Antibody-Mediated Rejection in Cardiac Transplantation: 
Emerging Knowledge in Diagnosis and Management

A Scientific Statement From the American Heart Association

Endorsed by the International Society for Heart and Lung Transplantation

Monica M. Colvin, MD, MS, Chair; Jennifer L. Cook, MD, Co-Chair; Patricia Chang, MD; Gary Francis, MD, FAHA; Daphne T. Hsu, MD, FAHA; Michael S. Kiernan, MD; Jon A. Kobashigawa, MD, FAHA; JoAnn Lindenfeld, MD, FAHA; Sofia Carolina Masri, MD; Dylan Miller, MD; John O’Connell, MD; E. Rene Rodriguez, MD; Bruce Rosengard, MD; Sally Self, MD; Connie White-Williams, RN, FAHA; Adriana Zeevi, PhD; on behalf of the American Heart Association Heart Failure and Transplantation Committee of the Council on Clinical Cardiology, Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation, Council on Cardiovascular Disease in the Young, Council on Cardiovascular Radiology and Intervention, and Council on Cardiovascular Surgery and Anesthesia

Antibody-mediated rejection (AMR) of the cardiac allograft is a poorly defined and challenging diagnosis for transplant recipients and their clinicians. Although even its very existence in heart transplantation was debated until relatively recently, improved immunopathologic and serological techniques to detect myocardiocapillary complement deposition and circulating anti-HLA (human leukocyte antigen) antibodies have led to the detection of a spectrum of newly uncovered immunologic changes that characterize AMR. The earliest standardized clinical and pathological criteria for the diagnosis of AMR in heart transplantation became available in 2004, the result of a task force assembled by the International Society for Heart and Lung Transplantation (ISHLT). In 2006, the criteria were refined by the ISHLT Immunopathology Task Force (Table 1). These revisions provide 4 categories of diagnostic criteria: clinical, histopathologic, immunopathologic, and serological assessment.1 Despite these published criteria, currently >50% of heart transplant centers make the diagnosis of AMR based on cardiac dysfunction and the lack of cellular infiltrates on the heart biopsy (preconference survey included in the ISHLT consensus article).2

More recently, the ISHLT Consensus Conference on AMR has redefined the pathological diagnosis of AMR.3 The 2013 ISHLT “Working Formulation for the Standardization of Nomenclature in the Pathologic Diagnosis of Antibody-Mediated Rejection in Heart Transplantation” was published in December 2013. This document provided an update to the 2010 consensus conference.4 It is anticipated that this update to the definition of AMR will reduce variations in the diagnosis of AMR, providing a platform for the development of standardized therapies. The goal of the present scientific statement is to provide the heart transplant professional with an overview of the current status of the diagnosis and treatment of AMR in the cardiac allograft based on recent consensus conferences and the published literature. We include recommendations to facilitate evolving standardization and strategies for future study.
Historical Perspective: Evolving Recommendations

The success of heart transplantation in the 1980s was enabled by the ability to diagnose rejection by transjugular right ventricular endomyocardial biopsy, a technique developed by Philip Caves in 1973. The diagnosis of acute cellular rejection (cytotoxic T-cell mediated) is made by histological identification of interstitial leukocyte infiltration with various degrees of myocyte damage. These features are sensitive and specific and correlate with allograft dysfunction. Furthermore, this immunopathologic and clinical state responds to anti–cellular rejection therapies with clinical improvement and resolution of histological rejection features. The subset of heart transplant recipients with graft failure and no evidence of cellular rejection were considered to have biopsy-negative rejection. Pathological changes observed in this setting were not included in the histological grading systems for cellular rejection, and these collective pathological changes were variably referred to as humoral, vascular, or antibody-mediated rejection (AMR).

The first limited description of humoral rejection was included in the 1990 ISHLT criteria defined as positive immunofluorescence, vasculitis, or severe edema in the absence of cellular infiltrate2 (Table 2). By the time of the 2004 ISHLT revision, the immunologic process underlying AMR was better described in the literature.4 Routine screening was still not advocated; however, a recommendation was made for every endomyocardial biopsy specimen to undergo histological evaluation for AMR. At that time, the classification AMR 0 was assigned in the absence of histological or immunopathologic features. Confirmation of AMR or AMR 1 was defined as histological evidence with identification of antibodies (directed against CD68, CD31, C4d) and serum presence of donor-specific antibody (DSA; Table 2). With the publication of the 2004 working formulation, the field moved toward almost exclusive use of the term AMR and use of more precise histological descriptors. In 2006, the ISHLT Immunopathology Task Force provided an expanded description of the histological evidence of acute capillary injury, the minimum requirement for immunopathologic evidence of antibody-mediated injury, and an improved definition of serological evidence of circulating antibodies1 (Table 1).

The persistent variations in the diagnosis and treatment of AMR were addressed in 2 related conferences: the Heart Session of the Tenth Banff Conference on Allograft Pathology (August 2009) and the ISHLT Consensus Conference on AMR (April 2010). These sessions undertook another revision in an attempt to refine the immunopathologic assessment of AMR. Our increased understanding of the pathological processes behind AMR enabled an evolution beyond the descriptive “biopsy-negative rejection” to AMR, a clinical entity with specific histopathologic, immunopathologic, and serological characteristics.

### 2010 ISHLT Consensus Conference on AMR

A consensus conference sponsored by the ISHLT convened transplant cardiologists, surgeons, pathologists, and immunologists on April 20, 2010, to advance the understanding of AMR.2 Participants represented 67 heart transplant centers from North America, Europe, and Asia. The most important

<table>
<thead>
<tr>
<th>Table 1. Findings in Acute AMR of the Heart</th>
</tr>
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<tbody>
<tr>
<td>Required Findings</td>
</tr>
<tr>
<td>1. Clinical evidence of acute graft dysfunction</td>
</tr>
<tr>
<td>2. Histological evidence of acute capillary injury (a and b required)</td>
</tr>
<tr>
<td>3. Immunopathologic evidence for antibody-mediated injury (a or b or c required)</td>
</tr>
<tr>
<td>4. Serological evidence of anti-HLA or anti-donor antibodies</td>
</tr>
</tbody>
</table>

AMR indicates antibody-mediated rejection; HLA, human leukocyte antigen; IF, immunofluorescence; and IH, immunohistochemistry.

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<table>
<thead>
<tr>
<th>Table 2. Historical AMR Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
</tr>
<tr>
<td>AMR 0</td>
</tr>
<tr>
<td>AMR 1</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

AMR indicates antibody-mediated rejection; IF, immunofluorescence; and IP, immunoperoxidase.

Modified from Stewart et al8 with permission from the International Society for Heart and Lung Transplantation. Copyright © 2005, International Society for Heart and Lung Transplantation.
issues included the need for a clinical definition of AMR, the significance of asymptomatic biopsy-proven AMR (without cardiac dysfunction), and the recognition that AMR may be caused by DSA as well as antibodies to non-HLA antigens. In cellular rejection, clinical descriptors such as recurrent, persistent, or hemodynamic compromise are used to illustrate clinical presentation or clinical severity. These clinical descriptors could also be used for AMR. Although AMR would be a pathological diagnosis, it was strongly recommended that at the time of suspected AMR, blood be drawn at biopsy and tested for the presence of donor-specific anti-HLA class I and class II antibodies. In the absence of detectable anti-HLA antibodies, the assessment of non-HLA antibodies may be indicated.

In contrast to the 2004 revision, screening for AMR was recommended. Specifically, recommendations were made regarding the routine timing for specific staining of endomyocardial biopsy specimens and the frequency by which circulating antibodies should be assessed (Table 3). Finally, recommendations for management and future clinical trials were given. The ongoing work by pathologists to refine the classification of pathological AMR (as commissioned by the ISHLT board of directors) was published by Berry et al in 2011 and more recently in 2013. The 2013 ISHLT working formulation for pathological diagnosis of AMR is shown in Tables 4 and 5.

Pathogenesis and Immunopathologic Features of AMR

Pathogenesis

AMR develops when recipient antibody is directed against donor HLA antigens on the endothelial layer of the allograft. Antibodies induce fixation and activation of the complement cascade, resulting in tissue injury. Complement activation, a key contributor to the pathogenesis of AMR, results in activation of the innate and adaptive immune responses. Complement and immunoglobulin are deposited within the allograft microvasculature, which results in an inflammatory process that is characterized by endothelial cell activation, upregulation of cytokines, infiltration of macrophages, increased vascular permeability, and microvascular thrombosis. This process ultimately manifests as allograft dysfunction.

AMR may present as hyperacute rejection within 0 to 7 days after transplantation in patients who are sensitized to donor HLA antigens. Early AMR may occur during the first month after transplantation because of the development of de novo DSA or preexisting DSA. Early AMR tends to be associated with a higher prevalence of allograft dysfunction and hemodynamic compromise. The reported prevalence of late AMR, occurring months to years after transplantation, has increased, most likely because of heightened recognition. Approximately 50% of heart transplant recipients who develop rejection >7 years after transplantation have evidence of AMR. Finally, AMR has been reported concurrent with cellular rejection in up to 24% of cases. As the definition of AMR has evolved and more sensitive diagnostic modalities have become available, there is increasing evidence that AMR is a spectrum of immunologic injury that ranges from subclinical, histological, immunologic, and/or serological findings without graft dysfunction (ie, subclinical AMR) to overt AMR with hemodynamic compromise.

Histopathologic Features

The vascular endothelium is the point of first contact for anti-donor antibody in the allograft and the primary locus of activity in AMR. The myocardial capillaries, arterioles, and venules are readily sampled at biopsy; however, changes in the epicardial coronary arteries have also been noted at autopsy and in the explanted allograft.

Enlarged or swollen endothelial cells, both cytoplasm and nuclei, are consistently seen, presumably reflecting endothelial activation as a consequence of intracellular signaling induced by antibody and subsequently complement, binding to surface antigen epitopes. The appearance of vasculitis or leukocytes infiltrating through the endothelium into the vessel wall demonstrates active humoral immunity with antibody-dependent cytotoxicity, cytokine- and chemoattractant-mediated homing, and circulating monocyte recruitment. Similar changes have been described in arterioles and venules, the closest contiguous segments upstream and downstream from myocardial capillaries, which are also lined by endothelium. Rarely, intravascular thrombi can be seen in these vessels, particularly in severe manifestations.

Table 3. ISHLT Recommendations for Monitoring for AMR

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Endomyocardial Biopsy</th>
<th>Circulating Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histological evaluation</td>
<td>C4d</td>
<td>Solid-phase assay and/or cell-based assays to assess for presence of DSA (and quantification if antibody present)</td>
</tr>
<tr>
<td>Immunoperoxidase: C4d</td>
<td>C4d and C3d</td>
<td></td>
</tr>
<tr>
<td>Immunofluorescent staining: C4d and C3d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histological evaluation</td>
<td>Every protocol biopsy</td>
<td>2 wk and 1, 3, 6, and 12 mo after transplantation</td>
</tr>
<tr>
<td>Immunoperoxidase/imunofluorescent staining: 2 wk and 1, 3, 6, and 12 mo after transplantation</td>
<td>When AMR is clinically suspected</td>
<td></td>
</tr>
<tr>
<td>When AMR is suspected on the basis of histological, serological, or clinical findings</td>
<td>Routine C4d(C3d) staining on subsequent biopsy specimens after a positive result until clearance</td>
<td></td>
</tr>
</tbody>
</table>

AMR indicates antibody-mediated rejection; DSA, donor-specific antibody; and ISHLT, International Society for Heart and Lung Transplantation.

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Interstitial edema and hemorrhage are also seen in AMR; however, interpretation of these findings is limited by the traumatic nature of procurement by the biopтоме, which may present a challenge in distinguishing interstitial hemorrhage from biopsy-related artifact. Squeezing and distortion during biopsy removal and handling may also obscure edema.6,19–21

Capillary changes indicative of AMR include endothelial cell swelling and intravascular macrophage accumulation coincident with pericapillary neutrophils.22

**Immunopathologic Features**

The primary evidence validating AMR as a distinct form of humoral immunity-induced rejection was the early demonstration of bound immunoglobulin and complement within the myocardial capillary bed. The role of immunoglobulins, complement activation, and the coagulation cascade in AMR is under constant study as diagnostic methods increase in sensitivity and specificity. Immunopathologic evidence, based on a variety of target antigens and immunopathologic assays, remains vital to the identification of AMR (Table 6).

**Immunoglobulin (IgG, IgM)**

For nearly a half a century, detection of tissue-bound immunoglobulin (and immune complexes) has been routine in kidney biopsies to diagnose immune complex glomerulonephritis. Detection assays for immunoglobulin heavy and light chains were therefore the first assays used to investigate AMR in cardiac transplant biopsies.17,22,23

Before 2000, the detection of tissue immunoglobulin was a defining characteristic of AMR. This technique has limited utility because of the considerable intra-assay, interobserver, and interinstitutional variability. The sensitivity of this test is poor because of dissociation of immunoglobulin from antigen in vitro and rapid degradation in vivo. Specificity is limited because of the abundance of immunoglobulin in serum, where “serum contamination” of tissue leads to nonspecific staining.

**Complement Components**

The complement components C3 and C1q have been demonstrated in kidney AMR; however, their detection is limited by a short half-life in vivo and consequently a short window of detection during a rejection episode. Nevertheless, complement deposition is the sine qua non of AMR, and the presence of C4d and C3d has been proposed as a diagnostic criterion for AMR.

The protein C4d is a complement split product that binds covalently to endothelium at the site of complement activation and persists longer than C3 or C1q (Figures 1 and 2). In 1998, this technique was adapted from experience in kidney transplantation and used to identify AMR.23–25 Currently, C4d is used frequently to diagnose AMR, and some authors suggest that C4d can be used as an immunopathologic surrogate for AMR.25–32 C4d positivity is also used in combination with histological features—with circulating DSA, or clinical graft dysfunction.23,33–36 Depending on how restrictive the pathological definition of AMR is (ie, number of criteria required), the reported incidence varies, with lower reported incidence with more criteria required. Early estimates using C4d alone ranged from 35% to 71%, whereas those using C4d in combination with other immunopathology markers, clinical graft dysfunction, and DSA reported AMR frequencies of 10%, 27%, and 12.5%, respectively.24,31,37–40

Although histological changes of AMR may be seen in any vessel type, C4d deposition is largely restricted to capillaries. Occasional staining of large-vessel endothelium (when present in a biopsy sample), perimyocytes, or sarcolemma may be seen; however, these patterns do not appear to indicate AMR.41

Presence of C3d, a complement split product, is also used to diagnose AMR.23,38,42–46 (Figures 1 and 2). Like C4d, C3d persists in tissues longer than C3 and C1q, but because C3d cleavage occurs further downstream in the complement cascade, it indicates progression of complement activation.35

The combination of C4d and C3d detected by immunofluorescence predicts graft dysfunction and mortality better than C4d alone.47 Because C3d staining of arterioles may be seen in

### Table 4. Proposed Scoring System for Pathological AMR

<table>
<thead>
<tr>
<th>Category</th>
<th>Immunohistochemistry</th>
<th>Immunofluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary distribution and intensity</td>
<td>Multifocal/diffuse weak or strong staining of C4d</td>
<td>Multifocal/diffuse weak or strong staining of C4d/C3d</td>
</tr>
<tr>
<td>Intravascular CD68 distribution</td>
<td>&gt;10% Focal/multifocal/diffuse intravascular macrophages</td>
<td>...</td>
</tr>
<tr>
<td>HLA-DR distribution and intensity</td>
<td>...</td>
<td>Multifocal/diffuse weak or strong staining</td>
</tr>
<tr>
<td>Caveats</td>
<td>Focal strong C4d staining is classified as negative but warrants close follow-up</td>
<td>Focal strong C4d staining is classified as negative but warrants close follow-up</td>
</tr>
</tbody>
</table>

AMR indicates antibody-mediated rejection; and pAMR, pathological antibody-mediated rejection category.

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Table 6. Immunopathologic Features of AMR

<table>
<thead>
<tr>
<th>AMR</th>
<th>Interpretation</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG/IgM</td>
<td>Immunoglobulin binding</td>
<td>+ Easily dissociated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3, C1q</td>
<td>Complement activation</td>
<td>+ Short half-life</td>
</tr>
<tr>
<td>C3d/C4d</td>
<td>Complement activation</td>
<td>+ Combination more predictive of AMR than C4d alone, long half-life</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>Endothelial integrity</td>
<td>+ Staining always present, but “frayed” pattern indicates capillary injury</td>
</tr>
<tr>
<td>Fibrin</td>
<td>Thrombotic environment</td>
<td>+ Interstitial extravasation suggests more severe AMR episode</td>
</tr>
<tr>
<td>CD55, CD59</td>
<td>Complement inhibitor</td>
<td>– Long incubation and granular staining pattern</td>
</tr>
<tr>
<td>CD31, CD34, CD68</td>
<td>Intravascular macrophages</td>
<td>+ CD68 confirms macrophage lineage of mononuclear cells</td>
</tr>
</tbody>
</table>

AMR indicates antibody-mediated rejection; and HLA, human leukocyte antigen.

normal native tissue and likely represents artifactual nonspecific binding of the antibody to connective tissue components, only capillary staining with C3d is significant.98,48

Immunoglobulin binding and complement activation are regulated in vivo by complement inhibitors. Two such regulators, CD59 and CD55 (decay accelerating factor), are used in conjunction with C4d and C3d to indicate aborted complement activation. The association with allograft function is unclear.47,49 Lengthy incubation times and a granular staining pattern render these assays impractical for clinical use.

HLA-DR staining is helpful to delineate the capillary endothelium and highlight any compromise to individual capillary integrity, in which a frayed, disrupted, or feathery pattern indicates endothelial damage.50 Venular thrombosis is present in hyperacute rejection, and intravascular thrombi are noted in severe rejection.51 Fibrinogen (factor II) staining, although readily available and routinely used in kidney transplant immunopathology, is less specific.8,19,37,52

Finally, the macrophage antigen CD68 allows identification of subtle accumulations of macrophages within vessels, which helps to differentiate intravascular/perivascular macrophages from lymphocytes, thereby excluding acute cellular rejection (ACR).17,33,53 Antigens CD34 and CD31 are endothelial cell markers, and like HLA-DR, they reflect the integrity of the capillary bed. CD34 and CD31 staining can be used to ascertain the intravascular location of macrophages/mononuclear cells, thereby supporting the diagnosis of AMR34 (Table 6; Figure 2).

Posttransplantation Antibodies

The development of anti-HLA antibodies after transplantation has been implicated in allograft injury. Tambur et al55 demonstrated that de novo production of antibodies during the first year after transplantation is significantly associated with cellular rejection and that class II antibodies significantly correlate with mortality and cardiac allograft vasculopathy (CAV). Posttransplantation panel reactive anti-HLA antibodies (PRAs) are associated with the development, frequency, and severity of CAV.56,57 Early and persistent anti-HLA antibody is associated with worse survival and CAV.58 DSAs, on the other hand, are associated with cellular rejection, AMR, and increased incidence of CAV.56,59,61 More recently, the demonstration of antibody specificity has provided greater prognostic determination. In a study of the relationship between complement deposition, HLA serology, and graft function, DSAs were found in 95% of biopsy samples that were positive for both C4d and C3d, compared with 35% in biopsy samples that were positive for C4d only. C4d+C3d+ biopsy samples demonstrated strong correlation with graft function and mortality; allograft dysfunction was present in 84% of patients with C4d+C3d+ compared with 5% of C4d+C3d+ (P<0.0001). Combined positivity had a mortality of 37%.57 The presence of DSAs alone is not diagnostic of AMR; however, in the presence of complement deposition or graft dysfunction, their presence supports alloimmune activation.

Non-HLA Antibody

Non-HLA and nontraditional antibodies may cause immune-mediated injury in the absence of detectable anti-HLA antibody. Non-HLA antibodies can be directed against autoantigens, polymorphic minor antigens, and polymorphic non-HLA antigens such as major histocompatibility complex class I chain–related antigens. These antibodies bind endothelium and result in apoptosis but not in complement-mediated lysis.1,62–65 Perhaps the most well described non-HLA antibodies are anti-endothelial cell antibodies, which have been implicated in acute humoral rejection, CAV, and poor graft survival.

Figure 1. A, C4d capillary staining by immunohistochemistry; B, C3d capillary staining by immunohistochemistry; C, C4d capillary staining by immunofluorescence; D, C3d, by immunofluorescence.
in heart transplantation. Production of anti-endothelial cell antibodies appears to be stimulated by cytomegalovirus infection, increasing 1 to 4 weeks after detection of cytomegalovirus DNA. Vimentin, a well-described autoantigen, is the most abundant immunoreactive endothelial cell antigen. A type III intermediate filament cytoskeletal protein, it is elaborated by damaged endothelial cells, proliferating smooth muscle cells, fibroblasts, and leukocytes. One- and 2-year anti-vimentin titers predict the development of CAV. Anti-vimentin antibodies have been detected alone or in conjunction with anti-HLA antibodies and appear to be linked to HLA-DQ2 antibodies in particular. Alvarez-Márquez et al tested the association between HLA antigens A and B (MICA and MICB) are polymorphic alloantigens that have been implicated as markers in transplantation, and MICA in particular has been linked with AMR. MICA is expressed on endothelial cell surfaces and can induce complement-lysing antibody. The appearance of anti-MICA antibodies after transplantation precedes the development of acute rejection and is more prevalent in patients who have rejection. Unfortunately, assays for measuring non-HLA antibodies are not widely available, which limits most centers’ abilities to comprehensively assess suspected AMR. Nevertheless, non-HLA antibodies should be suspected in patients who have no evidence of DSA by solid phase assay but have pathological or clinical evidence of AMR.

Clinical Features of AMR

Incidence

The true incidence of AMR is not known; because of the evolving diagnostic criteria and lack of routine screening by most programs, AMR is likely underreported. The reported incidence of AMR varies widely, between 3% and 85%, because of diverse diagnostic criteria and variations in screening frequency (Table 7). In published studies describing the incidence of AMR, the diagnostic criteria may include pathological findings, clinical findings, or both. To illustrate these disparities, Kfoury and colleagues evaluated histological and immunofluorescence findings in routine biopsy samples from 870 heart transplant recipients and reported an incidence of 85% at 100 days. This analysis included heart transplant recipients who received induction therapy with muromonab-CD3, which may have confounded the reported prevalence of AMR. Michaels and colleagues found AMR in 116 endomyocardial biopsy samples from 56 patients (≈600 patients followed up). Forty-four of the patients (77 biopsy samples) showed AMR without ACR (ISHLT grade 0). AMR was diagnosed by both immunofluorescence (immunoglobulin, C1q, and C3 deposition in capillaries or CD58+ cells on immunoperoxidase) and histological evidence as the criteria for AMR. Finally, Crespo-Leiro and colleagues reported an incidence of <3% when using the criteria of allograft dysfunction and C4d deposition. When ISHLT 2004 and 2006 criteria (ie, allograft dysfunction, serological evidence of DSA and biopsy evidence of complement deposition) were used, the incidence of AMR was 3% and 5%, respectively. It is anticipated that the establishment of standardized diagnostic criteria will improve consistency in the characterization of AMR.

Risk Factors

Reported risk factors for AMR in heart transplant recipients include elevated PRA, cytomegalovirus seropositivity, prior mechanical circulatory support, prior treatment with muromonab-CD3 and the development of antibodies against mouse monoclonal muromonab-CD3, history of retransplantation, multiparity, and positive T-cell flow-cytometry crossmatch. Women have a disproportionately higher incidence of AMR, comprising up to 50% of the heart transplant recipients with AMR in reported series. There is now clear evidence of a relationship between the presence of circulating anti-HLA antibody after transplantation and histological evidence of AMR.

Symptomatic AMR

Symptoms of acute allograft dysfunction are those of right and left ventricular (LV) systolic and diastolic dysfunction and include dyspnea, orthopnea, paroxysmal nocturnal dyspnea, elevated jugular venous pressure, edema and abdominal distention. In infants, symptoms related to AMR can include feeding intolerance, irritability, and poor weight gain. Acute AMR is reportedly associated with hemodynamic compromise in 10% to 47% of cases. The criteria for establishing hemodynamic compromise have been highly variable in the literature and include a decrease in LV ejection fraction, an unexplained elevation in intracardiac pressures with a concurrent decrease in cardiac output, and the need for inotropic therapy. The definition of hemodynamic compromise lacks uniformity, and the specific criteria for dysfunction may range from decreased ejection fraction to cardiogenic shock requiring inotropic support.
<table>
<thead>
<tr>
<th>Publication Year</th>
<th>First Author</th>
<th>Definition</th>
<th>n</th>
<th>Time to First AMR</th>
<th>AMR Rate</th>
<th>Outcome</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>Hammond⁴²</td>
<td>Immunopathology*</td>
<td>36</td>
<td>1–3 wk</td>
<td>55% of patients</td>
<td>Survival</td>
<td>Survival worse with AMR (57% vs 89% for mixed rejection and 95% for ACR at 3 y)</td>
</tr>
<tr>
<td>1991</td>
<td>Ensley⁴³</td>
<td>Immunopathology*</td>
<td>186</td>
<td>Mean 17 d</td>
<td>28% of patients</td>
<td>Survival</td>
<td>Survival worse with AMR (55% vs 92% at 2 y)</td>
</tr>
<tr>
<td>1992</td>
<td>Cherry⁴⁴</td>
<td>Immunopathology†</td>
<td>16</td>
<td>15–412 d</td>
<td>46% of 46 biopsies</td>
<td>…</td>
<td>Trend toward correlation between AMR and PRA</td>
</tr>
<tr>
<td>1992</td>
<td>Foerster⁴⁵</td>
<td>Histopathology and immunopathology*</td>
<td>108</td>
<td>…</td>
<td>1.6% of 1343 biopsies</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>1993</td>
<td>Hammond⁴⁶</td>
<td>Histopathology and immunopathology*</td>
<td>268</td>
<td>…</td>
<td>28% of patients</td>
<td>Graft survival; CAV</td>
<td>Graft survival worse with AMR (OR=2.1) AMR predictive of CAV (OR=9.5)</td>
</tr>
<tr>
<td>1993</td>
<td>Loy⁴⁰</td>
<td>Immunopathology†</td>
<td>16</td>
<td>…</td>
<td>0%</td>
<td>…</td>
<td>Note: no cases said to show histopathologic features of AMR</td>
</tr>
<tr>
<td>1993</td>
<td>Miller⁴⁷</td>
<td>Histopathology and immunopathology†</td>
<td>62</td>
<td>Mean 13 d</td>
<td>11% of patients</td>
<td>Graft dysfunction</td>
<td>More dysfunction with AMR (7% vs 6%)</td>
</tr>
<tr>
<td>1995</td>
<td>Lones⁴⁸</td>
<td>Histopathology and immunopathology†</td>
<td>81</td>
<td>3–6 wk</td>
<td>52% of patients</td>
<td>Survival; graft dysfunction</td>
<td>71% Survival with AMR 33% Dysfunction with AMR</td>
</tr>
<tr>
<td>1995</td>
<td>Bonnaud⁴⁹</td>
<td>Immunopathology†</td>
<td>18</td>
<td>6 wk</td>
<td>60% of biopsies (but also 63% of donor heart tissue controls)</td>
<td>…</td>
<td>Positive immunopathology is not rejection</td>
</tr>
<tr>
<td>1995</td>
<td>Caple⁵⁰</td>
<td>Immunopathology*</td>
<td>135</td>
<td>4 wk to 22 mo</td>
<td>7.4% of patients</td>
<td>Survival; graft dysfunction</td>
<td>No difference in survival 4 of 10 with hemodynamic compromise</td>
</tr>
<tr>
<td>1995</td>
<td>Ratliff⁵¹</td>
<td>Immunopathology*</td>
<td>53</td>
<td>4–6 wk</td>
<td>11% of patients</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>1998</td>
<td>Behr⁵²</td>
<td>Immunopathology*</td>
<td>45</td>
<td>&lt; 3 mo</td>
<td>60% of patients</td>
<td>Survival</td>
<td>No difference in survival</td>
</tr>
<tr>
<td>1999</td>
<td>Behr⁵³</td>
<td>Immunopathology*</td>
<td>56</td>
<td>&lt; 3 mo</td>
<td>71% of patients</td>
<td>Survival</td>
<td>Survival worse with AMR (6 deaths in C4d+ group vs 3 deaths in C4d– group and 6 deaths in fibrin-positive group vs 3 deaths in fibrin-negative group)</td>
</tr>
<tr>
<td>1999</td>
<td>Baldwin⁵⁴</td>
<td>Histopathology and immunopathology*</td>
<td>24</td>
<td>7–24 d</td>
<td>42% of 33 biopsies</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>2004</td>
<td>Chantranuwat⁵⁵</td>
<td>Immunopathology*</td>
<td>116</td>
<td>Majority &lt;3 mo</td>
<td>11% of 315 biopsies</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>2005</td>
<td>Garrett⁵⁶</td>
<td>Immunopathology*</td>
<td>53</td>
<td>6–51 mo</td>
<td>15% of patients</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>2005</td>
<td>Crespo-Leiro⁵⁷</td>
<td>Clinical graft failure with no ACR on biopsy</td>
<td>445</td>
<td>2–72 mo</td>
<td>2.6% of patients</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>2005</td>
<td>Hammond⁵⁸</td>
<td>Immunopathology*</td>
<td>681</td>
<td>1 day to 2 y</td>
<td>24% of 3170 biopsies</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>2005</td>
<td>Poelzl⁵⁹</td>
<td>Immunopathology*</td>
<td>17</td>
<td>…</td>
<td>53% of patients</td>
<td>CAV</td>
<td>AMR predictive of CAV (3 of 5 patients vs 1 of 12 patients without AMR)</td>
</tr>
<tr>
<td>2005</td>
<td>Rodriguez⁶⁰</td>
<td>Immunopathology*</td>
<td>165</td>
<td>60–163 mo</td>
<td>10% of patients</td>
<td>Survival; graft dysfunction; CAV</td>
<td>No difference in survival. 25% of AMR patients with hemodynamic compromise AMR predictive of CAV</td>
</tr>
<tr>
<td>2005</td>
<td>Smith⁶¹</td>
<td>Immunopathology†</td>
<td>38</td>
<td>&lt;100 d</td>
<td>23% of patients</td>
<td>…</td>
<td>Survival worse with AMR (79% vs 91% at 10 – extrapolated from Kaplan-Meier graphs)</td>
</tr>
<tr>
<td>2006</td>
<td>Kfoury⁶²</td>
<td>Immunopathology*</td>
<td>822</td>
<td>…</td>
<td>16%</td>
<td>Survival</td>
<td>No difference in survival AMR predictive of CAV development</td>
</tr>
<tr>
<td>2007</td>
<td>Almuti⁶³</td>
<td>Histopathology and immunopathology*</td>
<td>859</td>
<td>0–17 y</td>
<td>4.3% of patients</td>
<td>Survival; CAV</td>
<td>(Continued)</td>
</tr>
</tbody>
</table>
In accordance with past ISHLT recommendations, the diagnosis of AMR implies clinical evidence of allograft dysfunction, histological evidence of acute capillary injury, and immunopathologic evidence for antibody-mediated injury as C4d capillary positivity on endomyocardial biopsy samples.6 Studies report that complement activation (C4d deposition) can be present in the absence of organ dysfunction in renal and cardiac allograft recipients, and thus, the term subclinical AMR has been introduced.28,81,82 It is suggested that complement regulatory proteins can successfully terminate the complement cascade after activation in renal and heart allografts teleologically in an attempt to achieve a state of accommodation.83 In some heart transplant recipients, complement deposition without allograft dysfunction represents accommodation. This concept is supported by tissue expression of regulators of complement activation concomitant with C4d deposition.84 In other asymptomatic recipients, it is unclear whether complement deposition reflects accommodation or subclinical AMR. Patients with subclinical AMR are not generally treated, because more data regarding the significance of a positive biopsy in the absence of symptoms are needed. Two studies have shown the probable relationship between subclinical AMR and adverse outcomes. In the first study, published by Wu et al in 2009, 21 heart transplant recipients with subclinical AMR and 22 with treated AMR (LV dysfunction) were compared with a matched control group of 86 contemporaneous patients without AMR. In this study, the diagnosis of AMR was based on demonstration of capillary endothelial cell swelling, interstitial hemorrhage, interstitial edema and neutrophil infiltration, the presence of CD68+ macrophages within capillary cells, and

Table 7. Continued

<table>
<thead>
<tr>
<th>Publication Year</th>
<th>First Author</th>
<th>Definition</th>
<th>n</th>
<th>Time to First AMR</th>
<th>AMR Rate</th>
<th>Outcome</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 2007</td>
<td>Casarez27</td>
<td>Immunopathology*</td>
<td>111</td>
<td>Median 3 mo</td>
<td>14% of patients</td>
<td>Survival; graft dysfunction</td>
<td>No difference in survival</td>
</tr>
<tr>
<td>25 2007</td>
<td>Gonzalez-Stawinski25</td>
<td>Histopathology and immunopathology*</td>
<td>724</td>
<td>2 d to 8 y</td>
<td>8.9% of patients</td>
<td>Survival</td>
<td>No difference in survival</td>
</tr>
<tr>
<td>26 2008</td>
<td>Bayliss34</td>
<td>Histopathology and immunopathology†</td>
<td>76</td>
<td>2 wk to 1 y</td>
<td>15% of 152 biopsies</td>
<td>Survival; CAV</td>
<td>AMR predicted CAV development (OR=4.8)</td>
</tr>
<tr>
<td>27 2008</td>
<td>Cadeiras46</td>
<td>Histopathology, immunopathology, and DSA</td>
<td>40</td>
<td>…</td>
<td>12.5% of patients</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>28 2008</td>
<td>Fedson29</td>
<td>Immunopathology†</td>
<td>34</td>
<td>Mean 120 d</td>
<td>10% of 400 biopsies</td>
<td>Survival; graft dysfunction</td>
<td>No difference in survival</td>
</tr>
<tr>
<td>29 2008</td>
<td>Holt34</td>
<td>Immunopathology† and clinical graft failure</td>
<td>15</td>
<td>…</td>
<td>27% of patients</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>30 2009</td>
<td>Tan47</td>
<td>Immunopathology*</td>
<td>330</td>
<td>15–41 mo</td>
<td>19% of patients</td>
<td>Survival; graft dysfunction</td>
<td>C4d/C3d double-positive cases had highest mortality (8 of 19 patients vs 0 of 17 with only C4d+)</td>
</tr>
<tr>
<td>31 2010</td>
<td>Arias28</td>
<td>Immunopathology†</td>
<td>44</td>
<td>1 mo to 1 y</td>
<td>56% of patients</td>
<td>Survival; graft dysfunction</td>
<td>No difference in survival</td>
</tr>
<tr>
<td>32 2010</td>
<td>Fedrigo24</td>
<td>Immunopathology*</td>
<td>107</td>
<td>Median 9 mo</td>
<td>34% of patients</td>
<td>Survival</td>
<td>Survival worse with AMR (RR=18)</td>
</tr>
<tr>
<td>33 2010</td>
<td>Moseley20</td>
<td>Immunopathology†</td>
<td>43</td>
<td>1 wk to 2 y</td>
<td>41% of 280 biopsies</td>
<td>CAV</td>
<td>C4d/C3d double-positive predictive of CAV (58% vs 46%)</td>
</tr>
<tr>
<td>34 2010</td>
<td>Nath24</td>
<td>Histopathology and immunopathology*</td>
<td>43</td>
<td>&lt;12 mo</td>
<td>19% of patients</td>
<td>AMR/CAV</td>
<td>AMR predictive of CAV (indirectly, AMR correlates with DSA, DSA correlates with CAV)</td>
</tr>
<tr>
<td>35 2010</td>
<td>Nath72</td>
<td>Histopathology and immunopathology*</td>
<td>65</td>
<td>…</td>
<td>15% of patients</td>
<td>AMR/CAV</td>
<td>AMR predictive of CAV (indirectly, AMR correlates with anti-MICA, anti-MICA correlates with CAV)</td>
</tr>
</tbody>
</table>

*Immunopathology screening routinely on all cases.
†Immunopathology screening strategy outside of study not specified.

In accordance with past ISHLT recommendations, the diagnosis of AMR implies clinical evidence of allograft dysfunction, histological evidence of acute capillary injury, and immunopathologic evidence for antibody-mediated injury as C4d capillary positivity on endomyocardial biopsy samples.3 Studies report that complement activation (C4d deposition) can be present in the absence of organ dysfunction in renal and cardiac allograft recipients, and thus, the term subclinical AMR has been introduced.28,81,82 It is suggested that complement regulatory proteins can successfully terminate the complement cascade after activation in renal and heart allografts teleologically in an attempt to achieve a state of accommodation.83 In some heart transplant recipients, complement deposition without allograft dysfunction represents accommodation. This concept is supported by tissue expression of regulators of complement activation concomitant with C4d deposition.84 In other asymptomatic recipients, it is unclear whether complement deposition reflects accommodation or subclinical AMR. Patients with subclinical AMR are not generally treated, because more data regarding the significance of a positive biopsy in the absence of symptoms are needed. Two studies have shown the probable relationship between subclinical AMR and adverse outcomes. In the first study, published by Wu et al in 2009, 21 heart transplant recipients with subclinical AMR and 22 with treated AMR (LV dysfunction) were compared with a matched control group of 86 contemporaneous patients without AMR. In this study, the diagnosis of AMR was based on demonstration of capillary endothelial cell swelling, interstitial hemorrhage, interstitial edema and neutrophil infiltration, the presence of CD68+ macrophages within capillary cells, and

Immunopathology was conducted only on cases with suspicious histopathology or on clinical request. The following sets in parentheses represent multiple manuscripts from the same institutional transplantation program: (1, 2, 5, 18, 22); (10, 11, 24, 29); (28, 33, 34); (15, 23); (3, 26); (12, 13); (14, 20).

ACR indicates acute cellular rejection; AMR, antibody-mediated rejection; CAV, coronary allograft vasculopathy; DSA, donor-specific antibody; MICA, major histocompatibility complex class I chain–related antigen A; OR, odds ratio; PRA, panel reactive antibody; and RR, relative risk.

*Immunopathology screening routinely on all cases.
†Immunopathology screening strategy outside of study not specified.
C4d complement coating the walls of myocardial capillaries. The 5-year actuarial survival rates for the subclinical AMR (86%), treated AMR (68%), and control groups (79%) were not significantly different; however, patients with subclinical AMR were more likely to develop cardiac allograft vasculopathy than the control group and even tended to do worse than patients with treated symptomatic AMR. Khoury et al compared cardiovascular mortality between patients with subclinical AMR, cellular rejection, or mixed cellular rejection and AMR based on the pattern of rejection in the first 3 months after transplantation. AMR was diagnosed in endomyocardial biopsy samples that exhibited complement and immunoglobulin deposits on frozen section, as well as histological changes of endothelial activation and vascular adherence of macrophages, with or without hemorrhage. Patients with subclinical AMR had significantly worse cardiovascular mortality than patients with isolated cellular rejection (21.2% versus 12.6%, P = 0.009) and comparable mortality to those with mixed rejection (21.2% versus 18%, P = 0.9). When these data are considered, subclinical AMR appears to be associated with poor outcome; however, studies evaluating the management of subclinical AMR are yet to be performed.

Assessment of AMR

Imaging Modalities

Endomyocardial biopsy remains the “gold standard” for establishing the diagnosis of AMR. Multiple imaging modalities have been used to detect the rejection of allograft rejection. Most studies have focused on cellular rejection and have not specifically evaluated AMR. Diastolic dysfunction is one of the earliest features of acute rejection. Echocardiogram-derived Doppler tissue imaging as a measure of diastolic function has been reported to be an independent predictor of AMR in pediatric heart transplant recipients, although this was not supported by Sachdeva and colleagues. Although diastolic indices can be abnormal in acute rejection in adult heart transplant recipients, the sensitivity and specificity are low. Novel methods such as nuclear perfusion and cardiac magnetic resonance imaging may have an evolving role, especially in detecting myocardial edema and increased LV mass. One of the most promising cardiac magnetic resonance imaging modalities is T2 quantification. T2 relaxation time is the decay time constant of the magnetic signal after an excitatory pulse. T2 relaxation time lengths in proportion to the degree of myocardial edema. Prolonged T2 relaxation times have been demonstrated in models of myocarditis, myocardial infarction, and acute rejection. Of all cardiac magnetic resonance imaging modalities, T2 quantification appears to correlate best with biopsy-proven rejection; however, further evaluation is needed to determine whether noninvasive modalities can specifically distinguish AMR.

Immunopathology Assay Methods

Immunopathology assays use antibody that is developed to detect a specific antigen of interest. Assay methods may differ based on the method of tissue preparation (fresh frozen versus formalin fixed and paraffin embedded) and the reagents used. When performed on frozen sections, immunofluorescence is commonly used with antibody directed against C4d, C3d, and immunoglobulin heavy chains, as well as fibrin, HLA-DR, and CD55. Immunoperoxidase methods are performed on paraffin sections and are commonly used to stain for C4d and CD68, as well as C3d, CD34, CD31, CD3, and CD20. Each modality has its unique advantages and disadvantages. Immunoperoxidase staining preserves histological features and allows the immunopathologic assessment (ie, complement deposition or macrophage labeling) to be correlated with histopathologic changes (ie, endothelial cell swelling). A separate specimen is not required for histological examination. Immunoperoxidase-stained slides provide a permanent archival slide record that does not fade or dehydrate as quickly as immunofluorescence slides, and pathology laboratories are more often equipped to perform immunoperoxidase staining than immunofluorescence. Immunofluorescence, on the other hand, offers the distinct advantage of rapid result turnaround time and more favorable signal-to-noise ratio. Residual frozen tissue after immunofluorescence can also be a valuable resource for viral polymerase chain reaction or other molecular tests.

When one compares immunofluorescence to immunoperoxidase staining, diagnostic equivalence is an important factor. Comparison of C4d detection by immunofluorescence and immunoperoxidase on the same biopsy samples suggests very good to near perfect agreement (Figure 1). The prognostic value of C4d detection by immunoperoxidase was reported by Fedrigo et al., who found that C4d capillary staining was present in 36 of 107 patients (34%) and AMR (diagnosis based on 2005 ISHLT criteria) was present in 8 (7%). Over a median follow-up of 2.7 years, C4d-positive patients experienced higher mortality than C4d-negative patients, regardless of graft function. Conversely, C4d positivity alone detected by immunofluorescence is of unclear significance. The detection of C3d with C4d by immunofluorescence significantly enhances the diagnostic utility of testing for complement activation products. Tan et al demonstrated that in heart transplant recipients with diffuse linear capillary deposition of C4d, the presence of C3d deposition was associated with allograft dysfunction in 84% and with the presence of DSA in 95% of the patients. In contrast, only 1 patient with C4d staining alone (C3d negative) had concurrent allograft dysfunction. It is possible that detection of C4d by immunofluorescence is not a sufficiently specific indicator of AMR. Transient and nonimmunologic causes of C4d deposition, such as viral infections and reperfusion injury, have been reported and are not always associated with poor prognosis. To summarize, optimal staining for AMR by immunofluorescence should include both C3d and C4d, whereas demonstration of C4d by immunoperoxidase is sufficient. Interpretation by an experienced cardiac pathologist is preferred to ensure consistency. Several questions remain: (1) Is antibody-mediated allograft damage the result of a single episode, or does it represent a dynamic process that begins early after transplantation and continues at varying levels thereafter? (2) Do results of serial biopsies correlate better with outcomes? (3) What is the significance of late-appearing DSA? (4) What is the appropriate method and timing to monitor patients?
Donor-Specific Antibodies

There is growing interest in the monitoring of anti-HLA antibodies after heart transplantation. Solid-phase assays have allowed advancement beyond the mere detection of the presence of anti-HLA antibody to being able to quantify, even at low levels, anti-HLA antibody and allow the tracking of DSA strength over time. The presence of DSA is associated with poor graft survival, rejection, and CAV and is a marker of alloimmune activation. Although the appearance of DSA and a change in strength may occur with AMR, the optimal monitoring and subsequently management for this has not yet been elucidated. Studies evaluating specific monitoring protocols in heart transplantation will enable the clinical application of anti-HLA titers. The ISHLT has recommended routine monitoring of DSA as a marker of the alloimmune environment of the heart transplant recipient. As of yet, it is not part of the diagnosis of AMR but may serve as a supporting feature. As an example of its role in screening for AMR, DSA could be monitored after major decreases in immunosuppression to determine whether weaning of immunosuppression has resulted in increased alloimmune activation.

Current Management Strategies for AMR

Overview

The presentation of AMR may vary from mild heart failure to cardiogenic shock. General principles regarding the treatment of AMR involve halting the immune-mediated injury and the provision of supportive therapy for heart failure. In advanced cases, management of hemodynamic compromise may require inotropic and pressor support, as well as systemic anti-coagulation to reduce intravascular thrombosis. Stabilization of the hemodynamically compromised patient may extend to the use of mechanical circulatory support as described at the end of this section. Finally, retransplantation may be the only option for selected transplant recipients who do not respond to therapy. The ISHLT has recommended routine monitoring of DSA as a marker of the alloimmune environment of the heart transplant recipient. As of yet, it is not part of the diagnosis of AMR but may serve as a supporting feature. As an example of its role in screening for AMR, DSA could be monitored after major decreases in immunosuppression to determine whether weaning of immunosuppression has resulted in increased alloimmune activation.

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Overview

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The guiding principles for the management of AMR comprise removing circulating alloantibodies, reducing production of additional alloantibodies, and suppressing T-cell and B-cell responses. To date, there have been no large randomized trials evaluating therapies for AMR in heart transplant recipients. Guidelines for treatment have recently been suggested by the ISHLT, but currently there are no level I recommendations, and all recommendations are based on consensus (level of evidence C).106 The following section describes currently available treatment of AMR. Given the extremely limited resource of heart transplantation, the heart transplant community has typically taken its lead from experience in renal transplantation, adapting therapies that were originally designed to treat hematologic diseases, malignancies, and autoimmune disorders.81,107–113 Broadly speaking, the underlying mechanisms for these therapies are based on the following: (1) Suppression of the T-cell response (eg, corticosteroids, mycophenolate mofetil (MMF), anti-lymphocyte antibodies, photopheresis, or total lymphoid irradiation), (2) elimination of circulating antibodies (eg, plasmapheresis), (3) inhibition of residual antibodies (eg, intravenous immunoglobulins), (4) suppression or depletion of B cells (eg, corticosteroids, rituximab, or splenectomy), (5) suppression or depletion of plasma cells (eg, bortezomib), and (6) inhibition of complement (eg, eculizumab, intravenous gamma globulin [IVIg]). The discussion that follows examines the supporting evidence of efficacy for commonly used and novel therapies for AMR. Table 8 provides detailed information regarding mechanism of action, adverse effects, and commonly used doses of each therapy, whereas Table 9 provides a summary of the immune component affected by each therapy. A list of protocols used by several experienced centers is provided in the Appendix (Table A1).

Corticosteroids

First used in clinical renal transplantation in 1963, steroids remain a standard component of induction, maintenance, and antirejection therapy in heart transplantation.114,115 Corticosteroid pulse and taper regimens have been well-accepted therapy for ACR for decades and thus have been adapted for basic therapy for AMR.116 Corticosteroids are potent immunosuppressive and anti-inflammatory agents that affect the number, distribution, and function of all types of leukocytes and endothelial cells.117 The major effect on lymphocytes is mediated via the transcription factors nuclear factor-κB and activator protein-1.118,119 The numerous case reports and clinical studies describing new and old therapies for AMR all involve corticosteroids as part of the treatment regimen; therefore, one cannot draw conclusions regarding the clinical benefit of corticosteroids. Nevertheless, corticosteroids remain part of most AMR treatment regimens.

Intravenous Gamma Globulin

IVIg, a product of predominantly pooled IgG antibodies extracted from the plasma of thousands of donors, has been demonstrated to successfully reduce antibodies in sensitized transplant candidates. IVIg contains anti-idiotypic antibodies that inhibit HLA-specific alloantibodies in vitro and in vivo. Polyclonal preparations of human immunoglobulin have activity against class I and II HLA molecules, costimulatory molecules, cytokines and cytokine receptors, and T-cell receptors.120 The main immune effects of IVIg can likely be accounted for by blockade of Fc-γ receptors, inhibition of the complement system, neutralization of autoantibodies and cytokines, and downregulation of the B-cell receptor.121–123 IVIg is commonly used to treat the highly sensitized patient awaiting cardiac transplantation; however, it has never been systematically studied after transplantation to prophylactically reduce the incidence of AMR.124 When used for the management of AMR or in desensitization protocols, IVIg is frequently used in combination with other immune therapies. Very few data have been reported that support the use of IVIg for the treatment of acute AMR. In a study of 7 kidney and 3 heart transplant recipients with AMR, IVIg administered in combination with cyclophosphamide or tacrolimus was reported to reverse rejection in all patients within 2 to 5 days of infusion. The incidence of recurrence, however, was high.53 Similar findings have been reported in other small series.38
<table>
<thead>
<tr>
<th>Therapeutic Modality</th>
<th>Mechanism of Action</th>
<th>Adverse Events</th>
<th>Dose</th>
<th>Frequency</th>
<th>Duration</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Corticosteroids</strong></td>
<td>Upregulation of anti-inflammatory gene expression, mediated by activated protein-1 and NF-kB</td>
<td>Dyslipidemia, hyperglycemia, osteoporosis, leukocytosis</td>
<td>Oral: 1–3 mg/kg IV: 250–1000 mg</td>
<td>Daily</td>
<td>3 d</td>
<td>$</td>
</tr>
<tr>
<td><strong>IVIg</strong></td>
<td>Blockade of Fc-y receptor</td>
<td>Headache</td>
<td>1–2 g/kg in 2–4 divided doses</td>
<td>1–3 Times weekly</td>
<td>Variable</td>
<td>$$$$$</td>
</tr>
<tr>
<td>Complement inhibition</td>
<td></td>
<td>Chills</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Downregulates B-cell receptor</td>
<td></td>
<td>Rigors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutralizes circulating antibody and cytokines</td>
<td></td>
<td>Fever</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myalgia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tissue plasma exchange (plasmapheresis)</strong></td>
<td>Nonselective removal of circulating alloantibody, proteins, cytokines; IAP removes only immunoglobulins</td>
<td>Rebound antibodies</td>
<td>1–7 Sessions/wk, 1–4 weekly cycles</td>
<td>1–7 Sessions</td>
<td>Variable</td>
<td>$$$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bleeding diathesis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Hypotension</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Allergic reaction</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Transmission of blood-borne pathogens</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Photopheresis</strong></td>
<td>Upregulation of costimulatory molecules, downregulation of T cells, immunoregulation via T-regulatory cells</td>
<td>Vascular access complications, skin erythema, pruritus, nausea, rare drug-induced lupus or scleroderma-like syndrome</td>
<td>Oral: 0.6 mg/kg (target level ≥50 ng/mL 2 h after ingestion) or 25 mg/m²</td>
<td>Variable</td>
<td>Up to 6 mo</td>
<td>$$$$$</td>
</tr>
<tr>
<td><strong>Monomurab (OKT3)</strong></td>
<td>Binds CD3 antigen on T lymphocytes, leading to early activation of T cells, cytokine release, and blockade of T-cell function</td>
<td>Cytokine release syndrome, antimurine antibodies, anaphylaxis, hypersensitivity, infection (viral, fungal, bacterial), increased incidence of PTLD and lymphoma</td>
<td>5 mg (or 2.5 mg for ≤30 kg) IV</td>
<td>Once daily</td>
<td>10–14 d</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Rabbit ATG (thymoglobulin)</strong></td>
<td>Decrease circulating T lymphocytes</td>
<td>GI (diarrhea, abdominal pain, nausea, vomiting) myalgias, headache, dizziness, dyspnea, hypertension peripheral edema, tachyarrhythmia, hypokalemia, leukopenia, thrombocytopenia, fever, infection (viral, fungal, bacterial), increased incidence of PTLD and lymphoma</td>
<td>0.75–1.5 mg/kg IV</td>
<td>Daily</td>
<td>5–7 d</td>
<td>$ to $$$</td>
</tr>
<tr>
<td><strong>Equine anti-thymocyte globulin (ATGAM)</strong></td>
<td>Binds CD3 antigen on T lymphocytes</td>
<td>GI (diarrhea, abdominal pain, nausea, vomiting) myalgias, headache, dizziness, dyspnea, hypertension peripheral edema, tachyarrhythmia, hypokalemia, leukopenia, thrombocytopenia, fever, infection (viral, fungal, bacterial), increased incidence of PTLD and lymphoma</td>
<td>10–15 mg·kg⁻¹·d⁻¹ IV</td>
<td>Daily</td>
<td>5–7 d</td>
<td>$$$ to $$$$$</td>
</tr>
<tr>
<td><strong>Rituximab</strong></td>
<td>B lymphocyte depletion</td>
<td>Fever, chills, nausea, headache, myalgia, rash</td>
<td>375 mg/m² weekly</td>
<td>Weekly</td>
<td>1–4 wk</td>
<td>$ to $$$$$</td>
</tr>
<tr>
<td>Antibody depletion</td>
<td>Complement-induced cell lysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induction of apoptosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Alemtuzumab</strong></td>
<td>Monoclonal antibody against CD52 on surface of all B and T lymphocytes, absent on platelets, hematopoetic stem cells, and lymphoid progenitors; transient depletion of mature lymphocytes without myeloablation</td>
<td>Lymphopenia, pancytopenia, infusion-related effects*; increased CMV viremia, coagulopathy, cardiac toxicity (heart failure, arrhythmias) in patients receiving chemotherapy</td>
<td>Prophylaxis: 30 mg IV on day 1 and 4, 20 mg IV postoperatively within 24 h of transplantation, or 30 mg IV</td>
<td>Once</td>
<td>…</td>
<td>$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treatment: 20 mg IV or 30 mg SQ</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
Table 8. Continued

<table>
<thead>
<tr>
<th>Therapeutic Modality</th>
<th>Mechanism of Action</th>
<th>Adverse Events</th>
<th>Dose</th>
<th>Frequency</th>
<th>Duration</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bortezomib</td>
<td>Reversible 26S proteasome inhibitor present on plasma cells</td>
<td>Diarrhea, sensory neuropathy, fatigue, thrombocytopenia, conjunctivitis</td>
<td>1.3–1.5 mg/m²</td>
<td>Daily</td>
<td>4 doses</td>
<td>$</td>
</tr>
<tr>
<td>Eculizumab</td>
<td>Terminal complement (C5) inhibitor</td>
<td>Flu-like symptoms, sore throat, headache, back pain, nausea, neutropenia, extravascular hemolysis, increased risk of meningococcal infection</td>
<td>600–900 mg IV</td>
<td>Every 7–14 d</td>
<td>Until desired response</td>
<td>$$$$$</td>
</tr>
<tr>
<td>Mycophenolate</td>
<td>Reversible inosine monophosphatase dehydrogenase blocker that inhibits de novo guanosine synthesis, inhibits T- and B-cell proliferation</td>
<td>Diarrhea, esophagitis, increased lymphomas and other malignancies, leukopenia, anemia, thrombocytopenia, increased CMV infection, hypogammaglobulinemia</td>
<td>Adult: 1.5 g PO or IV BID Pediatric: 600 mg/m² to maximum 2 g or 10 mL</td>
<td>Twice daily</td>
<td>Indefinite</td>
<td>$</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Nitrogen mustard alkylating antineoplastic agent, targets B cells, inhibition of cholinesterase activity</td>
<td>Bone marrow toxicity, hemorrhagic cystitis, gonadal failure, malignancies, nausea, diarrhea, vomiting, stomatitis, mucositis, anorexia, pancytopenia, cardiotoxicity, interstitial pneumonitis, pulmonary fibrosis, hepatotoxicity, toxic epidermal necrolysis, teratogenicity</td>
<td>0.5–1 g/m² every 3 wk for 4–6 mo (with rituximab and plasmapheresis)</td>
<td>Monthly</td>
<td>2 mo</td>
<td>$</td>
</tr>
<tr>
<td>Total lymphoid irradiation</td>
<td>Suppression of activated T cells and the interleukin-2 pathway, eliminates circulating T and B cells</td>
<td>Bone marrow suppression, pancytopenia, nausea, PTLD, myelodysplasia, opportunistic infection, PTLD</td>
<td>80 cGy (1 cGy=1 rad) twice weekly over 5 wk to achieve a cumulative dose of 8 Gy</td>
<td>Twice weekly</td>
<td>Over 5 wk (cumulative dose of 8 Gy)</td>
<td>…</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>Diminishes antibody production by debulking plasma cells and activated B cells</td>
<td>Increased risk of sepsis and/or death (kidney transplant)</td>
<td>No results in heart transplantation</td>
<td>…</td>
<td>…</td>
<td>$$$$</td>
</tr>
</tbody>
</table>

Calculations of weight-based doses assume a weight of 70 kg or a body surface area of 2 m². IV Ig was based on 2 mg/kg for 2 doses; therapeutic plasma exchange and plasmapheresis, 5 days; and equine and rabbit ATG, 5-day course. Rituximab was based on 1 to 4 doses; alemtuzumab, 30 mg given twice; bortezomib, 4 doses; and eculizumab, 4 doses.

AMR indicates antibody-mediated rejection; ATG, anti-thymocyte globulin; BID, twice per day; CMV, cytomegalovirus; FFP, fresh-frozen plasma; GI, gastrointestinal; IAP, immunoadsorption with membrane plasmapheresis; IV, intravenous; IV Ig, intravenous gammaglobulin; NA, not applicable; NF, nuclear factor-xB; PO, by mouth; PTLD, posttransplantation lymphoproliferative disorder; SQ, subcutaneous; $, <$1000; $$, $1000 to $5000; $$$, >$5000 to $10,000; $$$$$, >$10,000 to $20,000; and $$$$$, >$20,000.

*Infusion-related effects: nausea, vomiting, diarrhea, headache, fatigue, dyspnea, rash, pruritus, fever, rigors, bronchospasms, and hypotension.

Plasmapheresis

Plasmapheresis mechanically removes circulating antibodies. Although there are variant modalities, including therapeutic plasma exchange, double-filtration plasmapheresis, and immunoadsorption plasmapheresis, because of lower cost and ease of use, plasma exchange has been the favored technique in the United States.116,123 Plasma exchange involves the extracorporeal separation of plasma from the cellular components of blood by use of membrane filtration or centrifugation. Blood is then reconstituted with exogenous albumin and/or fresh-frozen plasma or crystalloid and infused back into the patient.

Plasma exchange nonselectively removes proteins, whereas immunoadsorption plasmapheresis is designed to remove only immunoglobulins and avoids the need for replacement fluids. Immunoadsorption is less efficient in the removal of soluble cytokines and is also less widely available. The majority of reported experiences related to decreasing soluble alloantibody levels in transplantation are via plasma exchange.116,124 Although plasma exchange has become standard therapy for the management of AMR, there have been no randomized trials of plasma exchange for this indication.123 A number of small case series have reported on its use for the treatment of de novo and refractory AMR in cardiac transplantation.11,84,125

Additionally, there are several case reports describing the use of plasma exchange for decreasing alloantibody levels in highly sensitized patients awaiting heart transplantation.126,127 Plasma exchange has also been reported to facilitate transplantation across a positive crossmatch by decreasing the likelihood of subsequent allograft rejection.127,128

There is little support for the use of plasma exchange as monotherapy for the management of AMR. Treatment with plasma exchange for the reduction of alloantibody levels has always been reported in combination with other immunomodulatory therapies. Wang et al125 reported on the use of plasma exchange in 12 patients with biopsy-proven AMR. Biopsy specimens in patients with allograft dysfunction and hemodynamic compromise underwent immunofluorescent staining for IgG, IgM, C1q, C3d, C4d, and HLA-DR. Patients with AMR were treated with plasma exchange and intravenous corticosteroids (methylprednisolone 1 g/d for 3 days). Plasma exchange was performed to exchange twice the blood volume with fresh-frozen plasma daily for 5 days. Baseline immunosuppression was frequently changed with the substitution of tacrolimus for cyclosporine. Although survival in this group of hemodynamically unstable patients was decreased compared with other heart recipients, 1- and 5-year survival were 75% and 51%, respectively. Therapy with plasma
exchange and rescue immunosuppression appeared to be effective for this critically ill group of patients with acute AMR.

In a similar retrospective series of 445 patients undergoing heart transplantation, 12 were suspected of having acute AMR based on allograft dysfunction and hemodynamic compromise in the absence of cellular rejection on endomyocardial biopsy or CAV on coronary angiography. Diagnosis was confirmed if patients responded to therapy. Patients were treated for 3 days with methylprednisolone boluses (1 g/d for 3 days) and daily plasma exchange for a minimum of 7 days. Baseline immunosuppression was modified with the substitution of MMF or cyclophosphamide for azathioprine and tacrolimus for cyclosporine. Eleven of the 12 patients had recovery of allograft function with good long-term survival.

Grauhan et al compared the results of patients treated for AMR between 2 eras: 1986 to 1990 and 1991 to 1999. AMR was diagnosed based on hemodynamic instability in the presence of allograft dysfunction and the absence of significant cellular rejection (ISHLT grade 2 or less) or myocardial ischemia. Patients in the earlier period were managed with methylprednisolone boluses (1 g/d for 3 days) and daily plasma exchange for a minimum of 7 days. Baseline immunosuppression was modified with the substitution of MMF or cyclophosphamide for azathioprine and tacrolimus for cyclosporine. Eleven of the 12 patients had recovery of allograft function with good long-term survival.

The mechanism by which extracorporeal photopheresis works to prevent or treat transplant rejection has not been well defined, although irradiated T-helper cell-induced immunosuppression is the main theory. Unlike immunosuppressive drugs, extracorporeal photopheresis does not cause generalized immunosuppression. Although there are no published data to date of the efficacy of photopheresis in the management of AMR, photopheresis has been successfully used to treat recurrent rejection and ACR, with and without hemodynamic compromise. Kirklin et al studied 36 adult heart transplant recipients with recurrent/recalcitrant rejection (n=20), rejection with hemodynamic compromise (n=12), and anti-DSA (n=4) who received at least 3 months of photopheresis. Rejection free from hemodynamic compromise and death because of rejection were significantly reduced with photopheresis. It is likely, although not defined in this study, that some of the patients with hemodynamic compromise had AMR. Subsequent clinical trials supported the prophylactic use of photopheresis in posttransplantation patients. In a pilot study of 23 heart transplant recipients, Barr et al used photopheresis prophylactically beginning...
within 1 month of transplantation and for 2 successive days every 4 weeks during the first year, every 6 weeks during the first half of the second year, and every 8 weeks during the last half of the second year. PRA levels were significantly reduced during the first 6 postoperative months and coronary artery intimal thickness was significantly reduced in the photopheresis group at 1 year (0.23 versus 0.49 mm, P<0.04) and 2 years (0.28 versus 0.46 mm, P<0.02) compared with the control group. Given its relatively favorable risk profile and benefits in ACR, photopheresis might warrant further investigation in the treatment of refractory AMR. Although photopheresis may be costly, the Centers for Medicare & Medicaid Services will cover photopheresis as an indication for acute cardiac allograft rejection that is refractory to standard drug treatment (NCD [national coverage determination] 110.4).

Anti-Lymphocyte Globulins
Anti-lymphocyte globulins are antibodies directed at the T-cell lymphocyte or thymocyte. Two categories exist: Monoclonal antibodies, namely, muromonab-CD3 (brand name OKT3), and polyclonal antibodies, collectively known as lymphocyte immune globulin or ATG, of which there are 2 formulations widely available (rabbit anti-thymocyte globulin [RATG, brand name Thymoglobulin] and equine anti-thymocyte globulin [brand name ATGAM]). Supporting data for this class of drugs have been for active treatment of or prophylaxis for ACR; thus, these drugs have been adapted for AMR treatment. Anti-lymphocyte globulins have been used for AMR, but there are few data to support their role. The primary data supporting their use is for rescue therapy in severe ACR and as induction therapy. In sensitized patients, these drugs, particularly RATG, have been used as induction therapy in conjunction with IVIg, plasmapheresis, and rituximab. There have been direct comparisons between muromonab-CD3 and equine ATG and muromonab-CD3 and RATG, which have demonstrated similar efficacy in minimizing ACR, although polyclonal ATG probably compares more favorably to muromonab-CD3 with fewer side effects. Ironically, there has been concern for muromonab-CD3 sensitization and AMR in heart transplant recipients receiving muromonab-CD3 prophylaxis. Thus, because of the similar efficacy of the various antibody therapies and the higher risk profile associated with muromonab-CD3, polyclonal ATG regimens are commonly preferred. Muromonab-CD3 is no longer being marketed.

Prophylactic equine ATG has been associated with C4d and horse IgG deposition in capillaries of heart transplant recipients in the absence of clinical AMR. Prophylactic ATG (RATG and equine ATG) has been reported in 1 study to induce acute and hyperacute AMR in nonsensitized patients with renal allografts. Although there have been successful cases in which AMR has been treated successfully with ATG in combination with other immunosuppressive therapy, this class of drugs requires testing as part of a randomized trial in AMR.

Monoclonal Antibodies
Rituximab
Rituximab is a genetically engineered, chimeric murine-human monoclonal antibody against the pan B-cell marker CD20. CD20 phosphorylation is involved in regulation of B-cell development and differentiation. Rituximab was first introduced for the treatment of B-cell non-Hodgkin lymphoma but is also used for the treatment of autoimmune disease such as myasthenia gravis and posttransplantation lymphoproliferative disorder. When used for the management of AMR or as desensitization therapy, rituximab has largely been used in combination with other therapies, which hampers the evaluation of its efficacy as a solo agent. The most convincing evidence for the use of rituximab in heart transplantation comes from a series of patients with AMR treated with rituximab as monotherapy. AMR was defined as the presence of diffuse staining for IgG or complement on the vascular endothelium without evidence of cellular rejection in the setting of LV dysfunction (25% reduction in LV ejection fraction). Garrett et al treated 8 patients with 375 mg/m² per week for 4 weeks. All patients had normalization of LV function with complete histological resolution of AMR. There were no reports of significant infection or drug-related complications.

There are multiple case reports of the successful use of rituximab as salvage therapy for refractory AMR after failure of combination therapy with cytolytic antibodies, corticosteroids, plasma exchange, and cyclophosphamide. Similarly, rituximab decreased PRA in a sensitized patient who failed to respond to combination therapy with IVIg, MMF, and plasma exchange. Rituximab has been used successfully as combination therapy with plasma exchange and IVIg in other cardiac desensitization protocols.

Alemtuzumab (Campath)
Alemtuzumab is a humanized, lymphocyte-depleting rat monoclonal antibody that binds to CD52, a 12-amino acid glycosylphosphatidylinositol-anchored glycoprotein that is present on the surface of essentially all B and T lymphocytes, a majority of monocytes, macrophages, and natural killer cells. CD52 is absent on most granulocytes, erythrocytes, platelets, hematopoietic stem cells, and lymphoid progenitors, thus facilitating effective but transient depletion of mature lymphocytes without myeloablation. A proportion of bone marrow cells, including some CD34+ cells, express variable levels of CD52. The US Food and Drug Administration approved Campath-1H or alemtuzumab in 1999 for the treatment of lymphoma and leukemia, particularly chronic lymphocytic leukemia. It has also been used for multiple sclerosis and more recently as induction therapy in solid organ transplantation, with the most experience in abdominal and lung transplantation.

Alemtuzumab has been evaluated in desensitization protocols; however, there are few reports of its use for the treatment of rejection. Most are case reports describing alemtuzumab as salvage or induction therapy. In a small group of lung transplant recipients with rejection refractory to steroids and ATG, alemtuzumab appeared to be effective in reversing rejection and bronchiolitis obliterans syndrome. Treatment of rejection appears similarly effective in kidney transplant recipients but may be associated with increased early infection-related deaths. Woodside and Lick described successful reversal of cardiac rejection in a patient with recurrent and refractory hemodynamically significant rejection. To date, there are no studies evaluating alemtuzumab as treatment for rejection in heart transplantation.
Bortezomib
A potential limitation of available therapies for AMR is the lack of direct effect on the major alloantibody-producing cell, the mature plasma cell. Bortezomib is a reversible 26S proteasome inhibitor approved for the treatment of multiple myeloma that depletes plasma cell levels in addition to exhibiting other pleiotropic immunomodulatory effects.182–184

Early experience in renal transplantation has demonstrated variable efficacy of bortezomib in the treatment of AMR and desensitization.185–187 There are no published reports of the use of bortezomib alone for refractory AMR in cardiac transplantation. Evidence for bortezomib in preliminary reports has been confounded by the use of other therapies.110,187,188

Bortezomib has been used as a rescue therapy in combination with other immunotherapy for refractory AMR.110,188 Six kidney transplant recipients with refractory mixed AMR and ACR were treated by Every et al110 with a single cycle of bortezomib: 1.3 to 1.5 mg/m² × 4 doses over 11 days (days 1, 4, 8, and 11). AMR was diagnosed on renal biopsy samples using the updated 2005 Banff criteria and C4d immunostaining.189 All patients demonstrated reduction in DSA levels and improvement in allograft function after treatment with bortezomib.

Similar to other reports, bortezomib was given in combination with a number of immunomodulatory therapies, including cytolytic antibodies, corticosteroids, rituximab, plasma exchange, and IVlg, thereby preventing the conclusion that bortezomib alone was responsible for the reported findings. Perry et al188 demonstrated similar findings in 2 kidney transplant patients treated with a combination of bortezomib, plasma exchange, and IVlg. Both patients developed a transient decrease in bone marrow plasma cell levels and alterations in alloantibody specificities. Total IgG levels were unchanged.

In contrast, Sberro-Soussan et al186 found that a single cycle of bortezomib (1.3 mg/m² × 4 doses) as monotherapy did not decrease DSA levels in sensitized kidney transplant patients. The success of bortezomib in the treatment of AMR in other reports could thus be attributable to adjunctive therapies such as plasmapheresis and rituximab for active treatment of AMR.28 Blockade of anti-HLA and non-HLA antibodies, in heart transplant recipients treated with MMF,197,198 Weigel et al199 demonstrated that heart transplant recipients treated with MMF had a significant reduction in circulating alloantibody levels. Several studies have demonstrated a decrease in posttransplantation antibody levels, including both anti-HLA and non-HLA antibodies, in heart transplant recipients treated with MMF.195,196 MMF has not been systematically studied in the prevention or treatment of AMR.

Eculizumab (Complement Inhibitor)

Complement activation likely plays a major role in the pathogenic effects of circulating alloantibodies and is the predominant effector pathway of AMR.28 Blockade of antibody-mediated complement activation is an attractive target for therapies aimed at treating and preventing AMR. Clinical studies evaluating the role of complement inhibitors in the management of AMR have yet to be completed.

Eculizumab is a C5 inhibitor that is approved for use in paroxysmal nocturnal hemoglobinuria, which is a hematologic disorder characterized by clonal expansion of red blood cells that lack the ability to inhibit complement-mediated hemolysis. Eculizumab blocks serum hemolytic activity.190,191

Currently, complement inhibitors are not indicated for the treatment of AMR. The majority of evidence supporting a potential role for complement inhibitors in the management of AMR comes from preclinical studies. In a murine model of presensitized kidney transplant, Rother et al192 found that despite the continued presence of DSA, there was complete inhibition of intragraft terminal complement deposition and inhibition of AMR and ACR. Similar findings have been found in rat and murine models of cardiac transplantation in which treatment with C5 monoclonal antibody blocked terminal complement activity, preventing both AMR and ACR and allowing graft survival.190,193,194

A recent article by Stegall et al195 has shown that the use of eculizumab (given prophylactically immediate after transplantation) in highly sensitized kidney transplant patients reduced the incidence of antibody-mediated rejection. A single case has been reported in the literature of the use of eculizumab in the management of AMR in a kidney transplant recipient. AMR was biopsy proven with complement deposition and C4d positive immunofluorescence staining, capillary leukocyte margination, and arteriolar thrombi with graft dysfunction. The patient did not respond to initial attempts at therapy with IVlg and plasma exchange. Eculizumab was used as rescue therapy with rituximab and ongoing IVlg and plasma exchange. With this combination therapy, there was complete resolution of AMR on biopsy and normalization of allograft function. Before complete resolution of AMR, C5b-9 complement staining was reduced after treatment with eculizumab. Resolution of AMR cannot definitively be attributed to eculizumab therapy alone given the polytherapy used to treat this case of refractory rejection. Although promising, the use of eculizumab is limited because of cost and lack of coverage for heart transplant rejection by most insurers.

Mycophenolate Mofetil
Clinical and experimental data document the effect of MMF on reducing B-cell proliferation and antibody production, thus suggesting a role for MMF in the prevention and treatment of AMR.196 MMF has not been systematically studied in the prevention or treatment of AMR. The rationale for the use of MMF in the management of AMR is based on its ability to reduce circulating alloantibody levels. Several studies have demonstrated a decrease in posttransplantation antibody levels, including both anti-HLA and non-HLA antibodies, in heart transplant recipients treated with MMF.197,198

Cyclophosphamide (Cytoxan)
Cyclophosphamide is a nitrogen mustard alkylating antineoplastic agent that targets the B cells. It has been primarily used and approved (by the US Food and Drug Administration) for various cancers (particularly leukemias, lymphomas, breast cancer, multiple myeloma, ovarian cancer, neuroblastoma, and retinoblastoma). It is not currently approved by the US Food and Drug Administration for solid organ transplantation, but it has been used over the past few decades for refractory rejection and to reduce antibody levels in the highly sensitized pretransplantation patient.

There are modest data for the use of cyclophosphamide to treat AMR. It is generally used in combination with other therapies such as plasmapheresis and rituximab for active treatment of AMR.161 In the early era of heart transplantation, cyclophosphamide was used as a substitute for azathioprine for maintenance
immunosuppression in patients with AMR; this has since fallen out of favor because of potential serious and long-term side effects and its failure to prevent recurrence of AMR.205,206,209–215 Almuti et al33 reported a retrospective series of 37 patients with AMR defined by clinical symptoms that included graft failure and biopsy-proven rejection (immunohistochemical or immunofluorescence evidence including C4d). An initial episode of AMR was treated with 5 or 6 cycles of plasmapheresis over the course of 10 to 14 days and intravenous cyclophosphamide (0.5 to 1 g/m² every 3 weeks for 4 to 6 months). Repeat episodes of AMR were again treated with 5 or 6 cycles of plasmapheresis over the course of 10 to 14 days followed by an infusion of intravenous cyclophosphamide and immunoglobulin (250 mg/kg daily for 4 days, repeated every 3 weeks for 4–6 months). After 2002, this protocol was modified to include rituximab (375 mg/m²) weekly after the completion of plasmapheresis for a total of 4 infusions. One-year survival was 78%.

Total Lymphoid Irradiation
Total lymphoid irradiation (TLI) was originally developed as a nonmyeloablative treatment for Hodgkin disease, involving targeted irradiation to lymphoid tissue with sparing of solid organs. The use of TLI in clinical transplantation was first described as adjunctive treatment to induce prolonged renal allograft survival in humans 50 years ago.200 Its first report to induce cardiac allograft tolerance after transplantation was in rats in 1978 and later in humans.201,202 TLI became more commonly used for recurrent or refractory cardiac allograft rejection in the late 1980s and early 1990s.203–205 Since then, TLI has been rarely used for heart transplant recipients because of potential long-term radiation-related side effects, such as myelodysplasia and leukemia, the reports of which are somewhat conflicting.206,207

TLI (cumulative dose of 8 Gy) has been used in patients with biopsy-negative cardiac allograft dysfunction (“nonspecific graft dysfunction” and biventricular failure) with success, although AMR was not specifically diagnosed.208,209 Most studies in heart transplantation have used TLI as adjunctive or alternative therapy in patients with ACR.205,206,209–215 Less CAV has been reported in patients treated with TLI than in appropriately matched control subjects.216 The University of Alabama at Birmingham has one of the largest experiences with TLI. They reported a series of 73 adult recipients treated with TLI during the first 6 months after transplantation for recurrent rejection (71%), rejection with hemodynamic compromise (25%), and rejection with vasculitis (4%). TLI resulted in a decrease in rejection, but 7 patients developed myelodyplasia or acute myelogenous leukemia.217 Considering these concerns, the use of TLI for the treatment of AMR is not recommended.2

Splenectomy
The spleen houses the antibody-producing plasma cells and/or B cells that contribute to AMR. Plasma cells are the primary source of antibody production. Plasma cells do not express CD20 antigen and thus are not susceptible to ablation with the CD-20 antibody rituximab.157,218 Splenectomy may have the effect of debulking plasma cells and activated B cells, thereby diminishing antibody production to a level that can be managed with other immunomodulatory therapies.218 These cells, however, also exist outside of the spleen in the lymph nodes and bone marrow, and the role of splenectomy in the management of AMR remains unclear.

The rationale for splenectomy as a strategy for AMR derives historically from its use in adjunctive desensitization protocols in kidney transplantation.219–223 There are multiple reports of splenectomy as successful rescue therapy in a small number of patients with refractory AMR in kidney transplantation.218,221,224 There are no reports, to the best of our knowledge, of splenectomy in heart transplant recipients with AMR. Reports of death and infectious complications after splenectomy in kidney transplant recipients are conflicting.218,222 Splenectomy should likely be reserved as a last resort for patients with refractory AMR and who have exhausted all other therapies if used at all.

Combination Therapies and Therapies Under Consideration
Most of the above therapies are typically used in combination, either simultaneously or sequentially depending on the patient’s response.116 As detailed in the various clinical studies, incident AMR frequently has been treated with a corticosteroid pulse and taper, antilymphocyte globulin, plasmapheresis, IVIg, rituximab, and modification of the baseline maintenance immunosuppressive regimen. Refractory AMR is often treated with the newer agents, such as alemtuzumab, bortezomib, and eculizumab; photopheresis may also be considered. Older modalities, such as TLI and splenectomy, have been abandoned for the most part because of unclear efficacy and potentially more important adverse side effects.

Newer therapies on the horizon for AMR are derived from experiences in hematology and oncology. Carfilzomib is a recent irreversible proteasome inhibitor being investigated in hematologic malignancies but not yet studied in patients with AMR. Newer monoclonal antibodies directed at B cells include belimumab and atacicept, which both affect the B-lymphocyte stimulator, also known as B-cell activation factor of the tumor necrosis factor family.

Mechanical Circulatory Support in AMR With Hemodynamic Compromise
The use of mechanical circulatory support after transplantation has been reported in heart transplant recipients with primary graft failure (graft failure in the immediate postoperative period after heart transplantation) or with conditions unrelated to transplantation.226–235 The majority of published data describe the relatively successful use of extracorporeal membrane oxygenation (ECMO) in cases of primary graft failure. Although 30-day mortality is reduced in those who receive ECMO, in those who survive 30 days, long-term survival appears to be comparable to those who do not have primary graft failure.226,235 Successful use of other nondurable devices, including devices from Levitronix and Abiomed, has also been reported for primary graft failure.226,229 There are few reports specifically addressing use in the setting of AMR.336–339 Kittleson and colleagues239 described the use of ECMO in 32 patients with heart transplant rejection. Twenty-five of these patients had presumed rejection, and 15 had biopsy-proven rejection. Of these, 9 had grade 2R or 3R cellular rejection, and 6 had AMR. After support with ECMO, 5 of the 6 with AMR
improved. Preemptive use of ECMO, compared with salvage therapy, conferred a significant survival benefit. At 1 year, 26% of the preemptively treated patients were alive compared with only 7% of the salvage patients. It is not clear whether survival after mechanical circulatory support is different in those with acute rejection than in those with primary graft failure. In general, patients with primary graft failure and acute rejection with hemodynamic compromise constitute a high-risk group despite therapy. Thus, ECMO or other temporary mechanical support can be considered as salvage therapy in those with AMR and hemodynamic compromise refractory to medical therapy.

**Maintenance Immunosuppression**

In addition to treating AMR with cytotoxic or antibody-directed therapy, efforts should be made to optimize the background regimen. Although the literature remains sparse in this regard, there are data establishing MMF and sirolimus as potent B-cell inhibitors. Both sirolimus and MMF profoundly inhibit B-cell proliferation and immunoglobulin production in a dose-dependent manner, compared with calcineurin inhibitors; both drugs induce significant B-cell apoptosis. Likewise, methotrexate has been reported as successful in treating refractory rejection but is not commonly used currently.

On the basis of the limited data and the aforementioned principles underlying antibody-mediated rejection, the following steps can be taken: Patients with AMR who are taking azathioprine can be switched to MMF, and patients taking MMF can be switched to sirolimus. Although cyclophosphamide has been used, the significant toxicity and side effect profile renders it an unfavorable choice. Patients receiving cyclosporine should be given tacrolimus instead. The addition of corticosteroids or an increase in the dose of MMF may also be beneficial. In patients who remain resistant, escalation of therapy, as listed in the previous section on management, can be considered.

**When to Treat AMR**

The emergence of techniques to more elegantly define the alloimmune response and alloimmune injury has stimulated a renewed interest in heart transplant pathology and will provide the backdrop for improved diagnosis and subsequently improved treatment of AMR in the near future. In the short term, however, the ability to detect sophisticated immune markers and the availability of multiple diagnostic modalities has heightened the complexity of decision making. Perhaps the most significant result of the ISHLT Consensus Conference on AMR was the agreement on a pathological diagnosis of AMR (pAMR) based on a scale consisting of immunopathologic and histological features. The diagnosis of AMR using this scale would be supplemented by clinical descriptors and the presence of anti-HLA antibodies. When these are taken into account, up to 16 categories can be defined. Putting these into context for practical application is challenging because of the small amount of evidence using the new pathological criteria. The category of pAMR3, although uncommon, is associated with profound allograft dysfunction and poor outcomes. Therefore, this category of AMR should be treated regardless of clinical descriptors. Less clear is the management of milder forms of pAMR, such as pAMR 1 and 2 (with or without clinical evidence) or partial evidence of AMR; that is, pAMR1-h and pAMR1-I in the absence of clinical features. Furthermore, the benefit of treating subclinical AMR has not been established. It has been suggested that AMR is a clinical-pathological continuum that begins with a latent humoral response of circulating antibodies and then progresses through a silent phase of circulating antibodies with C4d deposition without clinical or histological alterations, to a subclinical stage, to symptomatic AMR. (Figure 3). On the basis of the limited evidence, few recommendations can be made at this point: pAMR3, because of its association with poor outcome, should be treated irrespective of clinical evidence. The decision to treat other categories of pathological AMR (pAMR0–2) should take into account the clinical evidence of rejection, such as symptoms or evidence of graft dysfunction, as well as supporting immunologic evidence, for example, increasing or new DSAs. The type of treatment has not been clarified, that is, one could consider full treatment for AMR using therapies such as IVlg, plasmapheresis, and rituximab in cases that are associated with clinical compromise or graft dysfunction. In cases of pathological AMR and no clinical evidence, perhaps optimization of baseline therapy with periodic evaluation is reasonable; this is not yet known. Finally, pAMR0 suggests the absence of AMR; however, cases of biopsy-negative allograft dysfunction are still reported and considered by many to represent AMR. In these cases, evaluation of other causes of LV dysfunction such as CAV with microvascular disease or determination of non-HLA antibodies might be warranted. A recent survey of transplant cardiologists who are members of ISHLT suggested that most would consider treatment of AMR in the presence of graft dysfunction regardless of the pathology finding. pAMR2 in the absence of graft dysfunction if DSAs are present, and pAMR3 regardless of the clinical scenario. We have proposed a similar strategy based on the different combinations of clinical scenarios and immunopathology findings (Figure 4).

**Consequences of AMR**

AMR is associated with allograft failure, decreased survival, increased incidence of CAV, and overall poor
Colvin et al  AMR in Cardiac Transplantation  1625

**Pathologic Assessment**

<table>
<thead>
<tr>
<th>Pathologic AMR Grade</th>
<th>Graft Dysfunction</th>
<th>DSAs</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−</td>
<td>−</td>
<td>No AMR, no treatment needed</td>
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<tr>
<td>−</td>
<td>+</td>
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<td></td>
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<td>increased surveillance</td>
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<td>Optimization of maintenance immunosuppression</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Consideration of AMR therapy</td>
</tr>
</tbody>
</table>

**pAMR0**

|                      | +                 | −    | Management unknown |
|                      | +                 | +    | Consider other causes of graft dysfunction (e.g. CAV) |
|                      |                   |      | Optimization of maintenance immunosuppression |
|                      |                   |      | Increased surveillance |
|                      |                   |      | Consider treatment for AMR; especially if complement-binding antibodies are present |

**pAMR1**

|                      | −                 | −    | Management unknown |
|                      | −                 | +    | Consider optimization of maintenance immunosuppression |
|                      | −                 | +    | Consider increased surveillance |
|                      | −                 | +    | Consider treatment for AMR; especially if complement-binding antibodies are present |

**pAMR2**

|                      | +                 | −    | Management unknown |
|                      | +                 | +    | Consider treatment for AMR; especially in the setting of complement-binding antibodies + increased surveillance and optimization of maintenance immunosuppression |

**pAMR3**

|                      | −                 | +    | Treat for AMR + increased surveillance and optimization of maintenance immunosuppression |
|                      |                   |      | Options: |
|                      |                   |      | Antibody removal/suppression: plasmapheresis/IVIg |
|                      |                   |      | Cell depletions: rituximab, thymoglobulin |
|                      |                   |      | Plasma cell depletions: bortezomib |
|                      |                   |      | Complement inhibition: ecocizumab |

*There is a lack of evidence supporting routine management of the gray zone, and management should be based on clinical presentation, however options are provided for consideration.*

**Figure 4.** Categories of antibody-mediated rejection (AMR) and possible therapies based on pathological, clinical, and immunologic presentation. CAV indicates cardiac allograft vasculopathy; DSA, donor-specific antibody; IVIg, intravenous gamma globulin; and pAMR, pathological antibody-mediated rejection category.

In a series reported by Michaels et al., AMR was associated with hemodynamic compromise in 47% of patients. One year after transplantation, patients with AMR had a greater incidence of CAV than control subjects (15% versus 5%, *P* = 0.09). After 5 years, 86% of patients with AMR had CAV compared with 22% of control subjects (*P* < 0.001). The incidence of CAV or death in the patients with AMR was twice that of the control subjects (*P* = 0.01).

**Clinical AMR in Pediatric Heart Transplant Recipients**

Similar to the adult heart transplant experience, the clinical hallmarks of AMR have been described in children since the earliest days of pediatric heart transplantation. The recognition of AMR as a cause of graft dysfunction and graft vasculopathy in the pediatric heart transplant population is also increasing as the criteria for the diagnosis of AMR have been refined and surveillance for AMR has been more widely adopted. In a large series (n=1217) reported by the Pediatric Heart Transplant Study Group, 15% of patients (<18 years of age) presented with severe acute ventricular dysfunction within 5 years of transplantation, and 30% of these had no or only mild evidence of cellular rejection (ISHLT grade 0 or 1R). Survival was only 50% at 2 years, and 26% of the deaths were attributed to graft vasculopathy, graft failure, or lethal arrhythmias. Pathological evaluation for AMR was not systematically performed in the study group; however, a positive crossmatch or the pretransplantation detection of circulating anti-HLA antibodies was not associated with these events. Although less common than in adult recipients, graft vasculopathy is an important cause of late graft failure in pediatric heart transplant recipients, with an incidence of 3% per year. Studies have not demonstrated that graft vasculopathy is associated with AMR in children, although analyses have been limited by small sample sizes and the lack of pathological evaluation for the manifestations of AMR. The importance of circulating anti-HLA antibodies in the development of AMR is well established, although the exact nature of the relationship between AMR and DSAs, nonspecific antibodies, and the timing of antibody formation is still unclear. A recent analysis of the United Network for Organ Sharing database has identified risk factors for the presence of PRA in children with end-stage heart failure, which potentially puts them at increased risk for AMR after transplantation. Overall, an elevated pretransplantation PRA (>10%) was detected in 11% of the pediatric population. The
proportion of patients with an elevated PRA increased from 7% in the earliest era (1987–1992) to 15% in the most recent era (1999–2004), perhaps reflecting a change in the methodology of detecting PRA or an increase in the proportion of patients at risk. Older age, the diagnosis of congenital heart disease, and a longer wait time for an organ were associated with an elevated PRA. There are few reports of de novo antibody production in children after heart transplantation. Yxdas et al reported an association of the posttransplantation formation of class II antibodies with graft vasculopathy, rejection, and survival.

**Risks of Anti-HLA Antibodies in the Pediatric Recipient**

The following conditions have been shown to increase anti-HLA antibodies in children: Congenital heart disease status with surgical repair, ventricular assist device support, and possibly retransplantation. An elevated PRA has been reported to occur in 12% to 19% of pediatric patients who underwent transplantation for end-stage congenital heart disease. The proportion of children with congenital heart disease who receive transplants is highest in the infant population (63%) but remains substantial in the adolescent age group (24%). These patients have often undergone multiple prior congenital heart surgeries and received blood transfusions that predispose them to antibody formation. Similar to the adult population, several small studies in children have reported elevated PRA levels in 30% to 90% of patients receiving long-term ventricular assist device support. Within 3 months of implantation, the mean class I and class II antibody levels reached 92±15% and 70±26%, respectively, and the high levels of circulating antibodies persisted for at least 12 months. The presence of allosensitizing material likely contributes to the higher incidence of anti-HLA antibodies detected in children with congenital heart disease.

There are conflicting reports regarding the incidence of an elevated PRA in children undergoing retransplantation. In an analysis from the multicenter Pediatric Heart Transplant Study, Chin et al reported no significant differences in the mean PRA level between patients undergoing primary and retransplantation (1.5±4.9% versus 8.5±2.7%, respectively, P=NS); however, the proportion of patients with an elevated PRA >10% was not presented for either group. Small single-center series have reported a 40% incidence of elevated PRA in patients undergoing retransplantation. The development of DSA after transplantation is associated with the development of AMR, and the presence of anti-HLA class I complement-fixing and cytotoxic antibodies before and after transplantation negatively impacts survival.

**Treatment and Outcomes of AMR in Children**

The lack of a standard definition of AMR, the heterogeneity of the pediatric transplant population, and the small number of patients who undergo transplantation at each center have thus far precluded large observational or randomized studies of AMR in pediatric heart transplantation. Diagnostic criteria are the same as those in adult heart transplantation and rely on histopathologic and immunopathologic changes. In addition, the appearance of DSAs is associated with AMR, and the pretransplantation and posttransplantation presence of class I anti-HLA antibodies that are complement-fixing and cytotoxic negatively impacts long-term survival. Therapies for the prevention and treatment of AMR in children are empiric and run the gamut of those described in adult patients. Therapies to remove circulating anti-HLA antibodies have included pretransplantation intravenous immunoglobulin or cyclophosphamide, intraoperative exchange transfusion or plasmapheresis, and postoperative immunoglobulin, plasmapheresis, or cyclophosphamide. Therapies described for hemodynamic compromise with suspected AMR include methylprednisolone, cytolytic agents, plasmapheresis, rituximab, and ECMO support.

A recent retrospective analysis by Casarez et al found that a pathological diagnosis of AMR was present in 32 of 103 pediatric heart transplant recipients within the first year of transplant. Congenital heart disease was found to be significantly associated with AMR. In the patients with AMR, there was a trend toward a higher proportion of patients with a positive flow-cytometry crossmatch and a trend toward worse graft survival. The establishment of a diagnosis of AMR is key to the management of graft dysfunction in pediatric recipients, particularly in patients with prior congenital heart disease who have a high level of circulating anti-HLA antibodies. No significant differences in the pathogenesis or manifestations of AMR have been demonstrated between pediatric and adult recipients. Thus, the ISHLT consensus criteria for the diagnosis of AMR are believed to be directly applicable to the pediatric heart transplant population.

**Future Directions in the Pediatric Population**

Heart transplantation continues to be the best treatment for end-stage heart failure in children. Presensitization and the development of de novo antibodies limit the long-term success of this therapy. The National Institute of Allergy and Infectious Diseases of the National Institutes of Health, in collaboration with the National Heart, Lung, and Blood Institute, is sponsoring an observational, multcenter, prospective cohort study of alloantibodies in pediatric heart transplantation. This study is designed to determine the clinical outcomes of sensitized pediatric heart transplant recipients with a positive donor-specific cytotoxicity crossmatch and to compare them with outcomes in nonsensitized heart transplant recipients (Allo-antibodies in Pediatric Heart Transplantation; ClinicalTrials.gov identifier: NCT01005316). The results of this study will quantify the risks and benefits of a protocol for the avoidance of a prospective crossmatch in presensitized pediatric patients and provide important outcome data for the design of intervention trials.

**Summary: Strategies for Standardizing Diagnosis and Management**

Standardization of management strategies for AMR is lacking in large part because of the absence of clinical trials that prospectively evaluate therapies for AMR. The definition of AMR is also in flux as more sensitive diagnostic modalities become available. Although the currently available gene expression
profile test for rejection (Allomap) is useful in the prediction of ACR, there is evidence that the fraction of circulating cell-free donor DNA may be useful in detecting both ACR and AMR. Experience in kidney transplantation, rheumatologic diseases, and oncology have provided a new armamentarium of potentially promising therapeutics that are more specific and have favorable side effect profiles. Clinical trials should include the evaluation of these newer therapies, desensitization protocols that include rituximab and other monoclonal antibodies and their effects on the development of AMR, and AMR protocols that incorporate combination therapies such as thymoglobulin and rituximab, rituximab and bortezomib, and rituximab and eculizumab. The natural history of subclinical AMR based on new criteria warrants further evaluation, as does the treatment of subclinical AMR. The development of widely accepted diagnostic and management protocols has the potential to improve the consistency of heart transplant outcomes across the country. In the following section, we have provided recommendations based on prior consensus conferences. Data are sparse, and many of the suggestions bear further study; however, these recommendations are intended to summarize what is currently accepted practice or consensus and to provide a framework for standardization and the development of research initiatives. A secondary goal is to provide the context for currently used therapies to delineate important knowledge gaps and highlight areas where there are clear supporting data.

**Recommendations for the Diagnosis and Management of AMR**

**Diagnosis and Surveillance**

The 2010 Consensus Conference on AMR developed recommendations for the pathological diagnosis and surveillance of AMR that were published in 2011 and subsequently as the ISHLT Working Formulation for the Standardization of Nomenclature in the Pathologic Diagnosis of AMR in Heart Transplant in 2013 (Tables 3, 4, 5, and 10). Specific recommendations were provided regarding the pathological criteria for AMR. It was also recommended that centers develop surveillance protocols based on their specific clinical and research indications. Protocols should include a minimum of immunostaining of 2 specimens during the first month and afterward, according to the circulating DSA evaluation schedule. Results of initial staining should guide subsequent immunostaining. For centers that do not perform routine immunofluorescence or immunohistochemistry on routine surveillance biopsies, immunostaining should be guided by histopathologic findings or clinical and serological data. On the basis of the available data and consensus of this writing group, we have not recommended specific intervals for surveillance but have provided a general guideline that encompasses the time during which the risk of AMR is highest (Table 11). ISHLT recommendations are in Table 3.

**Diagnosis Recommendations (Tables 4, 5, and 10)**

1. Diagnosis of AMR should be based on immunopathologic features and supported by clinical descriptors. Histological evidence of AMR, if concomitantly present, should be considered diagnostic (Class I; Level of Evidence C).

2. Immunofluorescent staining for C4d and C3d or immunoperoxidase staining for C4d and CD68 (or C3d) should be performed to evaluate for AMR (Class I; Level of Evidence B).

3. Determination of non-HLA antibodies, such as antiendothelial, anti-vimentin, and anti-MICA and anti-MICB antibodies, may be considered when anti-HLA antibodies are absent and AMR is suspected (Class IIb; Level of Evidence C).

**Surveillance Recommendations (Table 3)**

1. Immunopathologic assessment with staining for C4d and/or C3d should be performed during the first 90 days after transplantation or when AMR is suspected (Class I; Level of Evidence C).

---

**Table 10. Antibody Panels and Clinical Indicators for Pathological Diagnosis of Cardiac AMR**

<table>
<thead>
<tr>
<th>Immunopathologic Indicators</th>
<th>Required</th>
<th>Supporting Features</th>
<th>Recommended Clinical Indicators</th>
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<tbody>
<tr>
<td>Histology</td>
<td>Capillary endothelial changes and intracapillary macrophages</td>
<td>…</td>
<td>Clinical heart failure based on symptoms and signs of heart failure, Hemodynamics: PCWP ≥20 mm Hg and CI ≥2.0 L·min⁻¹·m⁻²</td>
</tr>
<tr>
<td>Frozen section: Immunofluorescence</td>
<td>C4d, C3d (2–3+ intensity), Anti-HLA-DR</td>
<td>C1q (2–3+), Ig, fibrin, IgG, and IgM HLA for cellular integrity</td>
<td>Requirement for inotropes or mechanical support during hospital stay, Systolic dysfunction: EF &lt;50% or ≥25% decrease from baseline</td>
</tr>
<tr>
<td>Paraffin section: Immunohistochemistry</td>
<td>C4d (2–3+ intensity), CD68</td>
<td>Pan-T-cell CD3, pan-B-cell CD20, complement C3d, endothelial cell CD31 or CD34, complement regulatory proteins</td>
<td>Restrictive physiology characterized by the following: EF &gt;50%, E/A &gt;2, IVRT &lt;60 ms, and DT &lt;150 ms and/or RAP &gt;12, PCWP &gt;25 mm Hg, and CI &gt;2.0 L·min⁻¹·m⁻²</td>
</tr>
<tr>
<td>Other</td>
<td>…</td>
<td>Presence of circulating donor-specific HLA antibodies, especially those that fix complement</td>
<td>…</td>
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</tbody>
</table>

AMR indicates antibody-mediated rejection; CI, cardiac index; DT, deceleration time; E/A, ratio of early to late mitral inflow velocities; EF, ejection fraction; HLA, human leukocyte antigen; IVRT, isovolumic relaxation time; PCWP, pulmonary capillary wedge pressure; and RAP, right atrial pressure.

Modified from Berry et al* with permission from the International Society for Heart and Lung Transplantation. Copyright © 2013, International Society for Heart and Lung Transplantation.
### Table 11. Applying Classification of Recommendations and Level of Evidence

<table>
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<th>LEVEL A</th>
<th>Multiple populations evaluated</th>
<th>Data derived from multiple randomized clinical trials or meta-analyses</th>
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<tr>
<td>Recommendation in favor of treatment or procedure being useful/effective</td>
<td>Some conflicting evidence from multiple randomized trials or meta-analyses</td>
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<tr>
<td>Recommendation’s usefulness/efficacy less well established</td>
<td>Greater conflicting evidence from multiple randomized trials or meta-analyses</td>
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<tr>
<td>Recommendation that procedure or treatment is not useful/effective and may be harmful</td>
<td>Sufficient evidence from multiple randomized trials or meta-analyses</td>
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<table>
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<th>Data derived from a single randomized trial or nonrandomized studies</th>
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<td>Recommendation that procedure or treatment is useful/effective</td>
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<tr>
<td>Recommendation in favor of treatment or procedure being useful/effective</td>
<td>Some conflicting evidence from single randomized trial or nonrandomized studies</td>
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<tr>
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<td>Greater conflicting evidence from single randomized trial or nonrandomized studies</td>
<td></td>
</tr>
<tr>
<td>Recommendation that procedure or treatment is not useful/effective and may be harmful</td>
<td>Evidence from single randomized trial or nonrandomized studies</td>
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</table>

<table>
<thead>
<tr>
<th>LEVEL C</th>
<th>Very limited populations evaluated</th>
<th>Only consensus opinion of experts, case studies, or standard of care</th>
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<tr>
<td>Recommendation in favor of treatment or procedure being useful/effective</td>
<td>Only diverging expert opinion, case studies, or standard of care</td>
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<tr>
<td>Recommendation’s usefulness/efficacy less well established</td>
<td>Only diverging expert opinion, case studies, or standard of care</td>
<td></td>
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<tr>
<td>Recommendation that procedure or treatment is not useful/effective and may be harmful</td>
<td>Only expert opinion, case studies, or standard of care</td>
<td></td>
</tr>
</tbody>
</table>

### SIZE OF TREATMENT EFFECT

- **Class I**: Benefit >> Risk
  - Procedure/Treatment SHOULD be performed/administered
- **Class IIa**: Benefit >> Risk
  - Additional studies with focused objectives needed
  - IT IS REASONABLE to perform procedure/administer treatment
- **Class IIb**: Benefit ≥ Risk
  - Additional studies with broad objectives needed; additional registry data would be helpful
  - Procedure/Treatment MAY BE CONSIDERED
- **Class III**: No Benefit or Class III Harm
  - Procedure/Treatment

### ESTIMATE OF CERTAINTY (PRECISION) OF TREATMENT EFFECT

- **COR III**: No Benefit
- **COR III**: Harm
- **COR II**: Excess Cost/No Benefit or Harmful

### Suggested phrases for writing recommendations

- should be recommended
- is indicated
- is useful/effective/beneficial
- may/might be considered
- may/might be reasonable
- usefulness/effectiveness is unknown/unclear/uncertain or not well established
- is indicated
- is useful/effective/beneficial

### Comparative effectiveness phrases

- treatment/strategy A is recommended/indicated in preference to treatment B
- treatment/strategy A should be chosen over treatment B
- treatment/strategy A is probably recommended/indicated in preference to treatment B
- it is reasonable to choose treatment A over treatment B
- it is not useful/effective
- it is not indicated
- should not be performed/administered/other
- should not be performed/other

### A recommendation with Level of Evidence B or C does not imply that the recommendation is weak. Many important clinical questions addressed in the guidelines do not lend themselves to clinical trials. Although randomized trials are unavailable, there may be a very clear clinical consensus that a particular test or therapy is useful or effective.

### Table 11.

<table>
<thead>
<tr>
<th>CLASS I</th>
<th>Benefit &gt;&gt; Risk</th>
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<tr>
<th>CLASS IIa</th>
<th>Benefit &gt;&gt; Risk</th>
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<tr>
<td>Additional studies with focused objectives needed</td>
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<tr>
<td>IT IS REASONABLE to perform procedure/administer treatment</td>
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<table>
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<tr>
<th>CLASS IIb</th>
<th>Benefit ≥ Risk</th>
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<td>Additional studies with broad objectives needed; additional registry data would be helpful</td>
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<td>Procedure/Treatment MAY BE CONSIDERED</td>
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<th>CLASS III</th>
<th>No Benefit or Class III Harm</th>
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<tbody>
<tr>
<td>Procedure/Treatment</td>
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</table>

### Management

As has been highlighted in this document, published data regarding the treatment of AMR are sparse. The ISHLT Consensus Conference on AMR in 2010 revealed that there is
significant variation in AMR protocols by centers. In a survey of 5 centers, the most common regimens included intravenous methylprednisolone, plasmapheresis, and IVIg with or without ATG. The most experience among the survey participants resides with IVIg, methylprednisolone, and ATG. Recently, there has been increasing use of rituximab and bortezomib. Despite an evolving pathological grading system for AMR, the decision regarding timing of treatment, given the multiple potential combinations of histopathologic findings, clinical manifestations, and antibody quantification, is not yet settled. Severe pathological AMR (pAMR3) is a high-risk finding, and therefore, treatment can be confidently recommended when pAMR3 is present, regardless of supporting clinical evidence. In the appropriate clinical setting, consideration can be given to treating lesser degrees of pAMR. In the absence of clinical symptoms with lesser degrees of pAMR, the presence or strength of DSAs may assist the clinician in determining whether to treat aggressively or to optimize baseline therapy and monitor periodically. Biopsy-negative graft dysfunction is still being reported despite current immunostaining techniques. Further characterization is needed to determine whether this entity truly represents AMR as opposed to advanced vasculopathy, another clinical manifestation along the continuum of AMR. Sample AMR management protocols are provided in the Appendix (Tables A1 and A2).

Management Recommendations

1. It is reasonable for primary therapy for AMR to include IVIg, plasmapheresis, anti-lymphocyte antibodies, and high-dose corticosteroids (Class IIa; Level of Evidence B).
2. It is reasonable for secondary therapy for AMR to include rituximab, bortezomib, and anti-complement antibodies (Class IIa; Level of Evidence C).
3. Consideration may be given to optimizing maintenance therapy by switching from cyclosporine-based immunosuppression to tacrolimus or by increasing the dose of MMF. Substituting MMF with sirolimus may also be considered (Class IIb; Level of Evidence C).
4. Consideration may be given to treatment of rising DSAs in the early posttransplantation period because this may represent a rapid amnestic antibody response. Supporting evidence that the antibodies fix complement may be useful (Class IIb; Level of Evidence C).
5. The significance of isolated appearance or increase in DSA >30 days after transplantation without clinical manifestations or pathological evidence of AMR is unclear, and treatment may be considered at the discretion of the clinician (Class IIb; Level of Evidence C).

Acknowledgment

The authors thank Cynthia DeKay at the Lillehei Heart Institute, University of Minnesota, for developing the graphics.
## Appendix

### Table A1. Examples of AMR Treatment Strategies for Adult Heart Transplant Recipients

<table>
<thead>
<tr>
<th>Center</th>
<th>AMR Treatment</th>
</tr>
</thead>
</table>
| **University of Utah** | Subclinical pAMR1: No treatment; consider slow steroid taper if early after transplantation and still taking steroids  
 pAMR2 without dysfunction or DSA: Pulse steroids only  
 pAMR2 with dysfunction and/or DSA: Steroids, IVlg, plasmapheresis, rituximab/bortezomib  
 pAMR3: Steroids, IVlg, plasmapheresis, rituximab/bortezomib (plus ATG or rATG if hemodynamically compromised) |
| Cedars-Sinai      | Methylprednisolone 500 mg QD × 3  
 rATG  
 Plasmapheresis for hemodynamic compromise  
 IVlg 2 g/kg on days 1 and 30 (first day after completion of rATG)  
 Rituximab 1g (375 mg/m² for smaller patients) on days 7 and 21  
 Refractory patients: Add bortezomib 1.3 mg/m² on days 1, 4, 7, and 10 |
| Cleveland Clinic | Methylprednisolone 1g IV QD × 3  
 Plasmapheresis 4–5 times over a week, then PRN  
 Unresolved: Consider the following:  
 • IVlg 2 g/kg  
 • Rituximab 375 mg/m² up to 4 doses  
 • Bortezomib 1.3 mg/m² IV for 4 doses over 2 wk  
 • Continue plasmapheresis  
 Refractory: Consider photopheresis or TLI |
| Columbia          | Methylprednisolone  
 Plasmapheresis 5–6 cycles over 10–14 d  
 Cyclophosphamide 0.5–1 g/m² every 3 wk for 4–6 mo |
| Stanford          | Low-risk patient: No treatment or augmentation of baseline immunosuppression with follow-up biopsy  
 High-risk patients (positive DSA, allosensitized): IV immune globulin or rituximab infusion  
 Hemodynamic compromise:  
 • Any patient presenting with unexplained graft dysfunction is presumptively treated with methylprednisolone sodium succinate IV 500 mg/d to 1000 mg/d for 3 consecutive doses during evaluation  
 • Plasmapheresis daily or every other day for a minimum of 5 sessions  
 • IVlg immediately after plasmapheresis 2 g/kg divided into 2 doses over 2 consecutive days (not to exceed 140 g) on days 1 and 2 and days 29 and 30; re-dose monthly based on response  
 • Consider ATG 1.5 mg/kg per day for 3 consecutive days with plasmapheresis in severe hemodynamic compromise  
 • Rituximab 1 g/d on days 7 and 22  
 Alternate modalities:  
 • Augmentation of baseline immunosuppression  
 • Change from cyclosporine to tacrolimus and/or addition of cyclophosphamide 1.5 mg/kg per day  
 • Bortezomib 1.3 mg/m² per day on days 1, 4, 8, and 11 |

AMR indicates antibody-mediated rejection; ATG, anti-thymocyte globulin; DSA, donor-specific antibody; IV, intravenous; IVlg, intravenous immune globulin; pAMR, pathological antibody-mediated rejection category; PRN, as needed; QD, once per day; rATG, rabbit anti-thymocyte globulin; and TLI, total lymphoid irradiation.
Table A2. Examples of AMR Treatment Strategies for Pediatric Heart Transplant Recipients

<table>
<thead>
<tr>
<th>Center</th>
<th>Treatment</th>
</tr>
</thead>
</table>
| St. Louis Children’s Hospital | Treatment of hemodynamic compromising rejection consists of 3 strategies: Antibody removal, inhibition of B-cell production, and T cell depletion.  
General: Echocardiogram and ECG on admission, DSA drawn before initiation of therapy, and endomyocardial biopsy for hematoxylin and eosin and C4d and right-sided heart catheterization within 24 h.  
T-cell depletion strategies:  
Thymoglobulin  
1. Administer 1.5 mg/kg IV daily for 5–7 d; first dose is given over at least 6 h, and subsequent doses may be given over 4 h if done through a central line.  
2. Premedications:  
a. Benadryl 1 mg/kg  
b. Tylenol 10–15 mg/kg  
c. Steroids  
Day 1: Methylprednisolone sodium succinate 20 mg/kg IV (maximum dose 1 g)  
Day 2: Methylprednisolone sodium succinate 10 mg/kg IV (maximum dose 500 mg)  
Day 3: Methylprednisolone sodium succinate 5 mg/kg IV (maximum dose 250 mg)  
Then prednisone or prednisolone 1 mg/kg for duration of treatment; taper dose after completion of course of thymoglobulin.  
Inhibition of B-cell production:  
Cyclophosphamide administration:  
1. Administer cyclophosphamide 2 mg/kg IV daily for 2 d and then orally at the same dosing.  
2. Continue course of cyclophosphamide for 28 d, then switch to mycophenolate.  
Antibody removal techniques:  
1. IVIg  
   Premedications:  
a. Benadryl 1 mg/kg  
b. Tylenol 10–15 mg/kg  
2. Plasmapheresis or exchange transfusions will be used to lower circulating antibody levels; both procedures will be a 2× volume exchange:  
a. Any patient <8 kg will undergo an exchange transfusion daily for 5–7 d  
b. Any patient >8 kg will undergo plasmapheresis daily for 5–7 d.  
Alternative therapy:  
Rituximab administration:  
1. Administer 375 mg/m² weekly for 4 wk; if patient’s body surface area is <0.5 m², the dose is 12.5 mg/kg.  
2. Premedications:  
a. Benadryl 1 mg/kg  
b. Tylenol 10–15 mg/kg  
Arkansas Children’s Hospital:  
1. Plasmapheresis on days 1, 4, 7, 10, and 14–16  
2. Bortezomib 1.3 mg/m² on days 1, 4, 7, and 10  
3. Rituximab 375 mg/m² on day 1, 60 min after bortezomib  
4. Methylprednisolone 1.5 mg/kg (maximum 100 mg) on days 1 and 4, 30 min before bortezomib; 0.7 mg/kg on days 7 and 10 before bortezomib.  

AMR indicates antibody-mediated rejection; DSA, donor-specific antibody; IV, intravenous; and IVIg, intravenous immune globulin.
## Disclosures

### Writing Group Disclosures

<table>
<thead>
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<th>Writing Group Member</th>
<th>Employment</th>
<th>Research Grant</th>
<th>Other Research Support</th>
<th>Speakers' Bureau/ Honoraria</th>
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<th>Ownership Interest</th>
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<td>Monica Colvin</td>
<td>University of Minnesota</td>
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*Modest.

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<tr>
<td>Linda Addonizio</td>
<td>Columbia University</td>
<td>NIH (University of Pittsburgh Primary Site PI for CTOTC Alloantibodies in Cardiac Transplantation-Intervention, Outcomes and Mechanisms)*</td>
<td>None</td>
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<td>Allan Gass</td>
<td>Westchester Medical Center</td>
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References


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on behalf of the American Heart Association Heart Failure and Transplantation Committee of the Council on Clinical Cardiology, Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation, Council on Cardiovascular Disease in the Young, Council on Cardiovascular and Stroke Nursing, Council on Cardiovascular Radiology and Intervention, and Council on Cardiovascular Surgery and Anesthesia

Circulation. 2015;131:1608-1639; originally published online April 2, 2015; doi: 10.1161/CIR.0000000000000093

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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