

The Pathology of Lung Allograft Rejection: A Concise Review

Akcığer Allograft Reddinin Patolojisi: Bir Kısa Derleme

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ABSTRACT

Lung transplantations in humans have been performed for almost 50 years. However, allograft rejection, non-rejection diseases such as harvest/reperfusion injury, infection, drug toxicity, post-transplant lymphoproliferative diseases, and recurrent disease are still significant complications. Although the clinical impression might suggest the possibility of any of these conditions, tissue diagnosis is usually necessary to establish a definitive diagnosis. This article mainly focuses on reviewing the morphological features of lung allograft rejection and its grading according to the revised 2007 ISHLT consensus classification of allograft rejection. Acute and chronic alloreactive injuries affect both the vasculature and the airways. Currently, the guidelines of the 2007 ISHLT consensus conference are used for the histopathologic assessment of rejection. Although antibody mediated rejection is recognized in heart and kidney transplants, at present, there is no consensus about its diagnosis in transplanted lungs. Mimickers of rejection and post-transplant diseases will also be discussed. The collaboration between the transplant clinician and pathologist cannot be overemphasized to establish an optimal treatment for the individual patient following lung transplantation.

(*Türk Toraks Derg* 2012; 13: 122-9)

Key words: Transplantation, lung, rejection, CMV

Received: 05.07.2012

Accepted: 17.07.2012

INTRODUCTION

The first human lung transplantation was performed in 1963. Advances in immunosuppression and surgical techniques led to a rapid increase in lung transplantations in the 1980s. However, despite progress in immunosuppression as well as operative management, lung preservation, and critical care, long-term survival in lung transplantation remains limited. The main barriers are: (i) harvest/reperfusion injury, (ii) acute rejection, and (iii) the development of bronchiolitis obliterans (chronic airway rejection). Most patients develop at least one episode of acute rejection within the first 3 weeks following transplantation and 35% of patients experience at least one episode in the first year [1]. Risk factors for acute

ÖZET

İnsanlarda akciğer nakli yaklaşık 50 yıldır yapılmaktadır, buna rağmen allograft reddi ve ürün/reperfüzyon hasarı, enfeksiyon, ilaç toksisitesi, nakil sonrası lenfoproliferatif hastalıklar gibi reddetme-dışı hastalıklar ve rekürren hastalık hâlâ önemli komplikasyonlardır. Klinik izlenim bu durumlardan birinin olasılığını düşündürebilmekle beraber, kesin tanıya ulaşmak için genellikle doku tanısı gereklidir. Bu makale esas olarak akciğer allograft reddinin morfolojik özelliklerinin gözden geçirilmesine ve allograft reddinin yenilenmiş 2007 ISHLT konsensus sınıflandırmasına göre derecelendirilmesine odaklanmaktadır. Akut ve kronik alloreaktif hasarlar hem damar hem de hava yollarını etkilemektedir. Halen, reddin histopatolojik değerlendirmesi için 2007 ISHLT konsensus konferansının kılavuzu kullanılmaktadır. Kalp ve böbrek nakillerinde antikor aracılı reddetme tanınmış olmakla birlikte, şu an için, nakledilmiş akciğerlerde bunun tanısı için bir konsensus bulunmamaktadır. Nakil sonrası hastalıklar ve reddin taklitçileri de tartışılacaktır. Akciğer naklini takiben bir hastanın optimal tedavisini sağlamak için, nakil doktoru ve patoloğ arasındaki işbirliği son derece önemlidir.

(*Türk Toraks Derg* 2012; 13: 122-9)

Anahtar sözcükler: Nakil, akciğer, red, CMV

Geliş Tarihi: 05.07.2012

Kabul Tarihi: 17.07.2012

rejection include HLA mismatch, immunosuppression, recipient factors and infections [2-4]. Acute rejection as well as non immune factors, including respiratory tract infection (CMV or non-CMV viral infection), gastroesophageal reflux with resultant aspiration, and increased eosinophils in post-transplant biopsies, are risk factors for obliterative bronchiolitis (OB) [5-8]. OB, the predominant form of chronic rejection, is the most common late cause of morbidity and mortality after lung transplantation and occurs in 49% of lung transplant patients by 5 years and 79% by 10 years [9].

Allograft rejection, the host's response to the foreign graft, affects both the vasculature and the airways of the donor lung [10]. Transbronchial biopsies are usually used

Presented in part at the Turkish Thoracic Society, Antalya Turkey, April 12, 2012

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to evaluate for rejection. Only occasionally, wedge biopsies, explants for retransplant or autopsy specimens are available for review. While acute rejection is characterized by perivascular mononuclear cell infiltrates and lymphocytic bronchiolitis, fibrous scarring of bronchioles and fibrointimal changes of pulmonary arteries and veins are the hallmark of chronic rejection.

Unfortunately, transbronchial biopsy has a low sensitivity (28%) and specificity (75%) for OB, probably due to sampling issues [11]. Therefore, the bronchiolitis obliterans syndrome (BOS) [11] is a clinically defined syndrome of chronic lung transplant rejection based on pulmonary function criteria, specifically FEF_{25-75} [12]. No particular marker is currently available that can predict the risk for OB [13].

Hyperacute Rejection

Hyperacute rejection, a type II hypersensitivity reaction, occurs within minutes to a few hours after perfusion of the transplanted organ. It is mediated by preexisting antibodies to ABO blood groups, human leukocyte antigens (HLA) class I, or antigens on graft vascular endothelial cells. Antigen-antibody binding initiates activation of complement and cytokines, which results in endothelial cell damage and platelet activation with subsequent vascular thrombosis and graft destruction. Overall, in 3 (of 6) patients with hyperacute rejection of a lung graft, pretransplant PRAs were negative, however, the crossmatch was positive in all cases with anti-A2, the most common identified antibody [14]. The outcome is usually fatal.

The graft grossly appears edematous and cyanotic. Histologically, platelet thrombi, neutrophilic infiltration, fibrin thrombi, necrosis of vessel wall, and morphologic features of diffuse alveolar damage (DAD) have been described [15]. Autopsy findings include red hepatization and firm consistency of the transplanted lung with microscopic evidence of acute lung injury [15].

2007 ISHLT Revised Consensus Classification for Lung Allograft Rejection [10] (Table 1)

The ISHLT consensus classification for lung allograft rejection was developed as a "working formulation" for the diagnosis of lung rejection by transbronchial biopsy [16].

It is solely based on histopathologic features as evaluated on hematoxylin & eosin (H&E) stained slides.

Acute rejection-A Grade

The A-Grade defines the absence or presence of perivascular chronic inflammatory (mononuclear) infiltrates with or without inflammation of the inner wall or endothelial lining of arteries or veins (intimitis, endotheliitis)

Table 1. Classification of allograft rejection according to 2007 revised ISHLT consensus classification of lung allograft rejection [10]

Acute rejection	
A0	None
A1	Minimal
A2	Mild
A3	Moderate
A4	Severe
Airway inflammation	
B0	None
B1R	Low grade
B2R	High grade
BX	Ungradeable
Chronic airway rejection (Obliterative Bronchiolitis)	
C0	None
C1	Present
Chronic vascular rejection	
D0	None
D1	Present

R: denotes revised

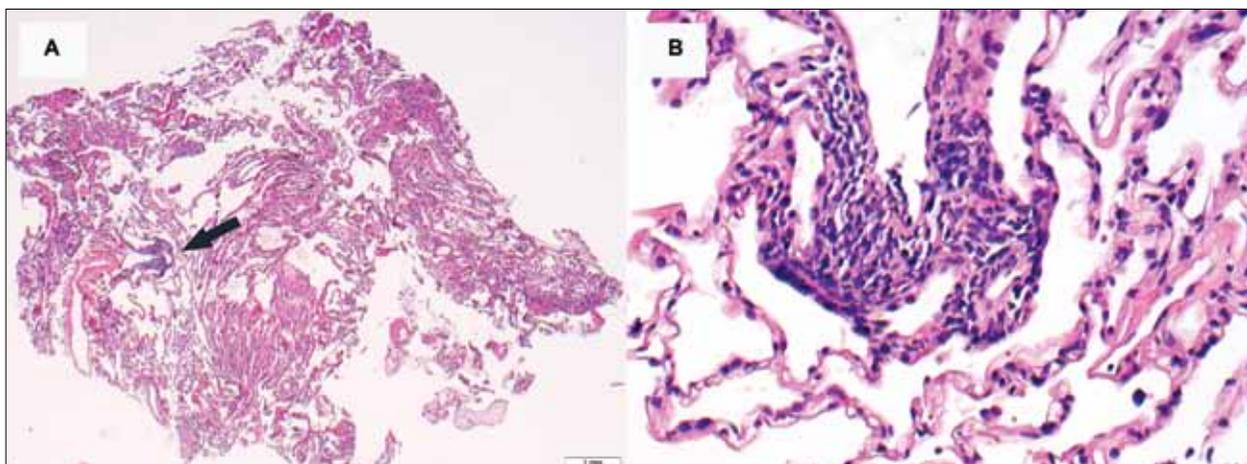


Figure 1. Mild acute rejection (ISHLT Grade A2): At low power, a cellular infiltrate surrounding small vessels is readily apparent (arrow) (A). High power view reveals multiple layers of chronic inflammatory cells surrounding a small vessel without extension into the adjacent interstitium (B). Hematoxylin&Eosin (H&E) staining. Magnification, x40 (A), x400 (B)

and may extend into the interalveolar septa and into the alveoli in higher grades. The majority of chronic inflammatory cells in acute rejection are T cells, although B cells and eosinophils have also been described [10].

No acute rejection (ISHLT Grade A0): Normal pulmonary parenchyma is present.

Minimal acute rejection (ISHLT Grade A1): A few blood vessels, particularly venules, in the alveolated lung parenchyma, are surrounded by a relatively thin but usually complete rim of chronic mononuclear infiltrate without extension into the adjacent interalveolar septa. Endotheliitis and eosinophils are absent.

Mild acute rejection (ISHLT Grade A2): The perivascular cellular infiltrate has more layers and there are more frequent infiltrates than in grade A1. The infiltrates are typically obvious at scanning magnification. The infiltrates usually consist of a mixture of lymphocytes, plasmacytoid lymphocytes, macrophages and rare eosinophils and generally do not extend into the interstitium (Figure 1A, B). Endotheliitis can occur.

Moderate acute rejection (ISHLT Grade A3): Easily recognizable dense perivascular mononuclear cells infiltrate cuff venules and arterioles and infiltrate into interalveolar septa (Figure 2A, B). Endotheliitis is quite frequent. Eosinophils and even occasional neutrophils are common [10]. Intra-alveolar macrophages are found in areas of septal cellular infiltrates. Type II pneumocyte hyperplasia and histologic features of acute lung injury may become apparent.

Severe acute rejection (ISHLT Grade A4): Diffuse perivascular, interstitial and air space infiltrates of mononuclear cells with prominent alveolar pneumocyte damage and endotheliitis characterize grade A4 (Figure 3A-C). Intra-alveolar necrotic epithelial cells, macro-

phages, eosinophils, hemorrhage and neutrophils may be apparent and morphologic evidence of acute lung injury in the form of organizing pneumonia or hyaline membranes are usually seen. Parenchymal necrosis, infarction or necrotizing vasculitis might be present, however, these features are more evident on surgical rather than transbronchial lung biopsies. A paradoxical diminution of perivascular infiltrates can occur as cells extend into alveolar septa. This grade can sometimes be difficult to distinguish from an infectious process, harvest/reperfusion injury or drug toxicity. However, the presence of perivascular inflammation is helpful in establishing the diagnosis.

Airway Inflammation-B Grade

This grade applies only to small airways. The pathology report should state whether or not small airways are present. Large airways, if present, should be described separately. The "R" behind grades 1 and 2 denotes the revised 2007 version.

No airway inflammation (ISHLT Grade B0): The small airways appear unremarkable.

Low-grade small airway inflammation (ISHLT Grade B1R): Lymphocytes are identified within the submucosa of the bronchioles (Figure 4A). The lymphocytic infiltrates can be infrequent and scattered or form a circumferential band. Intra-epithelial lymphocytic infiltration is usually not significant. Although occasional eosinophils may be seen within the submucosa, there is no evidence of epithelial damage, neutrophils, necrosis, ulceration or significant amount of nuclear debris.

High-grade small airway inflammation (ISHLT Grade B2R): A marked lymphocytic infiltrate of the airway epithelium and airway wall is apparent. A greater number of eosinophils and plasmacytoid cells are present within the submucosa. Epithelial damage is characterized by necrosis,

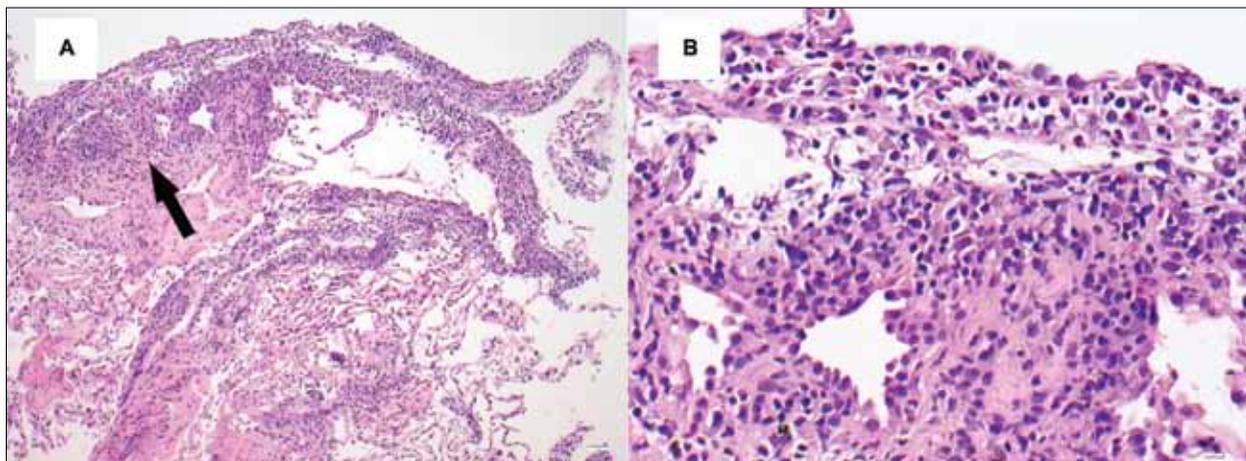


Figure 2. Moderate acute rejection (ISHLT Grade A3): At low power, a prominent cellular infiltrate cuffs vessels and extends into the adjacent interstitium (arrow) (A). The infiltrate is comprised of chronic inflammatory cells including eosinophils (B). H&E staining. Magnification, x100 (A), x400 (B)

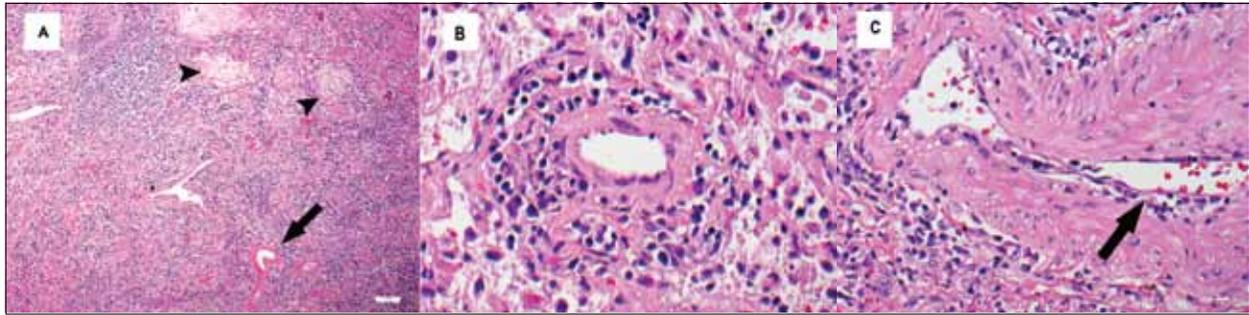


Figure 3. Severe acute rejection (ISHLT Grade A4): Low magnification shows thickened interstitium predominantly due to a cellular infiltrate. Intraalveolar plugs of proliferating fibroblasts (arrow head) are suggestive of an organizing process. Hyaline membranes (arrow) lining alveolar septae are indicative of diffuse alveolar damage (A). Although the cellular infiltrate is predominantly in the interstitium, perivascular infiltrates are also identified (B). Endotheilitis, characterized by small lymphocytes within the endothelial lining, is apparent (C). H&E staining. Magnification, x100 (A), x400 (B, C)

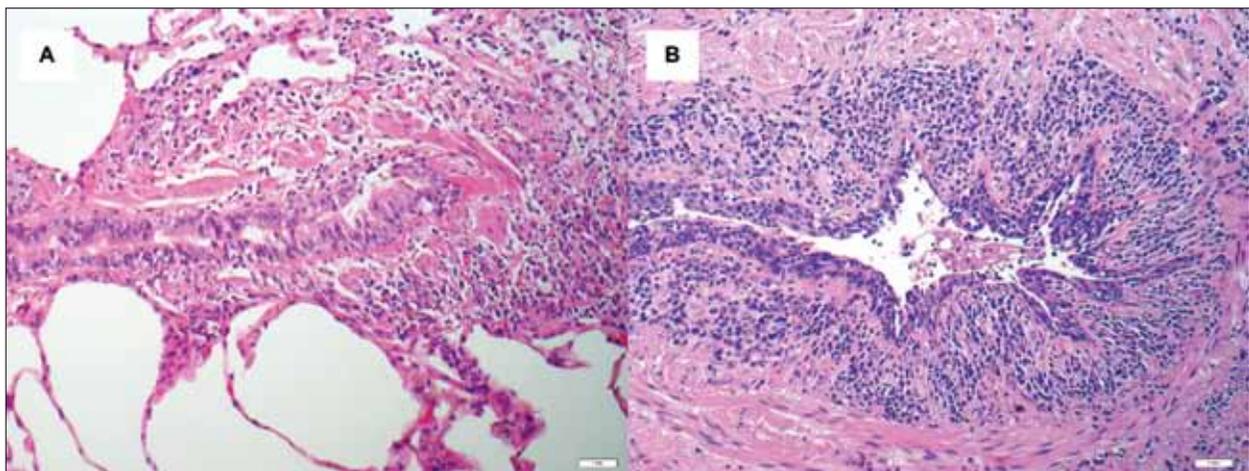


Figure 4. Airway inflammation. A. Low grade small airway inflammation (ISHLT Grade B1R): A chronic inflammatory infiltrate is present in the submucosa of a small airway largely sparing the respiratory epithelium. B. High grade small airway inflammation (ISHLT Grade B2R): The cellular infiltrate involves the submucosa and mucosa of the small airway. Neutrophils, plasma cells, and eosinophils accompany the infiltrate. Squamous metaplasia is present. H&E staining. Magnification, x200 (A, B)

metaplasia and marked intra-epithelial lymphocytic infiltration (Figure 4B). In its most severe form, high grade airway inflammation is associated with epithelial ulceration, fibrino-purulent exudate, cellular debris and neutrophils. It is important to exclude an infectious process [10].

Ungradeable small airways inflammation (ISHLT Grade BX): Small airways cannot be evaluated due to sampling problems, infection, tangential cutting, artifact etc.

Chronic Airway Rejection C-Grade

The working formulation has equated OB with the C-grade. This grade is restricted to submucosal and intraluminal scarring of small airways. When large tissue sections of the lung are examined, the process of OB is pan-lobar but patchy.

No chronic airway rejection (ISHLT Grade C0): The small airways appear similar in size to the accompanying artery with a ragged inner surface. Fibrosis is not present.

Chronic airway rejection (ISHLT Grade C1): Airways are narrowed due to fibrosis in the airway wall. The fibrosis is usually eccentric (Figure 5A, B). In the

acute phase, OB is characterized by loose myxoid granulation tissue with variable numbers of inflammatory cells filling or partially obstructing the airway lumen, resembling "organizing pneumonia". In later phases, OB may consist of eccentric, occasionally confluent plaques of dense hyalinized collagen attached to the wall of bronchioles. Metaplastic squamous or cuboidal epithelium may cover these bronchiolar scars. In other airways, a slit-like lumen may remain as a result of a confluent submucosal scar or intraluminal polyps of scar tissue. Capillaries supplying these intraluminal masses of collagen are occasionally prominent. In the most severe cases, the bronchiolar lumen can be entirely occluded by dense scar tissue and be recognizable only with the aid of an elastic stain, its location adjacent to an artery, and by the presence of residual circumferential smooth muscle. An elastic stain is invaluable in assessing the presence of OB and is particularly helpful in transbronchial biopsies specimens.

Chronic Vascular Rejection D-Grade

No chronic vascular rejection (ISHLT Grade D0): The pulmonary arteries appear of a similar size as the accompanying airway. The intima is slender, the media not thickened.

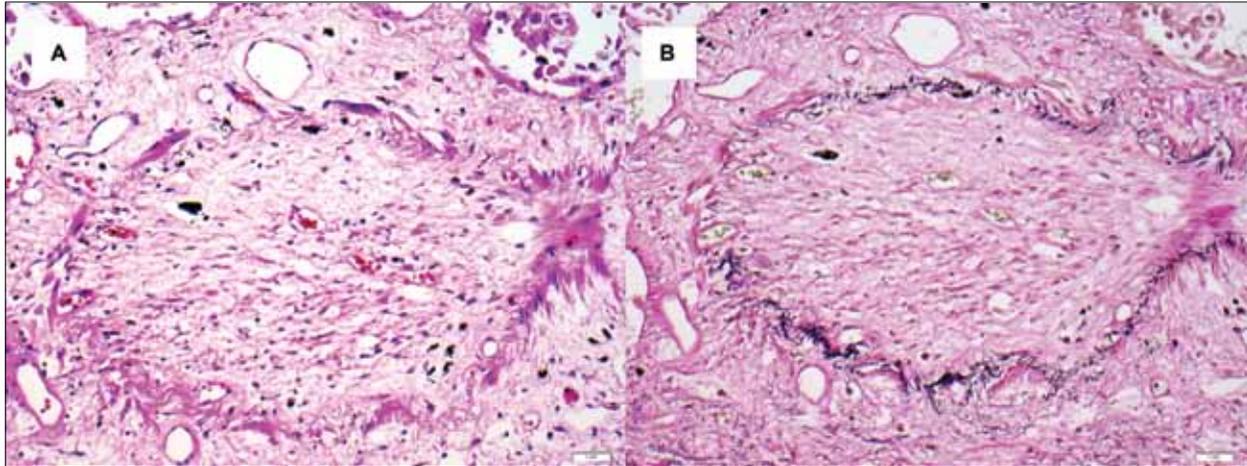


Figure 5. Chronic airway rejection (obliterative bronchiolitis) (ISHLT Grade C1): The lumen of the airway is occluded by scar (A). An elastic stain (Verhoeff-Van Gieson, VVG) helps to distinguish a bronchiole from a pulmonary artery. In contrast to the pulmonary artery which has 2 continuous elastic layers, a bronchiole has only 1 elastic layer which is usually also more irregular (B). H&E staining. Magnification, x200

Chronic vascular rejection (ISHLT Grade D1):

Chronic vascular rejection is rarely identified on biopsies since they usually lack vessels of sufficient size. Wedge biopsies, explants or autopsy material may reveal this. Pulmonary arteries, and more often veins, are thickened by fibrointimal connective tissue. Thickening is usually concentric, may be patchy and typically involves smaller vascular arteries and veins. The process is similar in pulmonary veins, although the intimal deposits may be less cellular and more waxy, eosinophilic and sclerotic. Chronic vascular rejection should be distinguished from recanalizing thrombi. An elastic stain is helpful in identifying the changes.

Chronic vascular rejection in lung transplants has not resulted in graft loss, however some patients develop pulmonary hypertension, particularly those with BOS [17].

Interobserver agreement of the A grade, using criteria of the 1996 revision, varies between poor to substantial (kappa, 0.18-0.73) while intraobserver agreement was good (kappa, 0.65 and 0.795) [18]. The interobserver variability for grading small airway inflammation (B-grades) was even worse, ranging from poor to only fair (kappa, 0.035-0.3) [18]. Therefore, the B-grading was simplified from the previous five (B0-B4) (1996) grades to the current three (B0-B2R) possible grades (2007 revision). The reasons for the interobserver variability are manifold and include the distinction of rejection and chronic airway inflammation from bronchus associated lymphoid tissue (BALT), infection and other mimickers of rejection. BALT is found in the vicinity of airways, usually contains black anthracotic pigment and presents as a rather nodular collection of chronic inflammatory cells which does not surround a vessel. Mimickers of severe acute rejection include conditions that present with acute lung injury or diffuse alveolar damage (DAD) such as infection, drug toxicity, antibody mediated rejection (AMR) or harvest/reperfusion injury. Features that help to distinguish acute rejection from mimickers thereof include perivascular inflammation, viral inclusions

and stains for microorganisms including Gomori-Grocott methenamine silver stain (GMS) and acid fast bacilli (AFB). However, although helpful, perivascular inflammation is not entirely specific for acute rejection and may also be seen in CMV pneumonitis, *Pneumocystis jiroveci* pneumonia, post-transplantation lymphoproliferative disease (PTLD) and recurrent primary diseases [19].

Antibody-Mediated Rejection

Antibody mediated rejection (AMR) or humoral rejection is thought to occur due to circulating preformed antibodies or antibodies that arise *de novo* after transplantation due to HLA-mismatch. Circulating antibodies are believed to target donor major histocompatibility (MHC) antigens or other endothelial and epithelial antigens expressed in the donor lung. The binding of preexisting antibodies to donor antigens can lead to hyperacute rejection or accelerated humoral rejection and BOS [20]. Moreover, studies have shown that patients with anti-HLA antibodies have an increased incidence of acute rejection, persistent rejection, increased BOS or, worse, overall survival [20-23].

Although well described in kidney and heart transplantation, no specific histopathologic features have been associated with AMR in lung transplantation. Capillary injury/small vessel intimitis, DAD and intra-alveolar hemorrhage should raise suspicion of AMR but these are non-specific morphologic findings that can also be seen in severe acute rejection, infection or harvest/reperfusion injury. The 2007 ISHLT revised consensus classification did not agree upon any AMR-specific histopathologic features in the lung [10] but recommended a multidisciplinary approach to diagnose AMR that includes the following: (i) The presence of circulating antibodies (HLA antibodies, antiendothelial and antiepithelial antibodies), (ii) Histologic features of acute lung injury or hemorrhage (DAD, capillary injury with neutrophils and nuclear debris), (Figure 6A, B), (iii) Focal or diffuse C4d deposition (Figure 6C), and (iv) Clinical signs of graft

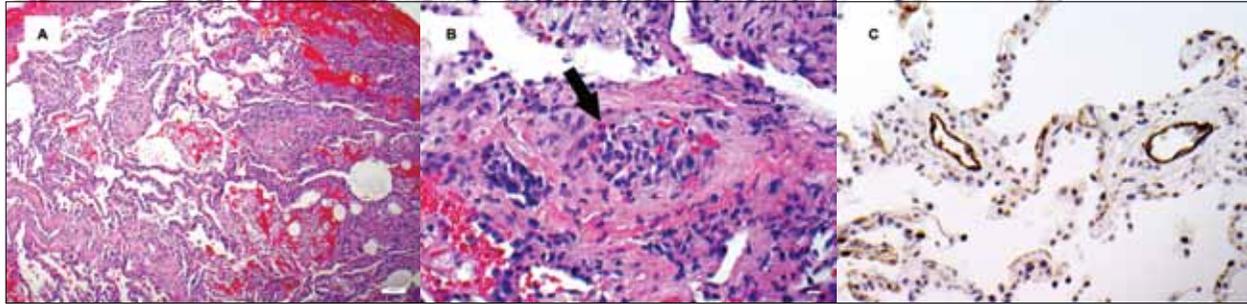


Figure 6. Antibody mediated rejection (AMR): At low power, the interstitium is thickened due to a chronic inflammatory infiltrate, fibroblasts and type II pneumocyte hyperplasia. The alveolar spaces are filled with macrophages and fibrin focally (A). A high power view reveals capillary injury with endothelial cell swelling and marginating neutrophils (arrow) (B). There is C4d deposition along the endothelium of small vessels and capillaries (C). Magnification, x100 (A), x400 (B, C)

dysfunction. If AMR is clinically, immunopathologically or histologically suspected, immunostains for C3d, C4d, CD68 and CD31 should be performed. However, the specificity of these stains in this setting has been extrapolated from kidney and heart transplant settings and, although there have been several studies advocating their use [10], their value is controversial.

Transbronchial Biopsy

Transbronchial biopsy is still the gold standard for evaluating the graft for acute and chronic rejection, infection, and possible recurrent disease since no reliable surrogate markers are clinically available to identify these patients.

The yield for acute rejection by transbronchial biopsies was reported as 6.1-31% and 25% or greater in studies performing surveillance biopsies, clinically indicated and follow-up bronchoscopies [24,25]. Grade A2 and higher acute rejection have been found in a relatively high percentage of asymptomatic patients, ranging from 22-39% [26]. Silent acute rejection appears most common within the first 3 months of transplantation (24.8% at 0 to 3 months; 16.7% at 3 to 12 months; 2.7% after 1 year) [27]. The rate of unsuspected but clinically significant infection was highest between 3 and 12 months post-transplantation but a relatively high rate (18.9%) was also detected after 1 year.

After lung transplantation, the total BAL fluid cell count is constantly increased even in the absence of infection or rejection [28]. In the early post-transplantation period (first 4 weeks), there is a dominance of neutrophils (up to 25-50% of total cell count) until about 3 months later when the cell count normalizes [28]. Acute rejection has been associated with elevated CD8+ T cells, activated CD4+ T cells, a trend toward increased NK T cells, increased B cells, and decreased NK cells [29]. Nevertheless, no study has proved the BAL cellular composition to be adequately sensitive or specific in the discrimination of rejection from infection [30].

Recent studies in heart transplantation (CARGO study) describe the use of peripheral blood gene expression profiling to identify a future risk of cardiac allograft rejection [31]. A similar study is now underway in lung

transplantation, known as the lung allograft rejection gene expression observational (LARGO) study. Preliminary data from almost 900 patients show differential gene expression in the lymphocyte priming and neutrophil homeostasis pathways for A0 versus \geq A2 acute lung rejection [32]. Such testing may hold promise for a non-invasive technique to monitor the status of the transplanted organs.

Specimen Adequacy and Handling

The 2007 ISHLT revised consensus classification of acute allograft rejection requires the evaluation of at least five pieces of well-expanded alveolated parenchyma [10]. To achieve this minimum number, the bronchoscopist may need to submit more than five tissue pieces. Furthermore, although there is no specific number of alveoli or small airways required by the ISHLT consensus classification, more biopsy pieces may improve the detection of OB. Gentle agitation of the tissue pieces in formalin to open up the alveoli may improve the histologic appearance of the fragments.

Histologic examination should include at least three levels from the paraffin block for H&E staining [10]. Connective tissue stains such as trichrome or Verhoeff-Van Gieson (VVG) stain for evaluating airways for the presence of submucosal fibrosis are essential for the diagnosis of OB, and vasculopathy. Silver stains such as GMS are recommended to evaluate for fungi, including pneumocystis, but have not been routinely mandated by the 2007 ISHLT revised consensus classification. BAL may be performed at the time of biopsy and is useful for the exclusion of infection.

Non-Rejection Related Allograft Pathology Harvest/reperfusion injury

Lung harvest/reperfusion (ischemia/reperfusion, I/R) injury remains the most common cause of early post-transplantation respiratory failure and manifests typically during the first 72 hours after transplantation [33]. Reported rates are as high as 41% [34]. The clinical equivalent of I/R injury is primary graft dysfunction (PGD), which usually presents with the immediate impairment in lung function after transplantation accompanied

by rapid development of pulmonary edema, increased pulmonary vascular resistance, and decreased airway compliance. I/R injury is a risk factor for OB [35].

Histologically, I/R injury presents as acute lung injury pattern including DAD. Perivascular infiltrates are usually not present and distinguish it from acute rejection. Several studies suggest that lung I/R injury is biphasic, with a distinct, acute injury characterized by macrophage activation followed by a later, neutrophil-dependent injury [35]. Again, infection can present similarly and needs to be excluded.

Recurrent Native Disease

Recurrent disease in the allograft has been reported in some allografts including cases of giant cell interstitial pneumonia, sarcoidosis, lymphangioleiomyomatosis, Langerhans' cell histiocytosis, allergic bronchopulmonary aspergillosis, desquamative interstitial pneumonia, bronchioloalveolar carcinoma, alveolar proteinosis and diffuse pan bronchiolitis. Morphological features are identical to the disease in non-transplanted lung.

Bronchiectasis

The majority of patients with OB have severe bronchiectasis. By specimen bronchograms, the bronchial tree shows alternating areas of dilatation and constriction. Microscopically, mucous plugging, goblet cell hyperplasia, squamous metaplasia, denudation of the bronchial epithelium, submucosal scarring, and acute and chronic inflammation of the bronchial wall may be apparent. Occasionally, foreign body giant cells are present, probably representing a manifestation of aspiration. Obliteration of the terminal respiratory bronchioles is often observed distal to these areas. The bronchiectasis of lung allografts is probably the result of several factors including immune-related injury, infection, mucostasis, aspiration and loss of innervation.

In conclusion, the pathologist plays a critical role in the management of lung transplant recipients. Communication with clinical care-givers is mandatory. While appropriate therapy rests on accurate pathologic diagnosis, it is not uncommon, when the differential diagnosis is acute infection vs. acute rejection, that the patient will be treated for both diagnoses. But, as alluded to above, it is chronic rejection that is the main complication leading to graft loss and most frequent patient death. New therapies e.g. the use of macrolide antibiotics, may offer hope that the scourge of obliterative bronchiolitis may be ameliorated. This gives both transplant care-givers and recipients renewed enthusiasm for this procedure.

Acknowledgements

This manuscript was presented in part at the Turkish Thoracic Society, Antalya Turkey, April 12, 2012. No author has any financial relations concerning the study. Both

authors scientifically contributed to the study. Dr. Anja Roden takes full responsibility for the content of the study.

Conflict of Interest

No conflict of interest was declared by the authors.

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