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MINIREVIEW

When tissue is the issue: A histological review of chronic lung allograft dysfunction

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Although chronic lung allograft dysfunction (CLAD) remains the major life-limiting factor following lung transplantation, much of its pathophysiology remains unknown. The discovery that CLAD can manifest both clinically and morphologically in vastly different ways led to the definition of distinct subtypes of CLAD. In this review, recent advances in our understanding of the pathophysiological mechanisms of the different phenotypes of CLAD will be discussed with a particular focus on tissue-based and molecular studies. An overview of the current knowledge on the mechanisms of the airway-centered bronchiolitis obliterans syndrome, as well as the airway and alveolar injuries in the restrictive allograft syndrome and also the vascular compartment in chronic antibody-mediated rejection is provided. Specific attention is also given to morphological and molecular markers for early CLAD diagnosis or histological changes associated with subsequent CLAD development. Evidence for a possible overlap between different forms of CLAD is presented and discussed. In the end, “tissue remains the (main) issue,” as we are still limited in our knowledge about the actual triggers and specific mechanisms of all late forms of posttransplant graft failure, a shortcoming that needs to be addressed in order to further improve the outcome of lung transplant recipients.

KEYWORDS

biopsy, bronchiolitis obliterans (BOS), lung (allograft) function/dysfunction, lung transplantation/pulmonology, pathology/histopathology, rejection: antibody-mediated (ABMR), rejection: chronic, translational research/science

1 | INTRODUCTION: A HISTORICAL PERSPECTIVE

At the time of implementation of human allogenic lung transplantation (LTx) by James Hardy in 1963,¹ survival was limited to a maximum of

weeks due to surgical and infectious complications. It took until 1971 for a posttransplant survival of 10 months to be reported by Derom et al.² Following several episodes of severe acute rejection, 6 months posttransplant their patient developed irreversibly decreased values of forced expiratory volume in 1 second (FEV₁) and FEV₁/forced vital

Abbreviations: AFOP, acute fibrinoid organizing pneumonia; BO, bronchiolitis obliterans; BOS, bronchiolitis obliterans syndrome; cAMR, chronic antibody-mediated rejection; CLAD, chronic lung allograft dysfunction; DAD, diffuse alveolar damage; DSA, donor-specific antibodies; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; HSCT, hematopoietic stem cell transplantation; ISHLT, International Society for Heart and Lung Transplantation; LTx, lung transplantation; PPFE, pleuroparenchymal fibroelastosis; RAS, restrictive allograft syndrome.

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capacity (FVC), suggestive of an obstructive airway disease. At autopsy, the bronchi displayed various degrees of fibrotic stenosis with infiltration by inflammatory cells. These findings from our current perspective represent the first described case of bronchiolitis obliterans (BO) as a manifestation of chronic lung allograft dysfunction (CLAD).

However, it took more than a decade until BO as an entity was formally defined by Epler and Colby as a fibrosing inflammatory process involving the terminal and respiratory bronchioles. At the time BO was thought to arise either idiopathically or following exposure, infection, or in the context of connective tissue disease.³ Subsequently, BO was also described in association with progressive obstructive pulmonary function defects following bone marrow transplantation⁴ and in more patients after LTx. Indeed, in biopsies and autopsies from 4 LTx recipients, Burke et al demonstrated extensive BO in 3, again associated with a progressive decline in pulmonary function, but they also found varying degrees of interstitial and pleural fibrosis.⁵ This report in conjunction with similar observations by other groups^{6,7} led to the acceptance of BO as the morphological correlate of what was at the time termed "chronic rejection." Subtotal and total airway obliteration as well as active and inactive remodeling based on the degree of lymphocytic airway inflammation were further used to categorize BO. At the time, it was already recognized that transbronchial biopsies generally have a low sensitivity to detect BO lesions due to the latter's heterogeneous and mostly peripheral distribution.⁸ Therefore, bronchiolitis obliterans syndrome (BOS), a clinical entity solely based on lung function tests, was introduced with 2 subcategories based on confirmation of the clinical diagnosis by histopathology.⁹ In later revisions, the relevance of histology faded further due to the aforementioned low sensitivity of biopsies, and BOS became a strictly clinical diagnosis based on the FEV₁. At that point, histology was mostly used to rule out other posttransplant complications, such as infection or acute cellular rejection. This definition was first challenged by the identification of a group of patients that recovered their FEV₁ following long-time treatment with azithromycin and on the histological level showed persistent chronic airway inflammation (lymphocytic bronchiolitis).¹⁰ However, interest in histopathology was really rekindled by observations of a primarily restrictive form of allograft dysfunction associated with an alternative form of fibrotic pulmonary remodeling.^{11,12} These observations eventually led to the definition of a restrictive variant of CLAD in 2011, restrictive allograft syndrome (RAS),¹³ an entity with its own specific clinical, radiologic, and histologic characteristics. As BO may also occur in RAS as a co-injury pattern, a fibrous alveolar obliteration with prominent elastic fiber deposition referred to as alveolar fibroelastosis (AFE), has been acknowledged as the main defining histomorphological feature.¹⁴ While lungs from patients with BOS typically show signs of airtrapping in computed tomography (CT) scans and by definition present with a strictly obstructive pulmonary function defect, patients with RAS show persistent radiological opacities and functional signs of restriction (decrease in total lung capacity $\geq 10\%$ or FVC $\geq 20\%$ compared to baseline).¹⁵

Based on these observations, the term CLAD has been (re-) implemented as an umbrella term, encompassing all subforms of chronic pulmonary graft failure.¹⁶ The more prevalent BOS subform (65%-75% of CLAD patients) has a median survival postdiagnosis of 3-5 years, while the less frequent RAS form (25%-35% of CLAD patients) shows an even worse prognosis limited to only 0.5-1.5 years. Although clinically well defined, major gaps in our understanding of CLAD remain, including the relationship between BOS, RAS, and antibody-mediated rejection (AMR). AMR is considered a process of immune activation, whereby B cells and plasma cells produce antibodies against donor antigens, a process clinically and morphologically well defined in kidney and heart transplant recipients, but poorly characterized in LTx recipients. The reported incidence of pulmonary AMR is highly variable, but unequivocally associated with a poor outcome.¹⁷

The process of correlating histological and molecular injury patterns with distinct clinical forms of CLAD is still ongoing and has been recognized to be of major prognostic importance. Therefore, in this review we will discuss the different presentations of CLAD, focusing on histopathological and molecular aspects of BOS, RAS, and AMR.

2 | BRONCHIOLITIS OBLITERANS SYNDROME

In an attempt to comprehensively quantify the degree of fibrotic airway remodeling in BOS lungs, post-LTx, CT, and microCT analysis were used on end-stage BOS explant specimens, demonstrating abundant small airway obstruction, starting from the fifth generation of airway branching and increasing to 40%-70% of airways being obstructed from the ninth generation of airways. Interestingly, next to the commonly known fibrotic BO lesions, evidence of airway obstruction in the form of an airway scar without luminal extracellular matrix accumulation was also identified.¹⁸ It was also evident that both types of airway obstruction were only segmental and that distally of BO lesions the airways reopen, an aspect that could be confirmed using serial sectioning¹⁹ (Figure 1).

BO is believed to be the result of persistent microinjuries (ie, toxic exposures, reflux, viruses, microbes, etc) to the epithelial lining, resulting in chronic inflammation, wound healing, and excessive (myo-)fibroblast recruitment and activation, ultimately leading to luminal obliteration of the peripheral small airways, although larger airways can also be affected. The adjacent alveolar tissue typically remains intact. A loss of the submucosal glands in cartilaginous airways and a subtotal loss of basal cells, which act as epithelial progenitor cells, are observed in large and small airways in BO lungs.²⁰ Chronic inflammation, submucosal extracellular matrix accumulation, muscle cell hyperplasia, and ultimately complete obliteration of the airway lumen with partial destruction of the original smooth muscle layer are often found. Evidence of aberrant angiogenesis is also seen in the form of an increase and enlargement of the microvasculature within and adjacent to BO lesions

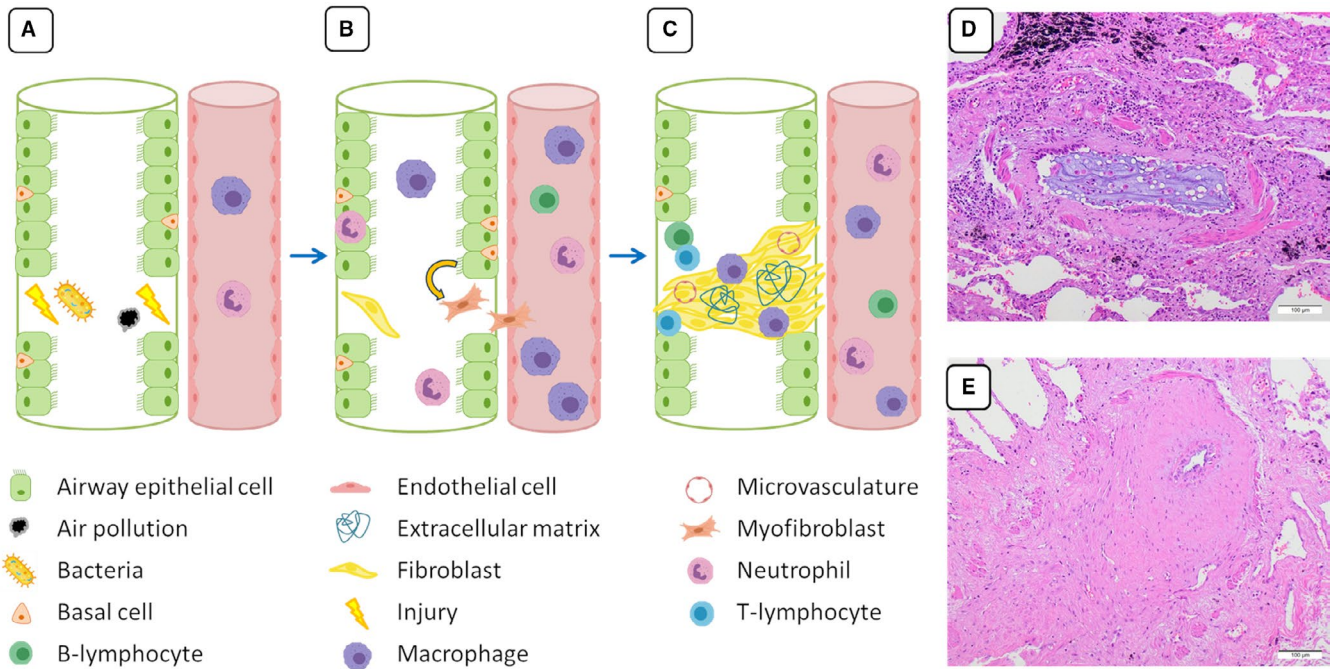


FIGURE 1 Pathophysiological mechanisms of airway fibrosis in bronchiolitis obliterans. A, Persistent airway microinjury induced by bacteria, viruses, gastroesophageal reflux, or air pollution lead to the destruction of the airway epithelial layer. B, The destruction of the airway epithelial layer induces the recruitment of inflammatory cells via the adjacent vessels leading to an excessive and uncontrollable inflammatory response, which subsequently drives recruitment of resident (myo-) fibroblasts, the transition of epithelial cells to a mesenchymal phenotype, and overall migration of (myo-)fibroblast progenitor cells to the site of the injury. C, Occlusion of a segment of the airway lumen by a plug of (myo-)fibroblasts and newly deposited extracellular matrix, further characterized by the presence of newly developed microvessels, macrophages, and loss of airway basal cells. D, Illustrative H&E of injured mucus-filled airway demonstrating fibrosis of the mucosa and airway epithelial injury. E, H&E demonstrating subtotal fibrotic obliteration of the airway typical for bronchiolitis obliterans [Color figure can be viewed at wileyonlinelibrary.com]

(Figure 1).^{18,21} Here, the amphiregulin-hyaluronan axis is considered a major driving force in the epithelial response to injury as it accumulates in the affected epithelium in fibrotic airways.^{22,23} While amphiregulin, an epidermal growth factor receptor ligand, induces an increased proliferative capacity, an initial mucous hyperplasia, and altered ciliated cell differentiation in airway basal cells, hyaluronan regulates the subsequent innate inflammatory responses by releasing cytokines, chemokines, and growth factors attracting predominantly neutrophils and macrophages. As this inflammatory response to injury spirals out of control, (myo-) fibroblasts from different sources accumulate, including epithelial to mesenchymal transition,²⁴ recruitment of fibrocyte progenitors from the peripheral blood,²⁵ accumulation of resident fibroblasts, while a diminished/alteration in the microvasculature has also been put forward as one of the main mechanisms towards BO development.²⁶

BO manifests in very different clinical settings, and some studies compared BO post-LTx to BO post-hematopoietic stem cell transplantation (HSCT). Although both patient groups share an allo-immune setting with additional infectious triggers, patients after HSCT lack the epithelial injury due to cold and warm ischemia associated with LTx. However, airway morphometric comparison revealed no differences between BO post-lung transplant and BO post-HSCT, while the molecular signatures consisting mostly of matrix

remodeling genes were also very similar in both conditions, resulting in the conclusion that “BO is essentially BO” whatever the underlying cause.²⁷

Given the strong signature of differentially expressed genes associated with BO, their analysis is thought to be usable for an early prediction and thus an earlier diagnosis of CLAD. In this respect, molecular profiling of transbronchial biopsies revealed a signature based on bone morphogenetic protein 4, interleukin-6, matrix metalloproteinases, mothers against decapentaplegic homolog 1, and thrombospondin1 as a reliable tool to separate patients with CLAD from stable patients.²⁸ Signatures that have proven to be valid in detecting rejection in other solid organ transplants²⁹ have been found to be generalizable in lung transplant biopsies for detecting T cell-mediated rejection, AMR, and other forms of lung injury. However, it yet remains to be confirmed whether these molecular signatures are specifically valid for BOS or even RAS, given the aforementioned low sensitivity of transbronchial biopsies for the diagnosis of CLAD³⁰ and also given that there are likely organ-specific mechanisms of rejection. Recently, mucosal biopsies, which are not routinely used in clinical diagnostics, have been shown to express a reasonably similar molecular signature to transbronchial biopsies and could therefore potentially be of use to detect and differentiate additional response-to-injury patterns.³¹ Lastly, the diagnostic use of cryobiopsies in lung allografts increases the amount of tissue obtained and

thus potentially improves the diagnostic yield. However, their universal implementation should be critically discussed considering the increased risks of bleeding and air leakage.³²

3 | RESTRICTIVE ALLOGRAFT SYNDROME

Following the first descriptions of lung injury patterns after LTx other than classical BOS^{11,12} and the subsequent original description of RAS with its characteristic scarring of the alveolar parenchyma,¹³ a number of papers addressed the actual morphological presentation of RAS. Initially, the main histopathological/radiological manifestation was thought to be pleuroparenchymal fibroelastosis (PPFE), defined by subpleural collagen deposition with a thickening of the alveolar septal elastic network, specifically affecting the (sub)pleural and paraseptal pulmonary compartment, especially in the upper lobes of the lung.¹⁴ However, in a further histopathological analysis of 21 RAS cases, von der Thüsen et al found PPFE only in 10 of 21 cases, while patterns of nonspecific interstitial pneumonia, fibrinous exudates, and emphysema were also described, similar to findings post-HSCT.³³ PPFE, however, is a terminology mostly utilized to describe a radiologic pattern, whereas the more accurate histologic description of the observed pattern of predominant subpleural and paraseptal collagenous obliteration of the alveoli with elastosis is AFE. Analogous to BO, AFE could also be shown to be a general reaction to injury, which can arise due to viral or bacterial infection, but also following acute AMR, leading to a sequence of vascular and epithelial injury, intra-alveolar fibrinous exudation, aberrant macrophage accumulation and activation, failed fibrin degradation, and ultimately fully

developed AFE.^{25,32} The relevance of fibrin deposition is provided by a study of Paraskeva et al, who demonstrated that acute fibrinoid organizing pneumonia (AFOP), an injury pattern defined by mesenchymal proliferation, loosely intermixed with fibrin and absence of interstitial fibrosis, is an important predictor of inferior posttransplant outcome.³⁴ In accordance with the nonobstructive pulmonary function defect, CT imaging showed varying degrees of fibrosis. It appears plausible that AFOP is an early histologic finding of RAS with a high specificity to predict poor outcome. A comparable association between late, but not early, new-onset diffuse alveolar damage (DAD) findings in transbronchial biopsies and increased incidence of RAS has also been described.³⁵ DAD and AFOP are similar in that both represent fibrin-rich acute injury patterns, but DAD is characterized by the deposition of hyaline membranes adjacent to alveolar walls, which is not seen in AFOP.

In addition to macrophages, myeloid (neutrophils, eosinophils, mast cells, and dendritic cells) and lymphoid cells are also abundantly present within the various stages of AFE, and specifically B cell-dominant lymphoid follicles³⁶ are thought to be crucially important for the development of AFE³⁷ and can be considered to be a response to the recognition of donor antigens, as donor-specific antibodies (DSA) are more frequently found in RAS^{38,39} (Figure 2). Montero et al furthermore demonstrated that compared to idiopathic PPFE, all patients with a clinical RAS diagnosis showed some degree of intra-alveolar fibrosis and elastosis, but also an increase in vascular lymphoplasmocytic inflammation with fibrointimal thickening was observed at the advancing edge of the fibrosis in RAS compared to idiopathic PPFE.⁴⁰

AFE as the main defining morphological feature of RAS is frequently also accompanied by BO lesions, indicating an—at least

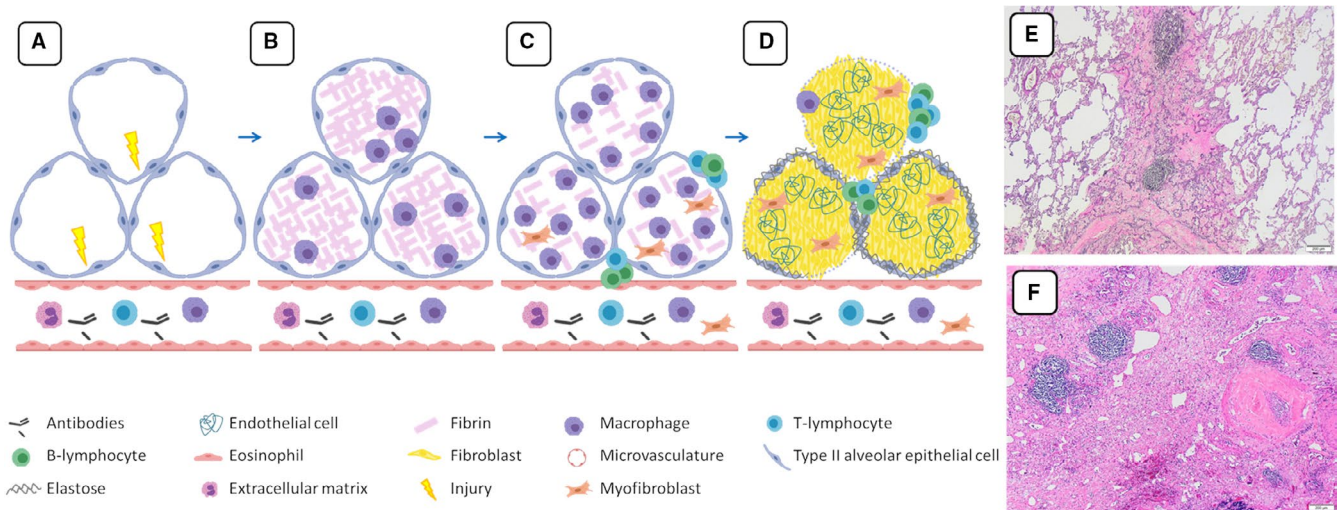


FIGURE 2 Pathophysiological mechanisms of alveolar fibroelastosis (AFE) in restrictive allograft syndrome. A, A multitude of potential injuries affect the alveoli. B, Antibody binding in the pulmonary capillaries or persistent injury to the alveoli lead to a disturbance of the air-blood interface and leakage of fibrin into the alveoli. C, This leads to an additional inflammatory response in which mainly macrophages seek to clear the fibrin, but fail to do, inducing an additional humoral response and recruitment of (myo-)fibroblasts. D, Finally, alveoli are being completely obliterated by (myo-)fibroblasts, resulting in the fully developed AFE. E, Elastica staining demonstrating paraseptal intra-alveolar fibrosis with septal elastosis. F, H&E showing consolidated fibrosis and oblitative airway remodeling with complete loss of the alveolar parenchyma and scattered lymphocytic aggregates [Color figure can be viewed at wileyonlinelibrary.com]

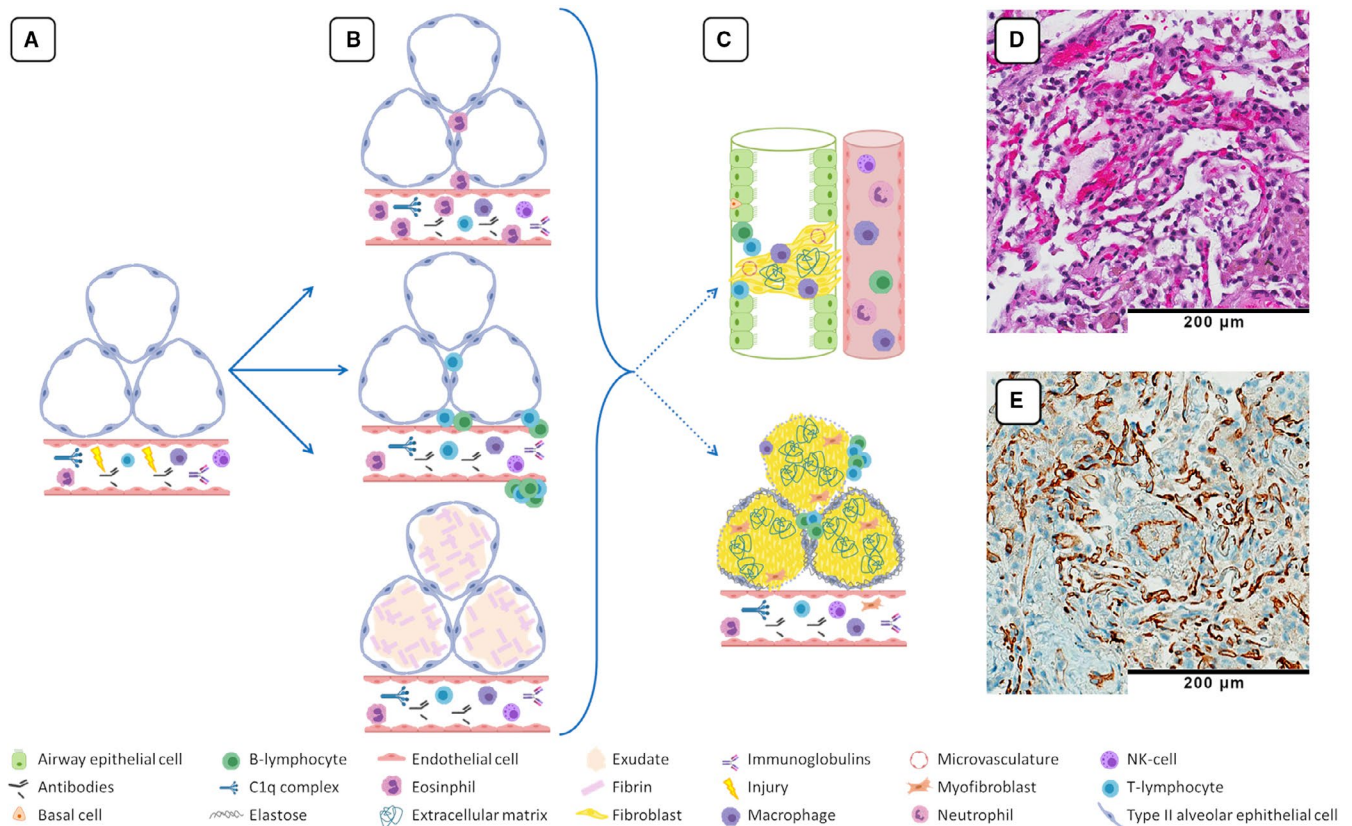


FIGURE 3 Pathophysiological mechanism of antibody-mediated rejection (AMR). A, Donor-specific antibodies (DSA) circulate in the bloodstream of the graft recipient and bind to the endothelial wall, which leads to complement activation with concomitant deposition of breakdown products such as C4d. B, Differential responses to persistent exposure to DSA, which induce persistent acute cellular rejection (top), neutrophilic capillaritis with eventual neutrophil extravasation, and migration to the alveolar air spaces (middle) and acute lung injury with fibrin accumulation and hyaline membrane formation (bottom). C, Ultimately, AMR will lead to bronchiolitis obliterans formation through peribronchial capillary invasion and/or alveolar fibroelastosis as seen in patients with bronchiolitis obliterans syndrome and restrictive allograft syndrome. D, H&E illustrating alveolar lung parenchyma with increase in marginalized macrophages and neutrophilic granulocytes. E, Complete circumferential endothelial positivity for C4d [Color figure can be viewed at wileyonlinelibrary.com]

partial–overlap between BOS and RAS. The relative number of airways per generation affected by BO is, however, lower in RAS compared to BOS, with 30%–40% of airways showing BO from generation 6 onwards. Next to these airways with BO, the absolute number of airways visible on CT was also lower in RAS due to alveolar fibrotic overgrowth extending into the terminal bronchioles.⁴¹

More comprehensive and preferably multicenter studies specifically assessing early histologic changes and possible predictive patterns in transbronchial biopsies of RAS patients are certainly needed. Further molecular signature studies should also focus on separating BOS from RAS early on. A common rejection gene expression panel that has been validated especially to detect all forms of rejection in kidney transplants found differences in CXCL-9 and CXCL-10 in RAS tissue vs controls, but failed to identify molecular differences between BOS and controls.⁴²

The common denominator of all these studies is probably that all focus is in some way on the clinical RAS phenotype and larger, multicenter trials are needed to further dissect the more subtle differences with regard to underlying mechanisms, but also patient outcome. The recently published International Society for Heart and

Lung Transplantation (ISHLT) consensus provides the correct framework for patient selection and recruitment to conduct such detailed studies by providing universal guidelines for patient diagnosis.^{15,16}

4 | ANTIBODY-MEDIATED REJECTION

An expert panel of the ISHLT established key diagnostic criteria for pulmonary AMR representing an under-recognized cause of CLAD. In that report, they acknowledged the lack of a proper definition of chronic AMR (cAMR) as a major limitation and one of the key areas for further research. While clinically evident AMR is associated with increased risk of CLAD, there is also evidence that subclinical AMR may be a precursor to CLAD. Diagnostic criteria for AMR include the presence of graft dysfunction, donor-specific HLA antibodies (DSA), aberrant lung histology with or without concurrent C4d deposition, a breakdown product from the complement system, within the graft leading to a “definite,” “probable,” or “possible” AMR diagnosis. In lung tissue specimens, common histologic findings include neutrophil margination, neutrophilic

capillaritis and arteritis, and alveolar septal widening, although all of these may also be seen in other forms of lung injury^{17,43} (Figure 3). A Banff study further correlated the presence of capillary inflammation, acute lung injury, and endothelialitis with the presence of circulating DSA,⁴⁴ while other (nonspecific) findings in lungs with AMR include microvascular thrombi, alveolar hemorrhage, accumulation of neutrophils within adjacent alveolar airspaces, but also persistent or recurrent high-grade acute cellular rejection, lymphocytic bronchiolitis, and BO.⁴⁵

The role for C4d in the diagnosis of AMR remains particularly questionable, as there are considerable technical and interpretation issues with C4d immunohistochemistry/immunofluorescence in pulmonary specimens, including poor reproducibility and prominent background staining.⁴⁶ The general consensus is that diffuse (>50%) C4d staining of the interstitial alveolar capillaries appears to be a strong indicator of acute (clinical) AMR, although in general this is considered very uncommon. Indeed, C4d particularly lacks the sensitivity to detect subclinical episodes of AMR.^{44,46,47} This has conclusively been proven by Aguilar and colleagues, who compared a large series of C4d⁻, probable AMR and C4d⁺, definite AMR cases. While the incidence of neutrophilic capillaritis was higher in the C4d⁺ group, there was no difference between the C4d⁺ and C4d⁻ group regarding other histological and clinical characteristics, with a similar (poor) freedom from CLAD and survival.⁴⁸

Besides the technical limitation of the C4d staining and limitation of C4d as a biomarker, it is now well accepted that AMR relies mainly on 3 different, nonexclusive mechanisms. The first is complement dependent, leading to split products liberation such as C3a and C5a (highly chemoattractive) and in few cases to membrane attack complex with subsequent cell lysis. The 2 others are noncomplement dependent, involving either antibody-dependent cellular cytotoxicity through NK cells,^{49,50} neutrophil, and macrophages⁵¹ or agonist role of HLA DSA through fixation to Class I and Class II MHC molecules leading to endothelial cell proliferation and remodeling.⁵²

Therefore, the diagnosis of pulmonary AMR requires a comprehensive multidisciplinary approach, including clinical, serological, and histopathological information.

5 | GRAFT FAILURE: ONE SIZE FITS ALL?

There seems to be considerable mechanistic and clinical overlap between BOS, RAS, and cAMR. BO lesions are observed in both BOS and RAS. Also in AMR, their presence is considered a histologic abnormality consistent with AMR. Therefore, BO appears to be an injury pattern due to a variety of both immunological and nonimmunological triggers leading to CLAD. Furthermore, patients can switch clinically from a BOS to a RAS phenotype (or vice versa) during their posttransplant trajectory (*mixed* CLAD).¹⁶ Similarly, the presence of DSA is an important indicator of AMR, but at the same time also forms a risk factor for both BOS and RAS.^{38,39} Therefore, the potential interrelation between RAS and cAMR deserves further attention. Patients surviving an acute AMR episode almost exclusively develop

RAS later on.⁵³ Moreover, in addition to the fact that a significant proportion of RAS patients show evidence of circulating DSA, recent evidence also suggests that DSA can be graft resident in the absence of circulating DSA, even with spatial heterogeneity within the lung.⁵⁴ Moreover, the concurrent presence of graft resident and circulating DSA correlates with poor transplant outcome.⁵⁵ Given these similarities and considering the fact that they are not mutually exclusive, it seems plausible that BOS-RAS and cAMR form an actual spectrum of injury and remodeling and that, depending on the site and the severity of injury (bronchial, alveolar, or vascular), different inadequate repair processes are at fault for the respective clinical manifestations.

In the end, "tissue remains the (main) issue," as we are still limited in our knowledge of the actual mechanisms of all late forms of posttransplant graft failure. Analysis of bronchoalveolar lavages, albeit clinically useful, has so far not proven to enable reliable differential diagnosis in CLAD phenotypes. Current therapies for CLAD such as extracorporeal photopheresis, montelukast, pirfenidone, or nintedanib at best slow down the progression.⁵⁶ Therefore, more comprehensive investigation of the processes involved will certainly improve our understanding of these diseases, and will bring us 1 step closer to adequate therapies, which are desperately needed.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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