Lung Transplantation

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Introduction

The therapeutic options for patients with advanced pulmonary parenchymal or vascular disorders are limited. Lung transplantation remains one of the few viable interventions, but on account of the insufficient donor pool only a minority of these patients actually undergo the procedure each year. Further, the projections for the number of lung transplant candidates will continue to increase with our expanding older patient demographic. Despite the increase in transplant procedures annually, the problem of organ availability and suitability will likely persist. A number of solutions for expanding the donor pool have been implemented or are under active experimental or clinical investigation, including changes in the allocation methods, interventions such as extracorporeal membrane oxygenation (ECMO) and ex vivo lung perfusion and utilizing older donor organs and organs procured following circulatory cessation or donation after circulatory determination of death (DCDD). Following transplantation there are a number of early and late allograft complications such as primary graft dysfunction, allograft rejection, infection, post-transplant lymphoproliferative disorder and late injury that is now classified as chronic lung allograft dysfunction. The pathologist plays an essential role in the diagnosis and classification of these myriad complications. Although the transplant procedures are performed in selected centers patients typically return home after a period of convalescence and observation. When complications arise thereafter it is often the responsibility of the local pathologist to evaluate the laboratory, cytopathologic and histopathologic studies. For these reasons familiarity with the pathology of lung transplantation is important.

History of Lung Transplantation

The initial experimental attempts at lung transplantation date back over a century ago when Guthrie and Carrel implanted the heart and lungs of a kitten into the neck vessels of an adult cat [1]. Forty years later, the Russian physiologist Demikhov developed canine models of combined heart–lung and single transplantation [2, 3]. In the early 1960s, Hardy and colleagues at the University of Mississippi performed the first human lung transplant in 1963 in a 58-year old man with advanced lung cancer. He survived 18 days, but succumbed to renal failure; no acute cellular rejection (ACR) was found at postmortem examination [4]. The first Canadian lung transplant was performed in 1966. The recipient was a

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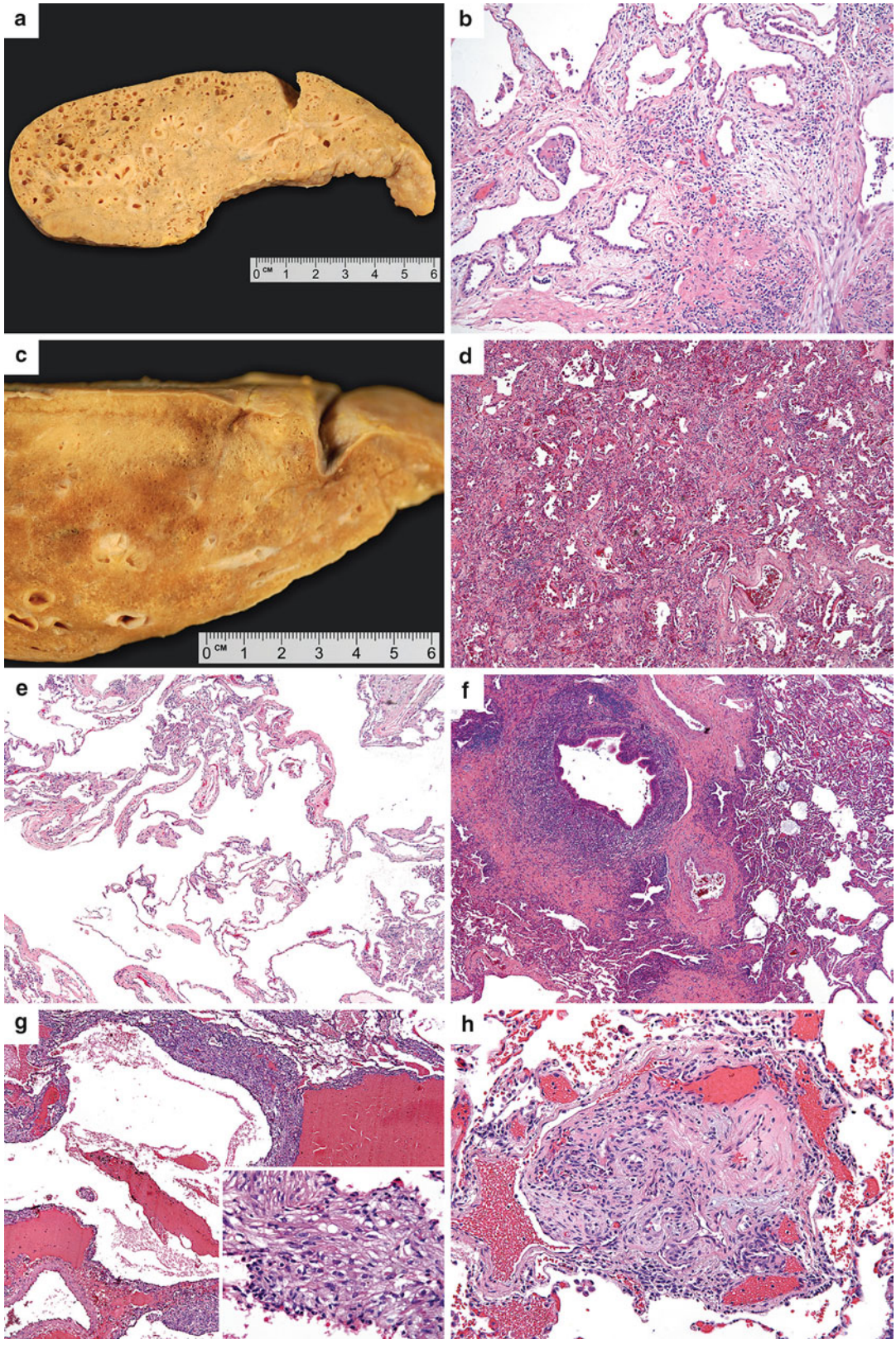
31-year old man with advanced silicosis and he survived only 7 days. At postmortem, the transplanted lung showed ACR, preservation injury, and necrosis of the proximal bronchus [5]. In 1968, Cooley attempted the first human combined heart–lung transplant on a 2-month old child with complex congenital heart disease, but she died 14 h later from respiratory failure [6]. Shortly thereafter, Lillehi and colleagues performed the procedure in an adult recipient with chronic obstructive pulmonary disease (COPD) and cor pulmonale with survival of only 8 days. Attempts at lung transplantation sputtered over the next decade with many centers abandoning their programs on account of dismal survival results attributable to insufficient immunosuppression and/or technical difficulties such as anastomotic dehiscence [7]. With the initiation of the cyclosporine era in 1980, there was resurgence in interest in combined heart–lung transplantation by the Stanford team and lung transplantation in the Toronto program. This novel form of immunosuppression permitted reduced corticosteroid dosages and led to a reduction in anastomotic complications. Reitz et al. at Stanford [8] overcame a series of technical and immunosuppressive liabilities utilizing a non-human primate model before achieving successful long-term survival in their clinical program. Four of the five patients transplanted for advanced pulmonary vascular disease (either idiopathic pulmonary arterial hypertension (IPAH) or complex congenital heart disease with Eisenmenger's physiology) survived beyond a 6-month period of follow-up. Shortly thereafter, Patterson and Cooper [9] at the University of Toronto reported the first successful single lung transplant program for patients with end-stage pulmonary fibrosis. The technique for double lung transplant evolved from the en bloc approach with tracheal anastomosis to the current bilateral sequential single lung transplant technique [10]. Lung transplantation in the pediatric population was introduced in 1986 and the first procedure in an infant was reported in 2000. The living-related lobar transplant program was initiated by Starnes and his colleagues in the mid-1990s and has now expanded to cadaveric lobar lung transplantation [11–14]. Excellent results have been reported from experienced centers.

Indications for Lung Transplantation

Over the last three decades, the **indications for combined heart–lung and lung transplantation** have expanded. In adults, the main indications are COPD including emphysema and alpha-1-antitrypsin deficiency, suppurative lung disease/cystic fibrosis (CF), fibrotic interstitial lung disease (ILD) including idiopathic pulmonary fibrosis (IPF), chronic hypersensitivity pneumonitis, and nonspecific interstitial pneumonia (NSIP), and pulmonary vascular disease including IPAH and Eisenmenger's physiology (Fig. 1) In the most recent published data from the ISHLT registry, there has been a sevenfold increase in the number of lung transplants since 1990, with 33,893 lung transplants reported in 2013 and a total of 51,440 lung and 3820 heart–lung procedures recorded up to mid-2014 [15]. The median survival is 5.6 years with bilateral/double lung recipients having much better median survival rates compared to single lung transplant patients (7.1 versus 4.5 years). Over the last 15 years, there has been a shift from single lung to bilateral/double lung transplant as

Fig. 1 (**a**) Axial section of right lung from patient with end-stage IPF/UIP. The periphery of the lobe shows honeycomb change. (**b**) Scanning magnification showing histopathologic features of temporal heterogeneity of UIP including fibroblastic foci, inflammation, fibrosis, and restructured airspaces. (**c**) Gross pathology from lung of patient with end-stage NSIP. Diffuse fibrosis is observed without honeycomb change. (**d**) Low power histopathology of NSIP with temporal and geographic uniformity of the process. (**e**) Severe centrilobular emphysema with dilated and distorted airspaces in patient with bullous disease. (**f**) Marked bronchiolectasis with luminal dilatation, intense inflammation, and bronchiolar fibrosis. (**g**) Young woman with LAM characterized by cystic spaces lined by smooth muscle cells. INSERT: High power magnification showing immature spindled smooth muscle cells. (**h**) Plexiform lesion in patient with idiopathic pulmonary arterial hypertension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the preferred surgical procedure. One hundred and twenty four pediatric lung transplant procedures were performed in 2013. In infants and children, there are unique pediatric conditions that require transplantation in addition to the suppurative and pulmonary vascular categories. In infants under the age of 1 year, the most common indications are IPAH or other vascular disorders, congenital heart disease, and surfactant deficiencies, while in the childhood group (1–5 years) IPAH and pulmonary fibrosis account for the majority of cases. In children older than 6 years, the most common indication is CF [16]. The median survival is comparable to the adult group at 5.3 years with bilateral/double recipients faring much better than single lung recipients. There has been a steady decline in the number of combined heart–lung transplants in adults since 2000 with median survival rates in patients transplanted in the most recent era now similar to the lung group (5.6 years). Patients with CF, congenital heart disease, and IPAH constitute the vast majority of these cases.

Historically, the "time on the wait list" has been the traditional approach to prioritizing and apportioning lung allografts. In 2005, the **lung allocation score (LAS)** was introduced in the United States to prioritize patients on the wait-list for lung transplants based on the urgency of the patient's condition and the likelihood of survival after transplant [17]. The LAS represents a paradigm shift in transplant medicine utilizing a statistical modeling approach that calculates a "waiting list urgency measure" and a "post-transplant survival measure." A numeric transplant benefit is calculated from the simple subtraction of these entities and then a normalized LAS is enumerated by a mathematical formula. The LAS score then determines the placement with higher scores reflecting sicker patients with greater expected benefit. Although still controversial, there are published data showing reduction of both the number of deaths on the waitlist and waitlist times (from years to months) [18–20]. It has also led to an increase in the proportion of patients transplanted for IPF and a decline in those transplanted for COPD [15]. There have been a number of recent studies that have shown that the overall survival time has not changed, reflecting the problem that sicker patients are now being transplanted. Not surprisingly, patients with high LAS scores have higher post-transplant complications [20]. The LAS approach has also had an impact on the selection of transplant procedure (single versus bilateral/double lung transplant) [21]. The impact of organ distribution in the pediatric setting is significantly more controversial, particularly in children under the age of 12 years [22, 23]. In this age group, the combination of group waitlist times and a Priority 1 scoring system is used [23, 24]. The LAS system is utilized for children older than 12 years of age in the US. Infants may be considered for an ABO-incompatible procedure as they have not yet developed preformed antibodies against ABO blood antigens, but there is very little published clinical experience with this approach [25].

Donor and Recipient Selection Criteria

Selected Donor-Related Issues

The current criteria for donor acceptability were recently summarized by the ISHLT [26]. These reflect in large part the accumulated historical experience of transplant programs and a conservative approach to donor evaluation. Briefly, the "ideal donor" would have most or all of the following characteristics: age under 55 years, ABO blood compatibility, normal chest X-ray, and absence or limited smoking history (<20 pack-years), comparable size matching, graft ischemia time of less than 6 h, and absence of aspiration or septic episodes, prior thoracic surgery, prior malignancy, organisms on a sputum gram stain, or purulent secretions at the time of bronchoscopy. Strict adherence to these requirements has traditionally resulted in limited utilization rates of potential allografts in the range of 5–20%. Over the past decade, a number of studies have questioned some or all of these criteria and many centers have focused on revised criteria to assess and potentially accept more donor lungs. Snell and colleagues have sub-classified this "extended donor pool" into two groups: **(1) General medi-**

cal issues such as prior history or age, and **(2) Specific medical issues** such as infection, pulmonary edema, etc. [27]. Both categories may have potential clinical consequences for the recipient such as late complications in the first group and primary graft dysfunction (PGD) and other early complications in the second group. On occasion, the pathologist may be asked to perform frozen-section examination of a pulmonary nodule or consolidation in a potential donor situation. Tissue should be sent for microbiologic testing in all cases and histochemical or immunohistochemical staining for bacterial, fungal, or viral organisms should be obtained on the permanent sections as warranted.

In addition to loosening many of the historic contraindications to donor suitability, a number of molecular and surgical techniques and potential donor sources have been adapted to the evaluation process.

Donation after cardiac death. Until recently, the vast majority of lung donor allografts were cadaveric organs retrieved following brain death. In addition to the living-related lobar donations described previously, attention is now focused on the potential role of cadaveric **donation after circulatory cessation/determination of death (DCDD)**. Based on a series of elegant experiments in a canine model by Egan et al. and other investigators, it was determined that the lung utilizes passive diffusion throughout the alveolar parenchyma rather than direct perfusion as a mechanism for aerobic metabolism. As a result, the lung has an extended period of viability following circulatory arrest [28–30]. In several centers, this method of organ procurement has been adopted into their clinical programs. There are limited published survival results, but most series suggest comparable or slightly better results at time points in the first 2 years after transplant [31–34]. A recent report raised concern for the diminished survival and freedom from bronchiolitis obliterans syndrome (BOS) in this group compared to the traditional group and therefore additional medium- and long-term studies are needed [35].

Ex vivo perfusion/resuscitation. Ex vivo lung perfusion (EVLP) represents another modality directed at expanding the donor pool. Lungs with traumatic injury, pulmonary edema, or infection can be evaluated for likelihood of the reversibility of the injury pattern and initiation of therapeutic interventions for potential salvaging and utilization. The technique has been used in selected centers in Europe and North America in both the experimental and clinical settings. Studies by Steen and colleagues in Sweden and Cypel and colleagues in Toronto utilize continuous anterograde perfusion with a hyperoncotic acellular preservation solution and ventilation of the lungs by ventilator at a normothermic setting [36, 37]. Deoxygenation of the perfusate is manipulated by an oxygenator containing hypoxic gas. The technique allows lung perfusion for up to 12 h. Recent studies by the Toronto group have also demonstrated the utility of gene therapy with adenovirus vector encoding human interleukin-10 to reverse the pro-inflammatory milieu of the injured lung [38]. Only a few limited long-term results have been reported in the literature, but they show similar outcomes in DCDD with EVLP compared to transplant following brain death [39–41].

Molecular evaluation of the donor. One of the early complications after transplantation is the development of primary graft failure (PGF). Its severity is variable, but it can lead to increased morbidity and mortality. Although some risk factors have been enumerated, there are no specific biomarkers. Recently, molecular techniques have been implemented and show promise for future clinical application. Kaneda and colleagues [42] investigated cytokine mRMA expression from lung biopsy samples obtained before implantation of the allograft. A step-wise logistic regression analysis for 30-day mortality found that the ratio of IL-6/IL-10 was most predictive ($p = 0.0013$). Using a gene expression profile technique, Anraku and colleagues [43] found that the overexpression of four genes (*ATP11B, FGFR2, EGLN1*, and *MCPH1*) was predictive for the development of PGF. In another study

from the Toronto group [44], lung allografts with low surfactant protein-A mRNA expression prior to implantation exhibited reduced survival. In their most recent molecular work, pre-implant-elevated levels of IL-6 mRNA correlated with the subsequent development of the BOS component of chronic lung allograft dysfunction (CLAD) [45]. Other molecular approaches to the lung allograft have identified innate immune and inflammatory pathways that may be responsible for post-transplant parenchymal injury [46, 47]. Further studies will be needed to confirm the predictive and prognostic utility of these molecular studies.

Selected Recipient-Related Issues

The ISHLT recently updated the guidelines for the selection and evaluation of patients for lung transplantation [48–50]. In addition to general criteria for candidate consideration such as the high risk of death from lung disease within 2 years and the high likelihood of survival for at least 3 months after transplant, the guidelines enumerate both absolute and relative contraindications to transplantation. As with all guidelines, these recommendations are based on current "state-of-the-art" and will likely undergo further revision with the emergence of new insights [51]. Special emphasis is placed on the indications for "bridging to transplant" using modalities such as mechanical ventilation, extracorporeal membrane oxygenation (ECMO), and other forms of extracorporeal life support systems [52–54].

As previously discussed, the **common indications for lung transplant in adults include ILD, COPD, CF, and pulmonary vascular disease**. A comprehensive evaluation must include not only the timing for the patient referral and the timing of listing after the evaluation, but also the likelihood of recovery and projected survival for 5 or more years. There are important issues in each of these categories for which the pathologist and the laboratory play an essential role. These will be briefly highlighted in the next section.

ILD. As previously discussed, the implementation of LAS system in the US and Eurotransplant programs has led to a sharp increase in the rates of lung transplants undertaken for ILD. There are unique issues related to ILD and the timing of the referral for transplant evaluation and the timing of listing for transplant. Many, but not all, patients will have had video-assisted thoracoscopic surgery (VATS) biopsies as part of their diagnostic work-up and it is important to have the biopsy reviewed for accurate classification. There is a difference in the rate of progression and prognosis of usual interstitial pneumonia (UIP) and NSIP. With the introduction of pirfenidone in the treatment of IPF, the pace of the disease as demonstrated by lung function, exercise tolerance, and survival has been altered [55]. Further, the diagnosis of chronic hypersensitivity pneumonitis should warrant a careful occupational, drug, and environmental exposure history to eliminate the risk of disease recurrence in the allograft. In some cases, the diagnosis of ILD is associated with a collagen vascular disorder and patients with scleroderma-associated ILD should be carefully evaluated for concurrent pulmonary hypertension or malignancy.

CF is a common indication for transplant in children, adolescents, and adults. It is a progressive disease characterized by repeated episodes of infection and may be complicated by acute respiratory failure, development of antibiotic resistance, pneumothorax, hemoptysis, and general nutritional decline. In general, these patients are not biopsied prior to transplant, but they are susceptible to two important infectious complications. There is an increased risk for non-tuberculous mycobacterial (NTM) infections such as *M. abscessus* [56]. Patients with culture-positive NTM are aggressively treated with antibiotic therapy. The presence of progressive pulmonary NTM despite medical therapy or extrapulmonary NTM is considered to be a contraindication to transplant by most programs.

Another infectious complication in CF patients is *Burkholderia cepacia* complex. It is investigated as part of the transplant evaluation. It is recognized as a relative contraindication to transplant, but many programs do not transplant this group of patients on account of the high rate of recurrence after transplant and resistance to

antibiotic therapy. These patients have poorer outcomes after transplant [57]. There are, however, selected centers with experience in the treatment of *B. cepacia* complex that will accept these patients for transplant.

COPD is another slowly progressive disease that has unique issues related to the timing of evaluation and listing for transplant. Frequent, severe clinical exacerbations, severely impaired PFTs, and the development of moderate or severe pulmonary hypertension are some of the indications for listing patients for transplant [50]. Patients with bullous disease may be candidates for lung-volume reduction surgery (LVRS) that may temporize the decline in pulmonary function. The surgical specimens should be carefully evaluated for smoking-related disorders such as pulmonary Langerhans cell histiocytosis, granulomatous infection, and pulmonary neoplasia as they can have potential consequences in the transplant setting [58].

Pulmonary vascular disease. The introduction of effective medical therapy for the treatment of pulmonary arterial hypertension has affected the timing for transplant referral and subsequent listing of patients. Over the course of the last two decades, the percentage of patients transplanted for IPAH has dropped from 11.8 % in 1991 to 2.7 % in 2011. Patients with IPAH and hypertension associated with other conditions (WHO Groups I and III) benefit from the prostanoids, endothelin receptor antagonists, and phosphodiesterase inhibitors [59]. Lung biopsies are rarely performed in these patients. The accurate classification into one or more pathologic categories such as pulmonary arteriopathy, pulmonary occlusive venulopathy, and pulmonary microvasculopathy is generally reserved for the explanted lung specimens [60]. There are case reports of recurrent pulmonary microvasculopathy (formerly pulmonary capillary hemangiomatosis) following lung transplant [61].

Multi-organ transplant. Over the last decade, the indications for multi-organ transplant besides combined heart–lung transplant have also expanded. Concurrent thoracic and abdominal transplants are performed on carefully selected patients in specialized centers. Wolf and colleagues recently reviewed the accumulated multicenter experience and identified 42 combined lung–liver (Lu–Li) transplants and 18 simultaneous lung–kidney (Lu–Ki) through 2010. Of the patients listed for Lu–Li transplant, CF and pulmonary hypertension accounted for the vast majority of lung disorders, while ILD and pulmonary hypertension were responsible for most of the pulmonary disorders in the Lu–Ki group [62]. Interestingly, the outcome for the two groups was similar to thoracic transplant patients in general (and slightly worse than their abdominal transplant counterparts). The pathologist is responsible for the careful histopathologic examination of each organ and should follow the guidelines for the pathologic examination of the explanted organ for each component.

Lung transplant for pulmonary adenocarcinoma. Patients undergoing lung transplant for COPD, ILD, and collagen vascular disorders such as scleroderma have an increased risk for bronchogenic carcinoma in the native lung. In most cases, these are discovered incidentally at the time of transplant during the pathologic examination of the explant [63, 64]. The prognosis depends on tumor stage. With the interest in extended donor and recipient criteria such as older age and light smoking history, the incidence of occult carcinomas is likely to expand. Other clinical scenarios for lung cancer in the transplant setting include lung cancer arising in the native lung after transplant and bronchogenic carcinoma occurring in the transplanted lung. An equally unique but controversial group is the cohort of patients transplanted specifically for pulmonary adenocarcinoma [65–70]. Prior to the recently adopted multidisciplinary classification of lung adenocarcinoma, these tumors were all grouped as "bronchioloalveolar carcinoma" or "BAC." In the new scheme, tumors formerly called BAC are now classified into nonmucinous lesions [adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), lepidic predominant adenocarcinoma (LPA)] and mucinous adenocarcinoma (Fig. 2). The majority

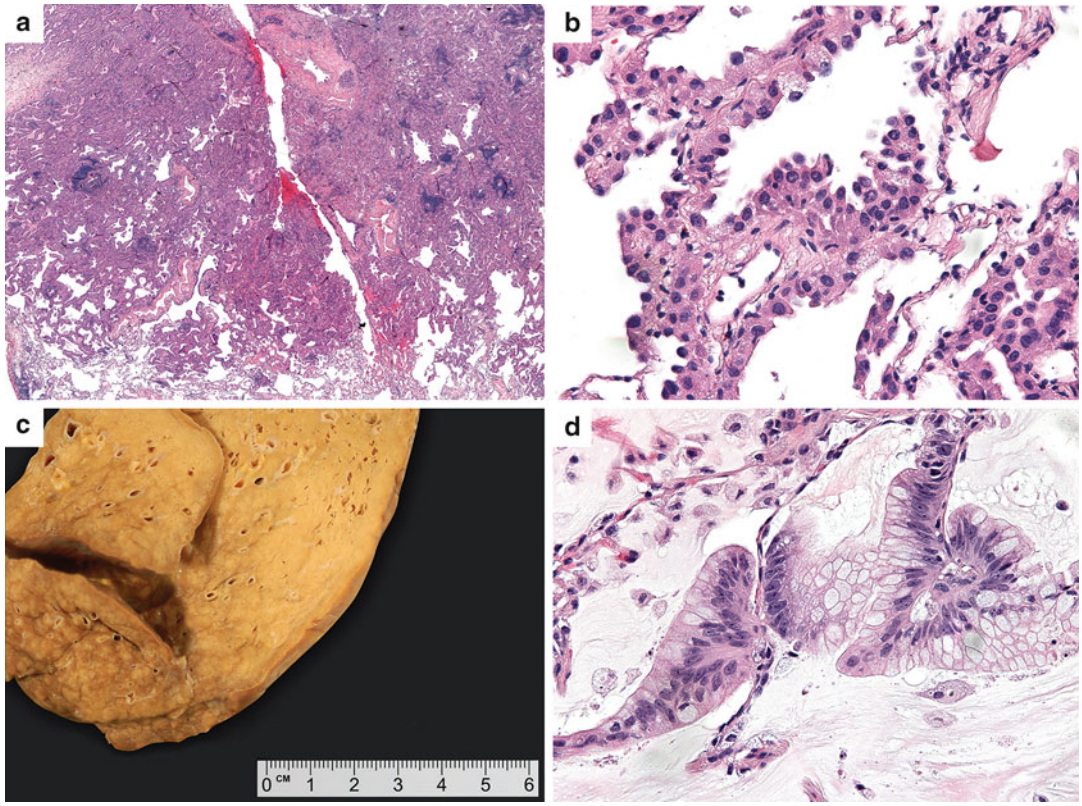

Fig. 2 (**a**) Predominantly lepidic adenocarcinoma at low magnification. (**b**) High power magnification showing enlarged, hyperchromatic neoplastic cells populating alveolar septa. (**c**) Gross photograph of axial section of lung from a patient with mucinous adenocarcinoma of the lung. The entire lobe was replaced by lepidic-predominant adenocarcinoma. (**d**) High power magnification showing neoplastic mucinous cells lining preexisting alveolar structures and foamy macrophages and mucin within the airspaces

of mucinous adenocarcinomas are thought to be invasive based on one or more of the current diagnostic criteria: size (>3 cm), amount of invasion (>0.5 cm), multiple nodules, or lack of a circumscribed border with miliary spread into the adjacent lung tissue [71]. In a recent review of the topic, Kachala and Murthy identify a total of 70 cases: two of the larger series represent a multicenter collection of data collected by the ISHLT Registry or the United Network for Organ Sharing (UNOS) database, and most of the reported studies are case reports or small case series [69, 70, 72]. None of the reports had rigorous application of the new nomenclature and applied terminology such as "diffuse" or "multifocal BAC." Nonetheless, there are important points that should be emphasized from the studies. Firstly, although lung transplantation may be considered for highly selected patients with AIS and MIA, there is a significant risk of local recurrence. De Perrot et al. reported recurrences in 13 of the 22 patients who survived the procedure ranging from 5 to 49 month; overall 5-year survival in the group was inferior to the lung transplant group (39% versus 53%) [69]. Bilateral lung transplant may be a superior option to limit recurrences. Secondly, the diagnosis must be established by surgical excision and not a small biopsy to firmly establish the pathologic classification (AIS versus MIA versus PLA). Interestingly, many of the transplant recipients showed a mucinous pattern that would be classified as invasive adenocarcinoma in the current scheme. Thirdly, all patients should be carefully staged to exclude mediastinal nodal involvement. Finally, transplantation should be considered after failure of conventional medical treatment. EGFR mutations can be

demonstrated in a minority of young, non-smokers with LPA and this group may benefit from tyrosine kinase inhibitors [73].

The Pathologist's Role in Lung Transplantation

The pathologist plays a critical role in the management of transplant recipients and is a key member of the multidisciplinary team (Table 1). This begins with the confirmation of the primary lung disease if a biopsy has been previously performed. Generally, this is limited to the surfactant deficiencies in the infant group and ILD in the adult group. The explanted lung specimen should be carefully evaluated to establish the primary diagnosis and to exclude secondary complications such as infections or occult malignancies [74, 75]. In the setting of pulmonary vascular disease, the distinction of IPAH from chronic recurrent thromboembolic disease (CRPE) is important as patients with CRPE are at risk for recurrent injury to the allograft. Patients with COPD can harbor occult malignancies such as bronchogenic carcinomas or other smoking-related lesions.

In the **perioperative and early postoperative period,** PGF must be distinguished from hyperacute rejection (HAR), severe ACR, and infection, as these will have different therapies and potential outcomes. In the later post-transplant setting, the pathologist is responsible for the diagnosis and grading of ACR. As we will discuss in more detail, ACR is a diagnosis of exclusion and a variety of morphological mimics need to be eliminated. Following the institution of augmented immunosuppressive therapy for the treatment of allograft rejection, follow-up biopsies are usually performed to determine treatment effect. Antibody-mediated rejection (AMR) has emerged as a clinicopathologic entity and a multidisciplinary approach for diagnosis and treatment is required.

The diagnosis of chronic airway rejection by transbronchial biopsy (TBBx) can be problematic on account of sampling issues. In the setting of diminishing pulmonary function after transplant, careful examination of the small airways is essential to identify obliterative bronchiolitis (OB). Less common findings include post-transplant lymphoproliferative disease (PTLD) and recurrence of the primary graft disease. Finally, the important role of the postmortem

Table 1 Role of the pathologist in lung transplantation

1.	Effective member of multidisciplinary team; openly communicate with clinicians and surgeons
2.	Establish pathologic diagnosis on transbronchial or open lung/video-assisted thoracoscopic specimens from native lung prior to transplant
3.	Thoroughly evaluate explanted lung(s) to confirm primary pathological diagnosis and identify additional lung lesions such as infection or occult malignancy
4.	Identify etiology of early graft dysfunction such as diffuse primary graft dysfunction/ischemia-reperfusion injury, hyperacute rejection, infection, anastomotic complications in post-transplant biopsy
5.	Diagnosis and grade acute cellular rejection using ISHLT criteria and exclude morphological mimics. Determine efficacy of anti-rejection or anti-infection therapy in follow-up biopsy specimens
6.	Identify histopathologic findings described in antibody-mediated rejection such as neutrophilic margination, neutrophilic capillaritis, or acute lung injury pattern
7.	Identify other morphologic causes of graft dysfunction such as aspiration pneumonia, drug toxicity, and infection
8.	Identify the presence of obliterative bronchiolitis in late biopsy specimens
9.	Diagnose and classify post-transplant lymphoproliferative disorder (PTLD)
10.	Establish recurrence of primary parenchymal diseases in the allograft such as sarcoidosis, LAM, hypersensitivity pneumonitis.
11.	Preserve tissue or bronchoalveolar lavage specimens for research protocols

examination for its clinical and educational functions must be emphasized. We attempt to obtain permission for autopsy evaluation of every deceased thoracic transplant recipient and discuss in detail the clinical and pathological findings in a multidisciplinary setting. It is not uncommon to find clinically significant missed diagnoses in postmortem studies. Akindipe and colleagues found a discrepancy between the autopsy findings and clinically suspected cause of death in 20 % of cases: the most common overlooked diagnoses were HAR, myocardial infarction, pulmonary embolism, high grade ACR, and disseminated fungal infection [76].

Pathologic Assessment of the Lung Explant

As discussed previously, there are a variety of indications for transplantation in the pediatric and adult populations. While we generally use a similar approach for handling explanted specimens, it is important to review the electronic clinical records and discuss unusual or uncommon diagnoses with the clinicians prior to the gross dissection. Specimen photography can be a helpful adjunct in the evaluation. The lungs are individually weighed and measured and any missing segments or lobes are noted. The lung explant is usually received in the fresh state and is inflated through the bronchus with 10 % neutral-buffered formalin or other standard fixative under slightly elevated passive pressures. We allow overnight fixation. The pleural surface is examined for adhesions, disruptions, cobblestone change, scars, or retractions. Following retrieval of hilar lymph nodes and an en-face section of the proximal bronchovascular margins, we cut the lung in an axial plane to allow correlation with CT imaging. Sectioning along a sagittal plane is alternative approach for cases of pulmonary hypertension as it enhances the gross alterations of the vasculature. Historically, specimen angiograms or bronchograms were performed primarily as a research protocol [77]. We recommend at least one section per lobe, all hilar and peribronchial lymph nodes and multiple sections of any gross abnormality found on examination. We routinely perform a silver stain such as a Gomori-methanamine-silver (GMS) to exclude fungal airway colonization or active infection. Additional histochemical stains such as elastin and collagen stains for connective tissue or vascular evaluation or stains for microorganisms are obtained on a case-by-case basis.

Pathologic Assessment of the Allograft at Postmortem or Retransplantation

The gross examination of retransplant and postmortem specimens is similar to the method described in the previous section. In addition to infection and malignancy, the explant is carefully examined for transplant-related changes including ACR, AMR, airway alterations including lymphocytic bronchiolitis (LB) and OB, and parenchymal changes of restrictive allograft syndrome (RAS). We generally submit multiple tissue sections from each transplanted lobe, as lesions can be patchy in distribution. Liberal use of connective tissue stains to evaluate airways for submucosal fibrous thickening and the vessels for transplant vasculopathy is essential. Further, histochemical staining for fungal and mycobacterial organisms and immunohistochemical staining for viral and parasitic inclusions are routinely performed. The histopathologic assessment of AMR is currently done by either immunohistochemistry on formalin-fixed paraffin-embedded sections or by immunofluorescence on fresh-frozen tissue samples. The diagnostic criteria will be discussed in detail in another section.

Diagnostic Techniques for Monitoring the Pulmonary Allograft

The post-transplant assessment and monitoring of recipients requires a comprehensive multidisciplinary approach. Not surprisingly, these patients are challenging and often have multiple interrelated clinical problems. Frequent clinic

visits, serial radiological imaging with radiographs and CT scans, daily spirometric measurements, interval pulmonary function studies, protocol fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) and TBBx, microbiological assessments, nutritional evaluations, measurement of immunosuppressive drug levels, and psychosocial assessments are all part of the complex postoperative management to detect changes in graft function, support patient compliance, augment quality of life, and other critical issues. The use of noninvasive techniques such as gene expression profiling and circulating cell-free donor-derived DNA (cfdDNA) to monitor the immunological status of cardiac transplant recipients has been used by some centers, but many of these techniques remain investigative at this time [78–81].

Transbronchial Biopsy and Cryobiopsy

In many centers, surveillance fiberoptic bronchoscopy with BAL and TBBx is a critical component of patient management and the topic was recently reviewed in detail by Glanville [82]. In some centers, asymptomatic patients are followed without protocol TBBx sampling and they reserve the biopsy for clinical indications such as new onset of symptoms or decline in pulmonary function [83, 84]. The goal of **surveillance biopsy** is the identification of treatable processes such as infection or acute rejection before allograft dysfunction develops, to identify potential risk factors for chronic airway rejection and ultimately to delay or prevent OB. This stems in large part from the poor sensitivity and specificity of clinical signs and symptoms, radiological techniques and functional techniques in distinguishing acute rejection, infection, and airway anastomotic complications. As a result, the TBBx is widely regarded as the "gold standard" for the evaluation of the pulmonary allograft. TBBx is a safe, invasive procedure in experienced hands, but there are potential serious complications such as bleeding and pneumothorax [85]. There are also technical and interpretative issues. Many of the pathologic processes affecting the allograft are patchy in distribution and may be "missed" by TBBx; these include ACR, LB, and OB. The criteria for the diagnosis and reporting of ACR and other forms of allograft rejection were established by the ISHLT and have undergone a series of modifications and revisions by the Lung Rejection Study Group in 1996 and 2007 [86–88]. Arcosoy et al. [89] recently reported the interobserver variability for the grading of ACR; a central panel of transplant pathologists reviewed over 1500 biopsies from 845 patients performed at 20 transplant centers. The kappa value for interobserver agreement was 0.183. Cases were upgraded from no rejection to ACR in 9 % of cases, downgraded from treatable rejection categories to no rejection or low-grade rejection in 35 % of cases, and cases of low-grade rejection were downgraded to no rejection in 36 % of cases and upgraded to treatable rejection in 19 % (95 % CI 0.147–0.220). In many cases, the biopsies were deemed "ungradeable" using the ISHLT criteria. In another interobserver study of 59 biopsies for ACR, only moderate agreement was shown between pathologists (kappa 0.470). There was less robust agreement for the diagnosis of either airway inflammation or OB. Excellent intraobserver agreement, however, was found (kappa 0.795) [90]. In addition to the interpretative challenges, there are a number of technical issues. Tissue atelectasis and artifactual distortion are found to varying degrees with every TBBx sample. Gentle agitation of the biopsy pieces by a swirling motion of the formalin container can reduce atelectasis. The liberal use of leveled sections and connective tissue stains may resolve the problem of crush artefactual distortion and render a biopsy fragment interpretable in some cases.

The Lung Rejection Study Group of the ISHLT proposed recommendations for tissue handling and processing in the original grading scheme and these have been reiterated in the subsequent revisions. Specifically, a minimum of five alveolated pieces that are not completely collapsed should be obtained and immediately fixed in a standard fixative such as 10 % neutral buffered formalin. Other fixatives such as B5 or

Bouin's may interfere with immunohistochemistry or other ancillary studies and each lab should carefully establish their optimal thresholds. In the 1990 and 1996 versions, it was recommended that each piece should contain bronchioles and greater than 100 air sacs, but this specific requirement was omitted from the 2007 document [86–88]. Additional pieces may be necessary if focal processes such as parenchymal nodules or OB are the primary diagnostic considerations. Further, centers that utilize IF staining for the diagnosis of AMR will require one or more additional tissue pieces in saline or another appropriate fixative before snap freezing. Electron microscopy has no role in routine transplant biopsy evaluation, but may be part of research protocols. It is important to emphasize that frequently five pieces alone will not be sufficient as one or more piece is composed of entirely of airway wall, strips of airway mucosa, blood vessel, or thrombus and so generous sampling is encouraged.

The transbronchial cryobiopsy (TCBx) was recently introduced into clinical practice as part of the multidisciplinary approach to the diagnosis of ILD. Among its advantages are larger pieces of lung tissue without procedure-related hemorrhage or atelectasis. Parajes reported a larger mean area of tissue by cryoprobe (14.7 ± 11 mm^2) compared to conventional forceps (3.3 ± 4.1 mm^2) ($P < 0.001$) [91]. There are limited published data of TCBx in the lung transplant setting, but early studies suggest a potential role for the procedure with the advantage of larger tissue volume and more airways for evaluation [92, 93] (Fig. 3).

The histological assessment of transplant pathology requires optimum handling and processing. Overnight processing in an automated processor is optimal, but a variety of rapid processing programs are available for handling emergent biopsies or **clinically indicated biopsies** that yield slides in 2–3 h. Following embedding in paraffin wax, a minimum of three "leveled" sections, each with multiple ribbons, are prepared at 4–5 μm thickness and routinely stained with hematoxylin–eosin (H&E). A connective tissue stain such as Masson's trichrome and/or an elastic stain is helpful for assessing airway and vascular integrity. We still routinely perform a silver stains such as GMS for fungal organisms, but some centers prefer other microbiological, serological, or molecular methods. Additional histochemical stains, immunohistochemistry, and molecular techniques are advocated on a case-by-case basis, e.g., viral infections such as cytomegalovirus (CMV) or for the diagnosis and classification of PTLD. Some centers alter the method of slide preparation and obtain seven levels, then stain levels 1, 4, and 7 with H&E, one with elastic trichrome (level 3), 1 with GMS (level 6), and 2 are left unstained for additional stains as necessary [94].

Bronchoalveolar Lavage

In many centers, BAL is used in conjunction with TBBx. It can provide rapid assessment of infection in a patient with clinical deterioration. The specific methodologies differ among institutions, but generally small aliquots of normal saline are instilled in the airways and then aspirated by manual or mechanical suction. Fractions of the fluid are sent for microbiological testing, cytopathologic evaluation, and for cell count and differential quantitation. The exclusion of bacterial or fungal infection is important in the early postoperative period and in patients with chronic airway rejection. Viral organisms can be seen on routine Papanicolaou or May-Grünwald-Giemsa-stained preparations and fungal organisms can be visualized with fungal stains such as GMS or predigested PAS (Fig. 4). Some centers analyze the functional characteristics of the cells retrieved from the lavage as an adjunct test for infection and acute and chronic rejection [95]. On occasion, ACR can present with increased levels of eosinophils in the BAL and may predict poorer outcomes [96, 97]. BAL characteristics such as the predominance of lymphocytes or neutrophils, the CD4:CD8 ratio, the mean percentage of neutrophils, and lavage levels of myeloperoxidase have been analyzed as predictors of patient outcomes and in patients with BOS [98, 99].

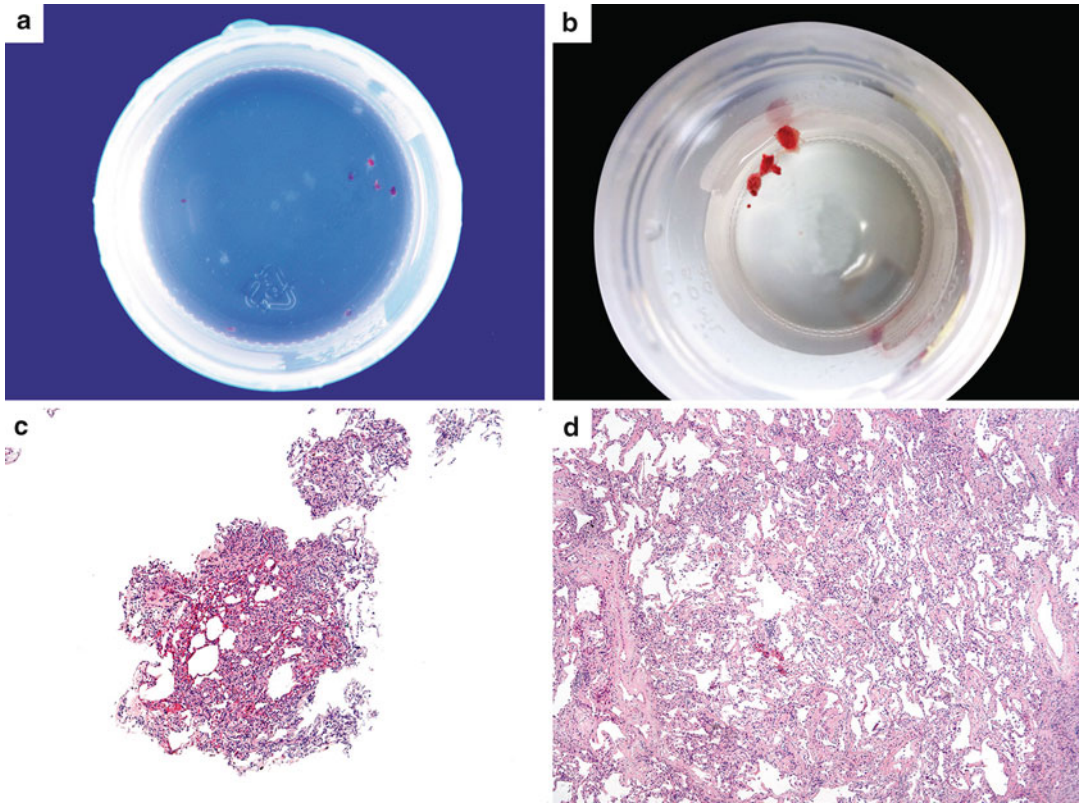

Fig. 3 (**a**) Transbronchial biopsy specimen composed of multiple small pieces measuring 1–2 mm in diameter. (**b**) Transbronchial cryobiopsy consisting of 3 large pieces of tissue, each 3–4 mm in diameter. (**c**) Transbronchial biopsy specimen at 40× magnification. (**d**) Transbronchial cryobiopsy also at 40× magnification exhibiting at least 3× the number of alveolar spaces required for morphologic evaluation

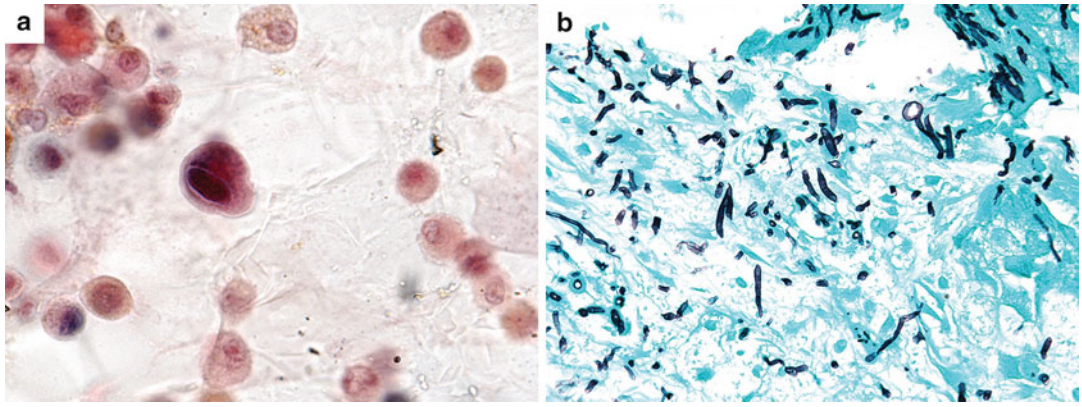

Fig. 4 (**a**) Papanicolaou stain of CMV inclusions in BAL preparation. (**b**) *Aspergillus* organisms in BAL preparation

Open Lung Biopsy or Video-Assisted Lung Biopsy

The role of open lung biopsy (OLB) and VATS biopsy in the management of transplant recipients has been controversial. Historically, concern for persistent air-leaks or bronchopleural fistulas limited its indication to seriously ill patients who had failed anti-rejection or anti-infection therapy or who suffered from multiple suspected concurrent processes such as infection and PTLD. A limited number of studies published in the pediatric and adult transplant populations have shown similar findings [100–103]. The majority of patients underwent OLB by way of a mini-thoracotomy incision and the biopsies were usually performed in the early or late transplant periods. The most common indications were unexplained deteriorating pulmonary dysfunction after thorough clinical, serological, and bronchoscopic evaluation or the onset of new or persistent pulmonary infiltrates or nodules. In 30–70 % of cases, a new diagnosis requiring therapeutic intervention or confirmation of the clinically suspected diagnosis was achieved including acute rejection, OB, organizing pneumonia pattern, infection, and malignancy.

The procedure was found to be most helpful in the early postoperative period to distinguish rejection from infection. There is a role for touch imprints stained with Diff-Quik (Dade-Behring, Newark, NJ) and frozen section examination to provide an initial impression of infection versus rejection versus PTLD and to direct the handling of tissue for special studies such as cytogenetics and molecular analysis. Tissue should always be sent for microbiological cultures. We gently inflate the wedge biopsy specimen with formalin using a 25- or 27-guage needle (Fig. 5). This promotes tissue expansion of the airspaces and airways and prevents tissue atelectasis and architectural distortion. Following adequate fixation time of at least 3 h, the wedge is thinly sectioned and submitted for overnight processing. Complications were reported in 5–25 % of cases and ranged from minor issues such as wound infection, postoperative pain, and prolonged air-leaks to more serious problems such as respiratory failure and intra-thoracic bleeding requiring surgical re-exploration. Importantly, resolution of air-leaks occurred in all patients, although the course was protracted in some cases. The results of these small numbers of studies and our own experience support the use of VATS biopsy for specific clinical indications.

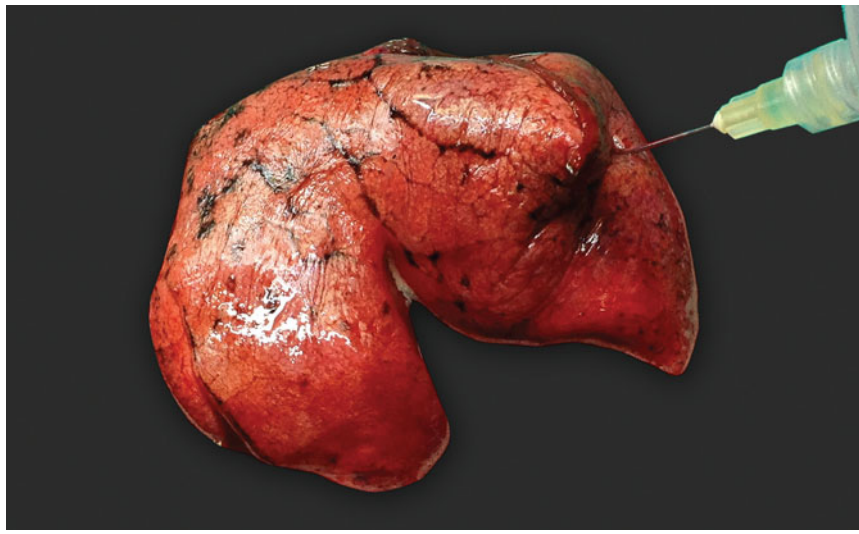

Fig. 5 Inflation of VATS biopsy specimen with 25-guage needle

A Temporal Approach to Lung Transplant Pathology

Akin to the clinical challenges that transplant clinicians face in managing these patients, pathologists recognize that there are a sundry of pathologic processes that can develop in the lung allograft. Fortunately, many of these issues occur within a reasonably narrow temporal framework. PGF and its morphological correlate of diffuse alveolar damage (DAD) occur in the early postoperative period. Acute rejection is uncommon during this early period and tends to develop within the first 3–6 months. OB is now uncommon in the first 6 months and typically presents after 1 year. We arbitrarily divide the pathological changes along various time points, but recognize that some disorders such as ACR, AMR, infection, or PTLD can occur anytime following transplantation (Table 2).

Table 2 Temporal paradigm for lung transplant pathology

1. Perioperative and early post-transplant period (up to 1 month)
Primary graft dysfunction/failure
Hyperacute rejection
Vascular or airway anastomic complications
Infection
2. Intermediate complications (1 month–1 year)
Acute cellular rejection
Airway inflammation
Antibody-mediated rejection
Infection
Post-transplant lymphoproliferative disorder
Drug toxicity
Aspiration-related changes
3. Late complications (after 1 year)
Obliterative bronchiolitis
Restrictive allograft syndrome
Neutrophilic-reversible allograft dysfunction
Transplant-associated vasculopathy
Post-transplant lymphoproliferative disorder
Recurrence of primary lung disease

Perioperative and Early Post-transplant Period (Up to 1 Month)

There is an overlap of clinical and radiologic features for alterations presenting within the first few days or weeks after transplant. These **include HAR, PGD, pulmonary venous obstruction, acute left ventricular dysfunction, and overwhelming pulmonary infection with sepsis.** The etiology, treatment, and prognosis differ substantially and prompt and accurate diagnosis is essential. Fortunately, other than PGD, most of the disorders in this group are uncommon nowadays.

Hyperacute Rejection

HAR is a rare, catastrophic complication presenting shortly after revascularization of the allograft. The presence of preformed circulating anti-HLA Type I or II or anti-ABO antibody against donor antigen triggers the complement system resulting in acute clinical dysfunction and morphological alterations. Currently, a handful of case reports have been published detailing the pathological changes [104–111]. Clinically, patients present with abrupt onset of respiratory failure with a sharp increase in mean pulmonary artery and airway pressures, release of copious amounts of bloody, frothy fluid into the airways, dramatic decline in pulmonary compliance, and a drop in both systemic blood pressure and arterial oxygenation. Radiological changes rapidly progress to "whiteout" of the transplanted lung. Of the eight cases detailed in the literature, all but one were female, six occurred in patients with emphysema, five had negative pretransplant studies for panel reactive antibodies (PRA), and three patients had elevated PRA. Death occurred in six patients despite aggressive intervention with plasmapheresis and potent immunosuppressive drugs. Currently, the primary goal is prevention of HAR by identifying patients at high risk, e.g., prior pregnancies, transfusions or transplantation, or underlying connective tissue disorders, and depletion of PRA by plasmapheresis or intravenous immunoglobulin (IVIg) prior to transplant in presensitized patients.

The lung in HAR is heavy, firm in consistency, and purplish-red in color. Microscopically, the changes range from florid pulmonary edema with fibrinous exudates in the airspaces, to conspicuous neutrophilic infiltrates within the septal walls with necrosis (acute capillaritis) and/or flooding of the airspaces with blood and neutrophils to classic DAD with hyaline membrane formation and endothelial and epithelial injury (Fig. 6). In the reported cases, platelet-fibrin thrombi in the capillaries and small vessels were inconspicuous or absent. Deposits of immunoglobulin or C4d in the interstitial septal capillaries have been reported.

Primary Graft Dysfunction

PGD is currently defined by the ISHLT Working Group as acute lung injury (ALI) that occurs within the first 72 h after transplant [112–114]. It affects 5–25 % of lung allografts and has previously been termed ischemia-reperfusion injury, reimplantation response and edema, reperfusion edema, early graft dysfunction, post-transplant acute respiratory distress syndrome (ARDS), and PGF. It exhibits both a clinical and morphological spectrum and the severe form is characterized by acute hypoxemic respiratory failure, diffuse pulmonary infiltrates on radiographs, and DAD in lung biopsy specimens. There is a clinical grading scheme (grades 0–3) for PGD severity based on the partial pressure of oxygen-to-the fraction of inspired oxygen ratio (PaO_2:FiO_2) and the presence or absence of radiological infiltrates at varying time points (0–6, 24, 48, and 72 h.) after transplant [112]. PGD, especially Grade 3 PGD, has a significant impact on mortality in the first 30-days and is a risk factor for long-term functional impairment and for the development of chronic allograft rejection in the form of histomorphologic lesion of OB or its clinical correlate of BOS [114–118].

Over the last decade, a number of potential molecular and genetic markers for PGD have emerged and may be helpful in assessing risk stratification before transplant [119–127]. Moreover, there are specific donor, recipient, and operative variables that promote a clinical predisposition to PGD [114, 115, 118]. Donor risk factors include both inherent and acquired variables such as older or very young donors, African-American race, female gender, history of smoking, prolonged mechanical ventilation, aspiration episodes, head trauma, and hemodynamic instability after brain death. The list of recipient risk factors includes patients with IPAH, elevated pulmonary artery pressures at transplant, obesity,

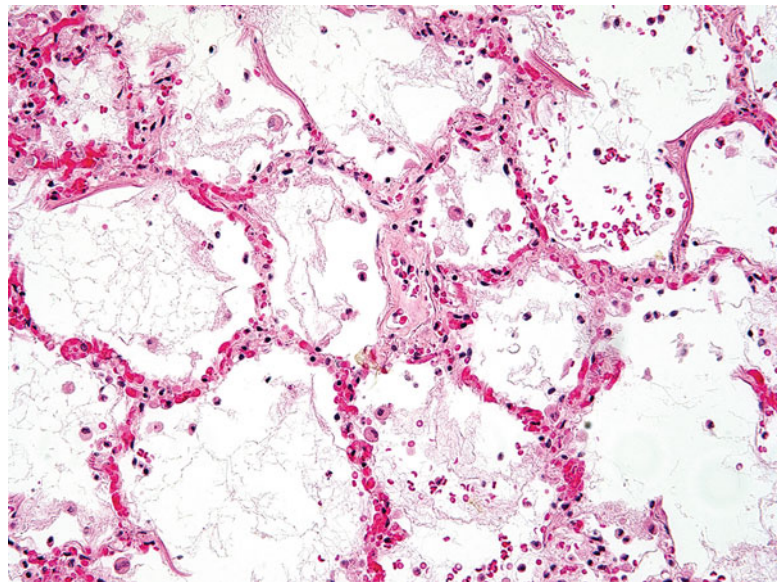

Fig. 6 Hyperacute rejection in patient who developed immediate graft dysfunction following completion of the lung transplant. Edema and scattered interstitial capillary neutrophils indicate early graft injury

and patients with diffuse parenchymal lung disease such as sarcoidosis. Prolonged ischemic time, the need for cardiopulmonary bypass, single lung procedures, high FIO_2 requirements at reperfusion, and excessive blood product administration are recognized operative risk variables. The pathogenesis is complicated and multifactorial, but involves free-radical formation, a host of cytokines, and the innate immune response. Treatment is directed at supportive measures, fluid and electrolyte balances, and prevention of infection and other complications. The clinical differential diagnosis includes HAR, cardiogenic pulmonary edema, venous anastomotic complications/obstruction, and infectious pneumonia, especially viropathic and bacterial types.

There is a morphological spectrum of PGD changes in transbronchial and OLBs. In its mild formulation, neutrophilic infiltration or sequestration of the alveolar septa, patchy alveolar fibrinous aggregates, and increased pulmonary macrophages are observed. At the severe end of PGD proliferative or organizing phase of DAD with fibroblastic foci, scattered residual hyaline membranes, sparse interstitial inflammation but widened, edematous septa with fibroblastic aggregates can be present (Fig. 7). The differential diagnosis includes HAR, vascular anastomotic problems with intravascular thrombi, infection, and severe ACR. We routinely perform staining for bacterial and fungal organisms and immunohistochemical staining for viropathic organisms and AMR in this setting and microbiological cultures and serologies (such as viral and donor-specific antibody (DSA)) are initiated.

Infections in the Early Post-transplant Period

As with anastomotic complications, a variety of donor, recipient, and operative and perioperative factors place the newly transplanted patient at risk for infection and sepsis [128, 129]. The intrinsic pulmonary components of lymphatic circulation, ciliary motility, mucous clearance, and neural connections are all disrupted and, together with ischemic injury in the proximal airways, can lead to breakdown of local defense mechanisms. Following transplantation extended ventilatory requirements, line placements, nutritional problems, and diminished ambulation contribute to the development of nosocomial infections. Likewise, recipient factors such as paranasal sinus and airway bacterial colonization in CF patients, smoking history, older age, nutritional and functional deconditioning, and the intense level of immunosuppression after transplant are significant risk factors. The evaluation of potential donors and recipients includes serologic screening for CMV, Epstein–Barr virus (EBV), varicella-zoster, human immunodeficiency virus (HIV), hepatitis B and C. A segment of the distal donor trachea or unused segment of

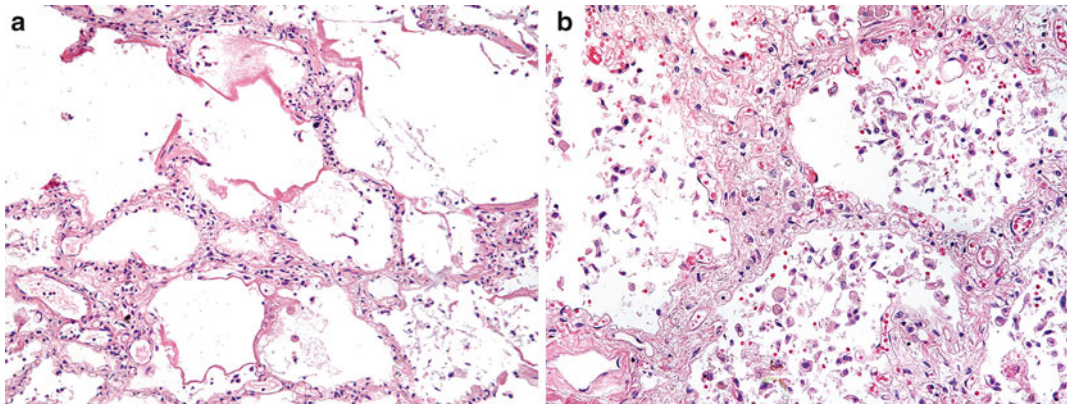

Fig. 7 (**a**) Severe primary graft dysfunction characterized by the exudative phase of diffuse alveolar damage with hyaline membranes. (**b**) High power magnification showing edematous, widened alveolar septa, sloughed epithelial lining cells, and intra-alveolar macrophages

bronchus is submitted for microbiologic culture. The most common pulmonary infections are bacterial infections and, specifically, nosocomial infections. Campos and colleagues [130] reported *Pseudomonas* and *Staphylococcus* as the most common organisms during this period. Nosocomial fungal infections also occur with Candida species making up the majority of cases. Viral, mycobacterial, and parasitic infections are uncommon in this period. Rare donor-derived viral infections that have been reported are HSV in the absence of prophylaxis, lymphocytic choriomeningitis virus, rhabdovirus (rabies), and West Nile virus [131–135].

The incidence of overwhelming infection/sepsis after transplant has greatly diminished over the last two decades. Careful patient (donor and recipient) selection and evaluation, judicious use of antimicrobial prophylaxis, and intensive post-transplant care are largely responsible. Fiberoptic bronchoscopy with BAL and culture are used to establish the diagnosis of most bacterial infections. The typical pattern of acute bronchopneumonia is seen in tissue specimens (Fig. 8). Because of the overlap of features with HAR, careful clinical correlation and histochemical and immunohistochemical staining may be warranted. The diagnosis of rare viral infections require sophisticated molecular testing, while more common viruses like HSV and CMV have characteristic cytopathic features.

Vascular and Airway Anastomotic Complications

Anastomotic complications have been recognized since the early days of lung transplantation [7]. Historically, rates as high as 60–80 % were recorded, but currently most programs report airway complications in the range of 10–20 % and related mortality rates of 2–3 % [136]. Vascular and/or airway complications are often multifactorial, influenced by donor-related, surgical, and immunosuppressive factors. Colonization of the donor airways by bacteria or fungi, size mismatch of donor and recipient airways or vessels, the use of positive pressure mechanical ventilation and the subsequent tension placed on the anastomosis, devitalization of the proximal airways by bronchial artery ligation, and the delay in graft healing due to immunosuppression all play a role in the initiation and progression of anastomotic problems. King-Biggs and colleagues reported high rates of airway dehiscence in patients receiving Sirolimus in combination with other immunosuppressive drugs when initiated early after transplant [137]. The overall incidence of airway complications is lower in combined heart–lung recipients than in single or double lung transplant patients.

Although vascular anastomotic complications are less frequent than airway problems, occurring in <2 % of recipients, the clinical

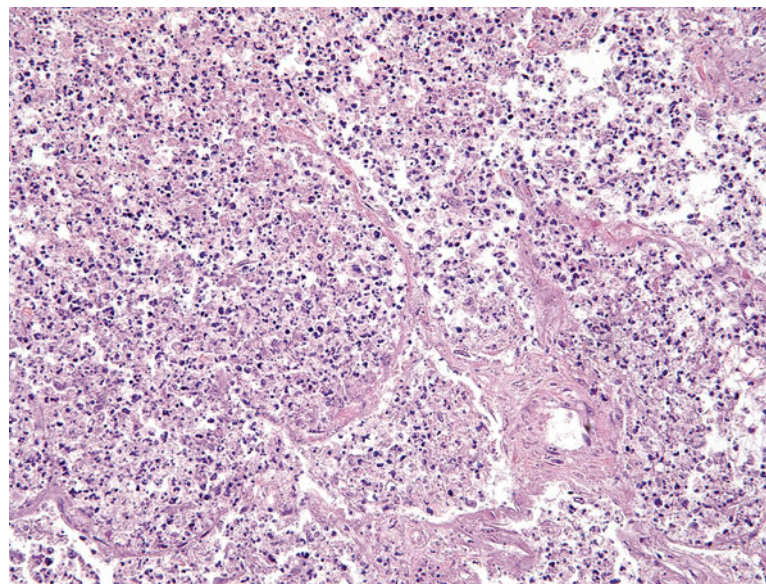

Fig. 8 Acute bronchopneumonia in a patient with cystic fibrosis with *Burkholderia cepacia* infection. The airspaces are filled with acute exudates and neutrophils

implications are more serious. In particular, partial or complete venous obstruction remains a significant cause of early morbidity and mortality if not recognized and urgently treated [138]. The majority of venous difficulties occur early after transplant and present with signs and symptoms of persistent pulmonary edema, pleural effusions, hemodynamic instability, high pulmonary capillary pressure, and parenchymal consolidation [139]. Systemic embolization and stroke are also potential complications of pulmonary vein thrombosis. Complete venous obstruction causes hemorrhagic infarction of the lung if surgical revision of the anastomosis is not performed promptly. Transesophageal echocardiography is an effective modality for assessing the anastomosis and for detecting obstruction. Other techniques that have been used include CT or MRI and CT angiography. Reports of late venous complications have also been reported [140]. Currently, therapeutic options for vascular disruptions range from medical therapy to either catheter-based interventions with stents or surgical revision [141].

Airway complications including **infarction, dehiscence, overgrowth by bacteria or fungi, luminal stricture by granulation tissue and scar, bronchomalacia, and fistula formation** are more commonly encountered than vascular problems. The bronchial arteries provide the primary blood supply to the distal trachea and initial 4–5 cm of the bronchi. Ligation of the bronchial arteries places the anastomosis at risk of ischemic injury for the first 2–4 weeks after transplantation. Luminal narrowing by granulation tissue and scar formation can be assessed and classified by a fiberoptic approach, and in some cases, requires laser excision and/or stent placement or even retransplantation (Fig. 9). Some centers advocate bronchial arterial re-anastomosis at the time of transplant to reduce this risk [142]. Devitalized airways are also sites for colonization of infectious organisms [143]. We have observed fungal tracheobronchitis and bacterial bronchitis in infarcted airway cartilage.

Intermediate Period After Transplantation (1 Month–1 Year)

The common complications encountered in this period are ACR, non-infectious airway inflammation, AMR, infections, and PTLD. It should be emphasized that there can be both an overlap of morphological findings of these entities and

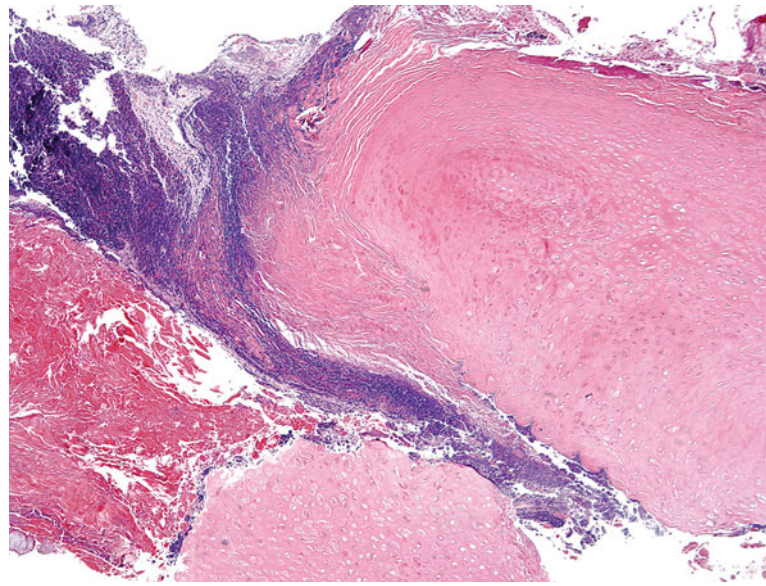

Fig. 9 Ischemic necrosis near the bronchial anastomosis showing devitalized cartilage and necrotic debris

synchronous presentation of one or more disorders in a biopsy specimen (e.g., concurrent ACR and AMR).

Acute Cellular Rejection

Most transplant recipients, pediatric and adult alike, will experience at least one episode of ACR. Further, it occurs in all types of transplant including lobar transplants and generally within the first 3–6 months. The Lung Rejection Study Group of ISHLT published the first classification for grading and reporting ACR in 1990 and the scheme was revised in 1996 and again in 2007 [86–88] (Table 3). Parenthetically, the current 2007 scheme for ACR is essentially unchanged from the 1990 scheme.

Most patients with ACR are asymptomatic and most biopsies are usually low grade in severity. Even some of our patients with moderate rejection present with limited symptoms. Signs and symptoms are nonspecific and include low-grade fever, cough, hypoxia, drop in expiratory flow rates (FEFs), or new onset and/or increase in pulmonary infiltrates or pleural effusions [144]. On account of the overlap of clinical and radiological findings with infection, imaging techniques are not sensitive or specific and PFTs are useful for patient surveillance but not as diagnostic tools [145, 146].

The TBBx has come to be regarded as the "gold standard" for the diagnosis and classification of ACR. As previously discussed, TBBx in most centers is performed as part of a surveillance protocol or for clinical indications with the latter typically demonstrating higher diagnostic yields. **The principal histopathologic features of ACR** center on the presence of mononuclear inflammatory cell infiltrates in the perivascular tissue planes with/without extension along adjacent alveolar interstitial structures and into airspaces. With increasing grades of rejection, the cellular infiltrates may become more polymorphous with eosinophils and scattered neutrophils and subendothelial mononuclear cell infiltrates or endothelialitis may be observed. Further, the conducting airways including cartilaginous bronchi and bronchioles can have concurrent lymphocytic mural infiltrates that are designated as lymphocytic bronchitis or bronchiolitis. The A grade designates the grade of ACR and the B grade designates the airway inflammation [88] (Table 3). The immunophenotypic composition of the infiltrate is CD4+ and CD8+ T-cells, CD68+ macrophages, and CD21+ dendritic cells; CD138+ plasma cells and CD20+ B-cells are uncommon.

Table 3 The 2007 International Society for Heart and Lung Transplantation grading for allograft rejection

1.	**Acute cellular rejection (grade A)**
	No evidence of acute rejection (grade 0)
	Minimal acute rejection (grade A1)
	Mild acute rejection (grade A2)
	Moderate acute rejection (grade A3)
	Severe acute rejection (grade A4)
2.	**Airway inflammation without scarring/ lymphocytic bronchiolitis (grade B)**[a]
	Ungradeable biopsy (grade BX)
	No lymphocytic bronchiolitis (grade B0)
	Low-grade lymphocytic bronchiolitis (grade B1R)
	High-grade lymphocytic bronchiolitis (grade B2R)
3.	**Chronic airway rejection/obliterative bronchiolitis (OB) (grade C)**
	Absent (grade C0)
	Present (grade C1)
4.	**Chronic vascular rejection/transplant-associated vasculopathy (grade D)**

Modified from reference [88]
[a]R designates revised grade

Grade AX (Ungradeable Specimen)
A numeric grade cannot be rendered because of insufficient (<4–5 adequate) pieces of tissue, crush artefactual distortion, etc.

Grade A0 (No Evidence of Rejection/NER)
No perivascular infiltrates are seen in an adequate biopsy sample.

Grade A1 (Minimal Acute Rejection)
Limited, infrequent circumferential cuffs of mononuclear inflammatory cells around one or a few venules or arterioles. Small and large, transformed lymphocytes with occasional plasmacytoid cells form perivascular rings of 2–3 cell layers (Fig. 10). These are generally inconspicuous at low power, but can be detected at high power magnification.

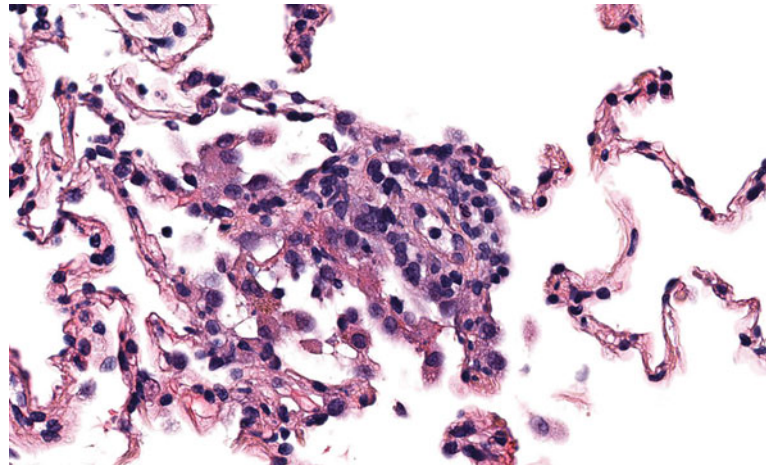

Fig. 10 Minimal (grade A1) acute cellular rejection showing a distinct perivascular cuff of mononuclear cells two cell layers- thick

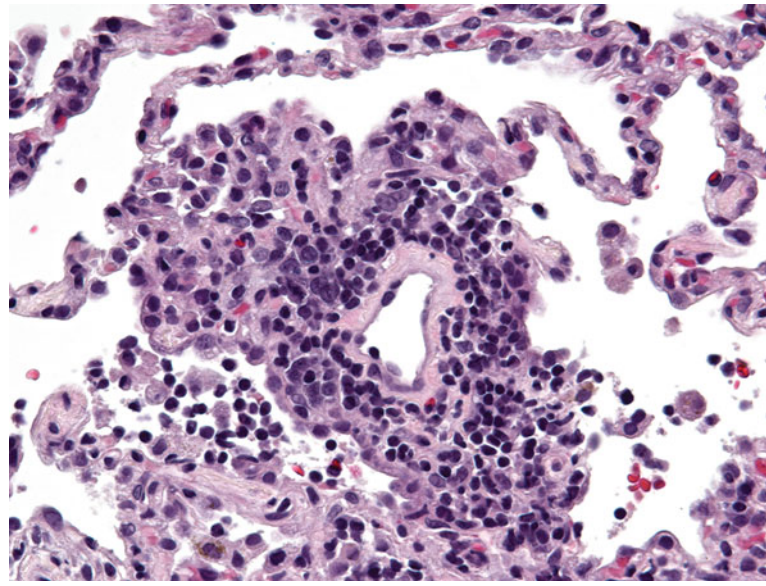

Fig. 11 Mild (grade A2) acute cellular rejection dense perivascular collection of mononuclear cells

Grade A2 (Mild Acute Rejection)

Perivascular infiltrates are readily observed at scanning magnifications and are composed of more than three cell layers in thickness. The composition includes small and transformed lymphocytes, macrophages and, not infrequently, eosinophils. The infiltrates are restricted to the perivascular spaces and do not extend along the alveolar septa (Fig. 11). Besides the extent of the infiltrates and the presence of eosinophils in Grade A2, another feature that distinguishes it from Grade A1 is the presence of endothelialitis or intimitis. Further, coexisting airway inflammation is more common in mild rejection than minimal rejection in our experience. The liberal use of leveled sections rather than immunohistochemistry is often helpful in borderline cases. In most centers, ACR Grade A2 is the threshold for instituting augmented immunosuppression on account of concern for immune-mediated damage of the allograft and the risk of developing CLAD [144].

Grade A3 (Moderate Acute Rejection)

The hallmarks of moderate rejection include the frequency and density of the perivascular cuffs and the extension of the inflammatory infiltrates along peribronchiolar alveolar septa and into alveolar spaces (Fig. 12). The latter is often characterized by macrophage and lymphocyte collections within alveolar spaces and type 2 changes of the alveolar epithelium. Endothelialitis is often present and concurrent airway inflammation may also be found.

Grade A4 (Severe Acute Rejection)

We rarely encounter this stage of ACR today. Most patients are symptomatic with marked dyspnea and acute hypoxemia respiratory failure. In addition to the perivascular, interstitial and airspace mononuclear infiltrates and endothelialitis ACR Grade A4 exhibit parenchymal damage such as alveolar damage, hyaline membranes, necrotic cellular debris, alveolar hemorrhage, and often a conspicuous neutrophilic component (Fig. 13). Vasculitis,

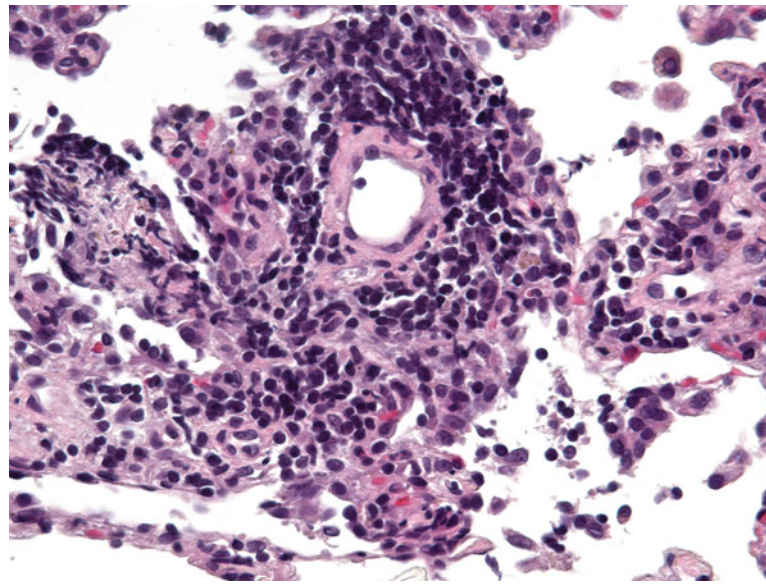

Fig. 12 Moderate (grade A3) acute cellular rejection showing perivascular cuff of mononuclear cells extending along adjacent alveolar septa

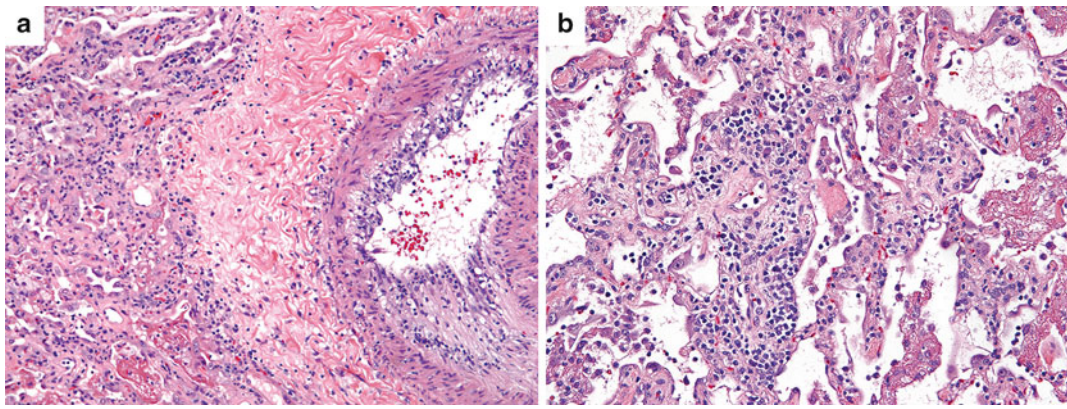

Fig. 13 (**a**) Severe (grade A4) acute cellular rejection characterized by fibrinous injury of alveolar structures and prominent endothelialitis of the muscular artery. (**b**) The adjacent alveolar tissue showing mixed perivascular infiltrates and an acute lung injury pattern

thrombosis, and parenchymal infarction are seen in the late stages.

Pitfalls in the Diagnosis and Grading of Acute Rejection

There are a number of morphological mimics of ACR. Firstly, perivascular inflammatory infiltrates can be found in viral, mycobacterial and fungal infections, and in PTLD. Classically, CMV and *Pneumocystis jiroveci* pneumonia (PJP) can show perivascular and/or interstitial components (Fig. 17). In **CMV pneumonitis**, however, there is a predominance of interstitial and septal changes over the perivascular findings. Secondly, prominent perivascular edema, small neutrophilic microabscesses, cytological atypia of the alveolar lining cells, and concurrent acute airway inflammation favor infection over ACR [147]. Consequently, the diagnosis of ACR is a diagnosis of exclusion and should be evaluated together with available clinical, serological, immunohistochemical, and microbiologic information. In cases where the distinction remains unresolved, the Lung Rejection Study Group recommends that the pathologist indicates which process is favored and recommend a prompt repeat biopsy after an appropriate period of antimicrobial therapy [87].

Bronchus-associated lymphoid tissue (BALT) is part of the normal airway immune defense mechanisms and is composed of mural nodular infiltrates in small airways (distal small cartilaginous bronchi and terminal bronchioles) at their branching points. In most cases, its nodular configuration and distribution confirm the diagnosis. Small vessels in the walls of the airways can display a circumferential inflammatory cell cuff as part of BALT and should not be confused with ACR. In some cases, the use of additional leveled sections is helpful in demonstrating the airway locale; alternatively, IHC shows collections of CD20+ B-cells in BALT foci. The confusion of BALT for ACR accounted for some of the discrepancy in the ACR readings between local and central pathologists in the study by Arcasoy and colleagues [89]. Mononuclear cellular collections within interlobular septa in the absence of a perivascular distribution likewise should not be classified as ACR. In some cases, the inflammatory cells are admixed with anthracotic pigment and macrophages.

Collections of mononuclear and other inflammatory cells can be seen in **biopsy site changes**. On occasion, the TBBx encompasses a focus of a healing or healed site of a prior biopsy. The presence of granulation tissue, hemosiderin-laden macrophages, fibrin, blood, and thrombus are histological clues to this diagnosis.

PTLD is defined as a proliferation of lymphoid and/or plasmacytoid cells and is usually EBV-associated. In most cases, these present as solitary or multiple lung masses. An interstitial component with or without perivascular involvement can be seen around the edge of the lesions. A TBBx specimen may yield the periphery of the lesion rather than the central diagnostic component. Communication between the clinical team and the pathologist is essential for proper specimen handling and selection of appropriate immunophenotypic and molecular panels. Interestingly, we have observed both ACR and PTLD in a VATS biopsy and used immunohistochemistry to delineate the different lesions (see also chapter "Transplantation and Malignancy").

Issues Related to the Diagnosis and Classification of Acute Rejection

A number of technical and interpretative issues related to the ISHLT grading scheme warrant further clarification. Firstly, it is not uncommon to find different patterns of severity of ACR in a transbronchial or VATS biopsy. For example, one piece of tissue may display a pattern of minimal rejection and another show mild or moderate ACR. The designated grade is based on the most advanced grade of rejection and not the predominant pattern. Secondly, some samples may exhibit ACR, but with less than the recommended five pieces of alveolated tissue. We advocate a descriptive diagnosis such as "Mild acute rejection in a suboptimal/borderline adequate sample" and refrain from a numeric grade to alert the clinician to the ACR. The concern for a higher grade of ACR in the lung must be considered in this setting. Thirdly, the diagnosis of ACR requires that

the inflammatory infiltrate is completely circumferential around the vessel. The arrangement can vary from compact, "tight" collections to more loosely arranged cuffs. The problem of a partial rather than complete cuff is not uncommon. Additional leveled sectioning of the paraffin block resolves many of these problematic cases. In equivocal cases, we report the findings and recommend careful clinical and microbiological correlation to exclude an infectious etiology. Fourthly, it is not uncommon to find a number of different pathologies in a TBBx sample [148]. For example, we have observed concurrent cases of ACR and OB in patients transplanted more than 1 year. All significant findings should be documented in the pathology report irrespective of the overall adequacy of the sample.

The clinical significance of Grade A1 or minimal ACR is controversial. Episodes of higher grades of ACR (mild or greater) have been shown to be an independent risk factor for the development of chronic rejection. Recent studies have raised concern that even asymptomatic minimal rejection (Grade A1), including patients with only a single episode, increases the risk for BOS [149–151]. As the current threshold for initiating treatment is mild rejection, it is important that the pathologist strictly adhere to the ISHLT diagnostic criteria. In addition, patients with minimal ACR warrant close follow-up and repeat biopsy after a designated interval.

Non-infectious Airway Inflammation Without Scarring

Both the large and small airways are the targets for inflammation and infection. As described previously, ACR can have concurrent airway mononuclear inflammatory cell infiltrates. If other etiologies (especially infection) are excluded, the infiltrates represent airway rejection. In the absence of ACR, active small airway inflammation without associated scarring is classified as **lymphocytic bronchiolitis**, **LB**. The grading of airway inflammation has been revised and simplified since the 1990 and 1996 ISHLT versions (Table 3). Currently, the previous minimal and mild LB grades (previously B1 and B2) are compressed to low-grade small airway inflammation and the numerical grade B1R is used to indicate the revised grade [88]. Low-grade LB is characterized by submucosal mononuclear inflammation. The patterns range from patchy or scattered infiltrates to circumferential bands of mononuclear cells (Fig. 14). Eosinophils are found occasionally, but in small numbers, and by definition, mononuclear cells are not seen within epithelial cells. High-grade small airway disease and its numeric counterpart B2R replaced the moderate and severe LB grades (originally B3 and B4) of the previous grading scheme. It is defined by the presence of dense mononuclear cells in the bronchiolar submucosa including small and trans-

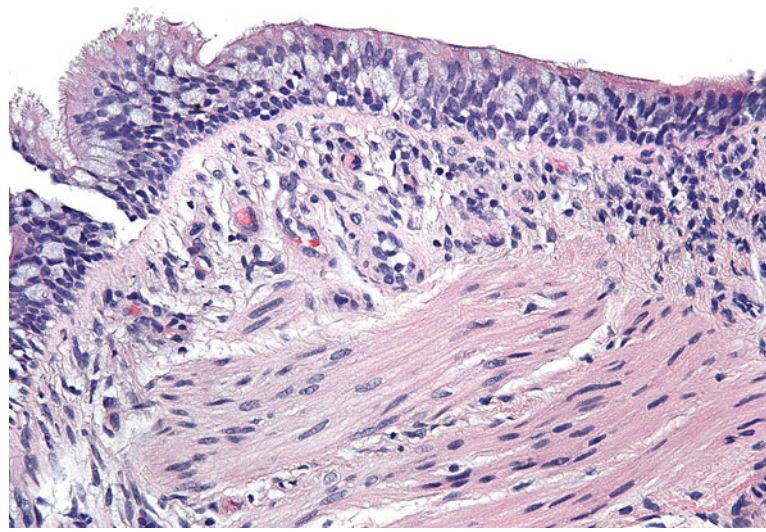

Fig. 14 Low-grade (grade B1R) lymphocytic bronchiolitis characterized by scattered mononuclear inflammatory cells within the wall of the bronchiole

formed lymphocytes admixed with other inflammatory cells such as eosinophils, neutrophils, and plasma cells in association with intraepithelial infiltrates and epithelial injury. The degree of epithelial injury ranges from respiratory cell necrosis and metaplasia to epithelial sloughing, ulceration, and cellular and fibrinous exudates (Fig. 15). Grade B0 is defined as the absence of airway inflammation and Grade BX indicates that the biopsy specimen is ungradeable on account of absence of airways, tangential sectioning, artifactual distortion of airways, or infection.

The clinical significance and management of LB is controversial. There is emerging evidence that LB is an independent risk factor for the development of CLAD [152]. Likewise, it may be a manifestation of AMR, perhaps as a surrogate marker of an activated immune system. Mechanistically, it involves an IL17+ T-cell-mediated pathway. The macrolide antibiotic, azithromycin, which has anti-inflammatory and immunomodulatory properties, has been shown to improve lung function in cases of LB [153, 154].

Antibody-Mediated Rejection

AMR is now formally recognized as a cause of morbidity and mortality in solid organ transplant recipients. The histopathological and immunophenotypic criteria have been recently enumerated in the cardiac allograft [155, 156]. Currently, pulmonary AMR is the focus of active multidisciplinary investigation, but the published morphologic experience is limited to case reports and small series [157–176]. There is increasing evidence that the 10–30 % of presensitized patients and the 10–50 % of patients who develop donor-specific antibodies (DSA) after transplant are at increased risk for ACR, LB, AMR, BOS, and diminished survival [177–181]. In addition to DSA, some patients develop non-DSA HLA antibodies and non-HLA antibodies against epithelial, endothelial, or connective tissue antigens such as Type V collagen or K-aplha-1-tubulin [182–184]. Some centers utilize prevention and treatment strategies with rituximab and IVIg, or IVIg alone or plasmapheresis alone. Multicenter trials are underway to further evaluate the efficacy of therapeutic intervention for the prevention chronic allograft dysfunction such as BOS in patients who develop anti-HLA antibodies after transplant. These studies emphasize the importance of serological monitoring of patients as part of routine surveillance.

The Pulmonary Working Group of the ISHLT recently convened a group of transplant pulmonologists, surgeons, immunologists, pharmacolo-

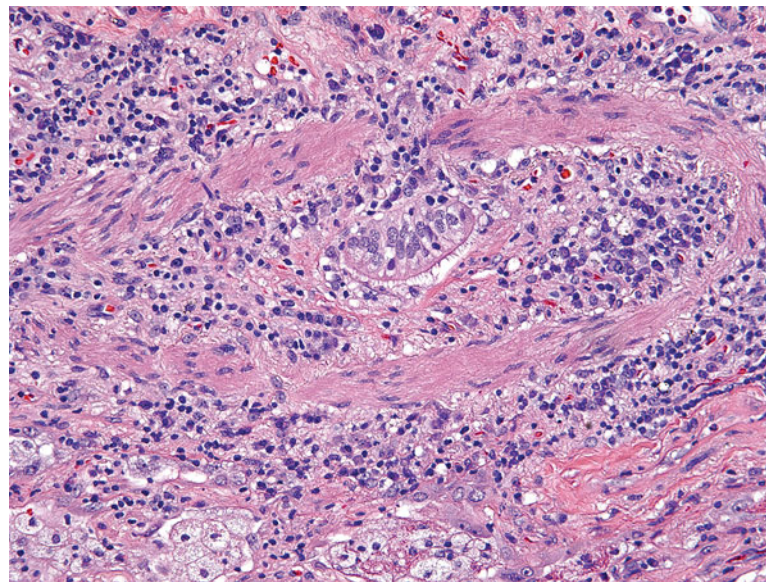

Fig. 15 High-grade (Grade B2R) lymphocytic bronchiolitis showing marked inflammation in all layers of the bronchiole and epithelial sloughing. This patient had concurrent severe ACR

gists, and pathologists to review the current state of knowledge and to establish working definitions and diagnostic criteria for pulmonary AMR [185]. With the 2005 NIH paradigm of clinical, subclinical, and latent forms of AMR, the group recognized levels of diagnostic certainty with "definite, probable and possible" categories of AMR. These categories reflect the aggregate of clinical, serologic, and pathologic support in a particular case. For example, **definite clinical AMR** is defined as the presence of allograft dysfunction, positive DSA, histology suggestive of AMR, and positive C4d staining by IHC or IF. In **probable clinical AMR,** two of the three features are present and the term **possible AMR** reflects the presence of only 1 feature.

The 2007 ISHLT Working Formulation reviewed the published literature and experience of pathologists at that time and proposed **recommendations for diagnostic terminology and antibody testing in AMR**. The term "acute capillary injury" replaced "acute capillaritis," "septal capillary necrosis," and other descriptions [88]. In 2012, the Pathology Council of ISHLT refined the terminology and proposed specific definitions for morphologic patterns that have been observed in patients with AMR and recommendations for biopsy and immunophenotypic protocols [186]. Neutrophilic capillaritis was defined as patchy or diffuse alveolar septal neutrophilic infiltrates containing cellular karyorrhetic debris with or without microvascular thrombi, alveolar hemorrhage, or airspace accumulations of neutrophils. Neutrophilic margination, in distinction, lacked the injurious components. These patterns were identified as infrequent, but reasonably specific for AMR. Additional morphologic patterns that were reported in AMR included persistent or recurrent ACR, high-grade LB, OB, ALI with or without hyaline membranes. As previously discussed, ALI can be seen in a variety of lung transplant conditions injury such as infection, high-grade ACR, drug toxicity, and ischemic/preservation injury. Other pathologic findings that warrant consideration for the possibility of AMR are recurrent/persistent low-grade LB, arteritis in the absence of viral infection, and graft dysfunction that does not reveal a morphologic explanation.

The role of C4d in pulmonary AMR is unsettled at this time. Some centers have shown an association of C4d immunoreactivity with patterns of histopathologic injury enumerated above, while others have suggested that C4d staining is unreliable as a marker of AMR, in distinction to the cardiac and renal transplant experience [172, 175, 176]. When C4d is present, the pattern is weak or strong continuous linear endothelial staining by IHC or IF (Fig. 16). Other structures such as arterial, arteriolar, or venular endothelium, airway basement membrane, vascular elastic membrane, and interstitial septal connective tissue often show C4d staining and serve as internal controls for the purpose of quality control, but are not indicative of AMR.

Differential diagnosis of AMR. As noted, the histologic features are nonspecific and can be seen in PGD, HAR, non-immunological causes of DAD, severe ACR, and infection. More importantly, these findings should warrant a thorough histochemical, immunohistochemical, molecular, serological (including DSA), and microbiological work-up for other possible causes.

There are a number of **unresolved issues** besides the utility of C4d staining. As with cardiac AMR, there is likely a morphological spectrum of changes that progress to a diffuse alveolar injury pattern in the allograft. Currently, we perform immunostaining on all TBBx specimens and utilize a limited antibody panel of C4d, CD31, and CMV. We routinely recommend serological DSA studies in this setting and think that the diagnosis of AMR should be made as a diagnosis of exclusion, and only in a multidisciplinary setting of clinical dysfunction, circulating anti-HLA antibodies, and positive pathologic findings. The role of C4d for the diagnosis of AMR is currently under investigation by a number of centers and more studies are needed to determine its utility.

Infections

The transplanted lung allograft is at risk for a variety of infectious complications beginning in the immediate post-transplant period and lasting throughout the lifetime of the organ. As previously discussed, a number of immunological, mechanical, and functional factors derived from

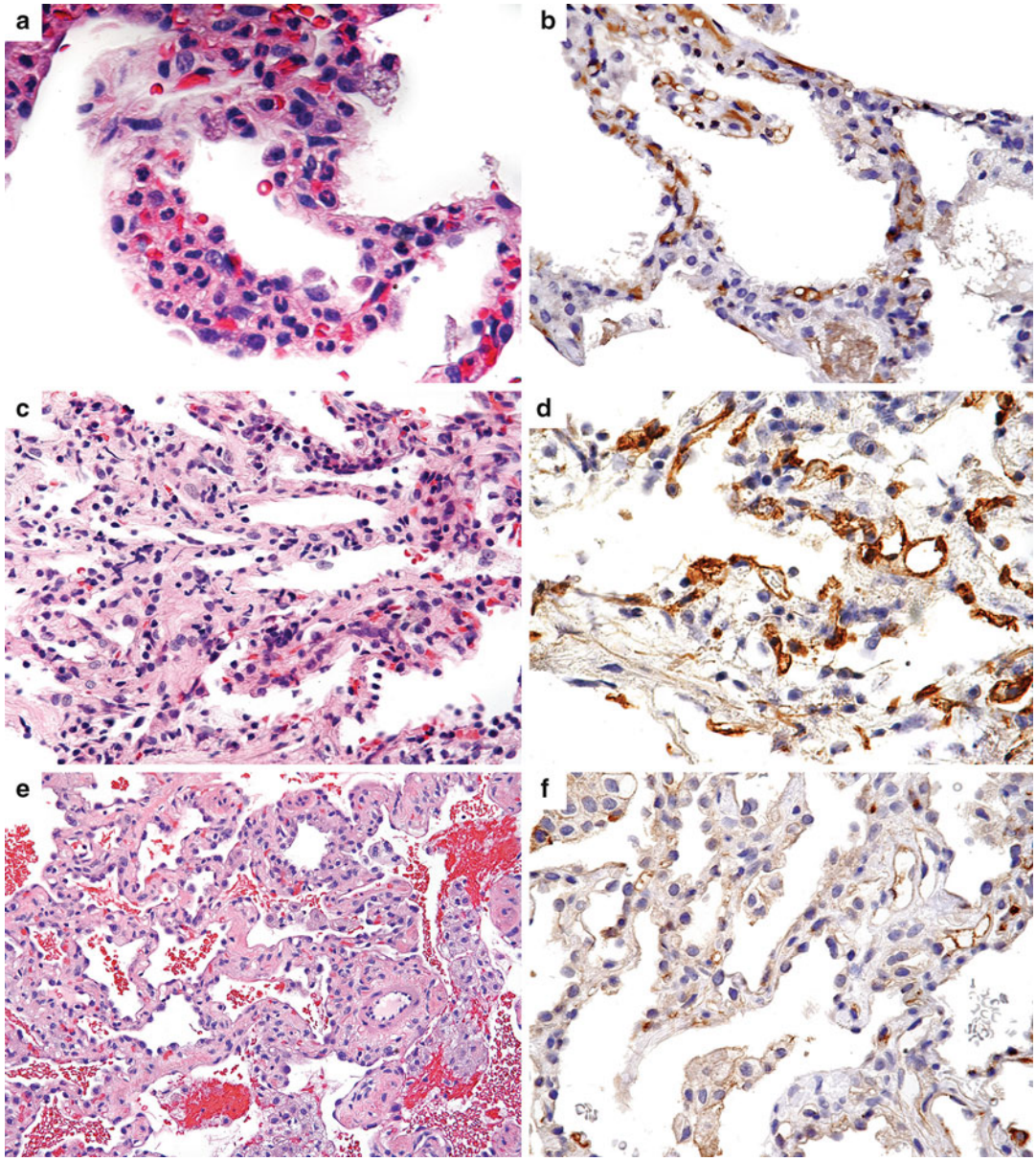

Fig. 16 Antibody-mediated rejection (AMR). (**a**) Neutrophilic margination in a patient with graft dysfunction and de novo DSA. Note the numerous neutrophils within the alveolar interstitial capillaries but absence of pyknotic debris or thrombi. (**b**) Concurrent C4d staining by IHC from same biopsy specimen. Distinct continuous endothelial staining of interstitial capillaries is seen. (**c**) Young man with new onset DSA and minimal changes in PFTs showing minimal alterations. (**d**) Corresponding C4d stain showing strong, diffuse staining of interstitial capillaries. (**e**) Acute lung injury pattern in patient with respiratory failure and positive DSA. (**f**) Corresponding C4d stain showing diffuse weak staining of the microvasculature

the donor, recipient, surgical procedure and post-transplant management account for many of these predisposing factors. Not surprisingly, lung transplant recipients are perhaps the most susceptible of the solid organ transplant group and this risk extends to all pediatric and adult patients. For example, respiratory viral infections affect more than half of the pediatric recipients [187].

Pulmonary infections, especially by the opportunistic group of agents, enhance the risk for BOS.

In our opinion, the most practical approach to the classification of **post-transplant infections** is the temporal paradigm of Fishman [131]. In the 1- to 6-month period after transplant when patients are maximally immunosuppressed, opportunistic infections are most commonly encountered. These include the immunomodulating viruses of the herpes group (CMV, EBV and others), fungal infections caused by *Aspergillus* species and *Pneumocystis jiroveci*, bacterial infections such as nocardiosis and mycobacterial infections, and rarely, parasitic infections [188]. After 6 months, most patients have achieved their lowest maintenance immunosuppression levels and are at risk primarily for community-acquired infections. Importantly, community-acquired respiratory viruses can cause serious clinical infections and trigger acute rejection episodes or promote the development of chronic rejection [189]. Opportunistic infections can also occur in the late period in patients treated with augmented immunosuppression for recurrent acute rejection episodes or to arrest the progression of BOS. With the widespread use of antimicrobial prophylaxis for specific bacterial, viral, and fungal organisms, new patterns and etiologies of infectious complication are now emerging [190].

Viral infections including CMV. CMV is the most common opportunistic infection in lung recipients as the lung is the principal reservoir for latent CMV virus. The risk is stratified by the CMV status of the donor and recipient. One scenario is primary infection in a seronegative recipient receiving an allograft from a seropositive donor (R-/D+) as this group is at highest risk. Seropositive recipients receiving a lung from either a seronegative donor (R+/D−) or seropositive donor (R+/D+) are at intermediate risk largely by either reactivation or reinfection with a new CMV strain. The group composed of seronegative donor and recipient is at the lowest risk (R-/D-) [128, 191–193]. That said, the terminology used in the literature can be confusing and our approach is based on the following definitions:

CMV infection: evidence of CMV replication regardless of symptoms (differs from latent CMV).

CMV disease: evidence of CMV infection with attributable symptoms. It can be further classified as CMV syndrome and tissue-invasive disease.

CMV viral syndrome: presence of fever (>38 °C for at least 2 days within a 4-day period), malaise, leukopenia, and thrombocytopenia and the detection of CMV in blood.

Tissue-invasive disease: presence of signs and/or symptoms of pulmonary disease combined with demonstration of CMV in BAL fluid or lung tissue specimens by virus isolation, histopathology, and immunohistochemical or in situ hybridization staining.

Prior to the era of CMV prophylaxis, transplant patients typically developed clinical CMV disease (fever, leucopenia, and end-organ involvement) or CMV infection between 1 and 3 months after transplant. CMV prevention is currently focused on prophylaxis strategies like antiviral agents, e.g., oral valganciclovir or oral/intravenous ganciclovir or CMV preemptive treatment. Prophylaxis programs vary in duration among centers with programs ranging from 1 to 12 months [128, 193]. Preemptive strategies are based on careful serologic monitoring with prompt treatment for new onset of CMV infection. Rather than completely eliminating CMV disease, some studies have shown that these programs delay the onset of disease and the problem of "late-onset CMV disease" has emerged in some patients [194]. In addition to the cost of prophylaxis programs, it should be noted that there are other complications such as drug toxicity, neutropenia, and the development of drug resistance in 10–15 % of patients.

The pathologic diagnosis of tissue-invasive CMV disease requires the identification of the classic amphophilic, large nuclear inclusion separated from the nuclear membrane by a translucent rim or halo with or without cytoplasmic inclusions amidst a variable inflammatory response (Fig. 17). CMV or "owl's eye" inclusions can be

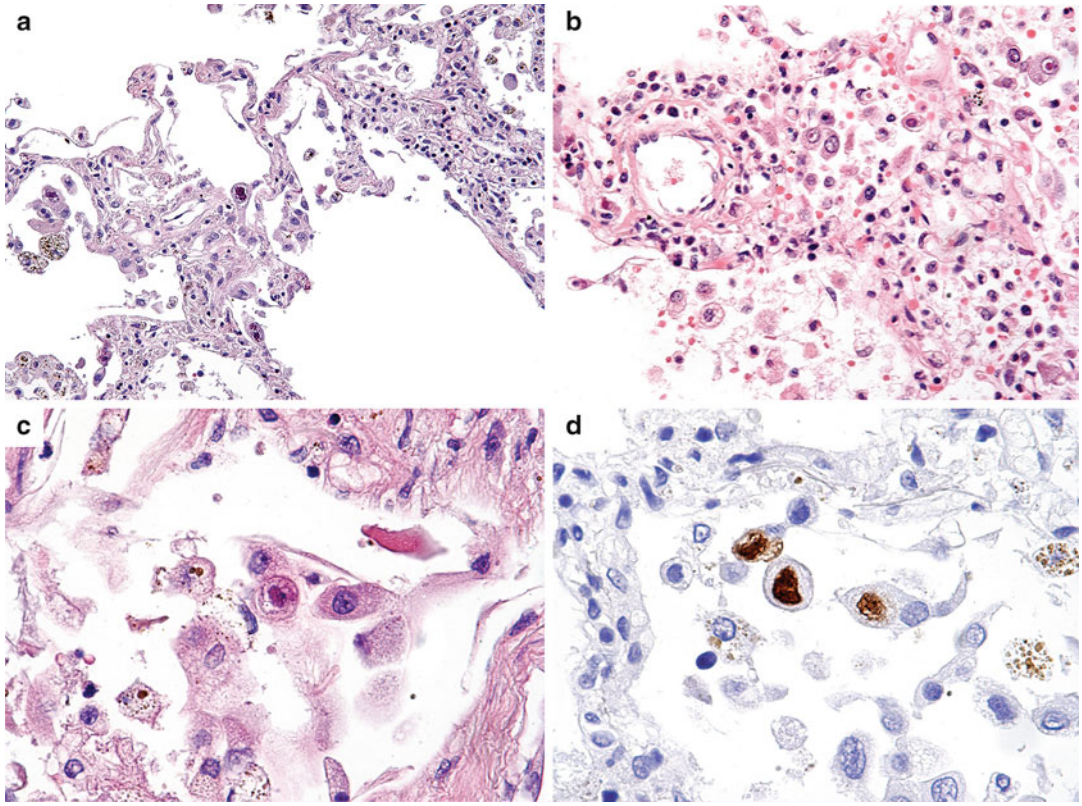

Fig. 17 CMV pneumonitis. (**a**) Perivascular and interstitial infiltrates admixed with characteristic nuclear inclusions of CMV. (**b**) High power magnification showing perivascular mixed inflammatory infiltrates and scattered inclusions. (**c**) High power magnification of CMV-infected cells with eosinophilic nuclear inclusion and surrounding halo. (**d**) CMV immunohistochemical stain highlighting infected cells

found in pneumocytes, endothelial cells, dendritic cells, macrophages, and smooth muscle cells. The inflammatory response in a TBBx specimen can range from a sparse interstitial pneumonitis of mixed inflammatory cells to an ALI pattern with hyaline membranes and microabscesses. Immunohistochemical or in situ hybridization techniques are useful for biopsies demonstrating equivocal findings. Following anti-viral therapy, cells infected by CMV can contain smudgy, inhomogeneous inclusions with irregular outlines and lose the characteristic perinuclear halos.

As discussed earlier, the problem of distinguishing ACR from CMV pneumonitis can be problematic. Perivascular infiltrates are reported in up to 45 % of biopsies with CMV pneumonitis; endothelialitis, another feature of ACR, can also be found [195]. In this setting, we recommend anti-viral therapy with an early follow-up biopsy to evaluate for ACR. Subtle clues that favor CMV pneumonitis and other viral infections over ACR include perivascular edema and mixed inflammatory infiltrates that contain neutrophils, more loosely arranged perivascular cuffs, and interstitial and septal infiltrates that predominate over the perivascular component [147]. Other viruses from the Beta-Herpes group causing graft injury that have been reported in lung recipients are human herpesvirus-6 (HHV-6) and human herpesvirus-7. PTLD and smooth muscle neoplasms are triggered by EBV [193].

Adenovirus and herpes simplex virus pneumonitis are less common opportunistic infections, but can cause severe acute infections. In our experience, adenovirus pneumonia can also progress to or promote chronic rejection. Adenoviral

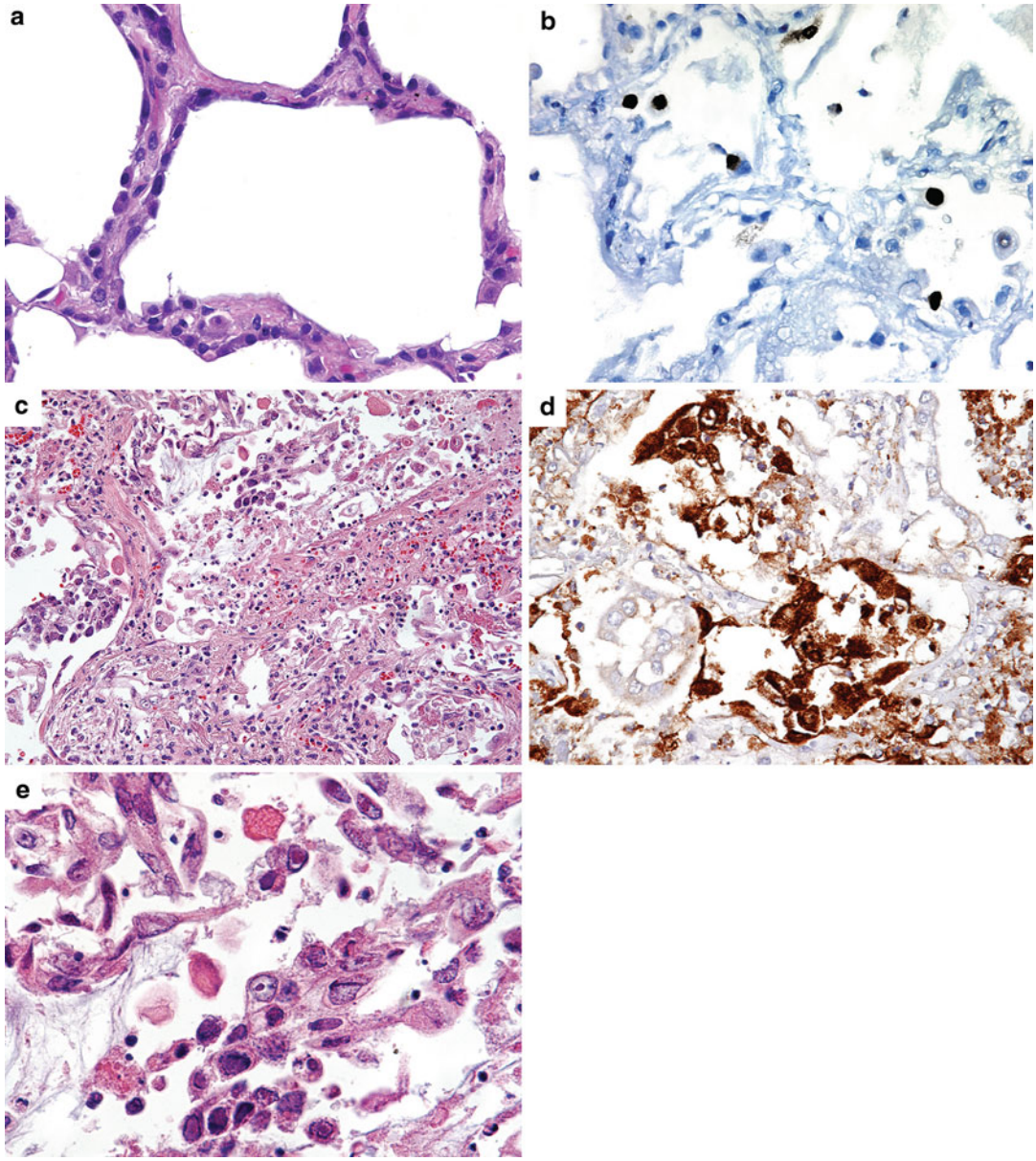

Fig. 18 (**a**) Adenovirus pneumonia characterized by small hyperchromatic nuclear inclusions within pulmonary epithelial lining cells. (**b**) Immunohistochemical staining for adenovirus highlights numerous positive cells. (**c**) Herpes virus pneumonia showing abundant inflammation, necrotic debris, and numerous inclusions in sloughed lining cells within the center of alveolar spaces. (**d**) Immunohistochemical staining of numerous herpetic inclusions. (**e**) High power showing mononuclear herpetic inclusions

pneumonia is characterized by necrotizing, hemorrhagic bronchopneumonia with irregular ground-glass intranuclear inclusions or smudge cells (Fig. 18). HSV infections create a variety of morphologic patterns such as ulcerative or necrotizing tracheobronchitis and bronchiolitis, hemorrhagic pneumonia, military nodules, and DAD patterns. Single inclusions or multinucleated giant cell inclusions are typically found within or adjacent to necrotic parenchyma.

Community-acquired viral infections. Community-acquired respiratory viral illnesses in lung recipients can be more serious and involve the lower respiratory tract more frequently than in immunocompetent patients. As such, they are associated with higher morbidity and mortality. The most common viruses are respiratory syncytial virus (RSV), influenza A/B, parainfluenza (serotype 3), metapneumovirus, and rhinovirus. The incidence ranges from 2 to 15 % of recipients [196, 197]. The histological findings are usually nonspecific and the diagnosis is established by viral culture, fluorescent antibody, or PCR tests. Another complication of these viruses (except for rhinovirus) is the risk of initiating episodes of acute and chronic rejection.

Fungal infections. Fungal infection, despite the reduction in overall incidence due to universal or targeted prophylaxis, remains a significant cause of mortality [128, 129]. In the first month after transplantation, infections are either donor-derived or related to surgical complications such as airway ischemia. Fungus was identified in 5 % of explanted lungs in one study, again emphasizing the need for comprehensive examination [198]. The patterns include chronic necrotizing pneumonia, mycetoma, and invasive fungal pneumonia. Fungal infections in the 1–6-month period fungal infections are opportunistic in type with relapsed or residual/persistent infection less frequent causes. Risk factors include recipient native disease, e.g., COPD, ILD, and CF, augmented immunosuppression for acute rejection, and concurrent or recent bacterial or viral infections. After 6 months, fungal infections usually arise after therapeutic interventions for allograft rejection.

The majority of infections are caused by *Aspergillus, Candida, Scedosporium,* or *Cryptococcus* species. Of these, *Aspergillus* infection is the most common. **The histopathologic spectrum** includes airway dehiscence, vascular anastomotic disruption, tracheobronchitis including ulcerative and pseudomembranous forms, angioinvasive/disseminated parenchymal disease, empyema, aspergilloma, endobronchial stent obstruction, mucoid bronchial impaction, and other patterns of allergic bronchopulmonary aspergillosis [199]. Fungal tracheobronchitis arises in ischemic segments and is characterized by necrotic cartilage containing hyphal forms (Fig. 19). In **angioinvasive fungal pneumonia,** parenchymal infarctive necrosis with intravascular plugs of fungi that spill out into the adjacent necrotic parenchyma is observed (Fig. 20). Widely disseminated aspergillosis is less common now, but involvement of the heart, CNS, thyroid, adrenal glands and other sites was typically encountered at postmortem in this setting. Subtle clues for the possibility of airway fungal colonization/infection include the presence of numerous mural eosinophils.

Serious *Candida* infections are less common today than in the early transplant experience where it accounted for the majority of infections in the first month [128, 129]. Identification of *Candida* mold in BAL fluid by culture or cytopathology is not uncommon and most represent upper airway colonization. *Scedosporium* species

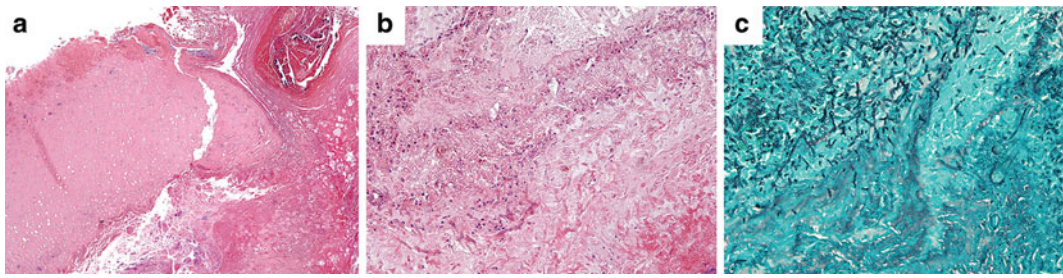

Fig. 19 Necrotizing fungal tracheobronchitis. (**a**) Low power of endobronchial biopsy showing necrotic cartilage and adjacent bronchial tissues. (**b**) Scattered fungal hyphae are present within the necrotic bronchial wall. (**c**) The GMS stain highlights numerous septate hyphae with acute angle branching consistent with *Aspergillus* species.

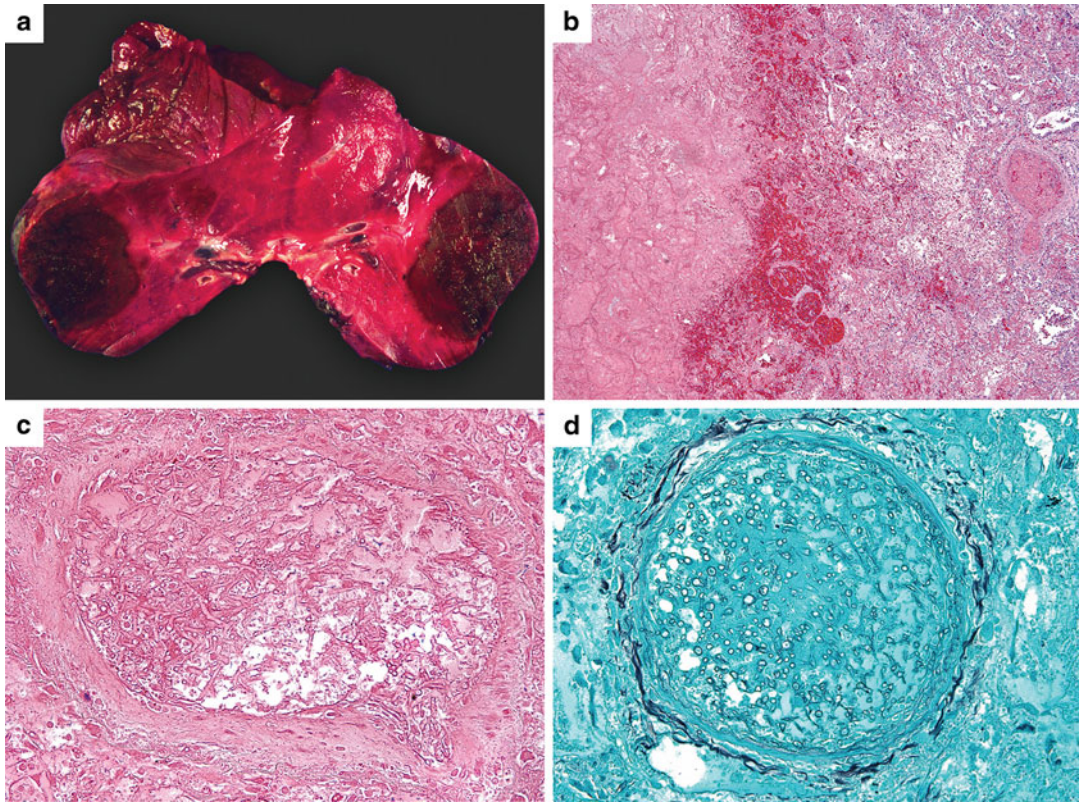

Fig. 20 Angioinvasive fungal pneumonia. (**a**) Gross image of hemorrhagic necrotic nodules in a VATS specimen. (**b**) Interface of necrotic parenchyma on left side and organizing pneumonia on right side. (**c**) Infarcted parenchyma showing muscular artery with fungal elements spilling out of the lumen into the adjacent lung parenchyma. (**d**) GMS stain of necrotic artery showing fungal hyphae of mucormycosis

(formally *Pseudoallescheria*) are indistinguishable from *Aspergillus* on morphology and by radiological techniques. They cause a similar spectrum of disease and have a poorer outcome. They are resistant to amphotericin-B and other polyene antifungal drugs, but respond to azole compounds. *Cryptococcus* infection is rare in pediatric lung recipients, but in adults is usually the result of reactivation. Unlike the nodular lesions found in immunocompetent hosts, alveolar or interstitial patterns are common in immunosuppressed hosts [200].

PJP (formerly *Pneumocystis carinii* or PCP) rarely occurs today on account of trimethoprim/sulfamethoxazole prophylaxis. In our experience, most cases today occur because of poor compliance or drug resistance. The classic radiological appearance is bilateral ground glass opacities. There are a number of histological patterns that range from sparse interstitial lymphoplasmacytic infiltrates, the classic frothy alveolar exudates, granulomatous lesions, to DAD. Perivascular mononuclear inflammatory cell infiltrates that can mimic ACR are reported, so we continue to routinely perform silver stains on TBBx specimens.

Post-transplant Lymphoproliferative Disorders and Other EBV-Related Disorders

PTLD is discussed in detail in the chapter "Transplantation and Malignancy". Nevertheless, a few features regarding post-transplant lymphop-

roliferative disorder (PTLD) in lung recipients deserve emphasis. In our experience, most PTLDs present as solitary or multiple lung masses. An interstitial component with or without a perivascular arrangement can be seen around the edge of the lesions resembling ACR. There should be open communication between the clinical team and the pathologist to assure proper tissue handling and to expedite the accurate diagnosis and classification of PTLD. **Histopathologic findings favoring PTLD** include monomorphic and atypical lymphoid cells, necrosis, and numerous mitotic figures [201].

The association of EBV and **smooth muscle neoplasms** in immunocompromised patients has been observed in congenital/primary and acquired immunodeficiency states including HIV and transplant recipients. Over 100 cases have been reported with half arising in solid organ transplant recipients [202–205]. Of the 40 cases in the lung, 7 were classified as leiomyosarcoma, 7 as benign leiomyomas, and the remainder as smooth muscle tumors of uncertain malignant potential. They can present as solitary or multiple solid parenchymal masses or as multiple polypoid endobronchial lesions with the morphology of benign leiomyoma. The smooth muscle cells express desmin and caldesmon by IHC and ISH for EBV RNA (EBER) shows strong nuclear staining (Fig. 21).

Drug-Induced Pulmonary Toxicity

Drug toxicity is an uncommon but frequently under-recognized problem. Of the many immunosuppressive drugs that lung recipients are required to take daily, the macrolide antibiotic **Sirolimus** is the one of the more commonly recognized for its pulmonary toxicity. Its immunosuppressive action is directed at inhibition of the mTOR pathway and it has been used clinically since 1999. Initially reported in renal transplant recipients, toxicity occurs in all solid organ recipients [206–211]. **The histopathologic findings** are often nonspecific and careful clinical-pathological correlation is required to establish the diagnosis. The histopathologic patterns of injury include an organizing pneumonia pattern, interstitial pneumonitis, pulmonary alveolar proteinosis, patchy fibrosis, alveolar hemorrhage, and granulomatous interstitial pneumonitis (Fig. 22). Withdrawal of the drug and addition of corticosteroid therapy are the mainstay of treatment and most patients improve promptly.

Everolimus is a proliferation signal inhibitor and a derivative of Sirolimus, although it has a shorter half-life and reportedly a wider bioavailability. Unfortunately, it is also associated with pulmonary toxicity with similar nonspecific patterns of injury such as cellular interstitial pneumonitis [212, 213].

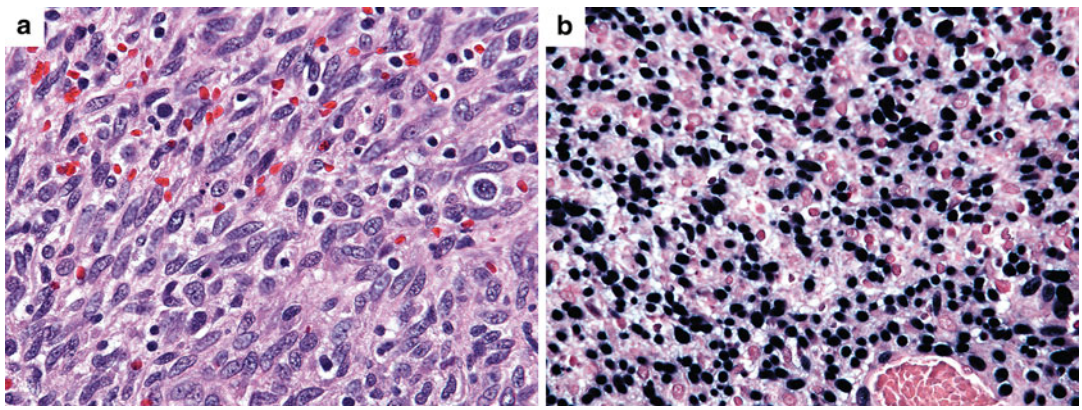

Fig. 21 EBV-associated post-transplant smooth muscle tumor. (**a**) High power magnification of cellular smooth muscle proliferation admixed with scattered lymphocytes. (**b**) In situ hybridization for EBV RNA (EBER) showing numerous positive cells

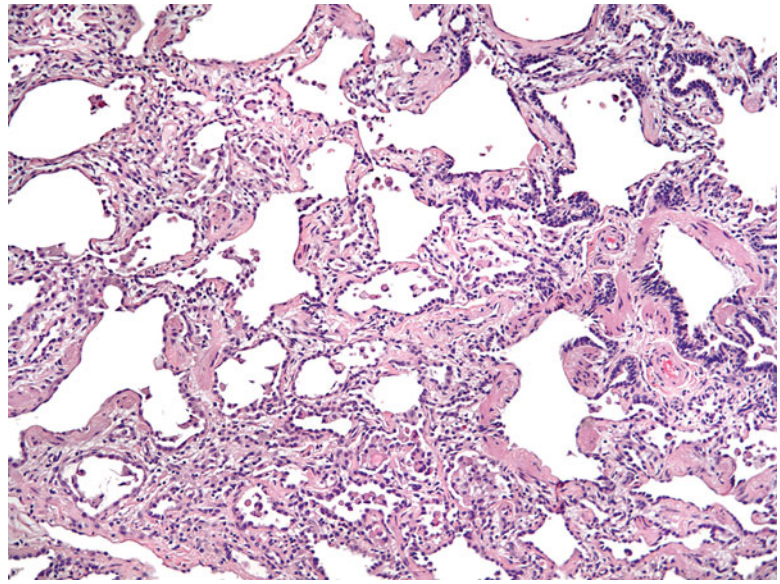

Fig. 22 Sirolimus toxicity. Organizing pneumonia pattern with scattered interstitial mononuclear cells and macrophages within the airspaces

Rituximab is a chimeric anti-CD20 monoclonal antibody used in the treatment of malignant lymphoma and a host of rheumatologic disorders. In lung transplantation, it is used for the treatment of PTLD and AMR and in desensitization protocols. Uncommon but reported pulmonary complications include bronchospasm, interstitial pneumonitis, organizing pneumonia pattern, DAD, ILD with UIP, lymphocytic interstitial pneumonia, or desquamative interstitial pneumonia patterns [214, 215].

Other Pulmonary Complications in Lung Transplant Recipients

The 2007 ISHLT report identified a number of "non-rejection" findings that can be seen in transbronchial or VATS biopsy specimens. These may be of either donor or recipient origin and could warrant further investigation or treatment in some cases. As discussed previously, oropharyngeal dysfunction with aspiration of food and other materials can elicit a foreign body type reaction along with bronchiolitis and organizing pneumonia patterns. Smoking-related lesions such as respiratory bronchiolitis or pulmonary Langerhans' cell histiocytosis could be from a donor with a heavy smoking history, but could be an indication that the recipient is actively smoking. The presence of intravascular talc granulomas or numerous intraalveolar macrophages with anthracotic pigment are indicators of illicit drug use.

Late Period After Transplantation (>12 Months)

In the late period after transplantation, **CLAD** continues to limit long-term survival in this group. Until recently, CLAD was essentially an obstructive process represented by the histopathologic lesion of OB and BOS as its clinical counterpart. The term has now been broadened to include RAS and a variety of allograft and non-allograft disorders not related to chronic rejection such as neutrophilic-reversible/azithromycin responsive allograft syndrome (NRAD/ARAD), GERD, diaphragm dysfunction, and problems in the remaining native lung, etc. [216, 217]. Overall, approximately 50 % of transplant recipients develop BOS- or RAS-related CLAD by 5 years following transplant [15]. The majority of cases of CLAD are associated with BOS (70 %), while nearly 30 % are caused by RAS and the remainder are composed of the non-chronic rejection forms of CLAD [218]. Other late complications include transplant-associated vasculopathy (TAV), PTLD, and recurrence of the primary lung disease.

Obliterative Bronchiolitis and Bronchiolitis Obliterans Syndrome

OB/BOS is the most common cause of death or indication for retransplantation in pediatric and adult recipients surviving at least one year. Further, the type of transplant does not provide immunity to this devastating complication. The pathogenesis is complex and a variety of alloimmune and non-alloimmune mechanisms, such as ACR, AMR, LB, increased number of HLA mismatches between donor and recipient, PGD, GERD with aspiration, development of de novo HLA antibodies after transplant, respiratory infections, and microvascular injury of small airways have all been identified as risk factors.

The term **bronchiolitis obliterans syndrome** was introduced in 1993 and is currently defined as an obstructive process characterized by persistent decline in the baseline forced expiratory volume in 1 second (FEV_1) or mid-FEF ($FEF_{25–75}$) over a specified period of time [219, 220]. In the latest revision, the concept of partial or complete reversibility is now officially recognized [221]. An essential principle is the exclusion of other causes of airway obstruction such as anastomotic narrowing, infection, acute rejection (ACR or AMR), or recurrent/progressive COPD. The numeric grades of BOS (grade 0–3) reflect increasing severity of obstruction. Two patterns have been identified on expiratory phase high-resolution computed tomography (HRCT) imaging, which predict to some degree of accuracy the responsiveness to azithromycin therapy. The component of bronchiolitis correlates with air trapping or hyperlucency, peribronchiolar thickening, and tree-in-bud alterations, while the OB-related form shows septal thickening, bronchiectasis, and mosaic patterns of parenchymal attenuation [222–225].

The 1990 ISHLT Working Formulation enumerated different patterns of airway luminal alteration along with the presence or absence of an inflammatory component. The lesions were classified as total or subtotal luminal narrowing and active or inactive depending on the presence or absence of bronchiolar and/or peribronchiolar mononuclear cell infiltrates [86]. In the 1996 ISHLT revision, the patterns of "total" and "subtotal" were eliminated, but the designation of inflammatory activity was retained [87]. In the 2007 ISHLT Grading Scheme, the classification was further simplified to the absence (grade C0) or presence (grade C1) of OB [88] (Table 3).

The macroscopic features of OB can be subtle and obscured by adjacent parenchymal infection or scarring. Nodular thickening of bronchovascular bundles can often be palpated. **The histopathologic hallmarks of OB** are fibroinflammatory lesions centered on membranous and respiratory bronchioles and mature, eosinophilic collagenous submucosal fibrosis producing partial or complete obliteration of the lumens (Fig. 23). When present, mononuclear inflammatory cells are distributed within the submucosal

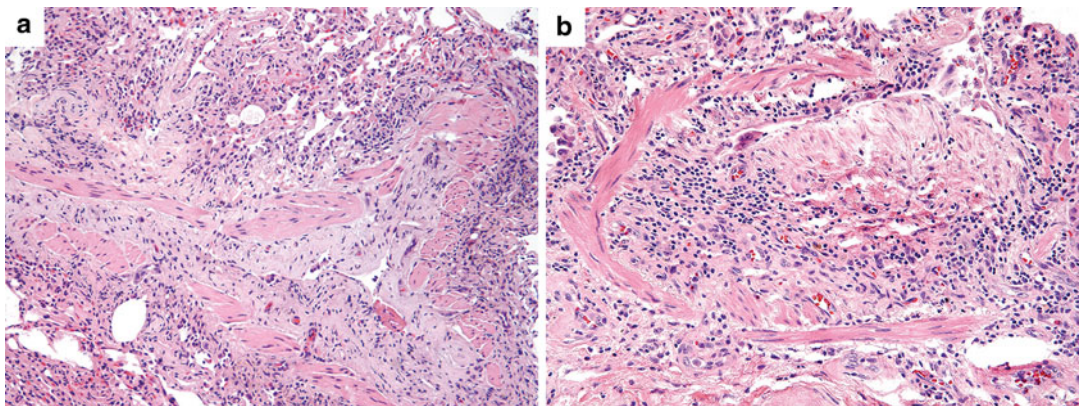

Fig. 23 Obliterative bronchiolitis. (**a**) Longitudinal section of bronchiole showing luminal narrowing by submucosal fibrous proliferation with only occasional admixed chronic inflammatory cells. (**b**) Near complete luminal obliteration by mature collagenous fibrosis and moderate numbers of chronic inflammatory cells. The epithelial lining cells are lost

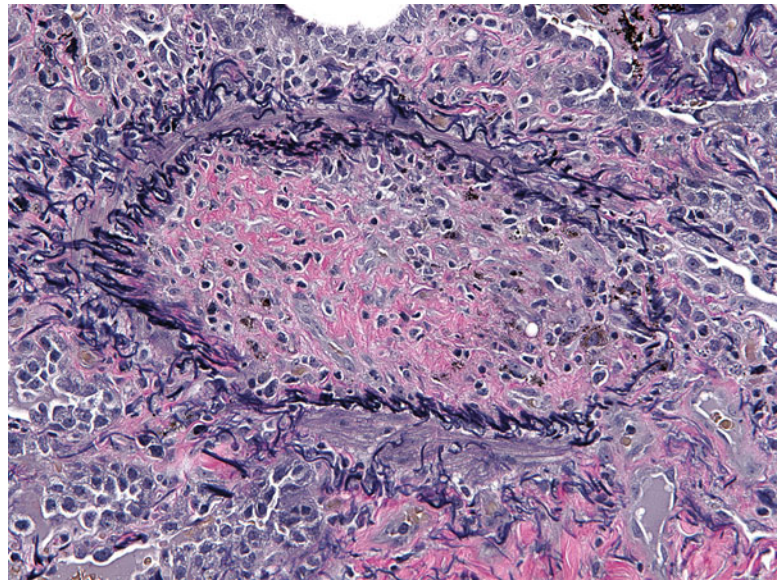

Fig. 24 Cicatricial phase of obliterative bronchiolitis characterized by complete luminal replacement by scar tissue. The elastic membranes and smooth muscle layer are preserved and are a clue to the presence of OB at scanning magnification

fibrosis and/or in the peribronchiolar tissue spaces. In early lesions, abundant inflammatory and fibromyxoid aggregates may overlie and even obscure the submucosal scarring. Late OB lesions with complete luminal obliteration may be skipped over on routine stains and connective tissue stains such as elastin-van Gieson (EVG), Masson's trichrome, or combined Masson's trichrome/elastic Verhoeff–van Gieson stains are helpful in identifying small airways. In the late cicatricial stages of OB, lumens are filled with mature collagenous tissue. Disruption of the elastic membrane and atrophy of the muscular layer of the airway are also seen (Fig. 24). The scar tissue can extend distally into the alveolar ducts and sacs and septal scarring is often present. Mucostasis and/or intraluminal foam cells in the distal airways are useful markers of airway obstruction (Fig. 25). Bronchiectasis and bronchiolectasis are usually found at postmortem or at the time of retransplantation in cases of advanced OB, but is not discernible in TBBx specimens [226]. Bronchiectasis/bronchioloectasis is characterized by mucous plugging, goblet cell hyperplasia, squamous metaplasia, and extensive mural inflammation.

The clinical course of patients with BOS is variable and difficult to predict. Some progress rapidly to end-stage pulmonary failure, while others show periods of stabilization of PFTs. Historically, patients were subjected to trials of high-dose corticosteroid therapy to arrest the pace of functional loss, but current guidelines proscribe against this practice on account of its detrimental side-effects and lack of effectiveness. Therapeutic approaches are now individualized and include substitution of tacrolimus for cyclosporine, a trial of 3 or more months of azithromycin antibiotic, surgical fundoplication for patients with GERD with aspiration, and retransplantation in carefully selected patients [217].

Restrictive Allograft Syndrome

Although there is currently no uniformly accepted definition of restrictive allograft syndrome (RAS), most patients present with restrictive physiology characterized by a persistent drop in total lung capacity (TLC) >10 % from baseline that is usually accompanied by a drop in FEV_1 of >20 % [227]. Some centers use forced vital capacity (FVC) and the FEV_1:FVC ratio as a surrogate marker of TLC [228]. The prognosis is poor and overall appears to be worse than BOS, although both share similar risk factors [229]. As this entity has only recently been recognized, the clinical, radiologic, and histopathologic details and diagnostic criteria remain to be fully elucidated.

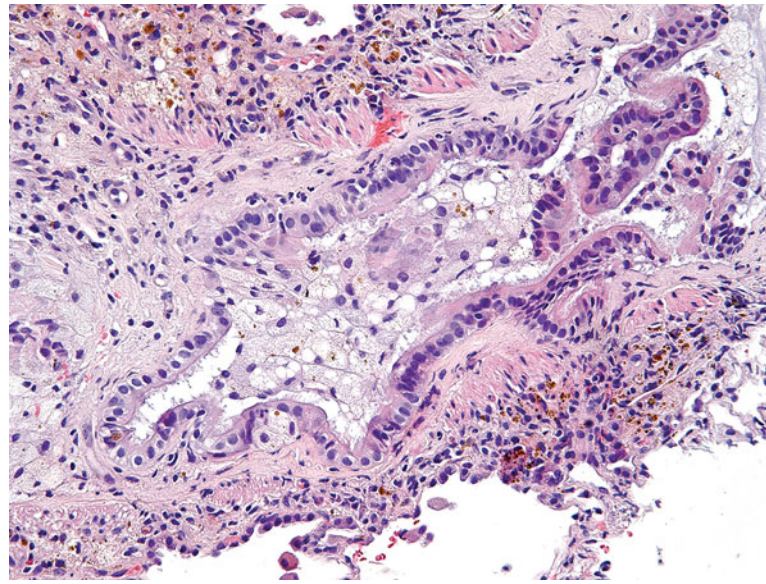

Fig. 25 Early OB with collections of foamy macrophages within the lumen and mild submucosal fibrosis. The epithelial lining cells are preserved and the lumen is nearly normal in diameter

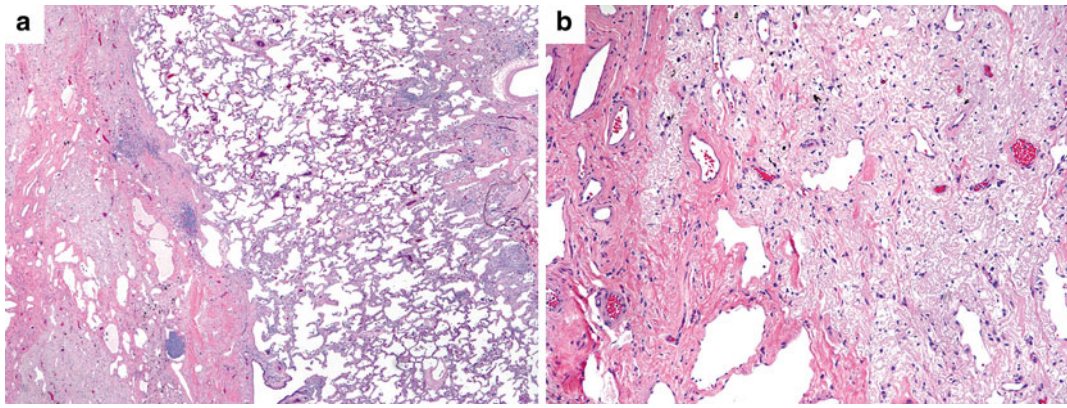

Fig. 26 (**a**) Restrictive allograft syndrome (RAS) showing a sharp delineation of the fibroelastotic tissue from the adjacent lung parenchyma. (**b**) High power of RAS lesion displaying admixtures of elastic and collagen fibers and variably sized vascular spaces

The radiologic findings reflect the ILD pattern of disease and include traction bronchiectasis, central and peripheral consolidation, pleural thickening, and volume loss in an upper lobe-predominant distribution [227, 228, 230]. Interestingly, these findings may be present prior to the onset of changes in pulmonary function tests. **The histopathologic findings** have been described by Hwang and colleagues from Toronto [231] from wedge biopsy, postmortem, and retransplant specimens: pleural fibrosis and subpleural fibroelastosis were the most common findings and most patients had concurrent OB either within or adjacent to the fibroelastotic regions and/or regions of DAD (Fig. 26). Paraseptal, centrilobular, and parenchymal fibroelastosis were found in around a third of the cases. There was a sharp delineation of the fibroelastosis from adjacent "normal" lung tissue with foci of organizing pneumonia/fibroblastic foci in the intervening regions. Although honeycomb change was distinctly uncommon, NSIP-like changes were found in up to a quarter of cases.

Currently, there is no directed or successful treatment for RAS. There are anecdotal reports of minor responses to pirfenidone and alemtuzumab. Retransplantation has shown poorer 3-year survival in RAS compared to BOS (34 % vs. 68 %) [232].

Acute Fibrinoid Organizing Pneumonia

Paraskeva and colleagues from Melbourne [233] recently reported a series of 22 patients who developed a clinicopathologic form of CLAD that differed from both BOS and RAS. The histopathologic findings were primarily peribronchiolar alveolar airspace accumulations of fibrin without the classic hyaline membranes (Fig. 27). Sparse inflammation and interstitial thickening were present. The radiologic findings reflected the morphology with ground glass opacities and interlobular septal thickening; peripheral fibrosis and consolidation were each observed in a quarter of the cases. Patients presented with a fulminant clinical course that rapidly progressed to respiratory failure. There are significant clinical and morphologic differences between acute fibrinoid organizing pneumonia (AFOP), RAS, and BOS (Table 4). It is unclear if there is a direct link between AFOP and RAS, but there is limited overlap between the two processes. To date, there are no predisposing risk factors or effective therapies and additional studies are needed. A recent report highlighted a similar histopathologic pattern in influenza A/H1N1 pneumonia [234].

Neutrophilic-Reversible/Azithromycin-Responsive Allograft Dysfunction

NRAD or ARAD is another form of CLAD characterized by clinical evidence of BOS, increased numbers of neutrophils in BAL samples, and improvement of PFTs (increased FEV_1) following a prolonged (2–3 month) course of neomacrolide antibiotics like azithromycin [216, 235]. Several studies have shown that BAL neutrophilia at 3 and 12 months following transplant is predictive for the development of BOS. The mechanism is likely through repetitive epithelial injury and fibroinflammatory airway injury by neutrophilic release of matrix metalloproteinases, chemokines, growth factors, and oxidative stress. Azithromycin exhibits both antimicrobial and anti-inflammatory effects, and in some studies, up to 40 % of patients with BOS showed sustained improvement in their FEV_1 of >10 % and a small number of patients reversed their BOS score to BOS 0 [236, 237]. Some investigators have recommended a trial of azithromycin therapy for all patients with BOS (regardless of BAL neutrophils counts).

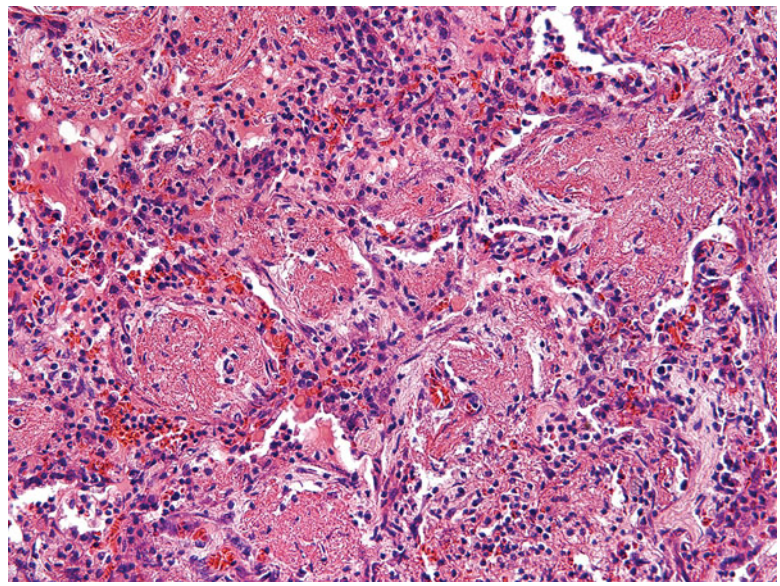

Fig. 27 Acute fibrinous pneumonia characterized by filling and expansion of alveolar spaces by fibrinous exudates. The alveolar lining cells are hyperplastic and a sparse interstitial inflammatory infiltrate may be present

Table 4 BOS/RAS/AFOP/NRAD

Entity	Classic BOS	RAS	AFOP	NRAD
Onset after transplant	Late	Late	Late	Early post-transplant
Clinical presentation	None until substantial drop in FEV_1	Coarse crackles	Rapid drop in FEV_1	Increased sputum; crackles
Radiologic findings	Air-trapping and mosaic attenuation	Upper lobe fibrosis, consolidation or reticular infiltrates	Bilateral GGO and intralobular septal thickening	Centrilobular nodules, tree-in-bud, +/– bronchiectasis
BAL findings	No inflammation	+/– Inflammation	Not reported	Increased neutrophilia
Pulmonary function testing	Obstructive	Restrictive	Nonobstructive	Obstructive
Histopathologic findings	OB lesions	Subpleural fibroelastosis +/– OB and DAD lesions	Alveolar and peribronchiolar fibrinous exudates	Airway inflammation
Response to azithromycin	None	None	None	Responsive
Clinical course & prognosis	Slowly progressive with protracted course	Progressive decline with periods of stabilization	Unrelenting clinical decline and early death	Good

Modified from Ref. [216, 225, 233]
AFOP acute fibrinoid organizing pneumonia, *BOS* bronchiolitis obliterans syndrome, *DAD* diffuse alveolar damage, *FEV_1* forced expiratory volume in 1 s; NRAD, neutrophilic-reversible/azithromycin-responsive allograft dysfunction, *OB* obliterative bronchiolitis, *RAS* restrictive allograft syndrome

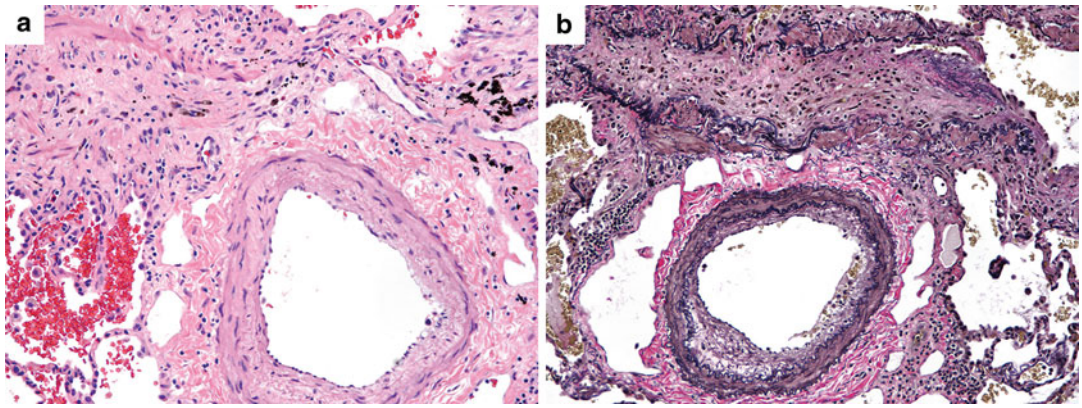

Fig. 28 (**a**) Chronic vascular rejection characterized by concentric intimal thickening of a muscular pulmonary artery. Note the OB lesion in the adjacent bronchiole (top left). (**b**) Corresponding EVG stain highlighting the intimal lesion in the artery and the complete luminal narrowing of the airway lumen in OB

Transplant-Associated Vasculopathy

The arteries and veins in the lung allograft are susceptible to the development of TAV. The lesions share the same fibrointimal alterations that are observed in other solid organ transplants and are currently designated as Grade D in the 2007 ISHLT Working Formulation. Coronary artery TAV is a frequent complication in combined heart–lung recipients [238]. In our experience, these lesions are easily found in retransplant and postmortem specimens, but are uncommon in TBBx specimens. A connective tissue stain is often helpful in outlining the components of vessels. TAV lesions usually accompany OB and are generally clinically insignificant (Fig. 28). A recent study reported hemodynamic evidence of pulmonary hypertension in some patients [239]. Concentric intimal thickening composed of

smooth muscle cells and myofibroblasts, variable lymphocytes, and macrophages including foam cells are found. On occasion, superimposed thrombosis complicates the lesions. Intimal sclerosis of veins and venules is commonly found in TBBx specimens and should not been designated as chronic rejection in isolation.

Recurrent Disease in the Allograft

As previously discussed, recurrence of lung adenocarcinoma has been documented in the allograft and is one argument against transplantation for this diagnosis. Other disorders have been reported in the transplanted lung including an array of smoking-related and different ILD patterns such as pulmonary Langerhans' cell histiocytosis, desquamative interstitial pneumonia (DIP), bronchiolitis-respiratory-interstitial lung disease (RBILD), and hypersensitivity pneumonitis. With the exception of PVOD and PCH recurrent pulmonary vascular disorders such as IPAH have not been recorded (Table 5). In our experience, sarcoidosis and lymphangioleiomyomatosis are the most common lesions and are usually detected as incidental findings on surveillance TBBx [61, 240–248]. The histopathological changes are similar to the native disease (Fig. 29). Awareness of the pretransplant diagnosis is essential prior to evaluating any TBBx. Infection should always be excluded in cases of granulomatous pneumonitis following transplantation.

Table 5 Recurrent pulmonary disorders in the lung allograft

Sarcoidosis
Lymphangioleiomyomatosis
Pulmonary adenocarcinoma
Desquamative interstitial pneumonia (DIP)
Giant cell interstitial pneumonia (GIP)
Hypersensitivity pneumonitis
Pulmonary alveolar proteinosis (PAP)
Idiopathic pulmonary hemosiderosis (IPH)
Diffuse panbronchiolitis
Pulmonary Langerhans' cell granulomatosis
Pulmonary veno-occlusive disease

Summary

Over the last 35 years, there has been tremendous progress in the field of lung transplantation. That said, there are a number of issues that need to be resolved if the survival rates at 1-, 5-, and 10-years are to reach the same level as other solid organ transplant programs. There continues to be refinement of selection criteria and expansion of both the indications for transplant and the overall number of candidates. The need for increasing the donor pool is critical. The use of "marginal" and reconditioned organs through EVLP has helped to identify organs that previously would have been considered unsuitable for transplant. Currently, there are only a few clinical EVLP programs in Europe and North America. With their continued achievements, the technology should become routine in most centers in the near future. This needs to be balanced with the risks of infection and PGD and their effects on overall survival, morbidity, and the risks for the development of CLAD. The therapeutic armamentarium must be expanded beyond conservative postoperative management for PGD and new interventions besides inhaled nitric oxide, inhaled surfactant, and C1-estaerase-inhibition must be found.

The diagnosis and treatment of ACR has become standardized and severe ACR is now uncommon. There have been very few new immunosuppressive drugs over the last decade in part because the challenges of drug development and multicenter trails are now daunting. Akin to the developments in PGD, the ideal approach would be to create personalized patient profiles through molecular technologies that would optimize immunosuppressive drug regimes, identify risks for infection, and CLAD. As the diminished costs and ever-increasing sophistication of molecular testing evolve, the concept of "precision transplant medicine" may become routine.

AMR has become a recognized but perplexing entity in lung transplantation. The role of de novo DSA in the development of acute graft failure and later complications such as BOS and RAS is firmly established from the medical literature. With the recent formulations of definitions and

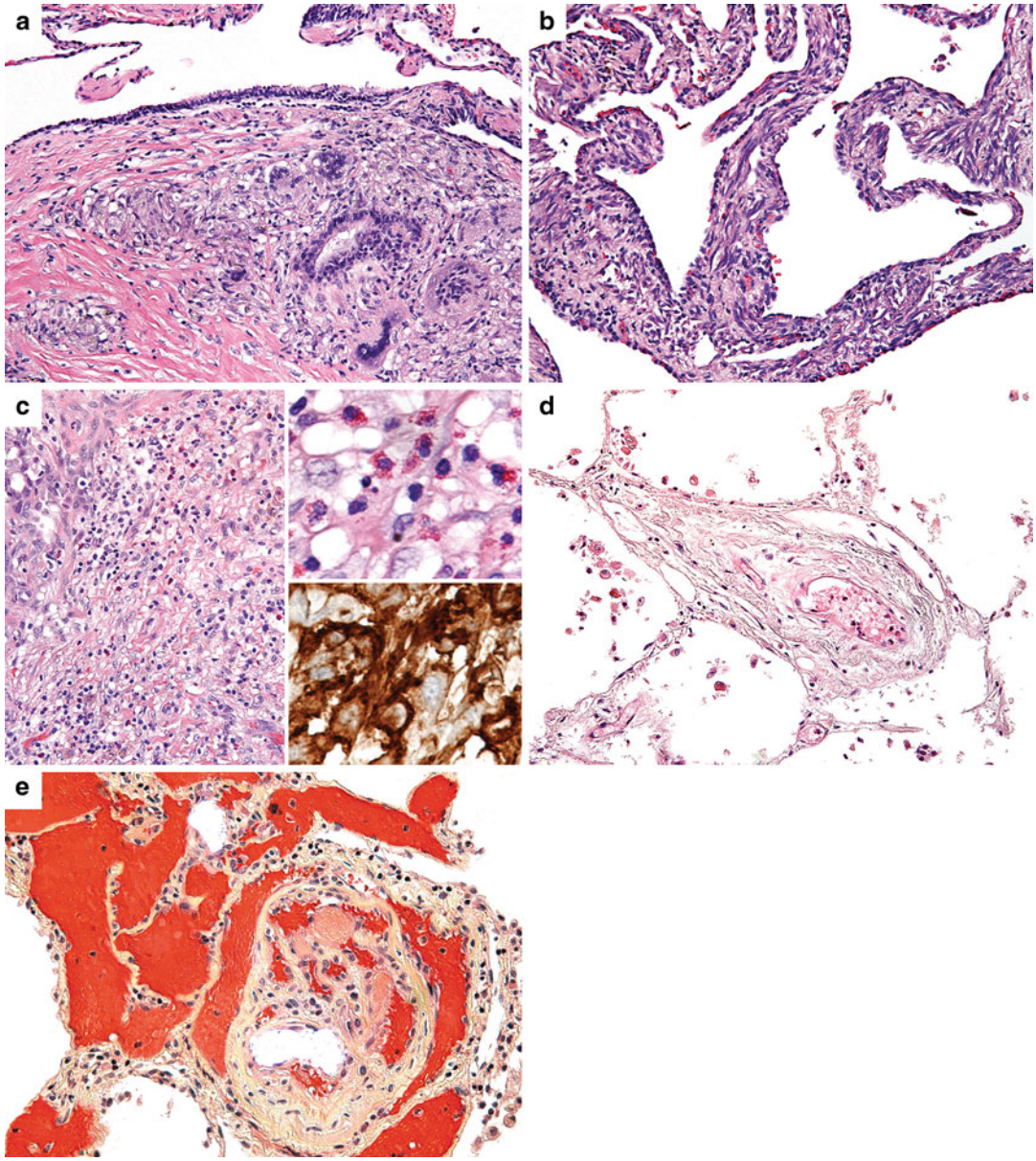

Fig. 29 Pulmonary disorders that can recur in the transplanted lung. (**a**) sarcoidosis. (**b**) lymphangioleiomyomatosis (LAM). (**c**) Pulmonary Langerhans' cell histiocytosis in the smoker. *Upper inset*: Classic angulated nuclei of Langerhans cells and eosinophils. *Lower Inset*: CD1a staining of Langerhans cells. (**d**) Pulmonary veno-occlusive disease. (**e**) Intravascular foreign material and associated hypertensive changes in an intravenous drug abuser

diagnostic criteria, clinicians now have the opportunity to investigate AMR at multiple levels. The role of histopathology and immunopathology in particular is unsettled at this time. To date, there are no diagnostic histopathologic features for AMR and those with acceptable specificity appear to be infrequent. Likewise, C4d immunostaining appears to be an infrequent finding in patients with allograft dysfunction and de novo DSA. A more specific set of pathologic markers will likely be required. At the present time, however, the pathologist plays an important

role in excluding other diagnostic possibilities and the diagnosis of AMR requires a multidisciplinary approach.

CLAD has now evolved from the singular BOS to a number of additional entities such as RAS and neutrophilic-reversible/azithromycin-sponsive allograft dysfunction. Unfortunately, CLAD continues to limit long-term survival and the pathogenesis is both complex and multifactorial. Like AMR, it requires a multidisciplinary approach for diagnosis and treatment options remain limited. The identification of azithromycin as a novel therapeutic intervention for airway inflammation in patients with BOS provides at least a temporizing step. Unfortunately, until more precise pathogenetic mechanisms are elucidated, preventative methods remain elusive.

The role of artificial lung technology and xenotransplantation in clinical lung transplant provides exciting theoretic options for addressing the shortage of donors. Improvements in ECMO technology and mounting clinical experience provide clinicians with opportunities to bridge patients to either recovery or transplant. Progress continues in bioartificial lung technology and tissue engineering through decellularization and reconstitution with stem cells such as induced pluripotent stem cells. There are a variety of technical and immunologic hurdles, but the concept of tissue engineered lung transplantation is an exciting endeavor [249]. Work on xenotransplantation continues in a limited number of centers. Like tissue engineering, there are daunting technical, immunologic, and ethical barriers that will need to be resolved in the future.

The future brings many challenges and the pathologist will continue to be an integral member of the multidisciplinary transplant team.

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