diameter of LV, DS – systolic diameter of LV, E/e´ – ratio of early diastolic velocities of LV filling and peak early diastolic velocity of mitral annulus, e´ –peak early diastolic velocity of mitral annulus movement, LVEF – left ventricular ejection fraction, EDV – end-diastolic volume of LV, ESV – end-systolic volume of LV, RV – right ventricle diameter, s´ – peak systolic velocity of mitral annulus movement, s´tri – peak systolic velocity of tricuspid annulus movement, TAPSE – tricuspid annulus plane systolic excursion, CD3+ – T-lymphocytes, LCA+ – mononuclear leukocytes.
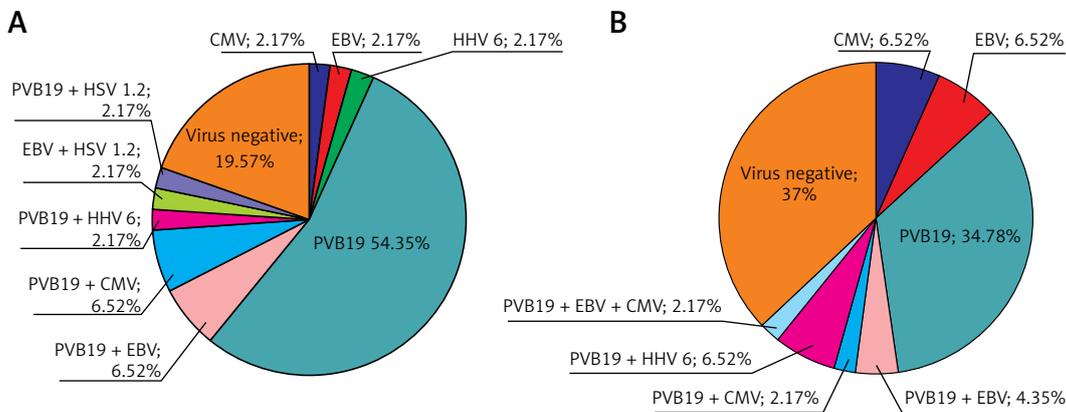

Figure 1. Viral genome distribution at baseline (**A**) and after 6 months (**B**) – standard heart failure therapy only
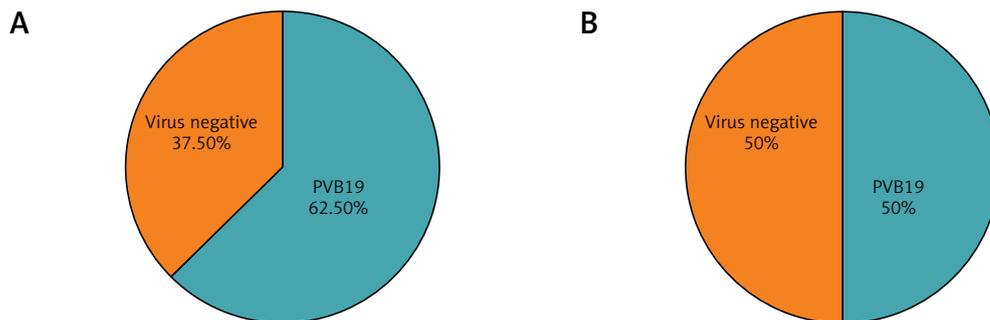

Figure 2. Viral genome distribution at baseline (**A**) and after 6 months (**B**) – immunosuppressive therapy added to standard heart failure therapy

benefit of administrating both immunosuppression therapy and standard heart failure medication in patients with chronic myocarditis [22, 33]. The TIMIC trial demonstrated the positive effect of immunosuppression on echocardiographic parameters in patients with myocardial inflammation and with absence of an infectious agent in the myocardium. In Frustaci's previous study, there was found no positive effect of immunosuppression in patients with viral presence [34]. But again, PVB19 was detected in only one patient in this study. In Wojnicz's study, the viral presence was not taken into consideration at all [33]. In our group of patients with immunosuppressive therapy, the only present virus was PVB19 in a low viral load, which was considered not to be able to create inflammation [35, 36]. We assumed that in this situation PVB19 is probably only an "inno-

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cent bystander" without any etiological relation to myocardial inflammation. We evaluated the change in viral presence after administration of immunosuppression therapy and compared it with the results of the group without immunosuppression. The only virus detected in the myocardium in this group was PVB19. To our best knowledge, such a study has never been published before. However, our pilot results from a small group of patients suggest that the administration of immunosuppression does not lead to a change in the viral presence or an increase in the viral load in follow-up biopsy samples.

The major limitation of this study is the low number of patients, which is particularly important in the group treated with immunosuppression, and which makes it impossible to perform a statistical analysis in this group of patients. We need to emphasize that in almost all positive bioptic findings PVB19 was present (and in all patients with immunosuppression). Because of contradictory opinions regarding its pathogenicity, it is not certain whether we can apply our findings to the presence of other viruses. In this respect, our pilot data provide new information about viral presence in patients with ICM that have not been published before. The proposed further follow-up of this group, as well as extending the number of patients, will bring more accurate information in the future. Better understanding of the role of viral persistence in the myocardium and the impact of immunosuppression could provide more precise prognostic stratification and thus contribute to more appropriate therapeutic decision making.

In conclusion, a decrease in the number of positive PCR findings in control EMB was observed. However, no significant difference was observed between the groups with viral clearance and with viral persistence in clinical and laboratory results or in the clinical development. Our results suggest that viral persistence did not affect further development of the disease in short-term follow-up.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

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