



# Clinical profile of patients with advanced age and inflammatory dilated cardiomyopathy on endomyocardial biopsy

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## Abstract

**Background** Endomyocardial biopsy (EMB) is an important tool when patients with inflammatory cardiomyopathy (DCMi) are evaluated. We aimed to assess the clinical profile of elderly patients with DCMi on EMB. **Methods** Retrospective study of all consecutive patients hospitalized from January 2007 to December 2011 with clinical suspicion of DCMi undergoing EMB. Patients with evidence of DCMi on EMB (Group 1  $\geq 70$  years,  $n = 85$ ; Group 3  $< 70$  years;  $n = 418$ ) were compared to patients of the same age group without evidence of DCMi on EMB (Group 2  $\geq 70$  years,  $n = 45$ ; Group 4  $< 70$  years;  $n = 147$ ). **Results** Among 24,275 patients treated at our institution during the study period, 695 had clinical suspicion of DCMi and underwent EMB; 503 (2.1%) patients had DCMi on EMB. There were more male patients in Group 1, mean age was  $74 \pm 2.8$  years, mean ejection fraction was  $38\% \pm 14\%$ . On presentation, signs of hemodynamic compromise (NYHA functional class III/IV, low cardiac output/index, and low cardiac power index) were more frequent in Group 1. EMB revealed viral genome in 78% of the patients, parvovirus B19 (PVB) was frequently encountered in both age groups (Group 1: 69.4% vs. Group 2: 59.6%); detection of more than one viral genome was more frequent in Group 1 (21.2% vs. 11.2%;  $P = 0.02$ ) whereas the extent of immune response was significantly lower in individuals with advanced age. **Conclusions** In patients  $\geq 70$  years with DCMi on EMB signs of hemodynamic compromise, detection of multiple viral genomes together with an overall lower extent of immune response were more frequently observed.

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**Keywords:** Advanced age; Clinical profile; Dilated cardiomyopathy; Endomyocardial biopsy; Inflammation factors

## 1 Introduction

Myocarditis/inflammatory cardiomyopathy (DCMi) is an inflammatory disease of the myocardium caused by different infectious and non-infectious triggers but often results from common viral infection and post-viral immune mediated responses.<sup>[1]</sup> In 1995, myocarditis was defined by the World Health Organization (WHO)/International Society and Federation of Cardiology (ISFC) as an inflammatory disease of the heart muscle, diagnosed by established histological, immunological, and immuno-histochemical criteria.<sup>[2]</sup>

With the development of new molecular techniques such as polymerase chain reaction (PCR) and *in situ* hybridiza-

tion, the spectrum of most frequently detected viruses in endomyocardial biopsies (EMB) shifted enteroviruses and adenovirus to mainly parvovirus B19 (PVB19) and human herpesvirus 6 (HHV 6).<sup>[3,4]</sup>

The aim of this study is to provide further information and understanding of the clinical characteristics of elderly patients ( $\geq 70$  years of age) with DCMi on EMB, and to compare those with (1): elderly patients without evidence of myocarditis on EMB, and (2): to compare younger patients ( $\leq 70$  years of age) with and without DCMi on EMB.

## 2 Methods

### 2.1 Study subjects

The study enrolled consecutive patients who underwent endomyocardial biopsy as part of an evaluation for clinically suspected myocarditis at our institution between January 2007 and December 2011. Patients were included if they experienced at least one of the following features not related to myocardial ischemia: impaired global or regional left ventricular systolic function, an increase in serum concen-

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trations of myocardial necrosis markers, pericardial effusion, or sustained or non-sustained ventricular tachycardia or ventricular fibrillation of unknown origin. Coronary artery disease as the cause of the reduced ejection fraction had to be excluded by means of coronary angiography before a patient was eligible to participate. All patients underwent a careful history and physical examination, as well as selected laboratory studies, including thyroid function testing and measurements of antinuclear antibodies. In each patient, left ventricular end-diastolic and end-systolic diameters were measured with 2-dimensionally guided M-mode echocardiography. The study was approved by the institutional ethics committee.

## 2.2 Cardiac catheterization and endomyocardial biopsy

Before endomyocardial biopsy, each patient underwent left heart catheterization with coronary angiography to exclude coronary artery disease. Left ventricular end-diastolic pressure was measured with standard fluid-filled catheters. Left ventricular ejection fraction was measured by contrast ventriculography in the 30° right anterior oblique and 60° left anterior oblique view. If renal failure or excessive end-diastolic pressures did not permit ventriculography, ejection fraction was estimated by echocardiography using the Teichholtz method. The biopsy sample sites (right *vs.* left ventricle, wall segment) were chosen according to the findings of echocardiography or magnetic resonance imaging of the heart with a 1.5-T Magnetom Sonata (Siemens Medical Solutions, Erlangen, Germany) to reduce the sampling error and to maximize the sensitivity and specificity of the method.<sup>[5]</sup> Biopsy specimens were taken with a dedicated biptome (B-18110-S; 4.5 mm<sup>3</sup>, Mohnheim, Germany) advanced through various 7F or 8F coronary guiding catheters (JR40/AL10/JL40, Medtronic, Danvers, Mass) to reach pre-specified regions of interest in the left and right ventricles. At least four biopsy specimens (median, *n* = 5) with a diameter of 1 to 3 mm were harvested immediately and under strictly sterile conditions: 2 to 3 biopsy specimens were fixed in 4% buffered formaldehyde for hematoxylin and eosin, Masson's trichrome, and Giemsa staining and performance of immunohistology; 2 to 3 cardiac tissue samples were quick-frozen or fixed in RNA later (Ambion Inc, Foster City, Calif) for PCR detection of viral genomes without a loss of sensitivity.<sup>[6,7]</sup> Biopsy specimens were investigated within 24 h.

## 2.3 Analysis of endomyocardial biopsies

Endomyocardial biopsy findings were classified in three ways: by histopathological analysis, by immunohistochemistry, and by the presence or absence of viral genomes.

## 2.4 Histopathological analysis

Histopathological examinations were done on 4 µm thick tissue sections from paraffin-embedded endomyocardial biopsies stained with hematoxylin and eosin, Masson's trichrome, and Giemsa and were examined by light microscopy. Histological analysis followed the Dallas criteria.<sup>[8]</sup> According to this classification, acute myocarditis is defined by lymphocytic infiltrates in association with myocyte necrosis. Borderline myocarditis is characterized by the presence of inflammatory infiltrates without microscopic signs of myocyte injury.

## 2.5 Immunohistochemistry

For immunohistological staining, paraffin-embedded tissue sections were treated with an avidin-biotin-immunoperoxidase method according to the manufacturer's protocol (Vectastain Elite ABC Kit, Vector, Burlingame, Calif). The following monoclonal antibodies were applied for identification, localization, and characterization of mononuclear cell infiltrates: CD3 for T cells (DAKO, Hamburg, Germany), CD 45RO clone UCHL1 for activated T-cells (DAKO, Hamburg, Germany), MAC 387 for macrophages and natural killer cells (Linares, Dossenheim, Germany), HLA-ABC clone w6/32, and HLA-DP clone CR3/43 (DAKO, Hamburg, Germany) to assess HLA class I or II expression in professional antigen-presenting immune cells, respectively. For assessment of CD54/ICAM clone 1304 (Biologo, Kronshagen, Germany) was used. The amount of endothelial or interstitial Human Leucocyte Antigen (HLA) activation was graded by the pathologist into five grades (1: low activation; 2: low-intermediate activation; 3: intermediate activation; 4: intermediate-strong activation; 5: strong activation).

## 2.6 Definition of myocarditis

According to the World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies, endomyocardial biopsies were considered to be inflamed after immunohistochemical detection of focal or diffuse mononuclear infiltrates with > 14 Leucocytes per 1 mm<sup>2</sup> (CD3 pos T lymphocytes and/or CD68 pos macrophages) in the myocardium, in addition to enhanced expression of HLA class II molecules.<sup>[1]</sup>

## 2.7 Molecular biological detection of viral genomes

Enterovirus species (comprising coxsackieviruses and echoviruses), parvovirus B19, adenoviruses, and human herpes virus type 6 were evaluated by nested PCR/RT-PCR from deep-frozen or RNA later-fixed endomyocardial biopsy specimens as described.<sup>[7]</sup> For RT-PCR analyses, RNA

was transcribed into cDNA by RT according to the protocol of the manufacturer (AGS, Heidelberg, Germany). The enzymatic amplification of cDNA respectively DNA was performed as nested PCR on a Perkin-Elmer GeneAmp PCR System 9600 (Applied Biosystems, Weiterstadt, Germany) in two 30-cycle programs. As an internal control for successful isolation of nucleic acids, the housekeeping gene GAPDH was detected by PCR. A biopsy was considered positive for viral infection if the viral genome was detected by PCR, and specificity was confirmed by automatic DNA sequencing of viral amplification products.

## 2.8 Statistical analysis

Continuous variables were reported as mean  $\pm$  SD or median and interquartile ranges (25<sup>th</sup>–75<sup>th</sup> percentiles) where appropriate. Categorical variables were presented as absolute (*n*) and relative (%) frequencies. Normal distribution of variables was assessed using the D'Agostino-Pearson omnibus normality test. Comparisons of continuous variables were made with the appropriate two-sample test; Student-t-test in cases where the variable was normally distributed. Otherwise, the Kruskal-Wallis test was used. A probability value of  $P < 0.05$  was considered to be statistically significant. Statistical analysis was performed using the GraphPad Prism version 6.02 for windows (GraphPad Software, La Jolla, California, USA).

## 3 Results

### 3.1 Patient population

During the study period 24,275 patients were treated in our institution, 695 (2.8%) of them underwent endomyocardial biopsy for clinically suspected DCMi and in 503 patients this suspicion could be confirmed [incidence of DCMi: 503/24,275 (2.1%)]. Among the 503 patients, 85 (16.9%) were  $\geq 70$  years and represented study Group 1, and 418/503 (83.1%) patients younger than 70 years represented the second study group (Group 3).

The 192 patients without evidence of DCMi on EMB served as controls [45/192 patients were  $\geq 70$  years (Group 2); 147/192 were younger than 70 years (Group 4)].

Group 1 compared to Group 2 patients were more frequently females, presented more frequently with dyspnoea grade 3 and 4 according to the NYHA classification, and presented less frequently with severe anginal symptoms. Group 3 compared to Group 4 patients had several significant differences of baseline characteristics (prevalence of hypertension, smoking status, and prior ICD implantation). On clinical presentation, Group 3 patients had significantly

more frequent fever, severe anginal symptoms, and signs of hemodynamical compromise with lower blood pressure measurements and higher incidence of pulmonary edema. The baseline data and characteristics at presentation of the four groups are found in Table 1.

In Table 2 laboratory values, electrocardiographic, echocardiographic, and data from cardiac catheterization and magnet resonance imaging (MRI) parameters are presented. Group 1 patients had more frequently higher serum levels of creatinine and creatinine kinase compared to Group 2 patients. Cardiac performance parameters assessed by cardiac catheterization (Cardiac output-index and cardiac power index) were significantly lower in elderly patients with DCMi (Group 1) compared to elderly patients without DCMi (Group 2). When Group 3 compared to Group 4 patients, almost no significant differences could be demonstrated. Only the end-systolic and end-diastolic volume indices on MRI were significantly larger in younger patients with DCMi.

Brain natrium peptide levels were higher on admission in the DCMi positive groups (Group 1 and 3) and corresponded in Group 1 with the higher incidence of hemodynamic compromise; however, this was not statistically significant. The results of the MRI studies regarding the DCMi diagnosis in our cohort of patients was un-sufficient as the frequency of positive studies was almost similar in the groups with or without DCMi on EMB.

### 3.2 Endomyocardial biopsy

The biopsy sample site was usually (in about 77%) the septum of the right ventricle. All other biopsies were taken from the left ventricle, and the left ventricular sampling site was chosen according to the findings of echocardiography or MRI of the heart to reduce sampling error. We never performed simultaneous left and right ventricular biopsies (Table 2). Histopathological examination of endomyocardial biopsies according to the Dallas criteria was positive in 265 (38.1%) patients, with the majority ( $n = 258$ ) of findings indicating borderline myocarditis. There were no significant differences in the positive Dallas criteria findings between Group 1 and Group 3 patients ( $P = 0.38$ ). Positive findings were more frequent after immuno-histochemical staining, which revealed significant inflammatory cellular infiltrates in the specimens of 432 subjects (85.9%).

Viral genome was detected in the myocardium of 67/85 Group 1 subjects (78.8%) compared to 315/418 Group 3 subjects (75.4%;  $P = 0.58$ ), respectively. Detection of more than one viral genome was significantly more frequent in Group 1 patients (21.2% vs. 11.2%;  $P = 0.02$ ), and co-detec-

**Table 1. Baseline characteristics and clinical presentation.**

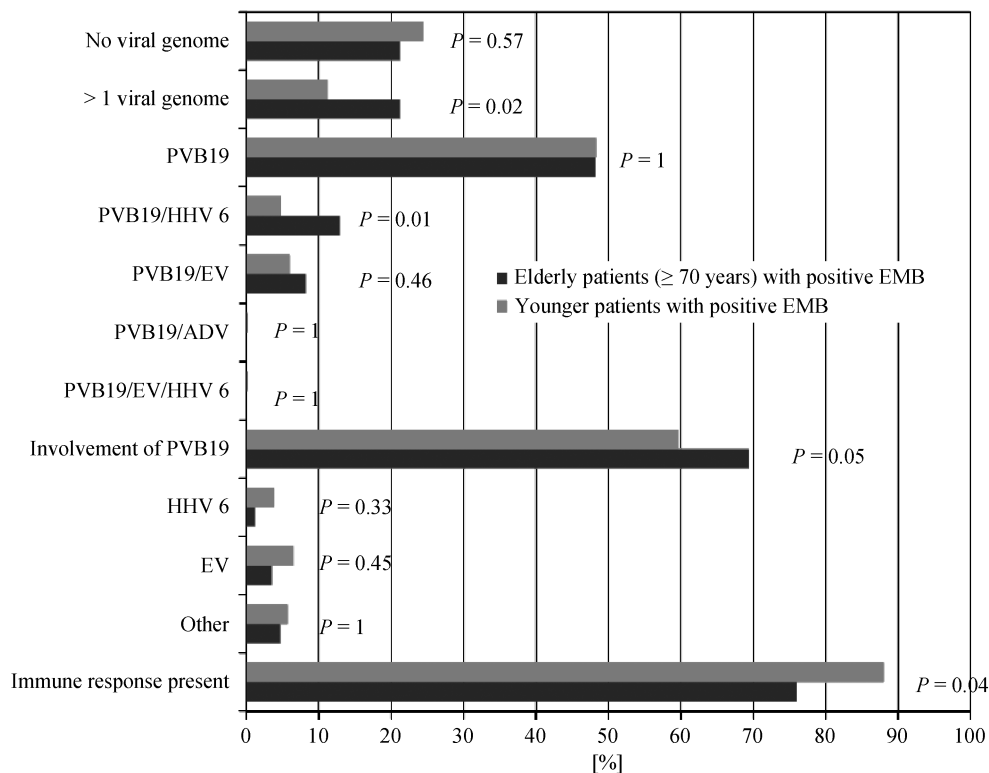
	Patients ≥ 70 years		P	Patients < 70 years		P
	Group 1 DCMi positive; n =85	Group 2 DCMi negative; n =45		Group 3 DCMi positive; n =418	Group 4 DCMi negative; n =147	
<b>Baseline characteristics</b>						
Age, yrs						
Mean ± SD	74 ± 2.8	74 ± 2.9	0.82	53 ± 13	54 ± 12	0.35
Median (IQR)	74 (72–76)	73 (71–76)		56 (45–62)	55 (47–65)	
Males	55/85 (64.7%)	37/45 (82.2%)	<b>0.004</b>	307/418 (73.4%)	108/147 (73.5%)	1.0
Coronary artery disease	7/85 (8.2%)	2/45 (4.4%)	0.50	14/417 (3.4%)	5/147 (3.4%)	1.0
Prior coronary artery bypass grafting	1/85 (1.2%)	0/45 (0.0%)	1.0	5/417 (1.2%)	0/147 (0.0%)	1.0
Prior percutaneous coronary intervention	5/85 (5.9%)	1/45 (2.2%)	0.66	7/418 (1.7%)	3/147 (2.0%)	0.73
History of myocardial infarction	7/85 (8.2%)	1/45 (2.2%)	0.26	29/418 (6.3%)	6/147 (4.1%)	0.32
Hypertension	74/85 (87.1%)	39/45 (86.7%)	1.0	248/416 (59.6%)	97/147 (66.0%)	<b>0.006</b>
Diabetes	38/84 (44.7%)	18/45 (40.0%)	0.58	94/416 (22.6%)	35/147 (23.8%)	0.82
Hyperlipidemia	46/84 (54.8%)	21/45 (46.7%)	0.46	159/416 (38.2%)	63/147 (42.9%)	0.92
Tobacco use						
Active	5/83 (6.0%)	5/45 (11.1%)	0.32	118/415 (28.4%)	36/147 (24.5%)	0.39
Former	29/83 (34.9%)	20/45 (44.4%)	0.34	134/415 (32.3%)	40/147 (27.2%)	0.30
Never	49/83 (59.0%)	20/45 (44.4%)	0.14	163/415 (39.3%)	71/147 (48.3%)	<b>0.004</b>
Prior pacemaker implantation	9/85 (10.6%)	4/45 (8.9%)	1.0	11/417 (2.6%)	4/147 (2.7%)	1.0
Prior ICD implantation	2/85 (2.4%)	0/45 (0.0%)	0.54	5/416 (1.2%)	6/147 (4.1%)	<b>0.004</b>
ICD-Shocks	1/85 (1.2%)	0/45 (0.0%)	1.0	2/417 (0.5%)	5/147 (3.4%)	<b>0.002</b>
Prior cardiac resynchronisation therapy	0/85 (0.0%)	0/40 (0.0%)	1.0	3/417 (0.7%)	1/147 (0.7%)	1.0
Peripheral artery disease	4/85 (4.7%)	2/45 (4.4%)	1.0	7/417 (1.7%)	5/147 (3.4%)	0.31
Prior transient ischemic attack or stroke	9/85 (10.9%)	5/45 (11.1%)	1.0	13/417 (3.1%)	6/147 (4.1%)	0.59
Chronic obstructive pulmonary disease	13/85 (15.3%)	8/45 (17.8%)	0.80	46/417 (11.0%)	15/147 (10.2%)	0.88
<b>Clinical presentation</b>						
Duration of symptoms, days						
Mean ± SD	80 ± 137	78 ± 114	0.93	87 ± 175	87 ± 168	0.94
Median (IQR)	30 (4.5–90)	30 (2.5–90)		28 (2–120)	21 (2–90)	
Fever	11/85 (12.9%)	5/45 (11.1%)	1.0	78/416 (18.8%)	16/147 (10.9%)	<b>0.003</b>
Acute coronary syndrome	22/85 (25.9%)	8/45 (17.8%)	0.38	104/418 (24.9%)	37/147 (25.2%)	0.87
CCS functional class						
I/II	26/85 (30.6%)	14/45 (31.1%)	1.0	88/418 (21.1%)	41/147 (27.9%)	0.11
III/IV	20/85 (23.5%)	21/45 (46.7%)	<b>0.009</b>	109/418 (26.1%)	24/147 (16.3%)	<b>0.002</b>
NYHA functional class						
I/II	23/85 (27.1%)	9/45 (20.0%)	0.40	123/418 (29.4%)	37/147 (25.2%)	0.34
III/IV	51/85 (60.0%)	8/45 (17.8%)	<b>&lt; 0.001</b>	182/418 (43.5%)	64/147 (43.5%)	1.0
Fatigue	32/85 (37.7%)	11/44 (25.0%)	0.17	141/416 (33.9%)	42/146 (28.8%)	0.31
Pulmonary edema	10/85 (11.8%)	6/45 (13.3%)	0.79	51/417 (12.2%)	10/147 (6.8%)	<b>0.009</b>
Syncope	6/85 (7.1%)	4/45 (8.8%)	0.74	25/417 (6.0%)	12/147 (8.2%)	0.34
Body-mass-index, kg/m <sup>2</sup>						
Mean ± SD	29 ± 4.9	28 ± 5.6	0.45	29 ± 5.3	28 ± 5.0	0.68
Median (IQR)	28 (26-32)	28 (26-31)		28 (25-31)	28 (25-31)	
Blood pressure, mmHg						
Systolic	138 ± 20	139 ± 20	0.93	132 ± 22	137 ± 24	<b>0.009</b>
Diastolic	80 ± 11	80 ± 12	0.69	80 ± 12	82 ± 14	<b>0.005</b>
Mean	98 ± 12	99 ± 14	0.91	96 ± 14	100 ± 16	<b>0.002</b>
Resuscitation	3/85 (3.5%)	2/45 (4.4%)	1.0	13/417 (3.1%)	5/147 (3.4%)	0.79
Peripheral edema	24/85 (28.2%)	12/45 (26.7%)	1.0	72/416 (17.3%)	28/147 (19.1%)	0.62
Jugular venous distension	2/85 (2.4%)	1/45 (2.2%)	1.0	6/416 (1.4%)	3/147 (2.0%)	1.0
Rales	20/84 (23.8%)	5/43 (11.6%)	0.16	41/412 (10.0%)	22/146 (15.1%)	0.09

CCS: Canadian Cardiovascular Society; DCMi: inflammatory cardiomyopathy; ICD: implantable cardioverter defibrillator; IQR: interquartile range; NYHA: New York Heart Association.

**Table 2. Laboratory values, resting ECG, and echocardiography, cardiac catheterization, and magnet resonance imaging data.**

	Patients ≥ 70 years		P	Patients < 70 years		P
	Group 1 DCMi positive; n = 85	Group 2 DCMi negative; n = 45		Group 3 DCMi positive; n = 418	Group 4 DCMi negative; n = 147	
<b>Laboratory on admission</b>						
Potassium, mmol/L	4.2 ± 0.5	4.3 ± 0.6	0.45	4.2 ± 0.5	4.2 ± 0.6	0.85
Creatinin, mg/dL	118 ± 75	105 ± 41	<b>0.001</b>	95 ± 52	94 ± 31	0.67
C-reactive protein, mg/dL	12 ± 19	7.5 ± 10	0.59	14 ± 29	15 ± 32	0.77
Creatinase, × 10 <sup>3</sup> U/L	8.0 ± 20	3.2 ± 2.7	<b>0.02</b>	3.8 ± 4.5	3.2 ± 3.3	0.23
CK-MB, U/L	2.0 ± 3.4	6.4 ± 6.2	0.28	4.4 ± 11	6.7 ± 14	0.41
BNP, pg/mL						
Mean ± SD	8896 ± 10395	5101 ± 11014	0.41	5268 ± 11014	2124 ± 2147	0.40
Median (IQR)	5280 (1556–14142)	5101 (356–5520)		1358 (356–5520)	1320 (308–4636)	
Hemoglobin, g/dL	8.4 ± 1.3	8.9 ± 6.8	<b>&lt; 0.001</b>	9.0 ± 1.1	9.0 ± 1.1	0.37
White blood cell, × 10 <sup>3</sup> /μL	8.3 ± 2.5	8.5 ± 2.7	0.36	8.6 ± 3.0	8.7 ± 3.0	0.92
Troponin, ng/mL	2.8 ± 7.1	2.9 ± 9.0	0.97	2.8 ± 9.7	1.2 ± 2.7	0.16
Troponin positive	18/33 (54.6%)	7/12 (58.3%)	1.0	83/155 (53.6%)	30/59 (50.9%)	0.76
<b>Electrocardiogram</b>						
Sinusrhythm	62/85 (72.9%)	31/45 (68.9%)	0.69	341/418 (81.6%)	120/140 (85.7%)	0.30
AV-Block	0/85 (0%)	0/34 (0.0%)	1.0	5/376 (1.3%)	1/147 (0.7%)	1.0
Heart rate at admission, min <sup>-1</sup>	76 ± 15	77 ± 18	0.66	78 ± 22	80 ± 18	0.43
PQ-interval, s	0.18 ± 0.035	0.18 ± 0.03	0.89	0.17 ± 0.03	0.15 ± 0.015	0.36
QRS-width, s	0.13 ± 0.013	0.15 ± 0.013	0.56	0.11 ± 0.013	0.12 ± 0.013	0.77
QT-interval, s	0.40 ± 0.044	0.41 ± 0.048	0.52	0.39 ± 0.043	0.38 ± 0.041	0.56
ST-segment alterations	32/79 (40.5%)	23/42 (54.8%)	0.18	145/405 (35.8%)	49/138 (35.5%)	1.0
Negative T-wave	41/79 (51.9%)	28/42 (66.7%)	0.13	214/408 (52.5%)	83/138 (60.1%)	.14
<b>Echocardiography</b>						
Ejection fraction, %	38 ± 14	37 ± 14	0.69	39 ± 15	39 ± 15	0.91
LA diameter, mm	44 ± 7.8	42 ± 5.7	0.17	42 ± 2.4	41 ± 6.5	0.68
LVEDD, mm	56 ± 7.6	57 ± 9.9	0.68	57 ± 9.1	56 ± 8.6	0.16
LVESD, mm	45 ± 11	49 ± 9.6	0.23	45 ± 11	44 ± 13	0.43
IVS, mm	12 ± 2.4	12 ± 3.8	0.85	11 ± 2.8	11 ± 3.1	0.47
PW, mm	12 ± 2.6	13 ± 4.2	0.18	11 ± 2.5	11 ± 2.2	0.71
RV-pressure, mmHg	34 ± 13	34 ± 9.1	0.99	29 ± 9.8	29 ± 14	0.74
Pericardial effusion	6/78 (7.7%)	1/40 (2.5%)	0.42	39/371 (10.5%)	10/123 (8.1%)	0.49
Valve dysfunction (> trivial)	19/85 (22.4%)	13/45 (28.9%)	0.75	56/418 (13.4%)	27/147 (18.4%)	0.12
Intracardiac thrombus	3/73 (4.1%)	0/38 (0.0%)	0.55	10/354 (2.8%)	3/117 (2.6%)	1.0
<b>Cardiac catheterization</b>						
LVEDP, mmHg	20 ± 7.1	20.0 ± 8.5	0.98	19 ± 8.2	20 ± 8.9	0.72
PCWP, mmHg	20 ± 9.1	20.0 ± 9.4	1.0	19 ± 8.9	19 ± 8.9	0.94
PA, mmHg	28 ± 11	27.0 ± 10	0.68	26 ± 11	28 ± 11	0.37
RV, mmHg	41 ± 14	42.0 ± 14	0.58	38 ± 15	40 ± 15	0.54
RA, mmHg	7.3 ± 5.4	6.8 ± 4.9	0.57	6.9 ± 4.5	7.2 ± 5.2	0.71
Cardiac output, L/min	4.0 ± 1.1	4.4 ± 1.1	<b>0.009</b>	4.1 ± 1.2	3.9 ± 1.1	0.59
Cardiac index, (L/min)/m <sup>2</sup>	2.0 ± 0.54	2.2 ± 0.47	<b>0.004</b>	2.0 ± 0.6	2.0 ± 0.53	0.79
Cardiac power index, W/m <sup>2</sup>	0.44 ± 0.13	0.49 ± 0.12	<b>0.002</b>	0.43 ± 0.14	0.44 ± 0.14	0.39
Pulmonary vascular resistance [dyn × s/m <sup>3</sup> ]	215 ± 144	195 ± 150	0.60	193 ± 141	209 ± 149	0.49
Right ventricular biopsy	69/85 (81.2%)	35/45 (77.8%)	0.65	325/418 (77.8%)	103/147 (70.1%)	0.07
<b>Magnetic resonance imaging</b>						
EF, %	40 ± 18	42 ± 19	0.79	43 ± 14	43 ± 16	0.85
LVESD, mm	45 ± 21	47 ± 17	0.70	46 ± 14	43 ± 13	0.23
LVESVI, mL/m <sup>2</sup>	119 ± 71	181 ± 114	0.23	136 ± 80	89 ± 82	<b>0.009</b>
LVEDD, mm	55 ± 17	57 ± 13	0.61	59 ± 12	57 ± 13	0.31
LVEDVI, mL/m <sup>2</sup>	198 ± 117	256 ± 133	0.20	219 ± 94	173 ± 97	<b>0.004</b>
MRI positive for myocarditis	8/31 (25.8%)	9/16 (56.3%)	0.12	90/195 (46.2%)	25/69 (36.2%)	0.16
Late gadolinium enhancement	8/32 (25.0%)	8/16 (50.0%)	0.11	86/195 (44.1%)	22/69 (31.9%)	0.09
Myocardial edema	3/30 (10.0%)	4/15 (26.7%)	0.20	36/181 (19.9%)	7/66 (10.6%)	1.0
Pericardial effusion	6/21 (28.6%)	3/15 (20.0%)	0.71	37/150 (24.7%)	15/58 (25.9%)	1.0
Intracardiac thrombus	1/20 (5.0%)	0/14 (0.0%)	1.0	2/130 (1.4%)	1/55 (1.8%)	0.98

AV: atrio-ventricular; BNP: brain natriuretic peptide; CK-MB: creatin kinase-MB; DCMi: inflammatory cardiomyopathy; EF: ejection fraction; LA: left atrium; LVEDD: left ventricular end-diastolic diameter; LVEDVI: left ventricular end-diastolic volume index; LVESD: left ventricular end-systolic diameter; LVESVI: left ventricular end-systolic volume index; IQR: interquartile range; IVS: interventricular septum; PW: posterior wall; RV: right ventricle.



**Figure 1. Distribution of viral genome on endomyocardial biopsy specimen.** ADV: adenovirus; EMB: endomyocardial biopsy; EV: enterovirus; HHV 6: human herpes virus type 6; PVB 19: parvovirus B19.

tion of parvovirus B19 prevailed (Group 1: 69.4% vs. 59.6%;  $P = 0.05$ ). Parvovirus B19 was also the most frequent virus in both groups. The most frequent combination of myocardial co-infection in both groups was parvovirus B19 and human herpesvirus type 6 (12.9% vs. 4.8%, Group 1 vs. Group 3;  $P = 0.01$ ). Additionally Group 1 patients had a significant lower immune response ( $>$  grade 1/5) compared to Group 3 patients (Figure 1).

## 4 Discussion

Our study represents a large data-set evaluating the clinical characteristics of patients with advanced age and DCMi on endomyocardial biopsy. In particular, the relevant findings of this study are (1) the presentation of patients with DCMi and advanced age is clinically more severe (NYHA functional class, more frequently signs of hemodynamic impairment [low cardiac output/index, low cardiac power index, and higher BNP levels]); (2) overall, the results of laboratory and/or instrumental tests did not differ significantly between patients with or without DCMi; and (3) on endomyocardial biopsy, the immunohistologic findings and the spectrum of viral genome significantly differed between older and younger age groups.

### 4.1 Patients characteristics

The initial clinical suspicion of viral DCMi is usually based on history, physical examination, and altered laboratory values such as troponin or elevated white blood cell count. The classical symptom of fever in our series was even lower than published in the literature,<sup>[9]</sup> and only differed between younger DCMi patients compared to younger patients without DCMi. Also, neither the number of troponin positive patients nor the level of troponin elevation differed significantly between the groups. Laboratory values indicating inflammation (CRP of white blood cell count) did not differ between the groups with or without DCMi on EMB. This can be partly explained by the duration of symptoms in our cohort (mean  $> 80 \pm 137$  days) which indicates a very mixed patient population with various stages of acute and more chronic phases of the disease. However, the clinical presentation of patients with advanced age was more severe with higher prevalence of NYHA functional class grade III/IV, signs of hemodynamic compromise, and higher BNP levels. As the NYHA functional class is a well recognized risk factor for worse prognosis in patients with DCMi,<sup>[10]</sup> this result of our study may be of importance.

### 4.2 Diagnosis of patients with myocarditis or inflammatory cardiomyopathy

The electrocardiogram (ECG) is widely used as a

screening tool despite low sensitivity and specificity.<sup>[1,11]</sup> The ECG findings in patients with myocarditis vary from non-specific T-wave and ST-segment changes to ST-segment elevation mimicking an acute myocardial infarction,<sup>[1,12]</sup> but were not significantly different among the four groups of younger and older patients in our series. The prognostic role of ECG parameters was investigated in patients with suspected myocarditis.<sup>[13]</sup> The ECGs recorded at the time of EMB were related to cardiac outcome during long-term follow-up. A QTc prolongation at 440 ms, an abnormal QRS axis, and ventricular ectopic beats were associated with poor clinical outcome. A prolonged QRS duration of 120 ms was found to be an independent predictor for cardiac death or heart transplantation. In our study, Group 1 and three patients both the QRS-width and the QT-interval were not significantly prolonged compared to the control groups of patients without DCMi.

In general, there are no specific echocardiographic features of DCMi. However, echocardiography allows the evaluation of cardiac chamber sizes and wall thickness as well as systolic and diastolic functions in patients with myocarditis.<sup>[1]</sup> The assessment of several echocardiographic parameters in our patients demonstrated reduced ejection fraction, dilated cardiac chambers [more pronounced of the left atrium and the left ventricular end-systolic diameter (LVESD) rather than the left ventricular end-diastolic diameter (LVEDD)]. Pericardial effusion and intracardiac thrombus was noted in less than 10% of the cases.

Cardiovascular magnetic resonance (CMR) in suspected DCMi can localize and quantify tissue injury, including edema, hyperaemia, and fibrosis.<sup>[14]</sup> In a series of 82 patients with myocarditis all of whom had biopsy-proven disease, CMR alone made the correct diagnosis in 80% (66 out of 82) cases.<sup>[5,15]</sup> However, both T2- and T1-weighted imagings are needed to achieve optimal sensitivity and specificity, and in contrast to older reports, CMR abnormalities do not correlate closely with EMB evidence of myocarditis.<sup>[16]</sup> When two or more of the three 'Lake Louise' criteria are positive, myocardial inflammation can be predicted with a diagnostic accuracy of 78%; if only delayed, post-gadolinium enhancement imaging is performed, the diagnostic accuracy drops to 68%.<sup>[14]</sup> However, the data on the encouraging diagnostic performance of CMR were derived in centers with extensive experience in DCMi diagnosis with this method. The sensitivity and specificity of CMR in the setting of DCMi is significantly lower in less experienced centers (43% and 65%, respectively).<sup>[17]</sup> Although the overall experience with MRI in our center is considerably high (> 14,000 studies annually of all body regions), the "heart specific" expertise is obviously not sufficient enough to obtain

adequate sensitivity and specificity to diagnose or rule out DCMi.

### 4.3 Endomyocardial biopsy

Confirmation of myocarditis still requires histological or immunohistological evidence of inflammation in heart tissue. Endomyocardial biopsy can be performed with a very low major complication rate when performed by experienced operators.<sup>[15,18]</sup> In experienced hands, left ventricular biopsy (which was performed more frequently in the younger patient population) is as safe as right ventricular biopsy.<sup>[16]</sup> The co-infection of PVB19 and HHV 6 genome as well as PVB19 involvement was significantly more prevalent in biopsies of patients with advanced age. In contrast to enteroviruses, which primarily infect and injure cardiomyocytes, PVB19 and HHV 6 infect the vascular endothelial cells.<sup>[15,19-21]</sup> Both viruses may reside asymptotically either in the bone marrow (PVB19) or endothelial cells and cardiomyocytes (HHV 6),<sup>[22]</sup> it has been suggested that often HHV 6 rather enhances the pathogenicity of other viruses than being a pathogen itself.<sup>[15]</sup> Both viruses become frequently reactivated especially in the setting of immunodeficiencies (acquired or drug induced) or in patients with autoimmune disorders.<sup>[15]</sup> Substantially, age-associated abnormalities in the function of neutrophils, macrophages<sup>[23]</sup> and B-cells<sup>[24]</sup> are described in the literature, although the observed abnormalities seem to be selective as certain signaling pathways and functions appear to be preserved in advanced age.<sup>[23]</sup> So the significantly lower immune response in myocardial tissue, the high prevalence of PVB19 and HHV 6 genome, and frequent presence of multiple viral genomes in patients with advanced age in our series might be explained, at least in part, by these facts.

Several limitations of the study merit further discussion. First, this study is subject to limitations inherent in retrospective studies. Second, the likelihood for patients with advanced age to undergo invasive diagnostic procedures like myocardial biopsy is probably lower which might introduce selection bias in this group.

In conclusion, this study further supports that in general medical history, clinical presentation, and results of non-invasive tests did not differ between DCMi positive patient groups on EMB with advanced/younger age and DCMi negative controls. However, the presentation of patients with DCMi and advanced age was clinically more severe in our cohort and the immunohistologic findings as well as the spectrum of viral genome was significantly different compared to younger patients. By recognizing these facts, clinicians can evaluate and work more effectively to ensure that treatment provided to individuals with myocarditis/DCMi covers their individual needs.

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