

ISHLT CONSENSUS

Report from a consensus conference on antibody-mediated rejection in heart transplantation

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BACKGROUND: The problem of AMR remains unsolved because standardized schemes for diagnosis and treatment remains contentious. Therefore, a consensus conference was organized to discuss the current status of antibody-mediated rejection (AMR) in heart transplantation.

METHODS: The conference included 83 participants (transplant cardiologists, surgeons, immunologists and pathologists) representing 67 heart transplant centers from North America, Europe, and Asia who all participated in smaller break-out sessions to discuss the various topics of AMR and attempt to achieve consensus.

RESULTS: A tentative pathology diagnosis of AMR was established, however, the pathologist felt that further discussion was needed prior to a formal recommendation for AMR diagnosis. One of the most important outcomes of this conference was that a clinical definition for AMR (cardiac dysfunction and/or circulating donor-specific antibody) was no longer believed to be required due to recent publications demonstrating that asymptomatic (no cardiac dysfunction) biopsy-proven AMR is associated with subsequent greater mortality and greater development of cardiac allograft vasculopathy. It was also noted that donor-specific antibody is not always detected during AMR episodes as the antibody may be adhered to the donor heart. Finally, recommendations were made for the timing for specific staining of endomyocardial biopsy specimens and the frequency by which circulating antibodies should be assessed. Recommendations for management and future clinical trials were also provided.

KEYWORDS:

heart transplant;
antibody;
rejection;
treatment;
outcomes

CONCLUSIONS: The AMR Consensus Conference brought together clinicians, pathologists and immunologists to further the understanding of AMR. Progress was made toward a pathology AMR grading scale and consensus was accomplished regarding several clinical issues. *J Heart Lung Transplant* 2011;30:252–69 © 2011 International Society for Heart and Lung Transplantation All rights reserved.

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A consensus conference was organized on April 20, 2010, to assess the current status of antibody-mediated rejection (AMR) in heart transplantation. The conference had 83 participants (transplant cardiologists, surgeons, immunologists and pathologists, see Appendix A) representing 67 heart transplant centers from North America, Europe, and Asia. Because of traveling difficulties imposed by Icelandic volcanic ash, participation by several Northern Europeans was facilitated by an Internet link.

Before the conference, survey data regarding clinical information about AMR was submitted by 46 of the 67 centers that participated in the conference and is summarized in Table 1. This survey provided background information on the pre-conference beliefs about AMR and contemporary practice considerations. AMR was reported in 6% of 5,406 heart transplant patients whose data was submitted. Criteria for diagnosis of AMR included various combinations of factors, including cardiac dysfunction, pathologic findings from endomyocardial biopsy specimens (from both histology and immunopathology stains), and circulating antibodies. However, of great concern was that 53% of centers diagnosed AMR on the basis of cardiac dysfunction accompanied by a negative endomyocardial biopsy specimen. This underscored the need for this conference to clearly define AMR so as to standardize the approach and management.

Many questions arise in regard to AMR in heart transplantation, specifically:

- Pathologic definitions: Can histology alone suffice? Are histology and immunopathology both needed for diagnosis?
- Clinical definition of AMR: Is it necessary? Is there a requirement for cardiac dysfunction? What is the meaning of asymptomatic AMR (biopsy specimen findings only, without symptoms or cardiac dysfunction)?
- Are circulating antibodies a prerequisite for AMR? What are the roles of donor-specific antibody (DSA), non-specific human leukocyte antigen (HLA) antibody, and non-HLA antibody?
- What are the appropriate monitoring intervals to detect AMR for endomyocardial biopsy specimens (eg, when do we perform immunopathology stains?) and blood draws for circulating antibodies?

The following is a summary of the AMR Consensus Conference, which addressed these questions. This sum-

mary will also include presentations given at the conference and the results of the breakout sessions that followed which helped to formulate the consensus points agreed on in the final session. This summary of the AMR Consensus Conference reflects the current state of AMR in heart transplantation and hopefully will lead to further understanding, clarification, and treatment options for patients experiencing this form of rejection.

Clinical background

Although the advent of immunosuppressants, such as cyclosporine, has significantly lowered the frequency of acute cellular rejection after heart transplantation, the incidence of AMR remains relatively unaffected.^{1,2} The problem of AMR remains unsolved because standardized schemes for diagnosis and treatment remain contentious, and current immunosuppressive regimens are largely intended to interfere in T-cell signaling pathways.³ As a result, AMR continues to appear in roughly 10% to 20% of heart transplant patients, correlating with factors of poor outcome such as increased incidence for hemodynamic compromise rejection, greater development of cardiac allograft vasculopathy (CAV), and higher incidence of death.^{4–7}

Evaluation of AMR first began with Herskowitz et al⁸ in 1987, who described it as a type of rejection characterized by arteriolar vasculitis and poor outcome in heart transplant recipients. Recognizing the importance of AMR, Hammond et al⁹ went on to supplement this literature, providing the initial immunohistochemical evidence that AMR involved antibody deposition with subsequent complement activation. Although some critics still doubt the existence of “antibody-mediated” or “vascular” rejection, the pathologic changes of AMR have been reviewed by the International Society for Heart and Lung Transplantation (ISHLT) and identified by a blueprint of capillary endothelial changes, macrophage and neutrophil infiltration, interstitial edema, and linear accumulation of immunoglobulins and complement, especially complement component C4d along capillary endothelium.¹⁰

Since this time, a number of studies have examined the many different features of AMR. From these mainly observational studies, AMR has been found to occur both early and late after transplantation and has been identified with risk factors such as female gender, elevated pre-transplant

Table 1 Antibody-Mediated Rejection in Heart Transplantation Pre-conference Survey Results (46 Participating Centers Represented)

The highlights from the antibody-mediated rejection (AMR) pre-conference survey (conducted January 2010 through April 2010) are the following:

- The total number of treated AMR patients was 324 of 5406 (6%)
 - The total number of AMR patients with PRA > 10% before transplant was 114 of 324 (35%)
 - The total number of sensitized patients treated pre-transplant to reduce circulating antibodies was 37 of 114 (32%)
- Other characteristics regarding AMR included:
 - 70% of treated AMR patients recover to left ventricular ejection fraction > 45%
 - 20% of AMR patients with recurrent AMR
 - 53% of centers diagnose AMR as a result of negative biopsy and reduced cardiac function
 - Of these centers, almost half of AMR episodes reported were diagnosed in this manner
- The criteria used by centers to detect AMR was:
 - Cardiac dysfunction: 79%
 - Immunohistochemistry: 68%
 - Immunofluorescence: 67%
 - Histologic findings: 57%
 - Donor-specific antibodies: 52%
- The assays used by centers to detect circulating antibodies were:
 - Luminex: 93%
 - Complement-dependent cytotoxicity: 43%
 - Flow cytometry: 43%
 - Enzyme linked immunosorbent assay: 21%
- The assessment of circulating antibodies was as follows:
 - 35% of centers routinely monitor for circulating antibodies post transplant
 - 79% of centers quantify amount of specific antibodies that are detected
 - 15% of centers treat asymptomatic donor-specific antibodies in post-heart transplant patients
 - 14% of centers evaluate for non-human leukocyte antigen antibodies
- The AMR treatment used by centers for the initial and secondary therapy, respectively, was:
 - Plasmapheresis: 81% and 16%
 - Intravenous Solu-Medrol: 79% and 18%
 - Intravenous immunoglobulin: 67% and 29%
 - Rituximab: 52% and 58%
 - Anti-thymocyte globulin: 36% and 29%
 - Photopheresis: 7% and 16%
 - Bortezomib: 7% and 8%
 - Total lymphoid irradiation: 0% and 21%
 - Other: 12% and 11%

panel-reactive antibodies (PRAs), development of de novo DSAs late after transplantation, positive donor-specific crossmatch, prior sensitization to OKT3, cytomegalovirus (CMV) seropositivity, prior implantation of a ventricular assist device, and retransplantation.^{4,10-13} In clinical practice, AMR is generally diagnosed and treated in patients with clinical symptoms of heart failure and evidence for left ventricular dysfunction in the absence of cellular infiltrates on endomyocardial biopsy (EMB) specimens. However, without clear pathologic and immunologic findings confirming AMR, these previous declared episodes of AMR would come into question.

Nonetheless, although such factors do provide some insight into the nature of the disease, much work is needed with respect to identification, clarification, and understanding of AMR, especially in patients with normal cardiac function and no symptoms of heart failure (asymptomatic) but with circulating DSAs or positive biopsy specimens, or both. In addition, the recommended frequency after heart transplant for monitoring circulating antibody or the impor-

tance of DSA titers has yet to be determined. Likewise, the criteria for diagnosis and categorization of AMR require further consideration. As a group, we aimed to resolve these issues and others in an attempt to guide the pursuit of future clinical trials and to improve the current quality of medicine.

Specific background topic presentations

I. Mechanisms of AMR: Adriana Zeevi, PhD

AMR in heart transplantation is associated with hemodynamic compromise, increased graft loss, CAV, and increased death.¹⁴ Rejection caused by an antibody is mediated by different mechanisms compared with T-cell rejection.³ Classically, antibody induces acute rejection through the fixation and activation of the complement cascade, resulting in tissue injury and coagulation.³ Comple-

ment, which is a multifunctional system of receptors, regulators, and effector molecules, is a very powerful amplifier of innate and adaptive immunity contributing to the pathogenesis of AMR.¹⁵

Activation of the complement cascade also generates biologically active complement split products, including C3a, C4a, and C5a, that can initiate vasoactive responses and are potent mediators of chemotaxis of neutrophils, monocytes, and macrophages.¹⁵ Furthermore, even sublytic amounts of the terminal complement components (C5b, C6, C7, C8 and C9) may initiate multiple pro-inflammatory changes in endothelial cells and smooth muscle cells.¹⁵ The vascular responses to C5a and membrane attack complex include release of von Willebrand factor, P-selectin, and CD63 from the Weibel-Palade storage granules.¹⁵ The interaction of platelets with endothelial cells is promoted through their receptors for P-selectin and vascular cell adhesion molecules expressed by activated endothelial cells.¹⁶ Adherent platelets release granules containing inflammatory molecules, such as regulated upon activation normal T-cell expressed and secreted (RANTES), interleukin-1b, and macrophage inflammatory protein-1, thereby further enhancing leukocyte localization and activation.¹⁶

High titers of anti-HLA antibodies bound to the target HLA antigen on endothelial cells can also up-regulate fibroblast growth factor receptor on endothelial cells, promoting fibroblast growth factor-mediated endothelial cell proliferation.¹⁷ Binding of HLA antibodies also triggers activation of mammalian target of rapamycin complex 1 and phosphorylation of downstream targets S6 kinase and S6 ribosomal protein (S6RP), resulting in protein synthesis and proliferation.¹⁷ Lepin et al¹⁸ demonstrated that p-S6RP staining of capillary endothelial cells is associated with AMR, C4d, and circulating donor-specific anti-HLA antibodies.

Recent studies have demonstrated a pattern of altered endothelial gene expression in biopsy specimens from patients with alloantibodies and acute or chronic renal dysfunction.^{19,20} The expression of these endothelial-associated transcripts, detected by microarray, were selectively higher in C4d-positive AMR than in T-cell mediated rejection.¹⁹ In addition, renal allograft recipients with active AMR and circulating alloantibodies expressed high endothelial-associated transcript scores even in the absence of C4d staining.²¹ Increased endothelial transcripts were also detected in cardiac biopsy specimens in the presence of circulating alloantibodies and were associated with graft dysfunction. This molecular endothelial cell phenotype in AMR indicates endothelial activation and may therefore be a sensitive and specific method to diagnose an antibody-mediated process in the presence or absence of C4d.^{19,20}

II. Standardization of the pathologic and immunologic criteria for the diagnosis of AMR in heart transplantation: Report in the framework of ISHLT and Banff: E. Rene Rodriguez, MD

The Heart Session of the Tenth Banff Conference on Allograft Pathology, held in August 2009, attempted to stan-

dardize the pathologic and immunologic criteria for the diagnosis of AMR in heart transplantation. This session was organized with mutual agreement of the ISHLT Board of Directors and the organizers of the Banff Conference on Allograft Pathology.

More than 60 participants attended the heart session, including immunologists, pathologists, and at least 10 clinical cardiologists (out of more than 250 attendees to the entire conference). Introductory presentations encompassed gene expression in heart biopsy specimens, with focus on the microvasculature, a brief review of the complement system and its regulators, and aspects of DSA testing in pre-sensitized patients and post-transplant testing recommendations.

These were followed by presentation of 2 surveys on the practice and diagnostic approach to AMR in Europe and in North America. Both surveys substantiated the lack of a uniform, standardized approach to the diagnosis of AMR in 29 centers surveyed in Europe and 94 in North America. The survey presentation was followed by presentation of specific data of evaluation for AMR in the United Kingdom and in Boston and Cleveland.

The afternoon session was dedicated to the presentation and discussion of the results of an exercise in the reproducibility of immunostaining human myocardium to detect products of complement activation C4d and C3d. These included 2 non-transplant control cases and 10 post-transplant autopsy cases in which AMR had been diagnosed. Thirteen centers participated by staining the exact same tissues with their local methods for detection of C4d deposition by immunoperoxidase. Seven centers also immunostained these tissues to detect C3d deposition. This exercise showed high reproducibility of the immunoperoxidase stains, with expected variations on the "extreme" cases (ie, very weak or very strong immunoreactivity). A rapid consensus was achieved in several points:

1. There is very good reproducibility between centers in North America and Europe in immunoperoxidase staining for C4d and C3d in the myocardium. Minor technical adjustment to the immunohistochemical techniques should provide close to 100% reproducibility.
2. There was consensus that the vascular territory to be evaluated should only include capillary vessels. Arterioles, veins, arteries, endocardium, vessels in Quilty lesions, myocyte sarcoplasm, and the interstitial connective tissue should not be considered in the immunohistochemical evaluation of AMR.
3. The use of immunostains with low sensitivity and low specificity reported in recent publications, such as immunoglobulins, should be obviated.
4. There seems to be good equivalence between immunofluorescence detection of C4d and C3d and immunoperoxidase detection of these two markers. Two ongoing studies should confirm reproducibility of these preliminary results in heart tissues.
5. Agreement was reached on a tiered system of light microscopic and immunohistochemical evaluation for markers of AMR.

6. There is some clinical evidence of protective mechanisms.
7. There should be a team approach to the evaluation and diagnosis of AMR in a patient, which includes the involvement of pathologists, immunologists, and cardiologists.
8. There should be minimum times in which serum is collected for storage in case it is needed for the diagnosis of AMR.
9. There is further reproducibility on the evaluation of C4d and C3d as markers between North American and European centers.

Additional work is needed in defining and standardizing the possible use of staining intensity scales for scoring C4d and C3d and their potential use as diagnostic criterion. Specific recommendations for this diagnosis in pediatric heart transplant recipients are imperative. Equivalence studies to compare immunofluorescence with immunoperoxidase are ongoing. Specific guidelines on reporting can then be crafted. The requirement that clinical dysfunction be present for the diagnosis of AMR, as the current ISHLT working formulation requires, was not discussed. The concepts of asymptomatic AMR/sub-clinical AMR/mixed AMR/and chronic AMR in heart transplantation were also not discussed, because it would be premature to define entities and processes when the basic definition of the acute underlying mechanism(s) and diagnosis has not yet been standardized.

In summary, the progress made during this session at the Banff conference was significant because it provided consensus on several important points. Further work and discussion is warranted.

III. A European approach to the pathologic diagnosis of AMR: Margaret Burke, MD, and Annalisa Angelini, MD, on behalf of the Association for European Cardiovascular Pathologists

A questionnaire-based survey of 51 centers in 15 European countries investigated how pathologists apply the 2004 ISHLT recommendations for a biopsy specimen diagnosis of AMR.²¹ It was presented at a 2010 ISHLT Scientific Session in Chicago.²² Information was sought on technical aspects of C4d immunostaining of routinely processed EMB specimens, on interpretation and reporting of the results for C4d using immunofluorescence (IF) of frozen sections or immunohistochemistry (IC) of paraffin sections, access to results of real-time serologic testing for DSAs, and access to clinical data at the time of biopsy reporting.

Completed questionnaires were received from 37 of the 51 centers (72%), with 32 (86%) of these performing C4d staining and 25 (78%) using paraffin IC. At the time of the survey, 5 centers (3%) did not assess C4d. Methodology was comparable: 23 of the 25 centers used the polyclonal Biomedica antibody at a dilution of between 1:10 and

1:120. Selection of additional antibodies in an AMR panel to supplement positive C4d staining was variable. Nearly 40% of centers routinely tested for C4d staining. Histologic and/or clinical abnormalities triggered testing in the remaining centers. All centers assessed C4d capillary positivity irrespective of other structures stained, but there was no uniformity of interpretation of intensity or distribution of staining, and hence, what should be considered as a positive result. Five centers (16%) adapted the Banff scoring system, with scores from 0 to 4+, and scores of 3+ and 4+ being considered positive; 12 centers (37%) used other grading systems. The remaining centers ignored distribution or intensity of staining, or used no grading system at all.

Twenty-two pathologists (69%) recommended to their clinicians that testing for DSAs be done if indicated from the biopsy specimen findings. However, DSA status at the time of the biopsy was known in only 17 centers (53%). Subsequent questioning about serology revealed that DSAs were assessed routinely in only 6 centers (19%) and for clinical indications in 14 (44%). HLA antibodies were investigated in 19 centers (59%) and non-HLAs in 10 (31%). Luminex methodology for DSA testing was used in 15 centers (47%), 11 also doing quantitative antibody assays. Serum was banked for future testing in 19% of centers. Pathologists regularly interacted with clinicians through clinical meetings in 26 centers (82%), but only 7 (22%) had easy access to expert immunologic advice. Perspectives from the European survey were:

1. The diagnosis of AMR requires input from the biopsy, serology, and clinical parameters of graft function; that is to say, a multidisciplinary approach as suggested by the 2004 National Institutes of Health consensus conference on AMR.²³
2. Validation of paraffin section IC against frozen section IF staining of capillaries for C4d is needed.
3. Should routine C4d staining be done and what other antibodies should be included in a primary panel?
4. A universally agreed grading system for biopsy C4d deposition by IC or IF should be established and must include definitions of a positive and a negative result.
5. The biopsy antibody panel should be widened if coexisting acute cellular rejection is suspected or if published evidence suggests that other markers, such as C3d and CD68 (for macrophages), give useful prognostic information.
6. Serum should be banked at pre-determined intervals to facilitate contemporaneous and retrospective DSA testing as required.

IV. What are the assays to specify and quantify circulating antibodies? Is quantity of antibody crucial? How often should they be monitored?: Nancy Reinsmoen, PhD

Cell-based and solid-phase assays are both used to specify and quantify circulating antibody. Laboratories use a combination of both procedures in a sequential and economi-

cally feasible approach to provide the information necessary regarding comprehensive antibody status and antibody to potential donors. Solid-phase antibody (SPA) detection assays are highly sensitive and specific and have revolutionized the approach to determining the specificity and strength of the antibodies detected. Usually, the most sensitive assay is used initially to determine whether HLA-specific antibody can be detected. The flow screening beads are commonly used to identify the percentage of binding observed with the class I and class II beads. The specificity of the antibodies is determined by the SPA single antigen or phenotype beads. Standard fluorescent intensity or mean fluorescence intensity can be used to report the strength of the antibodies.

Several caveats should be considered in the interpretation of the SPA results. The density of antigen on the beads is not standardized and differs among beads and between lots. The antigen density on the beads does not reflect the antigen density on the cells. HLA-C, HLA-DQ, and HLA-DP are at a higher density on the beads than on cells. The cell-based assays include both complement-dependent cytotoxicity (CDC) and flow cytometry crossmatches, which are used to determine donor antigen-specific reactivity.

The laboratory program must correlate the strength of binding obtained with the antibody detection methods with the concomitant CDC and flow cytometry crossmatch results. Several studies have investigated the ability to predict accurately crossmatch outcome from SPA data using single antigen bead, phenotype beads, or the summing of all SPA DSA binding.²⁴ These data provide the basis for assigning unacceptable antigens that will be program-specific and center-specific.²⁵

Finally, many studies have monitored the post-transplant antibody status of solid organ transplant recipients. De novo DSA antibody is more likely to develop after transplant in recipients who are sensitized before transplant.²⁶ Most of these antibodies appear in the first 60 days; thus, monitoring during this period appears critical. Development of de novo DSAs after this time is often class II-directed and precedes the development of chronic allograft dysfunction.

V. Non-HLA antibodies (against vimentin, endothelial cells, MICA/MICB) and clinical relevance: Marlene L. Rose, PhD, and Elaine F. Reed, PhD

This section discusses the clinical relevance of antibodies to major histocompatibility class I-related chain A (MICA), autoantigens (vimentin, cardiac myosin heavy and light chains, and nuclear antibodies), and endothelial cells.

A detailed study of early AMR in 433 renal transplant recipients suggests an incidence of 2.3%.²⁷ Although 3 of 10 patients with early AMR may have had antibodies to donor MICA, the antibodies in 7 of 10 cases of early AMR were unexplained. Since the introduction of solid-phase assays at this institution in 2004, 2 cases of early AMR have occurred in the absence of donor-specific HLA antibodies,

which is an incidence of 2 of 128. In one instance, anti-endothelial antibodies were detected in the patient by the XM-One crossmatch technique; in the second, anti-heart antibodies were detected in the patients by Western blotting. The XM-One assay uses magnetic beads coated with tie-2 antibodies to separate endothelial precursor cells from donor blood.²⁸ More work is required to understand whether these assays can be used to diagnose AMR.

MICA and MICB are polymorphic cell surface proteins expressed by human epithelial cells, endothelial cells, skin-derived fibroblasts, keratinocytes, and monocytes.²⁹ Two studies reported an effect of MICA antibodies on cardiac transplant outcome. One described an association with rejection episodes.³⁰ The other reported that although MICA antibodies were present in 14% before transplantation, these had no effect on patient survival, rejection episodes, or CAV.³¹ The latter report suggested that expression of MICA on donor organs may determine whether MICA antibodies are damaging.

There is a growing list of autoantibodies that have been detected in heart transplant recipients, including antibodies to cardiac myosin,³² anti-phospholipid antibodies,³³ anti-endothelial antibodies,³⁴ anti-vimentin antibodies,³⁵ and antibodies to K- α tubulin in lung transplant recipients.³⁶ Significantly worse 1-year survival of cardiac transplant recipients has been associated with pretransplant cytotoxic immunoglobulin (Ig) M non-HLA antibodies.³⁷ PRA reactivity was also found to be strongly associated with long-term graft loss in kidney transplants from HLA-identical sibling donors, suggesting non-HLA immunity is associated with chronic graft loss.³⁸ Unfortunately, the antigenic specificity of these low-level antibodies is not known.

Experimental studies have shown that although autoantibodies are secondary to the alloimmune response, autoantibodies are highly damaging.^{36,39-41} Recent data from Harefield has analyzed the contribution of autoantibodies to AMR, defined according to ISHLT criteria,²¹ occurring in 16 patients who received allografts between 2004 and 2009. The most common antibody found in the serum at the time of diagnosis was donor-specific HLA antibodies (14 of 16 patients); however, 4 patients had high titres of IgM anti-vimentin antibody, 8 had IgM or IgG anti-cardiac myosin antibodies, and 1 had anti-nuclear antibodies. This suggests that autoantibodies may contribute to AMR.

In conclusion, early rejection caused by non-HLA antibodies is a rare event in the modern era, and the nature of the antigens is not understood. Better techniques are required to elucidate and monitor autoantibody responses after cardiac transplantation.

VI. Does accommodation exist?: Jeffrey L. Platt, MD

Accommodation refers to the condition in which a graft remains structurally and functionally intact despite manifest immunity against it. It was first invoked in the 1980s to explain how ABO-incompatible kidney transplants might exhibit stable renal function in recipients with anti-blood

group antibodies that should have injured their grafts.^{42,43} The term “accommodation” was first used to explain how cardiac xenografts might survive in recipients with xenoreactive antibodies in the circulation.⁴⁴

Applying the original definition of accommodation (normal graft function in recipients with circulating anti-donor antibodies) might underestimate the prevalence of accommodation. Although normal graft function in the absence of anti-donor antibodies could reflect immunosuppression, ignorance, or tolerance, it could also reflect accommodation in some cases.

One problem with using circulating anti-donor antibodies as evidence of humoral immunity against a graft is that those antibodies can be bound in large quantities to functioning organ grafts. Recipients of accommodated xenografts can have little or no detectable xenoreactive antibody in the circulation, but production of antibody is easily demonstrated by removing the graft.⁴⁵ Consistent with this concept, cultured endothelial cells and intact organs can absorb appreciable amounts of xenoreactive antibody.^{46,47} Also consistent with this concept are preliminary observations of Lynch et al (unpublished data) that all renal allograft recipients have demonstrable B-cell responses to donor HLA even when the corresponding antibodies are not detectable in the blood. Thus accommodation might be much more prevalent than commonly thought.

What can accommodation explain besides normal graft function despite humoral immunity against a graft? Accommodation might explain the genesis of chronic rejection. By prolonging the period that T cells, antibodies, cytokines, and other factors can act on the graft and/or by invoking “protective” pathways that cause injury, accommodation might allow or facilitate chronic injury.^{48,49} Accommodation might explain how cytotoxic T cells, natural killer cells, complement, and tumor necrosis factor can control or eliminate intracellular microorganisms without overly damaging infected cells and may also explain how tumors evade control by immunity.

As one explanation for normal graft function despite humoral immunity against the graft, accommodation surely exists. Accommodation is not the only explanation, however. Given the challenges of detecting antibodies that actually attach to the graft, the condition could be explained by partial tolerance, such that B cells making antibodies of the highest affinity are deleted or anergic while other alloreactive B cells function. The condition might be explained by preferential production of antibodies of the IgG2 isotype that block binding of complement-fixing antibodies.⁵⁰ Such a phenomenon was originally ascribed to accommodation but now is better considered a type of immune regulation. Absence of graft injury despite humoral immunity against donor antigen could be explained by modulation of antigen⁵¹ or by unusually effective control of complement at the levels of C4, C3 or C5-9. Conditions modeling accommodation have been associated with control of complement at C9⁵² and C3-C4.⁵³ Whether heightened control of complement truly represents a broader biologic response (ie, accommodation), or simply complement regulation, is not yet

clear. Although these other explanations have not been shown to operate in organs grafted across major histocompatibility complex barriers, neither have these mechanisms been formally excluded.

VII. Should asymptomatic AMR be acknowledged and treated?: Abdallah G. Kfoury, MD

The current 2005 ISHLT guidelines include the requisite criterion of allograft dysfunction in the definition of AMR in heart transplantation.^{10,21} As such, it is debatably presumed that cardiac AMR is uniformly symptomatic or that it cannot be diagnosed in the absence of cardiac dysfunction. This definition has untowardly sustained the prior prevailing stance by many in the transplant community to disregard asymptomatic or sub-clinical AMR. The resulting lack of routine surveillance for cardiac AMR has also restricted our ability to identify its true incidence and, more important, to fully appreciate the spectrum of its progression from latent immunologic and pathologic stages to full clinical expression.

Until recently, only indirect evidence of the probable association of asymptomatic cardiac AMR with adverse outcomes existed, as most of the published work failed to separate it from symptomatic AMR.^{4,5,9,54} Last year, Wu et al⁵⁵ published a study comparing 5-year actuarial survival and freedom from CAV in 21 heart transplant recipients with untreated asymptomatic AMR and in 22 patients with treated AMR and left ventricular dysfunction. A matched control group of 86 contemporaneous patients without AMR was used for comparison. Survival was comparable, but CAV was more likely to develop in patients with asymptomatic untreated AMR than in the control group, and these patients even trended to do worse than patients with treated symptomatic AMR.⁵⁵ The study by Kfoury et al⁵⁶ reported cardiovascular mortality among 869 heart transplant recipients grouped as cellular (< 3 episodes of AMR), antibody-mediated (\geq 3 episodes of AMR), or mixed cellular and antibody-mediated (\geq 3 episodes of concurrent cellular and AMR) rejectors based on their predominant pattern of rejection type in the first 3 months after transplant. This study, which excluded symptomatic AMR or any rejection type with hemodynamic compromise, showed significantly worse rates of cardiovascular mortality among asymptomatic antibody-mediated and mixed rejectors compared with cellular rejectors.⁵⁶ These 2 studies were the first to directly associate asymptomatic AMR with worse clinical outcomes in heart transplantation.

The spectrum of cardiac AMR should be perceived as a clinical-pathologic continuum that starts with a latent *humoral response* of circulating antibodies alone and progresses through a *silent phase* of circulating antibodies with C4d deposition, without histologic or clinical alterations, to a *sub-clinical stage* with circulating antibodies, histologic, and immunopathologic to *symptomatic AMR* with clinical manifestations.²³ Acknowledging asymptomatic cardiac AMR can be accomplished by eliminating the requisite of allograft dysfunction from diagnostic guidelines. This

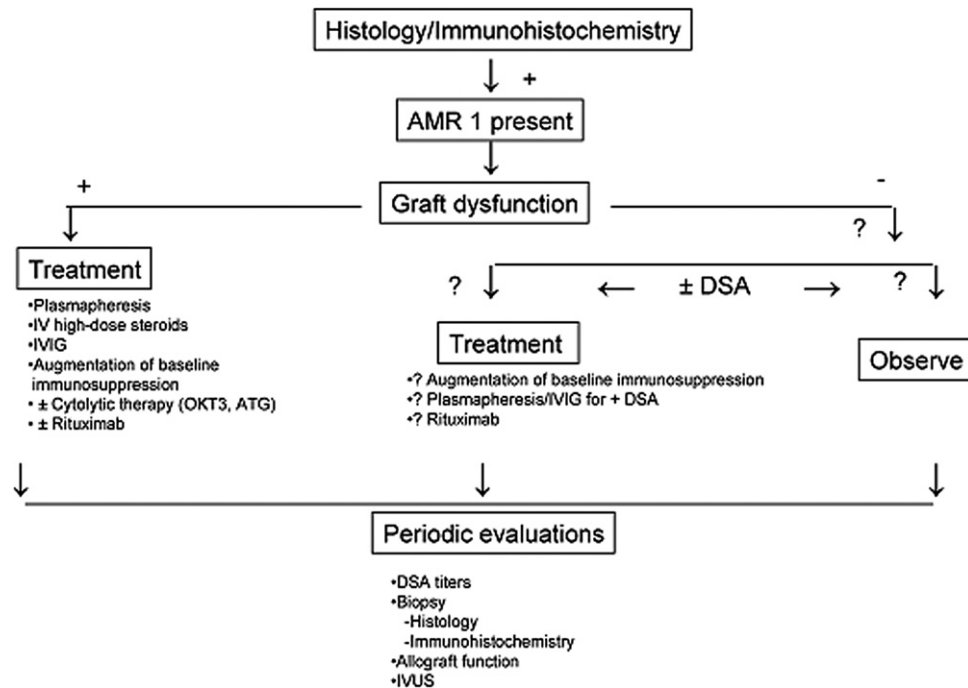


Figure 1 Previously proposed algorithm describes a therapeutic approach to the treatment of antibody-mediated rejection (AMR) in heart transplant recipients. ATG, anti-thymocyte globulin; DSA, donor-specific antibodies; IV, intravenous; IVIg, intravenous immune globulin; IVUS, intravascular ultrasound.⁵⁸ Reprinted from the American Journal of Transplantation with permission from John Wiley and Sons.

would make cardiac AMR a pathologic diagnosis to which clinical descriptors can be added, similar to what is done for acute cellular rejection.

Whether asymptomatic cardiac AMR should be treated is still unsettled. The evidence base for the related adverse outcomes is growing, but the compelling idea of intervening therapeutically in the hopes of preventing them has not yet been tested. The choice of whom to treat complicates the issue further because it is evident that some cardiac allografts develop an acquired resistance to humoral injury and do well long-term without any therapy. Risk-stratifying heart transplant recipients with asymptomatic AMR based on longitudinal monitoring of defining parameters, such as the nature and extent of complement deposition and antibody types and titers, would allow us to better identify a target population for prospective testing of various therapies. A large registry of patients routinely undergoing surveillance for asymptomatic AMR could be helpful in this regard and would be an ideal initial step.

VIII. The approach to treatment of AMR—Concepts of therapy and who should be treated?: Naveen Pereira, MD

It is difficult to recommend evidence-based guidelines for the treatment of AMR because the criteria for diagnosis have not been well established and have been used variably in different clinical series. The diagnostic tests used have lacked specificity and sensitivity, especially when applied to the most common and least controversial indication for treatment, acute cardiac allograft dysfunction in the absence

of acute cellular rejection.⁵⁷ Figure 1 outlines a algorithm for the treatment of AMR.

The goals of any treatment for this immune-mediated phenomenon are to improve allograft dysfunction, prevent development of CAV, a frequent long-term consequence of this condition, and minimize infection and the risk for malignancy while improving survival. The treatment of AMR has traditionally occurred in the period after transplant when a diagnosis has been established by EMB specimen or by the presence of graft dysfunction, or both. However, evidence from an animal model shows that occurrence of chronic sequelae, such as the development of CAV, can be attenuated by deletion of B lymphocytes by using rituximab before heart transplantation.⁵⁸ Whether the prophylactic use of rituximab in the pre-transplant period will prevent the development of AMR in human cardiac transplantation remains to be proven. Rituximab and other therapeutic modalities have been used to treat the sensitized heart transplant recipient who is at a high risk for development of AMR. The use of current desensitization protocols, although allowing successful transplantation, may not prevent AMR.⁵⁹

The improved detection of anti-HLA antibodies has thus enabled the use of a virtual crossmatch in performing heart transplantation.⁶⁰ Conceivably, the use of virtual crossmatch for transplantation in a patient with circulating anti-HLA antibodies by using a donor without unacceptable HLA antigens could prevent DSA-mediated AMR. The development of AMR could also be attenuated by identifying the high-risk patient in the immediate pre-operative or post-operative period by performing flow cytometry-based crossmatch, a technique that is more sensitive than complement-dependent lymphocytotoxicity techniques. A positive

flow cytometry-based crossmatch has been associated with subsequent development of AMR and reduced survival.⁶¹ Pre-emptive treatment guided by assessing graft function using echocardiography and for acute rejection by analyzing EMB specimens in recipients with a positive flow cytometry-based crossmatch may help attenuate the risk of subsequent development of AMR, but this approach remains to be proven.

The treatment of established AMR has been largely empiric and based on the combination of various therapies, dependent to a varying degree on the severity of illness at presentation. Different strategies have been used in various anecdotal reports; hence, accurate comparison of these strategies in efficacy and long-term outcomes cannot be made. The efficacy of treatment could be assessed by resolution of histologic changes, improvement of graft function, and potentially, suppression of DSA. The recovery of cardiac allograft function may be delayed, and resolution of C4d staining may occur weeks to months after recovery of allograft function.⁶² Echocardiography and single-antigen bead assays should be performed along with analysis of periodic biopsy specimens with appropriate staining after completion of treatment. The use of sensitive techniques to assess for early development of CAV, such as coronary flow reserve studies and intravascular ultrasound imaging, should be used especially in heart transplant recipients with asymptomatic AMR who can then potentially be targeted for therapy.

IX. Efficacy of specific therapies for AMR: Plasmapheresis and rituximab: Maria G. Crespo-Leiro MD

Current strategies for treatment of AMR focus on the modulation of antibody-induced injury, the blockade and elimination of alloantibodies, the downregulation of alloantibody production by plasma cells, and reduction of the levels of both naïve and memory B cells. New inhibitors of the complement system may prove to be effective adjuvants in the treatment of AMR.⁶³

Plasma exchange or plasmapheresis has been a commonly used method to remove circulating alloantibody. It involves the extracorporeal separation of plasma from cellular blood components by centrifugation or membrane filtration, which removes the alloantibody. The reconstituted blood is then infused back into the patient. It is a standard therapy for a number of autoimmune diseases, including idiopathic thrombocytopenic purpura, hemolytic uremic syndrome, Goodpasture syndrome, and Guillain-Barré syndrome.

There are 3 variant techniques of plasmapheresis. The first is therapeutic plasma exchange, where the plasma-depleted blood is reconstituted with exogenous fresh-frozen plasma or albumin solution. The use of heat-treated albumin for reconstitution ensures zero or minimal risk of viral transmission and anaphylactic reactions.

The second technique is called double-filtration plasmapheresis, where the low-molecular-weight fraction of the endogenous plasma is obtained by a second physical separation process and then reinfused into the patient.

The third technique is immunoadsorption plasmapheresis, where the second separation is immunochemical and is designed to remove only immunoglobulins. In immunoadsorp-

tion plasmapheresis, the immunoglobulin-binding immunoadsorbents are typically porous beads covalently coupled to polyclonal anti-human IgG antibody or to protein A, a natural component of *Staphylococcus aureus* with high affinity for circulating immune complex and IgG. Each adsorption column can typically bind 1 to 2 grams of antibody.

Therapeutic plasma exchange and double-filtration plasmapheresis are relatively inexpensive, easy to perform, and readily available, but involve non-selective removal of proteins, which can result in increased risk of bleeding and a risk of blood-borne infection if fresh-frozen plasma is used. Immunoadsorption plasmapheresis avoids replacement fluids and the associated risks. However, it is more expensive, not readily available, and does not remove circulating cytokines, which may play a role in AMR.

Plasmapheresis can be used before and after heart transplant surgery. Plasmapheresis may be performed in highly sensitized patients on the heart transplant waiting list to reduce these circulating antibodies, which can increase the chances of finding a negative crossmatch donor. One drawback is that antibody levels rebound shortly after treatment, which may necessitate adjuvant therapy. Plasmapheresis is commonly used after heart transplant to treat symptomatic AMR. A number of plasmapheresis protocols are used that differ in duration and treatment frequency. Duration of plasmapheresis ranges from 3 days to 4 weeks and frequency from daily to weekly.^{11,62,64,65}

CD20 protein is borne on the surface membrane of pre-B lymphocytes and mature B lymphocytes. It regulates the early steps of cell cycle initiation and differentiation. Rituximab is a chimeric, high-affinity monoclonal anti-CD20 antibody. It binds to CD20, which interferes with the activation and differentiation of B cells. It has U.S. Food and Drug Administration approval for treatment of B-cell lymphomas and rheumatoid arthritis^{66,67} but is also widely used for many other hematologic and autoimmune disorders.⁶⁸

Rituximab is beginning to be used for desensitization in highly sensitized patients awaiting heart transplantation. It is also used for treatment of AMR in lung, kidney, liver, and heart transplant patients.⁶⁹ In the case of heart transplantation, single patients or small series are mainly described. The rituximab dosage used against AMR includes 375 mg/m²/week for up to 4 weeks.⁷⁰⁻⁷² In the literature, this drug was always used in combination with other treatments, including steroids, anti-lymphocyte antibodies, mycophenolate mofetil (MMF), calcineurin inhibitors, plasmapheresis, and intravenous Ig administration. Unfortunately, this multiplicity of therapeutic agents has hampered evaluation of the efficacy of rituximab.

X. Efficacy of specific therapies for AMR—Maintenance immunosuppression, intravenous immune globulin, and anti-thymocyte globulin: Andreas Zuckermann, MD, and Stuart D. Russell, MD

No studies have evaluated the efficacy of any routine immunosuppressive regimen in preventing AMR. This is due partly to the lack of standardization in diagnosing AMR and

partly to the lack of clinical trials of any drugs to treat this disorder. In addition, no clinical trials evaluating a single immunosuppressive regimen or comparing different regimens have directly reported the incidence of AMR. In many trials, however, "any-treated rejection" has been reported and that incidence is usually higher than the incidence of biopsy specimen-proven cellular rejection (ISHLT grade 2R or 3R). From this information one can hypothesize that the difference in this incidence may actually be rejection related to AMR.

The standard immunosuppressive regimen for most post-transplant patients today includes a calcineurin inhibitor (cyclosporine or tacrolimus), anti-proliferative agent (MMF, sirolimus, or everolimus), and oral steroids.⁷³ One trial has evaluated the efficacy of 3 different drug regimens on outcomes, including the incidence of any-treated rejection. Kobashigawa et al⁷⁴ performed a 3-arm randomized trial of cyclosporine, MMF, and steroids vs tacrolimus, MMF, and steroids vs tacrolimus, sirolimus, and steroids. The study comprised 334 patients, and the primary end point was ISHLT >3A (2R) rejection plus hemodynamic compromised rejection. There was a strong trend towards a reduction in the primary end point with either of the tacrolimus arms. When any-treated rejection was examined, however, there was a significant reduction in the incidence of this rejection with both tacrolimus/sirolimus (35.1%) and tacrolimus/MMF (42.1%) compared with cyclosporine/MMF (59.6%; $p < 0.001$). Of note, although not directly examined, the rate of any-treated rejection exceeded the rate of biopsy-proven cellular rejection in the primary end point by 10% to 20%, depending on the arm, implying that there was a significant presence of AMR.

Intravenous immune globulin (IVIg) is often used to reduce the level of antibodies in patients who are sensitized before transplant. However, IVIg has never been systematically studied in patients after transplant to prophylactically reduce the incidence of AMR. Despite being routinely used for the treatment of AMR, only 1 study has reported the efficacy of the therapy in this setting.⁷⁵ Five patients with evidence of AMR were treated with a combination of IVIg and plasmapheresis. Hemodynamics initially improved in all 5 patients, but 2 patients later required further therapy with rituximab because of recurrent hemodynamic rejection.

In contrast to IVIg, 5 prospective trials have examined the efficacy of anti-thymocyte globulin (ATG) used as induction therapy to prevent rejection after transplant.^{76–80} Each trial had different end points, but all included the incidence of treated rejection at the end of the year. Renlund et al⁷⁶ reported the use of OKT3 vs ATG as induction therapy in 51 patients. OKT3 resulted in fewer treated rejection episodes at 6 months and fewer episodes of rejection requiring additional cytolytic agents. In contrast, Ladowski et al,⁷⁷ in a 3-arm study of ATG vs OKT3 vs anti-lymphocyte globulin in 34 patients, reported fewer rejection episodes in the ATG arm. Finally, Macdonald et al⁷⁸ studied ATG vs OKT3 and reported no difference in rejection episodes but noted reduced infections and other morbidity

with ATG.⁷⁸ Two trials have compared the use of 2 different forms of ATG for induction therapy. Schnetzler et al⁷⁹ in 50 patients and DeSanto et al⁸⁰ in 40 patients both reported no difference in the incidence of rejection between the 2 therapies. The evidence appears to show that ATG may reduce the incidence of rejection compared with other induction methods, but it is unclear if ATG reduces the incidence of AMR. In addition, it is unclear if the additive immunosuppressive effect of ATG induction decreased rejection and/or increased the risk of infections or other complications compared with no induction therapy.

Currently, it is suggested that the incidence of AMR is reduced with the use of tacrolimus compared to cyclosporine based immunosuppression. Although a number of different induction therapies have been used, no induction therapy has been shown to be superior to another for preventing AMR. Additionally, although IVIg is routinely used for the therapy of AMR, there are no randomized trials in heart transplantation using prophylactic IVIg to reduce the incidence of AMR.

XI. Efficacy of specific therapies for AMR—Bortezomib and eculizumab: **Jignesh Patel, MD, PhD**

Treatment to reduce circulating antibodies before transplant has had mixed results. Plasmapheresis, IVIg, rituximab, and high-dose cyclophosphamide have been demonstrated to successfully reduce circulating antibodies.⁸¹ Many patients remain refractory to these therapies, however, because these agents generally deplete or modulate antibodies or affect B-cell activity without affecting antibody production by plasma cells. Bortezomib is a 26S proteasome inhibitor that has proapoptotic effects on plasma cells and has been shown to decrease antibody production.⁸² It is approved in the United States for the treatment of multiple myeloma. Some early experience in renal transplantation has shown variable efficacy of bortezomib in the treatment of AMR and desensitization.^{83–85} We performed a pilot study to determine the efficacy of desensitization using bortezomib in patients refractory to IVIg, rituximab, and plasmapheresis and to assess its effectiveness in reducing calculated PRA (cPRA) for patients awaiting heart transplantation. We treated 7 patients with bortezomib awaiting heart transplantation who had cPRAs > 50%. Mean baseline cPRA was 62%, which reduced after treatment to a mean level of 35% ($p = 0.01$). Six patients exhibited a significant reduction in cPRA. The remaining patient demonstrated no reduction in cPRA after treatment. Bortezomib appeared to decrease cPRA in patients refractory to desensitization with IVIG/rituximab and plasmapheresis, thus increasing the chances that an acceptable donor heart would become available for the sensitized patient awaiting heart transplantation.⁸⁶

In accommodation, circulating DSA is present without evidence for graft injury. Complement activation appears to be important in the pathogenic effects of circulating DSA.⁵³ Therefore, blocking antibody-mediated complement activation may be effective in preventing AMR. Eculizumab is a

humanized monoclonal antibody that binds to and subsequently prevents activation of complement component C5 by the amplified C3 convertase molecules. This agent may therefore be effective in preventing AMR. It is approved in the United States for the treatment of paroxysmal nocturnal hematuria. In initial studies in renal transplantation by Stegall et al,⁸⁷ 10 patients with a positive crossmatch underwent desensitization with plasmapheresis and IVIg combined with eculizumab after renal transplantation. High levels of DSA developed in 5 patients, but after 12 months of follow-up, no AMR had developed.

Despite existing desensitization therapies, many patients demonstrate elevated levels of antibodies that preclude heart transplantation. Our data suggest bortezomib may be a useful supplement, but the most optimal desensitization strategy remains undetermined and begs randomized clinical trials. Eculizumab is a potentially promising agent that may promote accommodation, although clinical experience is sparse. Although bortezomib and eculizumab show promise in lowering antibodies and preventing AMR, respectively, routine use of these newer drugs for AMR treatment awaits further studies.

XII. Efficacy of specific therapies for AMR—Total lymphoid irradiation and photopheresis: Jose Tallaj, MD

Total lymphoid irradiation (TLI) has been used in transplantation for more than 20 years. The initial reports indicated a potential benefit from TLI in recurrent rejection,⁸⁸ but in subsequent years it has been largely abandoned. This presentation looked at our center's large experience in the use of TLI in the treatment of rejection during the early to mid-1990s.

Between 1990 and 1996, 73 adults received TLI during the first 6 months after transplant. The indication for TLI was recurrent rejection (71%), rejection with hemodynamic compromise (25%), and rejection with vasculitis (4%). The treatment consisted of 80 cGy twice weekly for 5 weeks, and 55 patients received at least 80% of the full dose (> 640 cGy). TLI resulted in a decrease in hazard for rejection (relative risk, 0.36 in the early stages). The beneficial effect achieved with TLI was maintained for approximately 4 years after transplantation, until increased rejection, especially hemodynamic-compromised rejection, and rejection death, were again observed. No differences were noted in the rates of infection, CAV, or malignancy, but myelodysplasia or acute myelogenous leukemia developed in 7 patients, 4 of those being the rare but uniformly fatal acute megakaryocytic leukemia-7. In conclusion, TLI may provide effective therapy for the reduction of subsequent rejection for 36 to 48 months after completion of TLI; however, there is concern for subsequent occurrence of myelodysplasia and acute megakaryocytic leukemia-7. Considering these concerns, the use of TLI for the treatment of AMR is not recommended.

Photopheresis therapy has gained clinical acceptance as an effective therapy for recurrent or persistent rejection after

cardiac transplantation. We previously reported that 3 months of photopheresis resulted in a significant decrease in the risk for rejection and rejection death in patients with hemodynamic compromise rejection or recurrent rejection.⁸⁹ Moreover, photopheresis is well-tolerated, with minimal side effects or long-term complications. In contrast to TLI, there are data indicating that its beneficial effect might be due to antigen-specific immunomodulation via regulatory T-cells. Its use for the treatment of AMR has not yet been established.

Summary of the breakout sessions from the consensus conference on AMR

Many clinically relevant issues arose during the consensus conference. These issues included the diagnosis, classification, and management of AMR. The 83 attendees of the consensus conference participated in smaller breakout sessions to address these topics and attempt to achieve consensus on the approach to the patient with AMR. The background AMR talks summarized above provided a framework and support for much of the discussion. A summary of these consensus points is provided. A separate pathology breakout session occurred during the morning lectures, as requested by the pathologists, to further discuss the pathology definition of AMR. A summary of this session follows.

Pathology breakout session: Annalisa Angelini, MD, Gerald Berry, MD, and Margaret Burke, MD

To place into perspective the discussion that occurred in the pathology breakout session, we present a modified and updated version of the report of Pathology and Basic Science Council presented to the ISHLT Council at the Chicago meeting in April 2010.

In the most recent ISHLT Working Formulation of 2005, the pathologic diagnosis of AMR included endothelial-cell swelling and accumulations of intravascular macrophages with immunophenotypic evidence of immunoglobulin (IgG, IgM, and/or IgA) and complement deposition (C3d, C4d, and/or C1q) in capillaries by immunofluorescence (IF) on frozen sections and/or CD68 staining of intravascular macrophages in capillaries and C4d staining of capillaries by paraffin immunohistochemistry (IC).²¹ This profile should be present in the setting of DSA positivity and graft dysfunction. However, a number of studies since 2005 have raised the question of asymptomatic AMR,^{55,56} questioned the sensitivity and specificity of the histologic features of AMR,⁹⁰ shown positive histology in the kidney in the absence of C4d,^{19,20} and shown C4d deposition in biopsy specimens without positive histology or intravascular macrophages.⁹¹⁻⁹³ These findings suggest that a morphologic and immunophenotypic spectrum of AMR-related changes may both exist and together with the published literature on AMR provided the basis for the reexamination of the 2005 criteria during this pathology breakout session. Participation

of the Northern European pathologists was facilitated by 2-way speakerphone.

Although agreement was reached on some issues, the group agreed that more detailed debate and investigation was required on others. *Thus, although we made considerable progress, we must emphasize that the outcome of our discussions represent "work-in-progress," which will be addressed in the coming months.* We are committed to producing a finished proposal that will be comprehensive with the goals of promoting patient care, accumulating necessary data for current and future studies, and enhancing reproducibility amongst pathologists.

Technical issues

As stated in Section II of this report, the work done in Banff 2009 by Rodriguez has shown acceptable results of IC on paraffin sections using a currently available polyclonal C4d antibody. There are no issues with the monoclonal C4d antibody currently available for IF. Recommended panels of antibodies and fixatives and/or fixation tissues for IC and IF will be proposed.

Interpretative issues

A small number of published validation studies have now demonstrated acceptable levels of equivalency between IF and IC techniques.^{94–97} Only staining of interstitial capillaries should be interpreted, whereas constituent biopsy structures, such as venules and arterioles, can be identified for the purpose of internal controls. External controls for each antibody should be used with appropriate antibody validation.

We identified issues with recognition of early histologic changes of AMR. Additional criteria to identify these will be sought and evaluated by centers. It will include further definition of intravascular macrophages in quantity and distribution, and the clarification of the distinction between intravascular and interstitial patterns will be further elucidated. We recognized that mixed acute cellular and antibody-mediated rejection occurs, but time constraints during the session limited further discussion.

Descriptive/reporting issues

The distribution and intensity of staining by IF and IC should be reported. Categories to be considered include negative, focal, multifocal, and diffuse staining patterns, and negative, faint, or strong intensity of staining (IC) or semiquantitative scoring on a scale of 0 to 3+ (IF). Each pattern and grade will be defined and photomicrographic examples provided. For the purpose of classification as positive or negative, only 2+ and 3+ IF staining and multifocal or diffuse strong staining in IC will be considered as positive. The significance of patterns such as diffuse and faint staining by IC will be studied prospectively. Other pattern combinations may also be subject to additional study by centers.

Currently, the minimum positive histopathologic findings of AMR are endothelial activation and intravascular macrophages. Interstitial hemorrhage, capillary fragmenta-

tion, mixed inflammatory infiltrates, endothelial cell pyknosis, and/or karyorrhexis and marked edema are recognized as findings in advanced or severe AMR. The group will consider these and other possible morphologic criteria.

A template will be constructed for standardized collection of histopathologic and immunophenotypic data, which will provide the basis for a pathologic diagnostic AMR registry to evaluate reproducibility and audit of results.

Tentative pathologic diagnosis

The group agreed that the combination of histopathologic and immunopathologic findings will be reported as the "pathological diagnosis of AMR" and will be designated by pAMR. The group then considered an initial framework for the diagnosis of subcategories of pAMR. This framework will be the subject of further discussion and potential modification before publication or implementation. The preliminary categories for the reporting of pAMR are:

- pAMR 0 = Negative for pathologic AMR; histologic and immunopathologic studies are both negative.
- pAMR 1 = Suspicious for pathologic AMR; histologic findings positive, immunopathologic findings negative (pAMR 1-h), or immunopathologic findings positive, histologic findings negative (pAMR 1-i).
- pAMR 2 = Positive pathologic AMR; histologic *and* immunopathologic findings both are present.
- pAMR 3 = Severe pathologic AMR; interstitial hemorrhage, capillary fragmentation, mixed inflammatory infiltrates, endothelial cell pyknosis, and/or karyorrhexis and marked edema.

General breakout sessions

The afternoon general breakout sessions included a mix of pathologists, clinicians, and immunologists divided into 3 groups to maximize discussion and interaction. Clinical issues of AMR were discussed in these groups, and each group presented their results in a reconvened meeting of all the conference participants. Specific AMR issues were openly discussed in this full session, with several consensus points being reached.

Summary of the consensus conference on AMR in heart transplantation

Dr Berry presented the results of the pathologists' breakout session held during the morning of the AMR Consensus Conference, including the tentative schema for pathologic diagnosis of AMR described in the previous section and emphasized that further discussion amongst the pathologists was needed before a formal recommendation for pathologic diagnosis of AMR to the ISHLT Board of Directors. A separate pathology report will follow.

The final session, which included all participants, centered on clinical issues discussed in the afternoon breakout sessions. One of the most important issues identified cen-

tered on whether a clinical definition of AMR is needed. In the past, the use of cardiac dysfunction or the presence of DSA, or both, have been included as criteria for the diagnosis for AMR. However, it became clear from Dr Kfoury's talk in the morning session that asymptomatic biopsy-proven AMR (without cardiac dysfunction) was associated with subsequently greater incidence of CAV⁵⁵ and greater mortality.⁵⁶ It was also noted that circulating DSA are not always detected in the serum at the time of AMR diagnosis. This may be caused by the absorption of the DSA to the allograft. Alternatively, AMR may be caused by antibodies to non-HLA antigens. Therefore, the consensus was that the diagnosis of AMR would be made according to specific pathologic findings.

Descriptors for immunologic and clinical presentation, such as DSA and cardiac dysfunction, would assist in diagnosis and management. This would be similar to cellular rejection, which is already established as a pathologic diagnosis. In cellular rejection, clinical descriptors, such as recurrent, persistent, or hemodynamic compromise, have been added to illustrate clinical presentation or clinical severity and have helped to guide management. These clinical descriptors could also be used for AMR. Although AMR will be a pathologic diagnosis, it is strongly recommended that at the time of suspected AMR, blood be drawn at biopsy and tested for the presence of donor-specific HLA class I and II antibodies. In the absence of detectable anti-HLA antibodies, the assessment of non-HLA antibodies may be indicated.

Another aspect of AMR discussed at length in the final session was how to monitor for this form of rejection. For EMB specimens, the question was when to use immunopathologic (immunoperoxidase or IF) stains. Another issue was when to assess for the presence of circulating antibody. Although DSA was no longer believed to be an absolute requirement for the diagnosis of AMR, it was felt that the presence of DSA is an important risk factor for AMR and should be assessed in a routine manner using a combination of cell-based and solid-phase assays.^{98–100}

After considerable discussion in the final session, the recommendation for monitoring for AMR included the following: Every EMB specimen should be reviewed for histologic evidence for AMR and immunopathologic staining for C4d and other markers as agreed should be performed at 2 weeks, and at 1, 3, 6, and 12 months after transplant. The timing for follow-up immunophenotypic studies after the diagnosis of AMR was briefly addressed and the recommendation made that IC should be delayed for at least 2 weeks because complement clearing is a protracted event. A positive result for C4d should trigger routine staining of subsequent specimens for that patient. The use of solid-phase and/or cell-based assays to assess for DSA—and quantification of antibody if present—should be performed at 2 weeks and at 1, 3, 6, and 12 months after transplant, and then annually thereafter and when AMR is clinically suspected.

Management of AMR was then discussed. Currently, there are no unanimously agreed upon treatments for AMR,

especially because newer therapies, such as bortezomib and the anti-complement antibodies, are currently being studied. Table 2 illustrates examples of AMR therapies from 6 experienced heart transplant centers. On the basis of current experience and efficacy, the initial therapies to treat AMR may include high-dose corticosteroids, plasmapheresis, and IVIg. Secondary therapies at this time, due to lack of clinical experience, include rituximab, bortezomib, and the anti-complement antibodies. These recommendations may change as the newer therapies demonstrate further benefit.

For AMR prevention after heart transplantation, the use of tacrolimus, MMF, and corticosteroids appeared to be most effective. Dr Russell's talk in the morning session reviewed the randomized clinical trials in heart transplantation. Although not conclusive, the 3-arm trial comparing tacrolimus/MMF with cyclosporine/MMF with tacrolimus/sirolimus immunosuppression regimens suggested that tacrolimus/MMF had the most advantage to reduce any-treated rejection (presumably inclusive of AMR) while exhibiting the least side effect profile.⁷⁴

Looking toward the future, a discussion of potential clinical trials pointed to the pursuit of answers to key questions about AMR. The trials suggested include a randomized trial to treat asymptomatic AMR with high-dose corticosteroids plus IVIg vs IVIg alone vs placebo; a randomized trial to prevent antibody production with the use of rituximab or bortezomib immediately after transplant; and a randomized trial with anti-thymocyte γ -globulin vs triple-drug therapy for pre-sensitized patients undergoing heart transplant. These future studies are not exhaustive: many other studies could be pursued to shed further understanding on the development, treatment, and prevention of AMR.

The AMR Consensus Conference brought together clinicians, pathologists, and immunologists to further the understanding of AMR. Progress was made toward a pathologic grading scale and consensus was accomplished regarding specific clinical issues (see consensus statements below). The understanding of AMR is still ongoing and it is incumbent among clinicians, pathologists, and immunologists to work together and continue efforts to clarify its existence, frequency, and clinical significance.

Consensus statements for AMR

1. A new pathology grading scale as delineated in the Pathology Breakout Session will be forthcoming. Meanwhile, the pathologic tests to be performed for AMR should include:
 - A. Histology
 - Evaluate for endothelial “activation” and intravascular macrophages, capillary destruction. Interstitial edema and hemorrhage, neutrophilic infiltrates, capillary fragmentation, and endothelial cell pyknosis should be recognized because these findings portend poor clinical outcomes.
 - B. Immunopathology

Table 2 Treatment Protocols for Antibody-Mediated Rejection: Experience from Clinical Centers

Center	Treated AMR Patients, <i>N</i>	Primary AMR Treatment
University Hospital (Inselspital) Bern (Switzerland) ^a	57	Repeated protein A immunoadsorptions IVIg Rituximab MMF
Cedars-Sinai Medical Center ^b	150	IV Solu-Medrol Plasmapheresis Anti-thymocyte globulin, followed by: IVIg 2.0 g/kg administered on treatment day 0 and 30 Rituximab 1 g on treatment day 7 and 21
Cleveland Clinic ^c	325	IV Solu-Medrol Plasmapheresis IVIg MMF/Tacrolimus
Columbia Presbyterian Medical Center ^d	352	IV Solu-Medrol Plasmapheresis (5–6 cycles over 10–14 days) Cyclophosphamide (0.5–1.0 g/m ²) every 3 weeks for 4–6 months
Hospital Universitario A Coruña (Spain) ^e	132	IV Solu-Medrol (1 g × 3) Plasmapheresis and/or immunoadsorption (7–10 sessions) Anti-thymocyte globulin IVIg Rituximab (4 weeks) MMF
Medical University of South Carolina ^f	33	IV Solu-Medrol Plasmapheresis IVIg Cyclophosphamide

AMR, antibody-mediated rejection; IV, intravenous; IVIg, intravenous immune globulin; MMF, mycophenolate mofetil. Data presented by: ^aPaul Mohacsi, MD; ^bLawrence Czer, MD; ^cDavid O. Taylor, MD; ^dDonna Mancini, MD; ^eMaria G. Crespo-Leiro, MD; ^fAdrian Van Bakel, MD, PhD.

- Immunofluorescence: C3d, C4d, HLA (HLA recommended to assess endothelial capillary integrity—not positive/negative);
Optional: Ig, fibrin
 - Immunoperoxidase: C4d, CD68
Optional: C3d (pending more experience), vascular marker (CD34, CD31), CD3, CD20.
2. The diagnosis of AMR will be made according to pathologic findings described above. When AMR is clinically suspected, blood should be drawn at biopsy and tested for the presence of donor-specific HLA class I and II antibodies. The test results should be interpreted by the clinician to assist in the diagnosis and specific management of the AMR episode. In the absence of detectable anti-HLA antibodies, the assessment of non-HLA antibodies may be indicated. Clinical presentation, such as cardiac dysfunction, should also be interpreted by the clinician to assess severity of AMR and used to guide management.
 3. The recommended frequency for routine monitoring for AMR includes:
 - A. Endomyocardial biopsy
 - Histologic evaluation of every protocol biopsy for AMR
 - Immunoperoxidase/immunofluorescent staining for C4d at 2 weeks and 1, 3, 6, 12 months after transplant and when AMR is clinically suspected
 - Interval testing for C4d should AMR be suspected on histologic, serologic, or clinical findings
 - Routine C4d staining on subsequent biopsy specimens after a positive result until clearance.
 - B. Circulating antibody

- Use of solid-phase assay and/or cell-based assays to assess for DSA (and quantification if antibody present) at 2 weeks and 1, 3, 6, 12 months, and then annually after transplant and when AMR is clinically suspected.
4. The initial recommended therapies to treat AMR may include high-dose corticosteroids, plasmapheresis, and IVIg. For now, secondary therapies include rituximab, bortezomib, and anti-complement antibodies.
 5. The use of tacrolimus/MMF maintenance immunosuppression appears to be most effective in AMR prevention with the least side effect profile after heart transplantation.
 6. Recommendation for clinical trials should be based on a final diagnosis of AMR using standardized criteria and may include:
 - A. Randomized trial to treat asymptomatic AMR with high-dose corticosteroids plus IVIg vs IVIg alone vs placebo
 - B. Randomized trial to prevent antibody production: use of rituximab or bortezomib immediately after transplant
 - C. Randomized trial with Thymoglobulin induction vs triple-drug immunosuppression therapy for presensitized patients undergoing heart transplant.

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Appendix A. Consensus Conference Attendees and Attendees in Absence

Juan Alejos, MD, UCLA Medical Center; Annalisa Angelini, MD, Universita di Padova (Italy); William Baldwin III, MD, PhD, Cleveland Clinic; David Baran, MD, Newark Beth Israel Medical Center; Eduardo Barge-Caballero, MD, Hospital Universitario A Coruña (Spain); Gerald Berry, MD, Stanford University; Patrick Bruneval, MD, Hopital European Georges Pompidou (France); Deborah Budge, MD, Intermountain Medical Center; Margaret Burke, MD, Harefield Hospital (UK); Charles E. Canter, MD, St. Louis Children's Hospital; Patricia P. Chang, MD, UNC Chapel Hill Div; Monica Colvin-Adams, MD, University of Minnesota; Maria Costanzo, MD, Midwest Heart Specialists; Maria G. Crespo-Leiro, MD, Hospital Universitario A Coruña (Spain); Lawrence Czer, MD, FACC, FACP, Cedars-Sinai Medical Center; Teresa De Marco, MD, UCSF Medical Center; Juan Delgado Jimenez, MD, Hospital Doce de Octubre (Spain); Mark Drazner, MD, UT Southwestern; Brooks S. Edwards, MD, Mayo Clinic; Howard J. Eisen, MD, Drexel University College of Medicine; Stephan M. Ensminger, MD, PhD, University of Erlangen-Nuremberg (Germany); Savitri Fedson, MD, University of Chicago Hospitals; David Feldman, MD PhD, Minneapolis Heart Institute; Giuseppe Feltrin, MD, Policlinico Universitario (Italy); Michael C. Fishbein, MD, UCLA School of Medicine; H. Edward Garrett, MD, Cardiovascular Surgery Clinic; James F. George, PhD, University of Alabama at Birmingham; Michael M. Givertz, MD, Brigham & Women's Hospital, Cardiovascular Division; Lee Goldberg, MD, University of Pennsylvania; Mark Haas, MD, Cedars-Sinai Medical Center; Elizabeth Hammond, MD, LDS Hospital; Sharon Hunt, MD, Stanford University Medical Center; Maryl R. Johnson, MD, FACC, University of Wisconsin School of Medicine & Public Health; Andrew Kao, MD, Mid America Heart Institute; Ronald H. Kerman, PhD, University of Texas Medical School; Abdallah G. Kfoury, MD, FACC, Intermountain Medical Center; Michael Kieran, MD, Tufts Medical Center; James Kirklin, MD, University of Alabama; Michelle Kittleson, MD, PhD, Cedars-Sinai Heart Institute; Jon Kobashigawa, MD, Cedars-Sinai Heart Institute; Robert L. Kormos, MD, University of Pittsburgh; Mary Leffell, PhD, Johns Hopkins University; JoAnn Lindenfeld, MD, University of Colorado; Donna M. Mancini, MD, Columbia Presbyterian Medical Center; David Markham, MD, UT Southwestern; Mandeep R. Mehra, MD, University of Maryland School of Medicine; Dylan Miller, MD, Intermountain Central Lab; Paul Mohacsi, MD, University Hospital (Inselspital Berne; Switzerland); Thalachallour Mohanakumar, PhD, Washington University School of Medicine; Takeshi Nakatani, MD, PhD, National Cardiovascular Center (Japan); Desley Neil, BMedSc, MB, University of Birmingham (UK); Jignesh K. Patel, MD, PhD, Cedars-Sinai Heart Institute; Samuel Pepkowitz, MD;

Naveen L. Pereira, MD, Mayo Clinic; Michael Pham, MD, Stanford University; Barbara Pisani, DO, St. Luke's Hospital; Jeffrey L. Platt, MD, University of Michigan; Elaine F. Reed, PhD, David Geffen School of Medicine at UCLA; Hermann Reichenspurner, MD, PhD, University Hospital Eppendorf, Hamburg (Germany); Nancy Reinsmoen, PhD, Cedars-Sinai Comprehensive Transplant Center; Monica P. Revelo, MD PhD, University of Utah; E. Rene Rodriguez, MD, Cleveland Clinic; Joseph G. Rogers, MD, Duke University Medical School; Marlene L. Rose, PhD, Harefield Hospital (UK); Eulalia Roig, MD, Hospital Santa Creu i San Pau (Spain); Bruce Rosengard, MD, Massachusetts General Hospital; Stuart D. Russell, MD, Johns Hopkins Hospital; Javier Segovia, MD, PhD, Hosp Puerto De Hierro (Spain); Mario Sénéchal; Susan Stewart, FRCP, Papworth Hospital (UK); Nicole Suciuc-Foca, PhD, Columbia University College of Physicians & Surgeons; Jose Tallaj, MD, University of Alabama at Birmingham; Anat R. Tambur, DMD, PhD, Northwestern University; Carmela D. Tan, MD, Cleveland Clinic Foundation; David O. Taylor, MD, Cleveland Clinic; Jeffrey Teuteberg, MD, University of Pittsburgh; Dolly Tyan, Stanford University; Patricia Uber, PharmD, University of Maryland; Walter Uber, PharmD, Medical University of South Carolina; Adrian B. Van Bakel, MD, PhD, Medical University of South Carolina; Johan Vanhaecke, MD, University Hospital Gasthuisberg (Belgium); Steve Webber, MB ChB, Children's Hospital of Pittsburgh; Lori J. West, MD, D.Phil, University of Alberta (Canada); Gayle L. Winters, MD, Brigham and Women's Hospital; Andrea A. Zachary, PhD, Johns Hopkins University; Adriana Zeevi, PhD, University of Pittsburgh; Andreas Zuckermann, MD, Medical University of Vienna (Austria).

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