Utility of Histologic Parameters in Screening for Antibody-Mediated Rejection of the Cardiac Allograft: A Study of 3,170 Biopsies

M. Elizabeth H. Hammond, MD,[a,b] Josef Stehlik, MD,[c] Gregory Snow, PhD,[d] Dale G. Renlund, MD,[e] James Seaman, MD,[a] Bashar Dabbas, MD,[a] Edward M. Gilbert, MD,[f] James C. Stringham, MD,[g] James W. Long, MD, PhD,[e] and Abdallah G. Kfoury, MD,[e] and the Utah Transplant Affiliated Hospitals (UTAH) Cardiac Transplant Program

Background: Diagnostic criteria for antibody-mediated rejection (AMR) of the cardiac allograft have recently been proposed as part of the International Society for Heart and Lung Transplantation (ISHLT) biopsy grading scheme. Histologic features of vascular adherence of macrophages (VASC) and endothelial activation or swelling in capillaries (ENDO) are proposed as criteria to prompt the immunohistochemical investigation of biopsies for AMR. The aim of this study was to determine whether VASC and ENDO are adequate to act as screening parameters to trigger further AMR investigation.

Methods: We examined our database of biopsy findings where histologic vascular parameters as well as immunofluorescence (IF) to detect AMR were collected (n = 3,170). Histologic parameters were graded semi-quantitatively on a scale from 1 to 5, where 1 = absence and 5 = obvious and generalized presence of the finding.

Results: Seven hundred sixty-eight of 3,170 biopsies had IF findings diagnostic of AMR in the absence of cellular rejection (ISHLT = 0). ENDO had a sensitivity of 63% and a specificity of 80%. VASC had a sensitivity of 30% and specificity of 99%. Combining the interpretation of the 2 tests did not result in a significant improvement of test sensitivity.

Conclusions: Neither ENDO, VASC nor the combination of the tests indicated sufficiently high sensitivity to serve as a screening tool before further diagnostic investigation for AMR. Immunohistochemical testing remains necessary in the majority of cases to identify AMR. J Heart Lung Transplant 2005;24: 2015–21. Copyright © 2005 by the International Society for Heart and Lung Transplantation.

Diagnostic criteria for cardiac antibody-mediated rejection (AMR) have recently been included as part of the revised International Society of Heart Lung Transplantation (ISHLT) grading scheme.[1] Publication of these criteria was the result of a consensus conference held in 2004 to modify the existing ISHLT grading scheme for cardiac transplant biopsies, which had been used routinely since 1991.[2] The revised scheme recommends that every endomyocardial biopsy be evaluated histologically for features suggestive of antibody-mediated rejection (AMR). The pathologic features that can be detected on light microscopy that are suggestive of AMR include myocardial capillary injury or endothelial cell swelling or activation and intravascular macrophage accumulation. These findings may be associated with interstitial edema and hemorrhage as well as with neutrophils in and around capillaries. Intravascular thrombi and myocyte necrosis without cellular infiltration are also features suggestive of AMR.[1,3–7]

The consensus recommendations specify that, if such features are *not* seen, further immunohistochemical testing is not warranted and the biopsy should be designated negative for antibody-mediated rejection (AMR 0). If features suggestive of antibody-mediated rejection are seen, the diagnosis of acute antibody-mediated rejection should be confirmed using immunohistochemistry, either by immunofluorescence on frozen tissue using antibodies directed against immunoglobulins (IgG, IgM, IgA) and complement components (C3d, C4d, C1q) or by immu-

From the [a]Department of Pathology, LDS Hospital; [b]Department of Pathology, University of Utah School of Medicine; [c]Department of Cardiology, Veterans Affairs Medical Center; [d]Statistical Data Center, LDS Hospital; [e]Cardiovascular Department, LDS Hospital; [f]Department of Cardiology, University of Utah School of Medicine; [g]Department of Cardiovascular Surgery, University of Utah School of Medicine, Salt Lake City, Utah.

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Reprint requests: M. Elizabeth H. Hammond, MD, Department of Pathology, LDS Hospital, 8th Avenue and C Street, Salt Lake City, UT 84143. Telephone: 801-408-1029. Fax: 801-408-5020. E-mail: elizabeth.hammond@ihc.com

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nohistochemical staining on paraffinized tissue, using antibodies directed against CD68, CD31 and C4d.[1,3–7] Patient serum should also be tested for donor-specific antibody.[6] If these markers are positive, a diagnosis of AMR should be made (AMR 1).

A further recommendation is that patients who have had several episodes of documented acute antibody-mediated rejection should be followed on future biopsies with one of these immunohistochemical methods and monitored for the production of donor-specific antibodies.[1,6] It was also recognized that acute cellular and antibody-mediated rejection can co-exist, although no specific criteria or recommendations about this combined entity were provided.[1] Figure 1 shows the algorithm recommended for determining the presence of AMR according to the new ISHLT grading scheme.

Since 1988, the UTAH Cardiac Transplant Program has routinely assessed endomyocardial biopsies for evidence of AMR.[3] Descriptions of the clinical findings and associated treatments of these patients have also been published.[8–11] All biopsies were evaluated by immunofluorescence for evidence of AMR for 6 to 8 weeks post-transplant, and later when indicated by clinical suspicion of AMR. Histologic parameters were graded in a semi-quantitative manner, including the presence of inflammatory infiltrates, capillary alterations, interstitial region features and presence of myocyte damage or ischemic injury. In addition, all biopsy samples were tested by immunofluorescence using a panel of antibodies that includes all of those recommended in the new grading scheme.[3,7] This information has been recorded prospectively into a database.

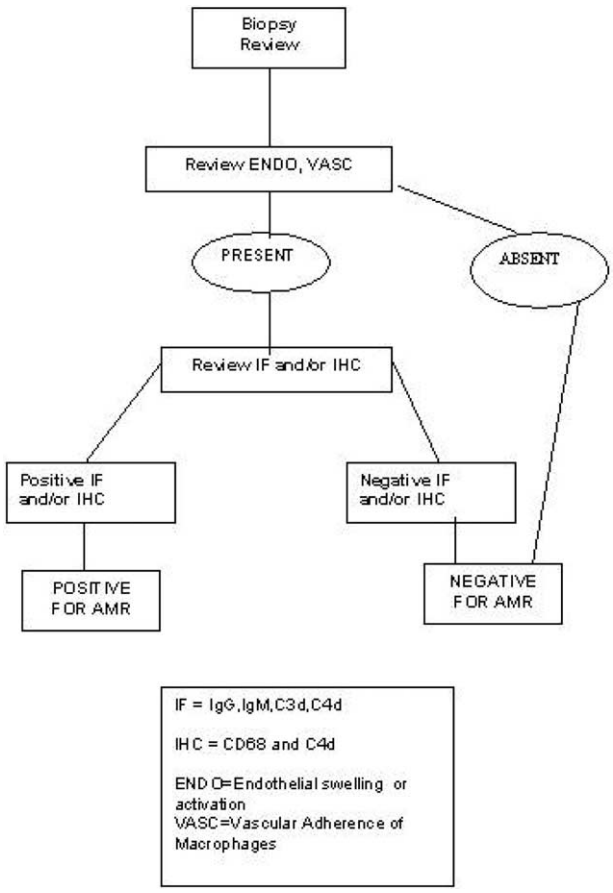

Figure 1. Algorithm showing decision steps in the evaluation of a cardiac biopsy for antibody-mediated rejection. Boxed information indicates the definitions of the various terms. AMR, antibody-mediated rejection.

The aim of this study was to determine whether histologic features alone are sufficiently sensitive to act as screening parameters to trigger AMR investigation by immunohistochemistry, as proposed in the revised ISHLT grading scheme.

METHODS

Data Source

Since 1988, a prospective database of endomyocardial biopsy findings on cardiac allograft biopsies has been maintained using a relational database program (FileMaker Pro, FileMaker, Inc., Santa Clara, CA). Table 1 shows the data entry document used to populate the database. Semi-quantitative assessment on a 1 to 5 scale (1 = represents an absent or normal finding, 5 = generalized abnormal finding) was performed for the histologic variables. Immunofluorescence (IF) findings were also recorded semi-quantitatively, where 0 = absent and 3+ = generalized deposition of the antibody being recorded. Pathologists reading the biopsies recorded their findings prospectively into the database. Histologic features were recorded independently from IF findings as they were independently observed. Figure 2 illustrates various semi-quantitative levels of histologic and IF features.

Study Group

Subjects in this study included adult and pediatric cardiac transplant recipients within our cardiac transplant program who were assessed between August 1988 and April 2004. Data from all biopsies on every patient during this time period were captured in the database. Only biopsies without evidence of cellular rejection (ISHLT = 0) for which both the histologic parameters and IF findings were available were used in the analysis.

Institutional Criteria for Antibody-Mediated Rejection

Antibody-mediated rejection was considered present if any immunoglobulin and any complement component were simultaneously demonstrated in capillaries of frozen sections of the biopsy fragments obtained along with the tissue for histologic examination. The IF staining was obtained during routine surveillance for AMR early post-transplant (6 to 8 weeks post-transplant) or as part