# Original Article

# Assessment of Cardiac Allograft Vasculopathy in Cardiac Transplantation: Experience of a Brazilian Center

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## Abstract

**Background:** Cardiac transplantation continues to be the treatment of choice for heart failure refractory to optimized treatment. Two methods have high sensitivity for diagnosing allograft rejection episodes and cardiac allograft vasculopathy (CAV), important causes of mortality after transplantation.

**Objective:** To assess the relationship between intravascular ultrasound (IVUS) results and endomyocardial biopsy (BX) reports in the follow-up of patients undergoing cardiac transplantation in a Brazilian reference service.

**Methods:** A retrospective epidemiological observational study was carried out with patients undergoing orthotopic cardiac transplantation from 2000 to 2009. The study assessed the medical records of those patients and the results of the IVUS and BX routinely performed in the clinical post-transplant follow-up, as well as the therapy used.

**Results:** Of the 77 patients assessed, 63.63% were males, their ages ranging from 22 to 69 years. Regarding the IVUS results, 33.96% of the patients were classified as Stanford class I, and 32.08%, as Stanford class IV. Of the 143 BX reports, 51.08% were 1R, and 0.69%, 3R. The Quilty effect was described in 14.48% of the BX reports. All patients used antiproliferative agents, 80.51% used calcineurin inhibitors, and 19.48% used proliferation signal inhibitors.

**Conclusion:** The assessment of cardiac transplant patients by use of IVUS provides detailed information for the early and sensitive diagnosis of CAV, which is complemented by histological data derived from BX, establishing a possible causal relationship between CAV and humoral rejection episodes. (Arq Bras Cardiol 2012;99(4):886-891)

**Keywords:** Vascular diseases / complications; vascular diseases / mortality; evaluation; heart transplantation / statistics & numerical data; ultrasonography; Brazil.

## Introduction

The International Society for Heart and Lung Transplantation (ISHLT)[1] registries have estimated that three thousand heart transplantations are annually performed in the world. This is mainly due to the survival benefits provided by the immunosuppressive therapy with antiproliferative agents, calcineurin inhibitors and corticosteroids. However, 50% of the patients are estimated to be alive ten years after transplantation. Co-morbidities, infections, neoplasias, sudden death, episodes of cellular and humoral rejection, and cardiac allograft vasculopathy (CAV) appear as risk factors with an important influence on post-transplant life expectancy. The objective of the clinical follow-up of those patients is to monitor and prevent the appearance of those risk factors, aiming at the continuous improvement of short- and long-term prognosis.

Cardiac allograft vasculopathy is one of the major causes of morbidity and mortality after heart transplantation[1-3]. The literature has suggested that CAV is an extreme case of immune-mediated arterial hyperplasia[2]. Thus, assessing the atherosclerotic lesions present in CAV has allowed the recognition of pathophysiological elements of the post-transplantation period, such as morphology of the atherosclerotic plaques, anatomy of the coronary vessels and cardiac allograft rejection processes.

Regarding diagnostic methods, the use of intravascular ultrasound (IVUS) provides a more sensitive and specific assessment of intimal thickening and vascular remodeling, which are considered to be early and independent predictors of CAV[2-9]. The intima layer can be visualized in details and its thickness, calculated (thickness over 0.3 mm diagnoses CAV)[2,3,7,10,11].

During diagnostic coronary catheterization, after removing the IVUS catheter, an endomyocardial biopsy (BX) catheter is introduced, and, through that catheter, material for histological analysis can be collected in the site previously assessed by use of IVUS. Information derived from the BX, such as the presence of tissue inflammation and its patterns and cellular and humoral graft rejection grading, can help in diagnosing CAV.

The objective of this study was to assess the morphology of the atherosclerotic plaque present in the cardiac allograft, by analyzing the results of the IVUS performed during the follow-up of cardiac transplant patients and distributed according to the Stanford classification (initially presented by Gao and then modified by the Stanford University)[2,3,6,10-15]. That assessment was complemented and compared with the histological results of BX.

## Methods

This study was performed according to the Declaration of Helsinki, and was submitted to and approved by the Committee on Ethics and Research of the Pontifícia Universidade Católica of the state of Paraná, on March 4, 2009 (protocol 0002474/09).

A retrospective epidemiological observational study was performed with non-consecutive patients submitted to orthotopic cardiac transplantation at the hospital Irmandade da Santa Casa de Misericórdia de Curitiba (ISCMC). The following characteristics were assessed: patients' clinical data; cardiovascular risk factors; ongoing medications; and complementary tests requested at the discretion of the transplant service. Those data were obtained from the medical records of the cardiac transplant outpatient clinic and from the follow-up visits of the patients.

The non-inclusion criteria were as follows: patients under the age of 18 years; and non-communicating patients whose data were not available for consultation at the cardiac transplant service. This study included 77 patients who underwent transplantation from February 2000 to December 2009, and were followed up according to the clinical routine already established at the service.

Regarding the complementary tests, the research team had access to 53 IVUS reports. The bidirectional tomographic images provided by IVUS allowed characterizing the arterial lumen dimension in regions difficult to access. The catheter was threaded at a fixed ratio, providing the reconstruction of the arterial wall and lumen[3,5,11,12,15]. The films resulting from the IVUS performed in patients submitted to cardiac transplantation are stored in a DVD® disk, DiCom® format, and filed in the CDCV® catheterization laboratory at the ISCMC. The images resulting from the exams were assessed by the major researcher, supervised by the advising authors, with specific software (ILAB®). The following were assessed: vessel luminal and total areas; plaque area; and presence of coronary calcifications. The lesions were distributed according to the Stanford classification[2,3,6,10-15] (Figure 1 and Table 1).

According to the transplant service protocol, a BX, considered reference standard for the diagnosis of acute rejection, should be performed during IVUS[16,17]. The material collected during BX should contain at least three distinct fragments, each with a minimum myocardium content of 50%. The fragments should be fixed in a 10% buffered formalin solution at room temperature and the sequential (three levels) histological sections should be stained with hematoxylin–eosin[3]. In this study, we had access to 143 printed BX reports, in which acute cellular rejection was histologically classified in four grades as follows: 0R (no rejection); 1R (mild rejection); 2R (moderate rejection); and 3R (severe rejection). The BX reports also described the Quilty effect, consisting in endocardial mononuclear inflammatory infiltrate, characteristically nodular[3]. Tissue analysis with immunohistochemistry has not been reported. The material collected was assessed at the cytopathology laboratory (CITOPAR®) affiliated to the ISCMC.

Simple or median percentages were used to analyze the following data: recipients' gender and age group; etiology of the underlying heart diseases; the year cardiac transplantation was performed; the treatments; the IVUS reports, and the biopsies.

## Results

This study assessed the data of 77 patients submitted to cardiac transplantation from February 2000 to December 2009. Most recipients (55.84%, n = 43) were under the age of 50 years when submitted to cardiac transplantation, and 44.15% (n = 34) were over that age. Forty-nine patients (63.63%) were of the male sex.

Several co-morbidities, mainly systemic arterial hypertension, diabetes, and dyslipidemia, were observed. The major heart diseases that generated the indication for cardiac transplantation were as follows: idiopathic dilated cardiomyopathy (n = 32; 41.55%); ischemic cardiomyopathy (n = 21; 27.27%); and Chagas' heart disease (n = 11; 14.28%).

This study collected data from 53 (68.83%) individuals who underwent IVUS after cardiac transplantation. Proximal lesions, defined as those located within 10 mm from the anterior descending coronary ostium, were observed in 31 (58.49%) IVUS exams assessed. The mean extension of the intracoronary lesions was 14.039 mm. The Stanford classification of the lesions observed was as follows: class I, 33.96% (n = 18); class II, 24.52% (n = 13); class III, 9.43% (n = 5); and class IV, 32.07% (n = 17) (Figure 1).

Regarding the BX reports, 143 results were analyzed as follows: 0R, 35.66% (n = 51); 1R, 50.34% (n = 72); 2R, 13.28% (n = 19); 3R, only 0.69% (1 report). Twenty-one BX reports (14.48%) described the Quilty effect (Figure 2) associated with the myocardial rejection grade.

According to the data obtained, 80.51% of the population (n = 62) were on calcineurin inhibitors as follows: 59 patients on ciclosporin (76.52%) and only three (3.89%) on tacrolimus. Association with antiproliferative agents [azathioprine in 11.68% (n = 9), and sodium mycophenolate in 88.31% (n = 68)] was observed; 19.48% (n = 15) of the patients studied used proliferation signal inhibitors (everolimus or sirolimus) associated with other immunosuppressive drugs. Corticosteroids, mainly prednisone, were used in 51 patients (66.23%). According to individual needs, other drugs were used as follows: antihypertensive agents; antidiabetic drugs; antidepressants; synthetic thyroid hormones; statins; inhibitors of the gastric secretion of hydrochloric acid; platelet aggregation inhibitors; diuretics; synthetic insulin; and anticoagulant drugs.
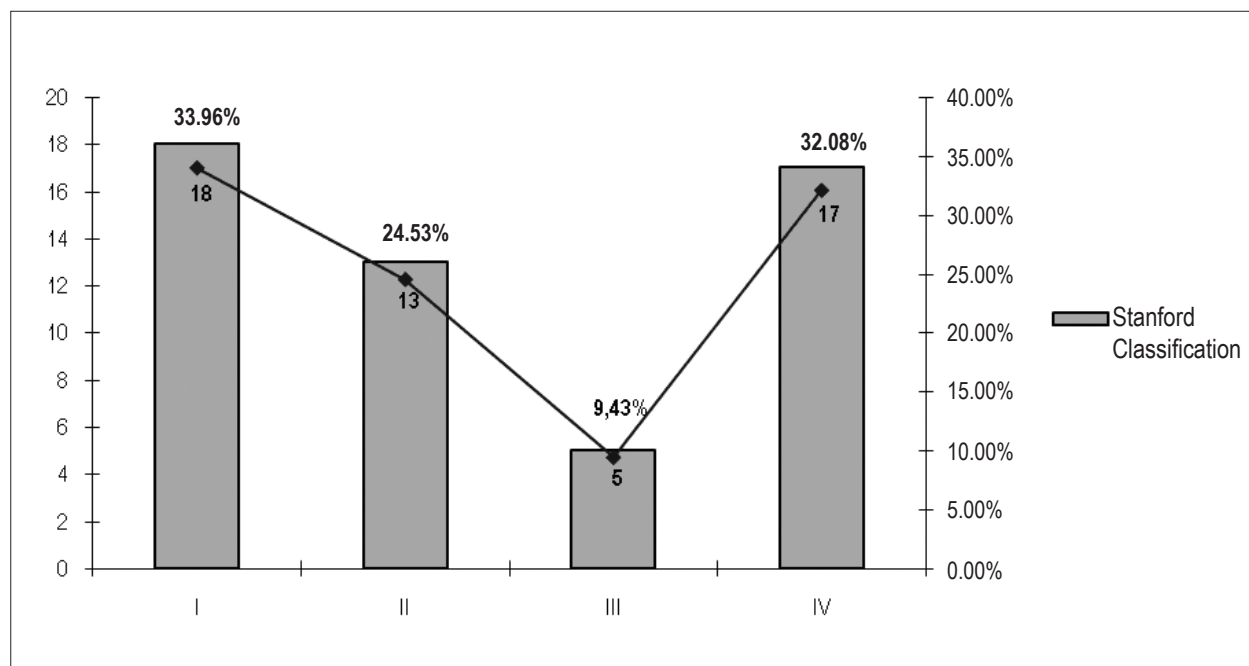
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**Chart 1 -** Stanford classification of intracoronary lesions assessed by use of IVUS

**Table 1 –** Stanford classification of cardiac allograft vasculopathy according to parameters assessed by use of IVUS

|  | Class I | Class II | Class III | Class IV |
|---|---|---|---|---|
| Severity | Minimum | Mild | Moderate | Severe |
| Intimal thickening | < 0.3 mm | > 0.3 mm | 0.3 – 0.5 mm | > 1.0 mm |
| or |  | or | or | or |
| Extension of the plaque | < 180 ° | > 180 ° | > 0.5 mm e < 180° | > 0.5 mm and > 180° |

*Modified from St Goar[13]*

## Discussion

Despite the great advances in immunosuppressant therapy worldwide, monitoring and preventing risk factors that can jeopardize the prognosis and quality of life of transplant patients continues to be a challenge for all cardiac transplant teams.

Within the first 30 days following cardiac transplantation, primary failure of the cardiac allograft can have many causes. But, over time, once the initial mortality of the first six months is overcome, allograft failure can be most often associated with chronic injury caused by immune-mediated rejection or CAV[1].

Consisting in the development of obliterative, anatomically diffuse and rapidly progressive premature coronary artery disease, CAV is currently one of the major complications that truly limit the long-term survival of cardiac transplant patients[2]. The deaths undoubtedly related to CAV occur between the first and third years after transplantation, and account for 10% to 15% of total deaths[1]. Deaths due to allograft rejection, CAV and late allograft failure frequently result from an ineffective change in the recipient's immunity[1], that is, non-adaptation of the recipient's immune system to the new immunosuppression status imposed after transplantation.

Non-invasive tests, such as echocardiography, show preserved function of the cardiac allograft years after transplantation, regardless of the number of rejection episodes[18]. However, angiographic evidence of the disease is present in the first year after transplantation in 10% of the patients, and 32.3% to 50% have some evidence in five years[3,7,10,15,19-21]. According to data from the ISHLT, 50% of cardiac transplant patients are estimated to reach a 10-year survival[1]; thus, half of cardiac transplant patients might have some degree of coronary artery lesion. In more recent multicenter studies, the incidence of intimal thickening and vascular remodeling detected by use of ultrasound was greater than 75% of the patients in one year of follow-up[3], and those data were considered early and independent predictors of CAV[3-9,21]. Endothelial dysfunction in CAV can be also associated with mechanical shear stress of the wall, donor's atherosclerosis or antibody-dependent lesion, especially in the first post-transplant year, when the immune response is more exacerbated[16,21].
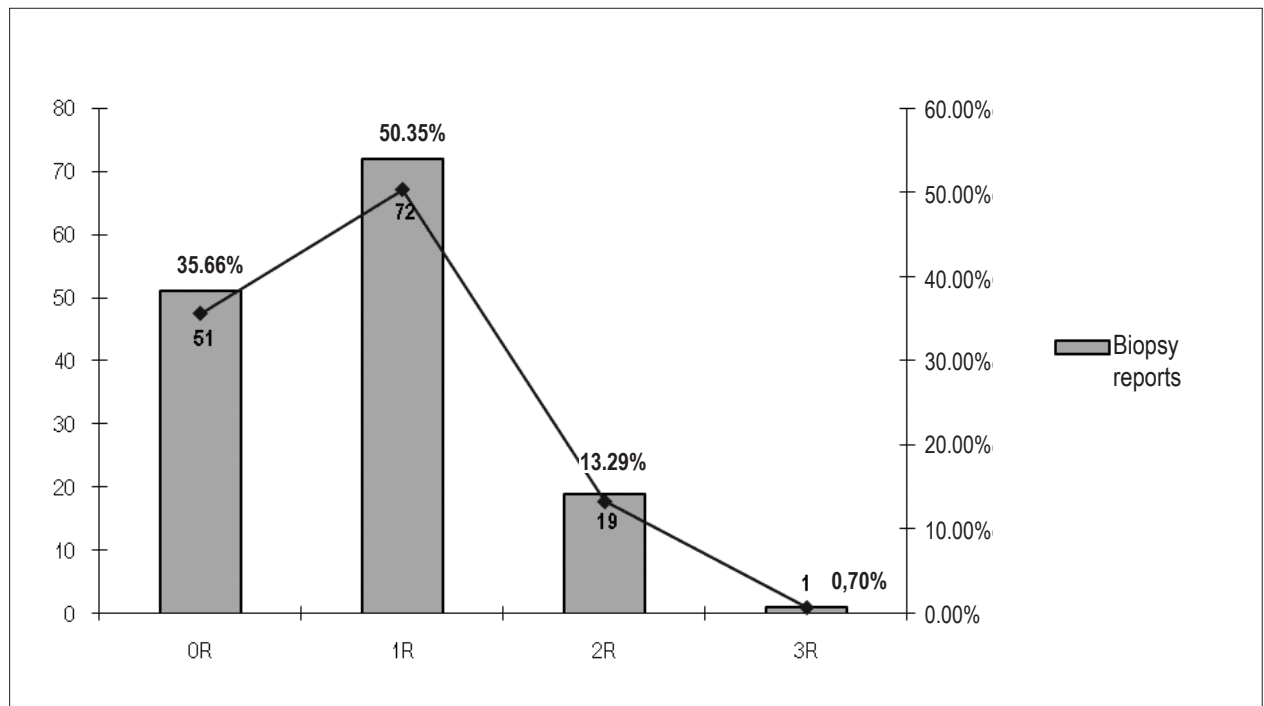
**Chart 2 -** Results of the endomyocardial biopsies according to the 2005 ISHLT classification

In the population studied, most IVUS exams assessed showed some degree of coronary lesion, while 27.69% showed none. Of those exams, 47.69% showed coronary artery lesion close to the anterior descending coronary artery ostium. Previous studies have shown that the prevalence of no lesion on the IVUS in the first two months was 22%, and that of major lesions was 26%[22]. In our population, the mean extension of the lesions assessed on IVUS was 14.039 mm, that is, more diffuse lesions than the focal pattern of the lesions associated with atherosclerosis. Studies have suggested that the site in which a concentric atherosclerotic lesion already exists differ from those where lesions associated with CAV occur[21-28], which does not prevent the existence of both in the same vessel. This could justify the finding of extensive and proximal lesions in our population. Studies have also shown that the intimal thickening of CAV would occur more rapidly in sites with no previous atherosclerotic lesions, within the first post-transplant year[28]. The most important factor might be the progression of the lesions and not their previous presence. In some cases, the donor's atherosclerotic lesions regress after transplantation, probably in association with changes in the patient's risk factors[28]. This can be associated with lesions occurring prior to transplantation (atherosclerosis, wall stress, donor's cellular dysfunction) or with humoral rejection mediated by antibodies against the donor's antigens, especially older donors, as reported by Kobashigawa et al.[20]. Further studies are required to better explain those pathological mechanisms.

No intracoronary calcification was identified on IVUS in the population studied, who had a mean post-transplant time of two years. According to the literature, vascular calcifications are more frequent in patients on a later post-transplant evolution. It has been suggested that the presence of calcifications would be a marker of allograft age, not associated with worse prognosis[22].

Regarding the ultrasound analyses, equivalent incidences were observed between minimum lesions (class I in 33.96%) and severe lesions (class IV in 33.96%), according to the previously cited classification. The population studied showed a predominance of 1R results in BX (51.08%), that is, presence of mild acute cellular rejection. Regarding the BX reports, 21 patients (14.48%) had the Quilty effect. Chantranuwat et al.[23] have reported an association between the Quilty effect on the histological analysis of BX and sudden death after transplantation. That effect is a type of low-grade rejection. That study has shown that CAV was not related to all cases of sudden death, and that 32.1% of the cases showed no coronary anatomic changes[23]. This could suggest the presence of other types of inflammatory infiltrate in the allograft, which had not necessarily developed intraluminal lesions such as CAV. In our study, while the BX reports showed a predominance of mild cellular rejection, the IVUS analyses showed from mild to significant lesions, possibly due to pathophysiological differences between them.

An ISHLT review has reported that humoral rejection is associated with endothelial dysfunctions of capillaries and accumulation of immunoglobulins and complement, especially the C4d fraction. The most dangerous antibodies for cardiac transplantation would be the complement-fixing ones[28-31]. Patients with episodes of humoral rejection are more exposed to CAV than those with no rejection,

differently from the cellular rejection whose relationship with CAV is still controversial[32]. Studies have reported that episodes of cellular rejection do not increase the risk of cardiovascular death (including myocardial infarction, arrhythmias, sudden death and CAV). However, patients with more than three episodes of humoral rejection would be at higher risk for cardiovascular death[13,16,23].

Recent studies have suggested that the direct activation of the recipient's immune system can induce cellular rejection episodes, and that the episodes of chronic rejection and CAV are more often associated with indirect activation of the immune system. Some studies have shown that CAV relates to the activation and deposition of C4d complement degradation products in tissues and to high circulating levels of the donor's specific antibodies against major histocompatibility antigens (HLA or human leukocyte antigen) of the allograft[33], that is, humoral rejection. However, it is still difficult for transplant teams to completely differentiate between episodes of humoral and cellular rejection, according to the ISHLT registries[1].

Despite the great advances in immunosuppressive therapy since the 1990's, which have managed to reduce the incidence of cellular rejections, the incidence of humoral rejection continues relatively unaltered and associates with a greater risk for developing CAV[1,16,29].

## Conclusions

The IVUS assessment of patients after cardiac transplantation provides detailed information for the early and sensitive diagnosis of CAV. That information is useful for the patients' follow-up, and can be complemented with the histological data provided by BX. New studies are necessary to specify the relationship between CAV and humoral rejection episodes.

## Acknowledgement

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