AHA Scientific Statement

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 and Stroke Nursing, 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 myocardial capillary 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.

The American Heart Association makes every effort to avoid any actual or potential conflicts of interest that may arise as a result of an outside relationship or a personal, professional, or business interest of a member of the writing panel. Specifically, all members of the writing group are required to complete and submit a Disclosure Questionnaire showing all such relationships that might be perceived as real or potential conflicts of interest.

This statement was approved by the American Heart Association Science Advisory and Coordinating Committee on June 2, 2014. A copy of the document is available at http://my.americanheart.org/statements by selecting either the "By Topic" link or the "By Publication Date" link. To purchase additional reprints, call 843-216-2533 or e-mail kelle.ramsay@wolterskluwer.com.

The American Heart Association requests that this document be cited as follows: Colvin MM, Cook JL, Chang P, Francis G, Hsu DT, Kiernan MS, Kobashigawa JA, Lindenfeld J, Masri SC, Miller D, O'Connell J, Rodriguez ER, Rosengard B, Self S, White-Williams C, Zeevi A; 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. Antibody-mediated rejection in cardiac transplantation: emerging knowledge in diagnosis and management: a scientific statement from the American Heart Association. *Circulation.* 2015;131:1608–1639.

Expert peer review of AHA Scientific Statements is conducted by the AHA Office of Science Operations. For more on AHA statements and guidelines development, visit http://my.americanheart.org/statements and select the "Policies and Development" link.

(*Circulation*. 2015;131:1608-1639. DOI: 10.1161/CIR.0000000000000093.)

***Circulation* is available at http://circ.ahajournals.org** **DOI: 10.1161/CIR.0000000000000093**

Table 1. Findings in Acute AMR of the Heart

	Required Findings	Optional
1. Clinical evidence of acute graft dysfunction		Recommended in combination with other evidence to support diagnosis of AMR
2. Histological evidence of acute capillary injury (a and b required)	a. Capillary endothelial changes b. Macrophages in capillaries	c. Neutrophils in capillaries (severe) d. Interstitial edema/hemorrhage (severe)
3. Immunopathologic evidence for antibody-mediated injury (a or b or c required)	a. IgG, IgM, and/or IgA + C3d and/or C4d or C1q (2–3+) by IF b. CD 68 for macrophages in capillaries (CD31 or CD34) and/or C4d (2–3+ intensity) in capillaries by paraffin IH c. Fibrin in vessels (severe)	
4. Serological evidence of anti-HLA or anti-donor antibodies		Anti-HLA class I and/or class II or other anti-donor antibody at time of biopsy (supportive of clinical and/or morphological findings)

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

Modified from Reed et al[1] with permission from the International Society for Heart and Lung Transplantation. Copyright © 2006, International Society for Heart and Lung Transplantation.

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 infiltrate[5] (Table 2). By the time of the 2004 ISHLT revision, the immunologic process underlying AMR was better described in the literature.[6] 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 antibodies[1] (Table 1).

Table 2. Historical AMR Definitions

	1990	2004
AMR 0		Negative for AMR
		No histological or immunopathologic features of AMR
AMR 1	Humoral rejection, positive IF, vasculitis or severe edema in the absence of cellular infiltrate	Positive for AMR
		Histological features of AMR
		Positive IF (C3d and/or C4d) or IP (CD68, C4d)

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

Modified from Stewart et al[6] with permission from the International Society for Heart and Lung Transplantation. Copyright © 2005, International Society for Heart and Lung Transplantation.

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

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.[2] 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.[3,4] 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.[7] 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.[8,9] The reported prevalence of late AMR, occurring months to years after transplantation, has increased, most likely because of heightened recognition.[8,10–13] Approximately 50% of heart transplant recipients who develop rejection >7 years after transplantation have evidence of AMR.[14] Finally, AMR has been reported concurrent with cellular rejection in up to 24% of cases.[15] 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.[16–18] Rarely, intravascular thrombi can be seen in these vessels, particularly in severe manifestations.[6]

Table 3. ISHLT Recommendations for Monitoring for AMR

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

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

Modified from Kobashigawa et al[2] with permission from the International Society for Heart and Lung Transplantation. Copyright © 2011, International Society for Heart and Lung Transplantation.

Table 4. Proposed Scoring System for Pathological AMR

Positive Biopsy	Immunohistochemistry	Immunofluorescence
Capillary distribution and intensity	Multifocal/diffuse weak or strong staining of C4d	Multifocal/diffuse weak or strong staining of C4d/C3d
Intravascular CD68 distribution	>10% Focal/multifocal/diffuse intravascular macrophages	...
HLA-DR distribution and intensity	...	Multifocal/diffuse weak or strong staining
Caveats	Focal strong C4d staining is classified as negative but warrants close follow-up	Focal strong C4d staining is classified as negative but warrants close follow-up

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

Modified from Berry et al[3] with permission from the International Society for Heart and Lung Transplantation. Copyright © 2011, International Society for Heart and Lung Transplantation.

Interstitial edema and hemorrhage are also seen in AMR; however, interpretation of these findings is limited by the traumatic nature of procurement by the bioptome, 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.

Table 5. Proposed Nomenclature for Pathological AMR

Category	Description
pAMR 0: Negative for pathological AMR	Both histological and immunopathologic studies are negative
pAMR 1 (H+): Histopathologic AMR alone	Histological findings present and immunopathologic findings negative
pAMR1 (I+): Immunopathologic AMR alone	Histological findings negative and immunopathologic findings positive
pAMR 2: Pathological AMR	Both histological and immunopathologic findings are present
pAMR 3: Severe pathological AMR	Severe AMR with histopathologic findings of interstitial hemorrhage, capillary fragmentation, mixed inflammatory infiltrates, endothelial cell pyknosis and/or karyorrhexis, and marked edema

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

Modified from Berry et al[3] with permission from the International Society for Heart and Lung Transplantation. Copyright © 2011, International Society for Heart and Lung Transplantation.

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.[27,41]

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 6. Immunopathologic Features of AMR

	Interpretation	AMR	Limitations
IgG/IgM	Immunoglobulin binding	+	Easily dissociated
			Short half-life
			Interobserver variability
C3, C1q	Complement activation	+	Short half-life
C3d/C4d	Complement activation	+	Combination more predictive of AMR than C4d alone, long half-life
HLA-DR	Endothelial integrity	+	Staining always present, but "frayed" pattern indicates capillary injury
Fibrin	Thrombotic environment	+	Interstitial extravasation suggests more severe AMR episode
CD55, CD59	Complement inhibitor	–	Long incubation and granular staining pattern
			Difficult to interpret
CD31, CD34, CD68	Intravascular macrophages	+	CD68 confirms macrophage lineage of mononuclear cells
			CD31/34 are endothelial markers which differentiate macrophages from endothelial cells and delineates intravascular localization

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.[39,48]

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.

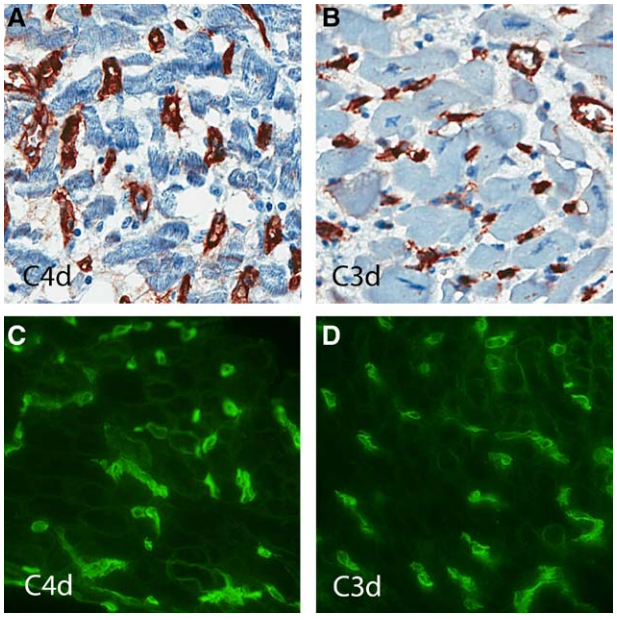

Figure 1. A, C4d capillary staining by immunohistochemistry; **B**, C3d capillary staining by immunohistochemistry; **C**, C4d capillary staining by immunofluorescence; **D**, C3d, by immunofluorescence.

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).[17,39,53] 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 AMR[54] (Table 6; Figure 2).

Posttransplantation Antibodies

The development of anti-HLA antibodies after transplantation has been implicated in allograft injury. Tambur et al[55] 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%.[47] 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

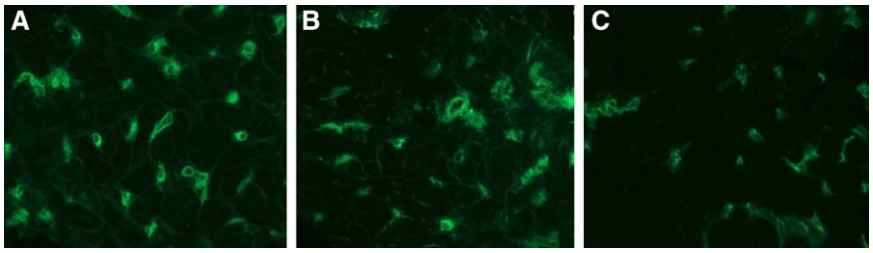

Figure 2. A, C4d capillary staining by immunofluorescence; **B**, C3d capillary staining by immunofluorescence; **C**, CD31 staining by immunofluorescence.

in heart transplantation.[66–68] 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.[62,69] 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.[69] 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[70] tested the association between various anti-cytoskeletal endothelial cell antibodies, including tubulin, vimentin, cytokeratin, and actin, and found that these antibodies were more frequent in heart transplant recipients who experienced rejection and that detection of these antibodies preceded the rejection episodes. Others have demonstrated an association between anti-vimentin antibodies and allograft injury.[71] Both anti-myosin and anti-vimentin antibodies are significantly elevated in patients with AMR compared with those without AMR, preceding the episodes by 3 to 4 months.[72]

Major histocompatibility complex class I chain–related 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[15] 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[8] 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.[8] Finally, Crespo-Leiro and colleagues[11] 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.[38,47] 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.[1,8,79] Women have a disproportionately higher incidence of AMR, comprising up to 50% of the heart transplant recipients with AMR in reported series.[8,38] There is now clear evidence of a relationship between the presence of circulating anti-HLA antibody after transplantation and histological evidence of AMR.[38,74]

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.[8,19,54,75,80] 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 7. Reported Pathological AMR Definitions With Frequency and Outcomes

	Publication Year	First Author	Definition	n	Time to First AMR	AMR Rate	Outcome	Effect
1	1989	Hammond[12]	Immunopathology*	36	1–3 wk	55% of patients	Survival	Survival worse with AMR (57% vs 89% for mixed rejection and 95% for ACR at 3 y)
2	1991	Ensley[73]	Immunopathology*	186	Mean 17 d	28% of patients	Survival	Survival worse with AMR (55% vs 92% at 2 y)
3	1992	Cherry[74]	Immunopathology†	16	15–412 d	46% of 46 biopsies	…	Trend toward correlation between AMR and PRA
4	1992	Foerster[22]	Histopathology and immunopathology*	108	…	1.6% of 1343 biopsies	…	…
5	1993	Hammond[50]	Histopathology and immunopathology*	268	…	28% of patients	Graft survival; CAV	Graft survival worse with AMR (OR=2.1) AMR predictive of CAV (OR=9.5)
6	1993	Loy[20]	Immunopathology†	16	…	0%	…	Note: no cases said to show histopathologic features of AMR
7	1993	Miller[75]	Histopathology and immunopathology†	62	Mean 13 d	11% of patients	Graft dysfunction	More dysfunction with AMR (7% vs 6%)
8	1995	Lones[19]	Histopathology and immunopathology†	81	3–6 wk	52% of patients	Survival; graft dysfunction	71% Survival with AMR 33% Dysfunction with AMR
9	1995	Bonnaud[52]	Immunopathology†	18	6 wk	60% of biopsies (but also 63% of donor heart tissue controls)	…	Positive immunopathology is not rejection
10	1995	Caple[76]	Immunopathology*	135	4 wk to 22 mo	7.4% of patients	Survival; graft dysfunction	No difference in survival 4 of 10 with hemodynamic compromise
11	1995	Ratliff[17]	Immunopathology*	53	4–6 wk	11% of patients	…	…
12	1998	Behr[24]	Immunopathology*	45	< 3 mo	60% of patients	Survival	No difference in survival
13	1999	Behr[25]	Immunopathology*	56	< 3 mo	71% of patients	Survival	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)
14	1999	Baldwin[23]	Histopathology and immunopathology*	24	7–24 d	42% of 33 biopsies	…	…
15	2004	Chantranuwat[27]	Immunopathology*	116	Majority <3 mo	11% of 315 biopsies	…	…
16	2005	Garrett[32]	Immunopathology*	53	6–51 mo	15% of patients	…	…
17	2005	Crespo-Leiro[11]	Clinical graft failure with no ACR on biopsy	445	2–72	2.6% of patients	…	…
18	2005	Hammond[44]	Immunopathology*	681	1 day to 2 y	24% of 3170 biopsies	…	…
19	2005	Poelzl[31]	Immunopathology*	17	…	53% of patients	CAV	AMR predictive of CAV (3 of 5 patients vs 1 of 12 patients without AMR)
20	2005	Rodriguez[38]	Immunopathology*	165	60–163 mo	10% of patients	Survival; graft dysfunction; CAV	No difference in survival. 25% of AMR patients with hemodynamic compromise AMR predictive of CAV
21	2005	Smith[77]	Immunopathology†	38	<100 d	23% of patients	…	…
22	2006	Kfoury[45]	Immunopathology*	822	…	16%	Survival	Survival worse with AMR (79% vs 91% at 10 – extrapolated from Kaplan-Meier graphs)
23	2007	Almuti[33]	Histopathology and immunopathology*	859	0–17 y	4.3% of patients	Survival; CAV	No difference in survival AMR predicted CAV development

(*Continued*)

Table 7. Continued

	Publication Year	First Author	Definition	n	Time to First AMR	AMR Rate	Outcome	Effect
24	2007	Casarez[37]	Immunopathology*	111	Median 3 mo	14% of patients	Survival; graft dysfunction	No difference in survival More graft dysfunction with AMR (47% vs 29%)
25	2007	Gonzalez-Stawinski[35]	Histopathology and immunopathology*	724	2 d to 8 y	8.9% of patients	Survival	No difference in survival
26	2008	Bayliss[36]	Histopathology and immunopathology†	76	2 wk to 1 y	15% of 152 biopsies	Survival; CAV	Survival worse with AMR (71.2 vs 44.6 mo) AMR predicted CAV development (OR=4.8)
27	2008	Cadeiras[40]	Histopathology, immunopathology, and DSA	40	…	12.5% of patients	…	…
28	2008	Fedson[29]	Immunopathology†	34	Mean 120 d	10% of 400 biopsies	Survival; graft dysfunction	No difference in survival No difference in graft dysfunction
29	2008	Holt[78]	Immunopathology† and clinical graft failure	15	…	27% of patients	…	…
30	2009	Tan[47]	Immunopathology*	330	15–41 mo	19% of patients	Survival; graft dysfunction	C4d/C3d double-positive cases had highest mortality (8 of 19 patients vs 0 of 17 with only C4d+) C4d/C3d double-positive cases had more graft dysfunction (16 of 19 vs 1 of 17 with only C4d+)
31	2010	Arias[26]	Immunopathology†	44	1 mo to 1 y	56% of patients	Survival; graft dysfunction	No difference in survival No difference in graft function
32	2010	Fedrigo[28]	Immunopathology*	107	Median 9 mo	34% of patients	Survival	Survival worse with AMR (RR=18)
33	2010	Moseley[30]	Immunopathology†	43	1 wk to 2 y	41% of 280 biopsies	CAV	C4d/C3d double-positive predictive of CAV (58% vs 46%)
34	2010	Nath[34]	Histopathology and immunopathology*	43	<12 mo	19% of patients	AMR/CAV	AMR predictive of CAV (indirectly, AMR correlates with DSA, DSA correlates with CAV)
35	2010	Nath[72]	Histopathology and immunopathology*	65	…	15% of patients	AMR/CAV	AMR predictive of CAV (indirectly, AMR correlates with anti-MICA, anti-MICA correlates with CAV)

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); (7, 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.

Subclinical AMR

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[85] 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

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. Kfoury et al[86] 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.[86] 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 evaluated in the detection 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.[87] 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.[88] Although diastolic indices can be abnormal in acute rejection in adult heart transplant recipients, the sensitivity and specificity are low.[88,89] 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.[90–97] 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 lengthens in proportion to the degree of myocardial edema.[98] Prolonged T2 relaxation times have been demonstrated in models of myocarditis, myocardial infarction, and acute rejection.[99–102] 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.[103]

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[27,77,104] (Figure 1). The prognostic value of C4d detection by immunoperoxidase was reported by Fedrigo et al,[28] 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[47] 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.[47] 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 anticoagulation 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 aggressive measures; however, retransplantation is associated with lower survival than with primary transplantation, particularly if performed for rejection or within the first 6 months of the initial transplantation.[105,106]

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 more 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 8. Summary of Commonly Used Agents for AMR

Therapeutic Modality	Mechanism of Action	Adverse Events	Dose	Frequency	Duration	Cost
Corticosteroids	Upregulation of anti-inflammatory gene expression, mediated by activated protein-1 and NF-κB	Dyslipidemia, hyperglycemia, osteoporosis, leukocytosis	Oral: 1–3 mg/kg IV: 250–1000 mg	Daily	3 d	$
IVIg	Blockade of Fc-q receptor Complement inhibition Downregulates B-cell receptor Neutralizes circulating antibody and cytokines	Headache Chills Rigors Fever Myalgia Volume overload	1–2 g/kg in 2–4 divided doses	1–3 Times weekly	Variable	$$$$
Tissue plasma exchange (plasmapheresis)	Nonselective removal of circulating alloantibody, proteins, cytokines; IAP removes only immunoglobulins	Rebound antibodies Bleeding diathesis Hypotension Allergic reaction Transmission of blood-borne pathogens	1–7 Sessions/wk, 1–4 weekly cycles Exchange 1–2 times the blood volume with FFP or albumin as replacement	1–7 Sessions	Variable	$$$
Photopheresis	Upregulation of costimulatory molecules, downregulation of T cells, immunoregulation via T-regulatory cells	Vascular access complications, skin erythema, pruritus, nausea, rare drug-induced lupus or scleroderma-like syndrome	Oral: 0.6 mg/kg (target level ≥50 ng/mL 2 h after ingestion) or 25 mg/m^2	Variable	Up to 6 mo	$$$$
Monomurab (OKT3)	Binds CD3 antigen on T lymphocytes, leading to early activation of T cells, cytokine release, and blockade of T-cell function	Cytokine release syndrome, anti-murine antibodies, anaphylaxis, hypersensitivity, infection (viral, fungal, bacterial), increased incidence of PTLD and lymphoma	5 mg (or 2.5 mg for ≤30 kg) IV	Once daily	10–14 d	NA
Rabbit ATG (thymoglobulin)	Decrease circulating T lymphocytes	GI (diarrhea, abdominal pain, nausea, vomiting) myalgias, headache, dizziness, dyspnea, hypertension peripheral edema, tachyarrhythmia, hypokalemia, leukopenia, thrombocytopenia, fever, infection (viral, fungal, bacterial), increased incidence of PTLD and lymphoma	0.75–1.5 mg/kg IV	Daily	5–7 d	$$ to $$$
Equine anti-thymocyte globulin (ATGAM)	Binds CD3 antigen on T lymphocytes	GI (diarrhea, abdominal pain, nausea, vomiting) myalgias, headache, dizziness, dyspnea, hypertension peripheral edema, tachyarrhythmia, hypokalemia, leukopenia, thrombocytopenia, fever, infection (viral, fungal, bacterial), increased incidence of PTLD and lymphoma	10–15 mg·kg^{-1}·d^{-1} IV	Daily	5–7 d	$$$ to $$$$
Rituximab	B lymphocyte depletion Antibody depletion Complement-induced cell lysis Induction of apoptosis	Fever, chills, nausea, headache, myalgia, rash	375 mg/m^2 weekly	Weekly	1–4 wk	$ to $$$$
Alemtuzumab	Monoclonal antibody against CD52 on surface of all B and T lymphocytes, absent on platelets, hematopoietic stem cells, and lymphoid progenitors; transient depletion of mature lymphocytes without myeloablation	Lymphopenia, pancytopenia, infusion-related effects*; increased CMV viremia, coagulopathy, cardiac toxicity (heart failure, arrhythmias) in patients receiving chemotherapy	Prophylaxis: 30 mg IV on day 1 and 4, 20 mg IV postoperatively within 24 h of transplantation, or 30 mg IV Treatment: 20 mg IV or 30 mg SQ	Once	…	$$

(*Continued*)

Table 8. Continued

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

Calculations of weight-based doses assume a weight of 70 kg or a body surface area of 2 m^2. IVIg 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; IVIg, intravenous gammaglobulin; NA, not applicable; NF-κB, nuclear factor-κB; 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, bronchospasm, 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 al[125] 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

Table 9. Immunosuppressive Agents and Therapeutic Targets

Therapeutic Modality	T Cells	B Cells	Plasma Cells	Circulating Antibody	Complement Activation	Other
IVIg	X	X		X	X	
Plasmapheresis				X		
Photopheresis	X					Upregulates regulatory T cells
Corticosteroids	X	X				
Cyclophosphamide		X				
Mycophenolate	X	X				
Anti-thymocyte globulin	X	X				
Rituximab		X				
Bortezomib			X			
Eculizumab					X	
Alemtuzumab	X	X				
Total lymphoid irradiation	X	X				
Splenectomy		X				

IVIg indicates intravenous immunoglobulin.

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.[11] 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[84] 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 (500 mg) and cytolytic antibodies (muromonab-CD3, anti-thymocyte globulin [ATG]) for at least 3 days. Patients in the later period were managed by adding plasma exchange (plasma exchange of 5% of body weight) to methylprednisolone and cytolytic antibodies. Cyclophosphamide was substituted for azathioprine for baseline immunosuppression in both periods. Compared with historical control subjects treated with cytolytic antibodies, patients with AMR treated with plasma exchange had improved survival.

Reports of the use of plasma exchange as desensitization therapy in a small number of highly sensitized patients suggest plasma exchange may allow successful transplantation in patients with high alloantibody levels and a positive crossmatch who would be expected to be at high risk of acute AMR.[127–129]

Photopheresis

Extracorporeal psoralen (P) and high-intensity, long-wavelength ultraviolet A irradiation (PUVA) or photopheresis is an apheresis technique in which the patient's leukocyte-rich plasma is treated with a photosensitizing agent (8-methoxypsoralen), exposed to ultraviolet A radiation, and then reinfused into the patient.[130] Peripheral blood lymphocytes exposed to ultraviolet A light in the presence of extracorporally administered liquid 8-methoxypsoralen undergo apoptosis and are subsequently phagocytized by activated monocytes, which are transformed into dendritic cells. The internalized apoptotic cells prevent the upregulation of costimulatory molecules. It also appears that the irradiated leukocytes release more HLA-G molecules, capable of downregulating T cells. Findings of multiple studies suggest that the reinfused photopheresis-treated leukocytes die over a 1- to 2-week period and stimulate an autologous suppressor response, mediated in part by T cells, that targets nonirradiated T cells of similar clones. In addition, these leukocytes enhance the protolerogenic function of dormant dendritic cells (and likely macrophages) that interact with them.

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.[130–134] 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.[2,135–139] Kirklin et al[140] 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.[141,142] In a pilot study of 23 heart transplant recipients, Barr et al[142] 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.[84,143,144] 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.[145] 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.[140,146–149] Ironically, there has been concern for muromonab-CD3 sensitization and AMR in heart transplant recipients receiving muromonab-CD3 prophylaxis.[44] 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.[116,148,150] 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.[151] Prophylactic ATG (RATG and equine ATG) has been reported in 1 study to induce acute and hyperacute AMR in nonsensitized patients with renal allografts.[152] 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.[108,143,153–156]

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.[157]

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[32] treated 8 patients with 375 mg/m^2 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.[32]

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.[43,158–161] Similarly, rituximab decreased PRA in a sensitized patient who failed to respond to combination therapy with IVIg, MMF, and plasma exchange.[162] Rituximab has been used successfully as combination therapy with plasma exchange and IVIg in other cardiac desensitization protocols.[163]

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.[164] A proportion of bone marrow cells, including some CD34$^+$ cells, express variable levels of CD52.[165] 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.[123,163,166–176] 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.[177] Treatment of rejection appears similarly effective in kidney transplant recipients but may be associated with increased early infection-related deaths.[85,109,115,178–180] Woodside and Lick[181] 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 Everly et al[110] with a single cycle of bortezomib: 1.3 to 1.5 mg/m^2 × 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 IVIg, thereby preventing the conclusion that bortezomib alone was responsible for the reported findings. Perry et al[188] demonstrated similar findings in 2 kidney transplant patients treated with a combination of bortezomib, plasma exchange, and IVIg. Both patients developed a transient decrease in bone marrow plasma cells and alterations in alloantibody specificities. Total IgG levels were unchanged.

In contrast, Sberro-Soussan et al[186] found that a single cycle of bortezomib (1.3 mg/m^2 × 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 plasma exchange, rituximab, and/or IVIg or to targeting multiple components of the immune system.[110,187,188]

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 al[192] 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.[180,193,194]

A recent article by Stegall et al[195] 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 IVIg and plasma exchange. Eculizumab was used as rescue therapy with rituximab and ongoing IVIg 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] Weigel et al[199] demonstrated that heart transplant recipients treated with MMF had a significant reduction in B-cell counts compared with healthy control subjects and heart transplant recipients treated with azathioprine. B cells declined at 3 months and were reduced by nearly half at 1 year.

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.[16] Almuti et al[33] 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/m^2 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/m^2) 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 myelodysplasia 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.[218,225] 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.[236–239] Kittleson and colleagues[239] 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.[240–243] Although no longer commonly used, cyclophosphamide suppresses B-cell activation, proliferation, and differentiation and has been reportedly effective in AMR.[8,75,80,244–247] Likewise, methotrexate has been reported as successful in treating refractory rejection but is not commonly used currently.[80,248,249]

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.[106] 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

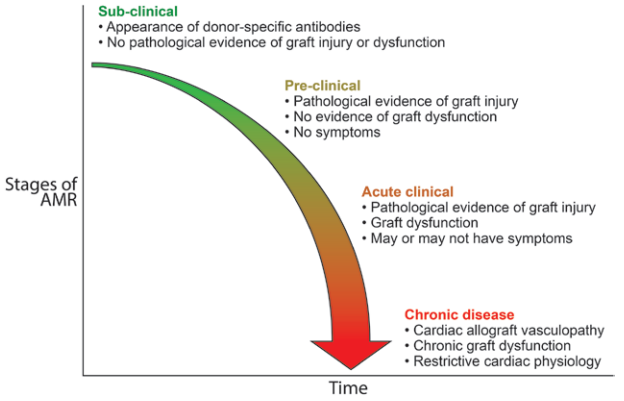

Figure 3. Clinical continuum of antibody-mediated rejection (AMR). Modified from Nair et al[250] with permission from the International Society for Heart and Lung Transplantation. Copyright © 2011, International Society for Heart and Lung Transplantation.

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[2,250] (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 IVIg, 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.[251] 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

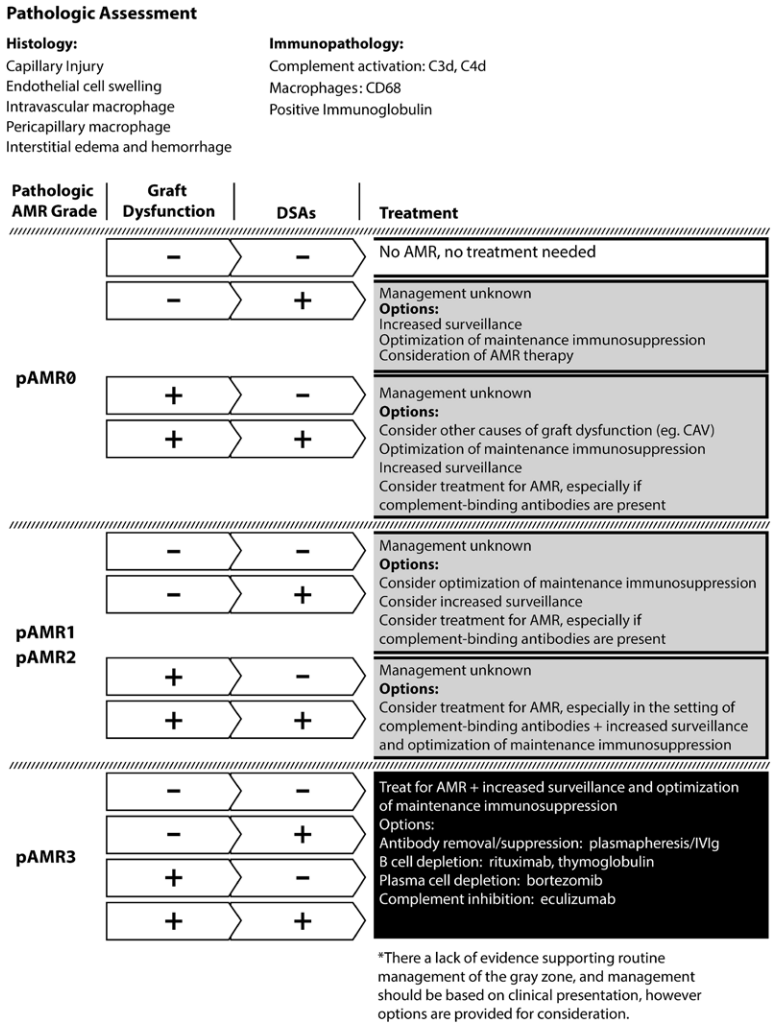

Figure 4. Categories of antibody-mediated rejection (AMR) and possible therapies based on pathological, clinical, and immunologic presentation. CAV indicates cardiac allograft vasculopathy; DSA, donor-specific antibody; IVIg, intravenous gamma globulin; and pAMR, pathological antibody-mediated rejection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

prognosis.[8,12,45,86,252] In a series reported by Michaels et al,[8] 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.[253–257] 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.[78,258,259] 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).[254] 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 systemically 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.[260,261] 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.[37]

Anti-HLA Antibodies in the Pediatric Recipient

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.[262–264] 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.[265] 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. Xydas et al[259] 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.[265,266] 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%).[267] These patients have often undergone multiple prior congenital heart surgeries and received blood transfusions that predispose them to antibody formation.[268] Shaddy and colleagues characterized the formation of anti-HLA class I and class II antibodies after implantation of cryopreserved allograft material for the repair or palliation of congenital heart disease.[261,269,270] 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.

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.[271–275] In contrast, circulatory assist support with ECMO does not appear to result in elevated PRA levels.[265,274]

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[275a] reported no significant differences in the mean PRA level between patients undergoing primary and retransplantation (1.5±4.9% versus 8.5±27.1%, 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.[134,276] 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.[264]

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.[6] 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.[264] 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.[277–279] Therapies described for hemodynamic compromise with suspected AMR include methylprednisolone, cytolytic agents, plasmapheresis, rituximab, and ECMO support.[130,277,278,280]

A recent retrospective analysis by Casarez et al[37] 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, multicenter, 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.[281] 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 anti-endothelial, 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

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

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[4] with permission from the International Society for Heart and Lung Transplantation.

Table 11. Applying Classification of Recommendations and Level of Evidence

ESTIMATE OF CERTAINTY (PRECISION) OF TREATMENT EFFECT	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/Test / Treatment COR III: No benefit — Not Helpful / No Proven Benefit COR III: Harm — Excess Cost w/o Benefit or Harmful / Harmful to Patients
LEVEL A Multiple populations evaluated* Data derived from multiple randomized clinical trials or meta-analyses	■ Recommendation that procedure or treatment is useful/effective ■ Sufficient evidence from multiple randomized trials or meta-analyses	■ Recommendation in favor of treatment or procedure being useful/effective ■ Some conflicting evidence from multiple randomized trials or meta-analyses	■ Recommendation's usefulness/efficacy less well established ■ Greater conflicting evidence from multiple randomized trials or meta-analyses	■ Recommendation that procedure or treatment is not useful/effective and may be harmful ■ Sufficient evidence from multiple randomized trials or meta-analyses
LEVEL B Limited populations evaluated* Data derived from a single randomized trial or nonrandomized studies	■ Recommendation that procedure or treatment is useful/effective ■ Evidence from single randomized trial or nonrandomized studies	■ Recommendation in favor of treatment or procedure being useful/effective ■ Some conflicting evidence from single randomized trial or nonrandomized studies	■ Recommendation's usefulness/efficacy less well established ■ Greater conflicting evidence from single randomized trial or nonrandomized studies	■ Recommendation that procedure or treatment is not useful/effective and may be harmful ■ Evidence from single randomized trial or nonrandomized studies
LEVEL C Very limited populations evaluated* Only consensus opinion of experts, case studies, or standard of care	■ Recommendation that procedure or treatment is useful/effective ■ Only expert opinion, case studies, or standard of care	■ Recommendation in favor of treatment or procedure being useful/effective ■ Only diverging expert opinion, case studies, or standard of care	■ Recommendation's usefulness/efficacy less well established ■ Only diverging expert opinion, case studies, or standard of care	■ Recommendation that procedure or treatment is not useful/effective and may be harmful ■ Only expert opinion, case studies, or standard of care
Suggested phrases for writing recommendations	should is recommended is indicated is useful/effective/beneficial	is reasonable can be useful/effective/beneficial is probably recommended or indicated	may/might be considered may/might be reasonable usefulness/effectiveness is unknown/unclear/uncertain or not well established	COR III: No Benefit: is not recommended; is not indicated; should not be performed/ administered/ other; is not useful/ beneficial/ effective COR III: Harm: potentially harmful; causes harm; associated with excess morbidity/mortality; should not be performed/ administered/ other
Comparative effectiveness phrases†	treatment/strategy A is recommended/indicated in preference to treatment B treatment 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		

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.

*Data available from clinical trials or registries about the usefulness/efficacy in different subpopulations, such as sex, age, history of diabetes, history of prior myocardial infarction, history of heart failure, and prior aspirin use.

†For comparative effectiveness recommendations (Class I and IIa; Level of Evidence A and B only), studies that support the use of comparator verbs should involve direct comparisons of the treatments or strategies being evaluated.

2. **Solid phase and/or cell-based assays to assess for DSA and quantification of antibody should be performed during the first 90 days after transplantation or when AMR is suspected *(Class I; Level of Evidence C)*.**
3. **It is reasonable to examine the endomyocardial biopsy specimen for histological evidence of AMR, particularly if there is a high clinical suspicion for AMR and no evidence of cellular rejection *(Class IIa; Level of Evidence C)*.**
4. **It is reasonable to perform immunopathologic assessment with staining for C4d and/or C3d at least 3, 6, and 12 months after transplantation or with the center's routine surveillance protocol *(Class IIa; Level of Evidence C)*.**
5. **It is reasonable to perform solid phase and/or cell-based assays to assess for DSA and quantification of antibody for surveillance at 3, 6, and 12 months after transplantation and annually thereafter or in accordance with the center's routine surveillance protocol *(Class IIa; Level of Evidence C)*.**
6. **It is reasonable to perform surveillance immunopathologic assessment after a positive result until clearance *(Class IIa; Level of Evidence C)*.**

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

Center	AMR Treatment
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, IVIg, plasmapheresis, rituximab/bortezomib
	pAMR3: Steroids, IVIg, plasmapheresis, rituximab/bortezomib (plus ATG or rATG if hemodynamically compromised)
Cedars-Sinai	Methylprednisolone 500 mg QD × 3
	rATG
	Plasmapheresis for hemodynamic compromise
	IVIg 2 g/kg on days 1 and 30 (first day after completion of rATG)
	Rituximab 1g (375 mg/m^2 for smaller patients) on days 7 and 21
	Refractory patients: Add bortezomib 1.3 mg/m^2 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: • IVIg 2 g/kg • Rituximab 375 mg/m^2 up to 4 doses • Bortezomib 1.3 mg/m^2 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/m^2 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 • IVIg 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/m^2 per day on days 1, 4, 8, and 11

AMR indicates antibody-mediated rejection; ATG, anti-thymocyte globulin; DSA, donor-specific antibody; IV, intravenous; IVIg, 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

Center	Treatment
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 Immune globulin IV (Gamunex) 10% 500 mg/kg IV once daily for 4 d 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/m^2 weekly for 4 wk; if patient's body surface area is <0.5 m^2, 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/m^2 on days 1, 4, 7, and 10 3. Rituximab 375 mg/m^2 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

Writing Group Member	Employment	Research Grant	Other Research Support	Speakers' Bureau/ Honoraria	Expert Witness	Ownership Interest	Consultant/ Advisory Board	Other
Monica Colvin	University of Minnesota	None	None	None	None	None	None	None
Jennifer L. Cook	Medical University of South Carolina	None	None	None	None	None	None	None
Patricia Chang	University of North Carolina At Chapel Hill	Novartis*	None	None	None	None	None	None
Gary Francis	University of Minnesota	None	None	None	None	None	None	None
Daphne T. Hsu	Montefiore Medical Center	None	None	None	None	None	None	None
Michael S. Kiernan	Tufts Medical Center	None	None	None	None	None	None	None
Jon A. Kobashigawa	Cedars-Sinai Heart Institute	Genzyme*; Novartis*; XDx*	None	None	None	None	Cylex*; GlaxoSmithKline*; Novartis*; XDx*	None
JoAnn Lindenfeld	University of Colorado	None	None	None	None	None	None	None
Sofia Carolina Masri	University of Washington	None	None	None	None	None	None	None
Dylan Miller	Utah Pathology Services, Inc.	None	None	None	None	None	None	None
John O'Connell	Thoratec	None	None	None	None	None	None	None
E. Rene Rodriguez	Cleveland Clinic	NIH*	None	None	None	None	None	None
Bruce Rosengard	Partners Healthcare/Harvard Medical School	None	None	None	None	None	None	None
Sally Self	Medical University of South Carolina	None	None	None	None	None	None	None
Connie White-Williams	University of Alabama at Birmingham	None	None	None	None	None	None	None
Adriana Zeevi	University of Pittsburgh	None	None	None	None	None	None	None

This table represents the relationships of writing group members that may be perceived as actual or reasonably perceived conflicts of interest as reported on the Disclosure Questionnaire, which all members of the writing group are required to complete and submit. A relationship is considered to be "significant" if (a) the person receives $10 000 or more during any 12-month period, or 5% or more of the person's gross income; or (b) the person owns 5% or more of the voting stock or share of the entity, or owns $10 000 or more of the fair market value of the entity. A relationship is considered to be "modest" if it is less than "significant" under the preceding definition.

*Modest.

Reviewer Disclosures

Reviewer	Employment	Research Grant	Other Research Support	Speakers' Bureau/ Honoraria	Expert Witness	Ownership Interest	Consultant/ Advisory Board	Other
Linda Addonizio	Columbia University	NIH (University of Pittsburgh Primary) Site PI for CTOTC Alloantibodies in Cardiac Transplantation-Intervention, Outcomes and Mechanisms*	None	None	None	None	None	None
Allan Gass	Westchester Medical Center	None	None	None	None	None	None	None
Shelley Miyamoto	University of Colorado	None	None	None	None	None	None	None

This table represents the relationships of reviewers that may be perceived as actual or reasonably perceived conflicts of interest as reported on the Disclosure Questionnaire, which all reviewers are required to complete and submit. A relationship is considered to be "significant" if (a) the person receives $10 000 or more during any 12-month period, or 5% or more of the person's gross income; or (b) the person owns 5% or more of the voting stock or share of the entity, or owns $10 000 or more of the fair market value of the entity. A relationship is considered to be "modest" if it is less than "significant" under the preceding definition.

*Significant.

References

1. Reed EF, Demetris AJ, Hammond E, Itescu S, Kobashigawa JA, Reinsmoen NL, Rodriguez ER, Rose M, Stewart S, Suciu-Foca N, Zeevi A, Fishbein MC; International Society for Heart and Lung Transplantation. Acute antibody-mediated rejection of cardiac transplants. *J Heart Lung Transplant*. 2006;25:153–159.
2. Kobashigawa J, Crespo-Leiro MG, Ensminger SM, Reichenspurner H, Angelini A, Berry G, Burke M, Czer L, Hiemann N, Kfoury AG, Mancini D, Mohacsi P, Patel J, Pereira N, Platt JL, Reed EF, Reinsmoen N, Rodriguez ER, Rose ML, Russell SD, Starling R, Suciu-Foca N, Tallaj J, Taylor DO, Van Bakel A, West L, Zeevi A, Zuckermann A; Consensus Conference Participants. Report from a consensus conference on antibody-mediated rejection in heart transplantation. *J Heart Lung Transplant*. 2011;30:252–269.
3. Berry GJ, Angelini A, Burke MM, Bruneval P, Fishbein MC, Hammond E, Miller D, Neil D, Revelo MP, Rodriguez ER, Stewart S, Tan CD, Winters GL, Kobashigawa J, Mehra MR. The ISHLT working formulation for pathologic diagnosis of antibody-mediated rejection in heart transplantation: evolution and current status (2005–2011). *J Heart Lung Transplant*. 2011;30:601–611.
4. Berry GJ, Burke MM, Andersen C, Bruneval P, Fedrigo M, Fishbein MC, Goddard M, Hammond EH, Leone O, Marboe C, Miller D, Neil D, Rassl D, Revelo MP, Rice A, Rene Rodriguez E, Stewart S, Tan CD, Winters GL, West L, Mehra MR, Angelini A. The 2013 International Society for Heart and Lung Transplantation Working Formulation for the standardization of nomenclature in the pathologic diagnosis of antibody-mediated rejection in heart transplantation. *J Heart Lung Transplant*. 2013;32:1147–1162.
5. Billingham ME, Cary NR, Hammond ME, Kemnitz J, Marboe C, McCallister HA, Snovar DC, Winters GL, Zerbe A. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: Heart Rejection Study Group: the International Society for Heart Transplantation. *J Heart Transplant*. 1990;9:587–593.
6. Stewart S, Winters G, Fishbein M, Tazelaar H, Kobashigawa J, Abrams J, Andersen C, Angelini A, Berry G, Burke MM, Demetris AJ, Hammond E, Itescu S, Marboe CC, McManus B, Reed EF, Reinsmoen NL, Rodriguez ER, Rose AG, Rose M, Suciu-Focia N, Zeevi A, Billingham ME. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *J Heart Lung Transplant*. 2005;24:1710–1720.
7. Brasile L, Zerbe T, Rabin B, Clarke J, Abrams A, Cerilli J. Identification of the antibody to vascular endothelial cells in patients undergoing cardiac transplantation. *Transplantation*. 1985;40:672–675.
8. Michaels P, Espejo M, Kobashigawa J, Alejos J, Burch C, Takemoto S, Reed E, Fishbein M. Humoral rejection in cardiac transplantation: risk factors, hemodynamic consequences and relationship to transplant coronary artery disease. *J Heart Lung Transplant*. 2003;22:58–69.
9. Taylor DO, Yowell RL, Kfoury AG, Hammond EH, Renlund DG. Allograft coronary artery disease: clinical correlations with circulating anti-HLA antibodies and the immunohistopathologic pattern of vascular rejection. *J Heart Lung Transplant*. 2000;19:518–521.
10. Kfoury A, Hammond M, Snow G, Stehlik J, Reid B, Long J, Gilbert E, Bader F, Bull D. Early screening for antibody-mediated rejection in heart transplant recipients. *J Heart Lung Transplant*. 2007;26:1264–1269.
11. Crespo-Leiro MG, Veiga-Barreiro A, Doménech N, Paniagua MJ, Piñon P, González-Cuesta M, Vázquez-Martul E, Ramirez C, Cuenca JJ, Castro-Beiras A. Humoral heart rejection (severe allograft dysfunction with no signs of cellular rejection or ischemia): incidence, management, and the value of C4d for diagnosis. *Am J Transplant*. 2005;5:2560–2564.
12. Hammond EH, Yowell RL, Nunoda S, Menlove RL, Renlund DG, Bristow MR, Gay WA Jr, Jones KW, O'Connell JB. Vascular (humoral) rejection in heart transplantation: pathologic observations and clinical implications. *J Heart Transplant*. 1989;8:430–443.
13. Hathout E, Beeson WL, Kuhn M, Johnston J, Fitts J, Razzouk A, Bailey L, Chinnock RE. Cardiac allograft vasculopathy in pediatric heart transplant recipients. *Transpl Int*. 2006;19:184–189.
14. Loupy A, Cazes A, Guillemain R, Amrein C, Hedjoudje A, Tible M, Pezzella V, Fabiani JN, Suberbielle C, Nochy D, Hill GS, Empana JP, Jouven X, Bruneval P, Duong Van Huyen JP. Very late heart transplant rejection is associated with microvascular injury, complement deposition and progression to cardiac allograft vasculopathy. *Am J Transplant*. 2011;11:1478–1487.
15. Kfoury A, Hammond M, Snow G, Drakos S, Stehlik J, Fisher P, Reid B, Everitt M, Bader FM, Renlund DG. Cardiovascular mortality among heart transplant recipients with asymptomatic antibody-mediated or stable mixed cellular and antibody-mediated rejection. *J Heart Lung Transplant*. 2009;28:781–784.
16. Abrams J, Amir O, Etheridge WB, Frazier OH. Histologic findings proving the existence of humoral rejection in a cardiac allograft. *Cardiovasc Pathol*. 2007;16:38–42.
17. Ratliff NB, McMahon JT. Activation of intravascular macrophages within myocardial small vessels is a feature of acute vascular rejection in human heart transplants. *J Heart Lung Transplant*. 1995;14:338–345.
18. Duong Van Huyen JP, Fornes P, Guillemain R, Amrein C, Chevalier P, Latremouille C, Creput C, Glotz D, Nochy D, Bruneval P. Acute vascular humoral rejection in a sensitized cardiac graft recipient: diagnostic value of C4d immunofluorescence. *Hum Pathol*. 2004;35:385–388.
19. Lones MA, Czer LS, Trento A, Harasty D, Miller JM, Fishbein MC. Clinical-pathologic features of humoral rejection in cardiac allografts: a study in 81 consecutive patients. *J Heart Lung Transplant*. 1995;14(pt 1):151–162.
20. Loy TS, Bulatao IS, Darkow GV, Demmy TL, Reddy HK, Curtis J, Bickel JT. Immunostaining of cardiac biopsy specimens in the diagnosis of acute vascular (humoral) rejection: a control study. *J Heart Lung Transplant*. 1993;12:736–740.
21. Trento A, Hardesty RL, Griffith BP, Zerbe T, Kormos RL, Bahnson HT. Role of the antibody to vascular endothelial cells in hyperacute rejection in patients undergoing cardiac transplantation. *J Thorac Cardiovasc Surg*. 1988;95:37–41.
22. Foerster A. Vascular rejection in cardiac transplantation: a morphological study of 25 human cardiac allografts. *APMIS*. 1992;100:367–376.
23. Baldwin WM 3rd, Samaniego-Picota M, Kasper EK, Clark AM, Czader M, Rohde C, Zachary AA, Sanfilippo F, Hruban RH. Complement deposition in early cardiac transplant biopsies is associated with ischemic injury and subsequent rejection episodes. *Transplantation*. 1999;68:894–900.
24. Behr TM, Richter K, Fischer P, Spes CH, Meiser B, Reichart B, Pongratz D, Feucht H, Theisen K, Angermann CE. Incidence of humoral rejection in heart transplant recipients treated with tacrolimus or cyclosporine A. *Transplant Proc*. 1998;30:1920–1921.
25. Behr TM, Feucht HE, Richter K, Reiter C, Spes CH, Pongratz D, Uberfuhr P, Meiser B, Theisen K, Angermann CE. Detection of humoral rejection in human cardiac allografts by assessing the capillary deposition of complement fragment C4d in endomyocardial biopsies. *J Heart Lung Transplant*. 1999;18:904–912.
26. Arias LF, Fernández R, Fernández D, Jaramillo JC, Gil M, López García-Asenjo JA. C4D immunostaining in surveillance endomyocardial biopsies from well-functioning heart allografts. *Transplant Proc*. 2010;42:1793–1796.
27. Chantranuwat C, Qiao JH, Kobashigawa J, Hong L, Shintaku P, Fishbein MC. Immunoperoxidase staining for C4d on paraffin-embedded tissue in cardiac allograft endomyocardial biopsies: comparison to frozen tissue immunofluorescence. *Appl Immunohistochem Mol Morphol*. 2004;12:166–171.
28. Fedrigo M, Gambino A, Tona F, Torregrossa G, Poli F, Benazzi E, Frigo A, Feltrin G, Toscano G, Caforio AP, Iliceto S, Valente M, Thiene G, Gerosa G, Angelini A. Can C4d immunostaining on endomyocardial biopsies be considered a prognostic biomarker in heart transplant recipients? [published correction appears in *Transplantation*. 2011;91:135] *Transplantation*. 2010;90:791–798.
29. Fedson SE, Daniel SS, Husain AN. Immunohistochemistry staining of C4d to diagnose antibody-mediated rejection in cardiac transplantation. *J Heart Lung Transplant*. 2008;27:372–379.
30. Moseley EL, Atkinson C, Sharples LD, Wallwork J, Goddard MJ. Deposition of C4d and C3d in cardiac transplants: a factor in the development of coronary artery vasculopathy. *J Heart Lung Transplant*. 2010;29:417–423.
31. Poelzl G, Ullrich R, Huber A, Ulmer H, Antretter H, Hoefer D, Mairinger T, Laufer G, Pachinger O, Schwarzacher S. Capillary deposition of the complement fragment C4d in cardiac allograft biopsies is associated with allograft vasculopathy. *Transpl Int*. 2005;18:313–317.
32. Garrett HE Jr, Duvall-Seaman D, Helsley B, Groshart K. Treatment of vascular rejection with rituximab in cardiac transplantation. *J Heart Lung Transplant*. 2005;24:1337–1342.
33. Almuti K, Haythe J, Dwyer E, Itescu S, Burke E, Green P, Marboe C, Mancini D. The changing pattern of humoral rejection in cardiac transplant recipients. *Transplantation*. 2007;84:498–503.
34. Nath DS, Angaswamy N, Basha HI, Phelan D, Moazami N, Ewald GA, Mohanakumar T. Donor-specific antibodies to human leukocyte antigens are associated with and precede antibodies to major histocompatibility complex class I-related chain A in antibody-mediated rejection and cardiac allograft vasculopathy after human cardiac transplantation. *Hum Immunol*. 2010;71:1191–1196.
35. Gonzalez-Stawinski GV, Cook DJ, Chui J, Gupta S, Navia JL, Hoercher K, Taylor DO, Yamani MH, Starling RC, Smedira NG. A comparative

analysis between survivors and nonsurvivors with antibody mediated cardiac allograft rejection. *J Surg Res*. 2007;142:233–238.
36. Bayliss J, Bailey M, Leet A, Stein AN, Thomson NM, McLean CA. Late onset antibody-mediated rejection and endothelial localization of vascular endothelial growth factor are associated with development of cardiac allograft vasculopathy. *Transplantation*. 2008;86:991–997.
37. Casarez T, Perens G, Williams R, Kutay E, Fishbein M, Reed E, Alejos J, Levi D. Humoral rejection in pediatric orthotopic heart transplantation. *J Heart Lung Transplant*. 2007;26:114–119.
38. Rodriguez E, Skojec D, Tan C, Zachary A, Kasper E, Conte J, Baldwin W 3rd. Antibody-mediated rejection in human cardiac allografts: evaluation of immunoglobulins and complement activation products C4d and C3d as markers. *Am J Transplant*. 2005;5:2778–2785.
39. Holt DB, Liapis H, Mohanakumar T, Phelan DR, Gandi SK, Huddleston CB, Canter CE. Complement fragment C4d and C3d deposition in pediatric heart recipients with a positive crossmatch. *J Heart Lung Transplant*. 2008;27:1073–1078.
40. Cadeiras M, Burke E, Dedrick R, Gangadin A, Latif F, Shahzad K, Sinha A, Tabak EG, Marboe CC, Califano AC, Deng MC. Gene expression profiles of patients with antibody-mediated rejection after cardiac transplantation [published correction appears in *J Heart Lung Transplant*. 2008;27:1190]. *J Heart Lung Transplant*. 2008;27:932–934.
41. Tan CD, Baldwin WM 3rd, Rodriguez ER. Update on cardiac transplantation pathology. *Arch Pathol Lab Med*. 2007;131:1169–1191.
42. Baldwin WM 3rd, Qian Z, Ota H, Samaniego M, Wasowska B, Sanfilippo F, Hruban RH. Complement as a mediator of vascular inflammation and activation in allografts. *J Heart Lung Transplant*. 2000;19:723–730.
43. Aranda JM Jr, Scornik JC, Normann SJ, Lottenberg R, Schofield RS, Pauly DF, Miles M, Hill JA, Sleasman JW, Skoda-Smith S. Anti-CD20 monoclonal antibody (rituximab) therapy for acute cardiac humoral rejection: a case report. *Transplantation*. 2002;73:907–910.
44. Hammond ME, Stehlik J, Snow G, Renlund DG, Seaman J, Dabbas B, Gilbert EM, Stringham JC, Long JW, Kfoury AG; Utah Transplant Affiliated Hospitals (UTAH) Cardiac Transplant Program. Utility of histologic parameters in screening for antibody-mediated rejection of the cardiac allograft: a study of 3,170 biopsies. *J Heart Lung Transplant*. 2005;24:2015–2021.
45. Kfoury A, Stehlik J, Renlund D, Seaman J, Gilbert E, Stringham J, Long J, Hammond M. Impact of repetitive episodes of antibody-mediated or cellular rejection on cardiovascular mortality in cardiac transplant recipients: defining rejection patterns. *J Heart Lung Transplant*. 2006;25:1277–1282.
46. Revelo MP, Stehlik J, Miller D, Snow GL, Everitt MD, Budge D, Bader FM, Alharethi RA, Gilbert EM, Reid BB, Selzman CH, Hammond ME, Kfoury AG. Antibody testing for cardiac antibody-mediated rejection: which panel correlates best with cardiovascular death? *J Heart Lung Transplant*. 2011;30:144–150.
47. Tan CD, Sokos GG, Pidwell DJ, Smedira NG, Gonzalez-Stawinski GV, Taylor DO, Starling RC, Rodriguez ER. Correlation of donor-specific antibodies, complement and its regulators with graft dysfunction in cardiac antibody-mediated rejection. *Am J Transplant*. 2009;9:2075–2084.
48. Okada M, Yoshioka K, Takemura T, Akano N, Aya N, Murakami K, Maki S. Immunohistochemical localization of C3d fragment of complement and S-protein (vitronectin) in normal and diseased human kidneys: association with the C5b-9 complex and vitronectin receptor. *Virchows Arch A Pathol Anat Histopathol*. 1993;422:367–373.
49. González-Stawinski GV, Tan CD, Smedira NG, Starling RC, Rodríguez ER. Decay-accelerating factor expression may provide immunoprotection against antibody-mediated cardiac allograft rejection. *J Heart Lung Transplant*. 2008;27:357–361.
50. Hammond EH, Hansen JK, Spencer LS, Jensen A, Riddell D, Craven CM, Yowell RL. Vascular rejection in cardiac transplantation: histologic, immunopathologic, and ultrastructural features. *Cardiovasc Pathol*. 1993;2:21–34.
51. Labarrere CA, Pitts D, Halbrook H, Faulk WP. Tissue plasminogen activator, plasminogen activator inhibitor-1, and fibrin as indexes of clinical course in cardiac allograft recipients: an immunocytochemical study. *Circulation*. 1994;89:1599–1608.
52. Bonnaud EN, Lewis NP, Masek MA, Billingham ME. Reliability and usefulness of immunofluorescence in heart transplantation. *J Heart Lung Transplant*. 1995;14(pt 1):163–171.
53. Jordan SC, Quartel AW, Czer LS, Admon D, Chen G, Fishbein MC, Schwieger J, Steiner RW, Davis C, Tyan DB. Posttransplant therapy using high-dose human immunoglobulin (intravenous gammaglobulin) to control acute humoral rejection in renal and cardiac allograft recipients and potential mechanism of action. *Transplantation*. 1998;66:800–805.
54. Fishbein MC, Kobashigawa J. Biopsy-negative cardiac transplant rejection: etiology, diagnosis, and therapy. *Curr Opin Cardiol*. 2004;19:166–169.
55. Tambur AR, Leventhal J, Kaufman DB, Friedewald J, Miller J, Abecassis MM. Tailoring antibody testing and how to use it in the calculated panel reactive antibody era: the Northwestern University experience. *Transplantation*. 2008;86:1052–1059.
56. Smith JD, Danskine AJ, Rose ML, Yacoub MH. Specificity of lymphocytotoxic antibodies formed after cardiac transplantation and correlation with rejection episodes. *Transplantation*. 1992;53:1358–1362.
57. Fenoglio J, Ho E, Reed E, Rose E, Smith C, Reemstma K, Marboe C, Suciu-Foca N. Anti-HLA antibodies and heart allograft survival. *Transplant Proc*. 1989;21:807–809.
58. Reed EF, Hong B, Ho E, Harris PE, Weinberger J, Suciu-Foca N. Monitoring of soluble HLA alloantigens and anti-HLA antibodies identifies heart allograft recipients at risk of transplant-associated coronary artery disease. *Transplantation*. 1996;61:566–572.
59. Kaczmarek I, Deutsch M, Kauke T, Beiras-Fernandez A, Schmoeckel M, Vicol C, Sodian R, Reichart B, Spannagl M, Ueberfuhr P. Donor-specific HLA alloantibodies: long-term impact on cardiac allograft vasculopathy and mortality after heart transplant. *Exp Clin Transplant*. 2008;6:229–235.
60. George JF, Kirklin JK, Shroyer TW, Naftel DC, Bourge RC, McGiffin DC, White-Williams C, Noreuil T. Utility of posttransplantation panel-reactive antibody measurements for the prediction of rejection frequency and survival of heart transplant recipients. *J Heart Lung Transplant*. 1995;14:856–864.
61. Leech S, Mather P, Eisen H, Pina I, Margulies K, Bove A, Jeevanandam V. Donor-specific HLA antibodies after transplantation are associated with deterioration in cardiac function. *Clin Transplant*. 1996;10:639–645.
62. Carter V, Shenton BK, Jaques B, Turner D, Talbot D, Gupta A, Chapman CE, Matthews CJ, Cavanagh G. Vimentin antibodies: a non-HLA antibody as a potential risk factor in renal transplantation. *Transplant Proc*. 2005;37:654–657.
63. Sumitran-Holgersson S, Wilczek H, Holgersson J, Soderstrom K. Identification of the nonclassical HLA molecules, MICA, as targets for humoral immunity associated with irreversible rejection of kidney allografts1. *Transplantation*. 2002;74:268–277.
64. Suárez-Alvarez B, López-Vázquez A, Gonzalez MZ, Fdez-Morera JL, Díaz-Molina B, Blanco-Gelaz MA, Pascual D, Martínez-Borra J, Muro M, Alvarez-López MR, López-Larrea C. The relationship of anti-MICA antibodies and MICA expression with heart allograft rejection. *Am J Transplant*. 2007;7:1842–1848.
65. Rose ML. Role of MHC and non-MHC alloantibodies in graft rejection. *Curr Opin Organ Transplant*. 2004;9:16–22.
66. Ferry BL, Welsh KI, Dunn MJ, Law D, Proctor J, Chapel H, Yacoub MH, Rose ML. Anti-cell surface endothelial antibodies in sera from cardiac and kidney transplant recipients: association with chronic rejection. *Transpl Immunol*. 1997;5:17–24.
67. Fredrich R, Toyoda M, Czer L, Galfayan K, Galera O, Trento A, Freimark D, Young S, Jordan SC. The clinical significance of antibodies to human vascular endothelial cells after cardiac transplantation. *Transplantation*. 1999;67:385–391.
68. Faulk WP, Rose M, Meroni PL, Del Papa N, Torry RJ, Labarrere CA, Busing K, Crisp SJ, Dunn MJ, Nelson DR. Antibodies to endothelial cells identify myocardial damage and predict development of coronary artery disease in patients with transplanted hearts. *Hum Immunol*. 1999;60:826–832.
69. Jurcevic S, Ainsworth ME, Pomerance A, Smith JD, Robinson DR, Dunn MJ, Yacoub MH, Rose ML. Antivimentin antibodies are an independent predictor of transplant-associated coronary artery disease after cardiac transplantation. *Transplantation*. 2001;71:886–892.
70. Alvarez-Márquez A, Aguilera I, Blanco RM, Pascual D, Encarnación-Carrizosa M, Alvarez-López MR, Wichmann I, Nuñez-Roldán A. Positive association of anticytoskeletal endothelial cell antibodies and cardiac allograft rejection. *Hum Immunol*. 2008;69:143–148.
71. Azimzadeh AM, Pfeiffer S, Wu GS, Schröder C, Zhou H, Zorn GL 3rd, Kehry M, Miller GG, Rose ML, Pierson RN 3rd. Humoral immunity to vimentin is associated with cardiac allograft injury in nonhuman primates. *Am J Transplant*. 2005;5:2349–2359.
72. Nath DS, Ilias Basha H, Tiriveedhi V, Alur C, Phelan D, Ewald GA, Moazami N, Mohanakumar T. Characterization of immune responses to cardiac self-antigens myosin and vimentin in human cardiac allograft recipients with antibody-mediated rejection and cardiac allograft vasculopathy. *J Heart Lung Transplant*. 2010;29:1277–1285.
73. Ensley RD, Hammond EH, Renlund DG, Yowell RL, Bristow MR, DeWitt CW, Menlove RL, Ratkovec RM, O'Connell JB. Clinical manifestations of vascular rejection in cardiac transplantation. *Transplant Proc*. 1991;23(pt 2):1130–1132.
74. Cherry R, Nielsen H, Reed E, Reemtsma K, Suciu-Foca N, Marboe CC. Vascular (humoral) rejection in human cardiac allograft biopsies: relation to circulating anti-HLA antibodies. *J Heart Lung Transplant*. 1992;11:24–29.

75. Miller L, Wesp A, Jennison S, Graham M, Martin T, McBride L, Pennington D, Peigh P. Vascular rejection in heart transplant recipients. *J Heart Lung Transplant*. 1993;12:S147–S152.
76. Caple JF, McMahon JT, Myles JL, Hook S, Ratliff NB. Acute vascular (humoral) rejection in non-OKT3-treated cardiac transplants. *Cardiovasc Pathol*. 1995;4:13–18.
77. Smith R, Brousaides N, Grazette L, Saidman S, Semigran M, Disalvo T, Madsen J, Dec G, Perez-Atayde A, Collins A. C4d deposition in cardiac allografts correlates with alloantibody. *J Heart Lung Transplant*. 2005;24:1202–1210.
78. Holt DB, Lublin DM, Phelan DL, Boslaugh SE, Gandhi SK, Huddleston CB, Saffitz JE, Canter CE. Mortality and morbidity in pre-sensitized pediatric heart transplant recipients with a positive donor crossmatch utilizing peri-operative plasmapheresis and cytolytic therapy. *J Heart Lung Transplant*. 2007;26:876–882.
79. Hammond EH, Wittwer CT, Greenwood J, Knape WA, Yowell RL, Menlove RL, Craven C, Renlund DG, Bristow MR, DeWitt CW, O'Connell JB. Relationship of OKT3 sensitization and vascular rejection in cardiac transplant patients receiving OKT3 rejection prophylaxis. *Transplantation*. 1990;50:776–782.
80. Olsen S, Wagoner L, Hammond E, Taylor D, Yowell R, Ensley R, Bristow M, O'Connell J, Renlund D. Vascular rejection in heart transplantation: clinical correlation, treatment options, and future considerations. *J Heart Lung Transplant*. 1993;12:S135–S142.
81. Loupy A, Suberbielle-Boissel C, Hill G, Lefaucheur C, Anglicheau D, Zuber J, Martinez F,Thervet E,Méjean A,Charron D,Duong van Huyen JP,Bruneval P,Legendre C,Nochy D. Outcome of subclinical antibody-mediated rejection in kidney transplant recipients with preformed donor-specific antibodies. *Am J Transplant*. 2009;9:2561–2570.
82. Kraus E, Parekh R, Oberai P, Lepley D, Segev D, Bagnasco S, Collins V, Leffell M, Lucas D, Rabb H, Racusen LC, Singer AL, Stewart ZA, Warren DS, Zachary AA, Haas M, Montgomery RA. Subclinical rejection in stable positive crossmatch kidney transplant patients: incidence and correlations. *Am J Transplant*. 2009;9:1826–1834.
83. Koch CA, Khalpey ZI, Platt JL. Accommodation: preventing injury in transplantation and disease. *J Immunol*. 2004;172:5143–5148.
84. Grauhan O, Knosalla C, Ewert R, Hummel M, Loebe M, Weng YG, Hetzer R. Plasmapheresis and cyclophosphamide in the treatment of humoral rejection after heart transplantation. *J Heart Lung Transplant*. 2001;20:316–321.
85. Wu G, Kobashigawa J, Fishbein M, Patel J, Kittleson M, Reed E, Kiyosaki K, Ardehali A. Asymptomatic antibody-mediated rejection after heart transplantation predicts poor outcomes. *J Heart Lung Transplant*. 2009;28:417–422.
86. Kfoury AG, Renlund DG, Snow GL, Stehlik J, Folsom JW, Fisher PW, Reid BB, Clayson SE, Gilbert EM, Everitt MD, Bader FM, Singhal AK, Hammond ME. A clinical correlation study of severity of antibody-mediated rejection and cardiovascular mortality in heart transplantation. *J Heart Lung Transplant*. 2009;28:51–57.
87. Yoshida S, Takeuchi K, Del Nido P, Ho C. Diastolic dysfunction coincides with early mild transplant rejection: in situ measurements in a heterotopic rat heart transplant model. *J Heart Lung Transplant*. 1998;17:1049–1056.
88. Sachdeva R, Malik S, Seib PM, Frazier EA, Cleves MA. Doppler tissue imaging and catheter-derived measures are not independent predictors of rejection in pediatric heart transplant recipients. *Int J Cardiovasc Imaging*. 2011;27:947–954.
89. Palka P, Lange A, Galbraith A, Duhig E, Clarke BE, Parsonage W, Donnelly JE, Stafford WJ, Burstow DJ. The role of left and right ventricular early diastolic Doppler tissue echocardiographic indices in the evaluation of acute rejection in orthotopic heart transplant. *J Am Soc Echocardiogr*. 2005;18:107–115.
90. Branch KR, Caldwell JH, Soine L, O'Brien KD. Vascular (humoral) cardiac allograft rejection manifesting as inducible myocardial ischemia on nuclear perfusion imaging. *J Nucl Cardiol*. 2005;12:123–124.
91. Almenar L, Igual B, Martínez-Dolz L, Arnau M, Osa A, Rueda J, Palencia M. Utility of cardiac magnetic resonance imaging for the diagnosis of heart transplant rejection. *Transplant Proc*. 2003;35:1962–1964.
92. Marie PY, Angioī M, Carteaux JP, Escanye JM, Mattei S, Tzvetanov K, Claudon O, Hassan N, Danchin N, Karcher G, Bertrand A, Walker PM, Villemot JP. Detection and prediction of acute heart transplant rejection with the myocardial T2 determination provided by a black-blood magnetic resonance imaging sequence. *J Am Coll Cardiol*. 2001;37:825–831.
93. Smart FW, Ballantyne CM, Cocanougher B, Farmer JA, Sekela ME, Noon GP, Young JB. Insensitivity of noninvasive tests to detect coronary artery vasculopathy after heart transplant. *Am J Cardiol*. 1991;67:243–247.
94. Mousseaux E, Farge D, Guillemain R, Amrein C, Vulser C, Bruneval P, Gaux JC, Carpentier A. Magnetic resonance imaging of human transplanted hearts with gadolinium-DOTA myocardial enhancement: potential use for the detection of rejection. *J Am Coll Cardiol*. 1991;17(2s1):A326. Abstract.
95. Rivard AL, Swingen CM, Blake D, Huang AS, Kanth P, Thomsen GF, Cordova EJ, Miller LW, Bianco RW, Wilke N. A comparison of myocardial perfusion and rejection in cardiac transplant patients. *Int J Cardiovasc Imaging*. 2007;23:575–582.
96. Lund G, Morin R, Olivari M, Ring W. Serial myocardial T2 relaxation time measurements in normal subjects and heart transplant recipients. *J Heart Transplant*. 1988;7:274–279.
97. Wisenberg G, Pflugfelder PW, Kostuk WJ, McKenzie FN, Prato FS. Diagnostic applicability of magnetic resonance imaging in assessing human cardiac allograft rejection. *Am J Cardiol*. 1987;60:130–136.
98. Butler CR, Thompson R, Haykowsky M, Toma M, Paterson I. Cardiovascular magnetic resonance in the diagnosis of acute heart transplant rejection: a review. *J Cardiovasc Magn Reson*. 2009;11:7.
99. Abdel-Aty H, Boyé P, Zagrosek A, Wassmuth R, Kumar A, Messroghli D, Bock P, Dietz R, Friedrich MG, Schulz-Menger J. Diagnostic performance of cardiovascular magnetic resonance in patients with suspected acute myocarditis: comparison of different approaches. *J Am Coll Cardiol*. 2005;45:1815–1822.
100. De Cobelli F, Pieroni M, Esposito A, Chimenti C, Belloni E, Mellone R, Canu T, Perseghin G, Gaudio C, Maseri A, Frustaci A, Del Maschio A. Delayed gadolinium-enhanced cardiac magnetic resonance in patients with chronic myocarditis presenting with heart failure or recurrent arrhythmias. *J Am Coll Cardiol*. 2006;47:1649–1654.
101. Aletras AH, Tilak GS, Natanzon A, Hsu LY, Gonzalez FM, Hoyt RF, Arai AE. Retrospective determination of the area at risk for reperfused acute myocardial infarction with T2-weighted cardiac magnetic resonance imaging: histopathological and displacement encoding with stimulated echoes (DENSE) functional validations. *Circulation*. 2006;113:1865–1870.
102. Sasaguri S, LaRaia PJ, Fabri BM, Fallon JT, Ayelsworth CA, D'Ambra MN, Newell JB, Brady TJ, Buckley MJ. Early detection of cardiac allograft rejection with proton nuclear magnetic resonance. *Circulation*. 1985;72(pt 2):II231-II236.
103. Estep JD, Shah DJ, Nagueh SF, Mahmarian JJ, Torre-Amione G, Zoghbi WA. The role of multimodality cardiac imaging in the transplanted heart. *JACC Cardiovasc Imaging*. 2009;2:1126–1140.
104. Miller DV, Roden AC, Gamez JD, Tazelaar HD. Detection of C4d deposition in cardiac allografts: a comparative study of immunofluorescence and immunoperoxidase methods. *Arch Pathol Lab Med*. 2010;134:1679–1684.
105. Johnson M, Aaronson KD, Canter C, Kirklin J, Mancini D, Mehra M, Radovancevic B, Taylor D, Webber S. Heart retransplantation. *Am J Transplant*. 2007;7:2075–2081.
106. Costanzo MR, Dipchand A, Starling R, Anderson A, Chan M, Desai S, Fedson S, Fisher P, Gonzales-Stawinski G, Martinelli L, McGiffin D, Smith J, Taylor D, Meiser B, Webber S, Baran D, Carboni M, Dengler T, Feldman D, Frigerio M, Kfoury A, Kim D, Kobashigawa J, Shullo M, Stehlik J, Teuteberg J, Uber P, Zuckermann A, Hunt S, Burch M, Bhat G, Canter C, Chinnock R, Crespo-Leiro M, Delgado R, Dobbels F, Grady K, Kao W, Lamour J, Parry G, Patel J, Pini D, Towbin J, Wolfel G, Delgado D, Eisen H, Goldberg L, Hosenpud J, Johnson M, Keogh A, Lewis C, O'Connell J, Rogers J, Ross H, Russell S, Vanhaecke J. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *J Heart Lung Transplant*. 2010;29:914–956.
107. Sivasankaran S, Pollard-Quintner S, Sachdeva R, Pugeda J, Hoq SM, Zarich SW. The effect of a six-week program of yoga and meditation on brachial artery reactivity: do psychosocial interventions affect vascular tone? *Clin Cardiol*. 2006;29:393–398.
108. Vieira CA, Agarwal A, Book BK, Sidner RA, Bearden CM, Gebel HM, Roggero AL, Fineberg NS, Taber T, Kraus MA, Pescovitz MD. Rituximab for reduction of anti-HLA antibodies in patients awaiting renal transplantation: 1: safety, pharmacodynamics, and pharmacokinetics. *Transplantation*. 2004;77:542–548.
109. Clatworthy MR, Friend PJ, Calne RY, Rebello PR, Hale G, Waldmann H, Watson CJ. Alemtuzumab (CAMPATH-1H) for the treatment of acute rejection in kidney transplant recipients: long-term follow-up. *Transplantation*. 2009;87:1092–1095.
110. Everly MJ, Everly JJ, Susskind B, Brailey P, Arend LJ, Alloway RR, Roy-Chaudhury P, Govil A, Mogilishetty G, Rike AH, Cardi M, Wadih G, Tevar A, Woodle ES. Bortezomib provides effective therapy for antibody- and cell-mediated acute rejection. *Transplantation*. 2008;86:1754–1761.
111. Lefaucheur C, Nochy D, Andrade J, Verine J, Gautreau C, Charron D, Hill G, Glotz D, Suberbielle-Boissel C. Comparison of combination plasmapheresis/IVIg/anti-CD20 versus high-dose IVIg in the treatment of antibody-mediated rejection. *Am J Transplant*. 2009;9:1099–1107.
112. Stegall M, Gloor J, Winters J, Moore S, Degoey S. A Comparison of plasmapheresis versus high-dose IVIG desensitization in renal allograft

recipients with high levels of donor specific alloantibody. *Am J Transplant*. 2006;6:346–351.
113. Twombley K, Thach L, Ribeiro A, Joseph C, Seikaly M. Acute antibody-mediated rejection in pediatric kidney transplants: a single center experience. *Pediatr Transplant*. 2013;17:E149–E155.
114. Venetz JP, Pascual M. New treatments for acute humoral rejection of kidney allografts. *Expert Opin Investig Drugs*. 2007;16:625–633.
115. Goodwin W, Kaufman J, Mims M, Turner R, Glassock R, Goldman R, Maxwell M. Human renal transplantation, I: clinical experiences with six cases of renal homotransplantation. *J Urol*. 1963;89:13–24.
116. Taylor D, Meiser B, Webber S, Baran DA, Carboni M, Dengler T, Feldman D, Frigerio M, Kfoury A, Kim D, Kobashigawa J, Shullo M, Stehlik J, Teuteberg J, Uber PA, West L, Zuckermann A. Task Force 2: Immunosuppression and Rejection. In: The International Society of Heart and Lung Transplantation guidelines for the care of heart transplant recipients. *J Heart Lung Transplant*. 2010;29:926–933.
117. Lindenfeld J, Miller G, Shakar S, Zolty R, Lowes B, Wolfel E, Mestroni L, Page R, Kobashigawa J. Drug therapy in the heart transplant recipient: part I: cardiac rejection and immunosuppressive drugs. *Circulation*. 2004;110:3734–3740.
118. Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science*. 1995;270:286–290.
119. Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS Jr. Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. *Science*. 1995;270:283–286.
120. Gelfand EW. Antibody-directed therapy: past, present, and future. *J Allergy Clin Immunol*. 2001;108(suppl):S111–S116.
121. Dalakas MC. Mechanisms of action of IVIg and therapeutic considerations in the treatment of acute and chronic demyelinating neuropathies. *Neurology*. 2002;59(suppl 6):S13–S21.
122. Dwyer JM. Manipulating the immune system with immune globulin. *N Engl J Med*. 1992;326:107–116.
123. Singh N, Pirsch J, Samaniego M. Antibody-mediated rejection: treatment alternatives and outcomes. *Transplant Rev (Orlando)*. 2009;23:34–46.
124. Velez M, Johnson MR. Management of allosensitized cardiac transplant candidates. *Transplant Rev (Orlando)*. 2009;23:235–247.
125. Wang SS, Chou NK, Ko WJ, Chi NH, Hung SC, Hsu RB, Yu HY, Chen YS, Chu SH, Tsao CI, Shun CT. Effect of plasmapheresis for acute humoral rejection after heart transplantation. *Transplant Proc*. 2006;38:3692–3694.
126. Leech SH, Rubin S, Eisen HJ, Mather PJ, Goldman BI, McClurken JB, Furukawa S. Cardiac transplantation across a positive prospective lymphocyte cross-match in sensitized recipients. *Clin Transplant*. 2003;17(suppl 9):17–26.
127. Leech SH, Lopez-Cepero M, LeFor WM, DiChiara L, Weston M, Furukawa S, Macha M, Singhal A, Wald JW, Nikolaidis LA, McClurken JB, Bove AA. Management of the sensitized cardiac recipient: the use of plasmapheresis and intravenous immunoglobulin. *Clin Transplant*. 2006;20:476–484.
128. Ratkovec RM, Hammond EH, O'Connell JB, Bristow MR, DeWitt CW, Richenbacher WE, Millar RC, Renlund DG. Outcome of cardiac transplant recipients with a positive donor-specific crossmatch–preliminary results with plasmapheresis. *Transplantation*. 1992;54:651–655.
129. John R, Lietz K, Burke E, Ankersmit J, Mancini D, Suciu-Foca N, Edwards N, Rose E, Oz M, Itescu S. Intravenous immunoglobulin reduces anti-HLA alloreactivity and shortens waiting time to cardiac transplantation in highly sensitized left ventricular assist device recipients. *Circulation*. 1999;100(suppl):II229–II235.
130. Marques M, Schwartz J. Update on extracorporeal photopheresis in heart and lung transplantation. *J Clin Apher*. 2011;26:146–151.
131. Marques MB, Tuncer HH. Photopheresis in solid organ transplant rejection. *J Clin Apher*. 2006;21:72–77.
132. Lamioni A, Parisi F, Isacchi G, Giorda E, Di Cesare S, Landolfo A, Cenci F, Bottazzo GF, Carsetti R. The immunological effects of extracorporeal photopheresis unraveled: induction of tolerogenic dendritic cells in vitro and regulatory T cells in vivo. *Transplantation*. 2005;79:846–850.
133. Meloni F, Cascina A, Miserere S, Perotti C, Vitulo P, Fietta AM. Peripheral CD4(+)CD25(+) TREG cell counts and the response to extracorporeal photopheresis in lung transplant recipients. *Transplant Proc*. 2007;39:213–217.
134. George JF, Gooden CW, Guo L, Kirklin JK. Role for CD4(+)CD25(+) T cells in inhibition of graft rejection by extracorporeal photopheresis [published correction appears in *J Heart Lung Transplant*. 2008;27:1190]. *J Heart Lung Transplant*. 2008;27:616–622.
135. Costanzo-Nordin MR, Hubbell EA, O'Sullivan EJ, Johnson MR, Mullen GM, Heroux AL, Kao WG, McManus BM, Pifarre R, Robinson JA. Photopheresis versus corticosteroids in the therapy of heart transplant rejection: preliminary clinical report. *Circulation*. 1992;86:II242–II250.
136. Dall'Amico R, Livi U, Milano A, Montini G, Andreetta B, Murer L, Zacchello G, Thiene G, Casarotto D, Zacchello F. Extracorporeal photochemotherapy as adjuvant treatment of heart transplant recipients with recurrent rejection. *Transplantation*. 1995;60:45–49.
137. Dall'Amico R, Montini G, Murer L, Andreetta B, Zacchello G, Gambino A, Feltrin G, Caforio A, Tursi V, Livi U. Extracorporeal photochemotherapy after cardiac transplantation: a new therapeutic approach to allograft rejection. *Int J Artif Organs*. 2000;23:49–54.
138. Wieland M, Thiede VL, Strauss RG, Piette WW, Kapelanski DP, Landas SK, Hunsicker LG, Vance SJ, Randels MJ. Treatment of severe cardiac allograft rejection with extracorporeal photochemotherapy. *J Clin Apher*. 1994;9:171–175.
139. Costanzo-Nordin MR, Hubbell EA, O'Sullivan EJ, Johnson MR, Mullen GM, Heroux AL, Kao WG, McManus BM, Pifarre R, Robinson JA. Successful treatment of heart transplant rejection with photopheresis. *Transplantation*. 1992;53:808–815.
140. Kirklin JK, Brown RN, Huang ST, Naftel DC, Hubbard SM, Rayburn BK, McGiffin DC, Bourge RB, Benza RL, Tallaj JA, Pinderski LJ, Pamboukian SV, George JF, Marques M. Rejection with hemodynamic compromise: objective evidence for efficacy of photopheresis. *J Heart Lung Transplant*. 2006;25:283–288.
141. Barr ML, Meiser BM, Eisen HJ, Roberts RF, Livi U, Dall'Amico R, Dorent R, Rogers JG, Radovančević B, Taylor DO, Jeevanandam V, Marboe CC. Photopheresis for the prevention of rejection in cardiac transplantation. *N Engl J Med*. 1998;339:1744–1751.
142. Barr M, Baker C, Schenkel F, McLaughlin S, Stouch B, Starnes V, Rose E. Prophylactic photopheresis and chronic rejection: effects on graft intimal hyperplasia in cardiac transplantation. *Clin Transplant*. 2000;14:162–166.
143. Ballester M, Obrador D, Carrió I, Bordes R, Augé JM, Crexells C, Oriol A, Padró JM, Aris A, Caralps JM. Reversal of rejection-induced coronary vasculitis detected early after heart transplantation with increased immunosuppression. *J Heart Transplant*. 1989;8:413–417.
144. Malafa M, Mancini MC, Myles JL, Gohara A, Dickinson JM, Walsh TE. Successful treatment of acute humoral rejection in a heart transplant patient. *J Heart Lung Transplant*. 1992;11:486–491.
145. Kobashigawa J, Miller L, Renlund D, Mentzer R, Alderman E, Bourge R, Costanzo M, Eisen H, Dureau G, Ratkovec R, Hummel M, Ipe D, Johnson J, Keogh A, Mamelok R, Mancini D, Smart F, Valantine H. A randomized active-controlled trial of mycophenolate mofetil in heart transplant recipients: Mycophenolate Mofetil Investigators. *Transplantation*. 1998;66:507–515.
146. Costanzo-Nordin MR, O'Sullivan EJ, Johnson MR, Winters GL, Pifarre R, Radvany R, Zucker MJ, Scanlon PJ, Robinson JA. Prospective randomized trial of OKT3- versus horse antithymocyte globulin-based immunosuppressive prophylaxis in heart transplantation. *J Heart Transplant*. 1990;9:306–315.
147. Griffith B, Kormos R, Armitage J, Dummer J, Hardesty R. Comparative trial of immunoprophylaxis with RATG versus OKT3. *J Heart Transplant*. 1990;9:301–305.
148. Deeb GM, Bolling SF, Steimle CN, Dawe JE, McKay AL, Richardson AM. A randomized prospective comparison of MALG with OKT3 for rescue therapy of acute myocardial rejection. *Transplantation*. 1991;51:180–183.
149. Kirklin JK, Bourge RC, White-Williams C, Naftel DC, Thomas FT, Thomas JM, Phillips MG. Prophylactic therapy for rejection after cardiac transplantation: a comparison of rabbit antithymocyte globulin and OKT3. *J Thorac Cardiovasc Surg*. 1990;99:716–724.
150. Weigensberg M, Morecki S, Weiss L, Fuks Z, Slavin S. Suppression of cell-mediated immune responses after total lymphoid irradiation (TLI), I: characterization of suppressor cells of the mixed lymphocyte reaction. *J Immunol*. 1984;132:971–978.
151. Baldwin WM 3rd, Armstrong LP, Samaniego-Picota M, Rahimi S, Zachary AA, Kasper EK, Conte JV, Hruban RH, Rodriguez ER. Antithymocyte globulin is associated with complement deposition in cardiac transplant biopsies. *Hum Immunol*. 2004;65:1273–1280.
152. Colovai AI, Vasilescu ER, Foca-Rodi A, Kim-Schulze S, Hussaini N, D'Agati VD, Markowitz GS, Wang C, Cohen DJ, Hardy MA, Suciu-Foca N. Acute and hyperacute humoral rejection in kidney allograft recipients treated with anti-human thymocyte antibodies. *Hum Immunol*. 2005;66:501–512.
153. Maldonado AQ, Davies NM, Crow SA, Little C, Ojogho ON, Weeks DL. Effects of plasmapheresis on mycophenolic acid concentrations. *Transplantation*. 2011;91:e3–e4.
154. Becker YT, Becker BN, Pirsch JD, Sollinger HW. Rituximab as treatment for refractory kidney transplant rejection. *Am J Transplant*. 2004;4:996–1001.
155. Bittner HB, Dunitz J, Hertz M, Bolman MR 3rd, Park SJ. Hyperacute rejection in single lung transplantation: case report of successful

management by means of plasmapheresis and antithymocyte globulin treatment. *Transplantation*. 2001;71:649–651.
156. Hammond EA, Yowell RL, Greenwood J, Hartung L, Renlund D, Wittwer C. Prevention of adverse clinical outcome by monitoring of cardiac transplant patients for murine monoclonal CD3 antibody (OKT3) sensitization. *Transplantation*. 1993;55:1061–1063.
157. Gopal AK, Press OW. Clinical applications of anti-CD20 antibodies. *J Lab Clin Med*. 1999;134:445–450.
158. Baran DA, Lubitz S, Alvi S, Fallon JT, Kaplan S, Galin I, Correa R, Courtney MC, Chan M, Spielvogel D, Lansman SL, Gass AL. Refractory humoral cardiac allograft rejection successfully treated with a single dose of rituximab. *Transplant Proc*. 2004;36:3164–3166.
159. Garrett HE Jr, Groshart K, Duvall-Seaman D, Combs D, Suggs R. Treatment of humoral rejection with rituximab. *Ann Thorac Surg*. 2002;74:1240–1242.
160. Kaczmarek I, Deutsch MA, Sadoni S, Brenner P, Schmauss D, Daebritz SH, Weiss M, Meiser BM, Reichart B. Successful management of antibody-mediated cardiac allograft rejection with combined immunoadsorption and anti-CD20 monoclonal antibody treatment: case report and literature review. *J Heart Lung Transplant*. 2007;26:511–515.
161. Keren A, Hayes HM, O'Driscoll G. Late humoral rejection in a cardiac transplant recipient treated with the anti-CD20 monoclonal antibody rituximab. *Transplant Proc*. 2006;38:1520–1522.
162. Balfour IC, Fiore A, Graff RJ, Knutsen AP. Use of rituximab to decrease panel-reactive antibodies. *J Heart Lung Transplant*. 2005;24:628–630.
163. McGee EC Jr, Cotts W, Tambur AR, Friedewald J, Kim J, O'Connell J, Wallace S, McCarthy PM. Successful bridge to transplant in a highly sensitized patient with a complicated pump pocket infection. *J Heart Lung Transplant*. 2008;27:568–571.
164. Frampton JE, Wagstaff AJ. Alemtuzumab. *Drugs*. 2003;63:1229–1243.
165. Gilleece MH, Dexter TM. Effect of Campath-1H antibody on human hematopoietic progenitors in vitro. *Blood*. 1993;82:807–812.
166. Morris PJ, Russell NK. Alemtuzumab (Campath-1H): a systematic review in organ transplantation. *Transplantation*. 2006;81:1361–1367.
167. Ciancio G, Burke GW, Gaynor JJ, Sageshima J, Herrada E, Tueros L, Roth D, Kupin W, Rosen A, Esquenazi V, Miller J. Campath-1H induction therapy in African American and Hispanic first renal transplant recipients: 3-year actuarial follow-up. *Transplantation*. 2008;85:507–516.
168. Ciancio G, Burke GW 3rd. Alemtuzumab (Campath-1H) in kidney transplantation. *Am J Transplant*. 2008;8:15–20.
169. Ciancio G, Gaynor JJ, Roth D, Kupin W, Hanson L, Tueros L, Zarak A, Ruiz P, Burke GW 3rd. Randomized trial of thymoglobulin versus alemtuzumab (with lower dose maintenance immunosuppression) versus daclizumab in living donor renal transplantation. *Transplant Proc*. 2010;42:3503–3506.
170. McCurry KR, Iacono A, Zeevi A, Yousem S, Girnita A, Husain S, Zaldonis D, Johnson B, Hattler BG, Starzl TE. Early outcomes in human lung transplantation with Thymoglobulin or Campath-1H for recipient pretreatment followed by posttransplant tacrolimus near-monotherapy. *J Thorac Cardiovasc Surg*. 2005;130:528–537.
171. Shyu S, Dew MA, Pilewski JM, Devito Dabbs AJ, Zaldonis DB, Studer SM, Crespo MM, Toyoda Y, Bermudez CA, McCurry KR. Five-year outcomes with alemtuzumab induction after lung transplantation. *J Heart Lung Transplant*. 2011;30:743–754.
172. van Loenhout KC, Groves SC, Galazka M, Sherman B, Britt E, Garcia J, Griffith B, Iacono A. Early outcomes using alemtuzumab induction in lung transplantation. *Interact Cardiovasc Thorac Surg*. 2010;10:190–194.
173. Thomas PG, Woodside KJ, Lappin JA, Vaidya S, Rajaraman S, Gugliuzza KK. Alemtuzumab (Campath 1H) induction with tacrolimus monotherapy is safe for high immunological risk renal transplantation. *Transplantation*. 2007;83:1509–1512.
174. Ciancio G, Gaynor JJ, Guerra G, Sageshima J, Chen L, Mattiazzi A, Roth D, Kupin W, Tueros L, Flores S, Hanson L, Vianna R, Burke GW 3rd. Randomized trial of three induction antibodies in kidney transplantation: long-term results. *Transplantation*. 2014;97:1128–1138.
175. Ciancio G, Sageshima J, Akpinar E, Gaynor JJ, Chen L, Zarak A, Hanson L, Tueros L, Guerra G, Mattiazzi A, Kupin W, Roth D, Ricordi C, Burke GW 3rd. A randomized pilot study of donor stem cell infusion in living-related kidney transplant recipients receiving alemtuzumab. *Transplantation*. 2013;96:800–806.
176. Ciancio G, Gaynor JJ, Sageshima J, Guerra G, Zarak A, Roth D, Brown R, Kupin W, Chen L, Hanson L, Tueros L, Ruiz P, Livingstone AS, Burke GW 3rd. Randomized trial of dual antibody induction therapy with steroid avoidance in renal transplantation. *Transplantation*. 2011;92:1348–1357.
177. Reams B, Musselwhite L, Zaas D, Steele M, Garantziotis S, Eu P, Snyder L, Curl J, Lin S, Davis R, Palmer SM. Alemtuzumab in the treatment of refractory acute rejection and bronchiolitis obliterans syndrome after human lung transplantation. *Am J Transplant*. 2007;7:2802–2808.
178. Basu A, Ramkumar M, Tan H, Khan A, McCauley J, Marcos A, Fung J, Starzl T, Shapiro R. Reversal of acute cellular rejection after renal transplantation with Campath-1H. *Transplant Proc*. 2005;37:923–926.
179. Das B, Shoemaker L, Recto M, Austin E, Dowling R. Alemtuzumab (Campath-1H) induction in a pediatric heart transplant: successful outcome and rationale for its use. *J Heart Lung Transplant*. 2008;27:242–244.
180. Wang H, Arp J, Liu W, Faas SJ, Jiang J, Gies DR, Ramcharran S, Garcia B, Zhong R, Rother RP. Inhibition of terminal complement components in presensitized transplant recipients prevents antibody-mediated rejection leading to long-term graft survival and accommodation. *J Immunol*. 2007;179:4451–4463.
181. Woodside KJ, Lick SD. Alemtuzumab (Campath 1H) as successful salvage therapy for recurrent steroid-resistant heart transplant rejection. *J Heart Lung Transplant*. 2007;26:750–752.
182. Eckman PM, Hanna M, Taylor DO, Starling RC, Gonzalez-Stawinski GV. Management of the sensitized adult heart transplant candidate. *Clin Transplant*. 2010;24:726–734.
183. Kyle RA, Rajkumar SV. Multiple myeloma [published correction appears in *N Engl J Med*. 2005;352:1163]. *N Engl J Med*. 2004;351:1860–1873.
184. Richardson PG, Barlogie B, Berenson J, Singhal S, Jagannath S, Irwin D, Rajkumar SV, Srkalovic G, Alsina M, Alexanian R, Siegel D, Orlowski RZ, Kuter D, Limentani SA, Lee S, Hideshima T, Esseltine DL, Kauffman M, Adams J, Schenkein DP, Anderson KC. A phase 2 study of bortezomib in relapsed, refractory myeloma. *N Engl J Med*. 2003;348:2609–2617.
185. Everly JJ, Walsh RC, Alloway RR, Woodle ES. Proteasome inhibition for antibody-mediated rejection. *Curr Opin Organ Transplant*. 2009;14:662–666.
186. Sberro-Soussan R, Zuber J, Suberbielle-Boissel C, Candon S, Martinez F, Snanoudj R, Rabant M, Pallet N, Nochy D, Anglicheau D, Leruez M, Loupy A, Thervet E, Hermine O, Legendre C. Bortezomib as the sole post-renal transplantation desensitization agent does not decrease donor-specific anti-HLA antibodies. *Am J Transplant*. 2010;10:681–686.
187. Trivedi HL, Terasaki PI, Feroz A, Everly MJ, Vanikar AV, Shankar V, Trivedi VB, Kaneku H, Idica AK, Modi PR, Khemchandani SI, Dave SD. Abrogation of anti-HLA antibodies via proteasome inhibition. *Transplantation*. 2009;87:1555–1561.
188. Perry DK, Burns JM, Pollinger HS, Amiot BP, Gloor JM, Gores GJ, Stegall MD. Proteasome inhibition causes apoptosis of normal human plasma cells preventing alloantibody production. *Am J Transplant*. 2009;9:201–209.
189. Solez K, Colvin RB, Racusen LC, Sis B, Halloran PF, Birk PE, Campbell PM, Cascalho M, Collins AB, Demetris AJ, Drachenberg CB, Gibson IW, Grimm PC, Haas M, Lerut E, Liapis H, Mannon RB, Marcus PB, Mengel M, Mihatsch MJ, Nankivell BJ, Nickeleit V, Papadimitriou JC, Platt JL, Randhawa P, Roberts I, Salinas-Madriga L, Salomon DR, Seron D, Sheaff M, Weening JJ. Banff '05 meeting report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am J Transplant*. 2007;7:518–526.
190. Hillmen P, Young NS, Schubert J, Brodsky RA, Socie G, Muus P, Roth A, Szer J, Elebute MO, Nakamura R, Browne P, Risitano AM, Hill A, Schrezenmeier H, Fu CL, Maciejewski J, Rollins SA, Mojcik CF, Rother RP, Luzzatto L. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 2006;355:1233–1243.
191. Hill A, Hillmen P, Richards SJ, Elebute D, Marsh JC, Chan J, Mojcik CF, Rother RP. Sustained response and long-term safety of eculizumab in paroxysmal nocturnal hemoglobinuria. *Blood*. 2005;106:2559–2565.
192. Rother RP, Arp J, Jiang J, Ge W, Faas SJ, Liu W, Gies DR, Jevnikar AM, Garcia B, Wang H. C5 blockade with conventional immunosuppression induces long-term graft survival in presensitized recipients. *Am J Transplant*. 2008;8:1129–1142.
193. Pruitt SK, Bollinger RR. The effect of soluble complement receptor type 1 on hyperacute allograft rejection. *J Surg Res*. 1991;50:350–355.
194. Wang H, Jiang J, Liu W, Kubelik D, Chen G, Gies D, Garcia B, Zhong R, Rother RP. Prevention of acute vascular rejection by a functionally blocking anti-C5 monoclonal antibody combined with cyclosporine. *Transplantation*. 2005;79:1121–1127.
195. Stegall MD, Diwan T, Raghavaiah S, Cornell LD, Burns J, Dean PG, Cosio FG, Gandhi MJ, Kremers W, Gloor JM. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *Am J Transplant*. 2011;11:2405–2413.
196. Allison AC, Eugui EM. Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology*. 2000;47:85–118.
197. Lietz K, John R, Schuster M, Ankersmit J, Burke E, Suciu-Foca N, Edwards N, Mancini D, Itescu S. Mycophenolate mofetil reduces anti-HLA antibody production and cellular rejection in heart transplant recipients. *Transplant Proc*. 2002;34:1828–1829.
198. Rose AG. Understanding the pathogenesis and the pathology of hyperacute cardiac rejection. *Cardiovasc Pathol*. 2002;11:171–176.

199. Weigel G, Griesmacher A, Karimi A, Zuckermann AO, Grimm M, Mueller MM. Effect of mycophenolate mofetil therapy on lymphocyte activation in heart transplant recipients. *J Heart Lung Transplant*. 2002;21:1074–1079.
200. Hamburger J, Vaysse J, Crosnier J, Auvert J, Lalanne CM, Hopper J Jr. Renal homotransplantation in man after radiation of the recipient: experience with six patients since 1959. *Am J Med*. 1962;32:854–871.
201. Slavin S, Reitz B, Bieber CP, Kaplan HS, Strober S. Transplantation tolerance in adult rats using total lymphoid irradiation: permanent survival of skin, heart, and marrow allografts. *J Exp Med*. 1978;147:700–707.
202. Kahn DR, Hong R, Greenberg AJ, Gilbert EF, Dacumos GC, Dufek JH. Total lymphatic irradiation and bone marrow in human heart transplantation. *Ann Thorac Surg*. 1984;38:169–171.
203. Winterland AW, Frist WH, Eisert DR, Stroup S. Total lymphoid irradiation to treat recurrent heart rejection. *Clin Transpl*. 1989:323.
204. Frist WH, Winterland AW, Gerhardt EB, Merrill WH, Atkinson JB, Eastburn TE, Stewart JR, Eisert DR. Total lymphoid irradiation in heart transplantation: adjunctive treatment for recurrent rejection. *Ann Thorac Surg*. 1989;48:863–864.
205. Hunt SA, Strober S, Hoppe RT, Stinson EB. Total lymphoid irradiation for treatment of intractable cardiac allograft rejection. *J Heart Lung Transplant*. 1991;10:211–216.
206. Salter SP, Salter MM, Kirklin JK, Bourge RC, Naftel DC. Total lymphoid irradiation in the treatment of early or recurrent heart transplant rejection. *Int J Radiat Oncol Biol Phys*. 1995;33:83–88.
207. Wolden SL, Tate DJ, Hunt SA, Strober S, Hoppe RT. Long-term results of total lymphoid irradiation in the treatment of cardiac allograft rejection. *Int J Radiat Oncol Biol Phys*. 1997;39:953–960.
208. Madden BP, Barros J, Backhouse L, Stamenkovic S, Tait D, Murday A. Intermediate term results of total lymphoid irradiation for the treatment of non-specific graft dysfunction after heart transplantation. *Eur J Cardiothorac Surg*. 1999;15:663–666.
209. Madden BP, Backhouse L, McClosky D, Reynolds L, Tait D, Murday A. Total lymphoid irradiation as rescue therapy after heart transplantation. *J Heart Lung Transplant*. 1996;15:234–238.
210. Evans MA, Schomberg PJ, Rodeheffer RJ, Katzmann JA, Schnell WA Jr, Tazelaar HD, McGregor CG, Edwards BS. Total lymphoid irradiation: a novel and successful therapy for resistant cardiac allograft rejection. *Mayo Clinic Proc*. 1992;67:785–790.
211. Ross HJ, Gullestad L, Pak J, Slauson S, Valantine HA, Hunt SA. Methotrexate or total lymphoid radiation for treatment of persistent or recurrent allograft cellular rejection: a comparative study. *J Heart Lung Transplant*. 1997;16:179–189.
212. Valentine VG, Robbins RC, Wehner JH, Patel HR, Berry GJ, Theodore J. Total lymphoid irradiation for refractory acute rejection in heart-lung and lung allografts. *Chest*. 1996;109:1184–1189.
213. Lim TS, O'Driscoll G, Freund J, Peterson V, Hayes H, Heywood J. Short-course total lymphoid irradiation for refractory cardiac transplantation rejection. *J Heart Lung Transplant*. 2007;26:1249–1254.
214. Trachiotis GD, Johnston TS, Vega JD, Crocker IR, Chesnut N, Lutz JF, Smith AL, Kanter KR. Single-field total lymphoid irradiation in the treatment of refractory rejection after heart transplantation. *J Heart Lung Transplant*. 1998;17:1045–1048.
215. Ghadjar P, Joos D, Martinelli M, Hullin R, Zwahlen M, Lossl K, Carrel T, Aebersold DM, Mohacsi P. Tailored total lymphoid irradiation in heart transplant patients: 10-years experience of one center. *Radiat Oncol*. 2010;5:3.
216. Pelletier MP, Coady M, Macha M, Oyer PE, Robbins RC. Coronary atherosclerosis in cardiac transplant patients treated with total lymphoid irradiation. *J Heart Lung Transplant*. 2003;22:124–129.
217. Salter MM, Kirklin JK, Bourge RC, Naftel DC, White-Williams C, Tarkka M, Waits E, Bucy RP. Total lymphoid irradiation in the treatment of early or recurrent heart rejection. *J Heart Lung Transplant*. 1992;11:902–911.
218. Locke J, Zachary A, Haas M, Melancon J, Warren D, Simpkins C, Segev D, Montgomery R. The utility of splenectomy as rescue treatment for severe acute antibody mediated rejection. *Am J Transplant*. 2007;7:842–846.
219. Takahashi K, Saito K, Takahara S, Okuyama A, Tanabe K, Toma H, Uchida K, Hasegawa A, Yoshimura N, Kamiryo Y; Japanese ABO-Incompatible Kidney Transplantation Committee. Excellent long-term outcome of ABO-incompatible living donor kidney transplantation in Japan. *Am J Transplant*. 2004;4:1089–1096.
220. Uchida J, Machida Y, Iwai T, Naganuma T, Kitamoto K, Iguchi T, Maeda S, Kamada Y, Kuwabara N, Kim T, Nakatani T. Desensitization protocol in highly HLA-sensitized and ABO-incompatible high titer kidney transplantation. *Transplant Proc*. 2010;42:3998–4002.
221. Ishida H, Omoto K, Shimizu T, Shirakawa H, Nishida H, Xiaobei L, Yamaguchi Y, Tanabe K. Usefulness of splenectomy for chronic active antibody-mediated rejection after renal transplantation. *Transpl Int*. 2008;21:602–604.
222. Sawada T, Fuchinoue S, Teraoka S. Successful A1-to-O ABO-incompatible kidney transplantation after a preconditioning regimen consisting of anti-CD20 monoclonal antibody infusions, splenectomy, and double-filtration plasmapheresis. *Transplantation*. 2002;74:1207–1210.
223. Gloor JM, Lager DJ, Fidler ME, Grande JP, Moore SB, Winters JL, Kremers WK, Stegall MD. A comparison of splenectomy versus intensive posttransplant antidonor blood group antibody monitoring without splenectomy in ABO-incompatible kidney transplantation. *Transplantation*. 2005;80:1572–1577.
224. Kaplan B, Gangemi A, Thielke J, Oberholzer J, Sankary H, Benedetti E. Successful rescue of refractory, severe antibody mediated rejection with splenectomy. *Transplantation*. 2007;83:99–100.
225. Alexander J. The late adverse effect of splenectomy on patient survival following cadaveric renal transplantation. *Transplantation*. 1984;37:467–470.
226. Marasco SF, Esmore DS, Negri J, Rowland M, Newcomb A, Rosenfeldt FL, Bailey M, Richardson M. Early institution of mechanical support improves outcomes in primary cardiac allograft failure. *J Heart Lung Transplant*. 2005;24:2037–2042.
227. Arpesella G, Loforte A, Mikus E, Mikus PM. Extracorporeal membrane oxygenation for primary allograft failure. *Transplant Proc*. 2008;40:3596–3597.
228. Leprince P, Aubert S, Bonnet N, Rama A, Léger P, Bors V, Levasseur J, Szefner J, Vaissier E, Pavie A. Peripheral extracorporeal membrane oxygenation (ECMO) in patients with posttransplant cardiac graft failure. *Transplant Proc*. 2005;37:2879–2880.
229. Santise G, Petrou M, Pepper JR, Dreyfus G, Khaghani A, Birks EJ. Levitronix as a short-term salvage treatment for primary graft failure after heart transplantation. *J Heart Lung Transplant*. 2006;25:495–498.
230. Tenderich G, Koerner MM, Stuettgen B, Minami K, El-Banayosy A, Arusoglu L, Mirow N, Wlost S, Gromzik H, Kleesiek K, Meyer H, Koerfer R. Mechanical circulatory support after orthotopic heart transplantation. *Int J Artif Organs*. 1998;21:414–416.
231. Listijono DR, Watson A, Pye R, Keogh AM, Kotlyar E, Spratt P, Granger E, Dhital K, Jansz P, Macdonald PS, Hayward CS. Usefulness of extracorporeal membrane oxygenation for early cardiac allograft dysfunction. *J Heart Lung Transplant*. 2011;30:783–789.
232. Chou N-K, Chi N-H, Ko W-J, Yu H-Y, Huang S-C, Wang S-S, Lin F-Y, Chu S-H, Chen Y-S. Extracorporeal membrane oxygenation for perioperative cardiac allograft failure. *ASAIO J*. 2006;52:100–103. 10.1097/01.mat.0000196514.69525.d9.
233. Kavarana MN, Sinha P, Naka Y, Oz MC, Edwards NM. Mechanical support for the failing cardiac allograft: a single-center experience. *J Heart Lung Transplant*. 2003;22:542–547.
234. Mihaljevic T, Jarrett CM, Gonzalez-Stawinski G, Smedira NG, Nowicki ER, Thuita L, Mountis M, Blackstone EH. Mechanical circulatory support after heart transplantation. *Eur J Cardiothorac Surg*. 2012;41:200–206.
235. D'Alessandro C, Aubert S, Golmard JL, Praschker BL, Luyt CE, Pavie A, Gandjbakhch I, Leprince P. Extra-corporeal membrane oxygenation temporary support for early graft failure after cardiac transplantation. *Eur J Cardiothorac Surg*. 2010;37:343–349.
236. Stendahl G, Berger S, Ellis T, Gandy K, Mitchell M, Tweddell J, Zangwill S. Humoral rejection after pediatric heart transplantation: a case report. *Prog Transplant*. 2010;20:288–291.
237. Saito S, Matsumiya G, Fukushima N, Sakaguchi T, Fujita T, Ueno T, Miyagawa S, Yamauchi A, Sawa Y. Successful treatment of cardiogenic shock caused by humoral cardiac allograft rejection. *Circ J*. 2009;73:970–973.
238. Morales DLS, Braud BE, Price JF, Dreyer WJ, Denfield SW, Clunie SK, Heinle JS, Fraser CD Jr. Use of mechanical circulatory support in pediatric patients with acute cardiac graft rejection. *ASAIO J*. 2007;53:701–705.
239. Kittleson MM, Patel JK, Moriguchi JD, Kawano M, Davis S, Hage A, Hamilton MA, Esmailian F, Kobashigawa JA. Heart transplant recipients supported with extracorporeal membrane oxygenation: outcomes from a single-center experience. *J Heart Lung Transplant*. 2011;30:1250–1256.
240. Heidt S, Roelen DL, Eijsink C, van Kooten C, Claas FHJ, Mulder A. Effects of immunosuppressive drugs on purified human B cells: evidence supporting the use of MMF and rapamycin [published correction appears in *Transplantation*. 2009;87:307]. *Transplantation*. 2008;86:1292–1300.
241. Schmid C, Garritsen HS, Kelsch R, Cassens U, Baba HA, Sibrowski W, Scheld HH. Suppression of panel-reactive antibodies by treatment with mycophenolate mofetil. *Thorac Cardiovasc Surg*. 1998;46:161–162.
242. Ishida H, Furusawa M, Murakami T, Tokumoto T, Nakajima I, Tanabe K, Fuchinoue S, Toma H. Outcome of an AB0-incompatible renal transplant without splenectomy. *Transpl Int*. 2002;15:56–58.

243. Mannami M, Mitsuhata N. Improved outcomes after ABO-incompatible living-donor kidney transplantation after 4 weeks of treatment with mycophenolate mofetil. *Transplantation*. 2005;79:1756–1758.
244. Taylor DO, Bristow MR, O'Connell JB, Ensley RD, Olsen SL, Hammond EH, Wagoner LE, Renlund DG. A prospective, randomized comparison of cyclophosphamide and azathioprine for early rejection prophylaxis after cardiac transplantation: decreased sensitization to OKT3. *Transplantation*. 1994;58:645–649.
245. Zhu LP, Cupps TR, Whalen G, Fauci A. Selective effects of cyclophosphamide therapy on activation, proliferation, and differentiation of human B cells. *J Clin Invest*. 1987;79:1082–1090.
246. Hammond EH, Yowell RL, Price GD, Menlove RL, Olsen SL, O'Connell JB, Bristow MR, Doty DB, Millar RC, Karwande SV. Vascular rejection and its relationship to allograft coronary artery disease. *J Heart Lung Transplant*. 1992;11(pt 2):S111–S119.
247. Crespo-Leiro MG. Calcineurin inhibitors in heart transplantation. *Transplant Proc*. 2005;37:4018–4020.
248. Bacal F, Sodré GL, Fernandes DA, Aiello VD, Stolf N, Bocchi E, Bellotti G. Methotrexate in acute persistent humoral rejection: an option for graft rescue. *Ann Thorac Surg*. 2003;76:607–610.
249. Costanzo-Nordin M, Grusk B, Silver M, Sobotka P, Winters G, O'Connell J, Pifarré R, Robinson J. Reversal of recalcitrant cardiac allograft rejection with methotrexate. *Circulation*. 1988;78(pt 2):III47–III57.
250. Nair N, Ball T, Uber PA, Mehra MR. Current and future challenges in therapy for antibody-mediated rejection. *J Heart Lung Transplant*. 2011;30:612–617.
251. Chih S, Tinckam KJ, Ross HJ. A survey of current practice for antibody-mediated rejection in heart transplantation. *Am J Transplant*. 2013;13:1069–1074.
252. Pahl E. Transplant coronary artery disease in children. *Prog Pediatr Cardiol*. 2000;11:137–143.
253. Zales VR, Crawford S, Backer CL, Lynch P, Benson DW Jr, Mavroudis C. Spectrum of humoral rejection after pediatric heart transplantation. *J Heart Lung Transplant*. 1993;12:563–571.
254. Everitt MD, Pahl E, Schechtman KB, Zheng J, Ringewald JM, Ecuyer TL, Naftel DC, Kirklin JK, Blume ED, Bullock EA, Canter CE. Rejection with hemodynamic compromise in the current era of pediatric heart transplantation: a multi-institutional study. *J Heart Lung Transplant*. 2011;30:282–288.
255. Pahl E, Naftel DC, Canter CE, Frazier EA, Kirklin JK, Morrow WR; Pediatric Heart Transplant Study. Death after rejection with severe hemodynamic compromise in pediatric heart transplant recipients: a multi-institutional study. *J Heart Lung Transplant*. 2001;20:279–287.
256. Phelps CM, Tissot C, Buckvold S, Gralla J, Dunbar Ivy D, Pietra BA, Miyamoto SD. Outcome of acute graft rejection associated with hemodynamic compromise in pediatric heart transplant recipients. *Pediatr Cardiol*. 2011;32:1–7.
257. Webber SA, Naftel DC, Parker J, Mulla N, Balfour I, Kirklin JK, Morrow R; Pediatric Heart Transplant Study Group. Late rejection episodes more than 1 year after pediatric heart transplantation: risk factors and outcomes. *J Heart Lung Transplant*. 2003;22:869–875.
258. Wright EJ, Fiser WP, Edens RE, Frazier EA, Morrow WR, Imamura M, Jaquiss RD. Cardiac transplant outcomes in pediatric patients with preformed anti-human leukocyte antigen antibodies and/or positive retrospective crossmatch. *J Heart Lung Transplant*. 2007;26:1163–1169.
259. Xydas S, Yang JK, Burke EM, Chen JM, Addonizio LJ, Mital SR, Itescu S, Hsu DT, Lamour JM. Utility of post-transplant anti-HLA antibody measurements in pediatric cardiac transplant recipients. *J Heart Lung Transplant*. 2005;24:1289–1296.
260. Kirk R, Edwards LB, Aurora P, Taylor DO, Christie JD, Dobbels F, Kucheryavaya AY, Rahmel AO, Stehlik J, Hertz MI. Registry of the International Society for Heart and Lung Transplantation: Twelfth Official Pediatric Heart Transplantation Report–2009. *J Heart Lung Transplant*. 2009;28:993–1006.
261. Pahl E, Naftel DC, Kuhn MA, Shaddy RE, Morrow WR, Canter CE, Kirklin J; Pediatric Heart Transplant Study. The impact and outcome of transplant coronary artery disease in a pediatric population: a 9-year multi-institutional study. *J Heart Lung Transplant*. 2005;24:645–651.
262. Chang AT, Platt JL. The role of antibodies in transplantation. *Transplant Rev (Orlando)*. 2009;23:191–198.
263. Ho EK, Vasilescu ER, Vlad G, Marboe CC, Addonizio LJ, Suciu-Foca N. HLA antibodies in pediatric heart transplantation. *Pediatr Transplant*. 2011;15:458–464.
264. Ho EK, Vlad G, Vasilescu ER, de la Torre L, Colovai AI, Burke E, Deng M, Schwartz J, Marboe C, Mancini D, Opelz G, Suciu-Foca N. Pre- and posttransplantation allosensitization in heart allograft recipients: major impact of de novo alloantibody production on allograft survival. *Hum Immunol*. 2011;72:5–10.
265. Rossano JW, Morales DL, Zafar F, Denfield SW, Kim JJ, Jefferies JL, Dreyer WJ. Impact of antibodies against human leukocyte antigens on long-term outcome in pediatric heart transplant patients: an analysis of the United Network for Organ Sharing database. *J Thorac Cardiovasc Surg*. 2010;140:694–699.e2.
266. Jacobs JP, Quintessenza JA, Boucek RJ, Morell VO, Botero LM, Badhwar V, van Gelder HM, Asante-Korang A, McCormack J, Daicoff GR. Pediatric cardiac transplantation in children with high panel reactive antibody. *Ann Thorac Surg*. 2004;78:1703–1709.
267. Kirk R, Edwards LB, Kucheryavaya AY, Aurora P, Christie JD, Dobbels F, Rahmel AO, Stehlik J, Hertz MI. The Registry of the International Society for Heart and Lung Transplantation: Thirteenth Official Pediatric Heart Transplantation Report–2010. *J Heart Lung Transplant*. 2010;29:1119–1128.
268. Lamour JM, Kanter KR, Naftel DC, Chrisant MR, Morrow WR, Clemson BS, Kirklin JK; Cardiac Transplant Registry Database; Pediatric Heart Transplant Study. The effect of age, diagnosis, and previous surgery in children and adults undergoing heart transplantation for congenital heart disease. *J Am Coll Cardiol*. 2009;54:160–165.
269. Shaddy RE, Hunter DD, Osborn KA, Lambert LM, Minich LLA, Hawkins JA, McGough EC, Fuller TC. Prospective analysis of HLA immunogenicity of cryopreserved valved allografts used in pediatric heart surgery. *Circulation*. 1996;94:1063–1067.
270. Hawkins JA, Breinholt JP, Lambert LM, Fuller TC, Profaizer T, McGough EC, Shaddy RE. Class I and class II anti-HLA antibodies after implantation of cryopreserved allograft material in pediatric patients. *J Thorac Cardiovasc Surg*. 2000;119:324–330.
271. Brancaccio G, Amodeo A, Ricci Z, Morelli S, Gagliardi MG, Iacobelli R, Michielon G, Picardo S, Parisi F, Pongiglione G, Di Donato RM. Mechanical assist device as a bridge to heart transplantation in children less than 10 kilograms. *Ann Thorac Surg*. 2010;90:58–62.
272. Amodeo A, Brancaccio G, Michielon G, Filippelli S, Ricci Z, Morelli S, Gagliardi MG, Iacobelli R, Pongiglione G, Di Donato RM. Pneumatic pulsatile ventricular assist device as a bridge to heart transplantation in pediatric patients. *Artif Organs*. 2010;34:1017–1022.
273. Gandhi SK, Huddleston CB, Balzer DT, Epstein DJ, Boschert TA, Canter CE. Biventricular assist devices as a bridge to heart transplantation in small children. *Circulation*. 2008;118:S89–S93.
274. Yang J, Schall C, Smith D, Kreuser L, Zamberlan M, King K, Gajarski R. HLA sensitization in pediatric pre-transplant cardiac patients supported by mechanical assist devices: the utility of Luminex. *J Heart Lung Transplant*. 2009;28:123–129.
275. O'Connor MJ, Menteer J, Chrisant MR, Monos D, Lind C, Levine S, Gaynor JW, Hanna BD, Paridon SM, Ravishankar C, Kaufman BD. Ventricular assist device-associated anti-human leukocyte antigen antibody sensitization in pediatric patients bridged to heart transplantation. *J Heart Lung Transplant*. 2010;29:109–116.
275a. Chin C, Naftel D, Pahl E, Shankel T, Clark ML, Gamberg P, Kirklin J, Webber S. Cardiac re-transplantation in pediatrics: a multi-institutional study. *J Heart Lung Transplant*. 2006;25:1420–1424.
276. Feingold B, Bowman P, Zeevi A, Girnita AL, Quivers ES, Miller SA, Webber SA. Survival in allosensitized children after listing for cardiac transplantation. *J Heart Lung Transplant*. 2007;26:565–571.
277. Solez K, Colvin RB, Racusen LC, Haas M, Sis B, Mengel M, Halloran PF, Baldwin W, Banfi G, Collins AB, Cosio F, David DS, Drachenberg C, Einecke G, Fogo AB, Gibson IW, Glotz D, Iskandar SS, Kraus E, Lerut E, Mannon RB, Mihatsch M, Nankivell BJ, Nickeleit V, Papadimitriou JC, Randhawa P, Regele H, Renaudin K, Roberts I, Seron D, Smith RN, Valente M. Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant*. 2008;8:753–760.
278. Frey BM, Frey FJ. Clinical pharmacokinetics of prednisone and prednisolone. *Clin Pharmacokinet*. 1990;19:126–146.
279. Dowling RD, Jones JW, Carroll MS, Gray LA Jr. Use of intravenous immunoglobulin in sensitized LVAD recipients. *Transplant Proc*. 1998;30:1110–1111.
280. Pahl E, Crawford SE, Cohn RA, Rodgers S, Wax D, Backer CL, Mavroudis C, Gidding SS. Reversal of severe late left ventricular failure after pediatric heart transplantation and possible role of plasmapheresis. *Am J Cardiol*. 2000;85:735–739.
281. De Vlaminck I, Valantine HA, Snyder TM, Strehl C, Cohen G, Luikart H, Neff NF, Okamoto J, Bernstein D, Weisshaar D, Quake SR, Khush KK. Circulating cell-free DNA enables noninvasive diagnosis of heart transplant rejection. *Sci Transl Med*. 2014;6:241–277.

KEY WORDS: AHA Scientific Statements ■ allograft ■ antibody-mediated rejection ■ cardiac transplantation ■ heart transplantation