



International Society for Heart and Lung Transplantation Scientific Statement on Pulmonary Antibody-Mediated Rejection and Proposed Graft, Antibody, and Pathology (GAP) Definition

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All authors participated in a comprehensive literature search, developing statements, voting on statements in multiple rounds, critical review and providing edits and final approval of the manuscript. FC, DJL, RRH, AMJ, JG, AZ, MH, MM, RV, ACR, GAF, KAWB, SAE, AR, ARG, CB, AC, JJ, LM, and SV participated in writing the manuscript.

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Abstract

Antibody-mediated rejection (AMR) is an increasingly recognized form of rejection and cause of graft failure after lung transplantation. AMR has been the focus of extensive research over the past decade. Despite growing awareness and recent advances in our understanding of AMR, outcomes remain dismal with a 2-year survival of only 20%. The International Society for Heart and Lung Transplantation convened a multidisciplinary workgroup of experts in AMR to review the most up-to-date research and clinical experience and to update the 2016 definition. The workgroup was divided into nine subgroups covering a broad range of topics pertaining to AMR and used the Delphi method to synthesize a cohesive summary of the literature. A multidimensional definition was developed to enhance precision by reporting the specific presenting features. This Graft, Antibody, and Pathology (GAP) definition is based on the presence of Graft dysfunction, the presence and characteristics of Antibodies, and Pathological findings. The workgroup emphasized that identifying better treatments for AMR is a critical unmet need and proposed that a more precise definition might allow better management by providing a platform for testing and developing new therapies.

Introduction

Antibody-mediated rejection (AMR) is an increasingly recognized form of lung rejection, in large part because of increased awareness and the development of the standardized International Society for Heart and Lung Transplantation (ISHLT) definition in 2016 (1). Indeed, the incidence of clinical and subclinical AMR after lung transplantation is nearly 50% (2, 3). Despite the increased awareness, outcomes after the diagnosis of AMR remain dismal. Approximately 20% of patients die within 30 days, 50% die within one year, and 80% die within two years of the diagnosis of AMR (2–5). Most AMR survivors develop chronic lung allograft dysfunction (CLAD) early after the diagnosis (2–7). In a prospective study, 73% developed severe CLAD, defined as >50% loss of lung function compared to baseline, within one year of the diagnosis (2). Moreover, the leading causes of death after AMR are refractory AMR and progressive CLAD (2–8). Clearly, identifying better diagnostic, preventative, and therapeutic approaches for AMR is a critical unmet need for lung transplant recipients.

The 2016 ISHLT Consensus Report on AMR after lung transplantation developed a standardized definition and nomenclature. This increased the awareness of AMR as a potential cause of graft dysfunction and facilitated important research in the field. However, this progress has not resulted in improved clinical outcomes, and important areas of uncertainty remain. The 2016 definition relied on a probabilistic approach which categorized cases based on hierarchical levels of diagnostic certainty. This yielded arbitrary categories comprised of heterogeneous cases and proved challenging to apply to complex clinical settings. The ISHLT assembled this international multidisciplinary group of experts in AMR to review progress in clinical, translational, and basic science research since the original report in 2016 and to update the definition of AMR based on these advances. The ultimate objective is to improve clinical outcomes through 1) enhancing our understanding of the mechanisms that lead to AMR and 2) identifying and testing novel treatments in multicenter clinical trials. The workgroup proposed that an alphanumeric classification that provides a detailed description of the presenting features would yield a more precise definition of AMR and would facilitate these objectives.

Methods

Workgroup Member Selection and Subgroup Development

The ISHLT Advanced Lung Failure and Transplantation Interdisciplinary Network (IDN) selected three co-chairs with expertise in pulmonary AMR for the workgroup (FC, DJL, and RRH). The IDN solicited interest from the community to identify subgroup leaders and writing members. The selection process was based on the appropriate areas of expertise and broad geographic and demographic representation to ensure that international perspectives were represented. Overall, the workgroup comprised 38 members with expertise in pulmonary medicine (n = 18, 47%), pediatric pulmonary medicine (n = 3, 8%), histocompatibility and immunogenetics (n = 5, 13%), pathology (n = 6, 16%), pediatric pathology (n = 1, 3%), pharmacy (n = 2, 5%), basic and translational science (n = 2, 5%), and cardiothoracic surgery (n = 1, 3%). Many members had overlapping expertise in multiple disciplines but are enumerated according to their primary discipline. Workgroup members represented North America (n = 21, 55%), Europe (n = 13, 34%), and Australia (n = 3, 8%). Nine subgroups were developed to span the spectrum of information and research relevant to AMR (Supplemental Table 1). Subgroup leaders were then selected based on experience and willingness to commit the effort. Institutional Review Board approval was waived because developing this document did not constitute human subjects research. The manuscript was prepared in compliance with the ISHLT ethics statement.

Modified Delphi Method

The Delphi method gathers expert opinion through successive questionnaires, each refined by feedback from the previous round (9). By enabling anonymous, asynchronous input from geographically dispersed specialists, it has become a staple in medicine for guiding decisions when evidence is scarce or conflicting (10). The process was conducted in three rounds and was coordinated by the co-chairs. In the first round (exploratory phase), each subgroup conducted a comprehensive literature review and formulated open-ended questions to explore key uncertainties and practice variations in their assigned topics. Details of

the literature review are described in the Supplemental Material. All subgroup members independently submitted written responses. These were anonymized and synthesized. Draft statements were formulated based on emerging themes from the synthesis of open-ended responses and were not derived from pre-existing opinions or specific publications. This ensured that the literature review and expert input jointly informed the development of statements rather than simply voting on pre-formed conclusions. During the second round (statement development and voting), the refined statements were presented to the full workgroup, and members rated their agreement on an eleven-point Likert scale (-5 = strongly disagree, $+5$ = strongly agree), with the option to abstain for topics outside their expertise. In the third round, each statement was redistributed together with individual initial scores and the group mean $\pm$ standard deviation (SD), enabling participants to reconsider their positions and narrow score dispersion. Consensus was predefined, as in prior studies, as a mean ≥ 2.5 or ≤ -2.5 with an SD that did not cross zero (9, 11–20). On an eleven-point Likert scale, a mean score ≥ 2.5 corresponds to $\geq 75\%$ agreement. The SD was included as an additional indicator of agreement to ensure that statements classified as having reached agreement were not only characterized by a high mean score but also by a shared direction of agreement among respondents. This safeguards against scenarios where the mean might suggest agreement, but the distribution of responses indicates polarization. All voting remained anonymous to minimize social-desirability bias, and final mean $\pm$ SD values are reported for transparency. Further details regarding the methodology are presented in the Supplemental Material file and Supplemental Table 1.

SECTION I

Graft, Antibody, Pathology (GAP) Definition

Key Workgroup Statements:

1. Scheduled surveillance monitoring for AMR should be performed after lung transplantation.	For clinical AMR, mean score = 4.32 ± 0.82 For subclinical AMR, mean score = 4.21 ± 0.88
2. AMR can co-exist with other causes of allograft injury including acute cellular rejection (ACR) and infection.	Mean score = 4.27 ± 0.74
3. A scoring rubric for HLA DSA that includes persistence, HLA class, number of specificities, relative strength (low, moderate, strong), and C1q or C3d positivity could improve its value as a diagnostic and prognostic criterion.	Mean score = 3.79 ± 0.83
4. There is evidence that HLA DSA may be pathogenic in the absence of C4d deposition, allograft dysfunction, or abnormal histology.	Mean score = 3.89 ± 0.93
5. Pathological findings frequently detected in AMR include diffuse alveolar damage (DAD), acute fibrinous and organizing pneumonia (AFOP), capillary injury, endothelialitis, microthrombi, and septal widening.	Mean score = 4.00 ± 0.96

Definition

The workgroup recognized that the diagnosis of AMR remains inherently difficult because it requires multiple clinicopathological criteria and a multidisciplinary approach. Consequently, the workgroup recommended scheduled surveillance monitoring for clinical and subclinical AMR and a multidisciplinary approach to facilitate earlier diagnosis and treatment. Surveillance protocols for AMR have not been studied. Thus, the workgroup could not recommend a specific protocol, but the workgroup encourages clinicians to have

increased awareness and vigilance of AMR as a potential cause of allograft dysfunction by developing protocols compatible with available local resources. In addition, AMR can present with a range of clinical and pathological features that may vary between individual cases. The basis of this variability remains unclear, but this may be related to time of onset, different phenotypes of AMR (e.g., complement-dependent, complement-independent), and the interval between onset and presentation. Therefore, the workgroup proposed that the definition should report the clinical features present to precisely characterize a specific case (Table 1). This would facilitate future research in the field and standardize the nomenclature across centers. The definition includes core elements that are essential for the evaluation and diagnosis of AMR, and non-core elements that provide supplemental information but are not routinely performed in clinical practice at many centers. The workgroup proposed the Graft, Antibody, and Pathology (GAP) definition based on the following criteria: the presence of graft dysfunction (G), the presence and characteristics of antibodies (A), and pathological findings (P). To align with the ISHLT statement on acute lung allograft dysfunction (ALAD), the AMR workgroup proposed that graft dysfunction, in the context of AMR, can be defined as new or worsening respiratory symptoms, >10% decline in spirometry from baseline, new or worsening hypoxemic respiratory failure, or new or worsening imaging abnormalities. Importantly, the workgroup proposed that AMR can co-exist with other causes of graft injury including ACR, obliterative bronchiolitis, and infection. This is an important distinction from the 2016 definition which considered exclusion of other causes of graft dysfunction as one of the five criteria for the diagnosis of definite AMR and required that the other four criteria be present if other causes of graft injury were not excluded for the diagnosis of probable AMR (1). This difference represents an evolution in the clinical approach to the evaluation of graft dysfunction, an increased recognition of mixed rejection (21, 22), and emerging evidence that infection can induce the development of DSA and AMR (23, 24). Table 2 outlines various combinations of features reported by the GAP definition and whether these represent a diagnosis of AMR or not. A “Need Further Assessment” designation is included as the workgroup’s recommendation to pursue additional studies that are not routinely performed at all centers to enhance diagnostic certainty. Figures 1–6 illustrate the GAP definition using 6 clinical cases.

Antibodies

The Antibodies subgroup focused on defining the assessment and characteristics of HLA and non-HLA antibodies that correlate with graft injury (Supplemental Table 2). Solid-phase single antigen bead (SAB) technology has revolutionized the field of HLA antibody detection, yet the mean fluorescent intensity (MFI) metric has been widely incorporated into clinical practice before the assay’s limitations were fully realized (25, 26). The literature on HLA antibody testing has been summarized in several consensus reports from the Sensitization in Transplantation: Assessment of Risk (STAR) evidence-based expert review (27–29), which demonstrated that MFI does not always reflect HLA antibody levels due to interfering substances within serum or saturation due to the assay’s limited dynamic range. Serum pre-treatments (e.g., EDTA, heat, DTT), complement binding/activation capacity, and the use of serum dilutions have greatly improved SAB performance for assessing HLA antibody level and correlating this with clinical outcomes (30–34). The workgroup agreed that a scoring rubric for HLA DSA that includes antibody characteristics,

such as persistence, HLA class, number of specificities, and relative strength, would enhance the assay's prognostic value and its contribution to the diagnosis of AMR. The workgroup recognized the limitations of MFI as an assessment of antibody strength and that interrogating HLA DSA beyond MFI is necessary to improve its correlation with AMR and its diagnostic application (Supplemental Table 2). DSA strength has been defined in the context of thresholds that would yield a positive or strongly positive T cell or B cell flow cytometry crossmatch. However, the HLA community has not endorsed specific cutoffs to define upper and lower MFI ranges because of interlaboratory variability and the lack of validated proficiency testing. Although the detection of strong (e.g., high MFI, high-titer, C1q-positive) DSA can make the diagnosis of AMR more compelling, finding weak (e.g., low MFI, low-titer, C1q-negative) DSA does not make the diagnosis less conclusive as a substantial amount of DSA could be bound to the graft causing significant tissue damage while only present in low titers in peripheral blood. To better characterize DSA, the workgroup proposed that if the HLA laboratory does not assess or report DSA strength, the presence or absence of HLA DSA would be noted as A = 1 or A = 0, respectively (Table 1). On the other hand, if DSA strength is assessed using MFI, weak DSA would be defined as $MFI < 2,500$ (A = 2), intermediate DSA would be defined as $MFI = 2,500 - 8,000$ (A = 3), and strong DSA would be defined as $MFI > 8,000$ (A = 4) (Table 1). Although titer is emerging as a more accurate assessment of DSA strength, specific cutoffs for weak, intermediate, and strong DSA are less well defined. Correlating DSA strength with clinical outcomes and validating different cutoffs are important topics for future research. C1q positivity indicates an antibody's ability to bind and activate complement and has important diagnostic and prognostic implications; however, because of practical limitations with accessibility to the assay, this was proposed as a non-core element in the definition of AMR (Table 1).

The workgroup recognized that DSA may be pathogenic in the absence of C4d deposition, clinically apparent graft dysfunction, or abnormal pathology. It is now widely accepted that C4d deposition is not a sensitive marker for pulmonary AMR (5, 35, 36). In addition, C4d-negative cases of AMR may be due to non-complement-activating DSA that bind to HLA molecules on endothelial cells and injure the graft through antibody-dependent cellular cytotoxicity (ADCC) or through mTOR signalling, which triggers endothelial cell activation and proliferation (37, 38). Furthermore, longitudinal studies have demonstrated that DSA can precede the development of abnormal pathology, subclinical AMR, and can lead to impaired lung function long-term (2, 7, 8, 39). Additional studies correlating DSA characteristics with subclinical AMR and time to AMR are needed to better understand the pathophysiology of injury and guide the development of effective clinical interventions (Supplemental Table 3).

Non-HLA antibodies specific for self-antigens, such as angiotensin II type I receptor (AT1R), endothelin type A receptor (ETAR), collagen V, and K- α 1 tubulin, and polymorphic alloantigens such as major histocompatibility complex class I-related gene A/B (MICA/MICB) have also been associated with CLAD and graft failure. However, the optimal clinical approach to testing for and intervening on these non-HLA antibodies remains unclear (40). Sensitization to sequestered self-antigens is clearly elicited through graft injury in the context of inflammatory insults such as ischemia-reperfusion injury,

rejection, and/or viral or bacterial infection (41–43). Selected mechanistic studies demonstrate that non-HLA antibodies can induce pathologic changes including complement activation and endothelial cell (EC) activation leading to increased expression of adhesion molecules and the release of proinflammatory cytokines (44). Luminex-based non-HLA antibody panels now offer higher throughput, sensitivity, and specificity, but require further studies to define their analytic validity and allow widespread adoption in clinical practice. A recent multicenter study utilizing a non-HLA bead panel correlated the breadth of non-HLA sensitization with an increased risk of CLAD (45). In addition, a recent study documented cases of AMR associated with non-HLA antibodies in lung transplant recipients (46). However, the workgroup recognized that non-HLA antibody testing has not been routinely used in clinical practice despite evidence suggesting that these antibodies can cause AMR. Consequently, non-HLA antibody testing is proposed as a non-core element in the definition (Table 1). Importantly, AMR without detectable HLA DSA is increasingly recognized in kidney transplantation (22, 47). While this might be due to non-HLA antibodies (46), other explanations include intragraft production and binding of DSA with low circulating levels (24, 48–52).

Pathology

The Pathology subgroup focused on specific areas to address the most important clinical issues: a) the pathological lesions compatible with pulmonary AMR, b) C4d immunostaining, c) other tissue surrogates of AMR, d) combined pathological grading (score), and e) other concomitant lesions (e.g., ACR or infection) (Supplemental Table 4). Based on previous reports (4–7, 53–55), the workgroup proposed that histologic findings frequently detected in AMR include DAD, AFOP, capillary inflammation, endothelialitis, microthrombi, and septal widening. The workgroup recognized that the diagnosis of AMR remains inherently difficult because it requires multiple clinicopathological criteria and a multidisciplinary approach especially when concomitant lesions are present. A particularly challenging clinical scenario arises when DSA and ACR are detected in the absence of histological features suggestive of AMR. The workgroup proposed that such cases be interpreted as AMR only if ACR is persistent or is severe (grade > A3) and refractory to steroid therapy.

Studies have confirmed the poor sensitivity of C4d immunostaining in pulmonary AMR (5, 35). Thus, the workgroup did not support the routine use of C4d immunostaining in surveillance transbronchial lung biopsies nor the use of immunostaining for other complement components such as C3d for the diagnosis of AMR (Supplemental Table 4). On the other hand, more recent studies demonstrate that the mTOR pathway, specifically the phosphorylated S6 ribosomal protein (p-S6RP), is associated with AMR in heart and lung transplantation (56–59). Indeed, a recent multicenter study showed that p-S6RP immunostaining can be a useful surrogate marker for improving the accuracy of the diagnosis of AMR (56, 57). Thus, the workgroup proposed that immunostaining for mTOR pathway activation markers, including p-S6RP, may be used in the diagnosis of AMR (Supplemental Table 4). However, because p-S6RP immunostaining is not yet routinely performed at many centers, this was proposed as a non-core element in the definition of

AMR (Table 1). Additional information is presented in the Supplemental Material file and Supplemental Tables 2–4.

SECTION II

Pre-transplant Allosensitization, Monitoring, Pediatric Considerations, and Management

Key Workgroup Statements:

6. Pre-transplant allosensitization negatively affects waitlist outcomes (i.e., death on the waitlist, delisting, longer waiting time).	Mean score = 3.80 ± 0.96
7. It is currently unclear how allosensitized lung transplant candidates are best managed from a diagnostic and therapeutic perspective, given the current lack of international practice guidelines, which is an unmet need.	Mean score = 3.96 ± 0.93
8. Surveillance monitoring for AMR after lung transplantation should include scheduled DSA testing.	Mean score = 4.32 ± 0.9
9. Surveillance monitoring for AMR after lung transplantation should include scheduled transbronchial biopsies.	Mean score = 3.26 ± 1.43
10. In the absence of existing data to suggest otherwise, the histopathologic findings to support the diagnosis of AMR are not different in children from those in adults.	Mean score = 3.65 ± 1.05
11. To date, there exists no rational basis that would require age-based thresholds for different AMR diagnostic categories or thresholds for AMR treatment.	Mean score = 3.74 ± 1.10
12. <i>De novo</i> DSA that is accompanied by otherwise unexplained allograft dysfunction should be treated.	Mean score = 3.88 ± 0.93
13. AMR with allograft dysfunction should always be treated with multiple drugs or interventions.	Mean score = 3.75 ± 0.85

Pre-transplant Allosensitization

The workgroup recognized that pre-transplant allosensitization adversely impacts waitlist outcomes as well as short- and long-term post-transplant outcomes (Supplemental Table 5). Consequently, the workgroup recommended that scheduled testing for HLA antibodies should be performed at standardized intervals of at least every 6 months before transplantation (Supplemental Table 5). Furthermore, the workgroup acknowledged that the optimal management of allosensitized lung transplant candidates remains unclear, representing a significant unmet need in the field. Avoiding donors with reactive HLA allele combinations can reduce the risk but may lead to prolonged waiting times and a higher mortality on the waiting list (60, 61). Pre-transplant desensitization may be considered; however, previous studies have reported disappointing results (62, 63). This approach may be more effective with refined and personalized protocols. Recent studies have reported good long-term outcomes with perioperative and early post-operative antibody depleting treatments in patients with preformed DSA (64–68), but one study reported a significantly higher risk of AMR with this approach (69). Future research should prioritize identifying antibody characteristics that might guide risk stratification and treatment (Supplemental Table 5).

Monitoring

Current graft surveillance testing includes pulmonary function testing, imaging studies, DSA testing, and bronchoscopy with BAL and transbronchial lung biopsies (70). Regarding specific monitoring for AMR, the workgroup recommended scheduled testing for DSA and recognized that identifying a *de novo* DSA offered valuable prognostic information

about the risk of graft failure (Supplemental Table 6). The workgroup also recommended routine monitoring with scheduled transbronchial biopsies. Pulmonary function tests and imaging studies were also regarded as essential in the evaluation for AMR. Although the workgroup recognized the lack of data supporting specific monitoring protocols, these statements emphasize that assessment for AMR should remain part of longitudinal follow-up to facilitate a timely diagnosis, recognizing that AMR can occur beyond the first year after transplant. Supplemental Table 7 is an example of a surveillance monitoring protocol for AMR; however, studies are needed to better define the optimal monitoring strategy. In addition to scheduled monitoring, the workgroup recommended testing for DSA after a sensitizing event as well as evaluating for a humoral component of CLAD at the time of CLAD diagnosis (Supplemental Table 6).

In addition to current assessments, emerging modalities including donor-derived cell-free DNA (dd-cfDNA) are increasingly used at some centers as monitoring strategies. dd-cfDNA has demonstrated a high negative predictive value for acute complications including AMR, ACR, and infection (71–73). Furthermore, dd-cfDNA can be a marker of the degree of immunosuppression and can predict long-term clinical outcomes among patients who have DSA, suggesting that this may have utility in surveillance monitoring (74, 75). Though the workgroup recognized that dd-cfDNA may be an appropriate assay to adopt into monitoring protocols in the future, the lack of practice guidelines defining strategies to incorporate dd-cfDNA into monitoring protocols has limited widespread international use (Supplemental Table 6).

Pediatric Considerations

AMR is recognized as a potential cause of ALAD and a risk factor for the development of CLAD across all ages including infants, older children, adolescents, and adults (76–80). The incidence of AMR among pediatric recipients remains unclear; however, the Clinical Trials in Organ Transplantation in Children (CTOTC-03), reported an incidence of 18% over a two year follow up period (70). Additionally, there are data reporting a higher prevalence of DSA associated with AMR in cystic fibrosis (CF) lung transplant recipients (8). No pediatric-specific guidelines exist for the diagnosis or management of AMR, and evidence is limited. However, a randomized, placebo-controlled trial, CTOTC-08, suggested that rituximab induction may reduce DSA development (81). The diagnostic approach and management of AMR in children are therefore, currently based on the adult experience. The workgroup proposed that the histopathological criteria for the diagnosis of AMR and the treatment thresholds for AMR were similar between children and adults. The workgroup called for children to be included in multicenter studies using standardized and mechanistic protocols with support from regulatory and funding agencies to improve clinical outcomes (Supplemental Table 8).

Current Treatments and Outcomes

Although an abundant amount of evidence has shown that the development of DSA and AMR are common after lung transplantation and that these are associated with worse clinical outcomes including graft failure and death, there have been no randomized-controlled trials, systematic reviews or head-to-head comparisons of different treatments to

guide clinical management. In the absence of high-quality evidence, expert opinion is based on the existing literature, its limitations, and clinical experience. The workgroup proposed that *de novo* DSA accompanied by otherwise unexplained graft dysfunction should be treated. However, in the absence of graft dysfunction, the workgroup could not recommend treatment for *de novo* DSA, even if the DSA was specific to HLA-DQ (Supplemental Table 9). Previous studies have used different metrics to assess outcomes after AMR treatment including changes in DSA MFI, loss of DSA complement activation, clearance of DSA, and survival. The workgroup agreed that AMR treatment success can be defined as stable graft function over 6 months after an episode of AMR or as clearance of DSA, improvement in spirometry, and reversal of pathological changes (Supplemental Table 9). The workgroup recommended that clinical AMR should be treated with multiple drugs or interventions. However, the workgroup recognized that there is lack of high-quality evidence to guide management and could not recommend a specific treatment regimen. This underscores the critical unmet need for clinical trials to improve the care of patients with AMR (82). Additional information is presented in the Supplemental Material file and Supplemental Tables 5–9.

SECTION III

Priorities for Future Research

Key Workgroup Statements:

14. The most urgent research priority is a consistent and uniform definition of AMR that can be implemented in clinical and translational studies.	Mean score = 4.23 ± 0.94
15. Understanding the pathophysiological mechanisms that lead to AMR is a research priority.	Mean score = 4.37 ± 0.61
16. Although dd-cfDNA is a marker for lung allograft injury, it is not specific for AMR.	Mean score = 4.12 ± 2.55

The workgroup identified the development of a standardized definition of AMR as a high-priority objective to advance research and clinical practice. A multicenter international project is currently aggregating a large number of AMR cases to apply both the 2016 and the newly proposed GAP definition to facilitate validation and rapid adoption of the new definition. A better understanding of the pathophysiological mechanisms that lead to AMR and identifying sensitive and specific biomarkers were also considered research priorities (Supplemental Table 10). The workgroup noted that while dd-cfDNA can be interpreted as a marker of lung allograft injury, it is not specific for AMR. In addition, molecular analysis of blood or tissue should be studied further to improve the diagnosis of AMR and identify specific phenotypes (Supplemental Table 10).

The workgroup identified other critical unresolved mechanistic questions in AMR. In particular, the cellular immunologic basis of AMR, including the roles of B cells, plasma cells, T cells, and NK cells remains unclear. NK cells can recognize DSA via the CD16 FcγRIII-receptor to activate ADCC (83). NK cell receptor-ligand interactions may also modulate recipient immune responses and allograft antigen presentation in the context of AMR (84, 85). The relative contributions of complement activation and cell-mediated cytotoxicity (e.g., NK cells) in AMR requires further research (Supplemental Table 10).

Additional information is presented in the Supplemental Material file and Supplemental Table 10.

Summary

Despite increased awareness and intensive research in pulmonary AMR over the past decade, clinical outcomes remain dismal, with most patients developing severe CLAD or dying within two years of the diagnosis. The ISHLT convened this international, multidisciplinary workgroup of experts to review the literature and update the definition of AMR with the objective of detailing the latest clinical, translational, and basic science research in the field. Using the modified Delphi method, the workgroup synthesized the evidence and developed statements covering all aspects of AMR. The workgroup used the 2016 ISHLT consensus report (1) as a foundation for the current document and focused on areas where the literature and clinical experience have evolved over time. The workgroup proposed a new definition of AMR, introducing an updated framework of a multidimensional GAP definition, which incorporates **G**raft status, **A**ntibody characteristics, and **P**athology findings to report the features of a given case. Notable important advances in the definition include the recognition that C4d immunostaining is of limited clinical utility in pulmonary AMR. Indeed, most cases in the literature have been C4d-negative (3, 5–7), and the workgroup did not endorse routine C4d immunostaining. Nonetheless, assessment of complement activation was still thought to be an important aspect of the definition that might better phenotype AMR although complement activation, and C4d immunostaining in particular, were not considered necessary criteria for the diagnosis of AMR. There is growing mechanistic research that suggests an important role for NK and other FcR expressing cells in the pathogenesis of AMR through ADCC. Whether this will lead to novel therapeutic targets remains to be seen. A major strength of the GAP definition is its emphasis on multidisciplinary collaboration and the use of a standardized alphanumeric nomenclature. Moving forward, center-level implementation will require clear roles and responsibilities for each specialty in establishing the center's protocol for monitoring and managing AMR. While there is enthusiasm for the use of dd-cfDNA as a biomarker for AMR and graft injury, the workgroup did not endorse its routine use for monitoring for AMR or for assessing the extent of graft injury or response to treatment. International studies to validate the performance of dd-cfDNA and growing clinical experience with dd-cfDNA may substantiate its potential utility in routine monitoring. Given the heterogeneity of AMR and the lack of clinical trials, it is not surprising that the workgroup could not identify an optimal therapeutic strategy. The proposed multidimensional GAP definition details the presenting features of a specific case of AMR with the goals of enhancing the diagnostic precision of the definition, standardizing the nomenclature, and providing a framework to advance research and support the development of novel treatments. The proposed definition will require validation, preferably in multicenter studies, and refinement as new evidence emerges in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

With this manuscript, all members of the workgroup wish to pay tribute and express their profound and heartfelt gratitude to Dr. Allan Glanville. His unwavering commitment to a standardized, universally accepted definition of AMR was instrumental in advancing the field, laying the foundation for more accurate diagnosis and more effective, shared therapeutic strategies for this complex and challenging complication.

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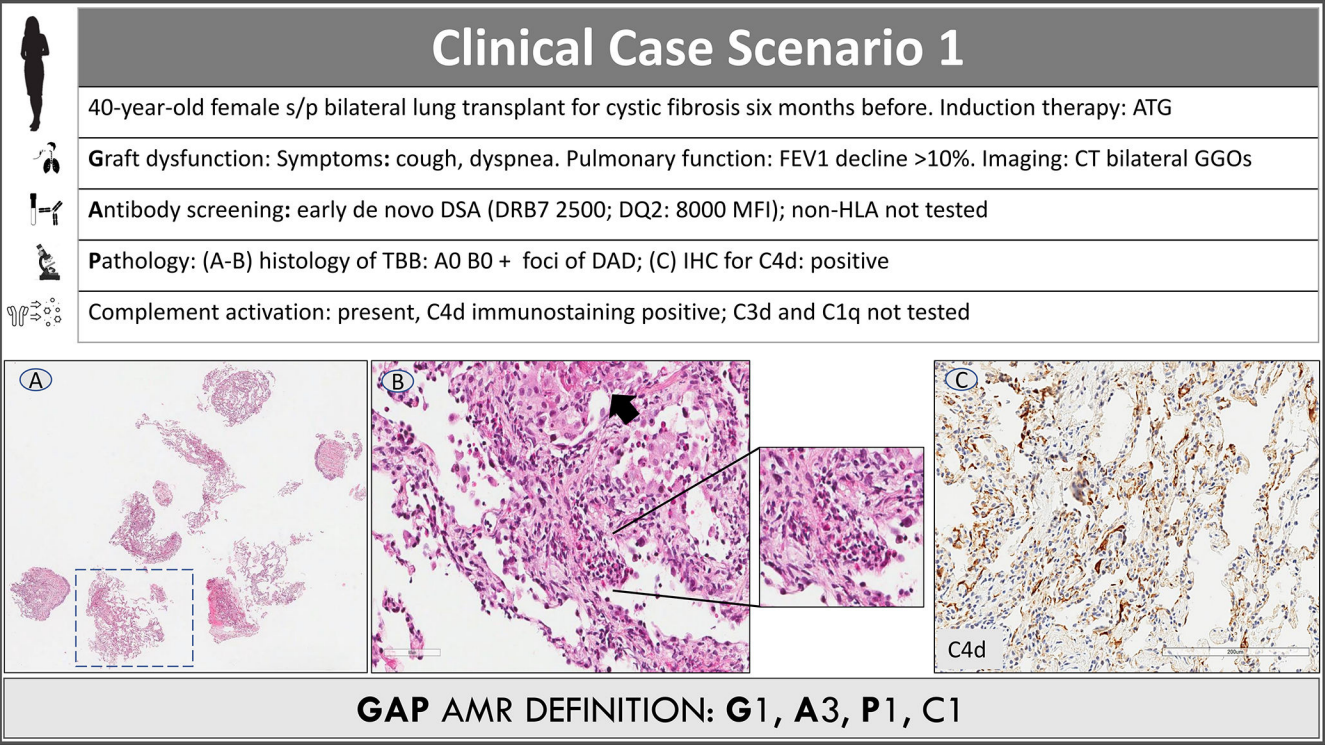


Figure 1.
Clinical Case Scenario 1. A: Hematoxylin & eosin-stained surveillance TBB. B: High magnification showing DAD with intra-alveolar hyaline membrane and macrophagic alveolitis (blue arrow) and neutrophilic capillaritis (insert). C: Diffuse strong C4d deposition. Case kindly provided by University of Padova (Padova; Italy). *Abbreviations:* ATG: Anti-thymocyte globulin; FEV1: Forced expiratory volume in one second; CT: computed tomography; GGO: ground glass opacity; DSA: donor specific antibody; HLA: human leukocyte antigen; DAD: diffuse alveolar damage, TBB: transbronchial biopsies

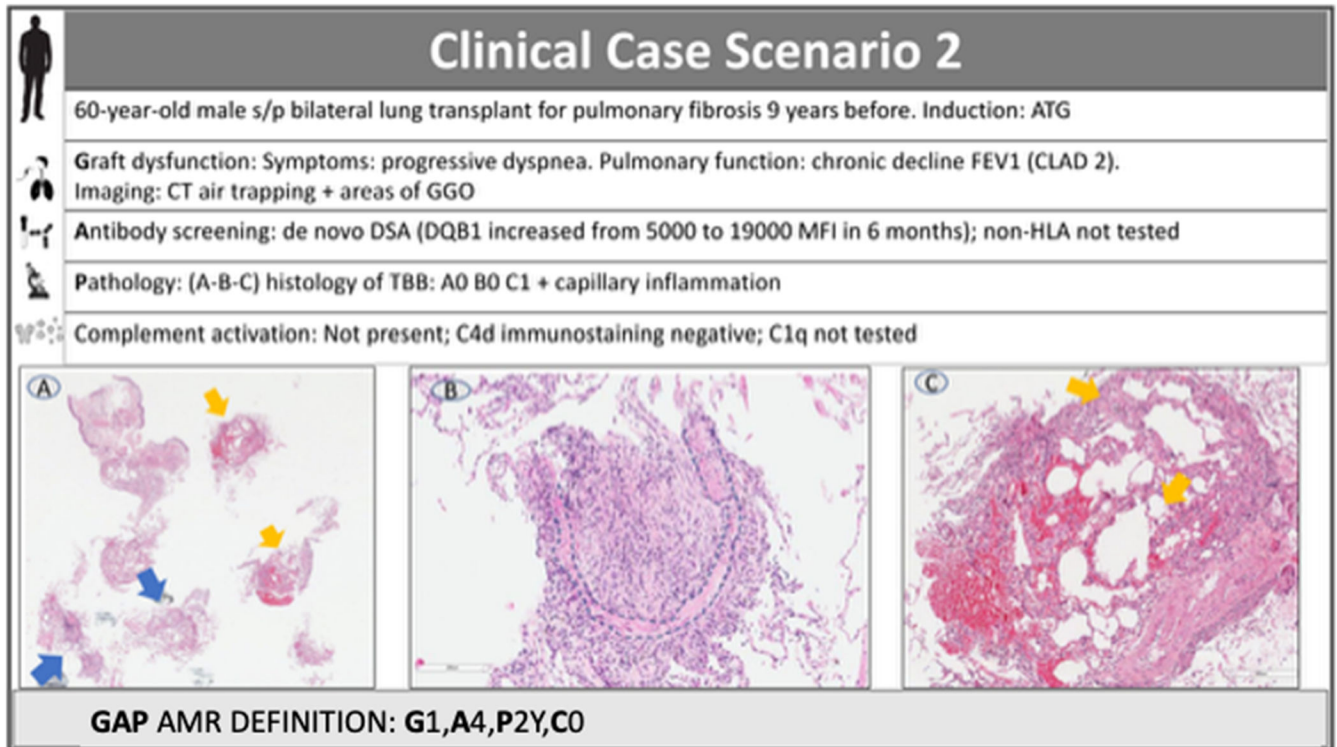


Figure 2.

Clinical Case Scenario 2. A: Hematoxylin & eosin-stained surveillance TBB. B: High magnification showing severe OB (the dotted lines mark the muscular component of the bronchiolar wall). C: High magnification showing foci of capillary inflammation (neutrophilic margination; yellow arrows) with intra-alveolar blood extravasation. Case kindly provided by provided by University of Padova (Padova; Italy). *Abbreviations:* ATG: Anti-thymocyte globulin; FEV1: Forced expiratory volume in one second; CLAD: chronic lung allograft dysfunction; CT: computed tomography; GGO: ground glass opacity; DSA: donor specific antibody; HLA: human leukocyte antigen, OB: obliterative bronchiolitis

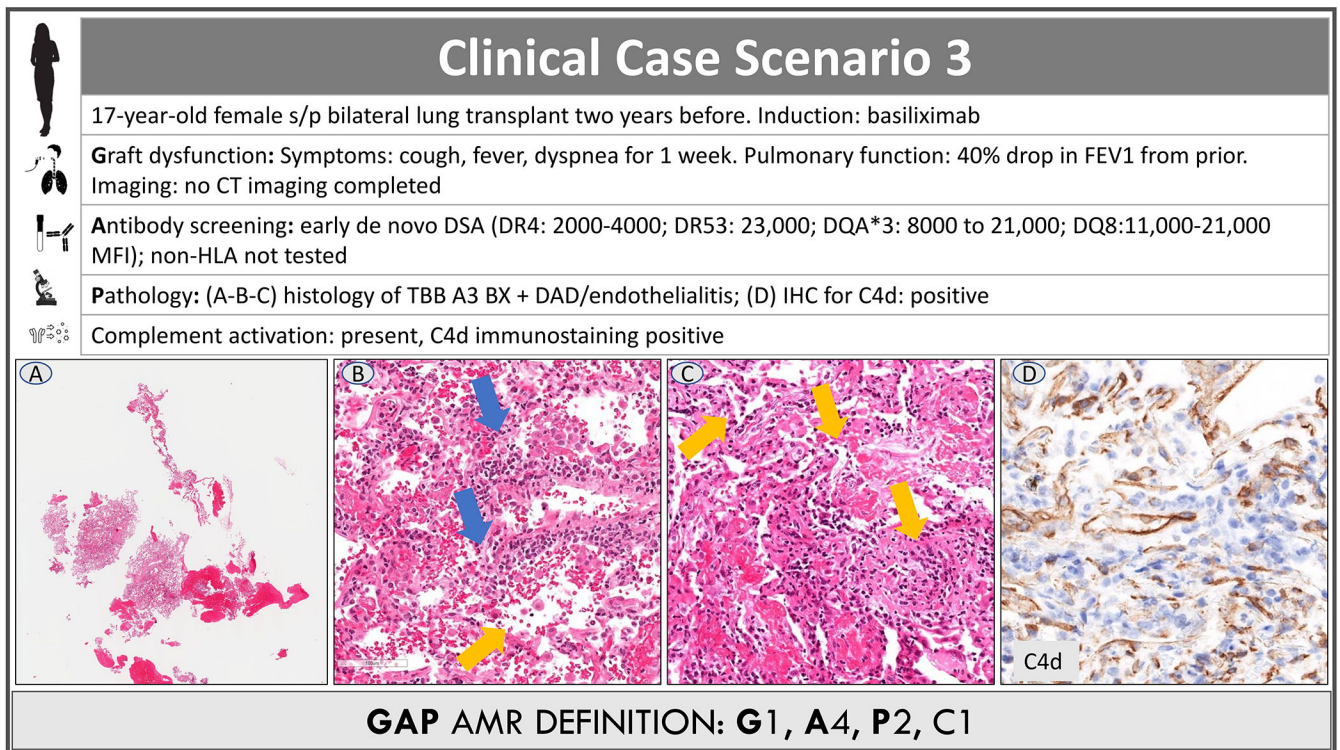
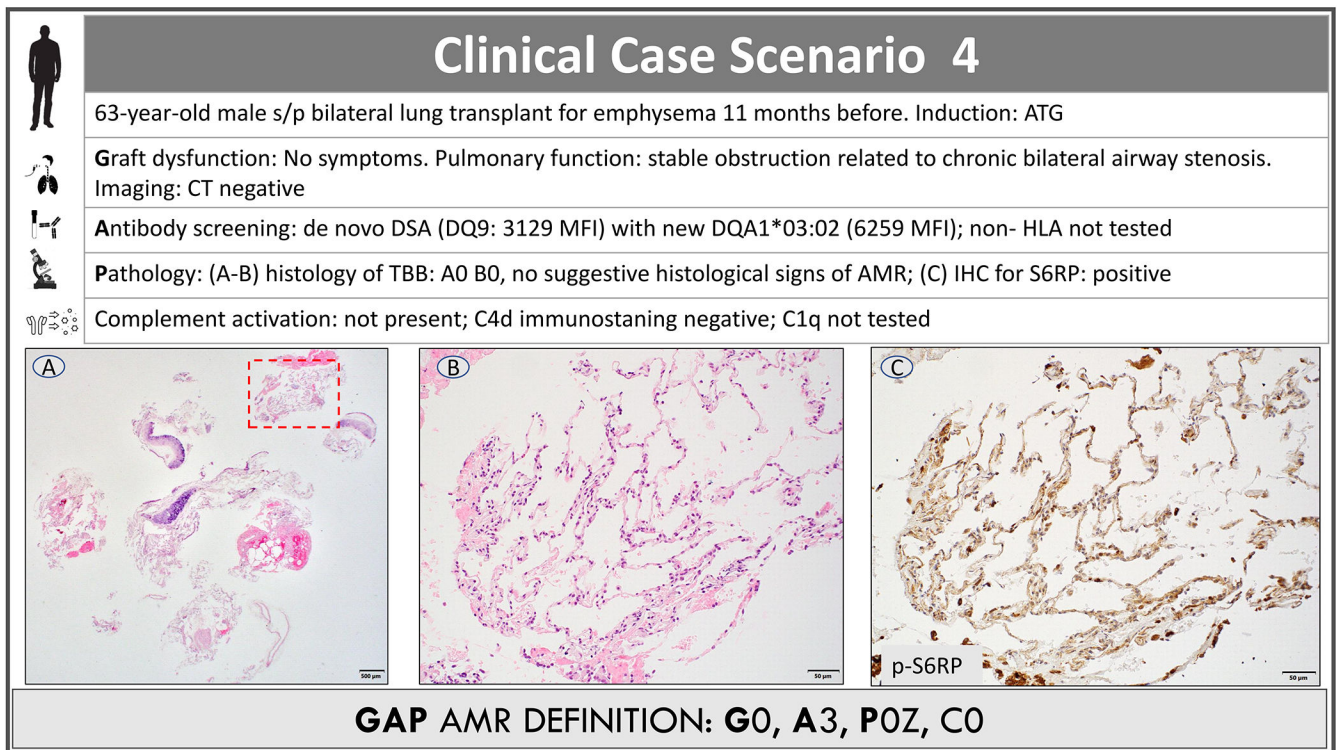


Figure 3.

Clinical Case Scenario 3. A: Hematoxylin & eosin-stained surveillance TBB. B and C: High magnification showing features of mixed rejection (Acute cellular rejection graded as A3 (blue arrows) and foci of DAD (yellow arrow and endothelialitis) (small yellow arrow). D: C4d immunoassay showing diffuse strong positivity in several capillaries. Case kindly provided by provided by University of Alberta (Alberta, Canada). *Abbreviations:* FEV1: Forced expiratory volume in one second; CT: computed tomography; DSA: donor specific antibody; HLA: human leukocyte antigen; TBB: transbronchial biopsy, DAD: diffuse alveolar damage.

**Figure 4.**

Clinical Case Scenario 4. A: Hematoxylin & eosin-stained surveillance TBB. B: high magnification showing no histological lesions. C: p-S6RP immunoassay section showing diffuse strong positivity in several cell types (endothelial, macrophages, epithelial cells). Case kindly provided by UCLA (Los Angeles, CA, USA). *Abbreviations:* ATG: Anti-thymocyte globulin; CT: computed tomography; DSA: donor specific antibody; HLA: human leukocyte antigen, TBB: transbronchial biopsy.

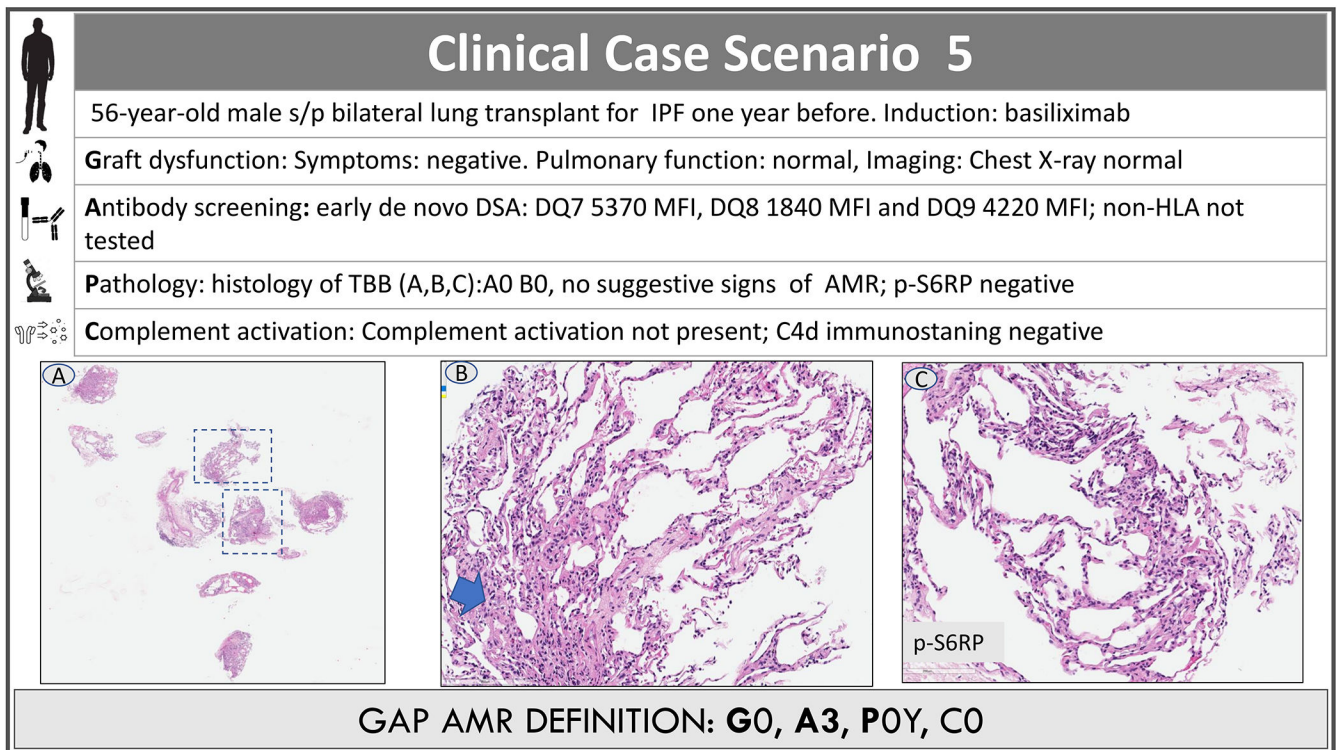


Figure 5.

Clinical Case Scenario 5. A: Hematoxylin & eosin-stained surveillance TBB. B and C: High magnification showing two samples without histological changes. Blue arrow showed interstitial widening with increased cellularity due to crush artifact. Case kindly provided by University of Padova (Padova, Italy). *Abbreviations:* IPF: idiopathic pulmonary fibrosis; DSA: donor specific antibody; HLA: human leukocyte antigen, TBB: transbronchial biopsy.

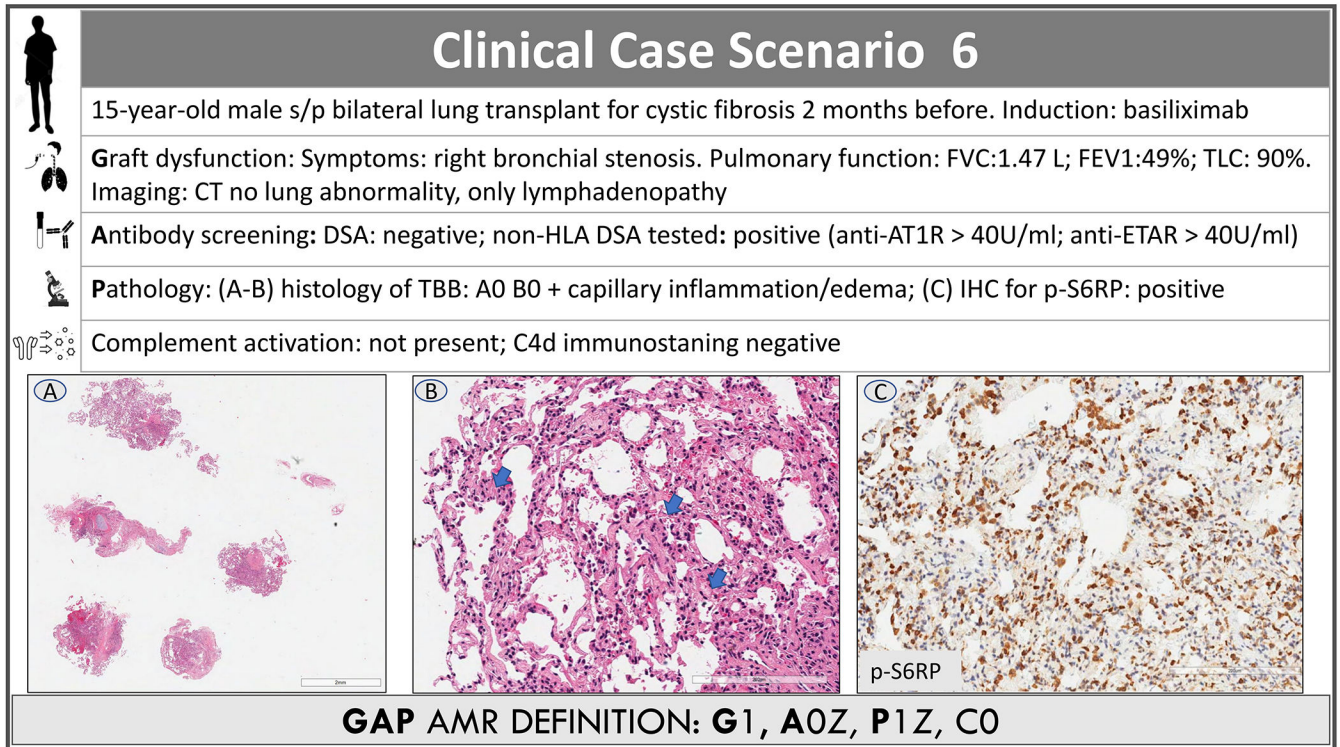


Figure 6.

Clinical Case Scenario 6. A: Hematoxylin & eosin-stained surveillance TBB.. B: High magnification showing edema and capillary inflammation (neutrophilic margination, blue arrow). D: p-S6RP immunoassay section showing diffuse strong positivity in several cell types (endothelial cells, macrophages, and epithelial cells). Case kindly provided by the University of Padova (Padova, Italy). *Abbreviations:* CT: computed tomography; DSA: donor-specific antibody; HLA: human leukocyte antigen, TBB: tranbronchial biopsy.

Table 1.
Graft, Antibody, Pathology (GAP) Definition of Antibody-Mediated Rejection

Parameter	Characteristics	Code
G: Graft dysfunction		
	Core Not present	0
	Core Present	1
A: Antibodies		
	Core No HLA DSA present	0
	Core HLA DSA present	1
	Weak HLA DSA (MFI: < 2,500) present	2
	Intermediate HLA DSA (MFI: 2,500 – 8,000) present	3
	Strong HLA DSA (MFI > 8,000) present	4
	<i>Non-core Non-HLA antibody not present</i>	<i>Y</i>
	<i>Non-core Non-HLA antibody present</i>	<i>Z</i>
P: Pathology		
	Biopsy not done or inadequate	X
	Core Histological findings frequently detected in AMR (e.g., DAD, OP, AFOP, capillary inflammation, septal widening, endothelialitis, microthrombi) not present	0
	Core Histological findings frequently detected in AMR present	1
	Core Histological findings frequently detected in AMR in addition to acute cellular rejection, chronic rejection, or lymphocytic bronchiolitis present	2
	<i>Non-core p-S6RP immunostaining negative</i>	<i>Y</i>
	<i>Non-core p-S6RP immunostaining positive</i>	<i>Z</i>
C: Complement activation		
	<i>Non-core Complement activation is not present (DSA C1q and/or C4d are/is negative)</i>	<i>0</i>
	<i>Non-core Complement activation is present (DSA C1q is positive and/or C4d deposition is positive)</i>	<i>1</i>

Abbreviations HLA: human leucocyte antigen; MFI: Mean Fluorescence Intensity; DSA: donor specific antibody; DAD: diffuse alveolar damage; OP: organizing pneumonia; AFOP: acute fibrinous organizing pneumonia; p-S6RP: phosphorylated S6 ribosomal protein.

Table 2.
Examples of Antibody-Mediated Rejection Classifications.

Code	AMR*	Comments
G0 A0 P0	Not AMR	No evidence of rejection or HLA DSA
G1 A0 P0	Not AMR	No evidence to implicate the role of HLA-DSA in graft dysfunction. Consider other etiologies (e.g., infection). To exclude other plausible humoral causes, consider testing for non-HLA antibodies.
G0 A1 P0	Not AMR	No evidence of rejection caused by HLA-DSA. Consider close follow-up for intermediate and strong HLA-DSA (A 3, A 4).
G0 A0 P1	Not AMR	Histologic findings frequently detected in AMR are not specific for AMR. Consider other etiologies (e.g., infection). To exclude other plausible humoral causes, consider testing for non-HLA antibodies.
G0 A0 P2	Not AMR	Histologic findings frequently detected in AMR are not specific for AMR; Consider other etiologies (e.g., infection). To exclude other plausible humoral causes, consider testing for non-HLA antibodies.
G0 A1 P1	AMR	Subclinical AMR
G0 A1 P2	AMR	Subclinical AMR
G1 A1 P1	AMR	Clinical AMR
G1 A1 P2	AMR	Clinical AMR
G1 A0 P1	NFA	Graft dysfunction with histologic findings frequently detected in AMR but no HLA DSA. Consider other etiologies (e.g., infection). Consider testing for non-HLA antibodies, C4d staining, p-S6RP staining.
G1 A0 P2	NFA	Graft dysfunction with histologic findings frequently detected in AMR but no HLA DSA. Consider other etiologies (e.g., infection). Consider testing for non-HLA antibodies, C4d staining, p-S6RP staining.
G1 A1 P0	AMR	Consider sampling problem/limitation, especially in cases with intermediate and strong HLA-DSA (A 3, A 4).

* AMR: this column indicates whether the combination of findings reported by the Code represents antibody-mediated rejection or not. Some combinations need further assessment (NFA) as noted in the Comments.

Abbreviations: AMR: antibody-mediated rejection; HLA: human leucocyte antigen; DSA: donor specific antibody; NFA: need further assessment; p-S6RP: phosphorylated S6 ribosomal protein.