Limited Utility of Endomyocardial Biopsy in the First Year after Heart Transplantation

Iman M. Hamour,[1] Margaret M. Burke,[2] Alex D. Bell,[2] Mathen G. Panicker,[1] Rajasi Banerjee,[1] and Nicholas R. Banner[1,3]

Background. Surveillance endomyocardial biopsies (EMBs) are used for the early diagnosis of acute cardiac allograft rejection. Protocols became standardized in an earlier era and their utility with contemporary immunosuppression has not been investigated.

Methods. We studied 258 patients after orthotopic heart transplantation comparing 135 patients immunosuppressed by mycophenolate mofetil (MMF) with 123 patients treated by azathioprine (AZA); both with cyclosporine and corticosteroids after induction therapy with rabbit antithymocyte globulin. Fifteen EMBs were scheduled in the first year. Additional EMBs were performed for suspected rejection, after treatment, or for inadequate samples. The MMF group had 1875 EMBs vs. 1854 in the AZA group.

Results. The yield of International Society for Heart and Lung Transplantation (ISHLT) grade≥3A biopsy-proven acute rejection (BPAR) was 1.87% per biopsy (35 of 1875) with MMF vs. 3.13% (58 of 1854) with AZA $P=0.024$. The number of clinically silent BPAR ISHLT grade ≥3A (the true yield of surveillance EMBs) was 1.39% (26 of 1875) of biopsies MMF vs. 2.1% (39 of 1854) AZA, $P=0.48$. There were five serious complications requiring intervention or causing long-term sequelae; 0.13% (5 of 3729) per biopsy and 1.94% (5 of 258) per patient. The incidence of all definite and potential complications was 1.42% (53 of 3729) per biopsy and 20.5% (53 of 258) per patient. There was no biopsy-related mortality.

Conclusion. The yield of BPAR was low in the AZA group and very low in the MMF group. The incidence of complications was also low, but repeated biopsies led to a higher rate per patient. Routine surveillance EMBs and the frequency of such biopsies should be reevaluated in the light of their low yield with current immunosuppression.

Keywords: Endomyocardial biopsy, Acute rejection, Heart transplantation, Diagnosis, Immunosuppression.

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Endomyocardial biopsy (EMB) was first introduced as a clinical method for diagnosing acute cardiac allograft rejection in 1974 (*1*, *2*). At that time, immunosuppression was restricted to corticosteroids, azathioprine (AZA), and antithymocyte globulin. As a consequence, the frequency of acute rejection was relatively high and rejection was an important cause of posttransplant mortality (*3*). Routine surveillance EMBs came to be used to detect rejection at an early stage before hemodynamic compromise had occurred (*2*). More recently, biopsy-proven acute rejection (BPAR) has been used as an endpoint in clinical trials of immunosuppression, and recent trials have mandated between 10 and 15 EMBs in the first year (*4–7*).

Advances in pharmacological immunosuppression regimens have resulted in a lower incidence and severity of acute rejection and a fall in rejection-related mortality (*8*). There has been a trend toward reducing the frequency of biopsies or even eliminating them completely after the first posttransplant year (*9*, *10*), but routine surveillance biopsies have remained the standard of care in most centers during the first year after heart transplantation. However, EMB is an invasive procedure that carries a risk of adverse effects and complications. In this study, we have examined the diagnostic yield and complications of routine surveillance EMBs performed in the first year after heart transplantation in patients treated with two immunosuppression regimens.

METHODS

Two hundred fifty-eight consecutive adult patients who had undergone orthotopic cardiac transplantation at our hospital between August 1997 and May 2005 were analyzed. During this period, two different immunosuppression regimens were used; 135 patients transplanted between August 2000 and May 2005 were immunosuppressed by mycophenolate mofetil (MMF, CellCept; Roche Products Ltd., Welwyn Garden City, Hertfordshire, UK) and 123 patients transplanted between August 1997 and July 2000 were treated with AZA. In both groups, the purine synthesis inhibitor selected was given in combination with cyclosporine (Neoral; Novartis Pharmaceuticals, Frimley, UK) and corticosteroids after induction therapy with rabbit antithymocyte globulin (Thymoglobulin; Genzyme Therapeutics, Oxford, UK) (*8*). Eighty percent (207) of the study group were men and their mean age was 50 years (range, 18–65).

Protocol EMBs were performed weekly for 6 weeks, every other week until 3 months, monthly until 6 months, and every other month until the end of the first year of transplantation. Additional EMBs were performed for inadequate samples or clinically suspected rejection or after treatment for rejection. Clinical features that were considered suggestive of

[1] Departments of Cardiology and Transplantation, the Royal Brompton and Harefield NHS Trust, Harefield Hospital, Harefield, Middlesex UB9 6JH, United Kingdom.

[2] Histopathology Department, the Royal Brompton and Harefield NHS Trust Harefield Hospital, Harefield, Middlesex UB9 6JH, United Kingdom.

[3] Address correspondence to: Nicholas R. Banner, F.R.C.P., The Royal Brompton and Harefield NHS Trust, Harefield Hospital, Hill End Road, Harefield, Middlesex UB9 6JH, UK.

E-mail: n.banner@rbht.nhs.uk

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rejection included symptoms and signs of heart failure (edema, breathlessness, fatigue, gallop rhythm, or a raised venous pressure), other evidence of cardiac dysfunction (fall in left ventricular ejection fraction, drop in electrocardiography voltage, new atrial arrhythmia), or nonspecific symptoms such as low-grade fever, malaise, flu-like symptoms in absence of an alternative explanation.

An adequate biopsy was defined as four myocardial samples each with at least 50% assessable myocardium (*11*). Endomyocardial biopsies were reported according to the 1990 International Society for Heart and Lung Transplantation (ISHLT) criteria, which grades rejection from 0 (no rejection) to 4 (severe rejection) (*11*). This system is compatible with the recent ISHLT revision of biopsy grading with regard to cellular rejection such that grades 1 and 2 in the 1990 system correspond to grade 1R in the new system whereas grade 3A corresponds to 2R and 3B and 4 together correspond to 3R (*12*). Our policy was to treat a biopsy grade of 3A (2R) or above with additional immunosuppression even in the absence of cardiac allograft dysfunction (*13*, *14*); this is the conventional approach that is used in most centers.

Patients were clinically assessed and had an electrocardiogram and echocardiogram before each biopsy. The biopsy procedure was performed under local anesthesia, using either the right internal jugular or femoral vein approach, under fluoroscopic control with either intracardiac electrogram or pressure monitoring using a 7-F bioptome.

Serious complications were recognized by symptoms and signs, such as chest pain, breathlessness, or hypotension, which prompted immediate investigation. Cardiac tamponade was recognized by the combination of chest pain, pulsus paradoxus, a raised venous pressure, and echocardiographic evidence of a pericardial collection with diastolic collapse of the right atrium or ventricle. Myocardial infarction after a biopsy was recognized by the development of chest pain, hypotension, and characteristic electrocardiographic changes, subsequently confirmed by coronary angiography.

Fistulae from septal branches to coronary arteries to the right ventricle were recognized retrospectively on routine coronary angiography performed 1 year after the transplant. Tricuspid regurgitation (TR) was assessed at routine echocardiography and attributed to an EMB if it suddenly increased by one grade or more on the subsequent routine echocardiogram. New pericardial effusions detected at the next routine echocardiogram were considered as a possible complication of the previous biopsy; such effusions were usually small, loculated, and asymptomatic.

Statistical Analysis

The results are presented as mean values±standard deviation or proportions as appropriate. Group comparisons were performed using chi-square, Mann-Whitney, Student, or Fisher's exact test as appropriate. Actuarial survival was calculated using the Kaplan-Meier method.

RESULTS

There was no difference in actuarial survival between the two groups at 1 year (80.5% in the AZA group vs. 81.5% MMF, $P=0.84$); one patient died of acute rejection in the AZA group whereas there were no deaths from acute rejection in the MMF group. The cumulative incidence of BPAR (grade≥3A) at 1 year was 24.35% for MMF and 35.6% for AZA ($P=0.038$).

A total of 3729 EMBs were performed in the first year after transplantation (MMF group 1875 and AZA group 1854). The incidence of BPAR grade≥3A varied by time after transplantation and between the two immunosuppression regimens. The MMF group had a lower incidence of BPAR per biopsy and per patient (Table 1). Most of the 93 BPAR grade≥3A rejection episodes were clinically silent; 74.2% (26 of 35) in the MMF group and 67.2% (39 of 58) in AZA group. Low-grade rejection (ISHLT grades 1 or 2) was evident in 19.4% of biopsies (722 of 3729); of these 1.15% (43 of 3729) were associated with clinical rejection and treated on the basis of the clinical and echocardiographic findings.

The incidence of low-grade rejection increased progressively until week 6 and then gradually declined; the pattern was similar with high-grade rejection (grade 3A or above), but at a much lower incidence (Fig. 1). Seventy-five percent (2801 of 3729) of biopsies showed no evidence of acute cellular rejection and 3% (113 of 3729) of the biopsies were inadequate for diagnosis.

We examined whether BPAR (grade≥3A) within the first 3 months predicted the incidence of subsequent rejection. Patients who experienced a BPAR episode within the first 90 days after transplantation were at a significantly increased risk of further rejection during the first year, $P=0.013$. This difference was significant in the AZA group ($P=0.033$) but not in the MMF group ($P=0.15$; Tables 2 and 3).

There was no evidence that the biopsy taken after a biopsy with low-grade rejection (ISHLT grade 1 or 2) was more likely to demonstrate a higher-grade rejection (grade≥3A) than one after a biopsy showing no rejection (grade zero), $P=0.33$ (Table 4) and there was no difference by drug group; AZA $P=0.5$ and MMF $P=0.42$.

TABLE 1. The incidence of BPAR ISHLT grade 3A or worse

	% BPAR per biopsy		% BPAR episode per patient	
Time after transplant (mo)	**AZA n=58/1,854 (3.13%)**	**MMF n=35/1,875 (1.87%)**	**AZA n=36/123 (29.3%)**	**MMF n=28/135 (20.7%)**
0–3	41 (2.21)	18 (0.96)	25 (20.3)	16 (11.8)
3–6	10 (0.53)	14 (0.75)	5 (4.1)	9 (6.67)
6–12	7 (0.38)	3 (0.16)	6 (4.9)	3 (2.22)

BPAR, biopsy-proven acute rejection; ISHLT, International Society for Heart and Lung Transplantation; AZA, azathioprine; MMF, mycophenolate mofetil.

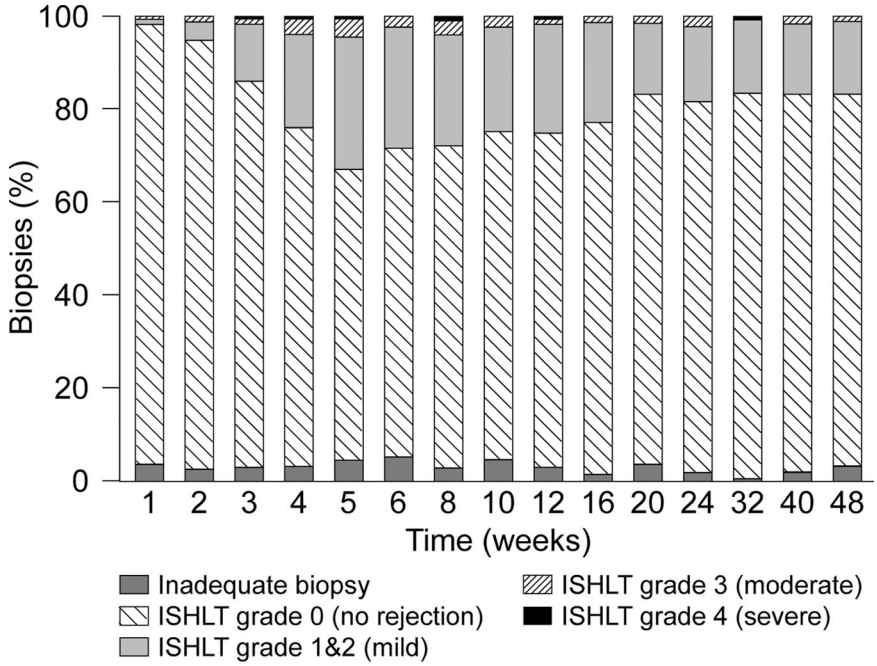

FIGURE 1. Diagnostic yield of endomyocardial biopsy during the first year after heart transplantation (n=3,729 biopsies).

Complications

One patient developed an anteroapical myocardial infarction because of injury of the left anterior descending artery during an EMB procedure. This patient remains alive and well but with impaired left ventricular function 7 years after this complication. Echocardiographically severe TR was detected in one patient who subsequently required treatment with loop diuretics (Table 5).

Three other patients developed complications that required a therapeutic invention but with no long-term sequelae. Two underwent pericardiocentesis for cardiac tamponade after EMB and one needed a chest drain after developing a pneumothorax complicating EMB performed through right internal jugular access.

Asymptomatic septal coronary branch to right ventricular fistulae were found incidentally in 12 patients at routine coronary angiography performed at 1 year after transplantation. One patient developed an arteriovenous fistula at a femoral access site, which was asymptomatic and was treated conservatively.

One patient experienced a biopsy-related Horner's syndrome after right internal jugular vein access, which resolved spontaneously. Nine patients developed echocardiographically moderate TR during the first transplant year that may have been related to EMB.

TABLE 2. Probability of a patient developing rejection (ISHLT grade≥3A) from 3 to 12 months according to whether rejection had occurred within the first 3 months in the AZA group

	Probability of repeated BPAR within 1 yr	
BPAR within 0–90 d	No (n=88)	Yes (n=12)
No (n=75)	69 (92%)	6 (8%)
Yes (n=25)	19 (76%)	6 (24%)
P	0.033	

BPAR, biopsy-proven acute rejection; ISHLT, International Society for Heart and Lung Transplantation; AZA, azathioprine.

TABLE 3. Probability of a patient developing rejection (ISHLT grade≥3A) from 3 to 12 months according to whether rejection had occurred within the first 3 months in the MMF group

	Probability of repeated BPAR within 1 yr	
BPAR within 0–90 d	No (n=96)	Yes (n=15)
No (n=95)	84 (88.4%)	11 (11.6%)
Yes (n=16)	12 (75%)	4 (25%)
P	0.15	

BPAR, biopsy-proven acute rejection; ISHLT, International Society for Heart and Lung Transplantation; MMF, mycophenolate mofetil.

TABLE 4. Probability of progression to high-grade rejection (≥3A) following a negative (grade 0) or low-grade (1 or 2) biopsy

	Incidence of ≥3A rejection in next biopsy	
First biopsy	AZA	MMF
No rejection (ISHLT grade 0), n=2801	89 (6.0%)	66 (4.7%)
Low-grade rejection (ISHLT grade 1 or 2), n=722	25 (7.0%)	23 (5.7%)
P	0.50	0.42

ISHLT, International Society for Heart and Lung Transplantation; AZA, azathioprine; MMF, mycophenolate mofetil.

TABLE 5. Endomyocardial biopsy complications

Complication	Number	Complication per biopsy (%) n=3,729	Complication per patient (%) n=258
Serious (with sequelae or requiring therapeutic intervention)			
Myocardial infarction	1	0.027	0.39
Severe tricuspid regurgitation	1	0.027	0.39
Cardiac tamponade	2	0.054	0.77
Pneumothorax	1	0.027	0.39
Less serious			
Horner's syndrome	1	0.027	0.39
Asymptomatic coronary septal branch to right ventricle fistulae	12	0.32	4.56
Arteriovenous fistula at access site	1	0.027	0.39
Chest pain of undetermined origin	1	0.027	0.39
Findings possibly related to EMB			
Moderate tricuspid regurgitation	9	0.24	3.49
Otherwise unexplained pericardial effusion	24	0.64	9.3

Twenty-four patients developed a new and otherwise unexplained pericardial effusion; most were detected in the early postoperative period and all were asymptomatic. One patient had postbiopsy chest pain of undetermined origin with normal cardiac investigations.

The incidence of serious complications with long-term sequelae per biopsy was low 0.05% (2 of 3729) per biopsy and 0.77% (2 of 258) per patient. The incidence of complications requiring therapeutic intervention was 0.08% (3 of 3729) per biopsy and 1.16% (3 of 258) per patient. The incidence of definite complications was 0.54% (20 of 3729) per biopsy and 7.75% (20 of 258) per patient. All definite and possible complications were 1.4% per biopsy (53 of 3729) or 20.5% (53 of 258) per patient. There was no biopsy-related mortality (Table 5).

DISCUSSION

The cumulative incidence of biopsy-proven rejection (ISHLT grade≥3A) in the AZA and MMF groups was similar to that reported in previous clinical trials (*4–7*); the incidence was lower with MMF than with AZA. However, this study has demonstrated a low incidence of rejection per biopsy in both the MMF and the AZA groups. Overall BPAR (ISHLT grade≥3A) constituted 2.49% (93 of 3729) of all biopsies, but the true yield of surveillance biopsies (clinically silent rejection) was only 1.74% (65 of 3729) This incidence was lower in the MMF group at 1.4% (26 of 1875) vs. the AZA group 2.1% (39 of 1854). Seventy-five percent (2801 of 3729) of biopsies showed no evidence of rejection and 19.4% (772 of 3729) of biopsies showed low-grade rejection (grades 1 or 2). The complication rate was low, at 1.4%, when related to the total number of biopsies performed (53 of 3729 biopsies) but when related to patient, increased to 20%; an increase in proportion to the number of biopsies carried out per patient.

The low diagnostic yield of routine surveillance EMBs is a cause for concern not only because of the invasive nature of the procedure and the risk of complications but also because patients are exposed to ionizing radiation and the procedure generates significant health-care costs.

Endomyocardial biopsies can be performed either because of a suspicion of allograft rejection, based on clinical features such as allograft dysfunction, or routinely in asymptomatic patients as surveillance for rejection. Ninety-seven percent (3607 of 3729) of biopsies (14 biopsies per patient) were performed when there was no clinical evidence of rejection. If biopsies had only been performed when patients were symptomatic only 3.0% (122 of 3729) biopsies (0.5 [122 of 258] per patient) would have been performed. Only 1.8% (65 of 3607) of these biopsies showed clinically silent rejection of grade 3A or worse. The rationale for surveillance biopsies is that early diagnosis and treatment of high-grade rejection, 3A or worse, minimizes the risk of mortality or long-term allograft dysfunction. However, this practice became established in an earlier era when immunosuppression regimens were less effective. The present study demonstrates that current immunosuppression regimens lead to a very low yield from surveillance biopsies performed in the absence of any other evidence or rejection. One of the histological criteria for rejection ≥3A is the presence of myocytolysis, which is regarded as evidence of ongoing myocardial damage, and conventionally, BPAR≥3A is considered an indication for augmented immunosuppression (*13*, *14*). However, lower grades (1 or 2) of rejection often resolve spontaneously under maintenance immunosuppression (*15–17*). Additionally, other studies have found that grade 3A rejection occurring more than 1 year after transplantation may not require treatment (*9*, *18*). This, combined with the low biopsy yield, has led some centers to decide that routine surveillance biopsies are unnecessary more than 1 year after transplantation (*9*, *19*, *20*). However, it seems biologically implausible that the nature of 3A rejection suddenly changes at 1 year, thus begging the question whether clinically silent 3A rejection episodes detected by routine surveillance biopsies within the first year require treatment. The

hypothesis that they do not require treatment needs to be confirmed in a prospective trial but, if correct, it would undermine the rationale for surveillance biopsies. Even if routine surveillance biopsies are indicated within the first year, our data raises a question about the frequency with which they should be performed. There is clearly a need to balance the incidence of adverse effects and complications against the diagnostic yield.

A large number of biopsies showed low-grade (1 or 2) rejection (n=722, 19.4% per biopsy and 2.8 biopsy per patient). This usually triggers closer follow-up that may include an earlier follow-up biopsy. However, our data indicate that the probability of detecting an asymptomatic rejection (3A or worse) was no greater after a grade 1 or 2 biopsy than after one of grade 0. Therefore, there is no indication to routinely perform an additional biopsy earlier than otherwise scheduled in asymptomatic patients.

The types of complications observed in this study were anticipated from the nature of the procedure and were similar to those reported in previous studies (*21–25*). The majority of complications were relatively minor, but five were serious with two having long-term sequelae (Table 5). None of the cases of TR demonstrated a flail leaflet on transthoracic echocardiography and so their relationship to EMBs is uncertain. The one case of severe TR was clinically considered to be definitely related to an EMB procedure. Transesophageal echocardiograms were not performed because none of the cases was severe enough to warrant surgical intervention. The overall incidence of TR in our study was 3.87% per patient (10 of 258), which is relatively low compared with other series that have reported an incidence of 7% to 12% (*26–29*). Pericardial effusion occurs frequently after heart transplantation, mostly with no adverse clinical outcome and usually resolves within 3 months; it has a number of etiologies (*30–34*). With the exception of the two cases of postprocedure tamponade, none of the pericardial effusions could be attributed definitely to EMBs.

The low rejection-related mortality observed here and in recent clinical trials has made it impossible to power trials of immunosuppression for a mortality endpoint after heart transplantation. The widespread use of routine surveillance EMBs as well as the availability of an internationally standardized reporting system (*11*, *12*) has led to the use of biopsy-proven rejection detected by surveillance EMBs as the primary endpoint or as part of a composite primary endpoint, in clinical trials; this approach has been by drug licensing authorities such as the Food and Drug Administration and the European Medicine Agency. Furthermore, such trials have mandated between 10 and 15 surveillance EMBs within the first year after transplantation (*4–7*). The findings of our study suggest that this approach now needs to be reviewed. We suggest that alternative outcomes such as medium-term survival, allograft function, and complications from immunosuppressive therapy (e.g., nephrotoxicity) should be considered as potential endpoints for future trials.

The risks associated with EMB could be avoided if there were satisfactory noninvasive methods to diagnose acute rejection. Methods that have been studied include echocardiographic assessment of systolic and diastolic function (*35*), Doppler and tissue Doppler studies (*36–38*), magnetic resonance spectrometry (*39*), intracardiac electrocardiograms (*40*, *41*), cardiac biomarkers such as troponin (*42*) and natriuretic peptides (*43*) as well as degraded membrane polyunsaturated peptides that release alkanes (*44*). However, none of these methods has been found to be sufficiently sensitive or specific to replace the EMB. More recently, microarray technology has been used to assess the expression of a panel of 11 genes in circulating peripheral blood mononuclear cells and so distinguish between patients with biopsy-proven rejection (≥3A) and those without rejection (grade 0) (*45*). Further studies are ongoing and this technology may reduce or even eliminate the need for EMBs in the future except in patients identified by microarray technology as being at risk for developing acute rejection.

Limitations

This was a retrospective analysis based on the contemporaneous biopsy reports made by two experienced cardiac pathologists (M.M.B., A.D.B.) using the1990 ISHLT grading system. Immunohistochemistry was not used routinely to assess biopsies for evidence of antibody-mediated rejection (AMR) (*12*). However, no patient died of AMR during the study; preliminary evidence from another study indicates that AMR is uncommon in the first year after transplantation (*46*). Furthermore, AMR is a clinicopathological diagnosis in which acute graft dysfunction is a cardinal feature, so AMR will not be diagnosed by routine EMBs in asymptomatic patients with good cardiac function.

CONCLUSION

Our data demonstrate that the yield of moderate to severe rejection (≥ISHLT grade 3A or 2R) in routine surveillance EMBs in heart transplant patients receiving contemporary immunosuppression is very low. Most of the episodes of cellular rejection that are detected are of low grade (ISHLT grades 1 and 2 or 1R) and do not warrant treatment in the absence of clinical evidence of rejection. The yield of surveillance biopsies is now of the same order of magnitude as the incidence of complications related to the procedure. Therefore, the role of EMBs in the first year after transplantation for clinical surveillance and for clinical trials needs to be reconsidered. Further studies are needed to determine whether clinically silent grade 3A or 2R rejection requires treatment in the first year after transplantation in patients receiving contemporary immunosuppression. A randomized trial comparing routine surveillance EMBs with individualized biopsies based on the clinical evidence of rejection or risk factors would be needed to clarify these issues.

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REFERENCES

1. Caves PK, Billingham ME, Schultz WP, et al. Transvenous biopsy from canine orthotopic heart allograft. *Am Heart J* 1973; 85: 525.
2. Caves PK, Stinson EB, Billingham ME, et al. Serial transvenous biopsy of the transplanted human heart. Improved management of acute rejection episodes. *Lancet* 1974; 1: 821.
3. Robbins RC, Barlow CW, Oyer PE. Thirty years of cardiac transplantation at Stanford University. *J Thorac Cardiovasc Surg* 1999; 117: 939.

4. Kobashigawa JA, Miller LW, Russell SD, et al. Tacrolimus with mycophenolate mofetil (MMF) or Sirolimus vs. cyclosporine with MMF in cardiac transplant recipients: 1-year report. *Am J Transplant* 2006; 6: 1377.
5. Kobashigawa J, Miller L, Renlund D, et al. A randomised active-controlled trial of mycophenolate mofetil in heart transplant recipients. Mycophenolate mofetil investigators. *Transplantation* 1998; 66: 507.
6. Hershberger RE, Starling RC, Eisen HJ, et al. Daclizumab to prevent rejection after cardiac transplantation. *N Engl J Med* 2005; 352: 2705.
7. Grimm M, Rinaldi M, Yonan NA, et al. Superior prevention of acute rejection by tacrolimus vs. cyclosporine in heart transplant recipients–a large European trial. *Am J Transplant* 2006; 6: 1387.
8. Hamour IM, Lyster HS, Burke MM, et al. Mycophenolate mofetil may allow cyclosporine and steroid sparing in de novo heart transplant patients. *Transplantation* 2007; 83: 570.
9. White JA, Guiraudon C, Pflugfelder PW, et al. Routine surveillance myocardial biopsies are unnecessary beyond one year after heart transplantation. *J Heart Lung Transplant* 1995; 14: 1052.
10. Sisson S, Jazzar A, Mischke L, et al. How many endomyocardial biopsies are necessary in the first year after heart transplantation? *Transpl Int* 1996; 9: 243.
11. Billingham ME, Cary NRB, Hammond EH, et al. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection study group. *J Heart Lung Transplant* 1990; 9: 587.
12. Stewart S, Winters GL, Fishbein MC, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *J Heart Lung Transplant* 2005; 24: 1710.
13. Beckman EN, Mehra MR, Park MH, et al. Utility of heart biopsy in transplant patients. *Ochsner J* 2001; 3: 219.
14. Winter GL, Costanzo-Nordin MR. Pathological findings in 2300 consecutive endomyocardial biopsies. *Mod Pathol* 1990; 4: 441.
15. Milano A, Caforio AL, Livi U, et al. Evolution of focal moderate (International Society of Heart and Lung Transplantation grade 2) rejection of the cardiac allograft. *J Heart Lung Transplant* 1996; 15: 456.
16. Fishbein MC, Bell G, Lones MA. Grade 2 cellular heart rejection: Does it exist? *J Heart Lung Transplant* 1994; 13: 1051.
17. Winters GL, Loh E, Schoen FJ, et al. Natural history of focal moderate cardiac allograft rejection. Is treatment warranted? *Circulation* 1995; 91: 1975.
18. Kubo BH, Naftel DC, Mills RM. Risk factors for late recurrent rejection after heart transplantation—A multiinstitutional, multivariable analysis. Cardiac Transplant Group. *J Heart Lung Transplant* 1995; 14: 409.
19. Eisen HJ, Kobashigawa J, Keogh A, et al. Three-year results of a randomized, double-blind, controlled trial of mycophenolate mofetil versus azathioprine in cardiac transplant recipients. *J Heart Lung Transplant* 2005 May; 24: 517.
20. Seth GK, Rosado LJ, Mc Carthy M, et al. Futility of yearly heart biopsies in patients undergoing heart transplantation. *J Thorac Cardiovasc Surg* 1992; 104: 90.
21. Albert DM, Osterhaus DVM, Margaret CV, et al. Transmission of Hepatitis B virus among heart transplant recipients during endomyocardial biopsy procedure. *J Heart Lung Transplant* 1998; 17: 158.
22. Pande AK, Gosselin G. Endomyocardial biopsy causing coronary artery –right ventricular fistula after cardiac transplantation. *Indian Heart J* 1995; 47: 253.
23. Henzlora MJ, Noth H, Bucy RP, et al. Coronary artery to right ventricular fistula in heart transplant recipients- a complication of endomyocardial biopsy. *J Am Coll Cardiol* 1989; 14: 258.
24. Quinton Gradek W, D'Amico C, Smith AL, et al. Routine surveillance endomyocardial biopsy continues to detect significant rejection late after transplantation. *J Heart Lung Transplant* 2001; 20: 497.
25. Williams MJ, Lee MY, Di Salvo TG, et al. Biopsy induced related tricuspid leaflet and tricuspid regurgitation following orthotopic cardiac transplantation. *Am J Cardiol* 1996; 77: 1339.
26. Huddleston CB, Rosenbloom M, Glodstein JA, et al. Biopsy-induced tricuspid regurgitation after cardiac transplantation. *Ann Thorac Surg* 1994; 57: 832.
27. Tucker PA, Jin BS, Goas CM, et al. Flial tricuspid leaflet after multiple biopsies following orthotopic heart transplantation: Echocardiographic and hemodynamic correlation. *J Heart Lung Transplant* 1994; 13: 466.
28. Dandel M, Hummel M, Loebe M, et al. Right atrial geometry and tricuspid regurgitation after orthotopic heart transplantation: Benefits of a modified biatrial surgical technique. *J Heart Lung Transplant* 2001; 20: 246.
29. Aziz TM, Burgess MI, Rahman AN, et al. Risk factors for tricuspid regurgitation after orthotopic heart transplantation. *Ann Thorac Surg* 1999; 68: 1247.
30. Al Dadah AS, Guthrie TJ, Posque MK, et al. Clinical course and predictors of pericardial effusion following cardiac transplantation. *Transplant Proc* 2007; 39: 1589.
31. Hauptman PJ, Couper GS, Aranki SF, et al. Pericardial effusions after cardiac transplantation. *J Am Coll Cardiol* 1994; 23: 1625.
32. Quin JA, Tauriainen MP, Huber LM, et al. Predictors of pericardial effusion after orthotopic heart transplantation. *J Thorac Cardiovasc Surg* 2002; 124: 979.
33. Ciliberto GR, Anjos MC, Gronda E, et al. Significance of pericardial effusion after heart transplantation. *Am J Cardiol* 1995; 76: 297.
34. Valantine HA, Hunt SA, Gibbons R, et al. Increasing pericardial effusion in cardiac transplant recipients. *Circulation* 1989; 79: 603.
35. Moidl R, Chevtchik O, Simon P, et al. Non-invasive monitoring of peak filling rate with acoustic quantification echocardiography accurately detects acute cardiac allograft rejection. *J Heart Lung Transplant* 1999; 18: 194.
36. Valantine HA, Fowler MB, Hunt SA, et al. Changes in Doppler echocardiographic indexes of left ventricular function as potential markers of acute cardiac rejection. *Circulation* 1987; 76(5 pt 2): V86.
37. Desruennes M, Corcos T, Cabrol A, et al. Doppler echocardiography for the diagnosis of acute cardiac allograft rejection. *J Am Coll Cardiol* 1988; 12: 63.
38. Dandel M, Hummel M, Muller J, et al. Reliability of tissue Doppler wall motion monitoring after heart transplantation for replacement of invasive routine screening by optimally timed cardiac biopsies and catheterisation. *Circulation* 2001; 104(12 suppl 1): I184.
39. Buchthal SD, Noureuil TO, den Hollander JA, et al. 31 P-magnetic resonance spectroscopy studies of cardiac transplant patients at rest. *J Cardiovasc Magn Reson* 2000; 2: 51.
40. Bourge R, Eisen H, Hershberger R, et al. Noninvasive rejection monitoring of cardiac transplants using high-resolution intramyocardial electrograms: Initial US multi-centre experience. *Pacing Clin Electrophysiol* 1998; 21: 2338.
41. Grasser B, Iberer F, Schreier G, et al. Intramyocardial electrogram variability in the monitoring of graft rejection after heart transplantation. *Pacing Clin Electrophysiol* 1998; 21(11 pt 2): 2345.
42. Dengler TJ, Zimmermann R, Braun K, et al. Elevated serum concentrations of cardiac troponin T in acute allograft rejection after human heart transplantation. *J Am Coll Cardiol* 1998; 32: 405.
43. Masters RG, Davies RA, Veinot JP, et al. Discoordinate modulation of natriuretic peptides during acute allograft rejection in humans. *Circulation* 1999; 100: 287.
44. Phillips M, Boehmer JP, Cataneo RN, et al. Heart allograft rejection: Detection with breath alkanes in low levels (the HARDBALL Study). *J Heart Lung Transplant* 2004; 23: 701.
45. Deng MC, Eisen HJ, Mehra MR, et al. Cargo investigators. non-invasive discrimination of rejection in cardiac allograft recipients using gene expression profiling. *Am J Transplant* 2006; 6: 150.
46. Hamour IM, Rose ML, Simpson D, et al. 'Normal' myocardial compliment deposition after heart transplantation. *J Heart Lung Transplant* 2007; 26(2S): S129.