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Banff Lung Report: Current knowledge and future research perspectives for diagnosis and treatment of pulmonary antibody-mediated rejection (AMR)

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The Lung session of the 2017 14th Banff Foundation for Allograft Pathology Conference, Barcelona focused on the multiple aspects of antibody-mediated rejection (AMR) in lung transplantation. Multidimensional approaches for AMR diagnosis, including classification, histological and immunohistochemical analysis, and donor-specific antibody (DSA) characterization with their current strengths and limitations were reviewed in view of recent research. The group also discussed the role of tissue gene expression analysis in the context of unmet needs in lung transplantation. The current best practice for monitoring of AMR and the therapeutic approach are summarized and highlighted in this report. The working group reached consensus of the major gaps in current knowledge and focused on the unanswered questions regarding pulmonary AMR. An important outcome of the meeting was agreement on the

1 | INTRODUCTION

Lung transplantation remains one of the few definitive therapeutic options for patients with end-stage lung disease. Currently, long-term allograft function and recipient survival remain disappointing, with a median survival of approximately 6 years.[1] Chronic lung allograft dysfunction (CLAD) remains the leading cause of allograft loss and patient death after the first-year posttransplant.[2]

Until recently, pulmonary allograft rejection was considered to be primarily a T cell–mediated process (acute cellular rejection, ACR). However, pulmonary antibody-mediated rejection (AMR) has become an increasingly recognized form of allograft rejection. AMR after kidney and heart transplant is well documented. The true incidence of pulmonary AMR is unclear because, until recently, there had been no standardized criteria or multicenter studies to identify and define this process. The literature suggests that presensitized patients and patients with de novo DSA after transplant are at increased risk for ACR, AMR, lymphocytic bronchiolitis, and CLAD.[3] The recent International Society for Heart and Lung Transplantation (ISHLT) consensus definition of pulmonary AMR is a significant step forward in standardizing the classification and reporting of AMR.[4] It is important to note that pulmonary AMR is associated with poor allograft and patient survival.[3,5,6]

The XIVth Banff meeting was held in Barcelona, Spain, in conjunction with the Societat Catalana de Transplantament from 27-31 March 2017. The lung transplant session, co-chaired by Drs. DM Hwang and EN Pavlisko, focused on current concepts, mechanisms, histopathologic criteria, classification, and therapeutic interventions of pulmonary AMR. Avenues for collaborative investigation to further integrate clinical, immunologic, histopathologic, and molecular diagnostics were explored. The group also reviewed current monitoring and treatment strategies. This report summarizes the contemporary concepts of AMR, current approaches to clinical care, and proposes avenues for further investigation.

2 | MULTIDISCIPLINARY APPROACH AND CLASSIFICATION

As in ACR, a recipient with acute AMR may present with a range of clinical severity and features, from being asymptomatic but having circulating donor-specific antibody (DSA) through the spectrum to fulminant hypoxemic respiratory failure. The challenge of making the diagnosis of AMR, prompted the ISHLT multi-disciplinary working group of clinicians, pathologists, and immunologists to create the consensus document on acute pulmonary AMR.[4]

This document identifies the following criteria to define acute pulmonary AMR: allograft dysfunction, DSA positivity, histopathology consistent with AMR, tissue C4d staining, and the exclusion of other causes of allograft dysfunction (Table 1). The degree of confidence in the diagnosis of AMR is based on the number of these criteria present. The certainty of AMR increases with the number of criteria. "Definite AMR" is identified when all 4 criteria are met, "Probable AMR" when 3 criteria are met, and "Possible AMR" when 2 criteria are met. AMR is further classified into clinical (with graft dysfunction) or subclinical (without graft dysfunction). For example, the diagnosis of subclinical AMR could be made with positive histologic features on surveillance biopsies in the absence of allograft dysfunction. In cases where there is an isolated circulating DSA without allograft dysfunction, the document stresses that heightened surveillance for allograft dysfunction is warranted. Based on experience in kidney and heart transplantation,[7,8] the natural history, clinical outcomes, and need for early treatment of subclinical AMR should be evaluated prospectively.

This initial set of criteria was created to standardize the diagnosis of acute pulmonary AMR, recognizing that a unified definition would foster further clarification of the natural history of AMR, promote collaboration and consistency between transplant centers, and facilitate engagement in multicenter trials that are evaluating therapeutic options. The expectation is that with additional experience and collaborative validation studies, the definition will be further refined and revised. For example, based on widespread experience and recent evidence, should C4d-negative AMR (probable in the current classification) be regarded as an additional phenotype of definite AMR?[9] Answers to this and other similar questions will require further adjudication of these criteria. Further classification of pulmonary AMR with regard to severity (mild, moderate, severe) will be a critical next step in defining this entity and in understanding the course and outcomes of AMR.

The consensus focused only on criteria required for defining acute AMR. The field is still lacking the data required to differentiate acute AMR from other phenotypes including hyperacute and chronic AMR. This classification will be important for clinical monitoring and management.

Currently, there is no published or standardized approach to monitoring patients with pulmonary AMR. The consensus noted

TABLE 1 Definition and diagnostic certainty of clinical and subclinical pulmonary antibody-mediated rejection

	Allograft dysfunction	Other causes excluded	Lung histology	Lung biopsy C4d	DSA	Certainty
Clinical	+	+	+	+	+	Definite
	+	+	+	−	+	Probable
	+	+	+	+	−	Probable
	+	+	−	+	+	Probable
	+	−	+	+	+	Probable
	+	+	+	−	−	Possible
	+	+	−	−	+	Possible
	+	+	−	+	−	Possible
	+	−	+	+	−	Possible
	+	−	+	−	+	Possible
	+	−	−	+	+	Possible
Subclinical	−	−	+	+	+	Definite
	−	−	+	−	+	Probable
	−	−	−	+	+	Probable
	−	−	+	+	−	Probable
	−	−	+	−	−	Possible
	−	−	−	+	−	Possible
	−	−	−	−	+	Possible

Adapted from Levine et al.[4]

aDSA, donor specific antibody.

that a standardized method to evaluate these patients before and after therapy is necessary to carry out controlled studies between centers.

3 | AMR PATHOLOGY

3.1 | Histopathologic criteria for AMR

The original 1990 ISHLT Working Formulation for the diagnosis and reporting of allograft rejection focused on ACR, lymphocytic bronchiolitis (LB), and obliterative bronchiolitis (OB) but did not consider AMR.[10] The 2007 revised ISHLT Working Formulation introduced terminology that refined the histopathologic descriptions of AMR from previous published reports (Table 2).[11]

Arteritis may be seen in the setting of high-grade ACR as well as with AMR (Figure 1A). The term "acute capillary injury" replaced terms such as "septal capillary necrosis" in recognition of the underlying mechanism of immunologic injury. In 2012, the Pathology Council of ISHLT proposed a series of definitions to further refine the morphologic continuum of AMR, with "neutrophilic margination" (Figure 1B) and "neutrophilic capillaritis" (Figure 1C-E) proposed as histopathologic patterns along with "acute lung injury with/without hyaline membranes" (Figure 1F-H) to reflect gradations of septal capillary injury.[12] Neutrophilic capillaritis is defined as patchy or diffuse septal capillary neutrophilic collections associated with cellular karyorrhectic debris. Other features can include microvascular thrombi, alveolar hemorrhage, and/or accumulations of neutrophilic

infiltrates within adjacent alveolar airspaces. Neutrophilic margination is characterized by increased numbers of neutrophils within septal capillaries but lacking capillary injury, in particular, the absence of karyorrhexis. To date, these findings have been evocative but rather insensitive markers of AMR.[6] Other patterns reported in AMR include persistent or recurrent high-grade ACR, LB, and OB.

The difficulty with enumerating specific histopathologic criteria for AMR is that the findings largely reflect nonspecific patterns of lung injury. These patterns can be seen in a host of other allograft-related injuries such as infection, severe ACR, aspiration, drug toxicity, and in the early posttransplant period, secondary to preservation or ischemic-reperfusion injury. The group emphasized the importance of the multidisciplinary assessment of AMR, as the morphologic features to this diagnosis were not specific to this process and concurrent diagnosis related to a given pathologic feature should be excluded for AMR diagnosis.

Of interest, a multicenter study about pathology associated with DSA highlighted the relatively low interobserver reliability for the different pathology patterns, with assessment of ACR and C4d having the best kappa value (0.4).[13]

Staining for the complement split product C4d (Figure 1D) as an adjunct for diagnosing AMR in lung biopsies continues to present challenges.[13] Although it was hoped that C4d staining in lung allografts would be as sensitive and specific as it is in cardiac and renal allografts, published studies have presented conflicting results with differing rates of positive C4d staining. Currently, C4d positivity is defined as more than 50% stained interstitial capillaries.[12] However,

TABLE 2 Histologic patterns evocative of AMR and differential diagnosis

Histologic pattern evocative of AMR[a]	Differential diagnosis[b]
Neutrophilic margination	Infection[c]
	Ischemia-reperfusion injury[d]
Neutrophilic capillaritis	Infection[c]
	Ischemia-reperfusion injury[d]
Acute lung injury pattern/diffuse alveolar damage	Infection[c]
	Toxic inhalation
	Ischemia-reperfusion injury[d]
Persistent/recurrent ACR (any A grade)	Persistent/recurrent ACR without AMR component[e]
High-grade ACR (≥A3)	High-grade ACR without AMR component[e]
	Infection[c]
Persistent low-grade LB (grade B1R)	Infection[c]
	GERD
	Low-grade LB without AMR component[e]
High-grade LB (grade B2R)	Infection[c]
	GERD
	High-grade LB without AMR component[e]
Obliterative bronchiolitis (grade C1)	Chronic rejection
Arteritis	Infection[c]
	ACR without AMR component[e]
Any histologic findings in setting of DSA positivity (eg, AFOP)	Infection[c]

ACR, acute cellular rejection; AFOP, acute fibrinous and organizing pneumonia; AMR, antibody-mediated rejection; BAL, bronchoalveolar lavage; DSA, donor-specific antibody; GERD, gastroesophageal reflux disease; LB, lymphocytic bronchiolitis.

[a]Only lesions proven to be associated with AMR in previous publications. This listing might be further completed.

[b]Nonexhaustive differential diagnoses for histologic pattern suspicious of AMR; only the more frequent are listed.

[c]Diagnosis of infection should integrate clinical presentation, BAL cellularity and microbiology results, and response to antimicrobial treatment.

[d]Diagnosis of ischemia-reperfusion injury should include clinical presentation according to primary graft dysfunction classification and chronological approach. Lesions compatible with ischemia-reperfusion present only on month 1 biopsy and not on prior biopsy are less likely related to ischemia-reperfusion injury.

[e]Antibody-mediated component should be discussed based on DSA presence, C4d staining, absence of other cause, and failure of T cell–targeted treatment. These lesions have been described but currently are thought to be due exclusively to cellular (T cell-mediated) rejection.

as in the heart and kidney Banff classification, a 3-category classification with 2 cut-offs (negative if <10%, weak if between 10% and 50%, and positive if >50%) may provide more precise depiction of the staining status.

The sensitivity and specificity of C4d positivity in the lung biopsy, however, is much less reliable than that published in the kidney and heart literature. A recent study to analyze this disparity revealed that lung transplant pathologists from multiple international centers (G. Berry, unpublished data) demonstrated reproducibility in their interpretation of a series of C4d-stained lung biopsies. These results suggest the issue of infrequent C4d staining is other than technical in origin.

Nonspecific binding of anti-C4d antibody to normal structures, such as elastin fibers of large vessels or hyaline membranes, is frequent. This can lead to difficulty in interpretation; however, this may also be regarded as an internal control for C4d staining (ie, unstained elastin fiber[s] reflect failure of C4d staining that preclude interpretation of C4d negativity). The specificity of C4d staining in the lung allograft is further limited by C4d deposition observed in infection and preservation injury. Thus C4d interpretation may require integration of clinical context and microbiology results.[14]

C4d-negative AMR, widely documented in the heart and kidney literature, has recently been demonstrated in lung transplantation.[9] Future work to refine the definition of positive C4d staining or consideration of C4d-negative AMR will facilitate further refinements of the AMR criteria. Thus C4d positivity could be considered a less mandatory key feature for AMR diagnosis and more of a supportive marker to assist in considering treatment strategies including anti-complement therapy.[15,16]

3.2 | Recent developments

Recent studies evaluating additional histologic features that may allow for further refinement of the diagnosis of pulmonary AMR were reviewed and discussed. Lepavec (Poster 958, ISHLT 2017) identified increased interstitial neutrophils by myeloperoxidase immunostaining in patients who were DSA positive, suggesting an association with the diagnosis of AMR. Reproducibility, tissue size, and the optimal assay all require further evaluation in larger cohorts. Calabrese presented results from a multicenter study utilizing computerized morphometric analysis. Increased alveolar septal widening was identified on light microscopy as a possible new feature of AMR. The results from a larger case series are expected to be available soon.

4 | DONOR-SPECIFIC ANTIBODY (DSA) IDENTIFICATION AND CHARACTERIZATION

4.1 | Human leukocyte antigen DSA

Development of HLA antibodies (Abs) in lung transplant recipients has been associated with the development of Bronchiolitis obliterans syndrome and Chronic Lung Allograft Dysfonction (CLAD).[17] Introduction of single-antigen bead array (SAB) to identify HLA Abs has significantly improved the sensitivity and specificity of circulating donor-specific HLA Ab (or DSA) detection[18] in all solid organ transplants. In lung transplantation, as in other organs, class II de
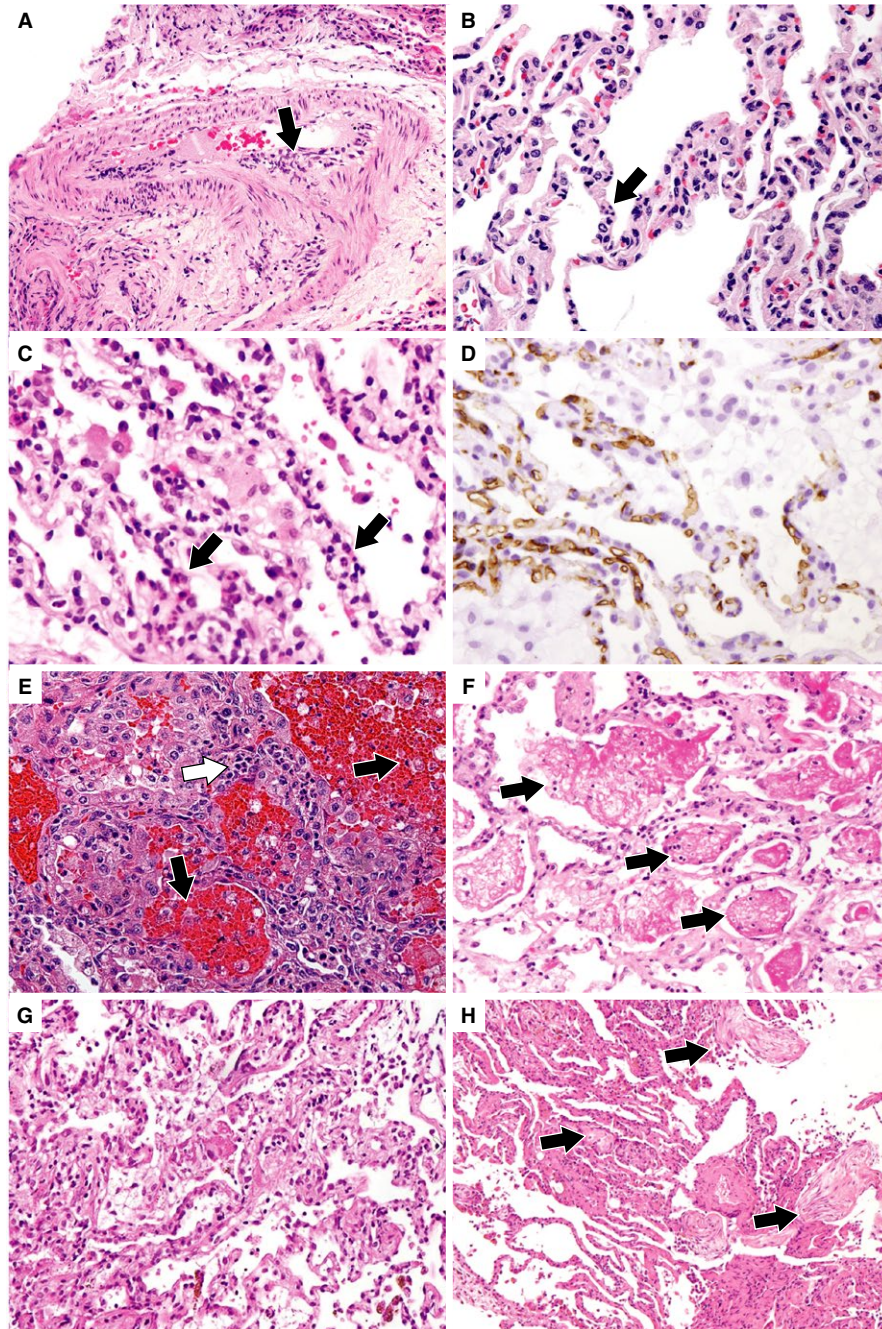
FIGURE 1 Panel of histologic features evocative of AMR. (A) Arteritis in a patient with definite AMR. Intimal inflammation and reactive changes in a small pulmonary artery (arrow). Hematoxylin & eosin, original magnification ×200. (B) Neutrophilic margination in an explanted lung from a patient with probable AMR and advanced obliterative bronchiolitis. Alveolar septa show a subtle increase in neutrophils (arrow) above baseline. Hematoxylin & eosin, original magnification ×300. (C-D) Capillary inflammation in a patient who developed early definite AMR (30 days after transplant). The histology shows excessive septal neutrophils with back-to-back features (C, arrows), and C4d deposition is seen in septal capillaries. Hematoxylin & eosin (C) and C4d immunostain (D), original magnification ×100. (E) Neutrophilic capillaritis in a patient with rapid chronic lung allograft dysfunction (obliterative bronchiolitis and chronic vascular rejection) along with probable AMR in the setting of prior episodes of AMR. Lung tissue shows diffuse neutrophilic infiltration (white arrow) with breakdown of the interstitial connective tissue and intraalveolar hemorrhage (black arrows). Hematoxylin & eosin, original magnification ×300. (F) Definite AMR manifesting histologically as acute fibrinous organizing pneumonia (AFOP) in a patient with multiple bilateral consolidative opacities on computed tomography scan. Alveolar spaces are filled with eosinophilic balls of fibrin (arrows) and lack an acute inflammatory infiltrate. Hematoxylin & eosin; original magnification ×200. (G-H) Two histologic patterns of acute lung injury from 2 different patients with definite and probable AMR (C4d-negative), respectively (developed 2 and 72 months after lung transplantation). (G) Exudative phase of lung injury with septal edema, alveolar fibrin, and mixed inflammation. (H) Organizing phase of lung injury with intra-alveolar plugs of organizing fibroblast (organizing pneumonia; arrows). Hematoxylin & eosin, original magnification ×200 and ×100, respectively

novo HLA Abs are most frequent, with a predominance of anti-DQ DSA.[19-21] Several reports describe the negative impact of DSA, especially early (within 3 months of transplantation), persistent, and de novo DSA after lung transplantation.[19,21]

Characterization of DSA (level, function, and IgG subclass) has improved risk stratification for allograft loss in heart and kidney transplantation, with fewer studies in lung transplantation.[4,21,22] Incorporating high-resolution typing for HLA antigens for donor/recipient pairs at the allele level can improve the characterization of DSA specificity and its impact on allograft outcome. There are, however, controversies regarding the clinical significance of DSA assessment by SAB. Further studies will need to address if all DSA detected by sensitive SAB are equally deleterious. In addition, we do not know the full impact of known limitations of the assays on DSA interpretation. The benefits and limitations of SAB have been addressed in many reviews.[18,23] In Table 3, we describe a few issues that may affect test interpretation and provide potential solutions to avoid false positive or incomplete results that may influence patient management. Detection of DSA must be interpreted within the context of assay limitations and clinical findings.[4,24] Characterization of DSA level and function may contribute to immunologic risk assessment and guide the clinical management of lung transplant recipients. Several groups are evaluating the association of DSA in lung tissue with AMR, both in clinical samples and in vivo models.[22,25] For example, a recent single-center study by Visentin et al reported a higher risk of graft loss with intragraft DSA than with serum DSA.[22]

4.2 | Non-HLA antibodies

More recent studies have demonstrated a potential role for non-HLA targets in AMR. Self-antigens that have received the most attention in lung transplantation are type V collagen, K-alpha 1 tubulin, and angiotensin type 1 receptor (anti-AT1R). These are expressed on both airway epithelial and endothelial cells, and antibodies against these self-antigens have been associated with primary graft dysfunction and bronchiolitis obliterans syndrome.[26] The presence of anti-AT1R and endothelin-1 receptor type A antibodies has been observed pretransplant and posttransplant,[27] with a negative impact on clinical outcomes[28] including a fatal case of hyperacute rejection.[29] These non-HLA Abs may lead to subclinical or clinical AMR, and should be kept in mind as possible causative agents when patients undergoing lung transplantation develop immediate and intractable pulmonary arterial hypertension, so that appropriate treatment measures can be implemented in a timely manner.

5 | TREATMENT OPTIONS

There is a dearth of high-quality evidence to guide the management of pulmonary AMR, with no randomized controlled trials and no head-to-head comparisons conducted to date. Treatment regimens have typically been individualized, and the specific treatments have

depended on the clinical course and response to first-line interventions. This makes it difficult to draw firm conclusions about the relative efficacy of any specific treatment or regimen. Nevertheless, the goals of treatment include depleting circulating DSA, suppressing additional antibody formation, and blocking antibody-mediated lung injury. Prevention of AMR using techniques such as organ allocation, perioperative desensitization, and preemptive treatment of DSA was not evaluated during the meeting and therefore is not detailed in this section. Relevant studies[3,5,6,30,31] of AMR treatment were discussed during the meeting and are summarized in Table 4. Despite the limitations of these studies, DSA clearance has been associated with superior survival.[5,6] This suggests that antibody depletion is critical for a favorable clinical response.

There is little experience with the use of complement inhibitors in pulmonary AMR. Although the ISHLT definition includes C4d deposition as a necessary criterion for the diagnosis of definite AMR, most cases are C4d-negative,[3,5,6,30,31] and preliminary data suggest that C4d-negative cases have presentation, DSA, histology, and outcomes similar to those of C4d-positive cases.[9] Beyond the diagnostic ramifications, C4d deposition may have important therapeutic implications if complement inhibitors are considered.[15,32,33]

To date, the optimal regimen for the treatment of pulmonary AMR is unknown. Specific recommendations of therapeutic agents and regimens are not included in this document because of the limited amount of evidence. Recognizing this lack of high-quality evidence supporting any one regimen, the committee concluded that it is necessary to collect both individual center clinical experience as well as to perform randomized controlled trials to better identify the best therapeutic options. Targeted trials focusing on specific clinical scenarios will be critical to resolve particular issues. For example, there is equipoise to conduct a randomized-controlled trial comparing rituximab to bortezomib (or carfilzomib) in addition to intravenous Ig (IVIg) in patients with AMR and mild allograft dysfunction. Another example would be to examine the role of therapy in subclinical AMR. This would further our understanding of the best monitoring strategies and the impact of persistent antibodies with and without therapy.

However, the committee suggested that therapeutic decisions be based on the severity of allograft dysfunction, clinical course, pathologic changes, presence of complement-binding DSA or C4d deposition, and presence or absence of other existing causes of allograft dysfunction.

The group discussed specific clinical situations and suggested options based on individual experience. For example, in patients with AMR resulting in CLAD, the committee considered the potential benefit of the combination of rituximab (given once or twice 30 days apart) and monthly IVIg for at least 6 months. For specific cases where there is mixed cellular- and antibody-mediated rejection, the group considered a potential role for the addition of anti-thymocyte globulin (ATG) or alemtuzumab. A possible role for ATG in pure AMR was also recognized because of its B-cell inhibitory effect. In cases of moderate or severe allograft dysfunction, the committee suggested that more intensive therapy is necessary. They proposed the addition of plasma

TABLE 3 Challenges for donor-specific antibodies assessment

Challenges	Interpretation	Resolution	References
False positive result	Clinically irrelevant HLA-Ab to denatured antigens	Perform surrogate crossmatch	18
	Nonspecific binding of IgG following IVIg	Repeat testing after acid treatment of SAB	
		Repeat testing after 2 weeks	
False low MFI or negative results	Inhibition of SAB assay due to intrinsic and extrinsic factors.	Removal of complement inhibition by addition of EDTA, heat treatment, dilution, and dithiothreitol for IgM	
	Lack of donor antigen in the Luminex bead assay	Identify the epitope of DSA and use if possible surrogate beads, or use alternative vendors	
Discordant results between SAB MFI and reactivity using cellular targets.	False low MFI: DSA to a shared target present on multiple beads	Adequate analysis of specific DSA allele/epitope	
Assessment of DSA specificity	Incorrect assignment when allele specific DSA is present and typing of donor allele is missing	Incorporate recipient and donor HLA typing for the allele level to properly assign presence or absence of DSA	40,41
	Incorporate all class I and class II HLA antigens including HLA –C, DRB3/4/5, DQB1, DQA1, DPB1, DPA1 for DSA determination	Typing of recipient and donor for all Class I and II HLA antigens if necessary retrospectively to improve DSA assignment	
		Consider DSA specificity for donor DQA/DQB pair	
Assessment of DSA burden based on single MFI level	Low or high MFI level of DSA may not correlate with: (1) risk of AMR, or (2) response to treatment following antibody removal therapies	Modified SAB assay to distinguish between complement and noncomplement-binding DSA and determining titer of DSA (serial dilutions of patient sera)	18,42,43
AMR features without serum HLA DSA	Presence of non-IgG DSA, of non-HLA Ab, of DSA against a nontyped HLA gene, or DSA against an HLA allele not represented in the SAB assay	See above. For non-HLA antigens, see text for targets reported in the literature	

Ab, antibody; DSA, donor-specific antibody; IVIg, Intravenous immune globulin; MFI, mean fluorescent intensity; SAB, single antigen bead array.

TABLE 4 AMR treatment efficacy in lung transplant patient

Reference	N	ISHLT definition diagnostic certainty	Treatments[a]	Outcomes
Lobo et al[3]	10	Definite and probable AMR cases	Steroids, IVIg, rituximab, PLEX, bortezomib	7/10 died: 5 died due to AMR and 2 died due to sepsis.
Otani et al[30]	9	Definite and probable AMR cases	Steroids, IVIg, rituximab, PLEX	5/9 had initial response: 4 died due to AMR; 2/5 subsequently developed progressive CLAD and died.
Witt et al[6]	21	Definite AMR cases	IVIg, rituximab, PLEX, bortezomib	15/21 had initial response: 6 died due to AMR; 13/14 developed CLAD and 15/21 died during the study period.
Roux et al[5]	22	Definite and probable AMR cases	IVIg, rituximab, PLEX	12/22 developed graft loss: 8 died and 4 required re-transplantation; 9/15 developed CLAD.
Ensor et al[31]	14	Definite, probable, and possible AMR cases	carfilzomib, IVIg, PLEX	10/14 responded to treatment by becoming DSA C1q negative; 7/14 died during the study period.

IVIg, intravenous immune globulin; PLEX, plasma exchange.
[a]In general, different combinations of the listed treatments were used in individual cases.

exchange (or immunoadsorption), proteasome inhibition (bortezomib or carfilzomib), and anti-complement therapy in cases where complement activation is evident (C1q-positive DSA or C4d-deposition). However, they noted that the addition of plasma exchange introduces certain complications regarding drug dosing particularly for IVIg, rituximab, and eculizumab. Finally, the committee acknowledged the emergence of novel agents including IDeS, tocilizumab, and others and recommended future trials evaluating these agents.

6 | EVALUATION AND OUTCOMES OF THERAPY

Currently, there are no evidence-based recommendations for monitoring after treatment. Expectations for improvement and its timing will depend on the treatment used (Table 5). Questions that require further study include the following: (1) What relevant endpoints to evaluate? (ie, DSA, pulmonary function tests [PFTs], histologic features, C4d staining), and (2) When and how frequently should these assessments occur? It is likely that the assessment after treatment should include short, intermediate, and long-term endpoints.

The diagnostic characteristics[4,12] used to define, classify, and stratify pulmonary AMR may also be useful for the evaluation of therapeutic outcomes. In the immediate posttherapeutic period, allograft dysfunction, histologic features, C4d staining, and circulating DSA can all be assessed for improvement or resolution. The diagnosis of CLAD or allograft/patient survival could be used to assess long-term outcomes of pulmonary AMR.

6.1 | Allograft dysfunction

Typically, acute allograft dysfunction is defined as a decline in Forced expiration volume in 1 seconde from baseline, radiographic infiltrates, change in oxygenation, or need for mechanical ventilation. Recovery or improvement of any of these features following treatment could be used as an outcome measure. In patients with subclinical AMR (ie, without allograft dysfunction), the diagnosis relies on histopathologic changes, C4d staining, and circulating DSA. In patients with CLAD secondary to AMR, a period of several months of follow-up after treatment may be necessary before any indications of stabilization or improvement are observed.

6.2 | Lung histology and C4d staining

As noted above, the histologic changes consistent with AMR, documented by the ISHLT working group in 2013, are nonspecific, and interobserver agreement for these features was recently described as slight to moderate with kappa values ranging from 0.14 to 0.4.[13] Beyond the challenge of reproducibility, the timeline for pathologic reassessment after therapy remains unknown.

6.3 | Circulating DSA

Clearance of DSA has been associated with improved allograft outcomes.[34] Complete DSA clearance, however, was found in only 40% to 60% of treated patients in 2 studies.[6,34] Clarification of what constitutes a clinically significant reduction of DSA is still needed. Future trials will need to assess how best to quantify the reduction (ie, mean fluorescent intensity vs titer, individual DSA vs all DSA) as well as the characteristics of the DSA (ie, HLA class, specificities, complement-binding, IgG subtypes).

7 | TISSUE GENE EXPRESSION FOR DIAGNOSIS OF ALLOGRAFT DYSFUNCTION

Several potentially complementary approaches for assessing the molecular phenotype of pulmonary allograft dysfunction include (1) gene expression analysis of prospectively collected biopsy

TABLE 5 Therapeutic options for AMR treatment in lung transplantation

Treatment	Target	Endpoint	Timing for action[a]	Length of action[a]	References
Plasmapheresis	DSA depletion	DSA decrease/clearance	immediate	Few weeks	44
Immunoadsorption	DSA depletion	DSA decrease/clearance	immediate	Few weeks	45
Rituximab	DSA production inhibition (B cell depletion)	DSA decrease/clearance	Immediate B cell depletion	Several months	46
			Delayed DSA decrease (few months)		
Proteasome inhibitors	DSA production inhibition (plasma cell depletion)	DSA decrease/clearance	Immediate plasma cell depletion	Several months	47
Human immunoglobulin	Downregulate B cells	DSA complement binding decrease	Few days for DSA complement binding	3-4 weeks	46
	Block effect of DSA on allograft	Possible C4d conversion to negative	Few weeks for C4d staining		
Complement inhibitors	Block effect of DSA on allograft	DSA Complement binding decrease	Few days for DSA complement binding	Few weeks	33,48,49
		C4d conversion to negative	Few weeks for C4d staining		

DSA, donor-specific antibody.
[a]Given only as an estimate.

samples using microarrays and (2) retrospective gene expression analysis of historical biopsies.

The molecular diagnosis of AMR in kidney transplantation has been more widely studied[35] than that in lung transplantation. It has facilitated insights into the nature of AMR, including changes in AMR subtypes with time posttransplant.[36] Rejection-associated transcripts (RATs) have been used to develop a liquid biopsy-type diagnostic platform for heart allografts[37] and are now being evaluated for lung allografts. This is timely, as there are concerns about reproducibility of interpretation of lung transplant histology.[38] A current study (NCT02812290) is prospectively enrolling lung transplant recipients with the objective of developing a lung-specific T cell–mediated rejection (TCMR), AMR, and all-rejection score.

Future work assessing the molecular phenotype of historical cases with definite, probable, and possible AMR has also been proposed. This would involve gene expression analysis of archival formalin-fixed paraffin-embedded biopsies[39] and include controls of other processes (eg, infection) and pure cellular rejection.

8 | FUTURE INVESTIGATIONS

Increased multicenter engagement to refine the histologic criteria for the diagnosis of pulmonary AMR is essential. The creation and utilization of a standardized grid with all the agreed-upon histologic, immunohistochemical (ie, C4d), and molecular features along with serologic and clinical data would be a significant step forward for the lung transplant community. This framework would provide a source for inter-institutional sharing of index cases for review and adjudication. This network of cases would then be shared with clinicians and immunologists for a multidisciplinary assessment. This network would be available not only for research purposes, but also would allow for an international database for all specialties to use as a clinical resource to both identify and manage pulmonary AMR. Survey of the different Trans Bronchial Biopsies and DSA testing strategies are mandatory for further evaluation of their impact on clinical outcome their respective cost-efficacy.

The group determined that future RCTs should focus on 2 critical clinical areas:

1. Prevention of AMR and its consequences. Future work is required to evaluate patients with isolated DSA (without allograft dysfunction or pathologic findings). This would be best studied in a multicenter clinical trial evaluating whether "preemptive therapy" of DSA will decrease the risk of pulmonary AMR or CLAD. Suggested endpoints include AMR, CLAD, and allograft loss.
2. Therapeutic options for AMR. Suggested trials would include comparative evaluation between therapies in addition to standard of care treatment. Short-term outcomes would include the recovery from AMR (clinically and histologically). Mid- and long-term outcomes would integrate CLAD and allograft loss, and recurrence of AMR.

9 | CONCLUSION

Our understanding of the pathogenesis, morphologic features, clinical presentation, treatment, and molecular expression in pulmonary AMR is still rudimentary, and there is much to be learned. Although the diagnosis and management of AMR in heart and kidney transplantation have evolved over the last 2 decades, the concept of pulmonary AMR has only been described in the literature over the last 10 years, and there has only recently been a consensus for the definition. The ISHLT consensus statement aggregated the available literature and clinical experience to permit uniform and systematic investigations. Further modifications will be forthcoming as we develop a better understanding. Ongoing collaborations between centers, ISHLT, and Banff will be necessary to work towards furthering these efforts as a community.

DISCLOSURE

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