Revision of the 1990 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Heart Rejection

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In 1990, an international grading system for cardiac allograft biopsies was adopted by the International Society for Heart Transplantation. This system has served the heart transplant community well, facilitating communication between transplant centers, especially with regard to patient management and research. In 2004, under the direction of the International Society for Heart and Lung Transplantation (ISHLT), a multidisciplinary review of the cardiac biopsy grading system was undertaken to address challenges and inconsistencies in its use and to address recent advances in the knowledge of antibody-mediated rejection. This article summarizes the revised consensus classification for cardiac allograft rejection. In brief, the revised (R) categories of cellular rejection are as follows: Grade 0 R—no rejection (no change from 1990); Grade 1 R—mild rejection (1990 Grades 1A, 1B and 2); Grade 2 R—moderate rejection (1990 Grade 3A); and Grade 3 R—severe rejection (1990 Grades 3B and 4). Because the histologic sub-types of Quilty A and Quilty B have never been shown to have clinical significance, the “A” and “B” designations have been eliminated. Recommendations are also made for the histologic recognition and immunohistologic investigation of acute antibody-mediated rejection (AMR) with the expectation that greater standardization of the assessment of this controversial entity will clarify its clinical significance. Technical considerations in biopsy processing are also addressed. This consensus revision of the Working Formulation was approved by the ISHLT Board of Directors in December 2004. J Heart Lung Transplant 2005;24:1710–20.

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At the request of the International Society for Heart and Lung Transplantation (ISHLT), a standardized grading system for the pathologic diagnosis of rejection in cardiac biopsies was developed in 1990 to address the proliferation of diverse grading systems that occurred during the 1980s. The goal was to develop a uniform description and grading scheme for acute cardiac rejection, to improve communication between clinicians and investigators, to enable comparison of treatment regimens and outcomes between transplant centers, to facilitate multicenter clinical trials, and to promote further studies to determine the clinical significance of the various histologic patterns. It was also intended to have a grading system that was easily learned, readily usable, reproducible, had defined clinical end-points, and could be modified as new information became available. The 1990 ISHLT grading system for cardiac biopsies was widely adopted and served the heart transplant community well for over a decade. However, several issues have arisen during this period requiring re-evaluation of the grading scheme.

First, it has become apparent that there were widespread inconsistencies in the use of the grading system as highlighted by multicenter therapeutic trials in which central pathology panel reviewers have been used for confirmation of endomyocardial biopsy diagnoses. Major areas of diagnostic difficulty have included: Grade 1A vs Grade 2; Grade 1B vs Grade 3B; Grade 2 vs Grades 3A or 3B; Quilty B vs Grade 2 or 3A; and ischemic injury vs Grades 2 or 3A. Less common and less problematic areas of difficulty have included biopsy site(s) vs Grade 2 or 3A, Quilty B vs post-
transplant lymphoproliferative disorder (PTLD) and infection or PTLD vs rejection.

When the 1990 grading system was proposed, the clinical importance of Grade 2 (focal moderate rejection) histology was unknown and, therefore, a separate rejection grade was assigned to allow studies to determine the clinical significance of this histologic pattern. The proposal was made at that time to meet again and review the data regarding the clinical correlations of the grades and to modify the system as necessary. It should also be noted that the 1990 grading system was defined in biopsies from patients generally receiving triple-drug therapy (steroids, cyclosporine, azathioprine) for immunosuppression. Since that time, immunosuppressive regimens have changed, the incidence of rejection has changed, and it is possible that the histology of rejection may also have changed.

The advances in the understanding of transplant rejection and new therapeutic options to prevent and/or treat rejection have warranted re-examination of the grading system. An attempt was made in 1994–1995 to fine-tune the 1990 grading system and clarify those areas that had caused difficulty in interpretation, including Grade 2 acute rejection. This revision drew mixed responses and was never officially adopted or published. The grading system was again discussed at the Sixth Banff Conference on Allograft Pathology in 2001, where a working group exchanged ideas and experience in using the 1990 grading system and recommended a review and update of the grading system, including the need to establish clear criteria for the pathologic diagnosis of humoral rejection.

In 2004, again under the direction of the ISHLT, a multidisciplinary review of the cardiac biopsy grading system was undertaken with task forces examining the areas of histopathology/cellular rejection, humoral (antibody-mediated) rejection, clinical issues and future research. In addition, comments solicited from the ISHLT membership at large were taken into account, which mainly concerned

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**Figure 1.** Myocardial biopsy showing acute cellular rejection with an inflammatory infiltrate composed of mainly lymphocytes in a perivascular distribution and not extending into interstitium or damaging myocytes. Hematoxylin and Eosin. (H&E)

**Figure 2.** Myocyte damage characterized by encroachment of mononuclear cells at the perimeter of myocytes resulting in irregular, scalloped borders and distorting the cellular architecture. Several myocytes are surrounded by infiltrating cells. (H&E)

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**Table 1.** ISHLT Standardized Cardiac Biopsy Grading: Acute Cellular Rejection

<table>
<thead>
<tr>
<th>2004</th>
<th>1990</th>
</tr>
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<tbody>
<tr>
<td>Grade 0 R</td>
<td>No rejection</td>
</tr>
<tr>
<td>Grade 1 R, mild</td>
<td>Interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage</td>
</tr>
<tr>
<td>Grade 2 R, moderate</td>
<td>Two or more foci of infiltrate with associated myocyte damage</td>
</tr>
<tr>
<td>Grade 3 R, severe</td>
<td>Diffuse infiltrate with multifocal myocyte damage ± edema, ± hemorrhage ± vasculitis</td>
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Where “R” denotes revised grade to avoid confusion with 1990 scheme.

The presence or absence of acute antibody-mediated rejection (AMR) may be recorded as AMR 0 or AMR 1, as required (see Table 3).
Grade 2 cellular rejection and humoral rejection. Consensus was reached and presented at the 24th Annual ISHLT scientific meeting. This study reports the consensus findings as a revision of the previous Working Formulation, which was approved by the ISHLT board in December 2004.

HISTOLOGIC DIAGNOSIS AND GRADING OF ACUTE CARDIAC ALLOGRAFT REJECTION

Biopsy-proven acute rejection on surveillance endomyocardial biopsies appears to be decreasing, due at least in part to improved immunosuppressive therapy. In addition, there has been a shift in clinical response to some grades of rejection. In the middle to late 1980s, most (but not all) transplant centers treated any biopsy with myocyte injury (1990 ISHLT Grade 2 and higher) with some form of augmented immunosuppression, regardless of the clinical presentation. Several studies in the early to mid-1990s showed that lower grades of rejection resolve without treatment in a majority of cases. Biopsies with 1990 ISHLT Grade 1, Grade 2 and even some sub-sets of Grade 3 rejection progress to high-grade rejection on the next biopsy in only 15% to 20% of cases. At the other end of the spectrum, Grades 3B and 4 are uniformly treated aggressively. Therefore, the consensus was to modify the 1990 ISHLT grading system as shown in Table 1. In brief:

- 1990 ISHLT Grades 1A, 1B and 2 would be combined into a new, revised 2004 ISHLT Grade 1 R.
- 1990 ISHLT Grade 3A would become 2004 ISHLT Grade 2 R; and
- 1990 ISHLT Grades 3B and 4 would become 2004 ISHLT Grade 3 R.

In addition, the Histopathology Task Force recommended that further characterization of the nature of the inflammatory infiltrate and definition of myocyte damage would be helpful in reducing inconsistencies in the application of the grading system (vide infra).

Inflammatory Infiltrate

Acute cellular rejection is characterized by an inflammatory infiltrate predominantly comprised of lympho-
cytes, as well as macrophages and occasional eosinophils (Figure 1). The presence of neutrophils (except in the most severe form of rejection) should raise the question of an alternative process, such as healing ischemic injury, antibody-mediated (humoral) rejection or infection. Plasma cells are also not typically present in acute cellular rejection and suggest a Quilty lesion, healing ischemic injury (often in response to allograft coronary disease) or a lymphoproliferative disorder (plasmacytoid lymphocytes).

**Myocyte Damage**

Damage or injury to the myocardium, originally termed “myocyte necrosis,” is an important but sometimes difficult feature to identify. Although readily distinguishable, cell death may be a feature of the most severe forms of rejection; myocyte damage in milder rejection is often characterized by myocytolysis and no contraction band or coagulation necrosis. Features of myocytolysis include clearing of the sarcoplasm and nuclei, with nuclear enlargement and occasionally prominent nucleoli. The presence of myocyte injury is frequently accompanied by encroachment of inflammatory cells at the perimeter of myocytes, resulting in irregular or scalloped myocyte borders, their partial or whole replacement, or distortion of the normal myocardial architecture (Figure 2). These features are often better appreciated by the examination of multiple levels of sectioning. It should also be noted that myocytolysis can be seen in both early and late ischemic injury.

**Figure 7.** Grade 1 R: Diffuse mononuclear cell infiltrate with an interstitial pattern of lymphocytes between and around myocytes without associated myocyte damage. Previously Grade 1B. (H&E).

**Figure 8.** Grade 1 R: High power view of a mononuclear infiltrate extending from a perivascular position into adjacent myocardium with damage to myocytes and distortion of architecture. This is a single focus in the biopsy series and therefore is included in the revised mild grade of acute rejection, previously described as Grade 2. (H&E).

**Figure 9.** Grade 2 R: Low power view showing three foci of damaging mononuclear cell infiltrate with normal myocardium intervening. Previously Grade 3A. (H&E).

**Figure 10.** Grade 2 R: Higher power view of one focus of figure 9 damaging infiltrate with myocyte damage and architectural distortion (a “space occupying lesion”). (H&E).
Grade 0 R (no acute cellular rejection)

In Grade 0 R there is no evidence of mononuclear (lymphocytes/macrophages) inflammation or myocyte damage (Figure 3).

Grade 1 R (mild, low-grade, acute cellular rejection)

Mild or low-grade rejection may manifest in one of two ways: (1) Perivascular and/or interstitial mononuclear cells (lymphocytes/histiocytes) are present. In general, these cells respect myocyte borders, do not encroach on adjacent myocytes, and do not distort the normal architecture (Figures 4, 5, 6 and 7). (2) One focus of mononuclear cells with associated myocyte damage may be present (Figures 2 and 8).

Grade 2 R (moderate, intermediate-grade, acute cellular rejection)

In Grade 2 R two or more foci of mononuclear cells (lymphocytes/macrophages) with associated myocyte damage are present. Eosinophils may be present. The foci may be distributed in one or more than one biopsy fragment. Intervening areas of uninvolved myocardium are present between the foci of rejection (Figures 9 and 10). Low-grade (Grade 1R) rejection can be present in other biopsy pieces.

Grade 3 R (severe, high-grade, acute cellular rejection)

A diffuse inflammatory process, either predominantly lymphocytes and macrophages or a polymorphous infiltrate, is present, involving multiple biopsy fragments (Figures 11 and 12). In most cases, the majority of biopsy fragments are involved, although the intensity of the infiltrate may vary between pieces. Multiple areas of associated myocyte damage are present. In the most severe forms of cellular (and humoral) rejection, diffusely infiltrating inflammatory cells are present (Figure 13).
edema, interstitial hemorrhage and vasculitis may be present.

**NON-REJECTION BIOPSY FINDINGS**

**Peri-operative Ischemic Injury**

Early (peri-operative) ischemic injury arises in the peri-operative period during the obligatory ischemic time that accompanies procurement and implantation of a donor heart (Table 2).\(^{15}\) Such injury may be exacerbated by prolonged hypotension due to poor graft function, hemorrhage during the peri-operative period, and the effects of prolonged high-dose inotrope therapy. Ischemic injury is characterized initially by contraction band necrosis or coagulative myocyte necrosis, often with myocyte vacuolization and fat necrosis, and frequently extends to the endocardial surface. As healing ensues, biopsies may contain mixed inflammatory infiltrates, including neutrophils as well as lymphocytes, macrophages and eosinophils, and it is at this point that confusion with acute rejection may occur. Ischemic injury, especially in its healing phase, is a common biopsy finding in the early post-transplant period (up to 6 weeks) and must be differentiated from acute rejection. In acute rejection, the inflammatory infiltrate frequently is proportionally greater than the degree of myocyte damage, whereas, in ischemic foci, it is usually the reverse (Figure 13). Peri-transplant injury with neutrophils may show overlapping features with antibody-mediated (humoral) rejection (*vide infra*).

**Late Ischemic Injury (related to allograft coronary disease)**

Assessing the arterial changes of allograft coronary disease in endomyocardial biopsy specimens is usu-
ally precluded by the lack of vessels large enough to permit such an evaluation. However, the ability to detect secondary myocardial changes, such as myocyte vacuolization and microinfarcts, may be helpful in determining the etiology of late cardiac failure.\textsuperscript{16} In addition, the diagnosis of late ischemic injury may be helpful in determining the etiology of cardiac failure in transplant recipients. It may be especially helpful in ruling out other potentially treatable etiologies that are part of the differential diagnosis, such as acute rejection or PTLD.

**Quilty Effect**

Nodular endocardial infiltrates, or Quilty effect, occur in approximately 10% to 20% of post-transplant endomyocardial biopsies.\textsuperscript{17,18} The infiltrates may be confined to the endocardium (1990 ISHLT Quilty A) or may extend into the underlying myocardium where associated myocyte damage may be present (1990 ISHLT Quilty B) (Figures 14, 15 and 16). The histologic sub-typing of Quilty A and Quilty B has never been shown to have any clinical significance and there is agreement that separating Quilty A from B has no clinical value.\textsuperscript{19} The designations “A” and “B” have therefore been eliminated and the lesion is referred to simply as the Quilty effect.

The relationship of Quilty effect to acute rejection, if any, remains unknown. Traditionally, this lesion has been considered distinct from acute rejection, requiring no treatment with intensified immunosuppression. Differentiation of Quilty effect from acute rejection is not usually a problem when the former is confined to the endocardium. However, when it extends into the underlying myocardium, a tangential cut through the biopsy may not show a connection between the myocardial lesion and the endocardial lesion, making differentiation from acute rejection more difficult.\textsuperscript{20} Cutting additional deeper sections may resolve this dilemma in some cases by demonstrating extension to the endocardium (Figures 17 and 18). In the absence of an endocardial extension, the density of the infiltrate, presence of B lymphocytes and plasma cells, background fibrosis and prominent vascularity favor a diagnosis of Quilty effect.

**Infection and PTLDs**

Infection and PTLDs remain important causes of post-transplant morbidity and mortality, but are relatively rare in post-transplant cardiac biopsies. Notable among these are cytomegalovirus (CMV) and toxoplasmosis, both of which may be accompanied by lymphocyte-predominant infiltrates, which may be misinterpreted as acute cellular rejection, leading to inappropriate augmentation of immunosuppression. More specifically, targeted immunosuppression and improved prophylaxis protocols, especially for CMV, have decreased the incidence of some infections. Recognition of the relationship between immunosuppression and post-transplant neoplasms, especially PTLD, has favored less aggressive immunosuppression protocols. Although infection and PTLD are less controversial than other post-transplant biopsy interpretations, they require continued awareness and vigilance.

**ACUTE ANTIBODY-MEDIATED (HUMORAL) REJECTION**

Acute humoral rejection is recognized as a clinical entity in the grafted heart (Table 3). It remains controversial, however, with a highly varied incidence be-
between different centers and no consensus has yet been reached on its recognition and diagnosis either histopathologically or immunologically. The 2004 ISHLT meeting reviewed evidence from the immunopathology and clinical task forces and felt able to suggest diagnostic criteria in specific circumstances so that further assessment of this entity could be encouraged. In the 1990 Working Formulation, there was an option to record immunofluorescence studies for those centers that sought to pursue these on biopsy specimens in the first 6 weeks after transplantation. Similarly, in utilizing the 2004 classification, pathologists can follow the guidance if they intend to investigate the possibility of antibody-mediated rejection as a cause of cardiac dysfunction. A separate companion study from the Immunopathology Task Force is available with a detailed discussion of antibody-mediated rejection. A summary of recommendations is provided here to allow incorporation, as required, into the revised Working Formulation.

Acute antibody-mediated rejection is associated with worse graft survival and is observed in allosensitized patients, including those with previous transplantation, transfusion or pregnancy and previous ventricular assist device use. The incidence may be up to 15% in the first year post-transplantation and the clinical presentation has no pathognomonic features. Pathologically, it can be recognized by myocardial capillary injury with endothelial-cell swelling and intravascular macrophage accumulation (Figures 19, 20 and 21). Interstitial edema and hemorrhage can be present together with neutrophils in and around capillaries. Intravascular thrombi and myocyte necrosis without cellular infiltration can also be identified. When these features are seen in the presence of unexplained cardiac dysfunction, typically early onset of hemodynamic compromise and myocardial dysfunction, it is proposed that immunostaining can be performed by immunofluorescence or immunohistochemistry as follows:

- Immunoglobulin (IgG, IgM and/or IgA) plus complement deposition (C3d, C4d and/or C1q) in capillaries by immunofluorescence on frozen sections (Figures 22 and 23); and/or

![Figure 19](image1.png) 
Figure 19. Antibody mediated rejection (AMR 1). Low power view of endomyocardial biopsy with scattered cellular infiltrates and intervening normal tissue. (H&E).

![Figure 20](image2.png) 
Figure 20. AMR 1. Higher power view shows that the cellular infiltrates are within vessels and include polymorphs. Endothelial cell swelling is present. The increased cellularity seen at low power is due to the presence of these intravascular cells and not perivascular inflammation. Compare with figures 1 and 5. (H&E).

![Figure 21](image3.png) 
Figure 21. AMR 1. High power view confirms the intravascular location of the cells which have the appearance of macrophages and illustrates the endothelial cell swelling. (H&E).
CD68 staining of macrophages within capillaries (CD31- or CD34-positive) by immunohistochemistry (Figure 24); and

- C4d staining of capillaries by paraffin immunohistochemistry (Figure 25).

It is recommended that patients with hemodynamic compromise undergo assessment for circulating antibodies.

The consensus meeting recommended that screening should not be advocated at this time, but every endomyocardial biopsy should undergo critical histologic evaluation for features suggestive of antibody-mediated rejection. If such features (as just detailed) are not seen, the biopsy should be designated negative for antibody-mediated rejection, or AMR 0. If features suggestive of antibody-mediated rejection are seen, the diagnosis of acute antibody-mediated rejection should be confirmed by immunohistochemistry, either immunofluorescence or immunoperoxidase, using antibodies directed against CD68, CD31 and C4d, and a serum should be drawn and tested for donor-specific antibody. If these markers are positive, a positive diagnosis for AMR should be made (AMR 1). Patients who have several episodes of documented acute antibody-mediated rejection should be followed on future biopsies with at least one of these immunohistochemical methods and monitored for the production of donor-specific antibodies. It is also recognized that acute cellular and antibody-mediated rejection can co-exist, but further studies will be required to delineate these.

This recommended approach to the diagnosis of acute antibody-mediated rejection—if there is either a clinical indication or a research need—should encourage clinicians, histopathologists and immunologists to work together and clarify its existence, frequency and clinical significance.
Table 4. Technical Considerations

<table>
<thead>
<tr>
<th>Minimum number of biopsy samples</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td>Number of hematoxylin and eosin slides</td>
<td>3</td>
</tr>
<tr>
<td>Number of levels</td>
<td>3</td>
</tr>
<tr>
<td>Routine special stains required</td>
<td>None</td>
</tr>
</tbody>
</table>

TECHNICAL CONSIDERATIONS

Due to the potential for sampling error in diagnosing acute rejection, multiple myocardial biopsy samples should be obtained from different right ventricle sites (Table 4). Samples should not be divided once procured in order to obtain the required number of pieces because this practice results in less representative sampling. Although the original ISHLT grading system required 4 samples of myocardium, the trend has been to accept 3 evaluable samples as the absolute minimum for interpretation. Therefore, a minimum of 3, and preferably 4 (or more), evaluable pieces of myocardium are now recommended for grading acute cellular rejection. An evaluable piece of myocardium contains at least 50% myocardium, excluding previous biopsy site, scar, adipose tissue or blood clot, which may comprise the remainder of the piece. Hematoxylin and eosin staining of at least 3 levels through the tissue samples are recommended. Additional spare slides may be saved unstained if additional studies are needed. Special stains are not routinely required and have been eliminated by many centers as the incidence of rejection has decreased. A trichrome stain may be helpful in selected cases for assessing myocyte damage and fibrosis, such as in the early post-operative period.

CONCLUSIONS

It is the intention of this consensus group that this revision of the grading system addresses and clarifies concerns that have developed in the 15 years since the adoption of the 1990 grading system. The plan is to supplement this revision with an educational program for pathologists and clinicians. As was the case for the 1990 grading system, the 2004 grading system will now be required for all ISHLT-sponsored meetings and publications.

There has been tremendous advancement in technology since the 1990 grading system was instituted, including many molecular techniques. Many of these advances have been used successfully in the research setting to further our knowledge of pathologic processes. The challenge will be to decide the appropriate time and choice of technique(s) to incorporate into routine clinical practice. For the ISHLT grading system to remain the standard worldwide, it must remain the lowest common denominator so that every transplant center has the technical ability and financial resources to incorporate any proposed changes. We must make sure, going forward, that we retain the universality of the grading system because this has always been a major component of its success. The consensus meeting task forces strongly urge the ISHLT to periodically review the grading system as immunosuppressive regimens evolve and as additional clinical and molecular monitors of cardiac function, coronary vasculopathy and immune responsiveness are developed and used in the management of heart transplant recipients.

REFERENCES

14. Brunner-LaRocca HP, Sutsch G, Schneider J, et al. Natural course of moderate cardiac allograft rejections (Interna-

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