Vasculitis and Eosinophils in Emdomyocardial Biopsies as Rejection Predictors in Heart Transplantation

Reginaldo Cipullo[2], Marco Aurélio Finger[2], João Manoel Rossi Neto[2], Carlos Mendes Contreras[2], Nádia Van Geen Poltronieri[2], Mabel de Moura Barros Zamorano[2], Lílian Mary da Silva[2], Paulo Chaccur[2], Jarbas Jakson Dinkhuysen[2], Noedir Antônio Groppo Stolf[1]

Instituto do Coração - Universidade São Paulo[1]; Instituto Dante Pazzanese de Cardiologia[2], São Paulo, SP - Brazil

Abstract

Background: The clinical significance of vasculitis, ischemic lesions, Quilty effect and the presence of eosinophils in endomyocardial biopsies of heart transplantation recipients with mild rejection has yet to be established.

Objective: To verify whether these histological findings observed in endomyocardial biopsies (eosinophils, vasculitis, Quilty effect and ischemic lesions) are capable of predicting acute graft rejection.

Methods: A total of 1,012 consecutive endomyocardial biopsies were reevaluated; of these, 939 were classified as OR or 1R according to the Nomenclature of the International Society of Heart and Lung Transplantation of 2005 and divided in two groups: (1) Predictive biopsies: those that preceded acute rejection; and (2) Nonpredictive biopsies: those that did not precede acute rejection. We compared the occurrence of the following histological findings: vasculitis, ischemic lesions, Quilty effect and eosinophils between the groups by uni- and multivariate analyses.

Results: The statistical analysis showed that the presence of severe vasculitis and eosinophils were the best predictors for future acute rejection, with the following odds ratios: 10.60 (95%CI: 3.62 - 31.06. p < 0.001) and 6.26 (95%CI: 3.16 - 12.43, p < 0.001).

Conclusion: Severe vasculitis and eosinophils in myocardial biopsies are the main predictive factors of acute graft rejection post-heart transplantation. (Arq Bras Cardiol 2011; 97(2) : 163-170)

Keywords: Vasculitis; eosinophils; myocardial; biopsy; graft rejection; heart transplantation.

Introduction

The endomyocardial biopsy (EMB) has been used as tool for the diagnosis of graft rejection in heart transplantation recipients for 35 years[1]. In spite of the progress achieved during this period, concerning the knowledge of graft rejection physiopathology, there is no current procedure capable of replacing it with advantage regarding the assessment of the inflammatory process in the graft[2] and the EMB is still considered the gold standard for the diagnosis of acute rejection[3-9].

Part of the success of the EMB is due to the standardization of the cardiac graft rejection nomenclature carried out in 1990[10], and a simple and easily comprehensible classification system was rapidly adopted by heart transplantation centers, allowing the progress of medical knowledge in the diagnosis and treatment of graft rejection[11].

In 2005, this classification was revised and simplified according to the clinical behavior of rejections, which is being currently used with good results[11]. However, some histological lesions observed in EMB have an unclear clinical significance, such as the presence of eosinophils in mild rejections, Quilty effect, vasculitis in intramyocardial vessels and ischemic lesions in myocardial muscle fibers.

Aiming at assessing the results obtained with optical microscopy in rejection prophylaxis, the objective of the present study was to determine whether the presence of Quilty effect, eosinophils, vasculitis in intramyocardial vessels and ischemic lesions in myocardial muscle fibers in biopsies with mild rejection or no rejection are capable of predicting rejection during the clinical evolution of these heart transplantation recipients.

Methods

The study was approved by the Ethics Committee of Instituto Dante Pazzanese, Protocol # 3,343, of March 31, 2005. The present was a retrospective, combined cohort study, carried out from 2002 to 2006 and prospectively from 2006 to 2009, which reassessed by optical microscopy all endomyocardial biopsies of 109 consecutive patients submitted to heart transplantation between January 10, 2002 and January 10, 2009, who underwent at least two endomyocardial biopsies during their clinical evolution.

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The population sample analyzed consisted of 109 consecutive patients submitted to heart transplantation in our institution, between January 10, 2002 and January 10, 2009, who underwent two or more endomyocardial biopsies after the aforementioned surgery; 80% of the patients were males and 73% of them were self-reported Caucasians. The youngest patient was 11 years and the oldest was 69, with a mean age in this group of 46 ± 13 years at the transplantation. Regarding the heart disease prior to the transplantation, 35% of the patients had idiopathic dilated cardiomyopathy; 33% had cardiomyopathy secondary to coronaropathy and 22% had cardiomyopathy secondary to Chagas' disease; 7% had cardiomyopathy secondary to valvulopathy and 3% due to other etiologies.

After the transplantation, all patients received immunosuppressive therapy with cyclosporine at a dose of 4 mg/kg/day, mycophenolate mofetil at a dose of 1.5 g/day and methylprednisolone at a dose of 10 mg/kg/day for five days, followed by prednisone, initially at a dose of 0.4 mg/kg. Alterations in the immunosuppressive therapy were carried out when necessary; however, 88% of the patients were treated with cyclosporine, mycophenolate mofetil and prednisone.

All patients were submitted to myocardial biopsies, according to the following protocol: during the first month after surgery, these examinations were performed weekly; in the second and third months, twice a month; in the fourth, fifth and sixth months, once a month and subsequently, every year after the surgery.

The biopsies were carried out in a surgical ward with a Stanford-Caves bioptome and under fluoroscopy. All fragments obtained during the biopsies were fixed in 10% formaldehyde soon after the procedure and paraffin-embedded in blocks, which were later divided in three planes: superficial, intermediate and deep. Each block yielded 30 slices that were 3 micrometers-thick, which were mounted on a glass slide and stained with Hematoxylin-Eosin (HE) for the three planes and Masson's trichrome only for the intermediate plane. All slides were reassessed using a Weiss™ optical microscope.

Each biopsy was reassessed aiming at identifying the following histological findings: Quilty effect, ischemic lesions and presence of eosinophils in the myocardial inflammatory infiltrate. The presence of these lesions in only one of the four reassessed slides was enough to consider it positive for the identified lesion.

The slides stained with HE of each biopsy were analyzed to measure the frequency of vasculitis. We designated the column of myocardial fragment slices of each slide with the most representative lesions and counted the vessels affected by the inflammatory process identified in each plane and subsequently, added up all vascular lesions in all assessed slides. This value was divided by the number of planes analyzed and the arithmetic mean of vasculitis per analyzed slide was obtained. Subsequently, this result was divided by the number of myocardial fragments obtained at the biopsy, thus yielding the mean number of vessels affected by the inflammatory process per analyzed fragment.

Vasculitis was defined as an inflammatory process affecting one or more vessels, of which extension was limited to adjacent structures, accompanied or not by hemorrhage or ischemic lesion at the area of vessel distribution caused by vascular damage[12] and these lesions were classified according to the characteristics shown by the inflammatory infiltrate found on the walls of affected vessels as follows (Figures 1, 2, 3 and 4):

1. *Mild vasculitis* - Focal inflammatory infiltrate, partially affecting the perimeter formed by the vessel walls, with no signs of vascular necrosis or thrombus formation in the vessel lumen.

2. *Moderate vasculitis* - Inflammatory infiltrate affecting the entire perimeter formed by the vessel walls, with no signs of vascular necrosis or thrombus formation in the vessel lumen.

3. *Severe vasculitis* - Inflammatory infiltrate present on the vessel wall associated with necrosis of the wall or thrombus formation inside the vessel.

The patients were followed through monthly outpatient clinic visits during the first year and bimonthly visits from the second year onward. A database was created using information obtained from outpatient visits and hospital admissions, which contained data on the clinical evolution of each heart transplantation recipient, from the date of the transplantation to the date of death or May 15, 2009, when the clinical follow-up of this study was finished.

The following outcomes were considered: moderate acute rejection (2R), diagnosed by EMB, characterized as two or more foci of mononuclear inflammatory infiltrate associated with myocardial fiber lesion or severe (3R), described as diffuse polymorphic inflammatory infiltrate, with multiple cell injury areas, and eventually, edema, hemorrhage and vasculitis according to the nomenclature of the International Society of Heart and Lung Transplantation[11] or clinical event that induced acute and immediate increase in the immunosuppression, which usually, but not always was accompanied by an abnormal result of the EMB[13].
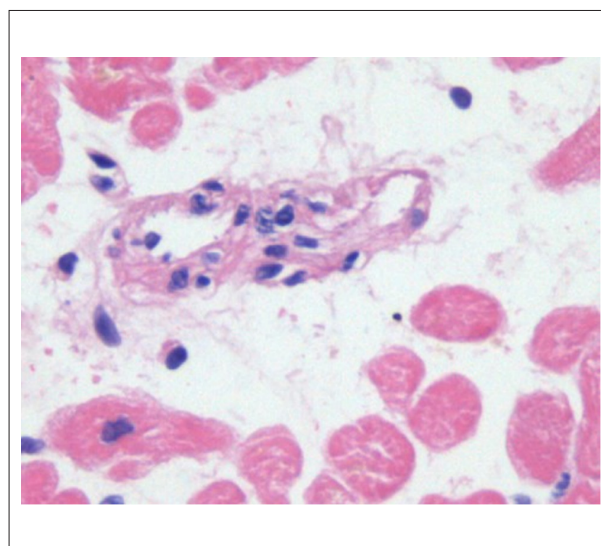

Figure 1 - Microphotography showing small artery with lymphocytic infiltrate in its walls.

All patients that during the study period had a decrease in left ventricular ejection fraction (LVEF), heart failure or those who died due to any other cause unrelated to acute rejection were excluded from the study.

Between January 10 2002 and February 28 2009, a total of 1,012 endomyocardial biopsies were performed at *Instituto Dante Pazzanese de Cardiologia*; 64% were obtained within six months after heart transplantation; 11.3% between the seventh and twelfth months after the surgery and 24.7% after the first year post-heart transplantation. Of the total number of biopsies, 31 (3.06%) were excluded: 9 for having signs of Chagas' disease reactivation and 22 for having fewer than four fragments suitable for the analysis. Of the remaining 981 biopsies, 28 were excluded (2.77%) for presenting histological signs of moderate (2R) or severe (3R) acute rejection.
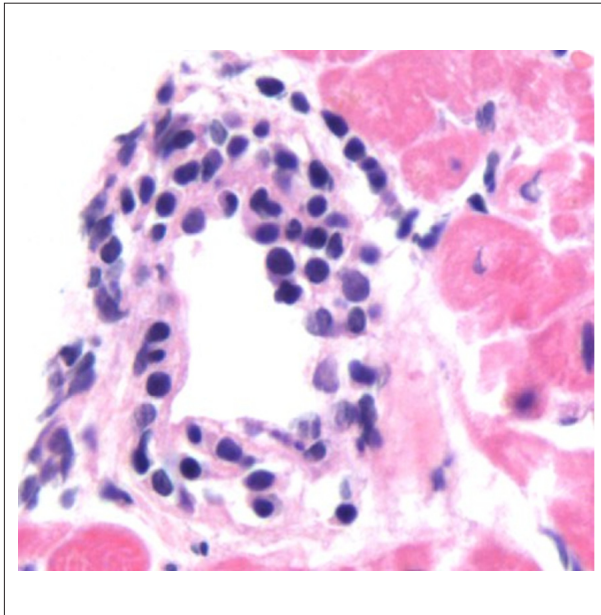

Figure 2 - *Microphotography showing inflammatory infiltrate affecting the entire wall perimeter of a small intramyocardial artery, characterizing moderate vasculitis.*
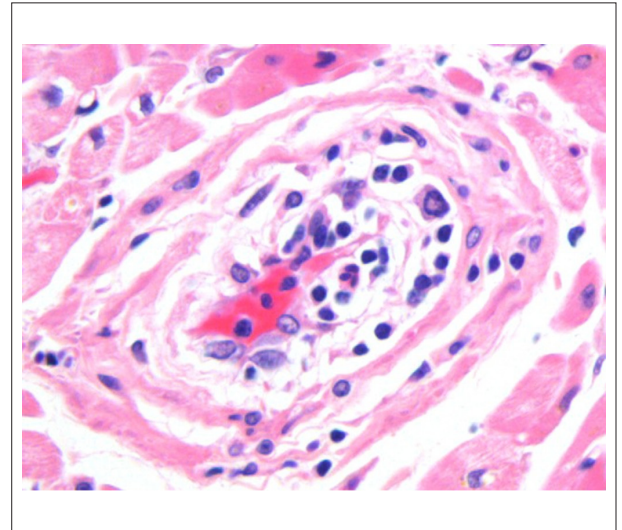

Figure 3 - *Microphotography showing a small intramyocardial artery affected by inflammatory infiltrate and delamination of vessel walls.*
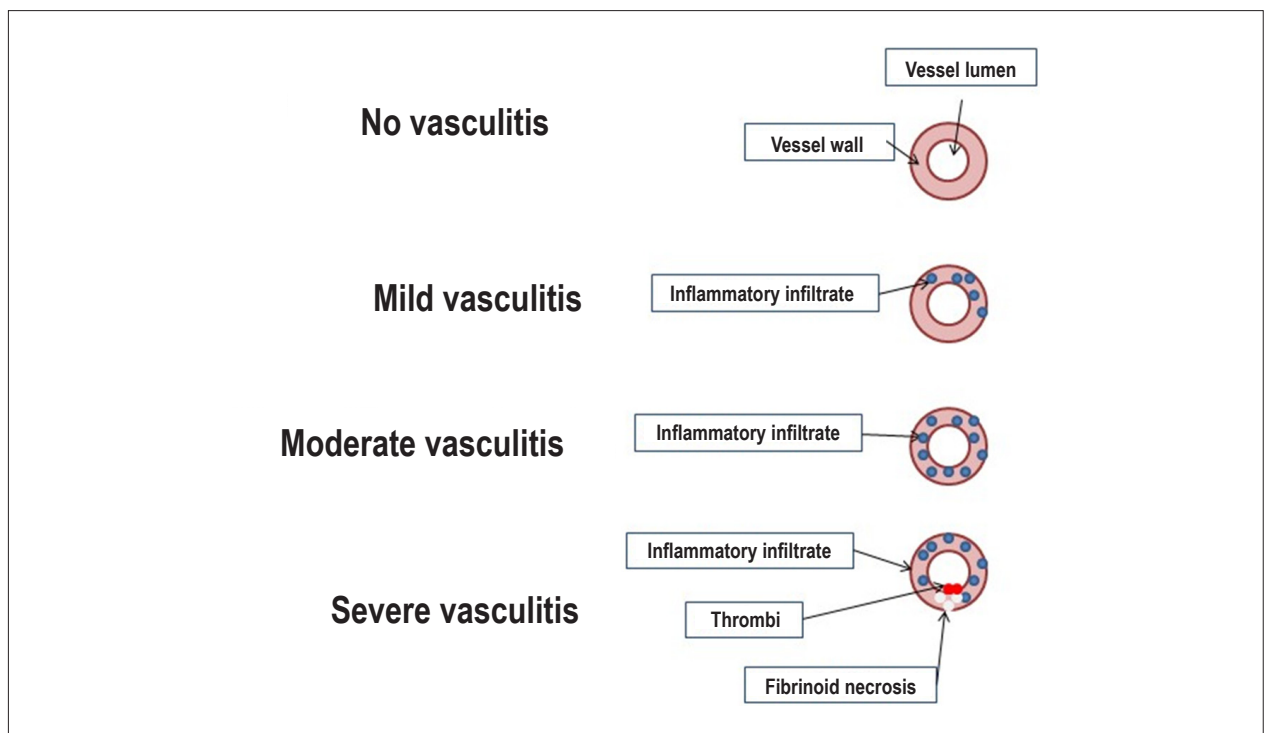

Figure 4 - *Schematic diagram showing vasculitis classification regarding their intensity.*

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After excluding biopsies with acute rejection and Chagas' disease recurrence, we divided the remaining biopsies in two groups: the predictive group, which consisted of biopsies that preceded an acute rejection episode and the nonpredictive group, consisting of biopsies that did not precede this complication.

Therefore, a total of 953 biopsies were then considered eligible for the study, which were divided in two groups, as follows: Predictive Group, consisting of 52 biopsies that preceded an episode of acute rejection, with 28 biopsies that preceded acute rejection episodes demonstrated by optical microscopy in asymptomatic patients plus 24 biopsies that preceded pulse therapy with immunosuppressive agents due to clinical and echocardiographic evidence of acute rejection; Nonpredictive Group, consisting of 901 endomyocardial biopsies that did not precede acute rejection episodes.

All data collected for the present study were stored in an Excel 2003 spreadsheet for Windows™, and the SPSS™ program release 10.0 was used to carry out all statistical calculations.

The univariate analysis was carried out with the following tests: Chi-square, Mann-Whitney, ANOVA or Fisher exact test for the categorical variables and Student's t test for normal variables. P values ≤ 0.10 were considered eligible for the multivariate analysis. The multivariate analysis used Cox proportional regression method to determine the risk offered by each studied parameter of acute rejection occurrence. To estimate survival and rejection-free survival (with or without hemodynamic involvement), the Kaplan-Meyer method was used and the differences between the groups were verified by the log-rank test. The multivariate analysis results that were considered significant were those with p values ≤ 0.05. P values ≤ 0.05 were considered significant at.

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Results

Univariate analysis

The univariate analysis compared the intensity and frequency of vasculitis, Quilty effect, eosinophils at the inflammatory infiltrate and ischemic lesions between the biopsies that preceded acute rejection episodes (predictive group) *versus* biopsies that did not precede this clinical complication (nonpredictive group). Table 1 summarizes the results of the univariate analysis for the clinical outcome of acute rejection.

Multivariate analysis

Of all studied variables, only vasculitis frequency per fragment did not correlate with acute rejection, showing an odds ratio of 1.174 (95%CI: 0.357 - 3.861. p = 0.792). After the removal of this variable from the equation, the best predictors of future rejection were severe vasculitis and presence of eosinophils, with the following odds ratios, respectively: 10.60 (95%CI: 3.62 - 31.06. p < 0.001) and 6.26 (95%CI: 3.16 - 12.43. p < 0.001). Chart 1 summarizes the results of the multivariate analysis for the clinical outcome of acute rejection.

Table 1 - Univariate analysis for the clinical outcome of acute rejection

Histopathological findings	Nonpredictive Group	Predictive Group	Significance
No vasculitis	463(51.4%)	11 (21.2%)	
Mild vasculitis	239 (26.5%)	24 (46.2%)	
Moderate vasculitis	163 (18.1%)	12 (23.1%)	
Severe vasculitis	36 (4.0%)	5 (9.6%)	< 0.001 between the studied groups
Ischemic lesions	190 (21.1%)	14 (26.9%)	0.319
Quilty effect	371 (41.2%)	29 (55.8%)	0.038
Eosinophils	47 (5.2%)	11 (21.2%)	< 0.001
Mean frequency of vasculitis per fragment (95%CI)	0.15 (0.13-0.16)	0.28 (0.20-0.36)	< 0.001

The group of patients with no vasculitis in their biopsies had an acute rejection-free survival of 96.3 ± 0.13% in the first year post-heart transplantation. However, we observed that patients with mild or moderate vasculitis had a similar decrease in the acute rejection-free survival in the same period, of 86.2 ± 0.32% and 86.4 ± 0.45%, respectively. Patients with severe vasculitis had a lower rejection-free survival at the end of the first year post-transplantation, with only 65.9 ± 16.8% of them free of this complication. As shown in Chart 2, the differences between the groups increase with time and after five years of follow-up, patients without vasculitis had 91.7 ± 0.3% of acute rejection-free survival. Among patients with severe vasculitis, on the other hand, only 49.4 ± 19% did not have this complication after the heart transplantation.

Patients that did not have eosinophils in the interstitial infiltrate had an acute rejection-free survival of 98.5% ± 0.05% in the first year after the transplantation. However, in the group that had this cell type in the myocardial interstitial inflammatory infiltrate, the rejection-free survival during this period was greatly reduced, being only 61.6% ± 10.2%. Chart 3 demonstrates rejection-free survival in patients with and without eosinophils in the inflammatory infiltrate.

Discussion

The use of the standardized nomenclature for the diagnosis[10,11] of cardiac rejection has been extremely important for the increase in medical knowledge in the heart transplantation area. However, the routine use of this classification gave rise to some gaps in the interpretation of histological findings in endomyocardial biopsies and their association with the physiopathology of rejection and its clinical significance. Among the knowledge gaps in heart transplantation is the clinical significance of vasculitis, ischemic lesions, Quilty effect and eosinophils in myocardial biopsies that demonstrate mild rejection.

The identification of findings in biopsies that can predict the onset of rejection and poor evolution is of great clinical
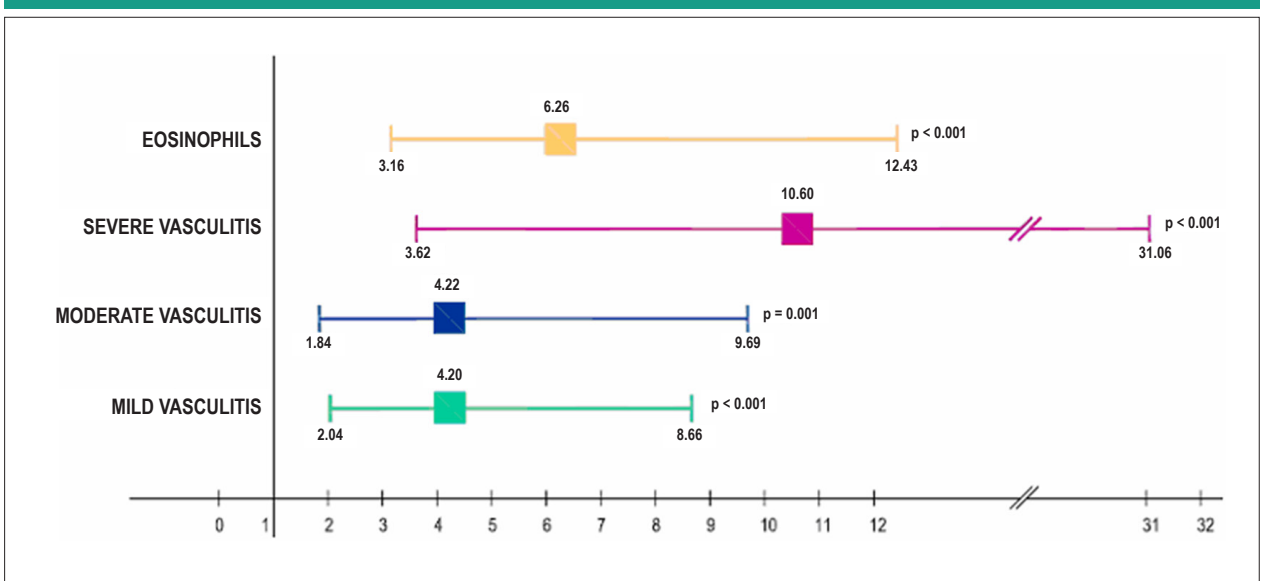
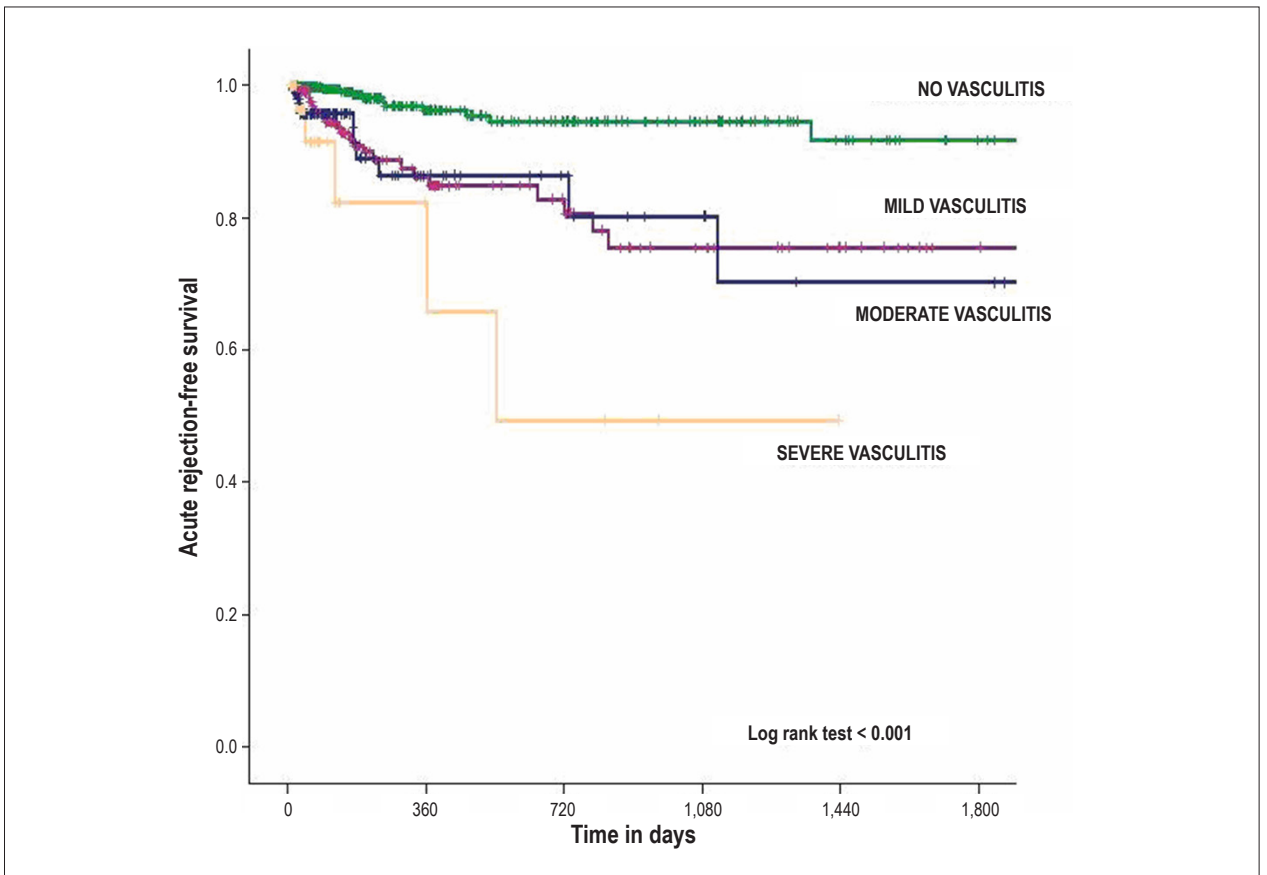*Chart 1 -* *Multivariate analysis results for acute rejection.*

Chart 2 - *Rejection-free survival in patients with vasculitis in endomyocardial biopsy.*
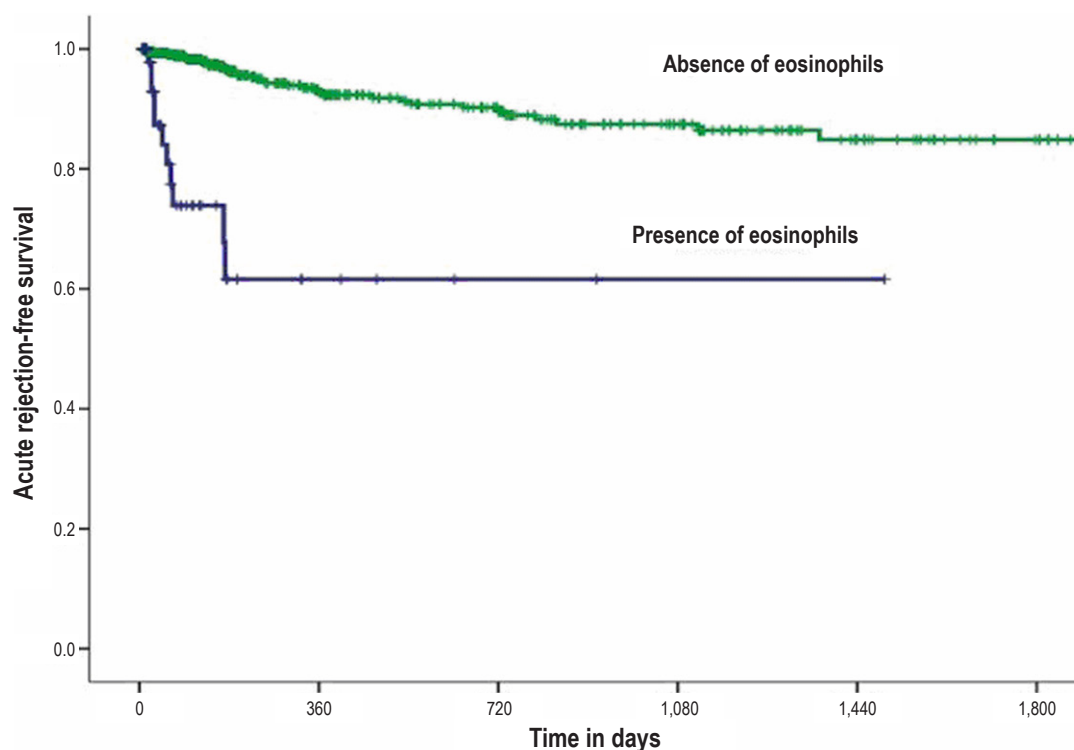
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Chart 3 - *Acute rejection-free survival in patients with eosinophils in interstitial infiltrate.*

importance and can be used as an important tool capable of identifying in which patients the immunosuppressive therapy must be enhanced, in order to prevent immunological aggression of the graft.

Small studies carried out between 1980 and 1990 considered vasculitis a dismal sign[14-17] in the interpretation of endomyocardial biopsies, being associated with severe rejection and death. However, this histological sign was not taken into account when establishing the standardized rejection nomenclatures of 1990[10] or 2005[11], and studies are currently needed to clarify its actual clinical importance.

The findings of this research corroborate the importance of vasculitis as independent predictive factors of acute rejection, acute rejection associated with severe hemodynamic involvement and death due to acute rejection. Vasculitis were morphologically classified according to the intensity of the inflammatory condition and the integrity of walls and their endothelium, which had not been performed before.

Among the studied histological alterations, vasculitis and the presence of eosinophils were capable of predicting acute rejection. Patients with mild to moderate vasculitis had a 4-fold higher chance of acute rejection than patients with no inflammation in vessel walls; those with severe vasculitis (accompanied by vessel wall necrosis or intravascular thrombus formation) had a 10-fold higher chance of acute rejection.

The presence of vasculitis significantly decreased survival free of this complication, especially in the group of patients

that had the severe form, where 50% of the patients had rejection up to the second year post-transplantation, *versus* only 5% of the patients from the group with no vasculitis that had this clinical outcome during the same period.

Considering the studies of systemic vasculitis that can be caused by cell immunity, the deposition of immune complexes or antibodies[18], it would be reasonable to infer that vasculitis, in the context of heart transplantation, can occur in cell rejection, as well as in antibody-mediated rejection; however, there is current clinical and experimental evidence that associates the presence of vasculitis to antibody-mediated rejection[19-21]. The association between vasculitis and the increased expression of HLA-DR, deposition of IgG or IgM on the vascular bed, associated with the fixation of C3d and C4d fractions of the complement and accumulation of fibrin capillary walls has been recently demonstrated in heart transplantation recipients and is correlated with an increase in cardiovascular mortality[20].

The mechanisms proposed to explain the vascular lesions are the fixation of the complement to antibodies deposited on the vascular bed, its activation leading to endothelial lesion, vascular thrombosis and neutrophil recruitment to the vascular inflammation site[22], thus originating tissue ischemia, which can be considered as the most decisive prognostic denominator in vasculitis, of which results range from heart dysfunction caused by metabolic alterations to myocardial infarction, causing definitive heart lesions[23].

The presence of eosinophils that comprise the interstitial inflammatory infiltrate is frequently associated with severe rejection, accompanied by hemorrhage, severe vasculitis, muscle fiber necrosis, leading to graft dysfunction and very often to the recipient's death; however, in the end of the 1980s, a series of four cases was reported, in which the presence of eosinophils in mild to moderate rejections was followed by severe hemodynamic consequences[24]. Since then, no new studies capable of elucidating the significance of eosinophils in mild rejections have been carried out.

In our population, the presence of eosinophil infiltration in the analyzed heart biopsies was uncommon, occurring in only 6.1% of the reassessed examinations; however, this finding had great clinical significance, as in patients that had eosinophil infiltration in the interstitial inflammatory infiltrate, the chance of rejection increased by six-fold in comparison with the group that had an exclusively lymphocytic inflammatory infiltrate.

It is known that eosinophils act as rejection effector cells when the cytotoxic T lymphocyte pathway is blocked or inoperative[25]. Triggered by auxiliary T lymphocytes type 2, the eosinophil pathway of rejection depends on interleukins 4, 5 and 9[26], ultimately causing an increase in vascular permeability and tissue destruction mediated by a series of proteins released from cytoplasmic granules of eosinophils, such as main basic protein, eosinophilic cationic protein, eosinophilic neurotoxin and eosinophilic peroxidase[27].

This is the first study in humans that correlated the eosinophilic infiltration in biopsies with no interstitial inflammation or with mild rejection, to acute rejection. One potential study limitation is its retrospective design.

Conclusions

After the assessment of the results obtained at the comparison of histological findings observed in the groups of Predictive *versus* Nonpredictive biopsies, we concluded that:

1. The presence of vasculitis in biopsies with no inflammatory infiltrate or with mild rejection is associated with an increased risk of developing acute rejection. Additionally, we found an association between the intensity of inflammation of affected vessels and patient evolution (the presence of severe vasculitis in endomyocardial biopsies was the independent predictive factor that best correlated with acute rejection).

2. The finding of eosinophils comprising the inflammatory infiltrate in patients with mild rejection was correlated with an increased chance of developing acute rejection.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

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Study Association

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