Is biopsy-proven cellular rejection an important clinical consideration in heart transplantation?

James K. Kirklin

Purpose of review

Immunosuppression strategies to prevent allograft rejection represent the cornerstone of long-term survival after heart transplantation. Endomyocardial biopsy has defined rejection in clinical cardiac transplantation and established a threshold for therapy. With the development of more effective immunosuppression modalities and the asymptomatic nature of most histologic rejection episodes, controversy exists regarding the need to augment immunosuppression based purely on histologic findings.

Recent findings

The frequency of histologic rejection has declined with current immunosuppression. Resolution of lower grades of histologic rejection without treatment is the norm in both pediatric and adult heart transplant studies. Recurrent rejection episodes have been linked to the subsequent development of allograft coronary artery disease, and late rejection (even if asymptomatic) is associated with decreased survival in pediatric heart transplant recipients. Black race is a risk factor for recurrent rejection and reduced survival after late cellular rejection. Apoptosis of inflammatory cells is more evident during and after histologic rejection treated with corticosteroids. Despite numerous noninvasive modalities evaluated for the detection of rejection, to date noninvasive methods cannot reliably predict histologic rejection.

Summary

Histologic rejection appears less common with current immunosuppressive strategies, and controversy exists about the need to treat asymptomatic rejection. It remains unproven whether non-treatment of moderate or greater rejection (≥3A) increases the likelihood of recurrent rejection, which if present, may increase the risk of allograft coronary disease and/or reduced long-term survival.

Keywords

allograft coronary artery disease, endomyocardial biopsy, heart transplantation, mycophenolate, rejection, tacrolimus

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University of Alabama at Birmingham, Alabama, USA

Correspondence to James K. Kirklin, Professor of Surgery, Zeigler Research Building, ZRB 740, 1530 3rd Avenue S, University of Alabama at Birmingham, Alabama 35294-0007, USA
Tel: 205 934 3368; fax: 205 934 5261; e-mail: jkirklin@uab.edu

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Introduction

The success of organ transplantation is based on the premise that immunosuppressive modalities can sufficiently suppress those aspects of the immune system, which when stimulated by donor HLA antigens, initiate the destruction of the transplanted organ. The current 1-year survival rate approaching 90% and 10-year survival exceeding 60% is largely attributed to an effective strategy of initial immunosuppressive therapy, a program of chronic maintenance immunosuppression, methods of monitoring the allograft for detection of rejection, and effective methods for treating rejection. Acute cellular rejection is a mononuclear inflammatory response, predominantly composed of lymphocytes, directed against the transplanted organ. Historically, identification of rejection in the transplanted heart has been based on direct histologic examination of allograft tissue samples, made possible by the development of techniques for safe endomyocardial biopsy [1–3]. After the development of a histologic grading system for rejection in 1990 by Billingham *et al.* at Stanford University [4], cardiac pathologists and heart transplant surgeons and physicians worked together to establish guidelines for standard methodology and criteria for histopathologic diagnosis of rejection [5]. More recently, a consensus conference convened at the 2004 meeting of the International Society for Heart & Lung Transplantation to reexamine the cardiac biopsy grading scale (Table 1). The consensus conference was prompted by several important observations that had been made over the past 5 years concerning acute cardiac rejection

1. despite great interest in noninvasive methods for detecting rejection, the endomyocardial biopsy remains the standard for rejection identification;
2. there continues to be considerable variability among pathologists in the interpretation of histologic grading of endomyocardial biopsies;
3. institutional protocols for frequency and duration of surveillance biopsies for rejection detection vary widely;
4. the threshold for treatment (augmentation of immunosuppression) based on biopsy grading scale remains controversial; and
5. the natural history of untreated, asymptomatic cellular rejection on biopsy has not been formally studied, particularly for ISHLT grade 3A or higher.

This review will focus on recent published studies that relate to the clinical impact and sequelae of cellular

Table 1. ISHLT Standardized Endomyocardial Biopsy Grading Scheme

Grade[a]	Description	Nomenclature
0	No lymphocytic infiltrate	No rejection
1A	Focal (perivascular or interstitial) lymphocytic infiltrate without myocyte necrosis	Focal mild acute rejection
1B	Diffuse but sparse lymphocytic infiltrate without myocyte necrosis	Diffuse mild acute rejection
2	One focus only with only with "aggressive" lymphocytic infiltrate and/or focal myocyte injury	Focal moderate rejection
3A	Multifocal aggressive lymphocytic infiltrates and/or myocyte necrosis	Multifocal moderate acute rejection
3B	Diffuse, inflammatory process with myocyte necrosis	Diffuse borderline severe acute rejection
4	Diffuse, aggressive, polymorphous infiltrate with necrosis (± edema; ± hemorrhage; ± vasculitis)	Severe acute rejection
	Additional information that should be reported	
	Biopsy less than 4 pieces	
	Resolving rejection–denoted by a lesser grade than prior biopsy	
	Humoral rejection (positive immunofluorescence, vasculitis, or severe edema in absence of cellular infiltrate)	
	'Quilty' effect	
	A = No myocyte encroachment	
	B = With myocyte enroachment	
	Ischemia	
	A = Up to 3 weeks posttransplant	
	B = Late ischemia	
	Infection present	
	Lymphoproliferative disorder	
	Other	

[a]Biopsy graded by worst infiltrate noted on at least 3 to 5 specimens reviewed. From [5].

rejection on biopsy, progress in noninvasive diagnosis, and special risk issues.

Clinical importance of lower grades of cellular rejection

Most of the available information regarding outcome after untreated acute rejection is inferential, since the standard practice in cardiac transplantation has been routine treatment for biopsy grade 3A or higher. If one loosens the histologic criteria to include biopsy grade 1B or 2, the situation is less controversial. In fact, it is well established that the natural history of grade 1B or 2 rejection is resolution without treatment. The benign natural history of grade 1B rejection is supported by a recent study in pediatric transplantation by Levi *et al.* [6]. Twenty-two patients treated with tacrolimus-based immunosuppression received no treatment for grade 1B rejection, with resolution of histologic rejection in all cases.

Is there a declining incidence of cellular rejection?

From a practical standpoint, the major impact of whether cellular rejection is "an important clinical consideration" may relate more to the low probability of its occurrence than the treatment or non-treatment of grade 3A rejection once it occurs.

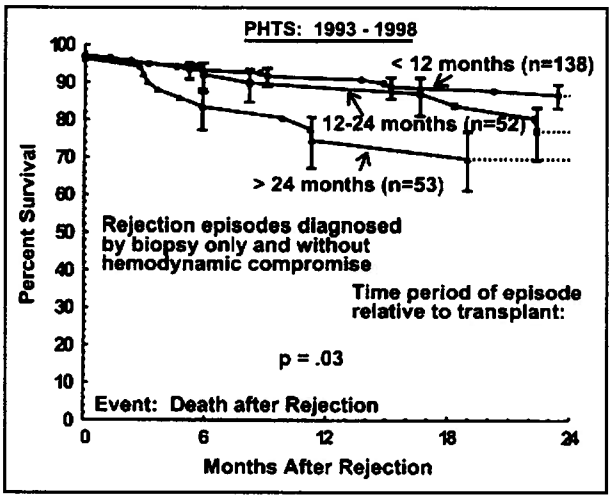
In an excellent clinical update by Garrity and Mehra [7••], the authors cite several studies that suggest that acute rejection is less frequent in a tacrolimus compared with cyclosporine-based regimen. Furthermore, tacrolimus is generally effective in halting recurrent rejection in patients who are switched from cyclosporine to tacrolimus. A combination of tacrolimus, mycophenolate, and steroids was superior to cyclosporine, azathioprine, and steroids in terms of frequency of biopsy-proven rejection. A recent single-institution study in pediatric heart transplant recipients treated with tacrolimus maintenance immunosuppression indicated an extremely low incidence (0.85%) of rejection grade 3A or higher on routine endomyocardial biopsy [6]. In a study of patients treated with cyclosporine, the addition of diltiazem and maintenance of cyclosporine trough levels greater than 362 nanograms per ml during the first month were independent predictors of a lower incidence of acute rejection on biopsy in the first posttransplant year [8]. With the availability of more effective combinations of immunosuppressive agents, the likelihood of important acute cellular rejection (and therefore the benefit of routine surveillance biopsies) may be less in the current era. Thus, for many patients, the *infrequency* of 3A or higher rejection has diminished its clinical importance.

Are there dangers of recurrent and/or late cellular rejection?

Despite these reports of a low frequency of grade 3A or greater rejection after pediatric heart transplantation with current immunosuppression, other recent studies indicate the danger of recurrent acute cellular rejection. In a multiinstitutional study, Chin *et al.* [9••] identified a progressive decrease in survival with more frequent rejection episodes and with rejection occurring later after transplantation. Even when rejection was diagnosed only by biopsy without clinical symptoms, subsequent survival was significantly reduced when rejection was identified after the first 24 months (Fig. 1). The major cause of death after late rejection was recurrent rejection (Table 2). These authors concluded that the 'use of surveillance biopsies

Figure 1. Actuarial survival

Actuarial survival among patients with rejection diagnosed by biopsy only and without hemodynamic compromise, stratified by the time period after transplantation. Reprinted with permission [9••].



appears warranted throughout the life of the transplant individual'.

At least one study among adult heart transplant patients portrays a conflicting view. Klingenberg *et al.* [10•] analyzed 307 grade ≥3A rejection episodes diagnosed up to 10 years after transplantation, 69 of which occurred greater than 2 years posttransplant. The authors noted that spontaneous resolution of grade 3A rejection beyond 2 years occurred in all 17 patients for whom specific anti-rejection therapy was electively withheld. In contrast to the adverse outcome reported after late rejection in pediatric patients [9••], this adult heart transplant analysis showed no decrement in survival among late rejectors.

TABLE 2. Pediatric Heart Transplant Study (PHTS), 1993 to 1998 (*n* = 847)

	Death within 1 year of recurrent rejection	
Cause of death	*n*	Percent% of 54
Acute rejection	19	35%
Infection	5	9%
Non specific graft failure	7	13%
Coronary artery disease/infraction	8	15%
Sudden cardiac death	9	17%
Arrhythmic	1	2%
Pulmonary hemorrhage	1	2%
Neurologic	1	2%
Lymphoma	1	2%
Cardiac failure	1	2%
Respiratory failure	1	2%
Total	54	100%

From [9••].

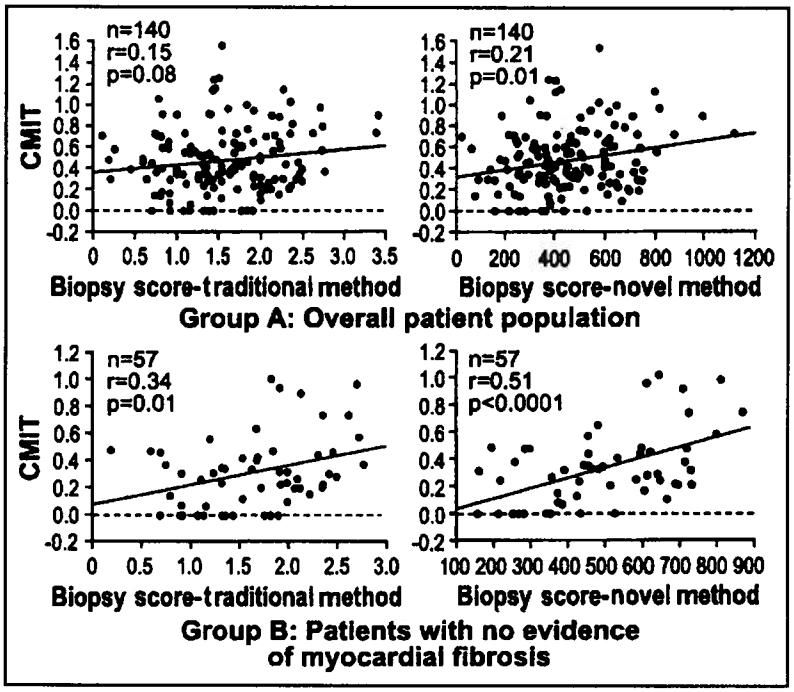
The most compelling evidence indicating the importance of acute rejection is the demonstrated association between recurrent cellular rejection and allograft coronary artery disease. This relation was examined in an excellent study by Yamani *et al.* at the Cleveland Clinic [11••], in which they observed a significant correlation between acute cellular rejection as indicated by the mean biopsy score and change in maximal intimal thickness on intravascular ultrasound at 1 year (Fig. 2). This correlation was masked in the presence of ischemic injury or fibrosis in biopsy specimens, and the authors speculate that myocardial fibrosis may be a marker for non-immune–meditated graft injury and an independent risk factor for allograft coronary disease. Other recent studies provide evidence for a link between repeated allograft rejection and subsequent allograft coronary disease [12•, 13].

Impact of patient risk profiles

The clinical importance of asymptomatic rejection (and therefore the benefit of treatment) may relate in part to the rejection risk profile of individual patients. In support of prior studies that have identified risk factors for rejection, the safety of non-treatment of 3A rejection may relate primarily to the patient's risk profile for rejection. In the multi-institutional pediatric analysis by Chin *et al.*, significant risk factors for recurrent rejection in children and adolescents included recipient black race (approximately 30% greater chance of recurrent rejection within 12 months compared with white recipients), Hispanic race, the number of prior rejection episodes, and a shorter time period since prior rejection. An interaction was noted between race, the presence of rejection with hemodynamic compromise and elapsed time since transplantation.

Figure 2. Regression plots

Regression plots showing the association between change in coronary maximal intimal thickness (CMIT) and acute cellular rejection score. Reprinted with permission [11••].



Histologic sequelae of acute rejection

An elegant study by Masri *et al.* [14••] provided some important insights into the changes induced by acute cellular rejection within the myocardium as well as the potential response to therapy. Apoptosis is known to occur during acute allograft rejection, but there is controversy regarding the cell types that undergo apoptosis, particularly after treatment of rejection. The authors observed that endomyocardial biopsy specimens obtained during and after an episode of moderate (grade 3A) rejection showed increased apoptotic activity, as indicated by increased caspase-8 and caspase-3 activity. Although prior studies have shown a correlation between increased cardiomyocyte apoptosis and grade of rejection, this study identified apoptosis primarily within the inflammatory cells, suggesting that rejection may be controlled in part by apoptosis of these inflammatory infiltrates. The patients in this study received oral prednisone as antirejection therapy, and corticosteroids have been shown to induce apoptosis in activated T-cells.

Diagnosis of acute rejection

Another goal in transplant management is the development of reliable noninvasive methods for identifying acute rejection, thereby reducing patient exposure to the invasive endomyocardial biopsy. This, of course, does not lessen the potential importance of identifying rejection, but rather would allow noninvasive methods for triggering either treatment or verification by endomyocardial biopsy. Unfortunately, little progress has been made in this area over the past year.

The use of brain natriuretic peptide (BNP) as a biochemical marker of rejection was investigated by Amau-Vives *et al.* [15]. The authors found that BNP concentrations remained elevated after heart transplantation, with significantly higher serum BNP levels among patients with allograft rejection than in those without. After the first 90 days, the BNP values were similar in patients with and without rejection. Unfortunately, BNP concentrations lacked sufficient discriminatory potential to serve as a trigger for endomyocardial biopsy in specific patients.

The importance of finding noninvasive methods that allow safe reduction or elimination of endomyocardial biopsies has been of particular interest in pediatric heart transplantation, where repeated vascular access in small recipients can limit the number of potential biopsy attempts. Unfortunately, recent publications continue to support the notion that echocardiographic parameters lack sufficient discrimination in the prediction of histologic rejection. An excellent study by Rosenthal *et al.* [16•] from Stanford evaluated a prospective blinded evaluation of formalized echocardiographic and standard right heart catheterization parameters to predict acute rejection as defined by histologic grading of endomyocardial biopsies. Although

echocardiographic left ventricular mass index was significantly different between rejecting and non-rejecting groups, none of the echocardiographic or hemodynamic variables had sufficient predictive value to replace or even predict the need for endomyocardial biopsy. It is noteworthy that the authors defined the 'rejection' group by biopsy scores of 2 or higher. This confounds the analysis somewhat, since grade 2 biopsy score does not meet the threshold for treatment in most institutions. The reason in this study for including grade 2 biopsy scores in the rejection group is apparent by looking at the frequency distribution of the individual biopsy scores; only 7 of 281 biopsies (2.5%) had grade 3A or higher biopsy grade (the standard threshold for treatment), which would not have provided a sufficient number of events for analysis.

Asante-Korang *et al.* [17] also examined echocardiographic indices in rejecting and non-rejecting patients. Although the study included only 37 patients, the authors found that diastolic performance, indexed by tissue Doppler imaging using the ratio of the peak early to late mitral valve annulus velocity (E_{max}/A_{max}) correlated with rejection.

Summary

In summary, available studies do not indicate with certainty whether asymptomatic acute cellular rejection identified on endomyocardial biopsy (particularly if isolated rather than recurrent) has a more favorable natural history with treatment than without. However, previous studies and more recent publications generally support the notion that acute rejection episodes (grade $\geq$3A) that are asymptomatic and diagnosed only on endomyocardial biopsy are not benign, and, particularly if recurrent, increase the probability of chronic rejection in the form of allograft coronary artery disease and possibly decreased survival when identified after the first year, particularly among pediatric heart transplant recipients. The ISHLT consensus conference for biopsy grading has recommended that grade 3A should be the threshold for treatment. Although echocardiography has proved useful in rejection surveillance for pediatric patients, endomyocardial biopsy remains the standard for identification of cellular rejection in older children and adults. Mycophenolate appears to provide superior protection against cellular rejection compared with azathioprine. The relation between more severe forms of rejection associated with hemodynamic compromise and prior unsuspected or inadequately treated acute cellular rejection is suspected, but unproven.

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- •• of outstanding interest

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