Pathology of Lung Rejection: Cellular and Humoral Mediated

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Introduction

Acute rejection is the host's response to the recognition of the graft as foreign. It can occur days, months, or even years after transplantation. Rejection can be divided into cellular and humoral forms. Acute cellular rejection is the predominant type of acute rejection of lung allografts. It is mediated by T lymphocytes that recognize foreign human leukocyte antigens (HLA) or other antigens [1, 2]. Humoral rejection is mediated by preformed or de novo recipient antibodies (therefore, also referred to as antibody-mediated rejection [AMR]) against antigens of the donor organ cells.

Acute rejection is an important complication in patients with lung allografts. Twenty-nine percent of adult patients have at least one episode of treated acute rejection between discharge from the hospital and 1 year after transplantation [3]. Moreover, 3.6% and 1.8% of all deaths that occur within the first 30 days or between 30 days and 1 year following lung transplantation are due to acute rejection, respectively [3]. In addition, the frequency and severity of acute rejections are thought to represent the major risk factor for the subsequent development of bronchiolitis obliterans syndrome (BOS) [1, 4–6].

HLA mismatch, genetic and recipient factors, type of immunosuppression, vitamin D deficiency, and infection are risk factors of acute rejection. For instance, the recipient alloimmune response is thought to be related to the recognition of differences to donor antigens leading to acute lung allograft rejection. Indeed a higher degree of HLA mismatch has been shown to increase the risk of acute rejection although this effect is not consistent across all HLA loci or studies [4, 7–10]. Mismatches at the HLA-DR, HLA-B [7], and HLA-A [8] loci, as well as a combination of all three loci [9], appear specifically important. For instance, acute rejection within 2 months after transplantation has been shown to be associated with HLA-DR mismatch, while acute rejection at 4 years has been found to be associated with HLA-B mismatch [11].

Several host genetic characteristics have been studied that may modulate acute lung rejection. For instance, a genotype leading to increased IL1- production may protect against acute rejection [12], while a multidrug-resistant genotype (MDR1 C3435T) appears to predispose to persistent acute rejection that is resistant to immunosuppressive treatment [13].

The incidence of acute rejection appears to be age-dependent, with the lowest incidence of

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acute rejection in infants (< age 2) [14]. However, children have a higher risk for acute rejection than adults [15]. Furthermore, the registry of the International Society of Heart and Lung Transplantation (ISHLT) showed that the incidence of acute rejection between discharge and 1-year follow-up was slightly higher in younger adult lung allograft recipients (age 18–34 years) (36%) [16] when compared to the entire adult population in which 29% had at least one acute rejection episode [3]. The incidence of acute rejection does not seem to change in older lung transplant recipients (age 65 and higher) [17].

Regimens of immunosuppression might also play a role in acute rejection. For instance, the rate of acute rejection in the first year after transplantation was highest among recipients who were on cyclosporine-based regimens and lowest among those on tacrolimus-based regimens [18].

Vitamin D deficiency might also play a role in acute rejection. A study found that 80% of lung recipients were 25(OH)D deficient around the time of transplantation and that vitamin D-deficient recipients had more episodes of acute cellular rejection and infection [19]. A similar association between vitamin D deficiency and acute rejection has been described in other solid organ recipients including the liver, kidney, and heart. Although the exact mechanism for this phenomenon is not entirely clear, it is speculated that (1) vitamin D might slow down the maturation of antigen-presenting cells as in vitro studies have shown, (2) vitamin D might induce dendritic cells to acquire tolerance, and/or (3) a synergistic effect between vitamin D analogs and immunosuppressants occurs [19].

Viral infections have also been thought to modulate the immune system and to increase alloreactivity. Indeed, a high incidence of acute rejection has been found in lung transplant recipients after community-acquired respiratory tract infections with human influenza virus, respiratory syncytial virus, rhinovirus, coronavirus, and parainfluenza virus [20–22]. *Chlamydia pneumoniae* infection has also been linked to the development of acute rejection in one study [23]. The significance of CMV infections and the impact of CMV prophylaxis strategies on acute rejection frequency are not clear at this time [24].

The clinical course of acute rejection can be variable. Acute rejection is often identified on surveillance transbronchial biopsy in an asymptomatic patient. If symptoms occur, they might be non-specific and overlap with those seen in other complications and diseases in this patient population. These symptoms might include dyspnea, fever, leukocytosis, and a widened alveolar-arterial oxygen gradient. Higher-grade rejection appears to cause more severe symptoms and can lead to acute respiratory distress [17]. In patients with rejection, pulmonary function testing may show a decrease in forced expiratory volume in 1 s (FEV_1) and vital capacity (VC). Although spirometry has a sensitivity of greater than 60% for detecting infection or rejection of Grade A2 and higher, it cannot differentiate between the two [25]. Furthermore, the usefulness of spirometry is diminished in single lung transplant recipients, as the dysfunction of the native lung confounds the pulmonary function test results [26].

Although in approximately half of the cases of acute rejection, chest X-ray studies are normal, ill-defined perihilar and lower lobe opacities, along with septal lines and pleural effusions, may be seen. Findings on CT scan might include ground-glass opacities, septal thickening, volume loss, nodules and consolidation, and pleural effusions. Infiltrates observed on imaging studies during the first week after lung transplantation are usually caused by the reimplantation response, i.e., reperfusion edema and other factors. Infiltrates that persist beyond the first week following transplantation suggest acute rejection or infection. However, although early, the authors of small studies have attempted to demonstrate the usefulness of chest X-rays and chest CT scans in the diagnosis of rejection, more recent data show a very low sensitivity for acute rejection (as low as 35%) and no discriminatory value between rejection and other processes [27].

Exhaled nitric oxide (NO) can also serve as a marker of lung injury; it is often increased in patients with lymphocytic bronchiolitis and acute rejection [28–30]. Furthermore, in a study of inert gas single-breath washout, the slope of

alveolar plateau for helium had a sensitivity of 68% for acute rejection [25].

Although the presentation of the patient and several ancillary studies may suggest the presence of acute allograft rejection, none of these findings are specific. Therefore, tissue diagnosis is necessary for a definitive diagnosis. Histopathology of adequate lung biopsy samples obtained from transbronchial biopsy is currently the gold standard to assess lung allografts for rejection and to distinguish rejection from its clinical mimickers such as aspiration, infection, drug toxicity, and recurrent disease.

Recently, the transbronchial cryobiopsy technique was introduced which yields larger biopsies containing more alveoli, small airways, and veins and venules while exhibiting less procedural alveolar hemorrhage and crush artifact than conventional forceps transbronchial allograft biopsies [31–33]. Although cryobiopsies appear to be as safe as forceps biopsies, complications can occur which is one of the reasons that this technique has so far not been universally performed for this purpose [31].

Other lung tissue specimens from lung allografts include wedge biopsies, explants for retransplant, or autopsy specimens from lung transplant recipients. Wedge biopsies, although seldom obtained in clinical practice, and specimens from explants provide useful histopathologic insights into the etiology of lung allograft dysfunction in advanced stages following all possible medical interventions.

Morphologic Features of Cellular Rejection

Cellular alloreactive injury to the donor lung affects both the vasculature and the airways [34]. Perivascular mononuclear cell infiltrates are the hallmark of acute cellular rejection. These infiltrates may be accompanied by subendothelial chronic inflammation (e.g., endotheliitis or intimitis) and also by lymphocytic bronchiolitis, which is characteristic of small airway rejection. The histologic changes are divided into grades based on intensity of the cellular infiltrate and the occurrence of an accompanying acute lung injury pattern.

In 1990, the ISHLT sponsored the Lung Rejection Study Group (LRSG), a workshop to develop a "working formulation" for the diagnosis of lung rejection by transbronchial biopsy [35]. Since then the grading scheme has been revised twice, in 1996 [36] and 2007 [34]. The grading scheme is strictly pathologic, based on morphologic features recognized in transbronchial biopsies of the allograft. Clinical parameters are not considered.

Due to overlapping histologic features between acute rejection and infection, the grading scheme relies on the absence of concurrent infection. Furthermore, infection and rejection may occur together. Therefore, the LRSG recommends grading rejection only after the rigorous exclusion of infection [34].

The most recent classification of lung allograft biopsies is the 2007 ISHLT consensus classification of allograft rejection [34] (Table 13.1). An attempt should be made to accurately distinguish the grade of rejection since treatment is largely dependent on the histologic grade assessed by an experienced pulmonary pathologist familiar with the histopathologic features and criteria used for grading. However, inter- and intra-observer variability in grading can impact treatment and outcome [37, 38]. Two studies using the 1996 grading system found relatively good interobserver agreements for the A grades (kappa of 0.65 and 0.73) [37, 38]; however, these results could not be replicated in another study in which the kappa was 0.47 in spite of dichotomization of the A grades to A0/A1 versus A2-4 [39]. Intraobserver agreement for acute rejection has been found to be good with kappa values of 0.65 and 0.79 [37, 39]. Using the revised 2007 ISHLT classification, Bhorade and colleagues showed an overall concordance rate of 74% for Grade A and 89% for Grade B specimens between a site pathologist and a central pathologist [40]. However, the weighted kappa scores in that study showed only fair to moderate agreement for A grades (kappa values varied between 0.22 and 0.48) and less than a chance agreement to moderate agreement for B grades (kappa values varied

Table 13.1 Classification of cellular allograft rejection according to the 2007 revised ISHLT consensus classification of lung allograft rejection

Type of rejection	ISHLT grade	Histomorphologic features
Acute rejection	A0 None	Normal pulmonary parenchyma
	A1 Minimal	Occasional blood vessels are surrounded by a thin chronic mononuclear cell infiltrate
	A2 Mild	Multiple blood vessels are surrounded by a more prominent mononuclear cell infiltrate Infiltrate confined to the perivascular adventitia Endotheliitis may occur
	A3 Moderate	Dense mononuclear cell infiltrates surround blood vessels and extend into interstitium Endotheliitis common Eosinophils and occasional neutrophils common Acute lung injury may be apparent
	A4 Severe	Diffuse perivascular, interstitial, and air space infiltrates of mononuclear cells Prominent alveolar pneumocyte damage and endotheliitis Intra-alveolar necrotic epithelial cells, macrophages, eosinophils, hemorrhage, and neutrophils may occur Acute lung injury
Small airway inflammation—Lymphocytic bronchiolitis	B0 None	Unremarkable small airways
	B1R[a] Low grade	Lymphocytes within the submucosa of the bronchioles
	B2R High grade	Marked lymphocytic infiltrate of the airway epithelium and airway wall Greater numbers of eosinophils and plasmacytoid cells Epithelial damage including necrosis, metaplasia, and marked intraepithelial lymphocytic infiltration Epithelial ulceration, fibrinopurulent exudate, cellular debris, and neutrophils can occur
	BX Ungradeable	Grading hampered by lack of definite small airways, presence of infection, tangential cutting, artifact, etc.
Chronic airway rejection—Obliterative bronchiolitis	C0 None	Small airways similar in size to the accompanying artery No fibrosis
	C1 Present	Fibrosis in the wall of small airways
Chronic vascular rejection	D0 None	No arterial or venous changes
	D1 Present	Pulmonary arteries and/or veins are thickened by fibrointimal connective tissue

Adapted with permission of Elsevier from Stewart S, Fishbein MC, Snell GI, Berry GJ, Boehler A, Burke MM, Glanville A, Gould FK, Magro C, Marboe CC, et al. Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. J Heart Lung Transplant 2007; 26:1229–1242

[a]R denotes the revised 2007 classification

between −0.04 and 0.46). Interestingly, the kappa values for A and B grades were dependent on the time that had elapsed between transplantation and biopsy. The best agreement occurred in biopsies taken within 6 weeks of transplant. Slightly higher agreements (81% and 93%, for A and B grades, respectively) were shown in a study that evaluated the interobserver agreement between two transplant pathologists from the same institution using the 2007 revision grading Scheme [31]. Although cryobiopsies are larger and appear to be easier interpretable, interobserver reproducibility did not improve with the use of cryobiopsies in that study [31].

2007 ISHLT Revised Consensus Classification of Lung Allograft Rejection

Acute Rejection: A Grade

Acute rejection is defined by the presence of perivascular mononuclear cell infiltrates with or without endotheliitis [34]. With progression, this infiltrate becomes more widespread and extends into the alveolar septa and, subsequently, into the alveoli. The majority of the mononuclear cells in acute rejection are T cells, although a few studies have described increased populations of B cells or eosinophils [34, 41, 42]. The histologic features of rejection are summarized in Table 13.1.

No Acute Rejection (ISHLT Grade A0)

Features of acute cellular rejection are lacking, although the biopsy may not be entirely normal.

Minimal Acute Rejection (ISHLT Grade A1)

Scattered infrequent blood vessels, particularly venules, in the alveolated lung parenchyma are surrounded by a relatively thin (ring of two to three layers) chronic mononuclear cell infiltrate (Fig. 13.1a, b). The lymphocytic rim can be loose or compact and is in general circumferential but does not spill into the adjacent interstitium. Endotheliitis and eosinophils are absent. In adequately alveolated and artifact-free specimens, the lymphocytic infiltrates may be detected at low magnification, but often higher power study is needed to identify the infiltrates.

Mild Acute Rejection (ISHLT Grade A2)

Although in mild acute rejection the perivascular infiltrate of lymphocytes is still confined to the perivascular adventitia without infiltrating the adjacent interstitium or air spaces, there are more layers of lymphocytes surrounding vessels (Fig. 13.2a, b). In addition, the perivascular mononuclear infiltrates surrounding venules and arterioles are more frequent than in Grade A1. They are typically recognizable at low magnification. These infiltrates usually consist of a mixture of small round lymphocytes, activated lymphocytes, plasmacytoid lymphocytes, macrophages, and eosinophils. The cellular infiltrates can be compact or loose. Subendothelial infiltration by mononuclear cells may be noted which can be associated with hyperplastic or regenerative changes in the endothelium. Concurrent lymphocytic bronchiolitis may be seen.

Moderate Acute Rejection (ISHLT Grade A3)

Venules and arterioles are cuffed by easily recognizable dense perivascular mononuclear cell infiltrates that are commonly associated with endotheliitis (Fig. 13.3a–c). Eosinophils and even occasional neutrophils are common. In

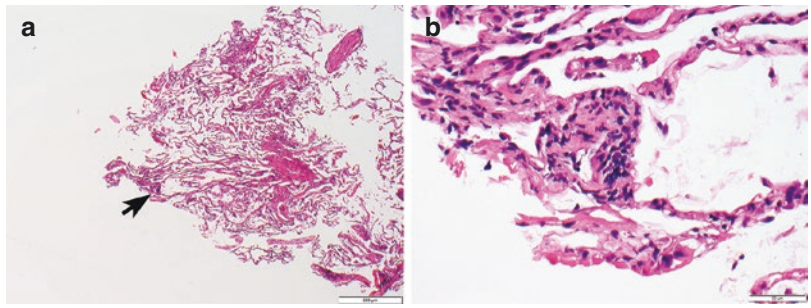

Fig. 13.1 (**a**, **b**) Minimal acute rejection (ISHLT Grade A1). (**a**) Low-power view of this well-expanded specimen with adequately open alveoli suggests a single focus of inflammatory infiltrate (arrow). (**b**) High magnification confirms a small vessel almost completely surrounded by a few layers of mononuclear cells. Magnification, ×40 (**a**), ×400 (**b**)

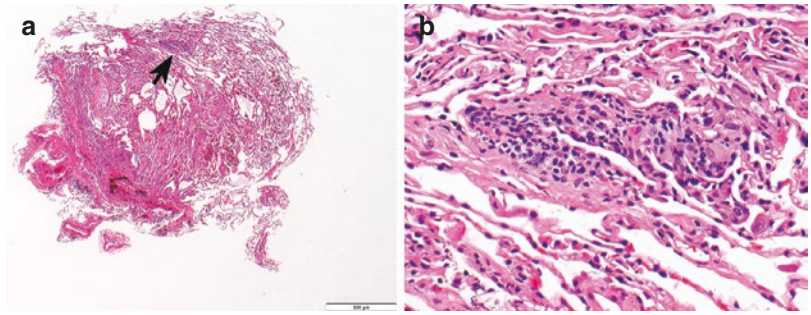

Fig. 13.2 (**a**, **b**) Mild acute rejection (ISHLT Grade A2). (**a**) At low magnification, an inflammatory infiltrate is easily identified (arrow) even though this specimen has crush artifact. (**b**) This mononuclear infiltrate completely surrounds a small vessel and is comprised of more than three layers without extending into the surrounding interstitium. Magnification, ×40 (**a**), ×400 (**b**)

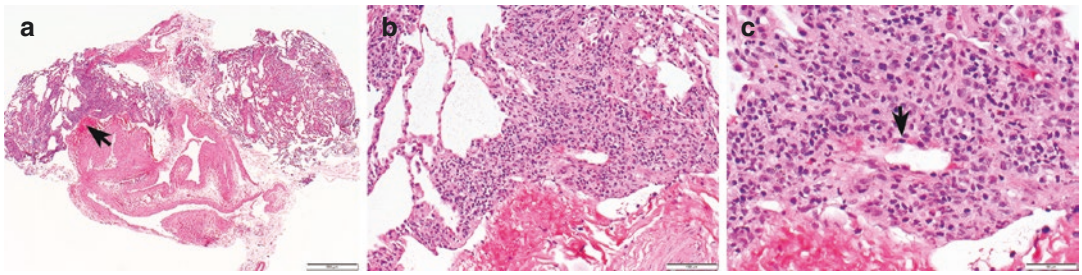

Fig. 13.3 (**a**–**c**) Moderate acute rejection (ISHLT Grade A3). (**a**) A prominent inflammatory infiltrate is apparent at low power (arrow). (**b**) This mononuclear infiltrate surrounds multiple small vessels and extends into the surrounding interalveolar septa. (**c**) Eosinophils are present, and a few lymphocytes within the endothelial lining are suggestive of endotheliitis (arrow). Magnification, ×40 (**a**), ×200 (**b**), ×400 (**c**)

moderate acute rejection, the inflammatory cell infiltrate extends into the adjacent alveolar septa where it can be associated with type II pneumocyte hyperplasia. The inflammatory infiltrate can also extend into adjacent airspaces and be associated with collections of intra-alveolar macrophages and lymphocytes. Histologic features of acute lung injury may become apparent in the form of airspace fibrin.

Severe Acute Rejection (ISHLT Grade A4)

In severe rejection, there are diffuse perivascular, interstitial, and air space infiltrates of mononuclear cells with prominent alveolar pneumocyte damage and endotheliitis (Fig. 13.4a–f). This may be associated with necrotic intra-alveolar epithelial cells, hemorrhage and neutrophils, and usually morphologic evidence of acute lung injury in the form of organizing pneumonia, fibrin deposition, or hyaline membranes. Parenchymal necrosis, infarction, or necrotizing vasculitis may be identified; however, these features are more evident on surgical rather than transbronchial lung biopsies. It should be noted that a paradoxical diminution of perivascular infiltrates can occur as cells extend into interalveolar septa and air spaces where they are admixed with macrophages.

Protocol surveillance biopsies of lung allografts are performed in many institutions. Even though these patients are in general asymptomatic and clinically stable, one study showed that 39% of surveillance biopsies reveal acute cellular rejection with 43% showing features of minimal rejection, 49% mild rejection, and 8% moderate rejection [43]. A more recent prospective study identified morphologic findings of acute cellular rejection only in 6% of surveillance biopsies [44], while a retrospective study of 592

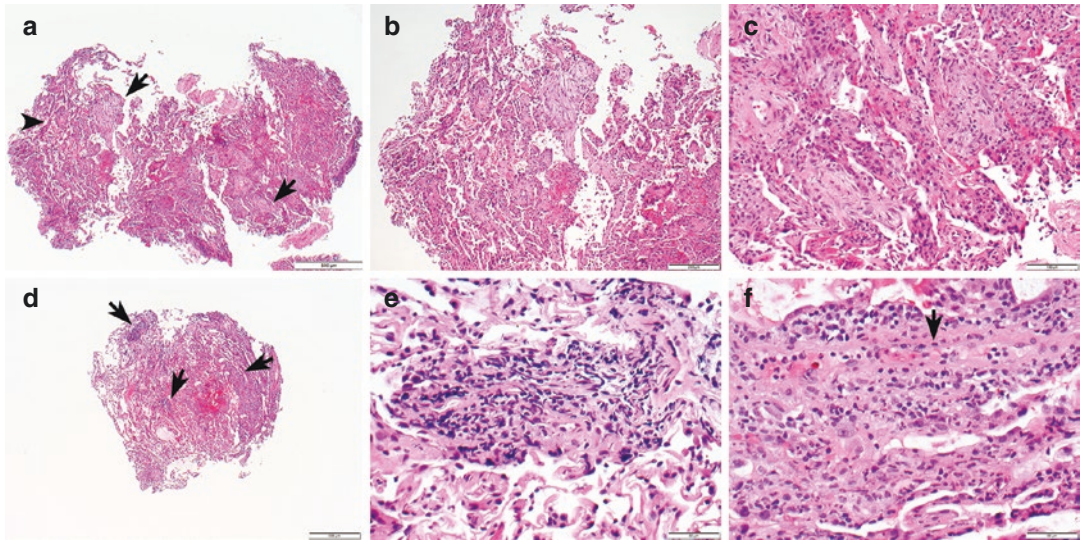

Fig. 13.4 (**a**–**f**) Severe acute rejection (ISHLT Grade A4). (**a**) At low magnification, organizing pneumonia (arrows) and interstitial thickening (arrowhead) are identified. (**b**) Intra-alveolar fibrin and blood with organization with proliferating fibroblasts are also present. (**c**) High-power view reveals more organizing pneumonia and interstitial thickening predominantly due to type II pneumocyte hyperplasia and scattered chronic inflammatory cells. Foamy macrophages and fibroblasts are forming clusters within alveolar spaces. (**d**) Another piece from the same biopsy reveals foci of inflammation (arrows). (**e**) These foci represent perivascular mononuclear cell infiltrates further suggestive of acute rejection. (**f**) Endotheliitis is also apparent (arrow points towards lymphocytes in between endothelial cells). Magnification, ×40 (**a**, **d**), ×100 (**b**), ×200 (**c**), ×400 (**e**, **f**)

surveillance biopsies taken within 400 days of transplantation revealed histologic findings of either acute cellular rejection or obliterative bronchiolitis in 31% of biopsies with 36% within the first 100 days and 25% between 100 and 400 days following transplantation [45].

Evidence suggests that acute cellular rejection is an important risk factor for the development of BOS [24]. Indeed, studies have demonstrated an increased risk of BOS with single episodes, increased frequencies, and increased severity of acute cellular rejection. Moreover, patients with multiple episodes of even minimal acute cellular rejection were shown to be at increased risk for BOS [46], and yet a single episode of minimal acute rejection without recurrence or subsequent progression to a higher grade has been identified as an independent significant predictor of BOS [47]. Because of these findings, patients who are asymptomatic but are found to have acute cellular rejection (even minimal acute cellular rejection) on a surveillance allograft biopsy might be treated accordingly. However, several centers do not utilize surveillance transbronchial lung biopsies and/or treat asymptomatic patients with no clinical evidence of allograft dysfunction. Prospective well-designed clinical studies are needed to provide evidence to support surveillance transbronchial lung biopsies and therapeutic interventions.

Small Airway Inflammation: Lymphocytic Bronchiolitis—B Grade

This grade applies only to small airways such as terminal or respiratory bronchioles. Bronchi, if present, should be described separately. It is important to mention in the pathology report whether or not small airways are present. If no small airways are identified or the biopsy has obvious infection, the grade "BX" should be used. The R behind grades 1 and 2 denotes the revised 2007 version.

No Airway Inflammation (ISHLT Grade B0)

The small airways appear unremarkable without evidence of bronchiolar inflammation.

Low-Grade Small Airway Inflammation (ISHLT Grade B1R)

Low-grade inflammation is characterized by lymphocytes within the submucosa of the bronchioles (Fig. 13.5a–c). The lymphocytic infiltrates can be infrequent and scattered or form a circumferential band; however, intraepithelial lymphocytic infiltration is not present. Occasional eosinophils may be seen within the submucosa. There is no evidence of epithelial damage, neutrophils, necrosis, ulceration, or significant amount of nuclear debris.

High-Grade Small Airway Inflammation (ISHLT Grade B2R)

In high-grade small airway inflammation, there is marked lymphocytic infiltrate of the airway epithelium and airway wall. The mononuclear cells in the submucosa appear larger, and a greater number of eosinophils and plasmacytoid cells can be seen (Fig. 13.6a–c). In addition, there is evidence of epithelial damage including necrosis, metaplasia, and marked intraepithelial lymphocytic infiltration. In its most severe form, high-grade airway inflammation is associated with epithelial ulceration, fibrinopurulent exudate, cellular debris, and neutrophils. It is important to exclude an infectious process, especially if the number of neutrophils is disproportionally high when compared to other mononuclear cells within the airway wall.

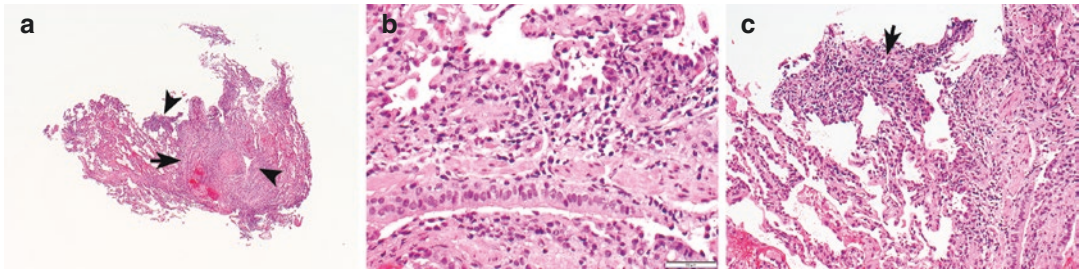

Fig. 13.5 (**a**–**c**) Low-grade small airway inflammation and moderate acute rejection (ISHLT Grade A3, B1R). (**a**) A small airway is surrounded by an inflammatory infiltrate (arrow). This biopsy also shows patchy inflammatory infiltrates away from the airway (arrowheads). (**b**) The mononuclear cell infiltrate is centered on the submucosa of the small airway, while the mucosa appears unremarkable consistent with low-grade small airway inflammation. (**c**) This biopsy also shows a marked mononuclear cell infiltrate around small vessels (arrow) and extending into the surrounding interstitium consistent with moderate acute rejection. Magnification, ×40 (**a**), ×400 (**b**), ×200 (**c**)

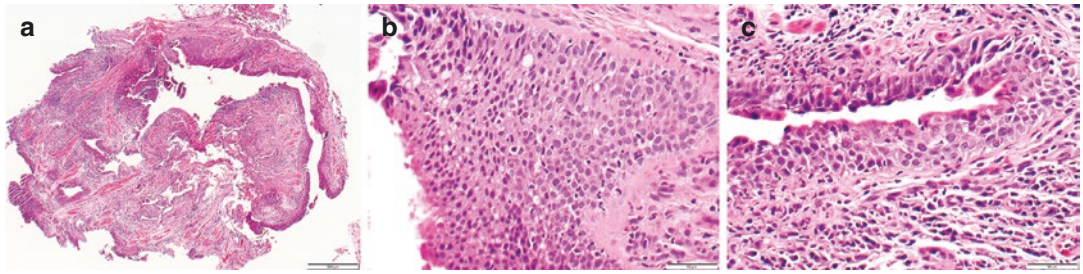

Fig. 13.6 (**a**–**c**) High-grade small airway inflammation (ISHLT Grade B2R). (**a**) A marked inflammatory infiltrate is noted within the wall of a small airway. (**b**) This chronic inflammatory infiltrate extends into the mucosa. (**c**) Squamous metaplasia is focally present. Magnification, ×40 (**a**), ×400 (**b**, **c**)

Ungradeable Small Airway Inflammation (ISHLT Grade BX)

Small airways might not be evaluable for several reasons including lack of small airways due to sampling problems, infection, tangential cutting, artifact, etc. In patients who are known to have an infection that could cause lymphocytic bronchiolitis, the allograft biopsy should also be classified as ungradeable for small airway rejection.

Chronic Airway Rejection: Obliterative Bronchiolitis—C Grade

Chronic airway rejection is restricted to submucosal and intraluminal scarring of small airways including terminal and respiratory bronchioles. When large tissue sections of the lung are examined, obliterative bronchiolitis may be recognized as a panlobar process but is usually patchy.

No Chronic Airway Rejection (ISHLT Grade C0)

The small airways appear similar in size to the accompanying artery with a ragged inner surface. Fibrosis is not present.

Chronic Airway Rejection (ISHLT Grade C1)

Narrowing of the small airways due to fibrosis in the airway wall is the hallmark of chronic airway rejection. The fibrosis may be eccentric or concentric. The type of fibrosis depends on the acuteness of the process, the degree of organization, and the amount of accompanying inflammation. The fibrosis can range from loose myxoid granulation tissue with variable numbers of inflammatory cells filling or partially obstructing the airway lumen in the more acute phase (Fig. 13.7a) to dense hyalinized collagen in the wall of bronchioles that is a characteristic of the chronic phase (Fig. 13.7b).

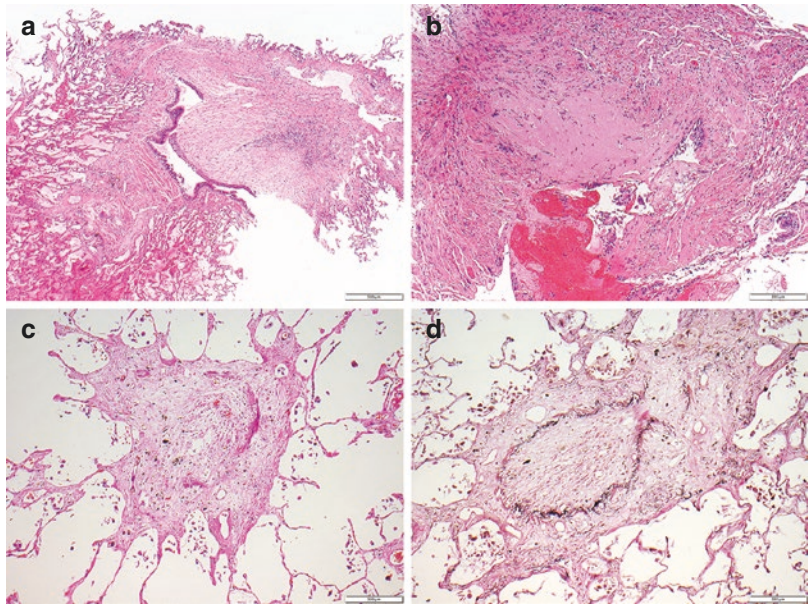

Fig. 13.7 (**a**–**d**) Chronic airway rejection (obliterative bronchiolitis) (ISHLT Grade C1). (**a**) Loose fibroblast proliferation with a patchy chronic inflammatory infiltrate is noted eccentric within the submucosa of a small airway narrowing its lumen. (**b**) In this example, submucosal collagen fibrosis eccentrically narrows the lumen of a small airway. (**c**) In this autopsy case of an allograft recipient, some airways are completely replaced by scar tissue. Focal smooth muscle might be suggestive of an airway. (**d**) A Verhoeff-Van Gieson stain highlights the remaining elastic fibers which helps to identify this scar replacing a former small airway. Magnification, ×40 (**a**), ×100 (**b**–**d**)

Metaplastic squamous or cuboidal epithelium may overly the bronchiolar fibrosis. Sometimes, only a slit-like lumen of the airway may remain as a result of a confluent submucosal scar or intraluminal polyps of scar tissue. There may be rather prominent capillaries supplying the intraluminal fibrotic areas. Ultimately, the bronchiolar lumen might be entirely occluded by dense scar tissue (Fig. 13.7c, d). In these cases, only an elastic stain highlighting residual elastic tissue, the vicinity of the scar to a pulmonary artery, and residual smooth muscle may indicate that a small airway has been replaced by fibrotic scar. In the chronic phase, inflammation may be minimal or absent. Usually, the scarring process is confined exclusively to respiratory bronchioles and terminal bronchioles, although it may occasionally involve adjacent alveoli.

Obliterative bronchiolitis is only infrequently identified in lung allografts by transbronchial biopsy, and the sensitivity of this morphologic finding for the presence of chronic rejection is only between 15 and 28% [48–50]. In a recent study, all seven conventional transbronchial biopsies that were included from patients clinically known to have BOS, the clinical equivalent to morphologic obliterative bronchiolitis, failed to reveal morphologic findings of obliterative bronchiolitis [31]. Although cryobiopsies contained more small airways, all nine cryobiopsies that were also included in that study from patients with clinically proven BOS did not reveal obliterative bronchiolitis in the tissue [31]. This low sensitivity is largely due to sampling and its patchy nature. Therefore, BOS is used and more reliable for the clinical assessment of chronic airway rejection. BOS is calculated as <80% FEV_1 in at least two consecutive lung function tests of the patient's maximum FEV_1 posttransplantation [51]. Despite the low sensitivity of transbronchial biopsies for obliterative bronchiolitis, the specificity of this morphologic finding in an allograft biopsy is high, ranging from 75 to 94% [49, 50]. Therefore, an attempt to diagnose obliterative bronchiolitis should be made in lung allograft biopsies.

Chronic Vascular Rejection: D Grade

No Chronic Vascular Rejection (ISHLT Grade D0)

The pulmonary arteries appear of a similar size as the accompanying airways. The intima is slender and the media not thickened.

Chronic Vascular Rejection (ISHLT Grade D1)

Chronic vascular rejection rarely is identified on biopsies since they usually lack vessels of sufficient size. Wedge biopsies, explants, or autopsy material may reveal it. Therefore, according to the ISHLT, the D grade of rejection is not applicable to allograft transbronchial biopsies. Although cryobiopsies contain a higher number of venules and small veins, in a recent small study, no difference was found in the number of cases with possible vascular rejection when compared to transbronchial biopsies [31].

Vascular rejection is characterized by thickened pulmonary arteries and more often veins, due to fibrointimal connective tissue (Fig. 13.8a, b). Also, thickening is usually concentric. Chronic vascular rejection may be patchy. Chronic vascular rejection usually starts with intimal proliferation. Subsequently, the internal elastic lamina may become fragmented and discontinuous. Occasionally the underlying muscular wall becomes thinned. In approximately half of the reported cases, a concurrent endovasculitis has been observed. The process is similar in pulmonary veins, although the intimal deposits may be less cellular and more waxy, eosinophilic, and sclerotic. Recanalized thrombi may mimic chronic vascular rejection. In contrast to heart allografts, chronic vascular rejection in lung transplants has not resulted in graft loss; however, some patients develop pulmonary hypertension particularly those with BOS [52, 53].

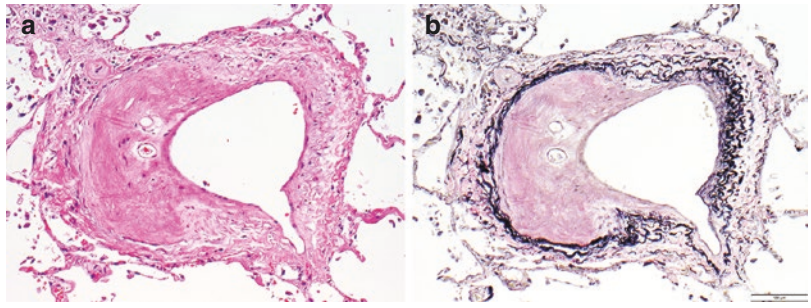

Fig. 13.8 (**a**, **b**) Chronic vascular rejection (ISHLT Grade D1). (**a**) A small vessel shows slightly eccentric intimal fibrosis. (**b**) A Verhoeff-Van Gieson stain delineates the internal elastic lamina which helps to better identify the extent of the intimal fibrosis. Magnification, ×200 (**a**, **b**)

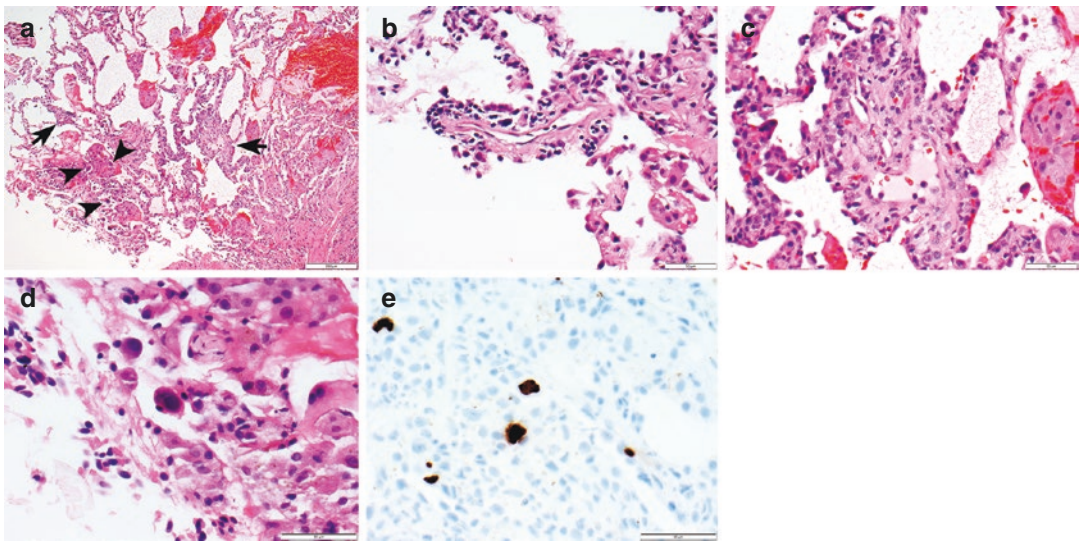

Fig. 13.9 (**a**–**e**) Cytomegalovirus (CMV) infection mimicking acute rejection. (**a**) This biopsy shows small chronic inflammatory infiltrates surrounding small vessels (arrows). In addition, large atypical cells are identified (arrowheads). (**b**, **c**) High-power view confirms mononuclear cells forming only a few layers around small vessels as can be seen in minimal acute rejection. (**d**) The large atypical cells have pink nuclear and cytoplasmic inclusions suggestive of CMV inclusions which were confirmed by a CMV immunostain (**e**). Magnification, ×100 (**a**), ×400 (**b**, **c**), ×600 (**d**, **e**)

Mimickers of Cellular Rejection

Infection can mimic acute cellular rejection. For instance, viral infection, particularly CMV (Fig. 13.9a–e) but also *pneumocystis jirovecii* pneumonia, can be associated with perivascular mononuclear cell inflammation mimicking acute cellular rejection [54]. Infection can also cause small airway inflammation imitating lymphocytic bronchiolitis.

Mimickers of severe acute rejection include conditions that might present with acute lung injury or diffuse alveolar damage. These conditions include infection, drug toxicity, aspiration,

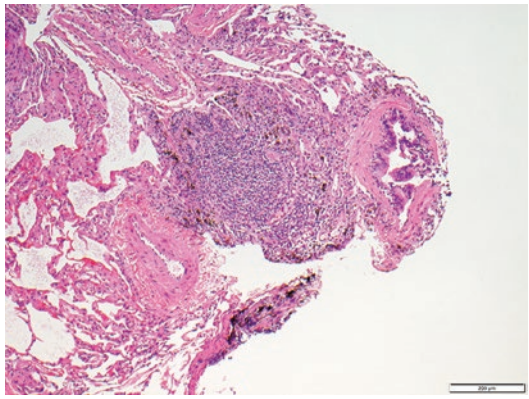

Fig. 13.10 Bronchiolar-associated lymphatic tissue (BALT). A nodule comprised of lymphocytes and anthracotic pigment is identified in the vicinity to an unremarkable small airway. Magnification, ×100

AMR, or harvest/reperfusion injury. The presence of perivascular inflammation is helpful in establishing the diagnosis of rejection. However, perivascular inflammation is not entirely specific for acute rejection, and many other conditions may simulate or mimic alloreactive lung injury [54].

Marked perivascular and/or peribronchiolar mononuclear infiltrates might also raise the possibility of posttransplantation lymphoproliferative disease (PTLD), and in such cases, an appropriate workup should be performed, including doing studies for Epstein-Barr virus, which is ubiquitous in PTLD. Further differential diagnosis of perivascular and interstitial infiltrates include recurrent primary diseases.

Small airway rejection and the perivascular infiltrates of Grade A rejection should be distinguished from bronchiolar-associated lymphatic tissue (BALT) . BALT is found in the vicinity of airways, usually contains black anthracotic pigment, and presents as a rather nodular collection of chronic inflammatory cells which does not surround a vessel (Fig. 13.10). Epithelial injury, neutrophils, or eosinophils should not be seen in BALT collections [34].

Antibody-Mediated Rejection

Originally recognized in kidney transplant patients who presented with acute allograft rejection, anti-donor antibodies, and poor prognosis [55], AMR is now well established in kidney and heart allografts. In lung transplantation, AMR is still an evolving concept but likely explains acute and chronic graft dysfunction/failure in a subset of patients. Evidence suggests that AMR occurs due to circulating antibodies that are either (1) preformed because of pregnancy, blood transfusion, or previous organ transplantation or (2) arise de novo after transplantation due to HLA mismatch. Furthermore, the recent development of very sensitive and specific solid-phase flow cytometry and Luminex-based methodologies has allowed for more accurate detection of antibody specificities in sensitized recipients, and it has become clear that more patients than previously expected have or develop preformed anti-HLA antibodies. Immune stimulation by prior infections or autoimmunity may also contribute to the development of antibodies in those patients with no identifiable risk factors.

Overall, these preexisting or de novo antibodies can react with donor antigens, leading to immediate graft loss (hyperacute rejection), accelerated humoral rejection, and/or BOS [56]. In addition, recent studies have consistently demonstrated an increased incidence of acute rejection (a threefold increase in one study) [57], persistent rejection, increased BOS [58], or worse overall survival [59] in patients with anti-HLA antibodies. This effect is seen both with pretransplant HLA sensitization and with the development of de novo anti-HLA donor-specific antibodies after transplantation [58].

About 10–15% of lung transplant recipients are pre-sensitized to HLA antigens [60].

Even though “unacceptable antigens” are avoided during the virtual crossmatch, patients with positive pretransplant PRA are at higher risk for posttransplant complications. Their posttransplant PRA can stay stable or increase via generation of either donor-specific or non-donor-specific anti-HLA antibodies. Similarly, patients that had negative PRA screening tests before transplantation can develop de novo non-donor-specific or donor-specific anti-HLA antibodies after transplantation.

The mechanisms by which antibodies promote lung allograft injury remain poorly understood. Antibody binding to allo-HLA or other

endothelial or epithelial targets in the lung allograft can activate the complement cascade. Complement deposits lead to endothelial cell injury, production of proinflammatory molecules, and recruitment of inflammatory cells. Complement-independent antibody-mediated mechanisms can also induce endothelial cell activation without cell injury, leading to increased gene expression and subsequent proliferation [56]. Furthermore, as demonstrated by in vitro studies, anti-HLA antibodies can cause proliferation of airway epithelial cells as well, producing fibroblast-stimulating growth factors [61], potentially contributing to the generation of obliterative bronchiolitis.

Although the diagnosis of AMR in lung allograft biopsies remains challenging, when the triple test criteria are met (graft dysfunction, positive panel reactive antibodies, and evidence of complement deposition in the graft), the disease can be life-threatening, and prognosis can be poor. Although the optimal treatment of AMR in the lung is currently not known due to the lack of clinical trials, treatment is typically comprised of plasmapheresis, possibly intravenous immunoglobulin (IVIG), and medications such as rituximab and bortezomib, among others. As such, the associated histopathologic and clinical parameters are the subject of intense investigation. Deposition of complement 4d (C4d), a complement split product, on the capillary endothelium has been suggested as a surrogate marker for AMR in heart, kidney, and pancreas transplants [62–71]. However, the role of C4d deposition in the diagnosis of AMR in lung allografts is still unclear. Moreover, reproducibility of C4d deposition in allograft lung TBBx is problematic, even among pathologists who routinely evaluate C4d in lung allograft biopsies [72]. Furthermore, there are currently no specific or sensitive morphologic features of AMR in lung allografts, although some features that are more commonly identified in these patients have emerged in some recent studies [73]. Studies have attempted to evaluate immunoglobulins (Ig) and complement deposits in the subendothelial space. Septal capillary deposits of Igs and complement products such as C1q, C3d, C4d, and C5b-9 have been described in association with anti-HLA antibodies [74, 75] as well as allograft dysfunction and BOS [76, 77]. However, except for C4d and in some institutions C3d, these studies have in general not been implemented for the workup of lung transplant biopsies for possible AMR. One of the reasons for the difficulties in lung is the relatively high background that is encountered in immunohistochemical as well as immunofluorescence studies. Often, C4d binds to the vascular elastic lamina or shows other non-specific binding such as intracapillary serum. Staining is commonly only focal, and, therefore, sensitivity and specificity have not been established. Only linear, continuous luminal endothelial staining of capillaries, arterioles, and/or venules by C4d should be interpreted as positive. In addition, C4d is not specific to AMR but also can be seen in infection, and harvest/reperfusion injury, or any process that is associated with complement activation.

In general, the concept of specific histopathologic features associated with AMR remains controversial in lung transplantation. The 2007 ISHLT revised consensus classification [34] did propose histopathologic features that might be specific for AMR. Because of the lack of specific histologic findings of AMR, a multidisciplinary approach to the diagnosis was recommended that includes the following: (1) the presence of circulating antibodies (HLA antibodies, anti-endothelial and anti-epithelial antibodies), (2) focal or diffuse C4d deposition (Fig. 13.11a–c), (3) histologic features of acute lung injury or hemorrhage (diffuse alveolar damage, capillary injury associated with neutrophils and nuclear debris, i.e., capillaritis), and (4) clinical signs of graft dysfunction [78]. In 2013, the Pathology Council of the ISHLT published findings in a summary statement with recommendations for the pathologic evaluation of AMR [78]. This report included suggestions for protocol biopsies with serologic evaluation for donor-specific antibodies (DSAs) at or near time of biopsy. In addition, this statement included recommendations for histopathologic patterns in AMR (Fig. 13.12a–e) and indications for immunohistochemical or immunofluorescence studies to further elucidate findings in AMR (Box 13.1). The morphologic features were confirmed by the 2016 consensus report of the ISHLT [79].

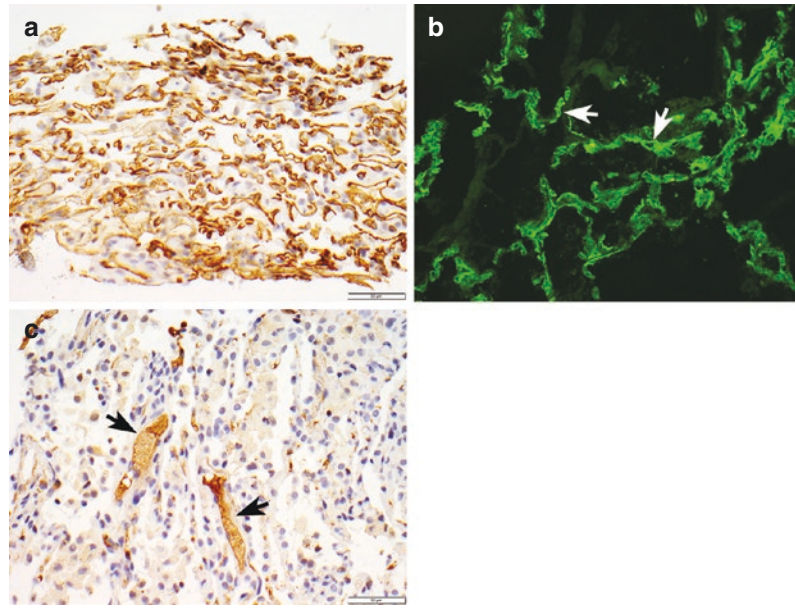

Fig. 13.11 (**a**–**c**) Complement 4d (C4d). Diffuse C4d deposition is defined by continuous subendothelial staining in more than 50% of capillaries either by immunohistochemistry (**a**) or immunofluorescence (**b**, arrows point toward capillary loops) often forming donut-shaped structures. The interpretation of C4d deposition can be complicated by non-specific staining including intracapillary serum (arrows) (**c**). Magnification, ×400 (**a**–**c**)

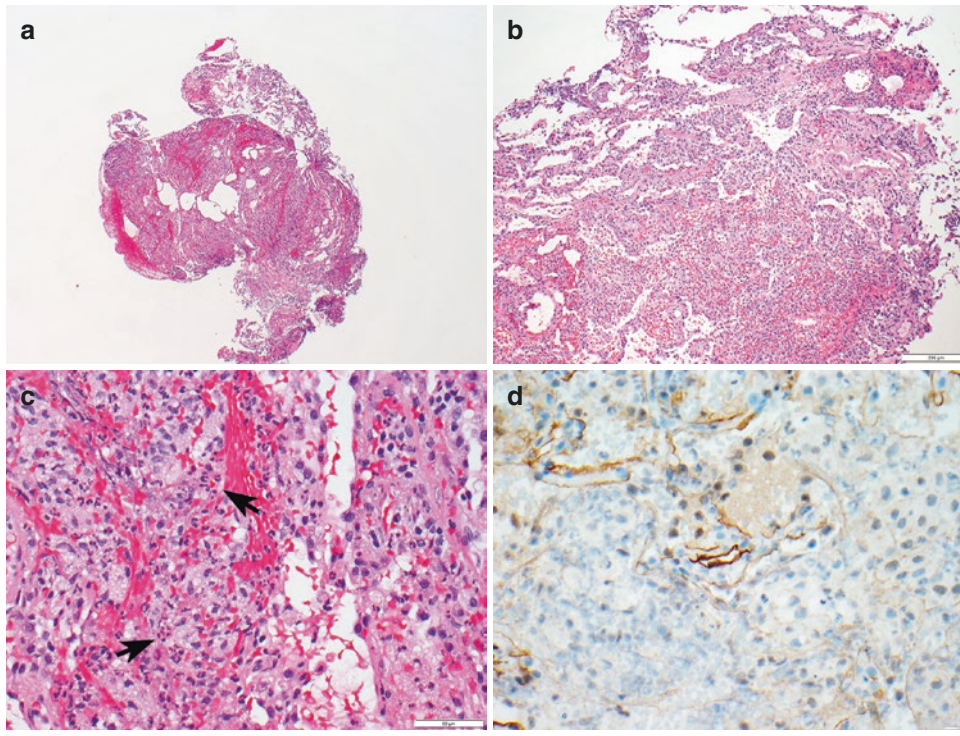

Fig. 13.12 (**a**–**e**) Possible antibody-mediated rejection (AMR). This patient presented with shortness of breath. Anti-DQ2 donor-specific antibodies were identified. The patient had undergone heart-lung transplantation 1 month prior to this biopsy. (**a**) A low-power view shows patchy inflammatory infiltrates and thickened interstitium. (**b**) At medium magnification, it becomes apparent that the interstitial thickening is due to neutrophilic inflammation and macrophages. Some alveoli are also filled with clusters of macrophages, scattered neutrophils, and blood. (**c**) There is neutrophilic margination in capillaries and neutrophilic capillaritis (arrows). (**d**, **e**) Complement 4d deposition was found in approximately 10–20% of capillaries by immunohistochemistry (**d**) and immunofluorescence (arrows) (**e**). Magnification, ×40 (**a**), ×100 (**b**), ×400 (**c**–**e**)

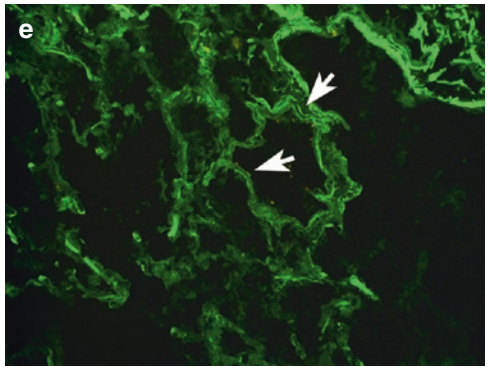

Fig. 13.12 (continued)

Box 13.1 Histomorphologic and Clinical Indications for Immunopathologic Evaluation (C4d Staining) of Lung Allograft Biopsies

Neutrophilic capillaritis
Neutrophilic septal margination
High-grade acute cellular rejection (≥ ISHLT Grade A3)
Persistent/recurrent acute cellular rejection (any ISHLT A grade)
Acute lung injury with or without diffuse alveolar damage
High-grade lymphocytic bronchiolitis (ISHLT Grade B2R)
Persistent low-grade lymphocytic bronchiolitis (ISHLT Grade B1R)
Obliterative bronchiolitis/chronic airways rejection (ISHLT Grade C1)
Arteritis in the absence of infection or cellular rejection
Graft dysfunction without morphologic explanation
Any histologic findings in setting of de novo DSA positivity

Used with permission of Elsevier from Berry G, Burke M, Andersen C, Angelini A, Bruneval P, Calbrese F, Fishbein MC, Goddard M, Leone O, Maleszewski J, et al. Pathology of pulmonary antibody-mediated rejection: 2012 update from the Pathology Council of the ISHLT. The Journal of Heart and Lung Transplantation 2013; 32:14–21

The 2016 consensus report confirmed the need for a multidisciplinary approach to establish a diagnosis of AMR in the lung that "integrates the clinical presentation with available immunologic and pathologic diagnostic tools" [79]. An AMR staging was also proposed (Table 13.2) [79].

Recently, Wallace and colleagues reported findings of the Banff study of the pathology of allograft lungs with DSA [73]. Nine experienced lung transplant pathologists from multiple institutions performed digital slide interpretation to study transbronchial biopsy specimens from patients with known antibody status (established within 30 days of biopsy) and negative infectious workup. The study demonstrated that biopsies from patients with DSA more commonly showed morphologic features of acute lung injury with or without diffuse alveolar damage than biopsies from patients with non-DSA or no circulating antibodies. Endotheliitis was more common in patients with DSA than patients without circulating antibodies. However, there was no difference in occurrence of endotheliitis between biopsies from patients with circulating non-DSA vs DSA or non-DSA vs no circulating antibodies. Specimens associated with DSA had a significant higher frequency of capillary inflammation, including neutrophilic margination, increased neutrophils, or capillaritis with karyorrhexis than patients with non-DSA or no circulating antibodies. C4d staining was positive in less than 50% of capillaries in 14% of biopsies and in more than 50% of capillaries in 7% of biopsies. While there was no difference between the groups in biopsies

Table 13.2 Staging of antibody-mediated rejection as proposed by the International Society for Heart and Lung Transplantation

Clinical antibody-mediated rejection	
Definite clinical AMR[a]	Allograft dysfunction DSA[b] present Histology suggestive of AMR C4d deposition Other causes of graft dysfunction were excluded except ACR[c] which can occur concurrently
Probable clinical AMR	Allograft dysfunction Two of the following 3 criteria: DSA present Histology suggestive of AMR C4d deposition When all 3 diagnostic criteria are identified, this grade can be applied even if infection or ACR is also present
Possible clinical AMR	Allograft dysfunction One of the following 3 criteria: DSA present Histology suggestive of AMR C4d deposition When 2 diagnostic criteria are identified, this grade can be applied even if infection or ACR is also present
Subclinical antibody-mediated rejection	
Histologic criteria of AMR identified on surveillance transbronchial biopsy with or without C4d deposition DSA present No allograft dysfunction	

Data from: Levine DJ, Glanville AR, Aboyoun C, Belperio J, Benden C, Berry GJ, Hachem R, Hayes D, Neil D, Reinsmoen NL, et al. Antibody-mediated rejection of the lung: A consensus report of the International Society for Heart and Lung Transplantation. The Journal of Heart and Lung Transplantation; 2016; 35:397–406

[a]*AMR* antibody-mediated rejection

[b]*DSA* donor-specific antibodies

[c]*ACR* acute cellular rejection

with <50% staining, biopsies with DSA more often had over 50% capillaries staining for C4d than biopsies without any circulating antibodies. There were no significant differences identified between HLA classes of the DSA and any of the evaluated pathologic findings. Taken together, this study identified capillary inflammation, acute lung injury, and endotheliitis as morphologic features in lung allograft biopsies that correlate with the presence of circulating DSA. However, none of these histopathologic features were specific to patients with DSA. Morphologic findings of acute lung injury with diffuse alveolar damage had the highest odds ratio for the presence of circulating DSA. This study also cautioned the usefulness of C4d immunohistochemical stain for the diagnosis of AMR in lung allografts because of its infrequent diffuse positivity. Although the study shows that some morphologic features correlate with the presence of circulating DSA and, therefore, might be histopathologic markers to at least suggest the possibility of AMR, the reproducibility of these morphologic features is quite problematic even among experienced lung transplant pathologists. In fact, the interobserver reproducibility kappa values ranged between 0.14 and 0.4, indicating a less than a chance to moderate agreement. The lowest agreement was noted for suspicion for aspiration (median kappa, 0.14) and the highest for acute cellular rejection, alveolar hemosiderosis, and C4d staining (median kappa, 0.4, all).

Although a definite diagnosis of AMR seems to elude pathologic interpretation at the current time, in a fully contextualized clinical environment, the findings from the biopsy specimen may aid the clinician to make a reasonable diagnosis of AMR if other relevant clinical and serologic features are present. The proposed "triple test" [78] of clinical features, serologic evidence of DSA, and pathologic findings supportive of AMR including capillary inflammation, acute lung injury with or without diffuse alveolar damage, and endotheliitis may currently be the best guide to the diagnosis of AMR.

There is no IHSLT recommendation at this time regarding the coexistence of AMR and acute rejection, but it clearly does occur.

Hyperacute Rejection

Hyperacute rejection is a severe form of AMR mediated by preexisting antibodies to ABO blood groups, HLA class I or II, or other antigens on graft vascular endothelial cells. This rejection

occurs within minutes to a few hours after the transplanted organ begins to be perfused. As in any form of AMR, the preexisting antibodies can result from previous pregnancies, blood transfusions, or previous transplant, and their binding to donor antigens provokes complement and cytokine activation resulting in endothelial cell damage and platelet activation with subsequent vascular thrombosis and graft destruction. The outcome is commonly fatal.

In hyperacute rejection, lungs are edematous, cyanotic, and heavy, have a firm consistency, lack crepitation, and show red hepatization [80–83]. The cut surface reveals patchy poorly defined areas of hemorrhagic consolidation. Anastomoses are intact and typically widely patent. Histologically, alveolar hemorrhage, platelet and fibrin thrombi, neutrophilic infiltration, necrosis of vessel walls, and diffuse alveolar damage are observed [76–80, 83, 84]. C4d deposition has been described.

Although hyperacute rejection is a well-known complication in kidney and heart transplantations, in lung transplantation, it appears to be rather rare with only eight cases reported. Six patients died within 1 h and 13 days after transplantation [80–85]. Only two patients survived [86, 87]. One of these two patients was treated with plasmapheresis, antithymocyte globulin, and cyclophosphamide immediately after hyperacute rejection was diagnosed [86]. The other patient was highly presensitized when he underwent double lung transplantation [87]. This patient was treated with multiple plasma exchanges and intravenous immunoglobulin pre- and posttransplantation together with posttransplant rituximab and bortezomib and later with anti-C5 antibody and eculizumab. Although in pretransplant, panel reactive antibodies (PRAs) were negative in four of the eight reported patients, crossmatch was positive in all reported cases.

Collectively, although hyperacute rejection is rare after lung transplantation, one should keep this reaction in mind given that false-negative PRAs may occur and pretransplantation crossmatch is not often possible [80].

Specimen Requirements

At least five pieces of well-expanded alveolated parenchyma are required for adequate evaluation of a transbronchial lung allograft biopsy specimen for acute rejection by the LRSG [34]. This specimen requirement was based on the "uniform opinion of the consensus meeting." To ensure that the minimum number of required pieces of alveolated lung parenchyma is available for pathology review, it is recommended that the bronchoscopist needs to take more than five pieces. Even more pieces might be necessary to provide small airways for review. Interestingly, a prospective 12-month single-operator study by Scott and colleagues [88] including 219 transbronchial allograft biopsies with 6 to 56 samples per procedure (mean 17.3 samples per procedure) taken from 3 lobes (or 2 lobes and the lingula of 1 lung) of 54 heart-lung transplant and 2 single lung transplant recipients revealed a sensitivity of 94% and a specificity of 90% for identification of rejection by histopathology. This study estimated that 18 samples per procedure are needed to have a 95% confidence of finding rejection. Therefore, false-negative results due to patchy distribution of acute rejection are likely not uncommon. The absence of histologic and immunophenotypic features of acute rejection or antibody-mediated rejection requires clinicopathologic correlation as a negative biopsy does not necessary rule out rejection. Furthermore, the bronchoscopist should be familiar with imaging studies, especially high resolution computed tomography studies if available, and aim to sample radiologically abnormal bronchopulmonary segments. If such imaging was not recently performed or the results are normal, then samples should be obtained from different lobes to try to minimize sampling error.

Specimens should be gently agitated in formalin to open up the alveoli. There is currently no recommendation for cryobiopsies. In a recent study using cryobiopsies to evaluate rejection in lung allografts, a median of three pieces provided twice as many alveoli and small airways than a median of ten pieces by conventional forceps biopsy [31].

The ISHLT recommends a minimum of three levels from the paraffin block for hematoxylin and eosin (H&E) staining for histologic examination [34]. In addition, "connective tissue stains" such as trichrome or Verhoeff-Van Gieson (VVG) stain are recommended to evaluate airways for the presence of submucosal fibrosis and vessels for graft vascular disease. Stains for microorganisms including Gomori-Grocott methenamine silver stain (GMS) and acid fast bacilli (AFB) may be added. While silver stains are routinely performed on lung allograft biopsies in some institutions, they are currently not mandated by the LRSG because many microbiologic, serologic, and molecular techniques are available and used to identify infections in these patients [34, 89]. BAL may be performed at the time of biopsy and is useful for the exclusion of infection but currently has no clinical role in the diagnosis of acute rejection.

Summary

The transbronchial allograft biopsy is currently the gold standard to evaluate the graft for cellular rejection and to exclude its clinical mimickers in lung transplant patients. When reviewing transbronchial biopsy material of these patients, attention must be paid not only to features of rejection but also to its morphologic mimickers, especially infection, PTLD, and abnormal drug effect. Before a diagnosis of acute cellular rejection can be rendered, an infectious process should be excluded by using stains for microorganisms and/or clinical tests including cultures of BAL and/or tissue and serology. While studies to identify histopathologic and immunophenotypic features of AMR are evolving, there are currently no specific morphologic findings, and clinical and serologic correlations are required for the diagnosis. Prospective, well-designed long-term studies with longitudinal data of therapeutic intervention of ACR on histopathology in totally asymptomatic patients with no physiological or HRCT evidence of allograft dysfunction are needed to determine the clinical significance and relevance of such interventions.

References

1. Martinu T, Chen DF, Palmer SM. Acute rejection and humoral sensitization in lung transplant recipients. Proc Am Thorac Soc. 2009;6:54–65.
2. Haque MA, Mizobuchi T, Yasufuku K, Fujisawa T, Brutkiewicz RR, Zheng Y, Woods K, Smith GN, Cummings OW, Heidler KM, et al. Evidence for immune responses to a self-antigen in lung transplantation: role of type V collagen-specific T cells in the pathogenesis of lung allograft rejection. J Immunol. 2002;169:1542–9.
3. Yusen RD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Goldfarb SB, Levvey BJ, Lund LH, Meiser B, Rossano JW, Stehlik J. The Registry of the International Society for Heart and Lung Transplantation: thirty-second official adult lung and heart-lung transplantation report—2015; focus theme: early graft failure. J Heart Lung Transplant. 2015;34:1264–77.
4. Martinu T, Pavlisko EN, Chen DF, Palmer SM. Acute allograft rejection: cellular and humoral processes. Clin Chest Med. 2011;32:295–310.
5. Glanville AR, Aboyoun CL, Havryk A, Plit M, Rainer S, Malouf MA. Severity of lymphocytic bronchiolitis predicts long-term outcome after lung transplantation. Am J Respir Crit Care Med. 2008;177:1033–40.
6. Verleden SE, Ruttens D, Vandermeulen E, Vaneylen A, Dupont LJ, Van Raemdonck DE, Verleden GM, Vanaudenaerde BM, Vos R. Bronchiolitis obliterans syndrome and restrictive allograft syndrome: do risk factors differ? Transplantation. 2013;95:1167–72.
7. Schulman LL, Weinberg AD, McGregor C, Galantowicz ME, Suciu-Foca NM, Itescu S. Mismatches at the HLA-dr and HLA-b loci are risk factors for acute rejection after lung transplantation. Am J Respir Crit Care Med. 1998;157:1833–7.
8. Quantz MA, Bennett LE, Meyer DM, Novick RJ. Does human leukocyte antigen matching influence the outcome of lung transplantation? An analysis of 3,549 lung transplantations. J Heart Lung Transplant. 2000;19:473–9.
9. Wisser W, Wekerle T, Zlabinger G, Senbaclavaci O, Zuckermann A, Klepetko W, Wolner E. Influence of human leukocyte antigen matching on long-term outcome after lung transplantation. J Heart Lung Transplant. 1996;15:1209–16.
10. Christie JD, Edwards LB, Kucheryavaya AY, Aurora P, Dobbels F, Kirk R, Rahmel AO, Stehlik J, Hertz MI. The Registry of the International Society for Heart and Lung Transplantation: twenty-seventh official adult lung and heart-lung transplant report—2010. J Heart Lung Transplant. 2010;29:1104–18.
11. Mangi AA, Mason DP, Nowicki ER, Batizy LH, Murthy SC, Pidwell DJ, Avery RK, McCurry KR, Pettersson GB, Blackstone EH. Predictors of acute rejection after lung transplantation. Ann Thorac Surg. 2011;91:1754–62.

12. Zheng HX, Burckart GJ, McCurry K, Webber S, Ristich J, Iacono A, Dauber J, McDade K, Grgurich W, Zaldonis D, et al. Interleukin-10 production genotype protects against acute persistent rejection after lung transplantation. J Heart Lung Transplant. 2004;23:541–6.
13. Zheng HX, Zeevi A, McCurry K, Schuetz E, Webber S, Ristich J, Zhang J, Iacono A, Dauber J, McDade K, et al. The impact of pharmacogenomic factors on acute persistent rejection in adult lung transplant patients. Transpl Immunol. 2005;14:37–42.
14. Ibrahim JE, Sweet SC, Flippin M, Dent C, Mendelhoff E, Huddleston CB, Trinkhaus K, Canter CE. Rejection is reduced in thoracic organ recipients when transplanted in the first year of life. J Heart Lung Transplant. 2002;21:311–8.
15. Scott JP, Whitehead B, de Leval M, Helms P, Smyth RL, Higenbottam TW, Wallwork J. Paediatric incidence of acute rejection and obliterative bronchiolitis: a comparison with adults. Transpl Int. 1994;7:S404–6.
16. Yusen RD, Christie JD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Dobbels F, Kirk R, Lund LH, Rahmel AO, Stehlik J. The Registry of the International Society for Heart and Lung Transplantation: thirtieth adult lung and heart-lung transplant report—2013; focus theme: age. J Heart Lung Transplant. 2013;32:965–78.
17. De Vito Dabbs A, Hoffman LA, Iacono AT, Zullo TG, McCurry KR, Dauber JH. Are symptom reports useful for differentiating between acute rejection and pulmonary infection after lung transplantation? Heart Lung. 2004;33:372–80.
18. Christie JD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Dobbels F, Kirk R, Rahmel AO, Stehlik J, Hertz MI. The Registry of the International Society for Heart and Lung Transplantation: 29th adult lung and heart-lung transplant report-2012. J Heart Lung Transplant. 2012;31:1073–86.
19. Lowery EM, Bemiss B, Cascino T, Durazo-Arvizu RA, Forsythe SM, Alex C, Laghi F, Love RB, Camacho P. Low vitamin D levels are associated with increased rejection and infections after lung transplantation. J Heart Lung Transplant. 2012;31:700–7.
20. Kumar D, Erdman D, Keshavjee S, Peret T, Tellier R, Hadjiliadis D, Johnson G, Ayers M, Siegal D, Humar A. Clinical impact of community-acquired respiratory viruses on bronchiolitis obliterans after lung transplant. Am J Transplant. 2005;5:2031–6.
21. Vilchez RA, Dauber J, McCurry K, Iacono A, Kusne S. Parainfluenza virus infection in adult lung transplant recipients: an emergent clinical syndrome with implications on allograft function. Am J Transplant. 2003;3:116–20.
22. Garantziotis S, Howell DN, McAdams HP, Davis RD, Henshaw NG, Palmer SM. Influenza pneumonia in lung transplant recipients: clinical features and association with bronchiolitis obliterans syndrome. Chest. 2001;119:1277–80.
23. Glanville AR, Gencay M, Tamm M, Chhajed P, Plit M, Hopkins P, Aboyoun C, Roth M, Malouf M. Chlamydia pneumoniae infection after lung transplantation. J Heart Lung Transplant. 2005;24:131–6.
24. Sharples LD, McNeil K, Stewart S, Wallwork J. Risk factors for bronchiolitis obliterans: a systematic review of recent publications. J Heart Lung Transplant. 2002;21:271–81.
25. Van Muylem A, Melot C, Antoine M, Knoop C, Estenne M. Role of pulmonary function in the detection of allograft dysfunction after heart-lung transplantation. Thorax. 1997;52:643–7.
26. Becker FS, Martinez FJ, Brunsting LA, Deeb GM, Flint A, Lynch JP III. Limitations of spirometry in detecting rejection after single-lung transplantation. Am J Respir Crit Care Med. 1994;150:159–66.
27. Gotway MB, Dawn SK, Sellami D, Golden JA, Reddy GP, Keith FM, Webb WR. Acute rejection following lung transplantation: limitations in accuracy of thin-section CT for diagnosis. Radiology. 2001;221:207–12.
28. Silkoff PE, Caramori M, Tremblay L, McClean P, Chaparro C, Kesten S, Hutcheon M, Slutsky AS, Zamel N, Keshavjee S. Exhaled nitric oxide in human lung transplantation: a noninvasive marker of acute rejection. Am J Respir Crit Care Med. 1998;157:1822–8.
29. Gashouta MA, Merlo CA, Pipeling MR, McDyer JF, Hayanga JW, Orens JB, Girgis RE. Serial monitoring of exhaled nitric oxide in lung transplant recipients. J Heart Lung Transplant. 2015;34:557–62.
30. De Soyza A, Fisher AJ, Small T, Corris PA. Inhaled corticosteroids and the treatment of lymphocytic bronchiolitis following lung transplantation. Am J Respir Crit Care Med. 2001;164:1209–12.
31. Roden AC, Kern RM, Aubry MC, Jenkins SM, Yi ES, Scott JP, Maldonado F. Transbronchial cryobiopsies in the evaluation of lung allografts: do the benefits outweigh the risks? Arch Pathol Lab Med. 2016;140(4):303–11.
32. Yarmus L, Akulian J, Gilbert C, Illei P, Shah P, Merlo C, Orens J, Feller-Kopman D. Cryoprobe transbronchial lung biopsy in patients after lung transplantation: a pilot safety study. Chest. 2013;143:621–6.
33. Fruchter O, Fridel L, Rosengarten D, Raviv Y, Rosanov V, Kramer MR. Transbronchial cryobiopsy in lung transplantation patients: first report. Respirology. 2013;18:669–73.
34. Stewart S, Fishbein MC, Snell GI, Berry GJ, Boehler A, Burke MM, Glanville A, Gould FK, Magro C, Marboe CC, et al. Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. J Heart Lung Transplant. 2007;26:1229–42.
35. Berry GJ, Brunt EM, Chamberlain D, Hruban RH, Sibley RK, Stewart S, Tazelaar HD. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: Lung Rejection Study Group. The International Society for Heart Transplantation. J Heart Transplant. 1990;9:593–601.
36. Yousem SA, Berry GJ, Cagle PT, Chamberlain D, Husain AN, Hruban RH, Marchevsky A, Ohori NP,

Ritter J, Stewart S, Tazelaar HD. Revision of the 1990 working formulation for the classification of pulmonary allograft rejection: Lung Rejection Study Group. J Heart Lung Transplant. 1996;15:1–15.
37. Chakinala MM, Ritter J, Gage BF, Aloush AA, Hachem RH, Lynch JP, Patterson GA, Trulock EP. Reliability for grading acute rejection and airway inflammation after lung transplantation. J Heart Lung Transplant. 2005;24:652–7.
38. Colombat M, Groussard O, Lautrette A, Thabut G, Marrash-Chahla R, Brugière O, Mal H, Lesèche G, Fournier M, Degott C. Analysis of the different histologic lesions observed in transbronchial biopsy for the diagnosis of acute rejection. Clinicopathologic correlations during the first 6 months after lung transplantation. Hum Pathol. 2005;36:387–94.
39. Stephenson A, Flint J, English J, Vedal S, Fradet G, Chittock D, Levy RD. Interpretation of transbronchial lung biopsies from lung transplant recipients: inter- and intraobserver agreement. Can Respir J. 2005;12:75–7.
40. Bhorade SM, Husain AN, Liao C, Li LC, Ahya VN, Baz MA, Valentine VG, Love RB, Seethamraju H, Alex CG, et al. Interobserver variability in grading transbronchial lung biopsy specimens after lung transplantation. Chest. 2013;143:1717–24.
41. Yousem SA, Martin T, Paradis IL, Keenan R, Griffith BP. Can immunohistological analysis of transbronchial biopsy specimens predict responder status in early acute rejection of lung allografts? Hum Pathol. 1994;25:525–9.
42. Reams BD, Musselwhite LW, Zaas DW, Steele MP, Garantziotis S, Eu PC, Snyder LD, Curl J, Lin SS, Davis RD, Palmer SM. Alemtuzumab in the treatment of refractory acute rejection and bronchiolitis obliterans syndrome after human lung transplantation. Am J Transplant. 2007;7:2802–8.
43. Trulock EP, Ettinger NA, Brunt EM, Pasque MK, Kaiser LR, Cooper JD. The role of transbronchial lung biopsy in the treatment of lung transplant recipients: an analysis of 200 consecutive procedures. Chest. 1992;102:1049–54.
44. Hopkins PM, Aboyoun CL, Chhajed PN, Malouf MA, Plit ML, Rainer SP, Glanville AR. Prospective analysis of 1,235 transbronchial lung biopsies in lung transplant recipients. J Heart Lung Transplant. 2002;21:1062–7.
45. Chakinala MM, Ritter J, Gage BF, Lynch JP, Aloush A, Patterson GA, Trulock EP. Yield of surveillance bronchoscopy for acute rejection and lymphocytic bronchitis/bronchiolitis after lung transplantation. J Heart Lung Transplant. 2004;23:1396–404.
46. Hopkins PM, Aboyoun CL, Chhajed PN, Malouf MA, Plit ML, Rainer SP, Glanville AR. Association of minimal rejection in lung transplant recipients with obliterative bronchiolitis. Am J Respir Crit Care Med. 2004;170:1022–6.
47. Hachem RR, Khalifah AP, Chakinala MM, Yusen RD, Aloush AA, Mohanakumar T, Patterson GA, Trulock EP, Walter MJ. The significance of a single episode of minimal acute rejection after lung transplantation. Transplantation. 2005;80:1406–13.
48. Kramer MR, Stoehr C, Whang JL, Berry GJ, Sibley R, Marshall SE, Patterson GM, Starnes VA, Theodore J. The diagnosis of obliterative bronchiolitis after heart-lung and lung transplantation: low yield of transbronchial lung biopsy. J Heart Lung Transplant. 1993;12:675–81.
49. Chamberlain D, Maurer J, Chapparo C, Idolor C. Evaluation of transbronchial biopsy in the diagnosis of bronchiolitis obliterans after lung transplantation. J Heart Lung Transplant. 1994;13:963–71.
50. Pomerance A, Madden B, Burke MM, Yacoub MH. Transbronchial biopsy in heart and lung transplantation: clinicopathologic correlations. J Heart Lung Transplant. 1995;14:761–73.
51. Meyer KC, Raghu G, Verleden GM, Corris PA, Aurora P, Wilson KC, Brozek J, Glanville AR. An international ISHLT/ATS/ERS clinical practice guideline: diagnosis and management of bronchiolitis obliterans syndrome. Eur Respir J. 2014;44:1479–503.
52. Saggar R, Ross DJ, Saggar R, Zisman DA, Gregson A, Lynch JP III, Keane MP, Weigt SS, Ardehali A, Kubak B, et al. Pulmonary hypertension associated with lung transplantation obliterative bronchiolitis and vascular remodeling of the allograft. Am J Transplant. 2008;8:1921–30.
53. Nathan SD, Shlobin OA, Ahmad S, Barnett SD, Burton NA, Gladwin MT, Machado RF. Pulmonary hypertension in patients with bronchiolitis obliterans syndrome listed for retransplantation. Am J Transplant. 2008;8:1506–11.
54. Tazelaar HD. Perivascular inflammation in pulmonary infections: implications for the diagnosis of lung rejection. J Heart Lung Transplant. 1991;10:437–41.
55. Takemoto SK, Zeevi A, Feng S, Colvin RB, Jordan S, Kobashigawa J, Kupiec-Weglinski J, Matas A, Montgomery RA, Nickerson P, et al. National conference to assess antibody-mediated rejection in solid organ transplantation. Am J Transplant. 2004;4:1033–41.
56. Colvin RB, Smith RN. Antibody-mediated organ-allograft rejection. Nat Rev Immunol. 2005;5:807–17.
57. Girnita AL, McCurry KR, Iacono AT, Duquesnoy R, Corcoran TE, Awad M, Spichty KJ, Yousem SA, Burckart G, Dauber JH, et al. HLA-specific antibodies are associated with high-grade and persistent-recurrent lung allograft acute rejection. J Heart Lung Transplant. 2004;23:1135–41.
58. Palmer SM, Davis RD, Hadjiliadis D, Hertz MI, Howell DN, Ward FE, Savik K, Reinsmoen NL. Development of an antibody specific to major histocompatibility antigens detectable by flow cytometry after lung transplant is associated with bronchiolitis obliterans syndrome. Transplantation. 2002;74:799–804.
59. Hadjiliadis D, Chaparro C, Reinsmoen NL, Gutierrez C, Singer LG, Steele MP, Waddell TK, Davis RD,

Hutcheon MA, Palmer SM, Keshavjee S. Pre-transplant panel reactive antibody in lung transplant recipients is associated with significantly worse post-transplant survival in a multicenter study. J Heart Lung Transplant. 2005;24:S249–54.
60. Appel JZ III, Hartwig MG, Davis RD, Reinsmoen NL. Utility of peritransplant and rescue intravenous immunoglobulin and extracorporeal immunoadsorption in lung transplant recipients sensitized to HLA antigens. Hum Immunol. 2005;66:378–86.
61. Jaramillo A, Smith CR, Maruyama T, Zhang L, Patterson GA, Mohanakumar T. Anti-HLA class I antibody binding to airway epithelial cells induces production of fibrogenic growth factors and apoptotic cell death: a possible mechanism for bronchiolitis obliterans syndrome. Hum Immunol. 2003;64:521–9.
62. Choi J, Cho YM, Yang WS, Park TJ, Chang JW, Park SK. Peritubular capillary C4d deposition and renal outcome in post-transplant IgA nephropathy. Clin Transpl. 2007;21:159–65.
63. Herman J, Lerut E, Van Damme-Lombaerts R, Emonds MP, Van Damme B. Capillary deposition of complement C4d and C3d in pediatric renal allograft biopsies. Transplantation. 2005;79:1435–40.
64. Moll S, Pascual M. Humoral rejection of organ allografts. Am J Transplant. 2005;5:2611–8.
65. Fedson SE, Daniel SS, Husain AN. Immunohistochemistry staining of C4d to diagnose antibody-mediated rejection in cardiac transplantation. J Heart Lung Transplant. 2008;27:372–9.
66. Mauiyyedi S, Crespo M, Collins AB, Schneeberger EE, Pascual MA, Saidman SL, Tolkoff-Rubin NE, Williams WW, Delmonico FL, Cosimi AB, Colvin RB. Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification. J Am Soc Nephrol. 2002;13:779–87.
67. Mauiyyedi S, Pelle PD, Saidman S, Collins AB, Pascual M, Tolkoff-Rubin NE, Williams WW, Cosimi AA, Schneeberger EE, Colvin RB. Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. J Am Soc Nephrol. 2001;12:574–82.
68. Rodriguez ER, Skojec DV, Tan CD, Zachary AA, Kasper EK, Conte JV, Baldwin WM III. Antibody-mediated rejection in human cardiac allografts: evaluation of immunoglobulins and complement activation products C4d and C3d as markers. Am J Transplant. 2005;5:2778–85.
69. Smith RN, Brousaides N, Grazette L, Saidman S, Semigran M, Disalvo T, Madsen J, Dec GW, Perez-Atayde AR, Collins AB. C4d deposition in cardiac allografts correlates with alloantibody. J Heart Lung Transplant. 2005;24:1202–10.
70. Tan CD, Baldwin WM III, Rodriguez ER. Update on cardiac transplantation pathology. Arch Pathol Lab Med. 2007;131:1169–91.
71. de Kort H, Munivenkatappa RB, Berger SP, Eikmans M, van der Wal A, de Koning EJ, van Kooten C, de Heer E, Barth RN, Bruijn JA, et al. Pancreas allograft biopsies with positive c4d staining and anti-donor antibodies related to worse outcome for patients. Am J Transplant Off J Am Soc Transplant Am Soc Transplant Surg. 2010;10:1660–7.
72. Roden AC, Maleszewski JJ, Yi ES, Jenkins SM, Gandhi MJ, Scott JP, Christine Aubry M. Reproducibility of Complement 4d deposition by immunofluorescence and immunohistochemistry in lung allograft biopsies. J Heart Lung Transplant. 2014;33:1223–32.
73. Wallace WD, Li N, Andersen CB, Arrossi AV, Askar M, Berry GJ, DeNicola MM, Neil DA, Pavlisko EN, Reed EF, et al. Banff study of pathologic changes in lung allograft biopsy specimens with donor-specific antibodies. J Heart Lung Transplant. 2016;35:40–8.
74. Ionescu DN, Girnita AL, Zeevi A, Duquesnoy R, Pilewski J, Johnson B, Studer S, McCurry KR, Yousem SA. C4d deposition in lung allografts is associated with circulating anti-HLA alloantibody. Transpl Immunol. 2005;15:63–8.
75. Miller GG, Destarac L, Zeevi A, Girnita A, McCurry K, Iacono A, Murray JJ, Crowe D, Johnson JE, Ninan M, Milstone AP. Acute humoral rejection of human lung allografts and elevation of C4d in bronchoalveolar lavage fluid. Am J Transplant. 2004;4:1323–30.
76. Magro CM, Abbas AE, Seilstad K, Pope-Harman AL, Nadasdy T, Ross P Jr. C3d and the septal microvasculature as a predictor of chronic lung allograft dysfunction. Hum Immunol. 2006;67:274–83.
77. Westall GP, Snell GI, McLean C, Kotsimbos T, Williams T, Magro C. C3d and C4d deposition early after lung transplantation. J Heart Lung Transplant. 2008;27:722–8.
78. Berry G, Burke M, Andersen C, Angelini A, Bruneval P, Calbrese F, Fishbein MC, Goddard M, Leone O, Maleszewski J, et al. Pathology of pulmonary antibody-mediated rejection: 2012 update from the Pathology Council of the ISHLT. J Heart Lung Transplant. 2013;32:14–21.
79. Levine DJ, Glanville AR, Aboyoun C, Belperio J, Benden C, Berry GJ, Hachem R, Hayes D, Neil D, Reinsmoen NL, et al. Antibody-mediated rejection of the lung: a consensus report of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2016;35:397–406.
80. de Jesus Peixoto Camargo J, Marcantonio Camargo S, Marcelo Schio S, Noguchi Machuca T, Adélia Perin F. Hyperacute rejection after single lung transplantation: a case report. Transplant Proc. 2008;40:867–9.
81. Campo-Canaveral de la Cruz JL, Naranjo JM, Salas C, Varela de Ugarte A. Fulminant hyperacute rejection after unilateral lung transplantation. Eur J Cardiothorac Surg. 2012;42:373–5.
82. Choi JK, Kearns J, Palevsky HI, Montone KT, Kaiser LR, Zmijewski CM, Tomaszewski JE. Hyperacute rejection of a pulmonary allograft. Immediate clinical and pathologic findings. Am J Respir Crit Care Med. 1999;160:1015–8.
83. Scornik JC, Zander DS, Baz MA, Donnelly WH, Staples ED. Susceptibility of lung transplants to pre-

formed donor-specific HLA antibodies as detected by flow cytometry. Transplantation. 1999;68:1542–6.
84. Frost AE, Jammal CT, Cagle PT. Hyperacute rejection following lung transplantation. Chest. 1996;110:559–62.
85. Masson E, Stern M, Chabod J, Thevenin C, Gonin F, Rebibou JM, Tiberghien P. Hyperacute rejection after lung transplantation caused by undetected low-titer anti-HLA antibodies. J Heart Lung Transplant. 2007;26:642–5.
86. Bittner HB, Dunitz J, Hertz M, Bolman MR III, Park SJ. Hyperacute rejection in single lung transplantation—case report of successful management by means of plasmapheresis and antithymocyte globulin treatment. Transplantation. 2001;71:649–51.
87. Dawson KL, Parulekar A, Seethamraju H. Treatment of hyperacute antibody-mediated lung allograft rejection with eculizumab. J Heart Lung Transplant. 2012;31:1325–6.
88. Scott J, Fradet G, Smyth R, et al. Prospective study of transbronchial biopsies in the management of heart-lung and single lung transplant patients. J Heart Lung Transplant. 1991;10:626–37.
89. Troxell ML, Lanciault C. Practical applications in immunohistochemistry: evaluation of rejection and infection in organ transplantation. Arch Pathol Lab Med. 2016;140(9):910–25.