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ORIGINAL PAPER

Predictors of outcome in patients with parvovirus B19 positive endomyocardial biopsy

Simon Greulich[1] · Ingrid Kindermann[2] · Julia Schumm[1,3] · Andrea Perne[4] · Stefan Birkmeier[1] · Stefan Grün[1] · Peter Ong[1] · Tim Schäufele[1] · Karin Klingel[5] · Steffen Schneider[6] · Reinhard Kandolf[5] · Michael Böhm[2] · Udo Sechtem[1] · Heiko Mahrholdt[1]

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Abstract
Objective Primary objective was to establish the prognostic value of the myocardial load of PVB19 genomes in patients presenting for work-up of myocarditis and/or unclear cardiomyopathy in comparison to clinical, and CMR parameters.
Methods 108 consecutive patients who underwent EMB because of suspected myocarditis and/or unclear cardiomyopathy, and had evidence of myocardial PVB19 genome, were enrolled. Primary endpoint was all-cause mortality; secondary endpoint was a composite of cardiac mortality and hospitalization for heart failure.
Results Mean LV-EF was 40 %. We found $n = 27$ patients to have a viral load ≥500 GE (IQR 559–846), $n = 72$ had 100–499 GE, and $n = 9$ had <100 GE. Immunohistology revealed chronic myocarditis in $n = 66$, acute myocarditis in $n = 1$, DCM in $n = 17$, PVB19 genome only in $n = 13$, and other pathologies in n = 11. During follow-up 11 patients died, two suffered SCD but were successfully shocked by ICD, and 21 were hospitalized for heart failure. Interestingly, not the viral load, but functional parameters such as LV-EF, LV-EDV (endpoint 2), as well as the histologic diagnosis of DCM and the presence of LGE (for all endpoints) reached statistical significance. In fact, the presence of LGE yields an odds-ratio for a lethal event of 8.56 (endpoint 1), and of 5.52 for endpoint 2. No patient with normal LV-EF, or the absence of LGE, suffered cardiac death during long-term follow-up.
Conclusion The viral load of PVB19 genomes in the myocardium is not related to the long-term outcome. Furthermore, this study suggests a growing role of imaging for risk stratification in non-ischemic myocardial disease.

Keywords Cardiovascular magnetic resonance · PVB19 · Myocarditis · Prognosis · Mortality

Abbreviations

CAD	Coronary artery disease
CMR	Cardiovascular magnetic resonance
DCM	Dilated cardiomyopathy
ECG	Electrocardiogram
GE	Genome equivalents per microgram of isolated nucleic acids
HR	Hazard ratio
ICD	Implantable cardioverter-defibrillator
IQR	Interquartile range
LGE	Late gadolinium enhancement
LV	Left ventricle
LV-EDV	Left ventricular end-diastolic volume
LV-EF	Left ventricular ejection fraction
LV-ESV	Left ventricular end-systolic volume
PVB19	Parvovirus B19
SCD	Sudden cardiac death

✉ Heiko Mahrholdt
Heiko.Mahrholdt@rbk.de

Simon Greulich
simon.greulich@rbk.de

[1] Division of Cardiology, Robert-Bosch-Medical Center, Auerbachstrasse 110, 70376 Stuttgart, Germany

[2] Universitätsklinikum des Saarlandes, Klinik für Innere Medizin III, Kardiologie, Angiologie und Internistische Intensivmedizin, Homburg/Saar, Germany

[3] Division of Cardiology, Horst-Schmidt-Kliniken, Wiesbaden, Germany

[4] Department of Medicine II, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany

[5] Universitätsklinikum Tübingen, Abteilung für Molekulare Pathologie, Tübingen, Germany

[6] Institut für Herzinfarktforschung Ruhr, Essen, Germany

Introduction

Viral myocarditis is a common cardiac disease that is identified in up to 9 % of post-mortem examinations [1, 2]. It appears to be a major cause of sudden, unexpected death, and may progress to dilated cardiomyopathy [3].

Several groups recently described possible predictors of clinical outcome in viral myocarditis and dilated cardiomyopathy, including clinical parameters [4], cardiovascular MR [5], myocardial inflammation [4], and the presence of viral genomes in the myocardium [6]. Nevertheless, risk stratification in these patients remains a difficult business [7], and especially the role of PVB19 genomes in the myocardium continues to be controversial [6, 8].

Consequently, the primary objective of this study was to establish the prognostic value of the myocardial load of PVB19 genomes in patients presenting for endomyocardial biopsy work-up of myocarditis and/or unclear non-ischemic cardiomyopathy in comparison to clinical and cardiovascular MR parameters. Specifically, we sought to identify the best predictors for adverse events in this patient group during a long-term follow-up.

Methods

Patient population

One-hundred-eight consecutive patients presenting at any of the participating institutions between January 2007 and June 2011 for endomyocardial biopsy work-up of suspected myocarditis and/or unclear non-ischemic cardiomyopathy (all comers) were enrolled in the long-term follow-up if they fulfilled the following criteria: (1) presence of PVB19 genome in the myocardium, AND (2) coronary artery disease ruled out by invasive angiography, AND (3) successfully underwent CMR or Echo imaging for assessment of ventricular size and function. Patients with valvular or congenital heart disease were not included. The study has been approved by the local ethics committee and the study protocol conformed to the ethical guidelines of the 1964 Declaration of Helsinki and its later amendments. All patients gave informed consent prior to their inclusion in the study. Some of the patients ($n = 36$) were part of a previous report [5].

Endomyocardial biopsy protocol

At least five biopsies were preferentially taken from the ventricle demonstrating LGE. Patients demonstrating LGE exclusively in the LV lateral wall underwent selective LV biopsies (21 %), while those demonstrating LGE in the septum or having no LGE at all underwent either biventricular (59 %), or selective RV biopsies (20 %).

Histopathological analysis

Endomyocardial biopsies were stained with Masson's trichrome as well as Giemsa and examined by light microscopy. For immunohistology, tissue sections were treated with an avidin–biotin-immunoperoxidase method (Vectastain Elite Kit, Vector, Burlingame, CA), applying the following monoclonal antibodies: CD3 (T-cells; Novocastra Laboratories, Newcastle, UK), CD68 (macrophages; DAKO, Hamburg, Germany), and HLA-DR (DAKO, Hamburg, Germany) as described previously. The presence of focal or diffuse mononuclear infiltrates with >14 leukocytes per mm^2 (CD3+ T lymphocytes and/or CD68+ macrophages) in the myocardium, in addition to enhanced expression of HLA class molecules, was used for the diagnosis of inflammation [5, 9].

Fulfilling the above criteria, acute myocarditis was diagnosed by extended interstitial infiltration of inflammatory cells (see above) with necrosis of adjacent myocytes.

Chronic myocarditis was characterized by ongoing degeneration of myocytes, persistent inflammation, and presence of myocardial remodeling/fibrosis.

DCM was characterized by the presence of remodeling/fibrosis with no signs of inflammation.

"PVB19 genome only" was diagnosed in the absence of myocyte necrosis and the absence of inflammation, e.g., consistent with healed myocarditis.

Detection of viral genomes

DNA and RNA were extracted with the use of proteinase-K digestion followed by extraction with phenol/chloroform. Nested polymerase chain reaction/reverse transcriptase–polymerase chain reaction was performed for the detection of viral genomes as described [6]. As control for successful extraction of myocardial nucleic acids, the housekeeping gene (GAPDH) was detected by PCR [4]. Specificity of all viral amplification products was confirmed by automatic DNA sequencing [9].

CMR protocol

ECG gated CMR imaging was performed in breath-hold using a 1.5T Magnetom Sonata, or Magnetom Aera (Siemens-Healthcare, Germany) in line with SCMR/EuroCMR recommendations [10]. Both cine and LGE short-axis CMR images were prescribed every 10 mm (slice thickness 6 mm) from base to apex. In-plane resolution was typically

Table 1 Baseline patient characteristics

		N or IQR
All patients with follow-up		108
Time to follow-up (days, median)	1319	1038–1575
Gender, female	35.2	38/108
Age (years, median)	55.0	41.5–65.0
Primary cardiac symptoms/findings leading to biopsy (%)		
Angina at rest	40.7	44/108
Angina at exertion	43.5	47/108
Dyspnea	84.3	91/108
Fatigue	40.7	44/108
Palpitations	21.3	23/108
Prior febrile infection	25.0	27/108
Ventricular extrasystoles	24.1	26/108
Abnormal ECG	68.5	74/108
LVEF	40	30–58
LVEF <45 %	55.6	60/108
Elevated C-reactive protein	39.8	43/108
Elevated Troponine	23.1	25/108
Pericardial effusion	23.1	25/108
CMR performed*	83.3	90/108
EDV (ml)	175	135–266
ESV (ml)	90	53–192
LGE present (%)	55.6	50/90
Biopsy (%)		
Right ventricle	78.7	85/108
Left ventricle	79.6	86/108
Both ventricles	58.3	63/108
Copy numbers (μg)		
Copy numbers LV	192	0–383
Copy numbers RV	245.5	50–448
Copy number <100	8.3	9/108
Copy number 100–499	66.7	72/108
Copy number > 500	25	27/108
Histology		
Acute myocarditis	0.9	1/108
Chronic myocarditis	61.1	66/108
DCM	15.7	17/108
PVB19 genome only	12	13/108
Other	10.2	11/108
NYHA class during follow-up		
NYHA I	41.2	40/97
NYHA II	36.1	35/97
NYHA III	22.7	22/97
NYHA > II	58.8	57/97
Events during follow-up (%)		
All cause death	10.2	11/108
SCD	45.5	5/11
Cardiac death, but no SCD	27.3	3/11
Non cardiac death	27.3	3/11
Aborted SCD	2.1	2/108
Hospitalisation with heart failure	19.4	21/108

Values shown are (%) and *n* or medians (25th–75th percentiles)

IQR interquartile range, *LVEF* left ventricular ejection fraction, *EDV* enddiastolic volume, *ESV* endsystolic volume, *LGE* late gadolinium enhancement, *LV* left ventricle, *RV* right ventricle, *PVB19 genome only* evidence of PVB19 genome in absence of any inflammation or fibrosis, *NYHA* New York Heart Association, *SCD* sudden cardiac death

* CMR performed in $n = 90$ patients

1.2 × 1.8 mm. Cine CMR was performed using a steady-state-free-precession-sequence. LGE images were acquired on average 5–10 min after contrast administration using segmented IR-GRE [11] constantly adjusting inversion time [12]. The contrast dose (Gadodiamide or Gadopentetat-Dimeglumin) was 0.15 mmol/kg.

CMR analysis

Cine and contrast images were evaluated by two experienced observers as described elsewhere [5, 9]. In brief, endocardial and epicardial borders were outlined on the short-axis cine images. Volumes and LV-EF were derived by summation of epicardial and endocardial contours. The LV-mass was calculated by subtracting endocardial from epicardial volume at end-diastole and multiplying by 1.05 g/cm^3 [13]. LGE was assessed using the Siemens Argus analysis software package.

Clinical follow-up

Clinical follow-up was performed using a standardized questionnaire at least 2 years after initial presentation. In case of a suspected event, all necessary medical records were reviewed by some of the authors (S.G., I.K., J.S., A.P., H.M.) acting as end-point committee.

Variables, endpoints, and definitions

All variables were collected directly from patients, and/or medical records except CMR parameters, which were evaluated as described above. Variables include general characteristics and follow-up results. Most variables are self-explanatory; all others are defined below.

There were two primary combined endpoints named endpoint 1 and endpoint 2. Endpoint 1 "all cause death" was defined as SCD, or cardiac death, or non-cardiac death. Thus, endpoint 1 could only be reached by suffering a lethal event. Endpoint 2 was defined as either cardiac

death, or aborted SCD, or hospitalization for heart failure. Consequently, endpoint 2 could be reached by suffering either a lethal or a non-lethal event such as hospitalization for heart failure. The explicit meaning of the events is described in the following paragraphs:

Death: death from any cause.

Cardiac death: death from all cardiac causes.

SCD: unexpected arrest of presumed cardiac origin within 1 h after onset of any symptoms that could be interpreted as being cardiac in origin.

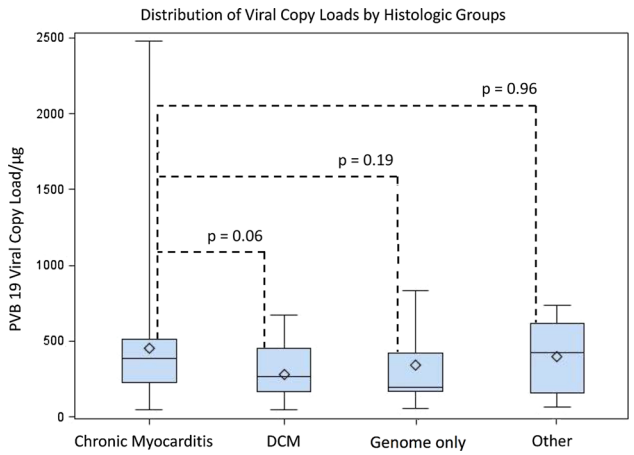

Fig. 1 Distribution of myocardial viral load. Final diagnosis "Chronic Myocarditis," "DCM," "PVB19 genome only," and "Other" was made on the basis of histological evaluation of all myocardial biopsy samples (see text for definitions). The only patient diagnosed with acute myocarditis is not displayed in this figure. Note that there seems to be a trend toward a lower viral load in the patient cohort with DCM and PVB19 genome only as final diagnosis made by histopathology

Aborted SCD: resuscitation after cardiac arrest defined as performance of the physical act of cardioversion and/or CPR in a patient who remains alive 28 days later.

Hospitalization for heart failure: Hospitalization as an in-patient >24 h, and heart failure as primary diagnosis according to the hospitals final report.

Statistical analysis

Absolute numbers, percentages, and medians (with quartiles) were computed to describe the patient population. Categorical variables were compared by Chi-square test or Fisher exact test as appropriate; continuous parameters by using Wilcoxon rank-sum test. The distribution of viral copy loads by histologic groups is presented as Box-plot. Kaplan–Meier curves were calculated for visualizing the cumulative event-free survival of patients for both endpoints. A log-rank test was performed to compare both survival curves. A multivariable Cox proportional hazard model was used for analyzing independent associations with mortality and other endpoints. p values <0.05 were considered significant. All p values are results of two-tailed tests. Statistical analyses were performed using the SAS© statistical package, version 9.2 (SAS, Cary, North Carolina).

Results

Patient characteristics

Chronic myocarditis according to the definition described above was the most frequent histopathologic diagnosis in this

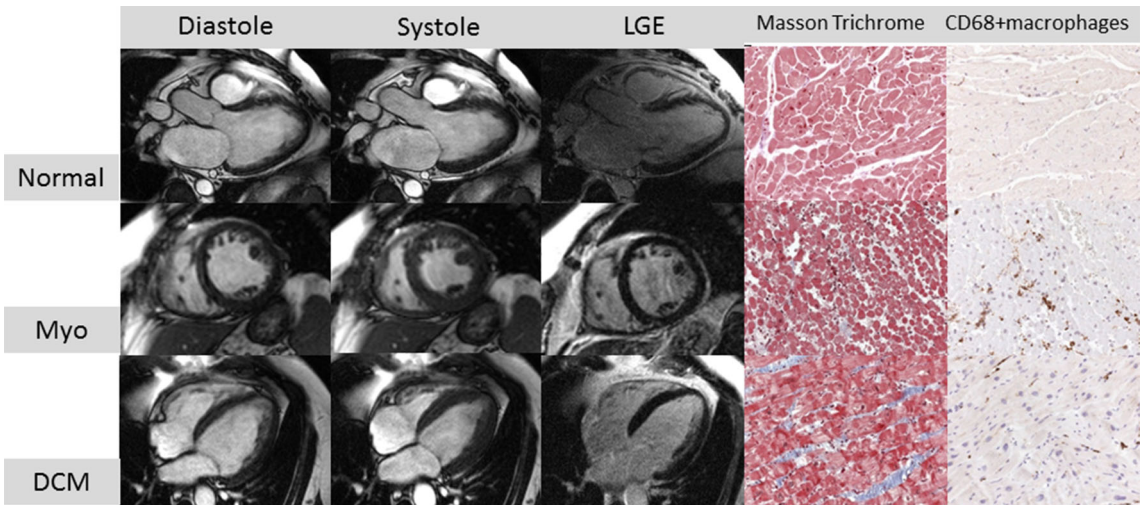

Fig. 2 Histological findings in patients with different entities. Three patients examples presenting with reduced LV-EF but different histological findings: *Top row* normal histology, but up to 720 GE of PVB19, LV-EF 15 %. *Middle row* Histology revealed chronic myocarditis with up to 1200 GE of PVB19, LV-EF 29 %. *Bottom row* diagnosed with DCM by histology (no inflammation but fibrosis), and 213 GE of PVB19, LV-EF 40 %

Table 2 Characteristics of patients with LVEF <45 % and LVEF >45 %

	LVEF <45 % ($n = 60$)	LVEF >45 % ($n = 48$)	p value	OR (95 % CI)
Gender, female	36.7 (22/60)	33.3 (16/48)	0.72	1.16 (0.52–2.57)
Age (years, median)	57.0 (48.0, 66.5)	45.0 (29.0, 61.5)	0.003	
Primary cardiac symptoms/findings leading to biopsy (%)				
Angina at rest	28.3 (17/60)	56.3 (27/48)	0.003	0.31 (0.14–0.68)
Angina at exertion	31.7 (19/60)	58.3 (28/48)	0.006	0.33 (0.15–0.73)
Dyspnea	95.0 (57/60)	70.8 (34/48)	0.0006	7.82 (2.10–29.21)
Fatigue	36.7 (22/60)	45.8 (22/48)	0.34	0.68 (0.32–1.48)
Palpitations	16.7 (10/60)	27.1 (13/48)	0.19	0.54 (0.21–1.37)
Prior febrile infection	20.0 (12/60)	31.3 (15/48)	0.18	0.55 (0.23–1.32)
Ventricular extrasystoles	28.3 (17/60)	18.8 (9/48)	0.25	1.71 (0.68–4.29)
Abnormal ECG	75.0 (45/60)	60.4 (29/48)	0.10	1.97 (0.86–4.47)
LVEF	30.5 (19–36)	58.5 (54–67)		
Pericardial effusion	20.0 (12/60)	27.1 (13/48)	0.39	0.67 (0.27–1.65)
CMR performed*	81.7 (49/60)	85.4 (41/48)	0.60	0.76 (0.27–2.14)
EDV (ml)	259 (197, 330)	135 (113.5, 163)		
ESV (ml)	188 (139, 248)	52.5 (39.5, 65)		
LGE present (%)	63.3 (31/49)	46.3 (19/41)	0.11	1.99 (0.86–4.64)
Biopsy (%)				
Right ventricle	81.7 (49/60)	75.0 (36/48)	0.40	1.48 (0.59–3.74)
Left ventricle	75.0 (45/60)	85.4 (41/48)	0.18	0.51 (0.19–1.38)
Both ventricles	56.7 (34/60)	60.4 (29/48)	0.69	0.86 (0.40–1.85)
Copy numbers (μg)				
Copy numbers LV	157 (0, 376)	217 (103, 400)	0.28	
Copy numbers RV	231 (50, 400)	271 (25, 516)	0.51	
Copy number <100	13.3 (8/60)	2.1 (1/48)	0.04	7.23 (0.87–60.0)
Copy number 100–499	66.7 (40/60)	66.7 (32/48)	1.0	1.0 (0.45–2.24)
Copy number >500	20.0 (12/60)	31.3 (15/48)	0.18	0.55 (0.23–1.32)
Histology (%)				
Acute myocarditis	0 (0/60)	2.1 (1/48)		
Chronic myocarditis	56.7 (34/60)	66.7 (32/48)	0.29	0.65 (0.30–1.44)
DCM	26.7 (16/60)	2.1 (1/48)	0.0005	17.09 (2.17–134.32)
PVB19 genome only	10.0 (6/60)	14.6 (7/48)	0.47	0.65 (0.20–2.08)
Other	6.7 (4/60)	14.6 (7/48)	0.18	0.42 (0.11–1.52)
NYHA class during follow-up				
NYHA I	28.0 (14/50)	55.3 (26/47)	0.006	0.31 (0.14–0.73)
NYHA II	40.0 (20/50)	31.9 (15/47)	0.41	1.42 (0.62–3.28)
NYHA III	32.0 (16/50)	12.8 (6/47)	0.02	3.22 (1.13–9.12)
NYHA >II	72.0 (36/50)	44.7 (21/47)	0.006	3.18 (1.37–7.40)
Events during follow-up (%)				
All cause death	16.7 (10/60)	2.1 (1/48)	0.01	9.40 (1.16–76.29)
SCD	50.0 (5/10)	0 (0/1)		
Cardiac death, but no SCD	30.0 (3/10)	0 (0/1)		
Non cardiac	20.0 (2/10)	100 (1/1)		
Aborted SCD	2.0 (1/50)	2.1 (1/47)	0.96	0.94 (0.06–15.45)
Hospitalisation with heart failure	30.0 (18/60)	6.3 (3/48)	0.002	6.43 (1.77–23.41)

Values are mean ± SD, n (%) or median (IQR)

CI confidence interval, *OR* odds ratio, other abbreviations as in Table 1

* CMR performed in $n = 90$ patients

Table 3 Characteristics of patients with LGE and no LGE

	LGE ($n = 50$)	No LGE ($n = 40$)	p value	OR (95 % CI)
Gender, female	22.0 (11/50)	47.5 (19/40)	0.01	0.31 (0.13–0.78)
Age (years, median)	55.0 (40.0, 65.0)	51.5 (41.0, 61.5)	0.98	
Primary cardiac symptoms/findings leading to biopsy (%)				
Angina at rest	36.0 (18/50)	47.5 (19/40)	0.27	0.62 (0.27–1.45)
Angina at exertion	42.0 (21/50)	50.0 (20/40)	0.45	0.72 (0.31–1.67)
Dyspnea	82.0 (41/50)	87.5 (35/40)	0.47	0.65 (0.20–2.12)
Fatigue	46.0 (23/50)	45.0 (18/40)	0.92	1.04 (0.45–2.40)
Palpitations	18.0 (9/50)	25.0 (10/40)	0.42	0.66 (0.24–1.82)
Prior febrile infection	36.0 (18/50)	20.0 (8/40)	0.10	2.25 (0.86–5.91)
Ventricular extrasystoles	32.0 (16/50)	22.5 (9/40)	0.32	1.62 (0.63–4.19)
Abnormal ECG	66.0 (33/50)	70.0 (28/40)	0.69	0.83 (0.34–2.03)
LVEF (%)	35.0 (21.0, 55.0)	52 (33.0, 60.0)	0.02	
EDV (ml)	200 (152, 279)	152 (107, 212)	0.002	
ESV (ml)	135 (65, 208)	60 (43, 142)	0.002	
Pericardial effusion	26.0 (13/50)	27.5 (11/40)	0.87	0.93 (0.36–2.37)
Biopsy (%)				
Right ventricle	76.0 (38/50)	80.0 (32/40)	0.65	0.79 (0.29–2.18)
Left ventricle	78.0 (39/50)	85.0 (34/40)	0.40	0.63 (0.21–1.87)
Both ventricles	54.0 (27/50)	65.0 % (26/40)	0.29	0.63 (0.27–1.49)
Copy numbers (μg)				
Copy numbers LV	148 (0, 377)	245 (103, 400)	0.24	
Copy numbers RV	231 (50, 369)	312 (56, 466)	0.25	
Copy number <100	10.0 (5/50)	7.5 (3/40)	0.68	1.37 (0.31–6.12)
Copy number 100–499	62.0 (31/50)	67.5 (27/40)	0.59	0.79 (0.33–1.88)
Copy number >500	28.0 (14/50)	25.0 (10/40)	0.75	1.17 (0.45–3.00)
Histology (%)				
Acute myocarditis	2.0 (1/50)	0 (0/40)	0.37	
Chronic myocarditis	62.0 (31/50)	67.5 (27/40)	0.59	0.79 (0.33–1.88)
DCM	24.0 (12/50)	7.5 (3/40)	0.04	3.89 (1.02–14.93)
PVB19 genome only	8.0 (4/50)	10.0 (4/40)	0.74	0.78 (0.18–3.35)
Other	4.0 (2/50)	15.0 (6/40)	0.07	0.24 (0.04–1.24)
NYHA class during follow-up				
NYHA I	51.2 (21/41)	38.5 (15/39)	0.25	1.68 (0.69–4.09)
NYHA II	29.3 (12/41)	38.5 (15/39)	0.38	0.66 (0.26–1.68)
NYHA III	19.5 (8/41)	23.1 (9/39)	0.70	0.81 (0.28–2.36)
NYHA >II	48.8 (20/41)	61.5 (24/39)	0.25	0.60 (0.24–1.45)
Events during follow-up (%)				
All cause death	18.0 (9/50)	2.5 (1/40)	0.02	8.56 (1.04–70.75)
SCD	55.6 (5/9)	0 (0/1)		
Cardiac death, but no SCD	33.3 (3/9)	0 (0/1)		
Non cardiac	11.1 (1/9)	100 (1/1)		
Aborted SCD	4.9 (2/41)	0 (0/39)		
Hospitalisation with heart failure	26.0 (13/50)	10.0 (4/40)	0.05	3.16 (0.94–10.61)

Values are mean ± SD, *n* (%) or median (IQR)

CI confidence interval, *OR* odds ratio, other abbreviations as in Table 1

patient cohort, followed by end stage DCM, and the presence of PVB19 genome only. Acute myocarditis was observed in one patient. Patients classified as "others" include hypertensive heart disease, hypertrophic cardiomyopathy after remodeling, Tako-Tsubo cardiomyopathy, and myocardial amyloidosis. Dyspnea and angina were the

Table 4 Characteristics of patients with different myocardial viral loads (<99 GE, 100–499 GE, >500 GE)

	<100 ($n = 9$)	100–499 ($n = 72$)	>500 ($n = 27$)	p value
Gender, female	33.3 (3/9)	34.7 (25/72)	37.0 (10/27)	0.97
Age (years, median)	50 (42, 60)	55 (42.5, 63.5)	52.0 (35, 68)	0.95
Primary cardiac symptoms/findings leading to biopsy (%)				
Angina at rest	33.3 (3/9)	40.3 (29/72)	44.4 (12/27)	0.83
Angina at exertion	33.3 (3/9)	41.7 (30/72)	51.9 (14/27)	0.54
Dyspnea	88.9 (8/9)	86.1 (62/72)	77.8 (21/27)	0.55
Fatigue	33.3 (3/9)	41.7 (30/72)	40.7 (11/27)	0.89
Palpitations	0 (0/9)	29.2 (21/72)	7.4 (2/27)	0.02
Prior febrile infection	33.3 (3/9)	22.2 (16/72)	29.6 (8/27)	0.63
Ventricular extrasystoles	66.7 (6/9)	23.6 (17/72)	11.1 (3/27)	0.003
Abnormal ECG	55.6 (5/9)	69.4 (50/72)	70.4 (19/27)	0.68
LVEF (%)	27 (20, 30)	40 (30.5, 57.5)	49 (33, 60)	0.04
EDV (ml)	233 (161, 343)	173 (135, 268)	160 (123, 217)	0.32
ESV (ml)	169 (64, 248)	90 (52, 194)	77 (46, 153)	0.19
LGE (%)*	62.5 (5/8)	53.4 (31/58)	58.3 (14/24)	0.85
Pericardial effusion	22.2 (2/9)	23.6 (17/72)	22.2 (6/27)	0.99
Biopsy (%)				
Right ventricle	77.8 (7/9)	76.4 (55/72)	85.2 (23/27)	0.63
Left ventricle	66.7 (6/9)	79.2 (57/72)	85.2 (23/27)	0.48
Both ventricles	44.4 (4/9)	55.6 (40/72)	70.4 (19/27)	0.28
Copy numbers (µg)				
Copy numbers LV	42 (0, 50)	164.5 (0, 329)	507 (231, 621)	
Copy numbers RV	50 (50, 50)	212 (25, 335)	572 (345, 827)	
Combined copy number	50 (50, 62)	282.5 (189, 406)	713 (559, 846)	
Histology (%)				
Acute myocarditis	0 (0/9)	1.4 (1/72)	0 (0/27)	0.78
Chronic myocarditis	33.3 (3/9)	61.1 (44/72)	70.4 (19/27)	0.14
DCM	44.4 (4/9)	15.3 (11/72)	7.4 (2/27)	0.03
PVB19 genome only	11.1 (1/9)	12.5 (9/72)	11.1 (3/27)	0.98
Other	11.1 (1/9)	9.7 (7/72)	11.1 (3/27)	0.98
NYHA class during follow-up				
NYHA I	25.0 (2/8)	43.9 (29/66)	39.1 (9/23)	0.57
NYHA II	37.5 (3/8)	34.8 (23/66)	39.1 (9/23)	0.93
NYHA III	37.5 (3/8)	21.2 (14/66)	21.7 (5/23)	0.58
NYHA >II	75.0 (6/8)	56.1 (37/66)	60.9 (14/23)	0.57
Events during follow-up (%)				
All cause death	11.1 (1/9)	8.3 (6/72)	14.8 (4/27)	0.63
SCD	100 (1/1)	33.3 (2/6)	50.0 (2/4)	
Cardiac death, but no SCD	0 (0/1)	50.0 (3/6)	0 (0/4)	
Non cardiac	0 (0/1)	16.7 (1/6)	50.0 (2/4)	
Aborted SCD	12.5 (1/8)	1.5 (1/66)	0 (0/23)	
Hospitalisation with heart failure	33.3 (3/9)	19.4 (14/72)	14.8 % (4/27)	0.48

Values are mean ± SD, n (%) or median (IQR)

CI confidence interval, *OR* odds ratio, other abbreviations as in Table 1

* *CMR* performed in $n = 90$ patients

primary reasons to seek medical attention, followed by fatigue and various combinations of heart failure and other symptoms (Table 1).

As per inclusion criteria, the genome of PVB19 was present in the myocardium of all patients. In nine patients, the viral load was <100 GE of PVB19. Seventy-two

Table 5 Characteristics of patients with inflammation and no inflammation

	Inflammation ($n = 67$)	No Inflammation ($n = 41$)	p value	OR (95 % CI)
Gender, female	34.3 (23/67)	36.6 (15/41)	0.81	0.91 (0.40–2.04)
Age (years, median)	55.0 (40.0, 63.0)	55.0 (43.0, 67.0)	0.36	
Primary cardiac symptoms/findings leading to biopsy (%)				
Angina at rest	49.3 (33/67)	26.8 (11/41)	0.02	2.65 (1.14–6.14)
Angina at exertion	49.3 (33/67)	34.1 (14/41)	0.12	1.87 (0.84–4.18)
Dyspnea	80.6 (54/67)	90.2 (37/41)	0.18	0.45 (0.14–1.49)
Fatigue	46.3 (31/67)	31.7 (13/41)	0.14	1.85 (0.82–4.19)
Palpitations	22.4 (15/67)	19.5 (8/41)	0.72	1.19 (0.45–3.12)
Prior febrile infection	26.9 (18/67)	22.0 (9/41)	0.57	1.31 (0.52–3.26)
Ventricular extrasystoles	25.4 (17/67)	22.0 (9/41)	0.69	1.21 (0.48–3.04)
Abnormal ECG	67.2 (45/67)	70.7 (29/41)	0.70	0.85 (0.36–1.97)
LVEF (%)	43.0 (33.0, 58.0)	34.0 (22.0, 56.0)	0.13	
EDV (ml)	161 (128, 212)	235 (144, 326)	0.01	
ESV (ml)	73 (52, 150)	193 (56, 248)	0.01	
LGE present*	54.2 (32/59)	58.1 (18/31)	0.73	0.86 (0.36–2.06)
Biopsy (%)				
Right ventricle	79.1 (53/67)	78.0 (32/41)	0.90	1.06 (0.41–2.74)
Left ventricle	82.1 (55/67)	75.6 (31/41)	0.42	1.48 (0.57–3.81)
Both ventricles	61.2 (41/67)	53.7 (22/41)	0.44	1.36 (0.62–2.99)
Copy numbers (μg)				
Copy numbers LV	227 (44, 404)	132 (0, 286)	0.12	
Copy numbers RV	273 (50, 449)	189 (50, 408)	0.41	
Copy number <100	4.5 (3/67)	14.6 (6/41)		0.27 (0.06–1.16)
Copy number 100–499	67.2 (45/67)	65.9 (27/41)		1.06 (0.47–2.41)
Copy number >500	28.4 (19/67)	19.5 (8/41)		1.63 (0.64–4.17)
NYHA class during follow-up				
NYHA I	43.5 (27/62)	37.1 (13/35)		1.31 (0.56–3.05)
NYHA II	35.1 (23/62)	34.3 (12/35)		1.13 (0.47–2.69)
NYHA III	19.4 (12/62)	28.6 (10/35)		0.60 (0.23–1.58)
NYHA >II	56.5 (35/62)	62.9 (22/35)		0.77 (0.33–1.79)
Events during follow-up (%)				
All cause death	7.5 (5/67)	14.6 (6/41)	0.24	0.49 (0.13–1.65)
SCD	60.0 (3/5)	33.3 (2/6)		
Cardiac death, but no SCD	40 (2/5)	16.7 (1/6)		
Non cardiac	0 (0/5)	50 (3/6)		
Aborted SCD	1.6 (1/62)	2.9 (1/35)		
Hospitalisation with heart failure	19.4 (13/67)	19.5 (8/41)	0.99	0.99 (0.37–2.65)

Values are mean ± SD, n (%) or median (IQR)

CI confidence interval, *OR* odds ratio, *Inflammation* acute or chronic myocarditis, *No inflammation* DCM, genome only, others

* CMR performed in $n = 90$ patients, other abbreviations as in Table 1

patients had between 100 and 499 GE of PVB19 in their myocardium, and more than 500 GE could be detected in 27 patients (IQR 559, 846, max. 2450 GE). Note that there seems to be a trend toward a lower viral load in the patient cohorts with DCM and the presence of PVB19 genome only (e.g., healed myocarditis) as final diagnosis (Fig. 1).

No patient was treated with anti-viral, or anti-inflammatory medication prior to inclusion or during follow-up, but all patients with heart failure received state of the art heart failure medication. If clinically indicated, ICD implantation was performed ($n = 15$ patients).

Table 6 Univariate analysis endpoint 1—all cause death

	Endpoint 1 ($n = 11$)	No endpoint ($n = 97$)	p value	OR (95 % CI)
Gender, female	27.3 (3/11)	36.1 (35/97)	0.56	0.66 (0.17–2.67)
Age (years, median)	59.0 (40.0, 65.0)	54.0 (42.0, 65.0)	0.63	
Primary cardiac symptoms/findings leading to biopsy (%)				
Angina at rest	18.2 (2/11)	43.3 (42/97)	0.11	0.29 (0.06–1.42)
Angina at exertion	36.4 (4/11)	44.3 (43/97)	0.61	0.72 (0.20–2.61)
Dyspnea	100.0 (11/11)	82.5 (80/97)	0.13	
Fatigue	54.5 (6/11)	39.2 (38/97)	0.33	1.86 (0.53–6.53)
Palpitations	9.1 (1/11)	22.7 (22/97)	0.30	0.34 (0.04–2.81)
Prior febrile infection	45.5 (5/11)	22.7 (22/97)	0.10	2.84 (0.79–10.20)
Ventricular extrasystoles	36.4 (4/11)	22.7 (22/97)	0.31	1.95 (0.52–7.27)
Abnormal ECG	63.6 (7/11)	69.1 (67/97)	0.71	0.78 (0.21–2.88)
LVEF	31 (21, 40)	43 (30, 58)	0.07	
Elevated C-reactive protein	63.6 (7/11)	37.1 (36/97)	0.09	2.97 (0.81–10.83)
Elevated troponine	18.2 (2/11)	23.7 (23/97)	0.68	0.71 (0.14–3.55)
Pericardial effusion	18.2 (2/11)	23.7 (23/97)	0.68	0.71 (0.14–3.55)
CMR performed*	90.9 (10/11)	82.5 (80/97)	0.48	2.13 (0.25–17.73)
EDV (ml)	274 (136, 390)	173 (135, 255)	0.15	
ESV (ml)	188 (79, 321)	80 (52, 184)	0.09	
LGE present (%)	90.0 (9/10)	51.3 (41/80)	0.02	8.56 (1.04–70.75)
Biopsy (%)				
Right ventricle	90.9 (10/11)	77.3 (75/97)	0.30	2.93 (0.36–24.19)
Left ventricle	90.9 (10/11)	78.4 (76/97)	0.33	2.76 (0.33–22.83)
Both ventricles	81.8 (9/11)	55.7 (54/97)	0.10	3.58 (0.74–17.46)
Copy numbers (μg)				
Copy numbers LV	286 (50, 596)	189 (0, 379)	0.43	
Copy numbers RV	345 (189, 453)	233 (50, 440)	0.34	
Copy number <100	9.1 (1/11)	8.2 (8/97)	0.92	1.11 (0.13–9.83)
Copy number 100–499	54.5 (6/11)	68.0 (66/97)	0.37	0.56 (0.16–1.99)
Copy number >500	36.4 (4/11)	23.7 (23/97)	0.36	1.84 (0.49–6.84)
Histology				
Acute myocarditis	0 (0/11)	1.0 (1/97)	0.74	
Chronic myocarditis	45.5 (5/11)	62.9 (61/97)	0.26	0.49 (0.14–1.73)
DCM	36.4 (4/11)	13.4 (13/97)	0.05	3.69 (0.95–14.39)
PVB19 genome only	9.1 (1/11)	12.4 (12/97)	0.75	0.71 (0.08–6.04)
Other	9.1 (1/11)	10.3 (10/97)	0.90	0.87 (0.10–7.52)
NYHA class during follow-up				
NYHA I	–	41.2 (40/97)		
NYHA II	–	36.1 (35/97)		
NYHA III	–	22.7 (22/97)		
NYHA >II	–	58.8 (57/97)		
Events during follow-up (%)				
All cause death	100 (11/11)	0 (0/97)		
SCD	45.5 (5/11)	–		
Cardiac death, but no SCD	27.3 (3/11)	–		
Non cardiac death	27.3 (3/11)	–		
Aborted SCD	–	2.1 (2/97)		
Hospitalisation with heart failure	36.4 (4/11)	17.5 (17/97)	0.13	2.69 (0.71–10.22)
Cardiac death	72.7 (8/11)	0 (0/97)		

Table 6 continued

	Endpoint 1 ($n = 11$)	No endpoint ($n = 97$)	p value	OR (95 % CI)
Cardiac death/hospitalisation with heart failure	81.8 (9/11)	17.5 (17/97)		21.18 (4.19–106.91)
Cardiac death/aborted SCD/hospitalisation with heart failure	81.8 (9/11)	18.6 (18/97)		19.75 (3.93–99.34)

Values are mean ± SD, n (%) or median (IQR)

CI confidence interval, *OR* odds ratio, other abbreviations as in Table 1

* CMR performed in $n = 90$, in one death there was no CMR performed

Imaging findings

The mean LV-EF was 40 %, and the mean LV-EDV was 175 ml. CMR imaging was performed in 90/108 patients (Table 1); in all others, ventricular function and size were evaluated by echo (18/108). LGE was present in 50/90 patients, most commonly occurring in a non-ischemic pattern located in the subepicardial or intramural areas of the LV. Typical patient examples are displayed in Fig. 2.

Dividing our patient population in subgroups with LV-EF above and below 45 % revealed that patients with LV-EF <45 % had larger ventricles, were more often diagnosed with DCM, had a higher prevalence of LGE, higher NYHA classes, and were more likely to suffer from dyspnea than from chest pain (Table 2). Comparing patients with LGE to patients without LGE demonstrates larger ventricles, poorer ventricular function, and a higher prevalence of DCM in the group with LGE (Table 3). When looking at different viral loads in the myocardium (Table 4), our data reveal the best ventricular function in the group with the highest viral load (>500 GE) and the highest incidence of DCM in the group with the lowest viral load. In addition, patients with myocardial inflammation by histology (Table 5) had more angina and a trend toward a higher viral load compared to patients without inflammation, who had larger ventricles and poorer LV-EF (mostly due to end stage DCM).

Follow-up results

During follow-up 11 of 108 patients died, two patients suffered SCD but were successfully shocked by their ICD, and 21 patients were hospitalized for heart failure (Table 1). Thus, eleven patients reached endpoint 1 "all cause death" as described above (Table 6). Most of the lethal events ($n = 8$) occurred for cardiac reasons. Of the remaining three patients, one died from severe cerebral hemorrhage, one from lung cancer, and one from lymphoma.

In addition, 27 patients reached endpoint 2 including cardiac death, aborted SCD, and hospitalization for heart failure (Table 7). Note that there is no relation between reaching endpoint 1 or endpoint 2 and the viral load in the myocardium, but 90 % of patients reaching endpoint 1 demonstrated myocardial LGE.

Predictors of events

For evaluation of predictors for adverse events, we looked at (1) all patients who reached endpoint 1 (Table 6) and (2) all patients who reached endpoint 2 (Table 7). There was no significant correlation between clinical presentation and endpoint 1 (Table 6). However, ventricular extrasystoles, which may be a surrogate parameter for undetected arrhythmias, symptoms of heart failure, which are a surrogate parameter of impaired LV-EF, and LV-EF itself were related to endpoint 2.

In addition, functional parameters such as LV-EF, LV-EDV (for endpoint 2), as well as the histologic diagnosis of DCM and the presence of LGE (for all endpoints) reached statistical significance in the univariate analysis. In fact, the presence of LGE yielded an odds-ratio for a lethal event of 8.56 (endpoint 1), and of 5.52 for endpoint 2.

Kaplan–Meier survival curves for endpoint 1 comparing LV-EF, the presence of LGE, and the myocardial load of PVB19 genomes are displayed in Fig. 3a–c. Note that only one patient without LGE and one with normal LV-EF died during follow-up (both from cancer as described above). Figure 4a–c displays the Kaplan–Meier survival curves for endpoint 2.

Multivariable Cox regression analysis including the presence of LGE, the initial LV-EF, the initial LV-EDV, the viral load, and the histologic diagnosis of DCM revealed a trend for LV-EDV at the initial presentation ($p = 0.07$, hazard ratio 1.02 per ml for endpoint 1) as a possible independent predictor of lethal events. In this model, the presence of LGE ($p = 0.17$), the viral load ($p = 0.16$), the histological diagnosis of DCM ($p = 0.16$), and the LV-EF upon presentation did not reach significance for endpoint 1. Looking at patients suffering, endpoint 2 revealed a trend for LV-EF at the initial presentation ($p = 0.07$, hazard ratio 1.04 per % EF for endpoint 2), and the presence of LGE ($p = 0.07$, hazard-ratio 8.9 for endpoint 2) as possible independent predictors of endpoint 2. All other parameters, including viral load ($p = 0.79$) were not significant. Typical patient examples are viewed in Fig. 5.

Table 7 Univariate analysis endpoint 2—cardiac death or aborted SCD or hospitalization for heart failure

	Endpoint 2 ($n = 27$)	No endpoint ($n = 81$)	p value	OR (95 % CI)
Gender, female	29.6 (8/27)	37.0 (30/81)	0.49	0.72 (0.28–1.83)
Age (years, median)	58.0 (46.0, 63.0)	52.0 (40.0, 66.0)	0.26	
Primary cardiac symptoms/findings leading to biopsy (%)				
Angina at rest	37.0 (10/27)	42.0 (34/81)	0.65	0.81 (0.33–1.99)
Angina at exertion	48.1 (13/27)	42.0 (34/81)	0.58	1.28 (0.54–3.08)
Dyspnea	96.3 (26/27)	80.2 (65/81)	0.05	6.40 (0.81–50.76)
Fatigue	40.7 (11/27)	40.7 (33/81)	1.0	1.00 (0.41–2.43)
Palpitations	11.1 (3/27)	24.7 (20/81)	0.14	0.38 (0.10–1.40)
Prior febrile infection	22.2 (6/27)	25.9 (21/81)	0.70	0.82 (0.29–2.30)
Ventricular extrasystoles	44.4 (12/27)	17.3 (14/81)	0.004	3.83 (1.48–9.93)
Abnormal ECG	70.4 (19/27)	67.9 (55/81)	0.81	1.12 (0.43–2.90)
LVEF	31.0 (17.0, 34.0)	49.0 (33.0, 60.0)	0.00006	
Elevated C-reactive protein	48.1 (13/27)	37.0 (30/81)	0.31	1.58 (0.66–3.80)
Elevated troponine	25.9 (7/27)	22.2 (18/81)	0.69	1.23 (0.45–3.36)
Pericardial effusion	18.5 (5/27)	24.7 (20/81)	0.51	0.69 (0.23–2.07)
CMR performed*	85.2 (23/27)	82.7 (67/81)	0.77	1.20 (0.36–4.02)
EDV (ml)	270 (170, 344.5)	165 (128, 217)	0.008	
ESV (ml)	194 (92.5, 288.5)	73 (50, 156)	0.002	
LGE present (%)	82.6 (19/23)	46.3 (31/67)	0.002	5.52 (1.69–17.96)
Biopsy (%)				
Right ventricle	85.2 (23/27)	76.5 (62/81)	0.34	1.76 (0.54–5.73)
Left ventricle	85.2 (23/27)	77.8 (63/81)	0.41	1.64 (0.50–5.37)
Both ventricles	70.4 (19/27)	54.3 (44/81)	0.14	2.00 (0.78–5.09)
Copy numbers (μg)				
Copy numbers LV	132 (44, 374)	213 (0, 397)	0.77	
Copy numbers RV	228 (50, 369)	250 (50, 453)	0.71	
Copy number <100	14.8 (4/27)	6.2 (5/81)	0.16	2.64 (0.66–10.67)
Copy number 100–499	66.7 (18/27)	66.7 (54/81)	1.00	1.00 (0.40–2.52)
Copy number >500	18.5 (5/27)	27.2 (22/81)	0.37	0.61 (0.21–1.81)
Histology				
Acute myocarditis	0 (0/27)	1.2 (1/81)	0.56	
Chronic myocarditis	59.3 (16/27)	61.7 (50/81)	0.82	0.90 (0.37–2.19)
DCM	29.6 (8/27)	11.1 (9/81)	0.02	3.37 (1.15–9.90)
PVB19 genome only	11.1 (3/27)	12.3 (10/81)	0.86	0.89 (0.23–3.50)
Other	0 (0/27)	13.6 (11/81)	0.04	
NYHA class during follow-up				
NYHA I	16.7 (3/18)	46.8 (37/79)	0.02	0.23 (0.06–0.85)
NYHA II	38.9 (7/18)	35.4 (28/79)	0.78	1.16 (0.40–3.32)
NYHA III	44.4 (8/18)	17.7 (14/79)	0.01	3.71 (1.24–11.10)
NYHA ≥II	83.3 (15/18)	53.2 (42/79)	0.02	4.40 (1.18–16.42)
Events during follow-up (%)				
All cause death	33.3 (9/27)	2.5 (2/81)		19.75 (3.93–99.34)
SCD	55.6 (5/9)	0 (0/2)	0.15	
Cardiac death, but no SCD	33.3 (3/9)	0 (0/2)	0.34	
Non cardiac death	11.1 (1/9)	100 (2/2)	0.01	
Aborted SCD	11.1 (2/18)	0 (0/79)	0.003	
Hospitalisation for heart failure	77.8 (21/27)	0 (0/81)		
Cardiac death	29.6 (8/27)	–		

Table 7 continued

	Endpoint 2 ($n = 27$)	No endpoint ($n = 81$)	p value	OR (95 % CI)
Cardiac death/Hospitalisation for heart failure	96.3 (26/27)	–		
Cardiac death/aborted SCD/hospitalisation with heart failure	100 (27/27)	–		

Values are mean ± SD, n (%) or median (IQR)

CI confidence interval, *OR* odds ratio, other abbreviations as in Table 1

* CMR performed in $n = 90$ patients

Discussion

This study is of clinical importance since we clearly demonstrate that the viral load of PVB19 genomes in the myocardium is not related to the long-term clinical outcome. Furthermore, our data suggest a growing role of non-invasive imaging parameters such as ventricular size and function, as well as LGE for risk stratification in patients with non-ischemic myocardial disease. Note that no patient with normal LVEF or the absence of LGE suffered cardiac death during long-term follow-up.

Patient characteristics

Dyspnea and angina were the primary reasons to seek medical attention, followed by fatigue and various combinations of heart failure and other symptoms, which is similar to other published patient cohorts presenting with PVB19-related myocardial disease [8, 14]. Furthermore, the mean myocardial viral load in the present study is in line with other recent German reports [6, 15].

Our data reveal a trend toward a lower viral load in the patient cohort with DCM as final diagnosis made by histopathology. This supports the results of other groups [6, 16], but challenges findings of Stewart et al. [8], who found the highest viral copy numbers in patients with DCM. A possible explanation could be the different patient populations studied, and the fact that PVB19 genome persistence in human tissues can be life-long [17], independent of active inflammation, representing a source of information about past and not necessarily of recent events.

CMR findings

Despite a median LV-EF of 40 %, there was a broad spectrum of LV impairment ranging from severely impaired to completely normal ventricular function (IQR 30–58 %). In the 90 patients undergoing CMR imaging, LGE was present in 55.6 % and was usually located in a non-CAD–pattern in the subepicardial or intramural areas of the LV, as described previously [9, 14, 18]. Patients with scar indicated by LGE had larger ventricles and poorer LV-EF compared to those without scar (Table 3). This finding also matches the results from other inflammatory [5] or non-ischemic cardiomyopathy populations [19].

Interestingly, the best ventricular function was seen in the group with the highest myocardial viral load (>500 GE), or in the presence of myocardial inflammation (Table 5). The highest incidence of the final diagnosis of end stage DCM was in the group with the lowest viral load, or in the group without myocardial inflammation. Whereas the high incidence of DCM in the group with the lowest viral load and without inflammation conceptually makes sense, reflecting some form of partial virus elimination over time [6], and the fact that DCM patients do not have myocardial inflammation by our definition above, the high load of viral genomes and the presence of inflammation in the group with the best LV-EF are more difficult to understand. However, the most likely explanation for the better LV-EF is that in this group with high viral load and/or active inflammation the end stage of post inflammatory heart disease with fibrous myocardial remodeling resulting in poor function has not yet been reached (as it has been reached in the DCM group). Stewart et al. [8] also described a better ventricular function for the PVB19 positive group compared to patients without myocardial PVB19 genome presence, concluding that the detection of PVB19 genome by PCR alone may not be sufficient to explain a pathologic effect [20].

Follow-up results and predictors of events

In our population with symptoms ranging from mild to severe, all-cause mortality was 10.2 %, and cardiac mortality was 7.4 %. SCD (including aborted SCD) occurred in 6.5 % of patients during follow-up. Thus, our event rate was much lower than in Mason's Myocarditis Trial [21], most likely due to different inclusion criteria and disease severity, but almost as high as in the non-ischemic cardiomyopathy group of the SCD-HEFT trial [22], although LV function was better in our cohort underscoring the importance of risk stratification and optimal clinical management in these patients.

Importantly, the present data clearly indicate that the viral load of PVB19 genomes in the myocardium is not related to the clinical outcome, as suggested by other

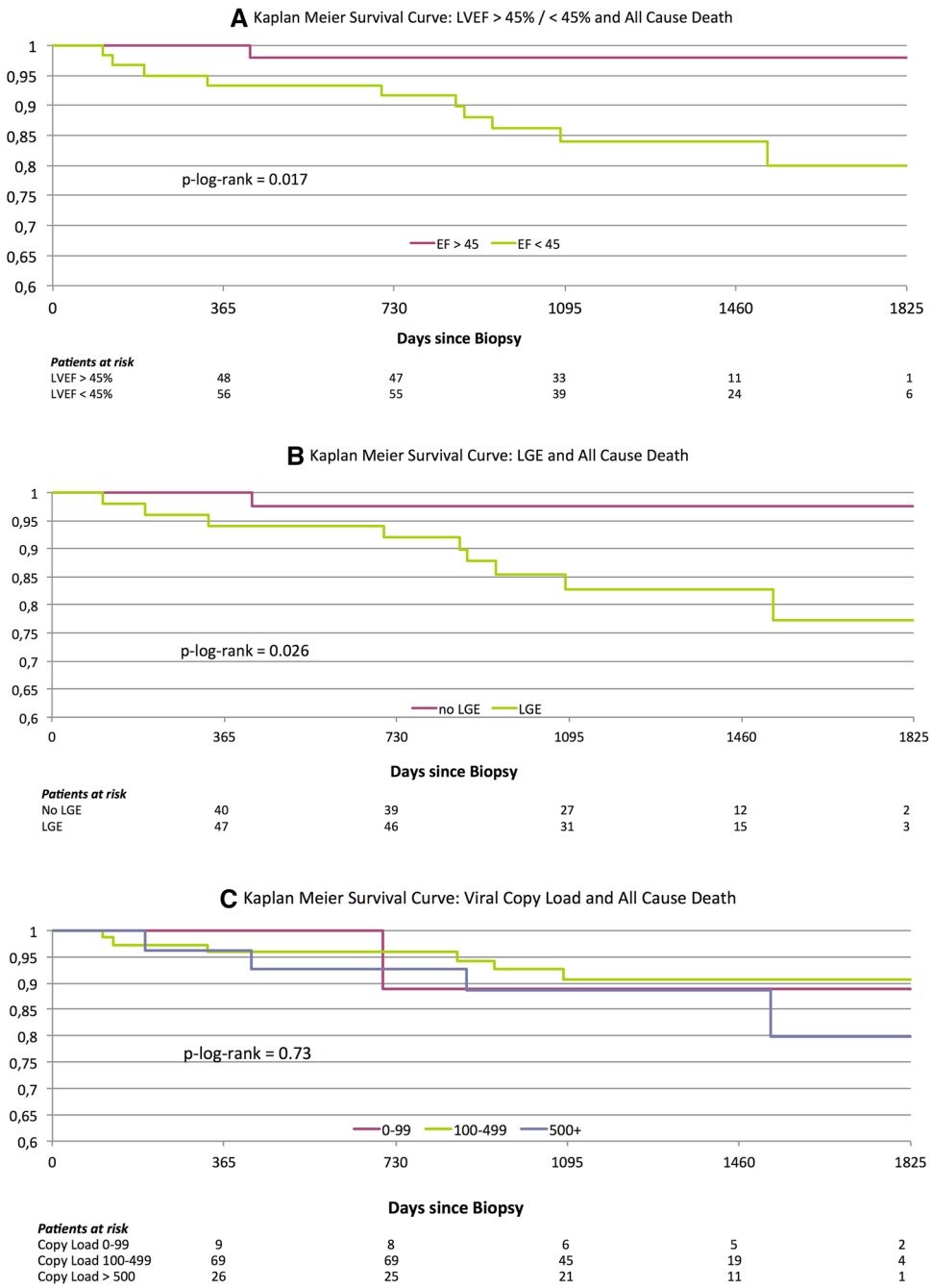

Fig. 3 Kaplan–Meier survival curves—Endpoint 1. Kaplan–Meier Survival Curves with regard to endpoint 1 (all cause death), displayed for LV-EF, presence of LGE, and myocardial PVB19 load. The number of patients at risk is shown at the bottom of the figure. Note that in the group without any LGE only a single patient suffered an event (death due to malignant disease, see text for details) during follow-up

studies [16]. The most likely explanation for this finding is based on the fact that PVB19 genome persistence in human tissues can be life-long without relevant activity and replication [17]. Thus, the detection of PVB19 genome by PCR alone is not sufficient to explain a pathologic effect. This is underscored by recent findings from Bock et al. [15] identifying PVB19 RNA replication intermediates demonstrated by RT-PCR amplification of the NS1 and VP1 regions of the PVB19 genome as a good surrogate parameter for active virus replication, which is (1) related to the inflammatory activity in the myocardium and (2) the clinical course (myocarditis vs. DCM). Furthermore, the authors discuss co-infection with other cardiotropic viruses like HHV6 in combination with host specific determinants as factors reactivating PVB19 replication from long-term persistent or latent infection. This idea is also supported by earlier data from our group describing a co-infection of PVB19 and HHV6 as a predictor for a poor clinical

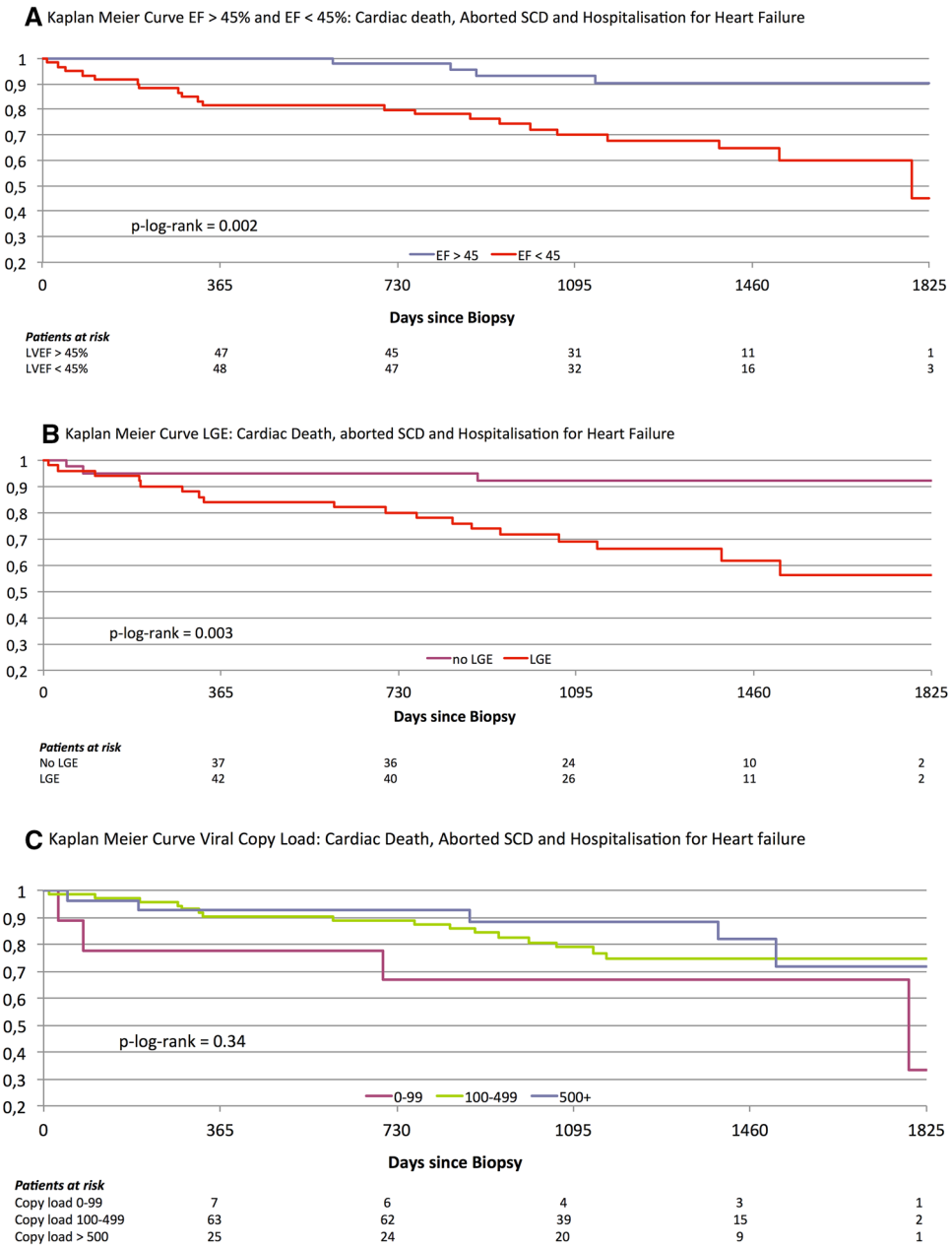

Fig. 4 Kaplan–Meier survival curves—Endpoint 2. Kaplan–Meier survival curves with regard to endpoint 2 (including cardiac death, aborted SCD and hospitalization for heart failure), displayed for LV-EF, presence of LGE, and myocardial PVB19 load. The number of patients at risk is shown at the bottom of the figure

outcome in myocarditis patients [14]. However, additional data are needed to clarify these issues in the future.

Interestingly, despite a trend toward more cardiac deaths in the group with active inflammation (Table 5), we could not confirm myocardial inflammation as a predictor of poor outcome (Table 6 and 7), as other groups have suggested [4]. However, we believe that this is explained by the high incidence of end stage DCM in our population, since end stage DCM patients by definition do not have myocardial inflammation, but are well known for a poor prognosis, which is underscored by our finding that the histologic diagnosis of DCM is a predictor of adverse events.

When looking at long-term predictors for adverse events, we found functional and morphological parameters determined by non-invasive imaging to be most promising. In fact, ventricular size and function upon initial presentation (assessed by echo or CMR) and the presence of LGE were potential predictors for adverse events, whereas the absence of LGE was a predictor for a favorable outcome without suffering any major adverse event. Importantly, no

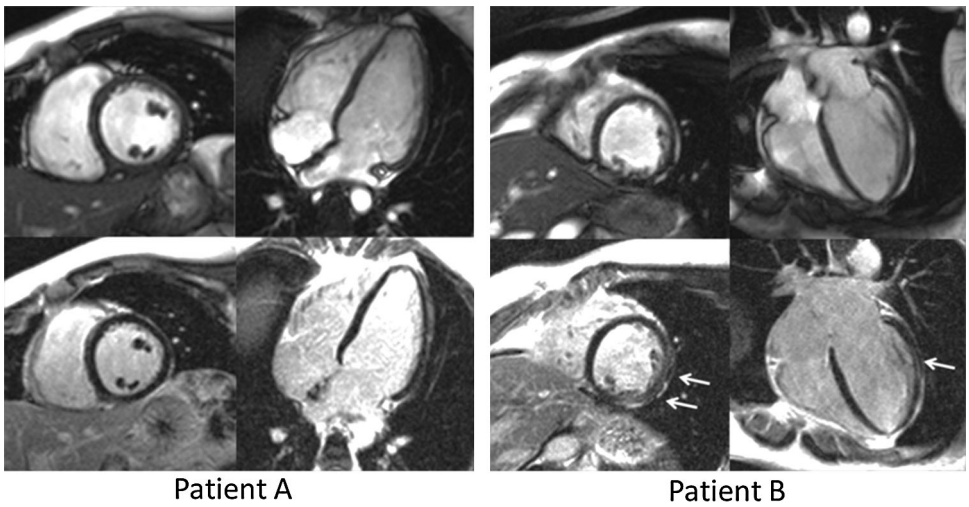

Fig. 5 Typical Example of Patients With and Without Major Adverse Event during Follow-up. The *left panel* (Patient A) shows CMR images of a 29-year-old female patient with normal LV-EF (67 %) and no LGE. Due to chest pain, prior febrile infection, frequent ventricular extrasystoles, and positive troponine, she underwent coronary angiography in which CAD could be ruled out. Myocardial biopsies revealed chronic myocarditis and a PVB19 load of 846 GE. Despite this relatively high viral load, she did not suffer any event during follow up. Patient B displays CMR results of a 40-year-old female presenting with palpitations, fatigue, chest pain, and dyspnea. One month ago she had a severe febrile infection. CMR showed a reduced LV-EF (40 %) and LGE in the posterolateral wall (white arrows), suggesting myocarditis. CAD was ruled out by coronary angiography. Myocardial biopsies revealed chronic myocarditis and 330 GE of PVB19. Despite this relatively low viral load, the patient had reduced LV-EF, positive LGE, and suffered SCD during follow-up

patient with normal LV-EF or the absence of LGE suffered cardiac death during long-term follow-up. This finding underscores the value of cardiac imaging in management and risk stratification of patients with non-ischemic myocardial disease, matching the results of earlier studies [5, 23, 24].

Clinical implications

Based on our data and the results from other groups discussed above, we believe that the detection of PVB19 genome by PCR alone, as well as the viral load in the myocardium determined by this technique does not allow risk stratification in patients suffering from non-ischemic myocardial disease. Whether additional parameters such as PVB19 RNA replication intermediates serving as a surrogate parameter for active virus replication or co-infections with other viruses may play a role in the clinical routine some time in the future needs to be determined by additional studies.

Non-invasive cardiac imaging, however, including ventricular morphology, function, and LGE in particular, appears to be a valuable tool for risk stratification of patients with myocardial disease, which is ready for the clinical routine and—if normal—can give suffering patients and worrying physicians some peace of mind. Note that 90 % of patients reaching endpoint 1 demonstrated LGE in the myocardium, and that no patient with normal LV-EF or the absence of LGE suffered cardiac death during long-term follow-up in the present study, matching earlier results [5, 23, 24].

As described above, we again identified impaired LV-EF and signs of heart failure as important predictors of adverse cardiac events. This reproducible finding [5, 23, 24] once more suggests that one should carefully optimize heart failure therapy in all patients with non-ischemic myocardial disease and any signs of heart failure.

Conclusion

Our data demonstrate that the viral load of PVB19 genomes in the myocardium is not related to the long-term clinical outcome. Furthermore, this study suggests a growing role of imaging parameters such as ventricular size and function, and LGE for risk stratification in patients with non-ischemic myocardial disease.

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Compliance with ethical standards

Conflict of interest None.

Informed consent All patients gave informed consent prior to their inclusion in the study; the study protocol conformed to the ethical guidelines of the 1964 Declaration of Helsinki and its later amendments.

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