

Evaluation of Biopsy Classification for Rejection: Relation to Detection of Myocardial Damage by Monoclonal Antimyosin Antibody Imaging

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Objectives. This study sought to compare the histologic grades of rejection in endomyocardial biopsy specimens with the global estimate of myocardial transplant-related cardiac damage detected by myocardial uptake of monoclonal antimyosin antibodies.

Background. The diagnosis and treatment of acute cardiac allograft rejection is based on the interpretation of endomyocardial biopsies. Because allograft rejection is a multifocal process and biopsy is obtained from a small area of the right ventricle, sampling error may occur. Global assessment of myocardial damage associated with graft rejection is now possible with the use of antimyosin scintigraphy. The present study was undertaken to compare the histologic grades of rejection in endomyocardial biopsy specimens with the global assessment of transplant-related myocardial damage detected by antimyosin scintigraphy.

Methods. Biopsies (n = 395) from 112 patients were independently interpreted by three pathologists in a blinded manner according to the original Stanford four-grade (normal, mild, moderate and severe) and the current International Society of Heart and Lung Transplantation (ISHLT) seven-grade (0, 1A, 1B, 2, 3A, 3B and 4) classifications. The results were correlated with 395 antimyosin studies performed at the time of the biopsies. The heart/lung ratio of antimyosin antibody uptake was used to assess the severity of myocardial damage.

Results. In the Stanford biopsy grade classification, significantly higher antimyosin uptake, indicating increasing degrees of myocardial damage, were associated with normal (1.78 ± 0.26), mild (1.88 ± 0.31) and moderate (1.95 ± 0.38) biopsy classifications for rejection ($p < 0.01$). In the ISHLT classification, significant differences were detected only for antimyosin uptake associated with grades 0 (1.77 ± 0.26) and 3A (1.98 ± 0.39) but not for intermediate scores (1A, 1B and 2). In view of the similar intensity of antibody uptake among the various grades, ISHLT biopsy scores were regrouped: normal biopsies in grade A; 1A and 1B as grade B; and 2 and 3A as grade C. Antimyosin uptake in grades A, B and C was 1.78 ± 0.26 , 1.88 ± 0.31 , 1.95 ± 0.38 , respectively ($p < 0.01$).

Conclusions. The current ISHLT seven-grade scoring system does not reflect the progressive severity of myocardial damage associated with heart transplant rejection. Because myocardial damage constitutes the basis of treatment for allograft rejection, there is a need to reevaluate the ISHLT grading system, given its importance for multicenter trials.

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The diagnosis of cardiac allograft rejection is made by histologic interpretation of endomyocardial biopsies (1-3). Billingham (2) initially established a four-grade system to classify rejection and provide a basis for treatment. After other grading schemes were proposed, it became apparent that a universally

accepted classification was necessary in order to meaningfully compare data from various centers (4). This led to the widely utilized seven-grade working formulation of the International Society of Heart and Lung Transplantation (ISHLT) (5).

Allograft rejection is a multifocal process and different regions of myocardium may simultaneously demonstrate varying severity of rejection. Because endomyocardial biopsy allows an exploration of only a small right ventricular apical area, sampling error may result. Only if the biopsy incidentally samples the focus of most severe rejection is an appropriate diagnosis of rejection likely to be made. For this reason, clinical trials for evaluation of rejection therapy based on endomyocardial biopsy diagnosis may be difficult to interpret (6-8).

Although in the past it has been difficult to evaluate larger areas of myocardium, global assessment of the heart for

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Abbreviations and Acronyms

HLR = Heart/lung ratio of antimyosin uptake
 ISHLT = International Society of Heart and Lung Transplantation

myocardial damage is now possible with the use of antimyosin immunoscintigraphy. Antimyosin antibody specifically binds to the regions of myocardial damage. The loss of sarcolemmal integrity in degenerating cardiomyocytes results in exposure of intracellular myosin to intravenously administered radiolabeled antimyosin antibodies (9–12). We have recently demonstrated the feasibility of indium-111 antimyosin scintigraphy for the detection of diffuse myocardial necrosis associated with heart transplant rejection (13–15). The intensity of antimyosin antibody uptake reflects the severity of acute rejection detected by biopsy (15). The present study was undertaken to compare the histologic grades of rejection in endomyocardial biopsy specimens with the global estimate of transplant-related myocardial damage detected by antimyosin scintigraphy. Biopsy interpretation was based on the original Stanford (2) and the currently accepted ISHLT (5) classifications.

Methods

A total of 395 biopsies from 112 patients who had received a heart transplantation at Hospital Santa Creu i Sant Pau, Barcelona, were retrospectively and independently interpreted by three experienced pathologists (referred to as X, Y and Z) in a blinded fashion. The results were correlated with antimyosin antibody studies concomitantly performed with the biopsies.

Interpretation of endomyocardial biopsies, diagnosis of graft rejection and interobserver variation. Multiple hematoxylin-eosin-stained sections of biopsies were reviewed and graded using both the original Stanford (2) and the currently accepted ISHLT (5) classifications for allograft rejection (Appendix 1). Independent interpretation of the biopsies by the three pathologists was used to assess interobserver variability. Discrepancies in interpretation between each pair of observers were classified as major when there was disagreement regarding the presence of myocardial damage, such as mild versus moderate rejection in the Stanford classification or grades 1A and 1B versus grades 2, 3A, 3B and 4 in the ISHLT classification. The discrepancy was considered minor when there was a lack of concordance in interpretation of a normal biopsy versus one showing a myocardial infiltrate without myocyte damage. The presence of focal endomyocardial infiltrates of lymphocytes (Quilty effect) was also assessed and classified as type A or B. A Quilty type A lesion is neatly localized to the endocardium; a type B lesion extends into the underlying myocardium and may be associated with myocardial damage (5,16,17). The discrepancy in the interpretation of various biopsy specimens was resolved by the consensus judgment of

Table 1. Correlation of Transplant Rejection Detected by Endomyocardial Biopsy and Antimyosin Scan

Classification	No. of Biopsies	HLR (mean \pm SD)
Stanford*	395	1.81 \pm 0.29
Normal	281	1.78 \pm 0.26
Mild	84	1.88 \pm 0.31
Moderate	30	1.95 \pm 0.38
Severe	0	0
ISHLT†	395	1.81 \pm 0.29
Grade 0	273	1.77 \pm 0.26
Grade 1A	48	1.88 \pm 0.31
Grade 1B	37	1.84 \pm 0.25
Grade 2	11	1.97 \pm 0.37
Grade 3A	26	1.98 \pm 0.39
Grade 3B	0	0
Grade 4	0	0

*Billingham (2). †Billingham et al. (5). HLR = heart/lung ratio of antimyosin uptake; ISHLT = International Society of Heart and Lung Transplantation.

all three pathologists and the resulting scores of both classifications compared with the intensity of antimyosin uptake.

Antimyosin scintigraphic studies. Antimyosin studies were performed by injecting patients with 500 μ g of monoclonal antimyosin Fab fragment (R11D10) coupled to DTPA labeled with 2 mCi of indium-111. The radiolabeled antibodies were injected intravenously and the planar images acquired in anterior and left anterior oblique projections 48 h later (18). Antibody uptake was assessed by a heart/lung ratio (HLR), calculated by dividing the average counts per pixel in a cardiac region of interest by the average counts in a pulmonary region of interest in the anterior view. In healthy persons the mean HLR is 1.39, and 1.55 (mean value plus 2 SD) is used as a cutpoint to discriminate between normal and abnormal studies. In transplant recipients HLR < 1.55 is associated with a virtually nil probability of detecting rejection by biopsy, whereas an HLR > 1.55 is associated with histologically verified rejection. In addition, a correlation between the intensity of uptake and the probability for detecting rejection at biopsy has been reported (15,18).

Statistical analysis. Analysis of variance was used to compare the mean intensity of antimyosin uptake (HLR) for the biopsy scores of each classification. When analysis of variance was statistically significant, comparison of pairs of mean values was performed using Tukey's correction for multiple comparisons. Chance-corrected agreement among the three independent observers (X, Y and Z) was assessed with the kappa statistic. A kappa score of 0 represents agreement by chance; the value of 1 reflects perfect agreement (19).

Results

Three hundred ninety-five biopsies were reviewed and the results correlated with 395 antimyosin scans (Table 1). Of the 395 scans, 339 were performed in the first year after heart transplantation (72 in the first month, 168 in months 2 to 3, 50

in 4 to 6, and 49 in months 7 to 12). Fifty-six studies were performed after the first year of heart transplantation. The median interval between biopsies and antimyosin scans was 2 days.

Interobserver variability in biopsy interpretation. The interobserver variability of biopsy interpretation for the two classifications is shown in Table 2. No statistically significant differences were observed between any pair of observers or between observations based on the two classifications. The chance-corrected agreement among the observers for the Stanford and ISHLT grading systems was 0.39 and 0.34, respectively. The proportion of major disagreement in 395 biopsy specimens based on the two classifications ranged from 7% to 17%. Minor discrepancies were observed in 15% to 20% of the biopsy specimens.

Intensity of antimyosin uptake and histologic grades of rejection. Using the original Stanford classification, 281 biopsies were graded as normal; mild graft rejection was detected in 84 specimens and moderate in 30; none of the biopsies were classified as severe (Table 1, Fig. 1). The intensity of antimyosin antibody uptake represented by the HLR was 1.78 ± 0.26 , 1.88 ± 0.31 and 1.95 ± 0.38 in biopsy specimens graded as normal, mild and moderate graft rejection, respectively (Fig. 2A, Table 1). Antimyosin uptake associated with mild and moderate rejection was significantly higher than with the normal biopsy specimens ($p < 0.01$).

Utilizing the ISHLT classification, uptake associated with grades 0, 1A, 1B, 2 and 3A biopsy specimens was 1.77 ± 0.26 , 1.88 ± 0.31 , 1.84 ± 0.25 , 1.97 ± 0.37 and 1.98 ± 0.39 , respectively (Table 1, Fig. 2B); none of the biopsies were classified as grade 3B or 4. No correlation was observed between antimyosin uptake and increasing severity of rejection by ISHLT grades (Fig. 2 and 3). The only statistically significant difference among various pairs of ISHLT grades was between grades 0 and 3A ($p < 0.01$).

In view of the similar intensity of antibody uptake among the various grades, ISHLT biopsy scores were regrouped: normal biopsies in grade A; 1A and 1B as grade B; and 2 and

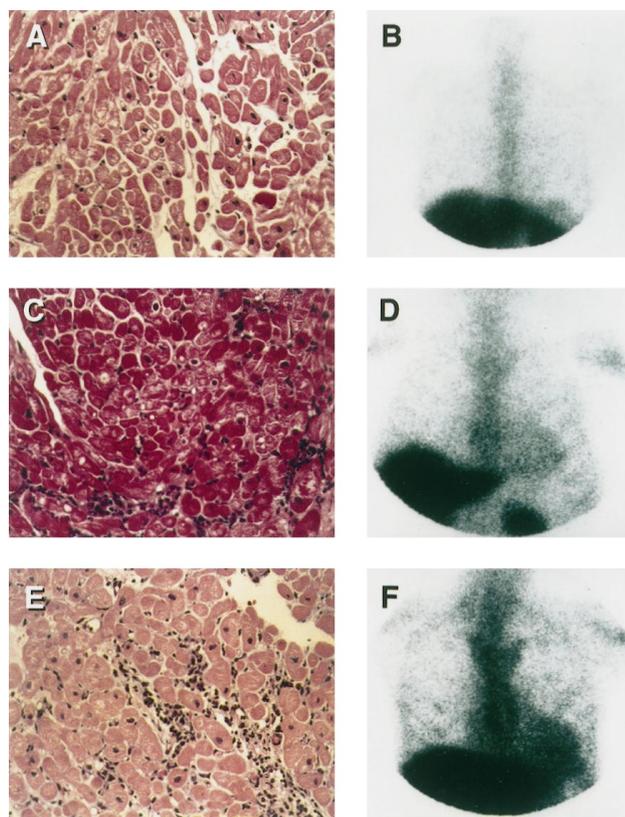


Figure 1. Antimyosin scans in increasing degrees of transplant rejection as detected by endomyocardial biopsies. **A**, Endomyocardial biopsy from a patient with no histologic evidence of transplant rejection was reported as normal by the original Stanford classification and grade 0 by the ISHLT classification. **B**, Antimyosin scan from the same patient (anterior view) demonstrates no uptake of antimyosin antibody in the cardiac region; the antimyosin uptake was 1.46. **C**, Endomyocardial biopsy from another patient who demonstrated mild transplant rejection by the Stanford classification and ISHLT grade 1B rejection. **D**, Antimyosin uptake in this patient can be clearly seen in the cardiac region, which is of mild intensity as reflected by an HLR of 1.65. **E**, Abnormal antimyosin scan with significant antibody uptake (HLR 1.98) was associated with moderate (Stanford) or ISHLT grade 3A transplant rejection **E**, in endomyocardial biopsy.

Table 2. Interobserver Variability in Interpretation of Endomyocardial Biopsy by Three Pathologists (X, Y and Z)

	Kappa†	Major Disagreement (%)*			Minor Disagreement (%)*		
		X-Y‡	X-Z	Y-Z	X-Y	X-Z	Y-Z
Stanford§	0.39	7	15	16	15	18	19
ISHLT	0.34	12	13	17	12	18	19

*Discrepancies in interpretation between each pair of observers were classified as *major* when there was disagreement regarding the presence of myocardial damage such as mild versus moderate rejection in the Stanford classification or grades 1A and 1B versus grades 2, 3A, 3B and 4 in the ISHLT classification. The discrepancy was considered *minor* when there was a lack of concordance in interpretation of a normal biopsy versus one showing a myocardial infiltrate without myocyte damage. †Chance-corrected overall agreement between three observers. ‡Pairs of pathologists interpreting endomyocardial biopsies. §Billingham (2). ||Billingham et al. (5). ISHLT = International Society for Heart and Lung Transplantation.

3A as grade C. Antimyosin uptake reflected by HLR in biopsy grades A, B and C was 1.78 ± 0.26 , 1.88 ± 0.31 and 1.95 ± 0.38 , respectively (Fig. 2C). Differences between A and B and between A and C were significant ($p < 0.01$), and a marginally significant difference was found between groups B and C ($p < 0.10$).

Relation of antimyosin antibody uptake to Quilty effect.

Antimyosin uptake was similar in patients with or without Quilty lesions, and in those demonstrating Quilty type a or type B lesions. The HLR of 1.81 ± 0.3 in 45 studies associated with a Quilty type a lesion was similar to that in 20 biopsy specimens with a Quilty type B effect (1.79 ± 0.24 ; $p = 0.79$). No differences in HLR were observed between the Quilty A effect (1.81 ± 0.30) and the remaining biopsy specimens (1.81 ± 0.27). The HLR was also similar in 20 studies coincident with biopsies showing a Quilty B effect (1.79 ± 0.24) compared with

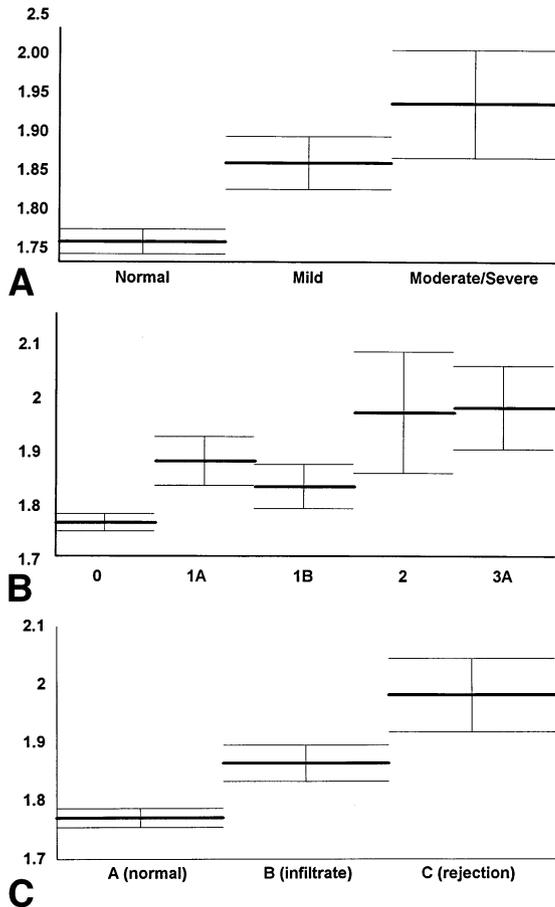


Figure 2. Relation between the intensity of antimyosin uptake (represented by HLR in the ordinate) and the histologic scores for rejection of three endomyocardial biopsy classifications for acute cardiac rejection (see Appendix). Results are expressed as the mean value \pm SD of HLR for each biopsy score. **A**, Original Stanford classification: HLRs for biopsies showing normal ($n = 281$), mild ($n = 84$) or moderate ($n = 30$) rejection. **B**, Current ISHLT biopsy classification: grades 0 ($n = 273$), 1A ($n = 48$), 1B ($n = 37$), 2 ($n = 11$) and 3A ($n = 26$). **C**, HLRs for recoded ISHLT scores A ($n = 273$), B ($n = 85$) and C ($n = 37$).

those not showing such effect (1.81 ± 0.29). Therefore, no differences were found in the degree of antimyosin uptake of hearts between those with or without Quilty effect, indicating that this histologic finding is unrelated to rejection.

Discussion

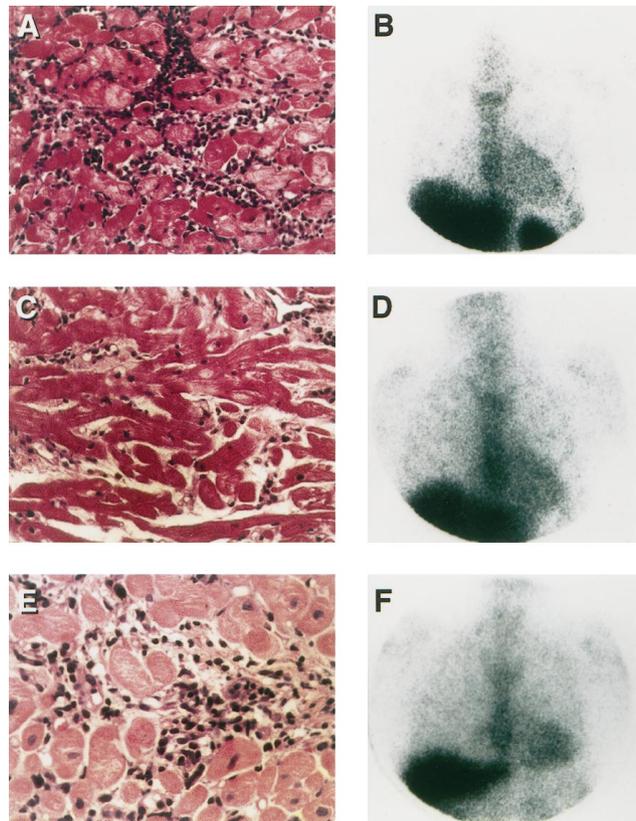
Cardiac allograft rejection manifests pathologically as interstitial mononuclear infiltration, which leads to an increasing degree of myocyte necrosis. The pathologic classifications of allograft rejection are designed to represent the severity of the immunologic process. The most critical feature of transplant rejection is myocyte necrosis, which constitutes the indication for augmentation of immunosuppressive therapy.

The present study utilized radiolabeled antimyosin imaging to identify the extent of myocardial necrosis *in vivo* and correlated it with the biopsy evidence of myocyte necrosis.

Antimyosin scintigraphy allows sensitive identification of myocardial necrosis. After heart transplantation antibody uptake can be equated with rejection activity: lack of myocardial uptake is associated with absent rejection activity detected at biopsy; antibody uptake directly correlates with the presence and severity of biopsy-proven rejection (14,15). In addition, very intense uptake is associated with a higher probability of occurrence of rejection-related complications (15). Therefore, this technology provides a unique opportunity to semiquantitatively assess the degree of myocardial damage after heart transplantation, eliminating the inherent limitation of assuming that the histologic changes in a small myocardial region of the right ventricular apex obtained at biopsy represents the phenomena occurring in the whole myocardium (20).

Comparison of myocardial antimyosin uptake in the scoring system suggests that there is a good correlation between antimyosin uptake and the original Stanford classification (Fig. 1A), but the correlation between increasing grades of histologic rejection in the present ISHLT scoring system and the intensity of antimyosin uptake is poor (Fig. 1B). However,

Figure 3. Discrepancy in the results of antimyosin scans and histologic transplant rejection can occasionally be explained by sampling error of endomyocardial biopsy. Three abnormal antimyosin scans (**B**, **D** and **F**) of approximately same intensity obtained 1, 2 and 3 months after heart transplantation from the same patient. HLRs were 1.80, 1.90 and 1.85, respectively. Endomyocardial biopsies obtained at the time of antimyosin scans demonstrated ISHLT grade 3A, 0 and 3A transplant rejection (**A**, **C** and **E**). Evidence of significant rejection in the first and third biopsy but persistent antimyosin uptake suggests that sampling error of endomyocardial biopsy is a distinct possibility.



regrouping intermediate grades of the latter into a three-score system, similar to the classification proposed initially, correlated well with the discriminate value of the biopsy (Fig. 1C). The poor results of the ISHLT classification are in keeping with the experience of 16 pathologists reported by Winters et al. (21) utilizing the ISHLT classification who found that the poorest correlation among the group was with grade 2 rejection and its differentiation from grades 1A and 3A. This suggests the need to reevaluate the ISHLT grading system given its importance for multicenter trials.

Conclusions. The current ISHLT seven-grade scoring system does not reflect the severity of myocardial damage associated with cardiac allograft rejection. A simple biopsy classification of heart transplant rejection into the categories "normal," "infiltration only" and "infiltration with myocyte necrosis," appears to better represent the severity of myocardial damage.

Appendix

Endomyocardial Biopsy Classification for Rejection

Stanford (Billingham [2]):

- Absent:* No rejection
- Mild:* Focal or diffuse lymphocytic infiltrates without myocyte damage
- Moderate:* Focal or diffuse lymphocytic infiltrates with myocyte damage
- Severe:* Extensive lymphocytic infiltrates associated with neutrophils and interstitial hemorrhage

International Society for Heart and Lung Transplantation (ISHLT) (Billingham et al. [5]):

- 0:* No rejection
- 1A:* Focal (perivascular or interstitial) lymphocytic infiltrates without necrosis
- 1B:* Diffuse but sparse infiltrate without necrosis
- 2:* One focus only with aggressive infiltration or focal myocyte damage, or both
- 3A:* Multifocal aggressive infiltrates or myocyte damage, or both
- 3B:* Diffuse inflammatory process with necrosis
- 4:* Diffuse aggressive polymorphous \pm infiltrate \pm edema \pm hemorrhage \pm vasculitis, with necrosis

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