

Randomized study on the efficacy of immunosuppressive therapy in patients with virus-negative inflammatory cardiomyopathy: the TIMIC study

Andrea Frustaci^{1,2*}, Matteo A. Russo^{3,4}, and Cristina Chimenti^{1,2,4}

¹Cardiovascular and Respiratory Sciences Department, La Sapienza University, Viale del Policlinico 155, 00161 Rome, Italy; ²Molecular and Cellular Cardiology Laboratory, National Institute for Infectious Diseases 'Lazzaro Spallanzani', Rome, Italy; ³Experimental Medicine Department, La Sapienza University, Rome, Italy; and ⁴IRCCS San Raffaele La Pisana, Rome, Italy

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Aims

To evaluate the efficacy of immunosuppression in virus-negative inflammatory cardiomyopathy.

Methods and results

This randomized, double-blind, placebo-controlled study included 85 patients with myocarditis and chronic (>6 months) heart failure unresponsive to conventional therapy, with no evidence of myocardial viral genomes. Patients received either prednisone 1 mg kg⁻¹ day⁻¹ for 4 weeks followed by 0.33 mg kg⁻¹ day⁻¹ for 5 months and azathioprine 2 mg kg⁻¹ day⁻¹ for 6 months (43 patients, Group 1) or placebo (42 patients, Group 2) in addition to conventional therapy for heart failure. Primary outcome was the 6 month improvement in left-ventricular function. Group 1 showed a significant improvement of left-ventricular ejection fraction and a significant decrease in left-ventricular dimensions and volumes compared with baseline. None of Group 2 patients showed improvement of ejection fraction, that significantly worsened compared with baseline. No major adverse reaction was registered as a result of immunosuppression.

Conclusion

These data confirm the efficacy of immunosuppression in virus-negative inflammatory cardiomyopathy. Lack of response in 12% of cases suggests the presence of not screened viruses or mechanisms of damage and inflammation not susceptible to immunosuppression.

Keywords

Inflammatory cardiomyopathy • Immunosuppressive therapy • Heart Failure • Ejection Fraction • NYHA class

Introduction

The role of immunosuppression in the treatment of myocarditis is still debated because of the controversial results obtained both in children^{1–2} and adults^{3–4} presenting with either cardiac arrhythmias⁵ or heart failure.⁶ These discrepancies may be due to heterogeneity of the pathogenic components, including host immune response, type of infectious agent, and mechanism of cell damage (directly cytopathic or immune-mediated). Nevertheless, it is recognized that, despite a spontaneous resolution may occur in up to 40% of patients with acute myocarditis,⁷ some patients with chronic heart failure do benefit from immunosuppression. Thus, it appears crucial identifying biological markers of potential

candidates to immunosuppression among the various forms of inflammatory myocardial disease. To this regard a retrospective analysis of virological and immunologic profile of patients with active lymphocytic myocarditis receiving immunosuppressive therapy revealed a 90% rate responsiveness in those with circulating serum cardiac auto-antibodies and negative cardiac polymerase chain reaction for the main cardiotropic viruses.⁸ Conversely, myocardial viral genomes were detectable in 85% of non-responders. These data indicate in the absence of cardiac viral genomes a prerequisite for the clinical use of immunosuppression while suggest a potential impact of antiviral agents for patients with virus-positive inflammatory cardiomyopathy. In this context, a pilot study⁹ has recently provided evidence of the efficacy of Interferon-beta in

* Corresponding author. Tel: +39 0655170575, Fax: +39 0655170577, Email: biocard@inmi.it

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inducing myocardial clearance of adeno and enteroviruses and a parallel improvement of left ventricular function.

Herein we report the results of the first prospective randomized study on immunosuppressive therapy in patients with virus-negative inflammatory cardiomyopathy [Tailored Immosuppression in Inflammatory Cardiomyopathy (TIMIC) study].

Methods

Study objectives

The objective of the present single-centre, randomized, double-blind, placebo-controlled study was to evaluate the benefit of immunosuppressive therapy combined with optimal conventional therapy vs. conventional therapy alone in patients with virus negative inflammatory cardiomyopathy.

Primary efficacy endpoint of the study was the 6 month improvement in left-ventricular function, based on the increase of left ventricular (LV) ejection fraction (EF) assessed by echocardiography.

Secondary objectives were the changes from baseline to month 6 in echocardiographic LV volumes and diameters, the changes in heart failure symptoms [New York Heart Association (NYHA) class] and the survival differences (cardiac death or heart transplantation).

Patients were recruited in Rome, Italy, coming from the South, and the centre of the country, either during hospitalization or as outpatients.

The study was approved by the Ethic Committee of our Institution and all patients provided written informed consent.

Patient population

Inclusion criteria were (i) dilatation and dysfunction of the left ventricle (LVEF <45%); (ii) age between 18 and 75 inclusive; (iii) chronic heart failure (lasting >6 months) unresponsive to conventional supportive therapy; (iv) Histologic and immunohistochemical evidence of active lymphocytic myocarditis; (v) absence of cardiotropic viruses at polymerase chain reaction (PCR) analysis; (vi) absence of congenital, valvular, and/or coronary artery disease that could justify the severity of cardiac dysfunction; (vii) written informed consent.

Exclusion criteria were (i) recent (less than 6 months) onset of heart failure; (ii) known causes of heart failure [such as hypertension, significant coronary artery disease, valvular heart diseases (but not relative mitral regurgitation), endocrine disease, significant renal disease, drug or alcohol abuse]; (iii) therapy with steroids within 6 months before the enrolment; (iv) contraindication to the treatment with steroids and/or azathioprine; (v) pregnancy or lactation; (vi) inability to understand the patient information or to give informed consent.

To be enrolled in the study patients complied with all the inclusion criteria and showed no exclusion criteria.

Therapeutic protocol and randomization

All patients were on optimal (maximal tolerable dose) continuous medication with conventional therapy including digitalis (0.25 mg daily), diuretics (furosemide 25–250 mg daily), ACE inhibitors (enalapril 10–20 mg bid), and carvedilol (25–50 mg daily) for at least 2 weeks 8 (>3 months from the initial dose) prior to enrolment in the study. In addition, the patients were randomly and blindly assigned to oral administration of immunosuppressive therapy (43 patients, Group 1) including prednisone 1 mg/kg daily for 4 weeks followed by 0.33 mg/kg daily for 5 months and azathioprine 2 mg/kg daily for 6 months or placebo (42 patient, Group 2). Immunosuppressive therapy was administered as two separated pills taken twice a day.

Placebo pills were identically supplied and formulated except that they contained no prednisone or azathioprine. Independent pharmacists prepared either active or placebo pills according to a computer generated randomization list.

Discontinuation of treatment was considered in case of adverse effects, including severe hypertension, infectious diseases, and leucopenia ($<3.0 \times 10^9/L$), while increase in body weight and fluid retention were treated with adjustment in diuretic dose.

The efficacy of therapy was evaluated at the end of the 6 month treatment.

All study personnel and participants were blinded to treatment assignment for the duration of the study.

Clinical studies and follow-up

Clinical assessment, resting electrocardiogram (ECG) Holter monitoring, and 2D-echocardiography were performed at baseline, weekly during the first month, every 4 weeks for the remaining 5 months. Cardiac catheterization, angiography, and bi-ventricular endomyocardial biopsy were performed at baseline and at 1 and 6 months. The NYHA class was used to assess functional capacity and determined by means of a questionnaire.

Echocardiographic studies were performed with Agilent Sonos 5500 (Hewlett-Packard, Palo Alto, CA). Patients were imaged and data were analysed offline by a single senior echocardiographer blinded to the treatment groups. Echocardiographic parameters were determined according to established criteria.¹⁰ In particular, EF was calculated in the apical 4 and 2-chamber views from three separate cardiac cycles using the modified Simpson's method.

Endomyocardial biopsy and histopathological studies

Endomyocardial biopsies (3–4 from each ventricular chamber) were performed in the septal–apical region of both ventricles, as previously described.⁸

The diagnosis of myocarditis was performed according with Dallas criteria¹¹ confirmed by immunohistochemistry.⁵ The histologic and immunohistological examination of biopsy specimens was made blindly to clinical features by an experienced pathologist.

To quantify the inflammatory infiltrates, CD45 positive leukocytes and T-lymphocytes (CD3+) were counted per high-power field (HPF) (400-fold magnification) in all available fields and the mean number was calculated.¹² More than 2.0 CD3-positive lymphocytes per HPF (7 per mm²) were considered as abnormal.¹³ The presence of an inflammatory infiltrate of a minimum of 14 infiltrating leukocytes/mm² was considered diagnostic for myocarditis.¹⁴

In particular, the presence of >14 infiltrating leukocytes/mm² and/or the presence of more than 2.0 CD3-positive lymphocytes per HPF, often adherent to the contour of cardiomyocytes and focally associated with cell necrosis, were considered diagnostic for active myocarditis.

For morphometric analysis, LV histologic sections were examined at 400× magnification with a reticule containing 42 sampling points (105844, Wild Heerbrugg Instruments, Gals, Switzerland)¹⁵ to determine the percent area occupied by fibrous tissue.

Molecular biology studies

Two frozen myocardial specimens from each patient were used for PCR and reverse transcriptase PCR analysis to detect the presence of cardiotropic viruses at baseline, as previously described.⁸ Briefly, 10 primer pairs were used to detect DNA and RNA viruses (i.e. adenovirus, Epstein Barr virus, Human herpes virus 6, Parvovirus B19,

Herpes simplex virus 1–2, Cytomegalovirus, enterovirus, influenza A and B viruses, Hepatitis C virus). Patients virus positive at baseline were not included in the study. Patient virus negative at baseline, in the absence of exclusion criteria, were enrolled in the trial. The same PCR analysis was performed in the 1 month and 6 months control biopsies.

Statistical analysis

According with our previous retrospective study, treatment of virus negative inflammatory cardiomyopathy patients with immunosuppressive therapy for 6 months leads to an improvement in EF in more than 85% of cases.⁸ On the other hand, a spontaneous resolution with recovery from heart failure has been described in up to 50% of patients with acute myocarditis,⁷ and a 12% relative improvement was observed in the placebo arm of the Wojnicz 2 trial.¹⁶ On this basis, we estimated a sample size of 80 patients (40 each treatment group) to be required for a two-tailed significance level of 0.05 and a power of 0.80.

Normal distribution of variables was assessed with Kolmogorov–Smirnov test.

Quantitative measurements were expressed as mean±SD. Categorical data were presented as absolute frequencies and percent values. Difference between the two groups was determined by unpaired *t*-test for continuous variables and Fisher's exact test for categorical data. Changes observed before and after immunosuppressive treatment were examined by paired *t*-test and McNemar test. Bivariate correlations were analysed by Spearman ρ coefficient computation.

Statistical analysis was performed using a BMDP package. The study statistician saw unblinded data at the end of the study.

Results

Patient population

At our institution, from January 2001 to January 2007, 901 patients underwent an endomyocardial biopsy, 512 because of LV dysfunction (ejection fraction <45%), 139 for idiopathic LV hypertrophy, 158 for severe ventricular arrhythmias, and 92 because of unexplained chest pain. A diagnosis of active myocarditis was made in 238 patients, 190 with LV dysfunction. Among the 190 patients with active myocarditis and LV dysfunction, 137 were characterized by chronic (lasting >6 months) heart failure unresponsive to supportive conventional therapy and were potentially eligible for the study. Among them, 36 were excluded because of the positive PCR for myocardial viral infection, 6 were excluded because of the presence of systemic disorders, 5 due to insulin-dependent diabetes, 2 due to renal failure, and 2 because of contraindication to steroidal treatment. One patient refused to participate. The remaining 85 patients (51 men and 34 women, mean age of 42.7 ± 15.4 years) were enrolled in the study and were randomly assigned to one of the two treatment groups.

There were no significant differences in baseline characteristics between the treatment groups (Table 1). No gender-based differences were present. The mean (\pm SD) LVEF for the overall group of patients was $27.1 \pm 6.5\%$, and most of the patients were in NYHA class III or IV.

All participants completed allocated treatment and planned follow-up and were analysed according to group assignment.

Table 1 Baseline characteristics of the 85 patients with active lymphocytic myocarditis randomized to immunosuppression (Group 1) and placebo (Group 2)

Variables	Group 1 (n = 43)	Group 2 (n = 42)	P-value
Demographic			
Age, years	44.2 \pm 15.8	41.1 \pm 15.1	0.374
Sex, n (%)			0.826
Male	25 (58)	26 (62)	
Female	18 (41)	16 (38)	
Time to onset, months	9 \pm 11	9 \pm 4	
Clinical			
NYHA class, n (%)			
I	0	0	
II	22 (51)	26 (62)	0.384
III	15 (35)	12 (29)	0.643
IV	6 (14)	4 (9)	0.738
LBBB, n (%)	5 (12)	8 (19)	0.382
Echocardiographic			
LVEDD, mm	68.4 \pm 7.0	68.9 \pm 7.5	0.751
LVEDV, mL	257.3 \pm 50.1	245.4 \pm 46.4	0.259
LVESV, mL	188.8 \pm 38.3	176.9 \pm 34.1	0.134
EF, %	26.5 \pm 6.6	27.7 \pm 6.4	0.397
Haemodynamic			
LVEDP, mmHg	20.4 \pm 3.1	19.5 \pm 3.1	0.184
Cardiac index, L min ⁻¹ m ⁻²	2.1 \pm 0.3	2.0 \pm 0.3	0.128
Morphometric			
Fibrous tissue, % area	21.6 \pm 5.1	22.5 \pm 6.0	0.458
CD3+ cells/HPF	4.1 \pm 1.1	4.2 \pm 1.3	0.703

Data are presented as mean \pm SD unless 'Time to onset' expressed as median \pm range.

NYHA, New York Heart Association; LBBB, left bundle branch block; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end diastolic volume; LVESV, left ventricular end-systolic volume; EF, ejection fraction; LVEDP, left ventricular end-diastolic pressure. HPF, high-power field (400-fold magnification).

Changes in left-ventricular function

At 6 months Group 1 patients as a whole showed a significant improvement of mean LVEF (Table 2, Figure 1). Similarly, LV volumes and dimensions significantly reduced compared with baseline (Table 2, Figure 1).

Specifically, 38 out of 43 patients on immunosuppressive therapy (88%) showed a improvement of cardiac function and dimensions, defined as an increase of >10 percentage points in the absolute EF and a reduction of LV end-diastolic volume (EDV) or LV end-diastolic diameter (EDD) $\geq 10\%$ (i.e. LVEF from 26.4 ± 6.9 to $48.0 \pm 7.3\%$, LVEDV from 258.0 ± 52.5 to 125.9 ± 29.6 , LVEDD from 68.6 ± 7.4 to 52.8 ± 6.3 mm). The remaining five patients maintained a stable clinical picture and cardiac function parameters. Remarkably, even patients with severe advanced disease (LVEDD up to 90 mm and LVEF <20%)

Table 2 Comparison of characteristics between baseline and 6 month treatment in the two groups of patients

Variables	Group 1 (n = 43)	P-value	Group 2 (n = 42)	P-value
Ejection fraction, %		<0.001		<0.001
Baseline	26.5 ± 6.7		27.7 ± 5.6	
Six month	45.6 ± 9.6		21.3 ± 5.3	
LVEDV, mL		<0.001		<0.001
Baseline	257.3 ± 50.1		245.4 ± 46.3	
Six month	140.7 ± 50.6		280.6 ± 48.9	
LVESV, mL		<0.001		<0.001
Baseline	188.8 ± 38.3		176.9 ± 34.1	
Six month	79.7 ± 43.9		223.4 ± 43.3	
LVEDD, mm		<0.001		<0.001
Baseline	68.4 ± 7.0		68.8 ± 7.5	
Six month	54.4 ± 7.4		74.0 ± 7.6	
NYHA class III/IV, n (%)		0.008		0.010
Baseline	21 (49)		16 (38)	
Six month	9 (21)		28 (67)	

Data are presented as mean ± SD unless stated otherwise.

LV, left ventricular; ESV, end-systolic volume; EDV, end-diastolic volume; EDD, end-diastolic diameter; NYHA, New York Heart Association.

significantly improved, being able to resume their previous work. The duration of heart failure did not correlate with the extent of recovery.

None of Group 2 patients showed at 6 month follow improvement of LVEF, that significantly worsened compared with baseline (Table 2). In particular, 35/42 Group 2 patient (83%) showed further impairment of cardiac function (LVEF from 27.6 ± 6.6 to 19.5 ± 4.8 , LVEDV from 244.7 ± 48.0 to 287.3 ± 48.0 , LVEDD from 69.2 ± 7.9 to 75.3 ± 7.4) while the remaining seven patients remained stationary. Specifically, after 1 month of treatment, control group exhibited in 38% a mild improvement of ejection fraction (from $27.8 \pm 5.5\%$ to $32.6 \pm 5.3\%$), that was maintained up to 3 months, but then it declined to baseline ($27.2 \pm 5.6\%$) or lower ($19.7 \pm 4.4\%$) values.

Clinical assessment and adverse events

The percentage of patients who improved by at least 1 NYHA class at 6 months was 49% in Group 1 and none in Group 2. The number of patients in NYHA class III/IV significantly reduced in Group 1 compared with baseline while increased in Group 2 (Table 2). In the 38 Group 1 patients who improved, a decline of heart rate, disappearance of gallop rhythm, increase in QRS voltages, and improvement in ECG repolarization abnormalities occurred within 1 week of treatment. Notably, in one patient with extreme LV dilatation and dysfunction a disappearance of left bundle branch block has been observed (Figure 3).

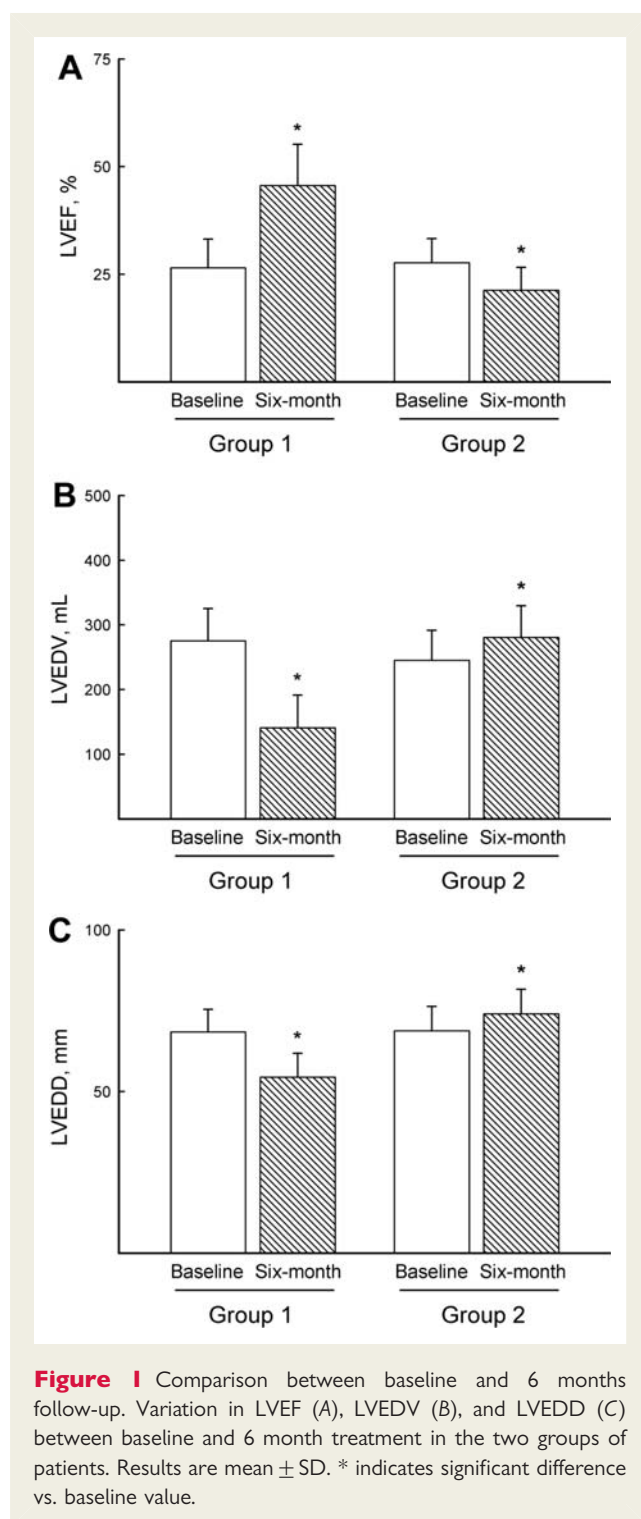
At the end of the study none Group 1 and 5 Group 2 patients (12%) experienced new hospitalizations because of the worsening of heart failure symptoms, and no patient had cardiac death or heart transplantation. All study population was followed-up after end of study protocol for a time range of 10–72 months.

In Group 2 cohort, two patients died and two were transplanted, while no major events occurred in the Group 1 patients. In the majority of the latter Group the benefit in terms of improvement of cardiac dimension and function was maintained. However, in three patients there was a new decline of cardiac contractility of which one had associated remarkable reduction of platelet count ($60 \times 10^3/\text{mm}^3$). In these three patients a new cardiac biopsy was obtained showing a reactivation of myocarditis process. Immunosuppression was, then, restored with new improvement of ejection fraction and normalization of platelets in the patient with piasrinopenia, suggesting the existence of a primary autoimmune disease. All these three patients are still on treatment with azathioprine 2 mg/kg/day.

Minor adverse reaction as increased body weight, glucose blood level elevation and fluid retention requiring diet, glucoactive drugs, insuline administration, and diuretic dose adjustment were reported in six patients on immunosuppression. No major drug-related side effects requiring therapy withdrawal were registered. Liver function test (AST, ALT) did not modify during treatment in Group 1 patients, while white blood count slightly increased (4783 ± 970 at baseline and 6218 ± 1270 at 6 months follow-up) remaining still within normal limits.

Endomyocardial-biopsy findings

Histological analysis showed an active myocarditis with diffuse inflammatory infiltrates associated with focal necrosis of the adjacent myocytes (meeting the Dallas criteria) with interstitial and focal replacement fibrosis in most of left and right ventricular specimens from all patients (Figures 2 and 3). Endocardial thickening with prominent smooth muscle cells suggesting long-lasting ventricular dilatation was observed in all cases. The infiltrates



included mainly activated T cells (CD45RO+, CD3+) with moderate amount of cytotoxic lymphocytes (CD8+) and macrophages (CD68+).

Morphometric analysis showed no differences in terms of extent of fibrosis and amount of inflammatory cells between Group 1 and Group 2 patients (Table 1).

Considering in Group 1 patients the extent of recovery at 6 months follow-up in terms of return of LV end diastolic diameter

to normal values, the percent of fibrous tissue correlated inversely (correlation coefficient=0.66, $P < 0.001$) while the severity of inflammation correlated directly (correlation coefficient=0.50 $P < 0.001$) with the normalization of LV dimensions.

Control histology at 1 and 6 month showed, in the 38 Group 1 patients who improved with immunosuppression, a healed myocarditis with disappearance of inflammatory infiltrates associated with interstitial and focal replacement fibrosis (Figures 2 and 3). In the five Group 1 patients who did not improve, myocardial inflammation reduced or disappeared in the control biopsies but some degenerative changes of myocardiocytes were observed.

In Group 2 patients, control biopsies were not dissimilar from baseline, showing persistence of myocarditis as well as expansion of interstitial and replacement fibrosis. PCR analysis of follow-up biopsies showed absence of cardiotropic viruses in both Group 1 and Group 2 patients.

Discussion

Currently there is no consensus on the treatment of inflammatory cardiomyopathy and the strategies used are those usually adopted for the management of heart failure.

Indeed several reports have emphasized the negative role of myocardial viral persistence as well as the benefit of myocardial viral clearance on LV function.^{9,17–20} Practically, detection and elimination of viral genomes from myocardium seems a key point for the treatment of virus-positive inflammatory cardiomyopathy. More debate is in this clinical context the use of immunosuppressive therapy since controversial results have been obtained both in children^{1–2} and in adults^{3–4} presenting with either cardiac arrhythmias⁵ or heart failure.⁶ To this regard a retrospective study on patients with inflammatory cardiomyopathy receiving immunosuppression showed a high prevalence (90%) of negative myocardial PCR for cardiotropic viruses among responders and a high rate of virus positive PCR (85%) among non-responders.⁸

Similarly, patients with dilated cardiomyopathy who showed an HLA upregulation on endomyocardial biopsy specimens received a long-term benefit from immunosuppressive therapy.¹⁶

These findings prompted us to evaluate the efficacy of immunosuppression in virus-negative inflammatory cardiomyopathy in a prospective randomized placebo-controlled study. In our study active myocarditis was observed in 37% (190/512) of patients with LV dysfunction exceeding the value reported from other groups (from 3 to 12%). This higher prevalence may be due to: (i) the more prominent severity of LV dysfunction of our patient population as the mean LV ejection fraction was 27% and (ii) a bi-ventricular endomyocardial biopsy approach allowing the histological evaluation of many samples from different sites of the heart and specifically from the usually more compromised and non-approached LV. Indeed judging from the many MRI studies obtained in patients with myocarditis, LV wall is a major site of delayed enhancement after Gadolinium infusion and the studies reporting a lower incidence of active myocarditis have a cardiac biopsy limited to RV. In addition, in the actual study only 36/137 patients (26.7%) were virus positive compared with the previous report⁸ where viral genomes were detected in about 45% of

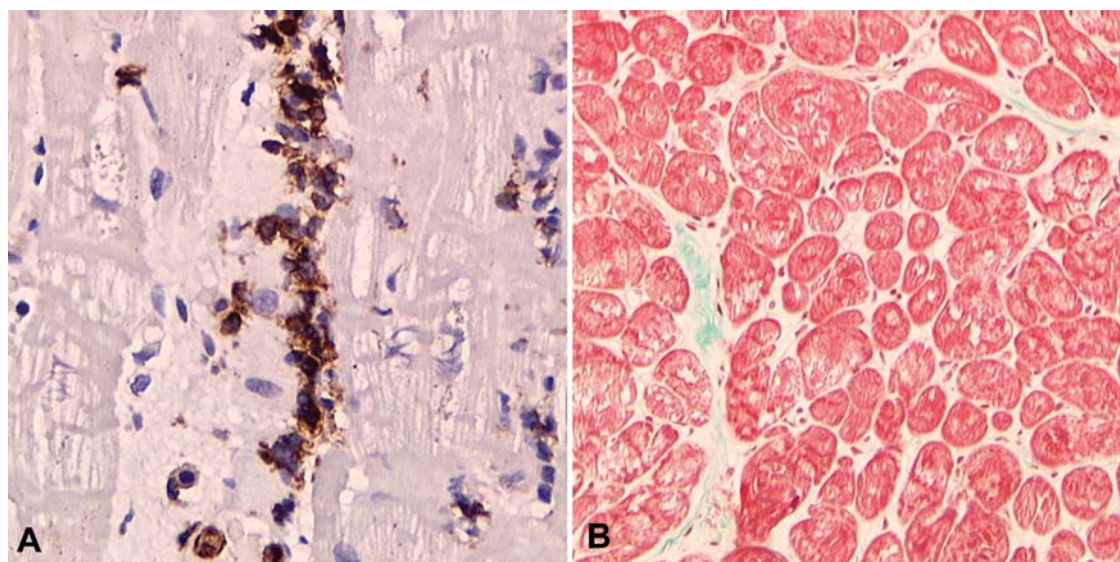


Figure 2 Improvement with immunosuppression. LV endomyocardial biopsy of a 19-year young boy before and after 6 months of immunosuppressive therapy. Marked reduction of left ventricular volumes and increase in LVEF (from 24 to 50%) were associated with disappearance of CD45RO positive activated T lymphocytes and myocyte necrosis present at baseline (A, immunoperoxidase, 200 \times) both replaced by fibrosis in control biopsy (B, Masson trichrome, 100 \times).

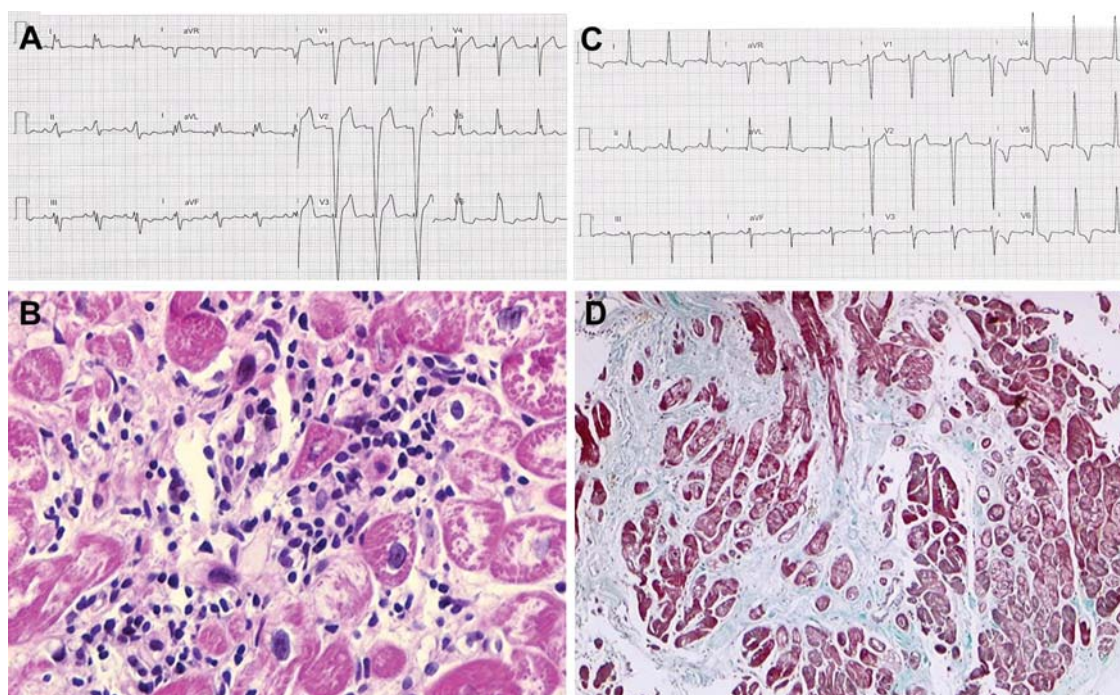


Figure 3 Improvement with immunosuppression. 12-lead ECG (A and C) and endomyocardial biopsy (B and D) from a 54-year-old man before (A and B) and after (C and D) 6 months of immunosuppression. Severely dilated (LVEDD = 90 mm) and hypokinetic (EF = 18%) left ventricle recovered significantly after treatment (EDD = 73 mm EF = 38%) and LV improvement was associated with disappearance of left-bundle-branch block (A and C), while an active lymphocytic myocarditis (B) progressed to a healed phase (D).

cases. This difference in otherwise clinically similar cohorts may be explained by epidemiologic variables and the specific immunologic characteristics of the patients enrolled.

Results of the present trial confirmed the positive impact of immunosuppression on recovery of LV function in a high rate (88%) of patients with no case of death or cardiac transplantation during treatment and in the following 6 months. Remarkably a striking improvement occurred even in patients with extreme LV dilatation and dysfunction and it was accompanied at histological examination by the disappearance of inflammatory infiltrates with progression of the disease from an active towards a healed myocarditis.

These encouraging results are at variance with those reported in previous studies^{1–4} documenting little or no impact of immunosuppression on inflammatory cardiomyopathy. We do believe that the main reason for the different results might reside in the different criteria used for selection of candidates to immunosuppressive therapy. In fact, while in other studies the inclusion criteria were essentially clinical (severity of cardiac compromise) and histological (Dallas criteria), we associated a negative PCR for an extensive panel of viral genomes including usual and uncommon cardiotropic agents. This allowed the identification of those patients in whom myocardial inflammation was most likely the result of an immune-mediated injury towards segregated (i.e. myosin) or new antigens shared with viral components (antigenic mimicry).²¹ Additionally our candidates presented at immunohistochemistry ≥ 14 leukocytes/mm²¹⁴ and >2 CD3+ lymphocytes/HPF¹³ of tissue section mostly adjacent to cardiomyocytes and associated with cell necrosis. These criteria identify the most aggressive forms of the disease excluding those with healing or healed myocarditis where the beneficial effect of immunosuppression can be minor or none.

Immunosuppression was, in general, well tolerated with minor side effects consisting in increased body weight and fluid retention requiring adjustment of diet and diuretics administration. Still, however, a consistent minority of patients (12%) failed to improve following immunosuppression. Histology of this cohort was characterized by reduction or disappearance of inflammatory infiltrates but occurrence of degenerative changes of cardiomyocytes. Possible explanation of this outcome can be the presence of myocardial viral genomes not included in our panel of PCR or mechanisms of damage and inflammation not susceptible to immunosuppression.

Among patients with virus-negative inflammatory cardiomyopathy receiving only supportive treatment and placebo, besides an initial improvement in some of them, 83% showed a further impairment of cardiac function of whom two patients were transplanted and two died in the 6 months after the end of the study. The remaining 17% maintained the degree of cardiac dysfunction detected at baseline. Histological analysis showed persistence of myocardial inflammation with evidence of myocyte degeneration and necrosis. The inefficacy of supportive treatment in such a large cohort can probably be explained with the type of patient selection and the unopposed chronic mechanism of cell death.

In conclusion, immunosuppressive therapy in patients with virus-negative inflammatory cardiomyopathy appears as an effective and safe option in addition to supportive treatment for recovery of

cardiac failure. Further studies are needed to detect the small cohort of non-responders for a more specific and tailored management.

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Conflict of interest: none declared.

References

- Matitau A, Perez-Atayde A, Sanders SP, Sluysmans T, Parness IA, Spevak PJ, Colan SD. Infantile dilated cardiomyopathy. Relation of outcome to left ventricular mechanics, hemodynamics, and histology at the time of presentation. *Circulation* 1994;**90**:1310–1318.
- Lee KJ, McCrindle BW, Bohn DJ, Wilson GJ, Taylor GP, Freedom RM, Smallhorn JF, Benson LN. Clinical outcomes of acute myocarditis in childhood. *Heart* 1999;**82**:226–233.
- Parrillo JE, Cunnion RE, Epstein SE, Parker MM, Suffredini AF, Brenner M, Schaer GL, Palmeri ST, Cannon RO III, Alling D. A prospective, randomized, controlled trial of prednisone for dilated cardiomyopathy. *N Engl J Med* 1989;**321**:1061–1068.
- Mason JW, O'Connell JB, Herskowitz A, Rose NR, McManus BM, Billingham ME, Moon TE. A clinical trial of immunosuppressive therapy for myocarditis: the Myocarditis Treatment Trial Investigators. *N Engl J Med* 1995;**333**:269–275.
- Chimenti C, Calabrese F, Thiene G, Pieroni M, Maseri A, Frustaci A. Inflammatory left ventricular microaneurysms as a cause of apparently idiopathic ventricular tachyarrhythmias. *Circulation* 2001;**104**:168–173.
- Feldman AM, McNamara D. Myocarditis. *N Engl J Med* 2000;**343**:1388–1398.
- Dec GW Jr, Palacios IF, Fallon JT, Aretz HT, Mills J, Lee DC, Johnson RA. Active myocarditis in the spectrum of acute dilated cardiomyopathies: clinical features, histologic correlates, and clinical outcome. *N Engl J Med* 1985;**312**:885–890.
- 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**:857–863.
- Kühl U, Pauschinger M, Schwimmbeck PL, Seeberg B, Lober C, Noutsias M, Poller W, Schultheiss HP. Interferon-beta treatment eliminates cardiotropic viruses and improves left ventricular function in patients with myocardial persistence of viral genomes and left ventricular dysfunction. *Circulation* 2003;**107**:2793–2798.
- Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography: American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr* 1989;**2**:358–367.
- Aretz H, Billingham ME, Edwards WD, Factor SM, Fallon JT, Fenoglio JJ Jr, Olsen EG, Schoen FJ. Myocarditis: a histopathologic definition and classification. *Am J Cardiovasc Pathol* 1986;**1**:3–14.
- Chimenti C, Pieroni M, Maseri A, Frustaci A. Histologic findings in patients with clinical and instrumental diagnosis of sporadic arrhythmogenic right ventricular dysplasia. *J Am Coll Cardiol* 2004;**43**:2305–2313.
- Kühl U, Noutsias M, Seeberg B, Schultheiss HP. Immunological evidence for a chronic intramyocardial inflammatory process in dilated cardiomyopathy. *Heart* 1996;**75**:295–300.
- Maisch B, Bultman B, Factor S. World Heart Federation consensus conference's definition on inflammatory cardiomyopathy (myocarditis): report from two expert committees on histology and viral cardiomyopathy. *Heartbeat* 1999;**4**:3–4.
- Loud AV, Anversa P. Biology of disease: morphometric analysis of biologic processes. *Lab Invest* 1984;**50**:250–261.
- Wojnicz R, Nowalany-Kozielecka E, Wojciechowska C, Glanowska G, Wilczewski P, Niklewski T, Zembala M, Polonski L, Rozek MM, Wodniecki J. Randomized, placebo-controlled study for immunosuppressive treatment of inflammatory dilated cardiomyopathy: two-year follow-up results. *Circulation* 2001;**104**:39–45.

17. Pauschinger M, Doerner A, Kuehl U, Schwimmbeck PL, Poller W, Kandolf R, Schultheiss HP. Enteroviral RNA replication in the myocardium of patients with left ventricular dysfunction and clinically suspected myocarditis. *Circulation* 1999;**99**:889–895.
18. Kuhl U, Pauschinger M, Seeberg B, Schwimmbeck PL, Poller W, Kandolf R, Schultheiss HP. Viral persistence in the myocardium is associated with progressive cardiac dysfunction. *Circulation* 2005;**112**:1965–1970.
19. Kühl U, Pauschinger M, Noutsias M, Seeberg B, Bock T, Lassner D, Poller W, Kandolf R, Schultheiss HP. High prevalence of viral genomes and multiple viral infections in the myocardium of adults with 'idiopathic' left ventricular dysfunction. *Circulation* 2005;**111**:887–893.
20. Bowles NE, Ni J, Kearney DL, Schultheiss HP, McCarthy R, Hare J, Bricker JT, Bowles KR, Towbin JA. Detection of viruses in myocardial tissues by polymerase chain reaction. evidence of adenovirus as a common cause of myocarditis in children and adults. *J Am Coll Cardiol* 2003;**42**:466–472.
21. Rose NR. Viral damage or 'molecular mimicry'-placing the blame in myocarditis. *Nat Med* 2000;**6**:631–632.