ELSEVIER

International Journal of Cardiology 104 (2005) 92 – 100

International Journal of Cardiology

www.elsevier.com/locate/ijcard

Myocardial biopsy based classification and treatment in patients with dilated cardiomyopathy

Oliver Zimmermann[a,*], Matthias Kochs[a], Thomas Paul Zwaka[b,1], Ziya Kaya[c], Philipp Moritz Lepper[a], Magdalena Bienek-Ziolkowski[a], Martin Hoher[a,2], Vinzenz Hombach[a], Jan Torzewski[a]

[a]*Department of Internal Medicine II-Cardiology, University of Ulm, Robert-Koch-Str. 8, 89081 Ulm, Germany*
[b]*Wisconsin Regional Primate Research Center, 1220 Capitol Court, Madison, WI 53715, USA*
[c]*Internal Medicine III-Cardiology, University of Heidelberg, 69115 Heidelberg, Germany*

Received 7 December 2004; received in revised form 16 February 2005; accepted 19 February 2005
Available online 5 May 2005

Abstract

Background: We investigated whether myocardial biopsy analysis for inflammation and viruses correlates with outcome in dilated cardiomyopathy.
Methods: Myocardial biopsies of 82 patients were analyzed for HLAI, HLAII, CD54, CD2, CD68 and entero-/adenovirus. Ejection fraction was determined by left ventriculography. NYHA classification, electrocardiogram (ECG) and echocardiography were analyzed at first admission and for follow up. Patients were attributed to three groups: (A) no inflammation/no virus (B) inflammation/no virus (C) virus with/without inflammation. Patients not responding to conventional treatment of heart failure received interferon β1b (group C) or prednisolone (group B). Median follow up was 7 months (group A), 11 months (group B) and 14.5 months (group C).
Results: Thirty nine patients (48%) belonged to group A, 33 patients (40%) to group B, 10 patients (12%) to group C. Only enterovirus was detected. Ejection fraction at admission was worse for group B compared to group A ($p=0.003$). Groups A and B improved for echochardiography and NYHA ($p \leq 0.001$). Group C improved for echocardiography only ($p=0.031$). Group B showed a better outcome for echocardiography ($p=0.014$) and NYHA ($p=0.023$) than group A.
Conclusions: Inflammatory cardiomyopathy shows the best outcome. Antiinflammatory or antiviral treatment may be an option in patients not responding to conventional therapy.

Keywords: Dilated cardiomyopathy; Prednisolone; Interferon β1b; Outcome

1. Introduction

Dilated cardiomyopathy is a myocardial disease of unknown aetiology [1,2]. The clinical course is characterized by chronic progressive heart failure, with a markedly reduced ventricular ejection fraction [3]. In symptomatic patients the course usually is one of progressive deterioration, with one quarter of newly diagnosed patients dying within a year and half dying within 5 years [4]. Prognosis within some subgroups is reported to be even worse, and greater ventricular enlargement and worse dysfunction tend to correlate with poorer prognosis [5,6]. In contrast, an enormous potential of left ventricular recovery exists in case of fulminant myocarditis [7].

Although the pathogenesis of DCM remains unclear, interest has focussed on three basic mechanisms: firstly,

* Corresponding author. Tel.: +49 731 500 0; fax: +49 731 500 24442.
E-mail address: oliver.zimmermann@medizin.uni-ulm.de (O. Zimmermann).
[1] Current address: Center for Cell and Gene Theraphy & Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA.
[2] Current address: Klinikum Bayreuth, Preuschwitzer Str. 101, 95445 Bayreuth, Germany.

0167-5273/$ - see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.ijcard.2005.02.052

genetic factors, secondly, viral infection and thirdly, immunological abnormalities. Familial linkage is common. In about 20% of patients, a first degree relative also shows evidence of DCM [8,9]. Viral infection is the most common cause of acute myocarditis. Subclinical viral myocarditis may initiate an autoimmune response that causes dilated cardiomyopathy. Particularly, entero- and adenoviruses are an important cause of left ventricular dysfunction [10–13]. The reported frequency of viral genome varies greatly [10,12,14], but the presence of virus within the myocardium is obviously associated with a poorer prognosis [15]. Abnormalities of both, humoral and cellular immunity have been demonstrated in dilated cardiomyopathy patients [16–22]. The reported frequency of inflammation varies and depends on patient selection and diagnostic criteria [23]. Recently, diagnostic markers including HLA I, HLA II, CD19, CD54 and the lymphocyte and macrophage markers CD2 and CD68 have been established to be ubiquitously present in the inflamed myocardium. By use of these markers, in almost 50% of patients with dilated cardiomyopathy a chronic inflammatory process is detectable [24,25]. Thus far only little information exists on whether myocardial biopsy findings correlate with outcome in dilated cardiomyopathy.

Treatment of dilated cardiomyopathy still relies on symptomatic therapy and conventional medication including ACE inhibitors, diuretics, digitalis, beta-blockers, aldosterone antagonists, etc. The value of immunosuppressive and antiviral therapy is not proven, because different studies revealed inhomogeneous results [26–34]. In some studies, patients with dilated cardiomyopathy were treated with immunosuppressive agents [26–29,32,33], and in others with interferon [28,30,31,34]. The very different success rates reported may have two major reasons: Firstly, viral infection was not excluded before immunosuppressive treatment [29,33]. Secondly, most of the studies applied light microscopy only for the diagnosis of inflammation.

In this study we retrospectively analyzed 82 patients with dilated cardiomyopathy who underwent myocardial biopsy in our hospital between 2000 and 2002. Immunohistochemistry of inflammatory markers and virological analysis of myocardial biopsies were used as a basis for diagnostic classification. According to this analysis patients not responding to conventional treatment received additional treatment with prednisolone or interferon β1b. We investigated whether evaluation of myocardial biopsies for inflammation and viral genome is helpful for determining clinical outcome in individuals with dilated cardiomyopathy.

2. Materials and methods

2.1. Patients

Eighty two patients with dilated cardiomyopathy admitted to Ulm University Medical Centre between 2000 and 2002 were included. Patients presented with symptoms of right or left sided heart failure. Acute myocarditis was clinically excluded by physical examination, lack of newly developed ECG changes and blood analysis.

Diagnostic evaluation included NYHA classification, ECG, echocardiography and cardiac catherization. Ejection fraction and right ventricular function were evaluated by left ventriculography and dextrocardiography. Coronary artery disease and valvular, congenital and hypertensive heart disease were excluded. Patients required a complete dataset of NYHA classification, ECG and echocardiography at the time of first diagnosis and for most recent follow up. Patients were seen in our outpatient's department in 3 to 6 months intervals depending on the course of disease and patient compliance. The median follow up period was 7 months (group A), 11 months (group B) and 14.5 months (group C).

2.2. NYHA classification

NYHA functional classification followed the guidelines of the Criteria Committee of the New York Heart Association [35].

2.3. ECG

The 12-lead ECG was analyzed for rhythm, QR-axis, heart rate, bundle branch block, ST deviation and Sokolow index I. Pacemaker triggered conduction was not assessed.

2.4. Echocardiography

Echocardiography was carried out according to international standards [36]. Left ventricular function (LVF) was assessed qualitatively in the apical view and classified as either normal, mildly, moderately or severely decreased. Fractional shortening (FS) was calculated by M-mode. Left ventricular enddiastolic diameter (EDD, mm) and endsystolic diameter (ESD, mm) were obtained from the parasternal view.

2.5. Follow up

The following parameters were assessed:

(A) NYHA-classification (I–IV)
(B) ECG-parameters:
- rhythm: for follow up within groups (Table 4): sinus rhythm (yes or no); for comparative follow up between groups (Table 5): from sinus rhythm to arrhythmia (−1), stable rhythm (0), from arrythmia to sinus rhythm (+1)
- QR-axis: for follow up within groups (Table 4): −30° to 60° (yes or no); for comparative follow up between groups (Table 5): change (+1), no change (−1)
- heart rate: absolute values

Table 1
PCR/nPCR primers

Primer name	Forward 5′→3′	Reverse 5′→3′
GAPDH	TACATGGTCGGGGTGTTG AA	AAGAGAGGCATCCTCACCCT
Adenovirus	ACTACAA(CT)ATTGGCTACCAGG	CAAAACATAAAGAAG(GT)GTGGG
Adenovirus-nested	AACTTCCAGCCCATGAGC(AC)G	CTCAAAAGTCATGTC(GCT)AGCGC
Enterovirus	CGGTACCTTTGTGCGCCTGT	CAGGCCGCCACCGCAGCC
Enterovirus-nested	CCCCGGACTGAGTATCAATA	GGCCGCCAACGCAGCCACCG

– bundle branch block: for follow up within groups (Table 4): bundle branch block (yes or no); for comparative follow up between groups (Table 5): from normal to bundle branch block (−1), no change (0), from bundle branch block to normal (+1)
– ST-deviation and/or Sokolow index I: for follow up within groups (Table 4): ST-deviation and/or Sokolow index I (yes or no); for comparative follow up between groups (Table 5): from normal to ST-deviation and/or Sokolow index I (−1), no change (0), from ST-deviation and/or Sokolow index I to normal (+1)

(C) Echocardiography: for qualitative assessment of left ventricular function (LVF), a rank was applied to each echocardiography: normal (0), mildly decreased (1), moderately decreased (2), severely decreased (3); for qualitative assessment of right ventricular size (RV) a rank was applied to each echocardiography: normal (0), mildly enlarged (1), moderately enlarged (2), severely enlarged (3), for fractional shortening (FS), left ventricular enddiastolic/-systolic diameter and left atrial diameter, absolute values were considered (see also Table 7).

2.6. Myocardial biopsies

All procedures were performed in accordance with the institutional committee on human research and patients gave informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. We used percutaneous transvenous femoral approach with a 1.8-mm Pilling Weck bioptome. Six right ventricular (septal) endomyocardial biopsies were obtained from each patient. Myocardial biopsies were placed in liquid nitrogen immediately, and 5 specimen were

Table 2
Main clinical characteristics of patient population

	No inflammation/ no virus A ($n=39$)	Inflammation B ($n=33$)	Virus C ($n=10$)	p value
Age (years)	54 (21;73)	54 (20;73)	54 (40;68)	A–C $p=0.953$
Sex (m:f)	27:12	24:9	7:3	A–C $p=0.947$
Clinical manifestation to biopsy (months)	3 (0;252)	3 (0;84)	1.5 (0;72)	A–C $p=0.617$
Duration from diagnosis to biopsy (months)	1 (0;96)	0 (0;38)	0 (0;72)	A–C $p=0.710$
Diagnosis=biopsy	27	25	6	A–C $p=0.606$
Follow up period (months)	7 (1;30)	11 (1;35)	14.5 (6;30)	A–C $p=0.022$ A vs. B $p=0.055$ A vs. C $p=0.015$ B vs. C $p=0.262$
EF at biopsy (%)	37 (14;69)	29.5 (7;48)	32 (12;61)	A–C $p=0.013$ A vs. B $p=0.003$ A vs. C $p=0.172$ B vs. C $p=0.594$
Clinical manifestation				
Symptomatic arrhythmia	10	9	3	A–C $p=0.960$
Decompensated heart failure	21	15	4	A–C $p=0.652$
Chronic progressive heart failure	7	8	3	A–C $p=0.656$
Random	1	1	0	A–C $p=0.860$
Administered drugs				
ACE-inhibitor/AT II receptor blocker	35→30	33→30	10→9	–
Diuretics	31→31	30→27	8→8	–
Digitalis	18→21	19→15	5→6	–
β-receptor blocker	32→34	30→30	8→10	–
Prednisiolone	–	2	–	–
Interferon β1b	–	–	3	–

Quantitative parameters: median (range). Qualitative parameters: absolute number of patients. A–C: overall test. "vs." indicates pairwise tests. →: follow up.

cryofixed. One sample was paraffin embedded and subjected to histomorphological analysis. Three specimens were used for virus PCR (2 for enterovirus and 1 for adenovirus), and 1 for immunohistochemical analysis. One biopsy remained as back-up.

Dilated cardiomyopathy was histomorphologically diagnosed by examination of interstitial fibrosis, cellular infiltrates, cellular hypertrophy and myocardial cell degeneration [37]. Active myocarditis was excluded according to the Dallas criteria [38].

Immunohistochemical analysis included staining for HLA I, HLA II, CD2, CD68 and CD54 (DAKO, Germany). Five sections from a single biopsy were analyzed. Peroxidase-conjugated horse anti-mouse antibody (Vector, Germany) served as secondary antibody. A semiquantitative score system [staining intensity from no discernible to strongly abundant immunoreactivity (0 to +++)] was applied for HLA I, HLA II and CD54. CD2 and CD68 positive cells were counted under the light microscope (cells/mm^2). Biopsies were classified as borderline inflammation (3 to 10 CD2 positive cells/mm^2, subgroup B1) or inflammatory cardiomyopathy (> 10 CD2 positive cells/mm^2, subgroup B2). Diagnostic classification was not based on CD68 count.

Virological analysis for enteroviral RNA and adenoviral DNA was performed by PCR (primers see Table 1) and confirmed by Southern blot analysis. Total RNA/DNA was

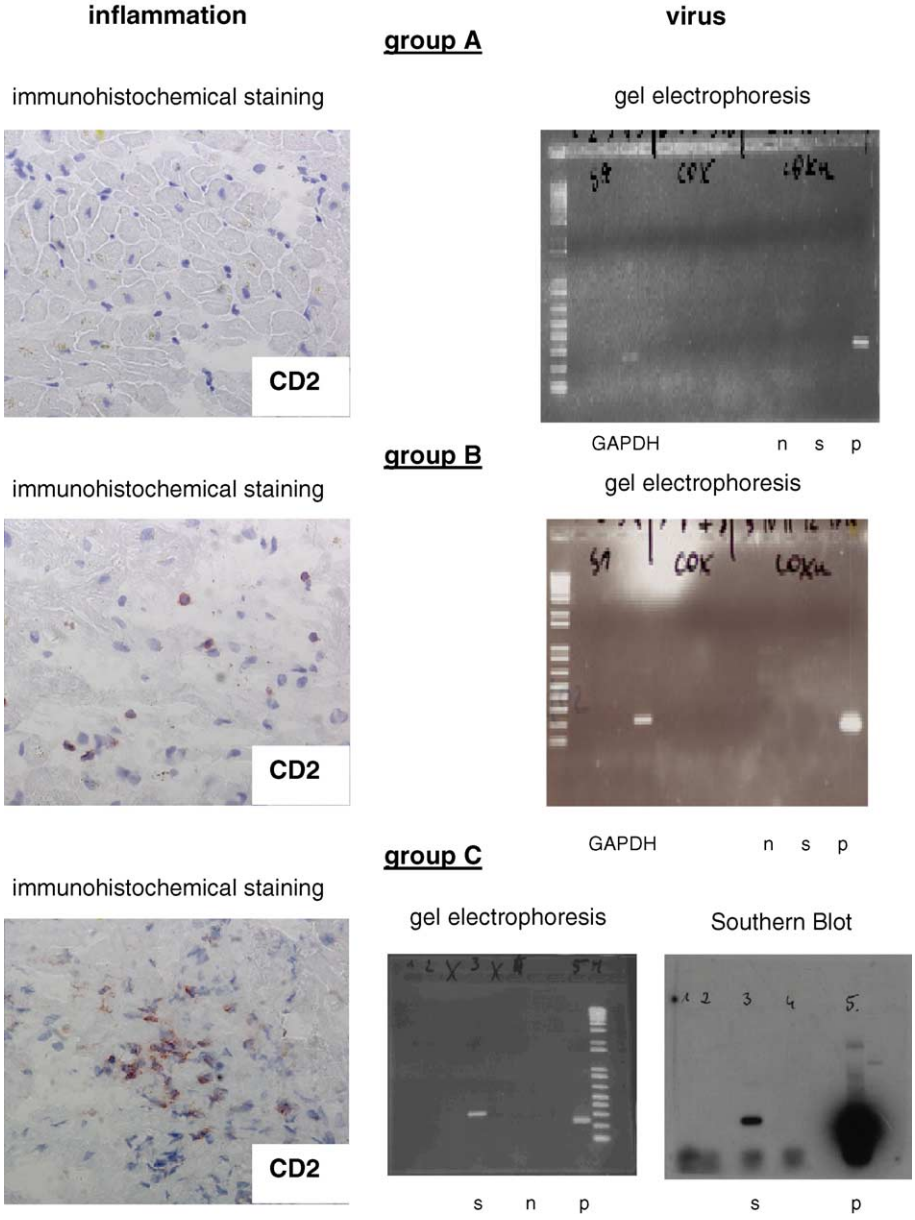

Fig. 1. Endomyocardial biopsy evaluation. Group A: no virus/no inflammation. Group B: inflammation/no virus. Group C: virus. Immunohistochemical staining for CD2 (left) and nPCR (right). n: negative control; p: positive control; s: sample. Bottom right shows virus positive sample and Southern Blot analysis.

extracted using RNeasy®-Protect-Mini-Kit/QIAamp®-DNA-Mini-Kit (Qiagen). For RNA viruses, RT-PCR was used to obtain cDNA. PCR products were amplified using HotStar®-Master-Mix-Kit (Qiagen). PCR was followed by nested PCR (nPCR). The GAPDH gene was used to demonstrate loading of intact DNA. Adenovirus DNA (Type 2) from GIBCOBRL (Cat. No. 15270-010) and a T-easy cloned enteroviral template kindly provided by Professor Dr. H.P. Schultheiss (Berlin, Germany) were used as positive controls.

2.7. Treatment protocol

Patients continuously received standard medication according to ACC/AHA guidelines [39]. Specific therapy with interferon β1b (virus positive) or prednisolone (inflammatory dilated cardiomyopathy) was applied to patients showing either no improvement or worsening of clinical symptoms, NYHA classification and echocardiography after 3 months.

Prednisolone (Decortin® H, Merk KGaA, Germany) was applied at an initial dose of 1 mg kg^{-1} body weight daily. Every 2 weeks the dose was reduced stepwise by 20 mg down to a maintenance dose of 7.5 mg/day. Treatment was continued until week 24.

2×10^6 units interferon β1b (Betaferon®, Schering AG, Germany) were applied subcutaneously three times a week during the first week. During the second week 4×10^6 units were applied per subcutaneous injection. At week 3 patients received the maintenance dose of 6×10^6 units per subcutaneous injection thrice weekly until week 24.

2.8. Statistical analyses

Statistical analysis was performed using SigmaStat version 2.0 software. Tests included One-Way Analysis of Variance (ANOVA), Kruskal–Wallis One-Way Analysis of Variance on Ranks, Mann–Whitney Rank Sum Test, Wilcoxon Signed Rank Test, McNemar's Test, *t*-Test and Chi-square Test. Tests were calculated two-sided. Paired tests were performed for follow up within groups (Table 4A), unpaired tests for comparative follow up between groups (Table 5). In Tables 2, 3, 5 and 6 overall tests were performed first. If these tests revealed a $p<0.05$, pairwise tests were calculated. Statistical significance (as highlighted in red colour) was assumed only in case of a $p<0.05$ for both, overall and pairwise analysis.

3. Results

3.1. Endomyocardial biopsy evaluation and clinical characteristics

Main demographic characteristics of the patient population are shown in Table 2. According to biopsy results patients were attributed to groups A to C. The following classification was applied to the results of biopsy analysis: (A) no virus and no inflammation (B) inflammation (borderline inflammation and inflammatory cardiomyopathy) but no virus (C) virus with or without inflammation. 39 (48%) patients belonged to group A, 33 (40%) patients to group B and 10 (12%) patients to group C. Within group C, the only virus detected was enterovirus. Six patients in group C showed borderline inflammation, one patient showed inflammatory cardiomyopathy, and three patients showed no inflammation. Fig. 1 depicts an example of biopsy analysis for each group.

Age, male:female ratio, clinical manifestation and median duration of symptoms before biopsy were not different between groups. Ejection fraction was worse for group B compared to group A at the time of myocardial biopsy ($p=0.003$). The follow up period was longer for group C compared to group A ($p=0.015$). Two patients in group B were treated with prednisolone and three patients in group C with interferon β1b.

3.2. Correlation of CD2 and additional markers of inflammation

Table 3 demonstrates the correlation between lymphocyte count and additional markers of inflammation. In general, a similar trend was observed in all groups showing an association between lymphocyte count and HLA I, HLA II, CD54 and CD68. This trend revealed statistical significance for group A versus group B1 (borderline inflammation) concerning HLA II, CD54 and CD68 and for group A versus group B2 (inflammation) concerning HLA II.

Table 3
Correlation of CD2 and additional markers of inflammation

	CD2<3 cells/mm²	CD2: 3–10 cells/mm²	CD2>10 cells/mm²	*p* value
	A (*n*=39)	B1 (*n*=25)	B2 (*n*=8)	
HLA I	1 (0;3.5)	1 (0;5)	3 (0,5;5)	A–B2 $p=0.081$
HLA II	2 (0;5)	2 (0;4)	3 (1;4)	A–B2 $p=0.013$
				A vs. B1 $p=0.017$
				A vs. B2 $p=0.032$
				B1 vs. B2 $p=0.437$
CD54	0.5 (0;2.5)	1 (0;2)	1 (0.5;3.5)	A–B2 $p=0.015$
				A vs. B1 $p=0.015$
				A vs. B2 $p=0.059$
				B1 vs. B2 $p=0.784$
CD68	13 (0;58)	32 (0;75.2)	24 (0;700)	A–B2 $p=0.003$
				A vs. B1 $p<0.001$
				A vs. B2 $p=0.102$
				B1 vs. B2 $p=0.817$

Quantitative parameter (interstitial CD68 cells/mm²): median (range). Semiquantitative staining for HLA I, HLA II and CD54 on cardiomyocytes and interstitial cells: no (0), mildly (1), moderately (2), severely (3); 0.5 indicates intermediate staining: median (range). A–C: overall test. "vs." indicates pairwise tests.

Table 4
Follow up within groups

A: Overall evaluation

	NYHA	ECG					Echocardiography	
		Sinus rhythm	HR (min)	QR-axis	Bundle branch block	ST-deviation/Sokolow I	LVF (qual.)	LVF (quant.)/FS (%)
A	I: 1→6	24→29	83 (52;250)	23→21	15→16	26→21	0: 5→8	20 (5;44)
	II: 3→20		→				1: 2→9	→
	III: 17→12		69.5 (44;100)				2: 9→14	25 (8;50)
	IV: 18→1						3: 23→8	
	$p \leq 0.001$	$p = 0.063$	$p \leq 0.001$	$p = 0.683$	$p = 0.813$	$p = 0.206$	$p \leq 0.001$	$p \leq 0.001$
B	I: 2→9	23→23	88 (50;160)	24→23	10→9	24→16	0: 0→13	17 (8;32)
	II: 1→21		→				1: 4→6	→
	III: 10→3		70 (50;172)				2: 7→9	28.5 (15;39)
	IV: 20→0						3: 20→3	
	$p \leq 0.001$	$p = 1.000$	$p = 0.001$	$p = 1.000$	$p = 0.813$	$p = 0.058$	$p \leq 0.001$	$p \leq 0.001$
C	I: 0→1	8→7	80.5 (65;135)	6→7	3→4	8→7	0: 0→1	16 (8;21)
	II: 2→4		→				1: 0→5	→
	III: 4→4		70.5 (44;106)				2: 2→0	20 (9;31)
	IV: 4→1						3: 8→4	
	$p = 0.094$	$p = 1.000$	$p = 0.322$	$p = 1.000$	$p = 0.750$	$p = 0.750$	$p = 0.031$	$p = 0.004$

B: Conventional and specific therapy vs. conventional therapy only

Patient	NYHA	LVF (qual.)	FS (%)	therapy
Prednisolone				
R.B., male, 65 years	4→2	3→3	20→16	conventional+prednisolone
W.U., female, 64 years	3→3	3→3	20→15	conventional+prednisolone
Others	4→2	3→1	17→29	conventional only
Interferon β1b				
N.H., male, 52 years	4→2	3→3	16→n.a.	conventional+interferon
A.H., male, 56 years	4→2	3→3	16→16	conventional+interferon
W.H., male, 69 years	3→3	3→3	8→9	conventional+interferon
Others	3→3	3→1	18.5→24	conventional only

Quantitative parameters: median (range). Semiquantitative parameters: absolute numbers; for left ventricular function (qual.): normal (0), mildly decreased (1), moderately decreased (2), severely decreased (3). Paired tests within the same group (time of diagnosis and most recent follow up).
FS: fractional shortening; LVF: left ventricular function. Single patients: absolute numbers. Others: median. n.a.: not assessable. →: follow up.

3.3. Follow up results

Table 4 shows the follow up results within groups. Overall evaluation of groups (Table 4A) revealed that: For NYHA classification, groups A and B improved significantly, whereas patients in group C reported no statistically significant improvement in clinical symptoms. For ECG parameters, the only significant results were observed for

Table 5
Comparative follow up between groups

		NYHA	ECG					Echocardiography	
			Sinus rhythm	HR (min)	QR-axis	Bundle branch block	ST-deviation/ Sokolow I	LVF (qual.)	LVF (quant.)/ FS (%)
Overall test	A–C	$p = 0.012$	$p = 0.038$	$p = 0.311$	$p = 0.111$	$p = 0.682$	$p = 0.709$	$p = 0.037$	$p = 0.026$
Pairwise tests	A vs. B	−1 (1;−2) vs. −2 (0;−3) $p = 0.023$	0 (0;1) vs. 0 (−1;1) $p = 0.364$	−19 (−169;30) vs. −16 (−92;62)	9 vs. 11	0 (−1;1) vs. 0 (−1;1)	0 (−1;1) vs. 0 (−1;1)	−1 (1;−3) vs. −1 (0;−3) $p = 0.016$	2 (−11;20) vs. 10 (−7;27) $p = 0.014$
	A vs. C	−1 (1;−2) vs. −0.5 (1;−2) $p = 0.280$	0 (0;1) vs. 0 (−1;0) $p = 0.134$	−19 (−169;30) vs. −5 (−60;23)	9 vs. 6	0 (−1;1) vs. 0 (−1;1)	0 (−1;1) vs. 0 (−1;1)	−1 (1;−3) vs. −1 (0;−3) $p = 0.358$	2 (−11;20) vs. 2.5 (−1;12) $p = 0.926$
	B vs. C	−2 (0;−3) vs. −0.5 (1;−2) $p = 0.026$	0 (−1;1) vs. 0 (−1;0) $p = 0.390$	−16 (−92;62) vs. −5 (−60;23)	11 vs. 6	0 (−1;1) vs. 0 (−1;1)	0 (−1;1) vs. 0 (−1;1)	−1 (0;−3) vs. −1 (0;−3) $p = 0.403$	10 (−7;27) vs. 2.5 (−1;12) $p = 0.105$

FS: fractional shortening; LVF: left ventricular function. First line (A–C): overall test. Following lines: pairwise tests. Quantitative parameters: median (range) of the differences between time of diagnosis and most recent follow up. Qualitative parameters: changes (QR-axis). Results relate to the changes from baseline.

Table 6
Cardiac events during follow up

	No inflammation/ no virus A ($n=39$)	Inflammation B ($n=33$)	Virus C ($n=10$)	p value
ICD implantation	2	2	3	A–C $p=0.034$ A vs. B $p=1.000$ A vs. C $p=0.051$ B vs. C $p=0.073$
Pacemaker implantation	1	4	0	A–C $p=0.166$
Cardiac resynchronisation (biventricular pacemaker)	1	1	0	A–C $p=0.860$
Cardiac decompensation	3	5	2	A–C $p=0.455$
Symptomatic arrhythmia	12	6	4	A–C $p=0.294$
Death	1	0	0	A–C $p=0.572$

Absolute number of patients is described. One patient may belong to more than one event group. A–C: overall test. "vs." indicates pairwise test.

reduction in heart rate within group A and B. Concerning echocardiographic parameters, a significant improvement was observed for all groups.

Table 4B shows an assessment of results for patients who received conventional and specific therapy vs. results for patients who received conventional therapy only.

Table 5 shows comparative follow up between groups. For NYHA classification, comparison between first diagnosis and most recent documentation revealed a better outcome for group B compared to group A ($p=0.023$) and group C ($p=0.026$). For ECG, no significant differences were observed. Regarding echocardiography, comparison between first diagnosis and most recent documentation revealed a better outcome for group B compared to group A ($p=0.016$ for LVF qual.; $p=0.014$ for FS).

3.4. Cardiac events during follow up

Table 6 shows cardiac events during follow up. No significant differences were observed for all parameters listed. One patient only died of cardiac death.

3.5. Additional echocardiographic parameters

Table 7 shows an evaluation of additional echocardiographic parameters (left ventricular endsystolic and enddiastolic diameter, left atrial diameter and right ventricular diameter).

4. Discussion

Eighty two patients with dilated cardiomyopathy were retrospectively analyzed. According to myocardial biopsy findings, patients were classified, outcome was evaluated and specific therapy was considered.

Three major observations were made:

(1) The best outcome was observed for patients with inflammatory dilated cardiomyopathy, although these patients presented with the worst left ventricular ejection fraction at the time of myocardial biopsy.

Table 7
Additional echocardiographic parameters

Echocardiographic parameter	No inflammation/no virus A ($n=39$)	Inflammation B ($n=33$)	Virus C ($n=10$)	p value
LVEDD	62 (44; 91) → 59 (46; 87) $p=0.034$	66.5 (51; 76) → 58 (43; 76) $p<0.001$	59.5 (53; 87) → 57 (48; 86) $p=0.005$	A–C (FD) $p=0.121$ A–C (FU) $p=0.584$
LVESD	49 (26; 82) → 44.5 (26; 69) $p=0.007$	56 (40; 70) → 42 (29; 61) $p<0.001$	53 (43; 73) → 43 (33; 72) $p=0.007$	A–C (FD) $p=0.166$ A–C (FU) $p=0.427$
LA size	45.5 (32; 59) → 42 (29; 62) $p=0.128$	48 (34; 67) → 45 (28; 64) $p=0.014$	49 (37; 59) → 46 (34; 62) $p=0.128$	A–C (FD) $p=0.329$ A–C (FU) $p=0.739$
RV size (qual.)	0 (0; 3) → 0 (0; 2) $p=0.820$	0 (0; 2) → 0 (0; 2) $p=0.018$	1.5 (0; 3) → 0 (0; 3) $p=0.031$	A–C (FD) $p=0.003$ A vs. B $p=0.218$ A vs. C $p=0.004$ B vs. C $p=0.019$ A–C (FU) $p=0.328$

LVEDD, left ventricular enddiastolic diameter; LVESD, left ventricular endsystolic diameter; LA, left atrium; RV, right ventricle; FD, first diagnosis; FU, follow up; →, follow up. "vs." indicates pairwise test.

(2) Independently from biopsy results all groups showed a significant improvement of echocardiographic parameters during follow up on standard oral treatment. Despite the bad prognosis attributed to dilated cardiomyopathy [4], only one death was reported in our study population.
(3) 48% of the patients had neither virus nor inflammation, 40% had inflammation without virus, and 12% had viral infection (enterovirus).

The observed distribution of patients is in line with earlier reports on the prevalence of inflammation and viral genome [2,10,11,21–24]. The fact that patients with inflammatory cardiomyopathy (group B) showed the best outcome concerning echocardiographic parameters and NYHA classification is a novel observation. Though it is possible that the greater extent of improvement could be related to a greater opportunity for improvement based solely on the initially greater severity of symptoms and dysfunction, the medians for fractional shortening after therapy support our interpretation that the greater extent of improvement is not only a relative, but an absolute advantage of group B.

As it is not possible to predict classification of patients in groups A–C from clinical symptoms, myocardial biopsy may provide important prognostic information. So far, only few information exists on that issue [40]. The observation is especially surprising in view of the fact that patients in group B started with a significantly worse ejection fraction at the time of myocardial biopsy. Up to now, in dilated cardiomyopathy greater ventricular enlargement and worse dysfunction were thought to correlate with poorer prognosis [5]. Inflammatory activity, however, may indicate repair mechanisms in the myocardium that support improvement under adjuvant treatment [7].

The benefit of immunosuppressive and antiviral therapy is discussed controversially, which may be explained either by lack of exclusion of viral infection before applying immunosuppressive drugs [29,33] or by use of light microscopy for the diagnosis of inflammation only.

This is the first study applying prednisolone and interferon β1b in addition to conventional treatment depending on biopsy results and clinical course within one study population. As only those patients who failed to improve or deteriorated under conventional medication received additional treatment with interferon β1b (virus positive) or prednisolone (inflammation without virus), few patients needed additional treatment. Concerning adverse drug effects we would like to refer to previous studies [31,32]. Patients reported flu-like side effects of interferon β1b during the first 3 weeks of treatment. During immunosuppressive therapy patients reported an increase in body weight. In order to evaluate the benefit of specific therapy, prospective multicenter studies in larger populations are warranted.

Our study has several limitations: (i) The retrospective study design. (ii) The relatively low number of patients with prevalent viral genome. As for these patients the longest follow up period was documented (most likely due to a better compliance in awareness of the biopsy findings) whereas the follow up was significantly shorter, in other groups a substantial bias for the statistical analysis of cardiac events can be assumed. (iii) Other studies used prednisolone in combination with azathioprine which may lead to more beneficial results of immunosuppressive treatment. (iv) As our data are limited to patients with dilated cardiomyopathy with enteroviral infection, no conclusions for other cardiotropic viruses such as adenovirus, parvovirus B19, Epstein-Barr virus, etc. can be drawn. Methodically, analysis for viral genome was limited to detection of adeno- and enterovirus. (v) The number of patients that received specific therapy is too small to draw valid conclusions on the outcome. Prospective multicenter studies in larger populations are warranted.

These limitations notwithstanding, our study demonstrates for the first time that patients with inflammatory cardiomyopathy show the best outcome. Myocardial biopsy based differential therapy may be an additional option in patients not responding to conventional treatment.

Acknowledgements

We acknowledge the staff of the catherization laboratory for support and the Department of Statistics for statistical advice.

References

[1] Richardson P, McKenna W, Bristow M, et al. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the definition and classification of cardiomyopathies. Circulation 1996;93:841–2.
[2] Calabrese F, Thiene G. Myocarditis and inflammatory cardiomyopathy: microbiological and molecular biological aspects. Cardiovasc Res 2003;60:11–25.
[3] Fuster V, Gersh BJ, Giuliani ER, Tajik AJ, Brandenburg RO, Frye RL. The natural history of idiopathic dilated cardiomyopathy. Am J Cardiol 1981;47:525–31.
[4] Dec GW, Fuster V. Idiopathic dilated cardiomyopathy. N Engl J Med 1994;331:1564–75.
[5] Coughlin SS, Neaton JD, Sengupta A, Kuller LH. Predictors of mortality from idiopathic dilated cardiomyopathy in 356,222 men screened for the multiple risk factor intervention trial. Am J Epidemiol 1994;139:166–72.
[6] Dries DL, Exner DV, Gersh BJ, Cooper HA, Carson PE, Domanski MJ. Racial differences in the outcome of left ventricular dysfunction. N Engl J Med 1999;340:609–16.
[7] McCarthy III RE, Boehmer JP, Hruban RH, et al. Long-term outcome of fulminant myocarditis as compared with acute (nonfulminant) myocarditis. N Engl J Med 2000;342:690–5.
[8] Baig MK, Goldman JH, Caforio AL, et al. Familial dilated cardiomyopathy: cardiac abnormalities are common in asymptomatic relatives and may represent early disease. J Am Coll Cardiol 1998; 31:195–201.

[9] McKenna CJ, Codd MB, McCann HA, et al. Idiopathic dilated cardiomyopathy: familial prevalence and HLA distribution. Heart 1997;77:549–52.
[10] Pauschinger M, Bowles NE, Fuentes-Garcia FJ, et al. Detection of adenoviral genome in the myocardium of adult patients with idiopathic left ventricular dysfunction. Circulation 1999;99:1348–54.
[11] Editorials. Dilated cardiomyopathy and enterovirus. Lancet 1990;336: 971–3.
[12] Pauschinger M, Doerner A, Kuehl U, et al. Enteroviral RNA replication in the myocardium of patients with left ventricular dysfunction and clinically suspected myocarditis. Circulation 1999;99:889–95.
[13] Fujioka S, Kitaura Y, Ukimura A, et al. Evaluation of viral infection in the myocardium of patients with idiopathic dilated cardiomyopathy. J Am Coll Cardiol 2000;36:1920–6.
[14] Rose NR, Hill SL. The pathogenesis of postinfectious myocarditis. Clin Immunol Immunopathol 1995;80:92–9.
[15] Why HJ, Meany BT, Richardson PJ, et al. Clinical and prognostic significance of detection of enteroviral RNA in the myocardium of patients with myocarditis or dilated cardiomyopathy. Circulation 1994; 89:2582–9.
[16] Luppi P, Rudert WA, Zanone MM, et al. Idiopathic dilated cardiomyopathy: a superantigen-driven autoimmune disease. Circulation 1998;98:777–85.
[17] Liu PP, Opavsky MA. Viral myocarditis: receptors that bridge the cardiovascular with the immune system? Circ Res 2000;86:253–4.
[18] Zwaka TP, Manolov D, Ozdemir C, et al. Complement and dilated cardiomyopathy: a role of sublytic terminal complement complex-induced tumor necrosis factor-alpha synthesis in cardiac myocytes. Am J Pathol 2002;161:449–57.
[19] Lauer B, Schannwell M, Kuhl U, Strauer BE, Schultheiss HP. Antimyosin autoantibodies are associated with deterioration of systolic and diastolic left ventricular function in patients with chronic myocarditis. J Am Coll Cardiol 2000;35:11–8.
[20] Neumann DA, Lane JR, Wulff SM, et al. In vivo deposition of myosin-specific autoantibodies in the hearts of mice with experimental autoimmune myocarditis. J Immunol 1992;148:3806–13.
[21] Fairweather D, Kaya Z, Shellam GR, Lawson CM, Rose NR. From infection to autoimmunity. J Autoimmun 2001;16:175–86.
[22] Kanzaki Y, Terasaki F, Okabe M, et al. Myocardial inflammatory cell infiltrates in cases of dilated cardiomyopathy as a determinant of outcome following partial left ventriculectomy. Jpn Circ J 2001; 65:797–802.
[23] Kasper EK, Agema WR, Hutchins GM, Deckers JW, Hare JM, Baughman KL. The causes of dilated cardiomyopathy: a clinicopathologic review of 673 consecutive patients. J Am Coll Cardiol 1994; 23:586–90.
[24] Kuhl U, Noutsias M, Seeberg B, Schultheiss HP. Immunohistological evidence for a chronic intramyocardial inflammatory process in dilated cardiomyopathy. Heart 1996;75:295–300.
[25] Kuhl U, Noutsias M, Seeberg B, Schannwell M, Welp LB, Schultheiss HP. Chronic inflammation in the myocardium of patients with clinically suspected dilated cardiomyopathy. J Card Fail 1994;1:13–25.
[26] Hufnagel G, Pankuweit S, Richter A, Schonian U, Maisch B. The European study of epidemiology and treatment of cardiac inflammatory diseases (ESETCID). First epidemiological results.. Herz 2000;25:279–85.
[27] Parrillo JE, Cunnion RE, Epstein SE, et al. A prospective, randomized, controlled trial of prednisone for dilated cardiomyopathy. N Engl J Med 1989;321:1061–8.
[28] Heim A, Stille-Siegener M, Kandolf R, Kreuzer H, Figulla HR. Enterovirus-induced myocarditis: hemodynamic deterioration with immunosuppressive therapy and successful application of interferon-alpha. Clin Cardiol 1994;17:563–5.
[29] Mason JW, O'Connell JB, Herskowitz A, et al. A clinical trial of immunosuppressive therapy for myocarditis. The myocarditis treatment trial investigators. N Engl J Med 1995;333:269–75.
[30] Lutton CW, Gauntt CJ. Ameliorating effect of IFN-beta and anti-IFN-beta on coxsackievirus B3-induced myocarditis in mice. J Interferon Res 1985;5:137–46.
[31] Kuhl U, Pauschinger M, Schwimmbeck PL, et al. Interferon-β treatment eliminates cardiotropic viruses and improves left ventricular function in patients with myocardial persistence of viral genomes and left ventricular dysfunction. Circulation 2003;107:2793–8.
[32] Wojnicz R, Nowalany-Kozielska E, Wojciechowska C, et al. Randomized, placebo-controlled study for immunosuppressive treatment of inflammatory dilated cardiomyopathy: two-year follow-up results. Circulation 2001;104:39–45.
[33] Frustaci A, Chimenti C, Calabrese F, Pieroni M, Thiene G, Maseri A. Immunosuppressive therapy for active lymphocytic myocarditis: virological and immunologic profile of responders versus nonresponders. Circulation 2003;107:857–63.
[34] Daliento L, Calabrese F, Tona F, et al. Successful treatment of enterovirus-induced myocarditis with interferon-alpha. J Heart Lung Transplant 2003;22:214–7.
[35] The Criteria Committee of the New York Heart Association. Diseases of the heart and blood vessels. Nomenclature and criteria for diagnosis, 6th ed. Boston, MA: Little Brown; 1964.
[36] Henry WI, Gardin JM, Ware JH. Echocardiographic measurements in normal subjects from infancy to old age. Circulation 1980;62: 1054–61.
[37] Edwards WD. Cardiomyopathies. Hum Pathol 1987;18:625–35.
[38] Aretz HT. Myocarditis: the Dallas criteria. Hum Pathol 1987;18: 619–24.
[39] Hunt SA, Baker DW, Chin MH, et al. American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1995 Guidelines for the Evaluation and Management of Heart Failure); International Society for Heart and Lung Transplantation; Heart Failure Society of America. ACC/AHA Guidelines for the Evaluation and Management of Chronic Heart Failure in the Adult: Executive Summary A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1995 Guidelines for the Evaluation and Management of Heart Failure): Developed in Collaboration With the International Society for Heart and Lung Transplantation; Endorsed by the Heart Failure Society of America. Circulation 2001;104:2996–3007.
[40] Angelini A, Crosato M, Boffa GM, et al. Active versus borderline myocarditis: clinicopathological correlates and prognostic implications. Heart 2002;87:210–5.