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Long-Term Evolution and Prognostic Stratification of Biopsy-Proven Active Myocarditis

Running title: *Anzini et al.; Active myocarditis: presentation and prognosis*

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Abstract

Background—Active myocarditis (AM) is characterized by large heterogeneity of clinical presentation and evolution. This study describes the characteristics and the long-term evolution of a large sample of patients with biopsy-proven active AM, looking for accessible and valid early predictors of long-term prognosis.

Methods and Results—From 1981 to 2009, 82 patients with biopsy-proven AM were consecutively enrolled and followed-up for 147±107 months. All patients underwent clinical and echocardiographic evaluation at baseline and at 6 months. At this time, improvement/normality of left ventricular ejection fraction (LVEF), defined as a LVEF increase > 20 percentage points and/or presence of LVEF≥50%, was assessed. At baseline, left ventricular (LV) dysfunction (LVEF <50%) and left atrium enlargement were independently associated with long-term heart transplantation (HTx)-free survival, regardless of the clinical pattern of disease onset. At 6 months, improvement/normality of LVEF was observed in 53% of patients. Persistence of NYHA III-IV classes, left atrium enlargement and improvement/normality of LVEF at 6 months emerged as independent predictors of long-term outcome. Notably, the short-term revaluation showed a significant incremental prognostic value when compared to the baseline evaluation (baseline model vs 6 months model: Area Under the Curve 0.79 vs 0.90, p=0.03).

Conclusions—Baseline LV function is a marker for prognosis regardless of the clinical pattern of disease onset and its reassessment at 6 months appears useful for assessing longer term outcome.

Key words: myocarditis, cardiomyopathy, biopsy, follow-up study, endomyocardial biopsy

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Introduction

Active myocarditis (AM) is an inflammatory disease of the myocardium, diagnosed by established histological[1], immunological and immunohistochemical criteria[2]. Heterogeneity of clinical manifestation is a peculiar feature of AM, which may variably present with recent onset heart failure (HF), arrhythmias, chest pain or a concurrence of these elements[3]. Similarly, natural history of AM is highly variable, ranging from full recovery to development of dilated cardiomyopathy or sudden cardiac death[3]. The prognostic stratification is critical in the management of AM. However, several studies including heterogeneous populations evaluated the prognostic values of different clinical and instrumental parameters in the mid-term risk assessment and reported conflicting conclusions[4,5,6]. Thus, currently there is no accordance on early independent long-term prognostic predictors for AM.

Our study describes the long-term natural history of a large sample of patients with AM diagnosed by endomyocardial biopsy (EMB), looking for accessible and valid early predictors of long-term prognosis. In particular, we estimated the prognostic role of early revaluation.

Methods

Study population

From 1981 to 2009, 82 patients with EMB proven AM were consecutively enrolled in the Trieste Heart Muscle Disease Registry. Informed consent was obtained from all subjects under the institutional review board policies of the Trieste Hospital administration. At baseline, all patients underwent a careful clinical evaluation, laboratory testing and echocardiographic assessment. Right ventricular catheterization was performed in presence of left ventricular (LV) dysfunction (LV ejection fraction - LVEF - <50%). All patients with cardiovascular risk factors, or older than 35, underwent selective coronary angiography to exclude coronary artery disease.

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AM was defined as “peripartum” when occurring during the last month of pregnancy or within 5 months after delivery.

Fulminant myocarditis was defined as the rapid onset of severe HF distinctly identifiable within the four weeks before the time of enrolment, associated with severe LV or biventricular dysfunction, hemodynamic instability and need for advanced pharmacological or mechanical circulatory assistance[7].

Laboratory testing consisted in: 1) evaluation of inflammation and myocytolysis markers, 2) titration of antiviral antibodies (Coxsackievirus, Adenovirus, Enterovirus, Citomegalovirus, Influenzavirus A and B), 3) search of antibodies for Mycoplasma and Toxoplasma, 4) study of blood lymphocytic subpopulations and 5) titration of antinuclear antibodies. When appropriate, serum antibody ELISA testing and cultural testing on blood and myocardial tissue for Borrelia Burgdorferi and for Rickettsiae were performed.

Details on echocardiographic assessment and measures are provided in Supplemental Material.

Cardiac magnetic resonance (CMR) and brain natriuretic peptide (BNP) testing were systematically performed since 2008 in patients with AM; given the small number of subjects that underwent these evaluations, CMR and BNP data were not included in the present analysis.

Endomyocardial biopsy and sample analysis

The criteria for indication to EMB in our Clinic have considerably changed over the time period considered in our study. From 1981 to 1992 all patients with LV dysfunction of unknown origin underwent EMB. Since 1993, in order to reach a more accurate selection of patients, EMB was performed only in those who presented with 1) recent onset HF (within 6 months from enrolment), and 2) severe LV dysfunction (LVEF <40%) without ventricular remodeling on

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echocardiography, and/or 3) idiopathic major ventricular arrhythmias (sustained ventricular tachycardias / ventricular flutter or fibrillation, aborted sudden death).

Similarly, technical procedures for EBM also changed over time. Whereas early EBMs were usually performed from the right ventricle, since 1989 they were performed from the LV, except for cases with suspected LV thrombus, or with probable endomyocardial fibrosis. In addition, whereas from 1981 to 1985 a "King's College" bioptome was used, since 1985 a "Cordis" bioptome was used. The median number of bioptic samples per patient was 4 [minimum-maximum: 1-6] (mean 4±1). In three patients with fulminant form, deceased soon after disease onset, diagnosis was confirmed with autopsy.

Details on the preparation of bioptic samples for traditional histopathological analysis, performed on all patients, are described in Supplemental Material. Since 1993 an additional sample was systematically frozen in liquid nitrogen and stored at -80°C[8]. The following antigens were tested using specific antibodies for identification of myocardial inflammation and for the identification, localization, and characterization of mononuclear cell infiltrates: HLA-DR; CD54 for adhesion molecules; CD4 for helper T-cells; CD8 for suppressor T-cells; CD25 for regulatory T-cells; CD2 for natural killer lymphocytes; CD45RO for memory T-cells; CD68-PGM1 for histiocytes.

Since 2000, bioptic samples were systematically evaluated for the presence of the genome of cardiotropic viruses (Parvovirus B19, Adenovirus, Enterovirus, Ebstein-Barr Virus, Herpes Simplex Virus 1, Herpes Simplex Virus 2) by Real Time Polymerase Chain Reaction using specific primers and probes; in case of virus-positive EMB, blood samples were also tested for the same virus.

Diagnosis of AM was made in accordance with Dallas Criteria[1]; cases with borderline

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myocarditis were excluded from the present study. Since 1992, the diagnosis of AM was established when immunohistochemical analysis detected myocardial immune activation in addition to positivity of Dallas Criteria.

Therapy

All patients with LV dysfunction and/or HF symptoms received tailored medical treatment, as indicated by guidelines at the time of enrolment.

From 1981 to 1991 all patients with AM received immunosuppressive therapy. From 1992 this therapy was provided only to the patients who presented 1) Dallas-positive AM in association with myocardial immune activation at immunohistochemical analysis, 2) persistent LVEF <35% despite optimal medical treatment, associated with persistent HF (NYHA classes III-IV) with hemodynamic instability despite maximal circulatory support, or 3) otherwise unexplained life-threatening ventricular arrhythmias. Immunosuppressive therapy consisted in administration of prednisone (50 mg/m^2 with progressive downscaling) and azathioprine (75 mg/m^2) for a 6 month period. Since 2003, immunosuppressive therapy was administered exclusively to virus-negative patients with evidence of myocardial immune activation at immunohistochemical analysis[9].

Study design and outcome measure

All patients (except one, who refused the examination) were followed with a scheduled revaluation at 6 (3 to 9) months from baseline in our HF Outpatient Clinic. Further assessments were planned according to the clinical and instrumental status of each patient.

The study outcome measure was long-term heart transplantation (HTx)-free survival. Patients were categorized in three groups according to the main pattern of disease onset:

Group 1. HF: NYHA II-IV; LVEF<50%.

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Group 2. Arrhythmias: electrocardiographic evidence of bradyarrhythmias or tachyarrhythmias.

Group 3. Chest pain.

Furthermore, the study population was categorized at the 6 months follow-up according to an improvement/normality criterion, defined as: LVEF increase > 20 percentage points or LVEF ≥50%. This criterion was selected as the most accurate for the end-point prediction in comparison with other criteria, set at different cut-off values (see "Statistical Analysis").

Information concerning the study end-point and the causes of death were obtained directly from the patient during the follow-up examinations, or by telephone contact with patients, their relatives, General Practitioner or by consulting the office of vital statistics at the place of residence of the patient. The end of follow-up was considered as 31st December 2012 (last check-date of status for alive patients) or the date of death or HTx.

Statistical Analysis

Summary statistics of clinical and instrumental variables were expressed as mean + standard deviation or median and first-third quartile or percentages, as appropriate.

Continuous variables were compared between groups by the Analysis of Variance (ANOVA), using the Brown-Forsythe statistic when the assumption of equal variances did not hold[10,11], whereas for discrete variables the Fisher's exact test was applied, with a Monte Carlo approximation to compute the p values when appropriate.

To define the improvement/normality criterion a grid of different cut-off values for LVEF variation between baseline and 6 months were 'a priori' selected and compared by means of the Receiver-Operating Characteristic analysis for the end-point death/HTx, and the best one in terms of Area under the Curve was selected **(Supplemental Table 1)**.

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To predict the improvement/normality at 6 months (LVEF increase > 20 percentage points, or LVEF ≥50%), a univariable screening of all clinical-laboratory baseline parameters of patients was initially made (estimating univariable logistic regression models variable by variable), followed by the application of a backward elimination based on likelihood ratio testing to the list of selected parameters (i.e., with $p<0.1$) at the univariable procedure, in order to estimate the multivariable logistic regression model.

Survival curves were calculated according to the Kaplan-Meier method, and the comparison between curves was carried out with the Log-Rank test.

Univariable Cox proportional hazards models were applied to find predictors of the endpoint. Multivariable Cox models were then estimated by means of a backward elimination based on likelihood ratio testing starting from the list of variables derived at the previous univariable screening.

The prognostic accuracy of the estimated survival models was estimated by using Receiver-Operating Characteristic analysis[12]: predicted probabilities of survival estimated by the baseline model were compared to the predicted probabilities of survival estimated by the 6 months model, re-estimating also the baseline model with a follow up starting at 6 months for the survived patients. All results were considered statistically significant when $p<0.05$.

The entire analysis was performed using the SPSS package, version 13.0 (SPSS Inc., Chicago, Illinois) and R statistical software version 2.7.2.

Results

Study Population

Baseline characteristics of study patients are summarized in **Table 1**. Patients were young (age 38±16 years) and predominantly males. The interval between the onset of cardiac symptoms and

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hospitalization was shorter than one month in 75% of patients and only 15% of subjects presented with left branch bundle block. On EMB, 75 patients (91%) had lymphocytic myocarditis; despite extensive investigation, in most cases (71%) the precise etiology could not be established by serological testing.

Patient characteristics according to the pattern of disease onset at enrolment and at 6 months are described in **Table 1**. Among the three groups, patients with HF presented more frequently LV dysfunction and enlarged left atrium and ventricle. The interval between the onset of cardiac symptoms and hospitalization was significantly longer in patients with HF, compared to the other groups; however, it was shorter than 2 months in 75% of HF patients.

Among patients with arrhythmic onset, 10 presented with tachyarrhythmias (6 with ventricular fibrillation/flutter or sustained ventricular tachycardia; 1 with frequent symptomatic ventricular premature beats; 3 with supraventricular arrhythmias) and 10 presented with bradyarrhythmias (2 with II degree atrioventricular block and 8 with III degree atrioventricular block).

At 6 months 41 subjects (53%) satisfied the LVEF improvement/normality criterion. Immunosuppressive therapy was administered to 56% of the patients of the entire cohort. In comparison with the remaining patients, they were characterized by a more severe functional impairment (NYHA III-IV 67% vs 33%, p=0.066), a larger LV end-diastolic diameter (37[33-42]mm/m vs 33[29-37]mm/m, p<0,001), a lower LVEF (28[21-37]% vs 43[30-56]%, p=0,004), and were more frequently treated with diuretics (74% vs 26%, p<0.001). The treated and untreated patients did not differ for the other baseline characteristics *(data not shown)*.

Long-term Outcome and prognostic stratification

During a mean follow-up of 147±107 [median 140; first-third quartile: 54-222] months, 23

(28%) patients died and 7 (9%) underwent HTx. HF was the most prevalent cause of death (14; 61%), followed by sudden cardiac death (3; 13%) and non-cardiovascular death (3; 13%). For 3 (13%) patients the cause of death remained unknown. Long-term HTx-free survival was significantly different among groups according to the pattern of disease onset, with the poorest outcome for HF patients, as shown in **Figure 1**. On the other hand, at multivariable analysis, the independent predictors of long-term HTx-free survival were the left atrium enlargement and the presence of LV systolic dysfunction at enrolment (**Table 2)**. The significant prognostic impact of LV dysfunction and left atrium dilatation at enrolment, regardless of the pattern of disease onset, is shown in **Figure 2**.

The independent prognostic role of short-term clinical and echocardiographic revaluation is shown in **Table 3**. The persistence of NYHA III-IV classes, the left atrium dilatation and the improvement or normality of LVEF at 6 months were selected as independent predictors of long-term outcome. As shown in **Figure 3**, the short-term revaluation showed a significant incremental prognostic value when compared to the baseline evaluation (for overall HTx-free survival after 6 months, baseline model vs 6 months model: Area Under the Curve 0.79 vs 0.90, p=0.03).

During the long-term follow up 9 (11%) patients (7 for primary and 2 for secondary prevention) received an implantable cardioverter-defibrillator (ICD), with a mean interval of 101±112 [median 59; first-third quartile: 14-193] months after the disease onset. Notably, all the 3 episodes of sudden death occurred when device therapy was not routinely used at our institution.

Predictors of Improvement/Normalization of Left Ventricular Ejection Fraction

At multivariable analysis, the presence of LV dysfunction at baseline was the only independent

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predictor for its own evolution at the short term follow-up, disfavoring improvement or normality of LVEF (OR 0.028, CI 0.003-0.225, p=0.001).

In the subgroup of patients with LV dysfunction at baseline, optimal medical therapy (with angiotensin-converting enzyme inhibitors and beta-blockers) was the only variable associated with 6 months improvement/normality of LVEF (OR 6.417, CI 1.081-34.861, p 0.031).

Fulminant Myocarditis

Out of the whole sample, 10 patients (12%) presented with fulminant myocarditis. Compared to the others, fulminant forms presented a more severe hemodynamic impairment at baseline and were more frequently treated with intravenous inotropes; moreover, fulminant patients were significantly younger, and 4 of them (40%) were younger than 13 **(Table 4)**. Histopathological analysis indentified lymphocytic myocarditis in 9 patients and eosinophilic myocarditis in one.

Five out of 10 patients with fulminant forms died or underwent HTx soon after the disease onset **(Figure 4)**. In particular, 4 among these patients accounted for all the events during the first 6 months follow-up in the whole population. All pediatric fulminant patients (age <13 years) met the study endpoint and in 3 cases diagnosis was obtained at autopsy.

After 6 months, patients surviving the acute phase presented clinical and echocardiographic features comparable to the others **(Table 4)** and demonstrated an excellent long-term survival free of HTx **(Figure 4)**.

Discussion

The prompt management and early long-term prognostic stratification of patients with AM represent a challenging task for clinical cardiologists, as both clinical presentation and long term evolution may present great variability. In this regard, the clinical course of AM may be dramatic

and physicians face challenging decisions, such as early referral for HTx or prophylactic placement of an ICD.

Current data provide a valuable clinical insight in the patterns of presentation and evolution of AM and underline the relevance of tight revaluation for the appropriate clinical management of such patients. In particular, relevant findings of this study are: 1) the independent association between poor prognosis and left atrium enlargement and LV dysfunction at diagnosis of AM; 2) the incremental predictive power of short-term revaluation for long-term risk stratification; 3) the poor survival of the subgroup of patients with fulminant myocarditis.

Baseline evaluation

Previous studies identified some parameters of severe hemodynamic impairment such as higher ventricular filling pressures[13,14], lower cardiac index[14] and intolerance to therapy with beta-blockers[5] as independent predictors of worse prognosis in the setting of AM. Moreover, the presence of Late Gadolinium Enhancement on CMR has been recently identified as a strong predictor of cardiovascular and sudden cardiac death in patients with EMB-proven AM[6]. These findings underline the prognostic value of the structural and functional impairment of the LV at initial presentation of patients with AM, whereas patterns of clinical onset contribute prognostically rather weakly. In this respect, data from our cohort interestingly demonstrate that a larger left atrium and LV dysfunction at baseline echocardiogram emerged as simple and clinically valid parameters to identify a subgroup of patients at higher risk of adverse events, regardless the pattern of clinical presentation **(Table 2, Figure 2)**.

Furthermore, our results confirm recent evidences on the futility of serological testing for the diagnosis of AM[15], confining a possible role for theses analyses in the context of suspected bacterial infection associated with advanced atrioventricular conduction defects **(Table 1)**.

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Role of short-term re-evaluation

AM represents a model of possibly reversible myocardial disease. Early spontaneous LV function recovery has been previously described in 39% of patients with EMB-proven AM[16], while significant early improvement of LV function has been demonstrated in 60% of patients with AM during immunosuppressive treatment[17]. In both series, LV function recovery was invariably associated with excellent mid-term prognosis.

In line with these results, early improvement or normality of LVEF was observed in 53% of our patients at 6 months. In the high-risk subgroup of patients with LVEF<50%, medical therapy with angiotensin-converting enzyme inhibitors and beta-blockers was the only variable independently associated with LV function recovery. Interestingly, as previously reported[18], immunosuppressive treatment was not associated with LVEF improvement or survival in our analysis. We assume that this finding may be confounded by earlier therapeutic strategies when immunosuppression was administered to all patients with AM regardless of LVEF and without the guidance of immunohistochemical[19] and virological analyses[20].

Importantly, the prompt improvement or normality of LVEF was independently associated with long-term outcome on multivariable analysis, regardless of LV function at disease onset **(Table 3)**. Moreover, the short-term revaluation presented additional incremental predictive value when compared to the baseline evaluation, thus allowing a more accurate long-term risk stratification **(Figure 3)**. In particular it is worthy of note that patients experiencing an increase of LVEF by 20 percentage points or a LVEF≥50% and a low NYHA functional class at 6 months demonstrated an excellent long-term prognosis.

Fulminant myocarditis

AM may abruptly present with severe HF rapidly progressing towards cardiogenic shock,

previously described as fulminant myocarditis[7,14,21]. In our sample this subgroup was characterized by extremely poor survival in the short term **(Figure 4)**. These data are in contrast with the experience previously published by McCarthy et al[14]. However, different from this study, our sample includes pediatric patients and necroscopic diagnoses. On the other hand, our findings are in line with recent data from the Pediatric Health Information System database, which demonstrate a significant association between pediatric myocarditis with need of advanced circulatory support and severe prognosis[22]. The reasons for the high prevalence of the fulminant form in the pediatric population with AM and the causes of its poor outcome remain unknown. We hypothesize that disproportionate and uncontrolled immune response in children may explain at least partially the fulminant course.

Finally, consistently with previous reports[21], patients that overcome the acute phase of the disease after 6 months presented clinical and echocardiographic features comparable to other patients **(Table 4)** and were characterized by an excellent long-term survival **(Figure 4)**.

Clinical implications

The fact that we observed only 82 AM over 28 years, despite the selection bias of a referral centre devoted to cardiomyopathies, might indicate either that AM is a rare disease, or, more reasonably, that it is often unrecognized. This could stem from different reasons. First, EMB remains the main tool to diagnose AM, although it presents well recognized accuracy limits, related to the number and sites of bioptic samples[23,24], proper timing of the procedure[25] and histopathological interpretation[26]. Consequently, the real incidence of suspected AM without criteria for EMB remains uncertain.

Our data indicate that AM patients with preserved LVEF at disease onset present a good long-term prognosis and should be treated conservatively. However, particular attention should

be devoted to patients with AM presenting with arrhythmias, in which the rhythm instability may indicate a diffuse myocardial involvement. Notably all the three endpoint events in the arrhythmic group occurred between the sixth and the eighth year of follow-up, underlining the importance of a periodic and structured reassessment of patients with AM **(Figure 1)**. Conversely, patients presenting with chest pain (all biopsied during the early enrolment period of our study, representing a "historical" subgroup), were invariably characterized by preserved LVEF and excellent prognosis in the long-term follow-up. These data support the belief that EMB is not indicated in this subgroup of patients[27,28].

Finally, patients with remodeled left atrium and LV dysfunction at baseline represent a high risk subgroup and require frequent revaluations under optimal medical treatment in order to improve the prognostic stratification and guide a tailored global management **(Figure 3)**. In this regard, patients showing a short-term improvement were characterized by benign long-term evolution**.** Accordingly, major clinical decisions such as referral for HTx or prophylactic ICD placement should be postponed and reconsidered, according to short-term evolution of clinical and instrumental parameters under optimal medical treatment.

Study Limitations and Distinctive Features

The study patients were enrolled in a tertiary referral centre for cardiomyopathies and HF, thus associated with a selection bias with respect to the characteristics of AM in the common clinical practice. Moreover, the patients were enrolled over a period of 28 years, during which several changes occurred in selection criteria for EMB, in EMB procedural protocols, in methods and techniques for biopsy evaluation as well as in criteria for administration of immunosuppressive therapy. Likewise, this period has been characterized by remarkable advances in the medical treatment of HF, LV dysfunction and arrhythmias. However, the historical phase of enrollment

did not affect the results of our analyses (pre/post 1992: HR 0.645; CI 0.270-1.538; p=0.322). Moreover, data regarding CMR imaging and BNP testing were not included in the present analysis. Finally, given the relative small sample size and event-rate of this study, our registry can be perceived as hypothesis generating and further multicentre studies with larger samples are needed to confirm the present findings and validate the proposed multivariable model of clinical assessment. Nevertheless, to our knowledge, this is the largest sample of patients with EMB-proven AM (according to Dallas criteria, excluding borderline myocarditis) described in literature with a homogeneous and average follow-up exceeding 12 years. In particular, the long-term follow-up provided valuable clinical insights on the evolution of such a variable disease. Although a fair prognosis is consistent with the spontaneous or therapeutically-induced reversibility of the pathological substrate, AM may recur, evolve to dilated cardiomyopathy or complicate with life-threatening arrhythmias coherently with its complex and not yet fully understood pathophysiology[29].

Conclusions

LV dysfunction at baseline evaluation defined a subgroup of patients characterized by poorer long-term prognosis in the setting of AM. On the other hand, the patterns of clinical presentation showed a weak prognostic power. Furthermore, the 6 months clinical/instrumental revaluation provided an incremental risk stratification with respect to the baseline assessment. In particular, early improvement/normality of LVEF was independently associated with a favorable long-term outcome. This study underscores the importance of clinical and echocardiographic early revaluation for the appropriate management of patients affected by a possibly reversible myocardial disease, such as AM.

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Conflict of Interest Disclosures: None.

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Table 1. Baseline and 6 months Characteristics of the Study Patients

Baseline Characteristics	N	Whole Population (N=82)	Heart Failure (N=53, 65%)	Arrhythmias (N=20, 24%)	Chest Pain (N=9, 11%)	p value*
Demographic findings						
Age – years	82	38±16	38±17	37±13	40±15	0.920
Age <13 years – no. (%)	82	6(7)	6(11)	0(0)	0(0)	0.262
Males – no. (%)	82	57(70)	35(66)	16(80)	6(67)	0.549
Enrolled after 1992 (%)	82	40(49)	23(43)	9(45)	2(22)	0.499
Anamnesis						
Recent virosis (<6 months) – no. (%)	82	58(71)	39(74)	13(65)	6(67)	0.711
Recent insect bite (<6 months) – no. (%)	82	12(15)	4(8)	8(40)	0(0)	0.002
Hypereosinophilia – no. (%)	82	4(5)	2(4)	1(5)	1(11)	0.416
Peripartum – no. (%)	82	4(5)	4(8)	0(0)	0(0)	0.736
Duration of cardiac symptoms – days	82	8[1-30]	15[5-54]	3.5[1-12]	1[1-14]	0.013
Clinical findings						
NYHA functional classes III-IV – no. (%)	82	39(48)	36(68)	3(15)	0(0)	<0.001
SBP – mm Hg	82	123±20	118±19	134±20	126±23	0.009
Resting heart rate – beats/min	82	88±28	98±26	64±19	84±22	<0.001
BMI – kg/m^2	82	24±4	24±4	24±3	25±4	0.624
ECG / Holter-ECG findings						
AF / other non-sinus rhythms – no. (%)	82	5(6)	4(8)	1(5)	0(0)	1
LBBB – no. (%)	82	12(15)	10(19)	2(10)	0(0)	0.407
NSVT – no. (%)	78	22(28)	20(40)	2(11)	0(0)	0.007
Echocardiographic findings						
LADI – mm/m	75	23±4	25±4	20±3	20±3	<0.001
LVEDDI – mm/m	81	35[31-40]	39[35-42]	30[28-33]	31[29-33]	<0.001
LVEF – %	82	32[24-52]	28[21-32]	57[49-64]	56[53-64]	<0.001
LVEF < 50% – no. (%)	82	59(72)	53(100)	5(25)	1(11)	<0.001
Restrictive pattern – no. (%)	41	18(44)	16(61.5)	1(8)	1(33)	0.003
Moderate-severe MR – no. (%)	82	15(18)	13(25)	2(10)	0(0)	0.146
RVFS < 33%– no. (%)	82	30(37)	27(51)	2(10)	1(11)	0.001
Hemodynamic findings						
Cardiac index – ml/min/m²	62	3336±1276	3121±1200	3994±1308	4272±1441	0.043
Mean PAP– mm Hg	65	18±9	20±9	13±5	11±4	0.008
PCWP – mm Hg	65	12±8	14±9	8±4	6±3	0.022

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RAP – mm Hg	61	4[2-5]	3[2-5]	4[1.5-5.5]	3[1-5]	0.422
Laboratory findings						
Positive serology – no. (%)						
Virus	82	3(4)	1(2)	1(5)	1(11)	0.168
Rickettsia	82	5(6)	0(0)	5(25)	0(0)	<0.001
Borrelia	82	3(4)	1(2)	2(10)	0(0)	0.281
CRP	60	8[1-39]	6[1-38]	6[1-15]	75[1-139]	0.111
Serum CPK > 160 UI/l – no. (%)	72	15(21)	8(17)	4(24)	3(38)	0.399
Medical therapy						
ACE-inhibitors/sartans – no. (%)	82	37(45)	30(57)	5(25)	2(22)	0.018
Beta-blockers – no. (%)	82	33(40)	26(49)	6(30)	1(11)	0.058
Diuretics – no. (%)	82	46(56)	45(85)	0(0)	1(11)	<0.001
Digoxin – no. (%)	82	40(49)	38(72)	1(5)	1(11)	<0.001
Inotropes – no. (%)	82	11(13)	11(21)	0(0)	0(0)	0.034
Amiodarone – no. (%)	82	18(22)	15(28)	3(15)	0(0)	0.130
Immunosoppressants – no. (%)	82	46(56)	35(66)	8(40)	3(33)	0.043
Histopatology						
Lymphocytic – no. (%)	82	75(91)	49(92)	19(95)	7(78)	0.315
Eosinophilic – no. (%)	82	5(6)	3(6)	1(5)	1(11)	0.616
Rheumatic – no. (%)	82	1 (1)	0(0)	0(0)	1(11)	0.106
Giant cell – no. (%)	82	1 (1)	1(2)	0(0)	0(0)	1
Six-months Characteristics	**N**	**Whole Population (N=77)**	**Heart Failure (N=49, 64%)**	**Arrhytmias (N=19, 24%)**	**Chest Pain (N=9, 12%)**	**p value***
Clinical findings						
NYHA III-IV – no. (%)	77	3(4)	4(8)	0(0)	0(0)	0.703
Echocardiographic findings						
LADI – mm/m	72	21±3	22±3	20±4	20±3	0.128
LVEDDI – mm/m	73	35±7	38±6	30±4	29±4	<0.001
LVEF – %	76	46±14	40±13	54±11	61±6	<0.001
LVEF < 50% – no. (%)	77	40	35(71)	5(26)	0(0)	<0.001
Improved/Normal LVEF – no. (%)	77	41(53)	18(37)	14(74)	9(100)	<0.001

ACE: angiotensin-converting enzyme; AF: atrial fibrillation; BMI: body mass index; CRP: C reactive protein; CPK: creatine phosphokinase; LADI: left atrium diameter indexed to height; LBBB: left branch bundle block; LVEDDI: left ventricular end-diastolic diameter indexed to height; LVEF: left ventricular ejection fraction; MR: mitral regurgitation; NSVT: non-sustained ventricular tachycardia; NYHA: New York Heart Association; PAP: pulmonary artery pressure; PCWP; pulmonary capillary wedge pressure; RAP; right atrium pressure; RVFS: right ventricular fractional shortening; SBP: systolic blood pressure.
*between groups

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Table 2. Univariable and Multivariable analyses at baseline for HTx-free survival

	Univariable analysis			Multivariable analysis			Missing [n. (%)]
	HR	CI 95%	p	HR	CI 95%	p	
Demographic findings							
Age < 13 years	3.554	1.226-10.301	0.020	3.316	0.965-11.389	0.057	0(0)
Clinical findings							
NYHA functional class III to IV	2.941	1.391-6.220	0.005				0(0)
Heart failure	7.192	2.172-23.820	0.001				0(0)
ECG / Holter-ECG findings							
NSVT	2.330	1.078-5.032	0.031				4(5)
Echocardiographic findings							
LADI – mm/m (for 1 mm/m increase)	1.205	1.092-1.330	<0.001	1.141	1.022-1.274	0.019	7(8)
LVEDDI – mm/m (for 1 mm/m increase)	1.069	1.021-1.119	0.005				1(1)
LVEF – % (for 5-U decrease)	1.271	1.108-1.458	<0.001				0(0)
LVEF < 50%	9.088	2.148-38.458	0.003	8.029	1.010-63.860	0.049	0(0)
RVFS < 33%	2.130	1.038-4.371	0.039				0(0)
Hemodynamic findings							
RAP – (for 1 mm Hg increase)	1.231	1.099-1.380	<0.001				21(26)
Mean PAP – (for 1 mm Hg increase)	1.056	1.008-1.107	0.021				17(21)
PCWP – (for 1 mm Hg increase)	1.099	1.049-1.152	<0.001				17(21)
Cardiac Index – (for 500 ml/min/m^2 decrease)	1.649	1.634-1.663	0.003				20(24)
Medical therapy							
Diuretics	3.365	1.434-7.897	0.005				0(0)
Digoxin	4.266	1.816-10.021	0.001				0(0)
Inotropes	4.155	1.664-10.374	0.002				0(0)

LADI: left atrium diameter indexed to height; LVEDDI: left ventricular end-diastolic diameter indexed to height; LVEF: left ventricular ejection fraction; NSVT: Non-sustained ventricular tachycardia; NYHA: New York Heart Association; PAP: pulmonary artery pressure; PCWP: pulmonary capillary wedge pressure; RAP; right atrium pressure; RVFS: right ventricular fractional shortening.

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Table 3. Univariable and Multivariable analyses at 6 months for HTx-free survival

	Univariable analysis			Multivariable analysis			Missing [n. (%)]
	HR	CI 95%	p	HR	CI 95%	p	
Clinical findings							
NYHA functional class III to IV	37.153	7.362-187.507	<0.001	16.237	3.193-30.572	0.001	1(1)
Echocardiographic findings							
LADI – mm/m (for 1 mm/m increase)	1.229	1.081-1.397	0.002	1.178	1.030-1.348	0.017	5(6)
LVEF – % (for 5-U decrease)	1.560	1.347-1.808	<0.001				0
LVEF < 50%	41.175	5.523-304.625	<0.001				0
LVEDDI – mm/m (for 1 mm/m increase)	1.091	1.037-1.148	0.001				4(5)
Improved/Normal LVEF	0.020	0.003-0.152	<0.001	0.028	0.004-0.213	0.001	0

LADI: left atrium diameter indexed to height; LVEDDI: left ventricular end-diastolic diameter indexed to height; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association.

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Table 4. Baseline and 6 months characteristics of patients with fulminant and non-fulminant myocarditis

Baseline Characteristics	N	Non-fulminant (N=72, 88%)	Fulminant (N=10, 12%)	p value *
Demographic findings				
Age – years	82	39±15	28±18	0.034
Age <13 years – no. (%)	82	2(3)	4(40)	0.002
Males – no. (%)	82	51(71)	6(60)	0.484
Clinical findings				
NYHA functional classes III-IV – no. (%)	82	29(40)	10(100)	<0.001
SBP – mm Hg	82	126±19	102±14	<0.001
Resting heart rate – beats/min	82	84±25	119±32	<0.001
BMI – kg/m^2	82	24±4	22±4	0.032
Echocardiographic findings				
LADI – mm/m	75	23±4	24±8	0.143
LVEDDI – mm/m	81	35[31-40]	34[32-41]	0.438
LVEF – %	82	35[26-54]	22[18-24]	<0.001
LVEF < 50% – no. (%)	82	23(32)	0(0)	0.029
Restrictive pattern – no. (%)	41	12(34)	6(100)	0.004
Moderate-severe MR – no. (%)	82	13(18)	2(20)	1
RVFS < 33%– no. (%)	82	23(32)	7(70)	0.032
Hemodynamic findings				
Cardiac index – ml/min/m^2	62	3.6±1.2	2.1±0.6	<0.001
Mean PAP– mm Hg	65	17±8	25±11	0.027
PCWP – mm Hg	65	11±8	20±9	0.001
RAP – mm Hg	61	3[2-5]	7[4-16]	0.094
Medical therapy				
ACE-inhibitors/sartans – no. (%)	82	31(43)	6(60)	0.335
Beta-blockers – no. (%)	82	28(39)	5(50)	0.513
Diuretics – no. (%)	82	40(56)	6(60)	1
Digoxin – no. (%)	82	34(47)	6(60)	0.514
Inotropes – no. (%)	82	2(3)	9(90)	<0.001
Amiodarone – no. (%)	82	16(22)	2(20)	1
Immunosoppressants – no. (%)	82	42(58)	4(40)	0.322
Six-months Characteristics	**N**	**Non-fulminant (N=72, 92%)**	**Fulminant (N=6, 8%)**	**p value ***
Clinical findings				
NYHA III-IV – no. (%)	76	2(3)	1(17)	0.221
Echocardiographic findings				
LADI – mm/m	72	20±3	20±7	0.629
LVEDDI – mm/m	73	32[28-37]	33[28-46]	0.605
LVEF – %	77	49[36-56]	48[29-52]	0.531
LVEF < 50% – no. (%)	77	37(51)	3(50)	1
Improved/Normal LVEF – no. (%)	77	38(53)	3(50)	1

ACE: angiotensin-converting enzyme; BMI: body mass index; LADI: left atrium diameter indexed to height; LVEDDI: left ventricular end-diastolic diameter indexed to height; LVEF: left ventricular ejection fraction; MR: mitral regurgitation; NYHA: New York Heart Association; PAP: pulmonary artery pressure; PCWP: pulmonary capillary wedge pressure; RAP: right atrium pressure; RVFS: right ventricular fractional shortening; SBP: systolic blood pressure.
*between groups

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Figure Legends:

Figure 1. Long-term HTx-free survival according to the pattern of disease onset. HTx: heart transplantation

Figure 2. Estimated probability of HTx-free survival according to baseline prognostic Cox model independent variables. HTx: heart transplantation. LADI: left atrium diameter indexed for height. LVEF: left ventricular ejection fraction

Figure 3. ROC curve indicating the comparison of accuracy between baseline survival model (LADI, LVEF<50%) vs 6 months survival model (NYHA III-IV, LADI, improved/normal LVEF). AUC: Area Under the Curve. LADI: left atrium diameter indexed for height. LVEF: left ventricular ejection fraction. NYHA: Hew York Heart Association. ROC: receiver operating characteristics

Figure 4. Long-term HTx-free survival according to fulminant vs non-fulminant presentation. HTx: heart transplantation.

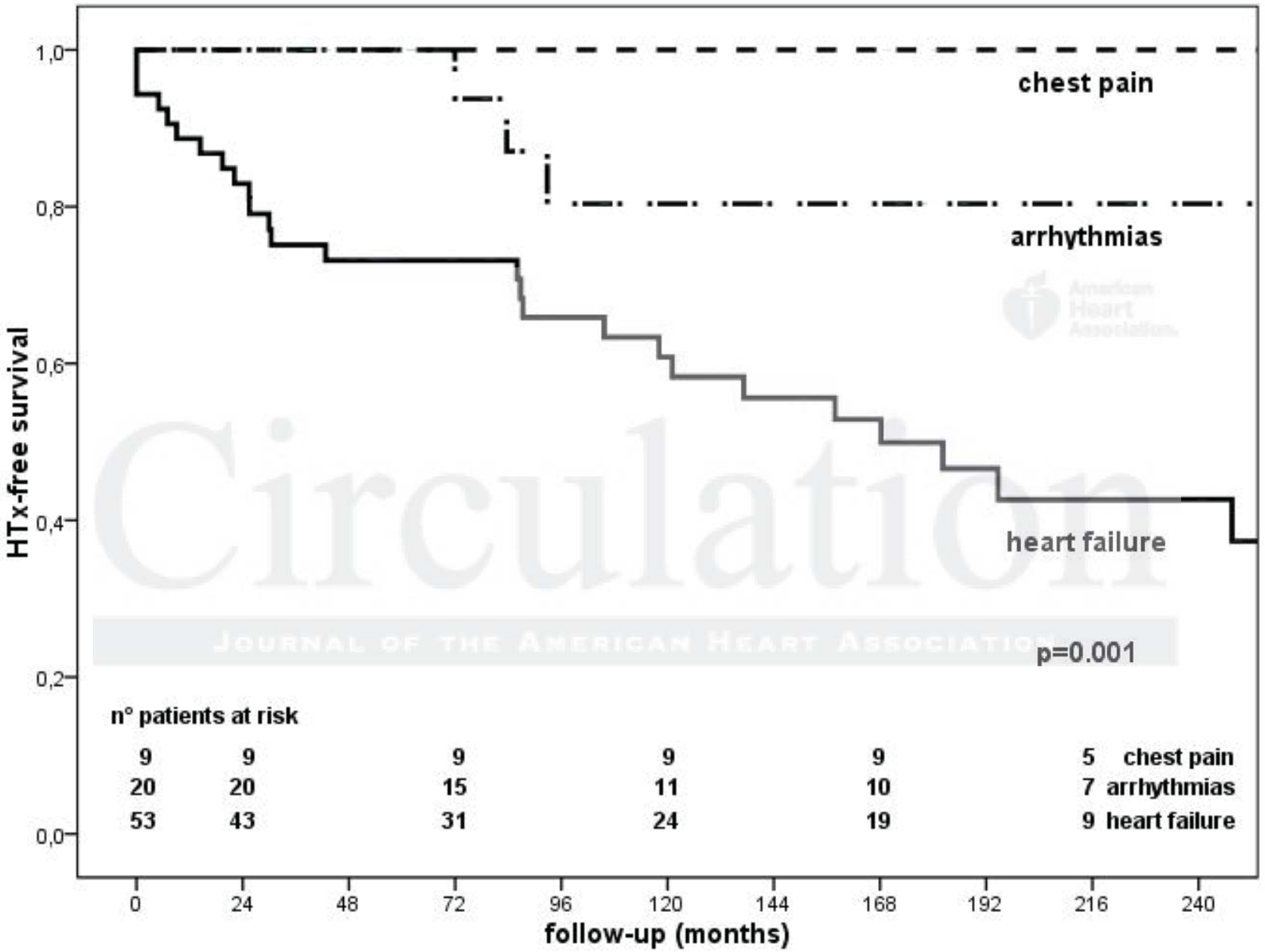
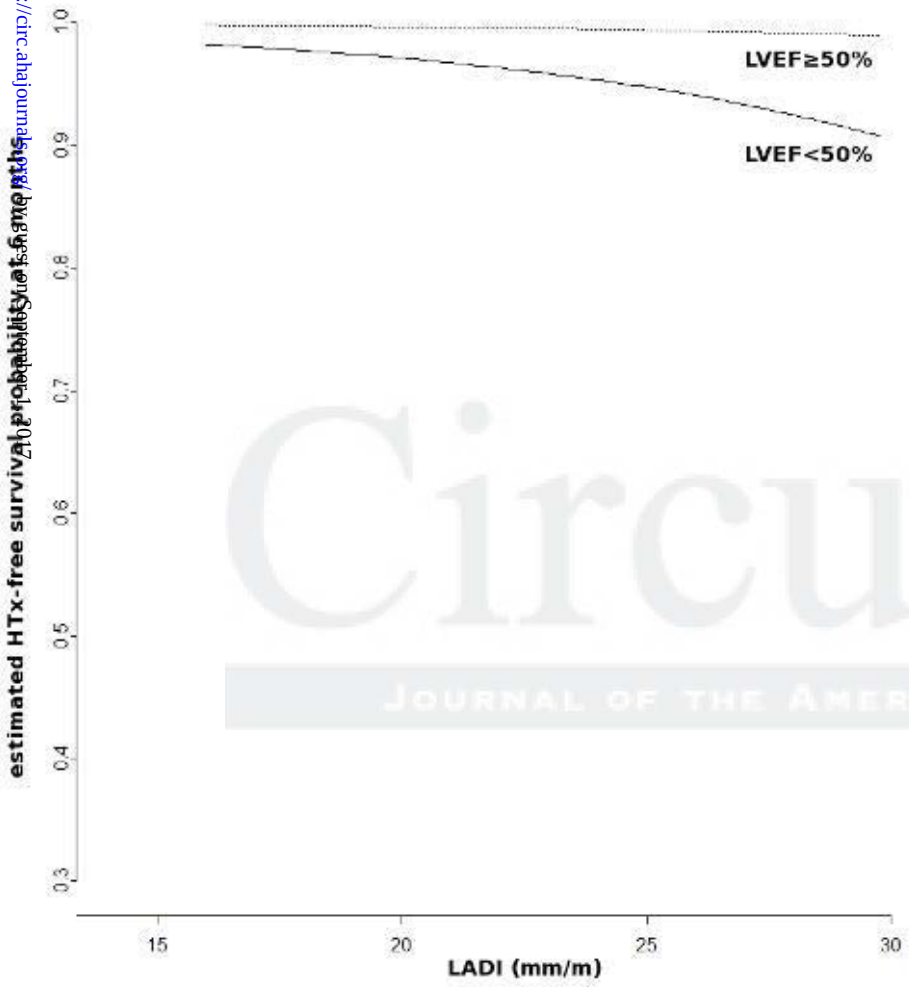


Figure 1



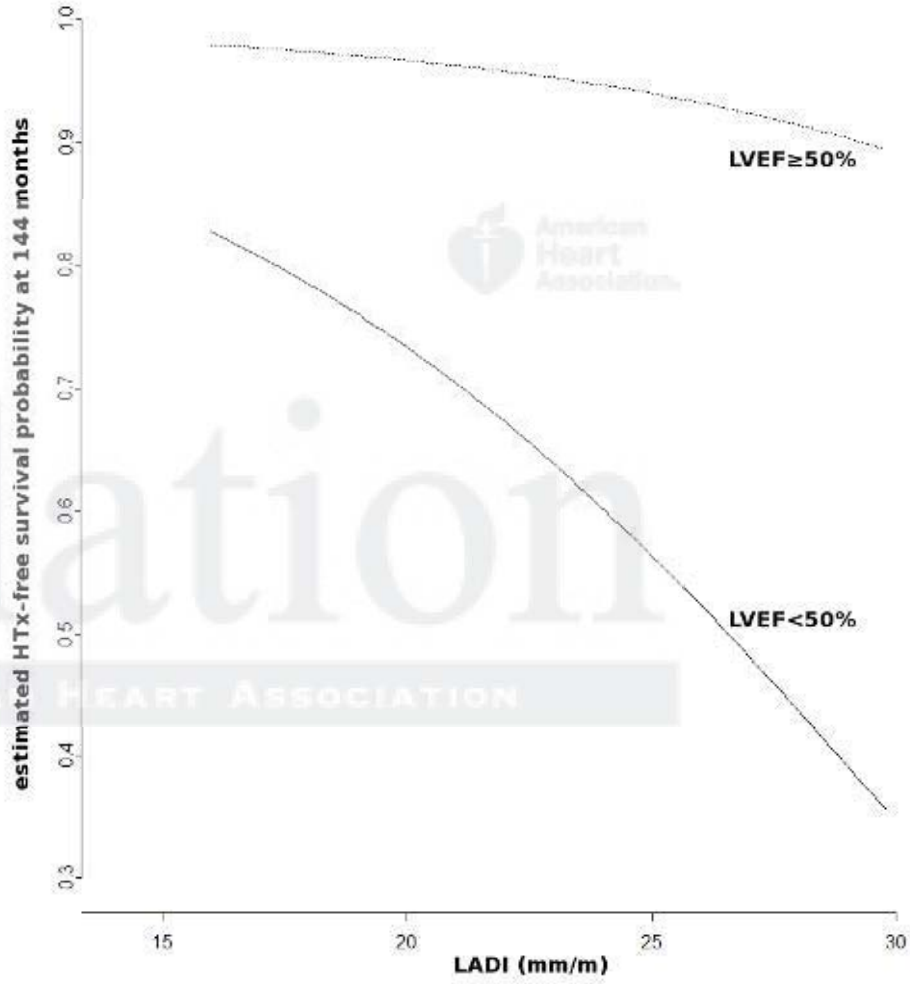


Figure 2

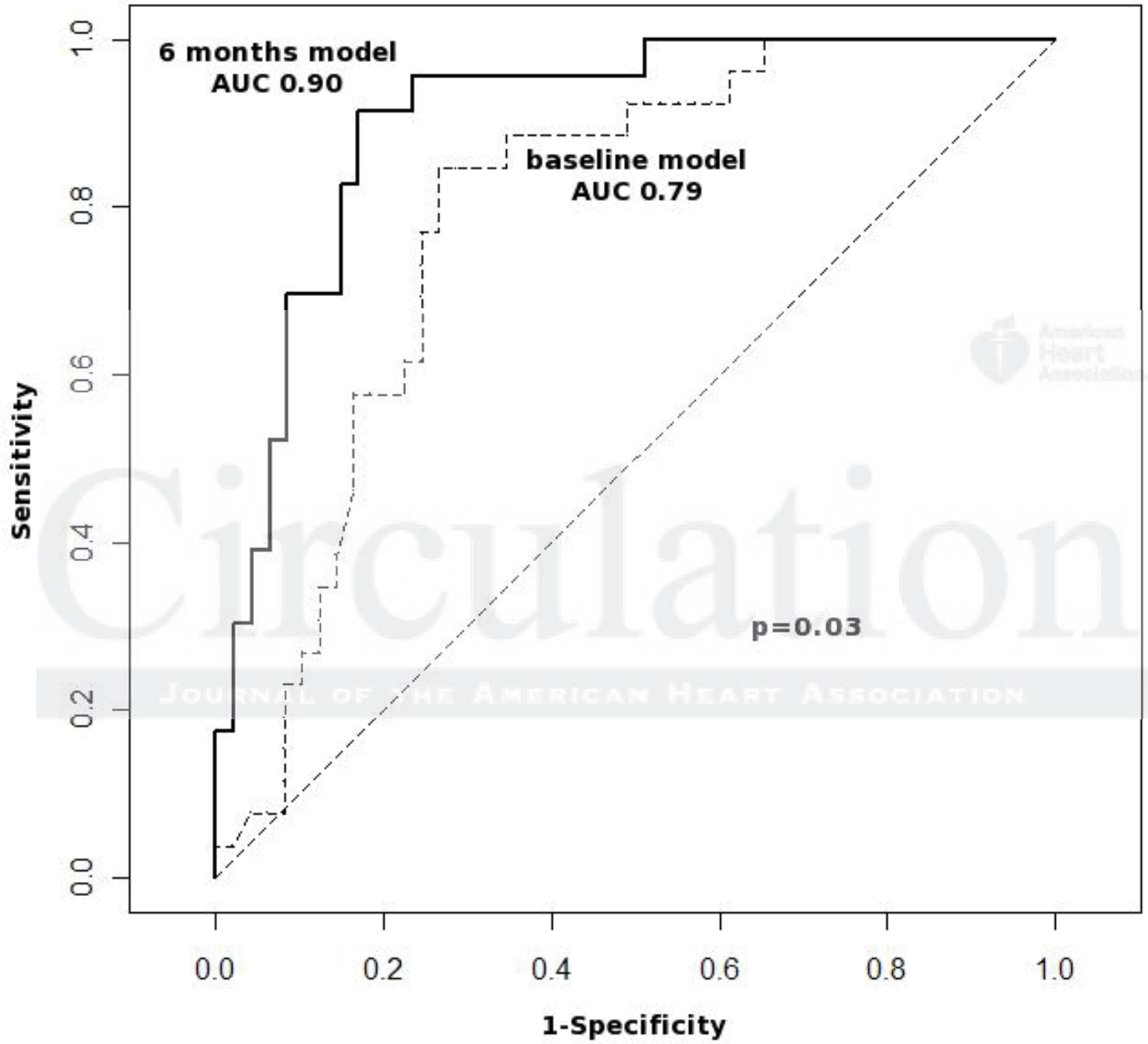


Figure 3

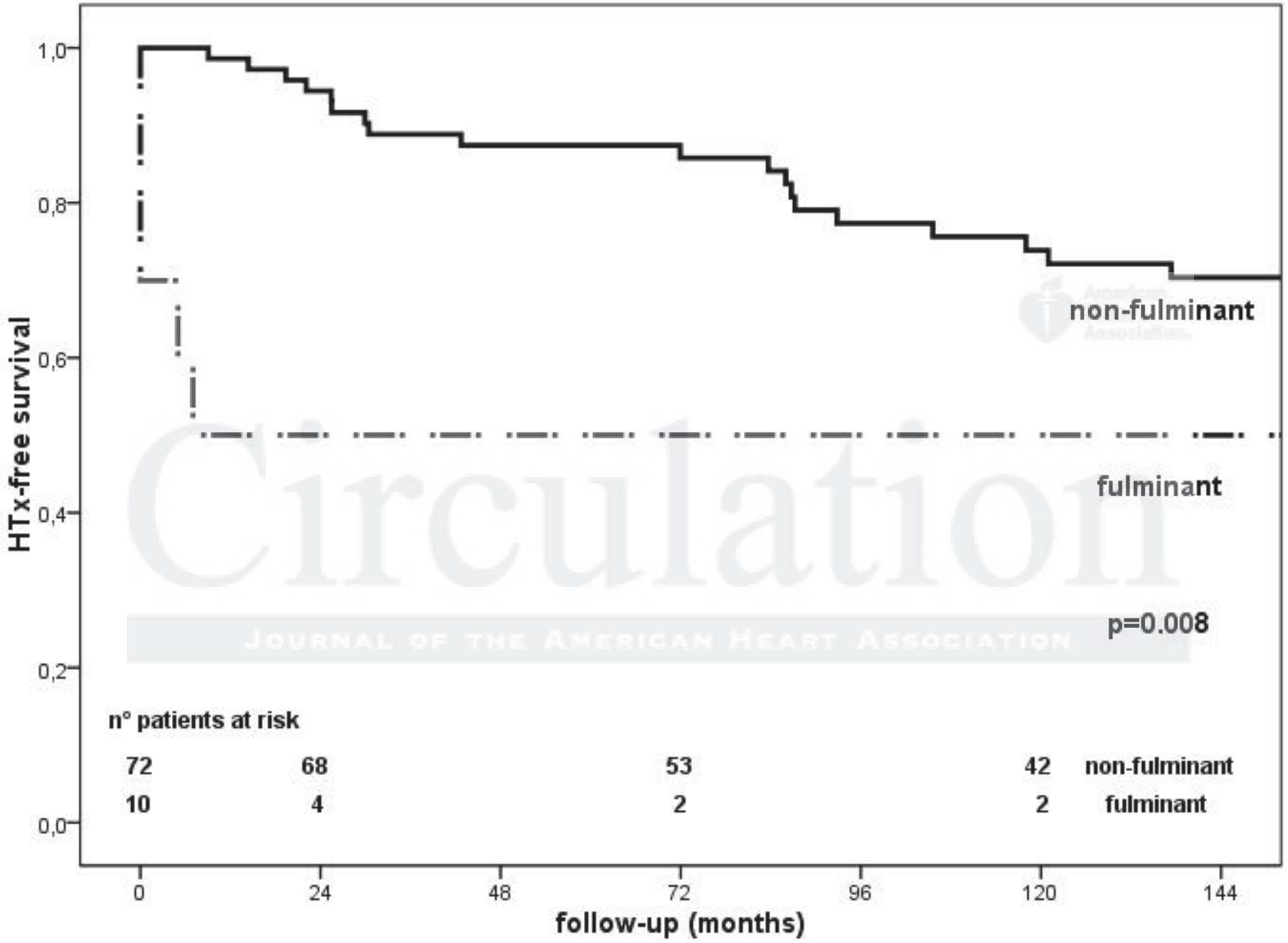


Figure 4

Long-Term Evolution and Prognostic Stratification of Biopsy-Proven Active Myocarditis

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SUPPLEMENTAL MATERIAL

Supplemental Methods

Echocardiographic assessment

Echocardiographic assessment consisted in comprehensive M-mode, 2-dimensional and Doppler studies. Systolic and diastolic functions were evaluated according to international guidelines[1,2]. Left ventricular (LV) volumes and LV ejection fraction were calculated from 2-dimensional apical 4 and 2 chambers approach using the biplane method of discs (modified Simpson's rule)[1]. The extent of mitral regurgitation was semiquantitatively assessed considering the size of the jet at Color flow Doppler[3]. Mitral regurgitation was considered moderate with a jet area between 4 cm2 and 8 cm2 and severe with values > 8 cm2. LV filling was evaluated by means of pulsed wave Doppler study of transmitral flow. The LV filling pattern was classified as restrictive in the presence of an E wave deceleration time <120 ms or ≤150 ms with an E/A ratio ≥ 1.5[4]. For patients with atrial fibrillation we considered only E-wave data.

Endomyocardial biopsy and sample analysis

Bioptic samples for histopathological analysis were fixed in 10% formaldehyde and then embedded in paraffin; multiple slices (5 micron thickness) were stained with hematoxylin-eosin, Azan Mallory and Weigert Von Gieson methods. Congo Red staining for amyloid, Perls staining for iron deposits, Giemsa staining for eosinophils, Warthin Starry staining for Borrelia Burgdoferi and Von Kossa staining for intramyocellular calcifications were used when appropriate. Samples for electron microscopy were fixed with Karnovsky solution, post-fixed with osmium tetroxide and embedded in Epon.

Supplemental Tables

Table 1. Area under the curve and 95% confidence interval comparison between different improvement/normality criteria to predict long-term HTx-free survival.

Model - LVEF at 6 months	AUC	CI 95%
LVEF increase > 5 percentage points, or LVEF ≥50%	0.7300	0.6254-0.8346
LVEF increase > 10 percentage points, or LVEF ≥50%	0.8160	0.7206-0.9113
LVEF increase > 15 percentage points, or LVEF ≥50%	0.8156	0.7220-0.9092
LVEF increase > 20 percentage points, or LVEF ≥50%	**0.8729**	**0.8046-0.9413**
LVEF ≥50%	0.8337	0.7602-0.9073
LVEF increase > 5 percentage points, or LVEF ≥45%	0.7300	0.6254-0.8346
LVEF increase > 10 percentage points, or LVEF ≥45%	0.8258	0.7319-0.9197
LVEF increase > 15 percentage points, or LVEF ≥45%	0.8254	0.7332-0.9176
LVEF increase > 20 percentage points, or LVEF ≥45%	0.8348	0.7466-0.9231
LVEF ≥45%	0.8250	0.7354-0.9146
LVEF increase > 5 percentage points, or LVEF ≥55%	0.6810	0.5699-0.7921
LVEF increase > 10 percentage points, or LVEF ≥55%	0.7572	0.6549-0.8595
LVEF increase > 15 percentage points, or LVEF ≥55%	0.7760	0.6812-0.8708
LVEF increase > 20 percentage points, or LVEF ≥55%	0.8333	0.7680-0.8987
LVEF ≥55%	0.7353	0.6661-0.8045

AUC: area under the curve; CI: confidence interval; LVEF: left ventricular ejection fraction.

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