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ORIGINAL PAPER

Long-term outcome of patients with virus-negative chronic myocarditis or inflammatory cardiomyopathy after immunosuppressive therapy

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Abstract
Aim To analyze the long-term outcome after immunosuppressive treatment of patients with virus-negative chronic myocarditis or inflammatory cardiomyopathy (CMi).
Methods and results We investigated 114 patients with endomyocardial biopsy (EMB)-proven virus-negative chronic myocarditis or CMi, who were treated with prednisone and azathioprine for 6 months. Myocardial inflammation was assessed by quantitative immunohistology. We examined hemodynamic measurements after 6 months and long-term follow-up periods of up to 10 years {median 10.5 months [95 % confidence interval (CI) 11.69–59.16]}. At follow-up, the patients showed a significant improvement of left ventricular ejection fraction (LVEF) compared to baseline after 6-month period (LVEF rising from 44.6 ± 17.3 to 51.8 ± 15.5 %, $p = 0.006$) and in the long-term follow-up (LVEF 52.1 ± 15.6 %, $p = 0.006$). Simultaneously, EMB-analysis revealed significant reduction of quantified inflammatory infiltrates ($CD3^+$ cells 16.03 ± 29.09–8.2 ± 9.0/mm^2, $p = 0.002$; $CD2^+$ cells 12.62 ± 20.01 to 6.61 ± 8.47/mm^2, $p = 0.001$; perforin$^+$ cells 3.94 ± 4.65–1.03 ± 1.47/mm^2, $p = 0.0001$), and cell-adhesion molecule HLA-1 [9.91 ± 5.55–6.65 ± 2.81/area fraction (AF), $p = 0.0001$]. In a subgroup analysis, patients with initial LVEF ≤45 % ($n = 53$) significantly increased with LVEF at follow-up (29.3 ± 8.8–41.7 ± 13.2–42.1 ± 13.1 %, $p < 0.0001$, Group I), defined as CMi. Patients with initial LVEF >45–60 % ($n = 25$) significantly improved further or recovered completely, regarding LVEF (53.0 ± 3.6–59.0 ± 9.4–59.8 ± 10.0 %, $p = 0.03$, Group II). Patients with initial LVEF >60 % ($n = 36$) remained stable and did not deteriorate over long-term follow-up (68.8 ± 6.7–67.5 ± 10.9–68.8 ± 10.7 %, $p = 0.5$, Group III). Groups II and III were defined as chronic myocarditis.
Conclusions In patients with virus-negative chronic myocarditis or CMi, we could show the effectiveness and beneficial effects of immunosuppressive treatment. Based on the normalization of the inflammatory process LVEF improvement is lasting for a long-term period of time.

Keywords Inflammatory cardiomyopathy · Immunosuppressive therapy · Endomyocardial biopsy · Intramyocardial inflammation · Long-term follow-up

Abbreviations

AF	Area fraction
CAMs	Cell-adhesion molecules
CMi	Inflammatory cardiomyopathy
DIA	Digital image analysis
EMB	Endomyocardial biopsy
HLA-1	Human leukocyte antigen-1

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IH	Immunohistology
LVEDD	Left ventricular end-diastolic diameter
LVESD	Left ventricular end-systolic diastolic diameter
LVEF	Ejection fraction
Npcr	Nested polymerase chain reaction
RT-PCR	Reverse transcription PCR

Introduction

Inflammatory cardiomyopathy (CMi) represents a major cause of heart failure with a potential of transition to the clinical picture of end-stage heart failure [1–3].

Despite the advancement of diagnostic techniques based on endomyocardial biopsies (EMBs) in defining the etiology and pathophysiology of CMi [4, 5], a specific standardized treatment is not yet available. From the perspective of pathogenesis, chronic and insidiously progressive inflammation-mediated tissue injury will result if an inflammatory response against the myocardium triggered by infectious agents or other tissue injuries is not adequately controlled [6–12]. The primary purpose of the inflammatory response aiming at adequate tissue repair or reparative remodeling will then have failed and the need for immunosuppression arises.

Immunosuppressive treatment, including prednisolone and azathioprine, are reportedly effective for the recovery of left ventricular (LV) systolic function in patients who exhibit virus-negative CMi [13–15]. The first randomized clinical trials by Mason et al. that sought to determine whether anti-inflammatory treatment is effective for the treatment of myocarditis did not see changes in the left ventricular ejection fraction after treatment [24]. However, they just analyzed the EMBs by histology according to the Dallas criteria and did not look for evidence of viral infection at this time. To this end, in a retrospective analysis of Frustaci et al. a virological and immunological profile of patients with active lymphocytic myocarditis receiving immunosuppressive therapy revealed 90 % rate responsiveness in those patients with a negative cardiac polymerase chain reaction to the main cardiotropic viruses. Conversely, myocardial viral genomes were detectable in 85 % of non-responders [25].

Moreover, Frustaci et al. reported the randomized placebo-controlled TIMIC trial of immunosuppressive treatment in 85 patients. Results of the TIMIC trial confirmed the positive impact of immunosuppression on recovery of LV function in a high rate (88 %) of patients. A striking improvement occurred even in patients with extreme LV dysfunction and it was accompanied at histological examination by the disappearance of inflammatory infiltrates. However, the efficacy of therapy was evaluated solely at the end of the 6 month treatment [16, 17].

These findings prompted us to validate the efficacy of immunosuppressive treatment in chronic myocarditis or CMi in a long-term follow-up. The aim of the present study was to confirm the positive effect of an immunosuppressive therapy and to evaluate the hemodynamic long-term outcome of virus-negative chronic myocarditis or CMi patients after immunosuppressive therapy.

Patients and methods

Patients

In the current study we included 114 patients with EMB-proven virus-negative chronic myocarditis or CMi.

All patients admitted to our hospital in the time period from 2005 to 2008 with EMB-proven virus-negative chronic myocarditis or CMi, who were treated immunosuppressive, were included into the study, consecutively. Data were analyzed retrospectively.

All patients complained for symptoms of heart failure of unknown cause for at least 6 months, despite more than 2 months' stable clinical status and stable optimal conventional heart failure medication with angiotensin-converting-enzyme inhibitors, β-adrenergic blocking drugs, and diuretics. Coronary artery disease and other possible causes of myocardial dysfunction (hypertension, valvular heart disease) had been excluded by angiography prior to EMB in all patients. Patients were screened by EMB for suspected CMi and included in the treatment phase if virus-negative inflammatory intramyocardial inflammation was detected in EMB samples. Only virus-negative chronic myocarditis or CMi positive patients were treated and further evaluated. Beside histology to exclude active myocarditis by detection of myocytolysis, immunohistological criteria with different monoclonal antibodies were used to increase the sensitivity of EMB-analysis and to characterize the different immune-cells.

According to the statement of the ESC [3], EMBs were obtained from the right ventricular septum using a flexible biotome (Fa. Westmed, Germany) [18, 19]. LV ejection fraction (LVEF) was determined by echocardiography. Patients presenting with signs of acute myocarditis with very recent onset of symptoms (e.g., mimicking acute myocardial infarction with elevated serum markers of troponin T and creatine kinase/creatine kinase-MB) were excluded, as well as those with proof of intramyocardial genomes of enterovirus, adenovirus, Epstein-Barr virus, erythrovirus, and human herpes virus 6 [20, 21].

Other exclusion criteria were antiviral therapy within the past 6 months, clinical or biochemical evidence for concomitant chronic inflammatory disease, or any malignant disease.

We analyzed the hemodynamic course of all patients retrospectively after 6 months and in long-term follow-ups subsequent to immunosuppressive therapy: 1 mg/kg body weight daily of prednisone for 4 weeks, followed by 0.33 mg/kg body weight daily for 5 months, and azathioprine 2 mg/kg body weight daily for 6 months. Thereafter, all patients underwent follow-up EMB. No relevant changes in medication for chronic heart failure were allowed that either would have been expected to be given to further improve the patient's clinical symptoms at the time of enrollment, or that would have become necessary due to a marked deterioration of chronic heart failure within 8 weeks before enrollment. In all patients immunosuppressive treatment was started in between 4 weeks after receiving the EMB results.

The patients were clinically and echocardiographically followed by a period of up to 10 years {median 10.5 month [95 % confidence interval (CI) 11.69–59.16]}.

Ethical approval

The study was performed within the CRC Transregio 19 and was approved by the local ethics committees of the participating clinical center as well as by the committees of the respective federal states. An informed written consent is obtained from each study patient.

Detection of viral genomes by nested PCR (nPCR) and reverse transcription-PCR (RT-PCR)

Four EMBs were subjected to molecular biological investigation of cardiotropic viral genomes according to the published techniques [20]. In brief, a polymerase chain reaction (PCR) was performed on RNA extracted from EMBs for enterovirus, adenovirus, and on DNA for Epstein-Barr virus, Erythrovirus genomes and human herpesvirus 6.

Histological and immunohistochemical staining for assessment of fibrosis and inflammation

EMBs were obtained from the right ventricular septum. Histology was developed by hematoxylin-eosin staining in light microscopy. For immunohistological evaluation, specimens were embedded in Tissue Tec (SLEE, Mainz, Germany) and immediately snap-frozen in methyl butane which had been cooled in liquid nitrogen, and then stored at −80 °C until processing. Embedded specimens were cut serially into cryosections of 5-mm thickness and placed on 10 % poly-L-lysine-precoated slides. Immunohistochemistry was used for the characterization of inflammatory infiltrates and myocardial inflammation was diagnosed according to [3], by threshold cell count >14 leucocytes/mm^2, including >7 CD3$^+$ lymphocytes/mm^2, or >2.9 perforin$^+$ cytotoxic cells/mm^2. Antibodies used: anti-CD3 (Dako, Glostrup, Denmark, dilution 1:25), anti-CD2 (Mybiosource, San Diego, USA, dilution 1:50), anti-perforin (BD Bioscience, San Jose, USA, dilution 1:150), anti-HLA-1 (Dako, dilution 1:2000).

As a secondary antibody we used enhancing EnVision™ peroxidase-conjugated anti-mouse antibody (DakoCytomation, Hamburg, Germany). Immunohistological staining was visualized using 3-amino-9-ethylcarbazole (Merck, Darmstadt, Germany) as chromogenic substrate. Finally, slides were counterstained in hematoxylin and mounted with Kaiser's gelatinR (Merck, Darmstadt, Germany). The staining and peroxidase reactions in all samples were carried out identically and in parallel for all samples. Immunoreactivity was quantified by digital image analysis (DIA). The images for the quantification of infiltrates were grabbed at 200× magnification. The calculated objects were related to the unit HA (mm^2) [20].

For histology, multiple 5-µm-thick sections were cut and stained with hematoxylin-eosin (HE), Azan, and Van Gieson, and examined by light microscopy.

Statistical analyses

Data are shown as mean values and standard deviation or median with 95 % CI. After having established that any data were not distributed normally, the non-parametric Mann–Whitney U test for group comparisons, and Wilcoxon's signed rank test for comparisons between baseline and follow-up were utilized. Differences were considered to be statistically significant at a value of <0.05. All statistical analyses have been performed with SPSS.22, and Prism7.

Results

Total study population

The clinical hemodynamic and immunohistological characteristics of all immunosuppressive treated patients are summarized in Table 1.

At 6thmonth after immunosuppressive treatment the total study population showed a significant improvement of LVEF compared to baseline and this effects are lasting for the extended long-term follow-up period (LVEF rising from 44.6 ± 17.3 to 51.8 ± 15.5 %, $p = 0.006$, and finally to 52.1 ± 15.6 %, $p = 0.006$) (Fig. 1A).

At baseline EMB histological analysis showed borderline myocarditis according to the Dallas criteria [22]. On immunohistological staining, enhanced diffuse CD3$^+$ lymphocytes infiltration with a median number of

Table 1 Clinic, hemodynamic, and immunohistological characteristics of total study population at baseline and follow-up

Patients	
No., *n*	114
Gender, m/f	68/46
Follow-up period/months	10.5 month (95 % CI 11.69–59.16)
LVEF, %, baseline	44.6 ± 17.3*
LVEF, %, 6-month follow-up	51.8 ± 15.5
LVEF, %, long-term follow-up	52.1 ± 15.6
LVEDD, mm, baseline	59.12 ± 9.87
LVEDD, mm, long-term follow-up	57.42 ± 11.02
LVESD, mm, baseline	43.77 ± 12.86
LVESD, mm, long-term follow-up	41.83 ± 13.46
Atypical angina, baseline, *n*	21
Atypical angina, long-term follow-up, *n*	12
Dyspnea at exertion, baseline, *n*	62
Dyspnea at exertion, long-term follow-up, *n*	35
Dyspnea at rest, baseline, *n*	13
Dyspnea at rest, long-term follow-up, *n*	3
Atrial fibrillation, *n*	14
Supraventricular extrabeats, *n*	4
Ventricular extrabeats, *n*	9
Atrioventricular block, *n*	8
Pacemaker, *n*	6
Implantable cardioverter defibrillator, *n*	3
Immunohistochemistry in EMB	
$CD3^+$ cells/mm^2, baseline	16.03 ± 29.09*
$CD3^+$ cells/mm^2, follow-up	8.25 ± 9.09
HLA class I/AF, baseline	9.91 ± 5.55*
HLA class I/AF, follow-up	6.65 ± 2.81
$CD2^+$ cells/mm^2, baseline	12.62 ± 20.01*
$CD2^+$ cells/mm^2, follow-up	6.61 ± 8.47
$Perforin^+$ cells/mm^2, baseline	3.94 ± 4.65*
$Perforin^+$ cells/mm^2, follow-up	1.03 ± 1.47

The data are presented as mean ± standard deviation or median and median with 95 % Confidence Interval (CI)

LVEF left ventricular ejection fraction, *LVEDD* left ventricular end-diastolic diameter, *LVESD* left ventricular end-systolic diameter, *CD3* T-lymphocytes, $CD45R0^+$ T memory cells, *HLA* human leukocyte antigen, *Perforin* cytotoxic cells, *AF* area fraction

* Significantly different; baseline vs. follow-up

16.03 ± 29.9 $CD3^+$ lymphocytes/mm^2 was detected in all of the 114 patients' samples, and concurrent abundance of the HLA-1 (HLA-1 9.9 ± 5.5/AF) was confirmed. $Perforin^+$ cells and $CD2^+$ cells—expressed on most human cytotoxic T cells and natural killer cells—acting as co-stimulatory molecules, were also increased at baseline in the myocardial tissues ($Perforin^+$ cells 3.9 ± 4.6/mm^2, $CD2^+$ cells 12.6 ± 20.0/mm^2).

At follow-up-EMB a significant decrease of $CD3^+$ lymphocytes/mm^2 (8.2 ± 9.0/mm^2, $p = 0.002$), as well as abundance of the HLA-1 (HLA-1 6.6 ± 2.8/AF, $p = 0.0001$) could be observed. In all of the patients, $perforin^+$ cells and $CD2^+$ cells decreased significantly in a similar manner, in comparison to baseline EMB ($perforin^+$ cells 1.03 ± 1.47, $p = 0.0001$; $CD2^+$ cells 6.6 ± 8.4/mm^2, $p = 0.001$) (Fig. 1B).

Subgroup analysis

Hemodynamic classification at baseline

In a subgroup analysis, patients with initial LVEF ≤45 % ($n = 53$) significantly increased with LVEF at follow-up (29.3 ± 8.8–41.7 ± 13.2–42.1 ± 13.1 %, $p < 0.0001$),

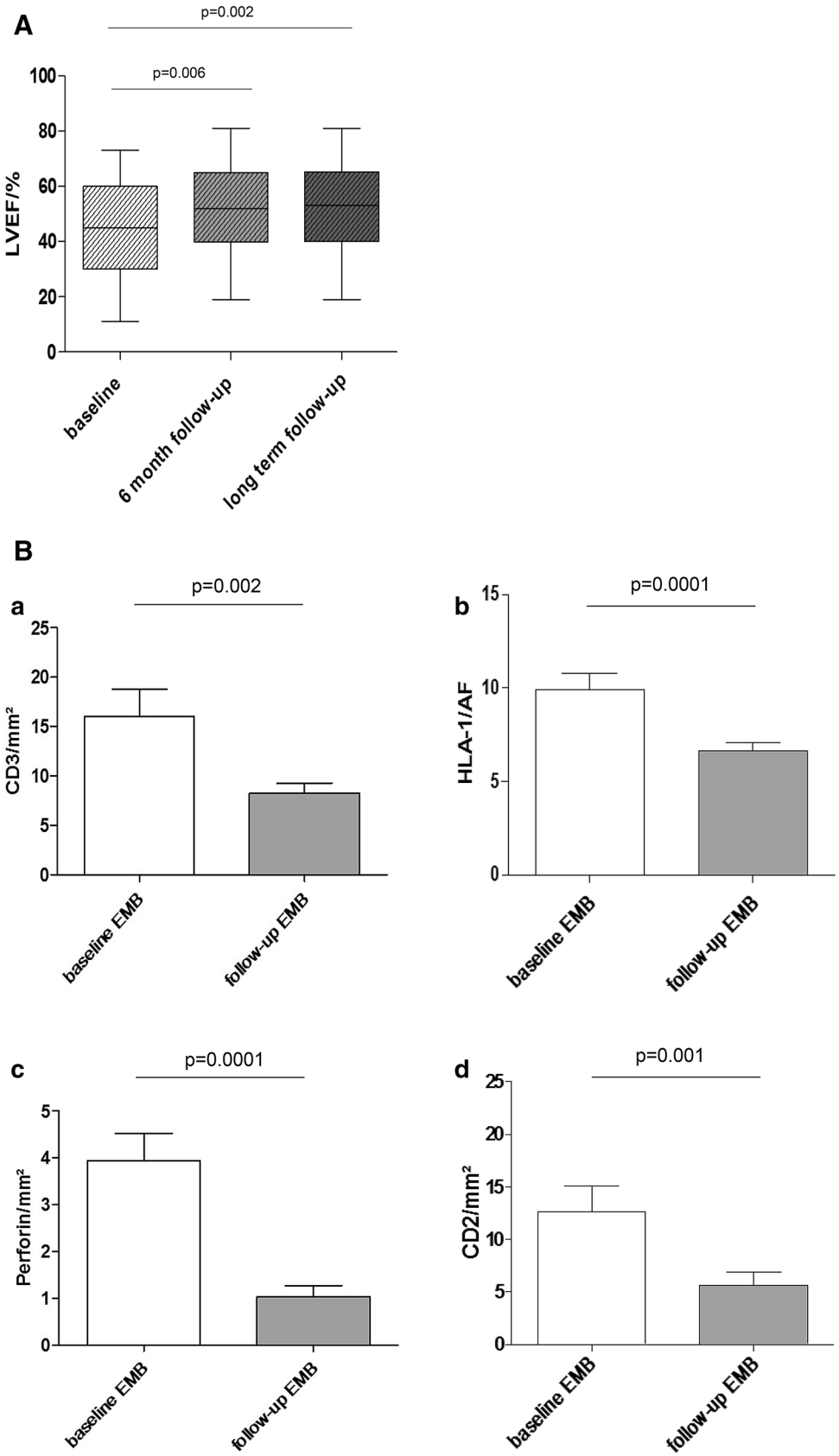

Fig. 1 **A** Hemodynamic course of total study population. Measurement of LVEF (%) at baseline, at 6 months and at a long-term follow-up period of median at 10.5 months (95 % CI 11.69–59.16). Mean values ± standard deviation are shown; $*p < 0.05$ (compared to baseline EMB).
B Immunohistochemical detection of intramyocardial inflammation (cardiac immune cell infiltration and cell-adhesion molecules) of total study population: **a** $CD3^+$ cell infiltration (mm^2). **b** HLA-1 expression (area fraction/AF). **c** $Perforin^+$ cells (mm^2). **d** $CD2^+$ cells (mm^2). In baseline EMB and follow-up EMB of total study population. Mean values ± standard deviation are shown; $*p < 0.05$ (compared to baseline EMB)

accompanied by decrease of LVEDD from 65.0 ± 8.2 to 60.8 ± 14.2 mm, $p = 0.2$ (Group I, Fig. 2A, a). We defined this group as inflammatory cardiomyopathy. Patients with initial LVEF >45–60 % ($n = 25$) significantly improved further or recovered completely, regarding LVEF (53.0 ± 3.6–59.0 ± 9.4–59.8 ± 10.0 %, $p = 0.03$), accompanied by decrease of LVEDD from 55.0 ± 6.3 to 53.1 ± 10.7 mm, $p = 0.8$ (Group II, Fig. 2A, b). Patients with initial LVEF >60 % ($n = 36$) remained stable and did not deteriorate over long-term follow-up (68.8 ± 6.7–67.5 ± 10.9–68.8 ± 10.7 %, $p = 0.5$), and this was parallel to the LVEDD (50.8 ± 7.2–49.3 ± 7.9 mm, $p = 0.8$) (Group III, Fig. 2A, c). We defined Groups II and III as chronic myocarditis.

In each patient group, $CD3^+$ lymphocytes were significantly decreased in follow-up EMBs in contrast to baseline EMBs [Group I $CD3^+$ cells 21.5 ± 39.7 vs. 6.9 ± 4.9/mm^2, $p = 0.02$ (Fig. 2B, a); Group II $CD3^+$ cells 12.8 ± 16.9 vs. 6.9 ± 5.7/mm^2, $p = 0.03$ (Fig. 2B, b); Group III $CD3^+$ cells 10.2 ± 11.1 vs. 6.2 ± 4.7/mm^2, $p = 0.03$ (Fig. 2B, c)].

Moreover, evaluation of the follow-up EMBs revealed a significant reduction of perforin$^+$ cells in contrast to baseline EMB in all three groups of patients [perforin$^+$ cells 3.6 ± 4.6 vs. 0.6 ± 0.8/mm^2, $p = 0.01$ (Group I, Fig. 2B, d); perforin$^+$ cells 2.9 ± 4.36 vs. 0.7 ± 1.1/mm^2, $p = 0.04$ (Group II, Fig. 2B, e); and perforin$^+$ cells 4.9 ± 4.8 vs. 1.3 ± 1.7/mm^2, $p = 0.02$ (Group III, Fig. 2B, f)].

Representative aspects of immunohistologically detected infiltrates, and CAMs expression are shown in Fig. 3.

Discussion

The present study demonstrates again the effectiveness and beneficial effects of immunosuppressive therapy in virus-negative chronic myocarditis or CMi patients, and, moreover, we show for the first time that these positive results are lasting for an extended period of time. To our knowledge this is the first report of validation of immunosuppressive therapy in CMi for 6 months elucidating the hemodynamic course of 114 treated patients over the long-term follow-up period of up to 10 years [median 10.5 month (95 % CI 11.69–59.16)]. The total study population showed a significant improvement of LVEF compared to baseline.

Our study has a direct clinical impact, as the results imply that an immunosuppressive treatment of patients with chronic myocarditis or CMi resulted in improvement of LVEF in long-term follow-up with significant reduction of intramyocardial inflammation in 6 month EMB.

In previous studies, intramyocardial inflammation was demonstrated to predict worse outcome [7, 23, 37]. Nonetheless, the function of immunosuppression in the treatment of inflammatory cardiomyopathy is still debated because of the controversial results obtained in recovery of treated patients [24, 25]. Results of the TIMIC trial confirmed the positive impact of immunosuppression on recovery of LV function in a high rate (88 %) of patients [16]. The efficacy of therapy was evaluated at the end of the 6 month treatment. That is consistent with the long-term follow-up results found in the present study. After immunosuppressive treatment with prednisone and azathioprine for 6 months, a significant improvement with either a complete or partial recovery of LVEF was observed. $CD3^+$ lymphocytes and HLA-1 expression were both markedly increased in baseline EMBs. Immunohistological evaluation of the follow-up EMBs revealed significant reductions of $CD3^+$ lymphocytes and HLA-1. Therefore, at the 6 month EMB control, all patients had an almost complete resolution of the intramyocardial inflammation.

In our study the substantial improvement in LVEF was observed, especially in patients with inflammatory cardiomyopathy with an initial LVEF ≤45 % at study entry. However, even in Group II with an initial LVEF >45–60 % a significant improvement of LVEF was observed. In Group III, with an initial normal LVEF (>60 %), no hemodynamic improvement was expected. However, the aim of the treatment was to stop further inflammatory processes before hemodynamic deterioration continued. Our data imply that virtually all patients can benefit from an immunomodulatory treatment, irrespectively of baseline LVEF, and even in cases where initially only a slightly impaired LV function is present in cases of chronic myocarditis.

It appears crucial to identify single biological markers of potential candidates for immunosuppression among the various forms of inflammatory myocardial disease [26–30]. Although the exact mechanisms of immune-mediated myocyte injury are not fully understood, a growing body of evidence showed that cytotoxic cells expressing cytotoxic effector molecules may play a pathogenetic key role regarding progression myocarditis [31–33]. In multiple inflammatory diseases perforin acts as an effector of tissue destruction [34–36]. In the setting of experimental myocarditis, perforin is known to mediate myocytolysis contributing to loss of contractile units [33], and ultimately leading to the observed failure of recovery. In murine models perforin-positive infiltrates cause extensive myocarditis, damaging the host. In a previous clinical study we were able to show in a large cohort of 495 patients with EMB-proven myocardial inflammation that LV function deteriorates in patients with detection of perforin$^+$

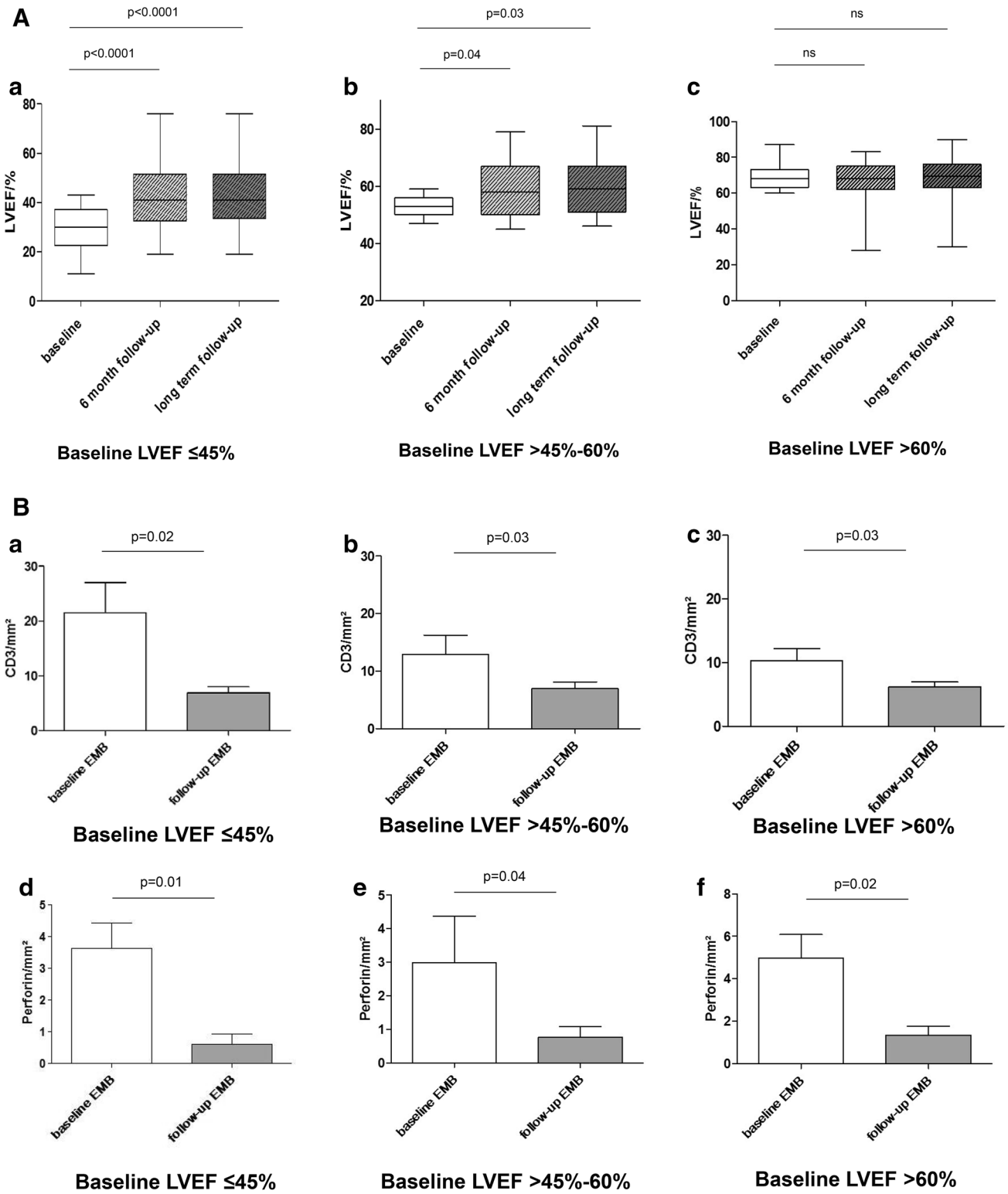

Fig. 2 **A** Hemodynamic course at 6 months and at a long-term follow-up period in **a** $n = 53$ patients with baseline LVEF ≤45 % (Group I), **b** n = 25 patients with baseline LVEF >45–60 % (Group II), and **c** $n = 36$ patients with baseline LVEF >60 % (Group III). Mean values ± standard deviation are shown; $*p < 0.05$ (compared to baseline EMB). **B** Immunohistochemical detection of intramyocardial inflammation of subgroup analysis in patients with baseline LVEF ≤45 %, LVEF >45–60 %, and LVEF >60 %. **a–c** $CD3^+$ cell infiltration (mm^2), **d–f** $perforin^+$ cell infiltration (mm^2)

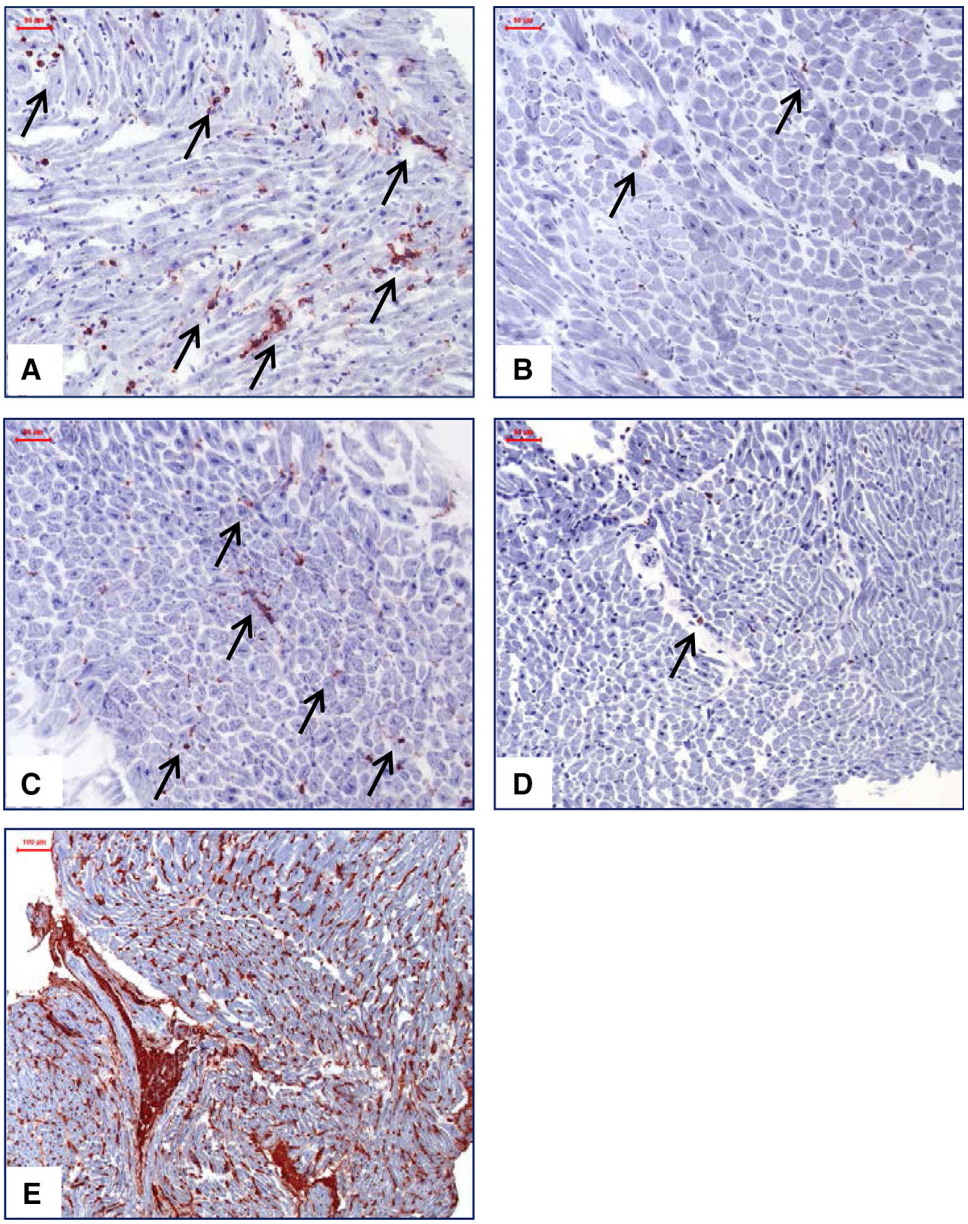

Fig. 3 Representative images of immunohistological staining from frozen samples. **a** Increased $CD3^+$ infiltration (*arrow*) with diffuse infiltration pattern at baseline (×200). **b** Significantly reduced $CD3^+$ infiltration after treatment at follow-up EMB (×200). **c** Increased $perforin^+$ infiltration (*arrow*) with diffuse infiltration pattern at baseline (×200). **d** Follow-up EMB with significantly reduced $perforin^+$ infiltration after treatment (×200). **e** Extensive HLA-1 abundance (*red*) with homogenous expression pattern at baseline (×100)

infiltration above 2.9 cells/mm^2 and progresses towards substantial cardiac dysfunction over a long-term follow-up period despite continued heart failure medication [37].

In the present study we could show for the first time that patients with increased perforin expression responded favorably to immunosuppressive therapy. Of note, immunohistological evaluation of the follow-up EMBs revealed significant reduction of $perforin^+$ cells in patients showing LVEF improvement with a significantly increased perforin expression at baseline. The same could be observed for $CD2^+$ cells. Interestingly, CD2 is the cell surface glycoprotein expressed on most human T cells and natural killer cells and plays an important role in mediating cell adhesion in both T-lymphocytes and in signal transduction [38]. Hence, we hypothesize that intervention in patients with increased cytotoxic cells could be a new decisive axis in treatment of CMi.

However, therapies need to be installed at a time when no irreversible myocardial damage has yet occurred, since they are able to influence active pathogenic processes with cell migration and tissue remodeling still going on. For this reason, timely prediction of the probable disease course is of particular importance with regard to clinical CMi therapy. A therapeutic regime considers that over and above any standard heart failure medication, an early-enough installment of such a therapy will logically reduce the extent of any irreversible cardiac injury, especially, in high risk patients (i.e., with increased perforin in EMBs).

We conclude that in this EMB-based analysis of chronic myocarditis and CMi patients, immunosuppressive treatment showed a significant effectiveness and beneficial effect even after a long-term follow-up period of median 10.5 month (95 % CI 11.69–59.16). The patients improved significantly with either a complete or partial recovery of LVEF. Follow-up EMBs revealed elimination of perforin$^+$ and CD2$^+$ cells in these patients. This was accompanied by significantly reduced numbers of CD3$^+$ lymphocytes and cell-adhesion molecules.

Therefore, our study has a direct clinical impact, as the results imply, that an immunosuppressive treatment of patients with virus-negative chronic myocarditis or CMi resulted in improvement of LVEF in long-term follow-up with significant reduction of intramyocardial inflammation in 6 month EMB, especially in high risk patients (i.e., with high concentrations of perforin in EMBs). In this sense we believe that our results should be integrated into the routine clinical practice for cardiologists in the future.

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Compliance with ethical standards

Conflict of interest None declared.

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