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.

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 antimyosin studies performed at the time of the biopsies. The heart/lung ratio of antimyosin antibody uptake was used to assess myocardial damage detected by antimyosin scintigraphy.

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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myocardial damage is now possible with the use of antimiycin immunoscintigraphy. Antimiycin 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 antimiycin antibodies (9–12). We have recently demonstrated the feasibility of indium-111 antimiycin scintigraphy for the detection of diffuse myocardial necrosis associated with heart transplant rejection (13–15). The intensity of antimiycin 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 for the regions of myocardial damage. The loss of sarcolemmal integrity in degenerating cardiomyocytes results in exposure of intracellular myosin to intravenously administered radiolabeled antimiycin antibodies (9–12). We have recently demonstrated the feasibility of indium-111 antimiycin scintigraphy for the detection of diffuse myocardial necrosis associated with heart transplant rejection (13–15). The intensity of antimiycin 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 antimiycin 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 antimiycin 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 all three pathologists and the resulting scores of both classifications compared with the intensity of antimiycin uptake.

**Antimiycin scintigraphic studies.** Antimiycin studies were performed by injecting patients with 500 μg of monoclonal antimiycin 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 antimiycin 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

Table I lists the correlation of transplant rejection detected by endomyocardial biopsy and antimiycin scan.

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

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

*Billingham (2). †Billingham et al. (5). HLR = heart/lung ratio of antimiycin uptake; ISHLT = International Society of Heart and Lung Transplantation.
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). 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 (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. 1). 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 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

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

<table>
<thead>
<tr>
<th>Major Disagreement (%)*</th>
<th>Minor Disagreement (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kappa†</td>
</tr>
<tr>
<td>Stanford§</td>
<td>0.39</td>
</tr>
<tr>
<td>ISHLT†</td>
<td>0.34</td>
</tr>
</tbody>
</table>

*Discrepancies in interpretation between each pair of observers were classified as minor 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 major 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.
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,
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 ± infiltrate ± edema ± hemorrhage ± vasculitis, with necrosis

References