Revision of the 1995 Working Formulation for the Standardisation of Nomenclature in the Diagnosis of Lung Rejection.

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Abstract

In 1990 an international grading scheme for the grading of pulmonary allograft rejection was adopted by the International Society for Heart Transplantation and was modified in 1995 by an expanded group of pathologists. The original and revised classifications have served the lung transplant community well, facilitating communication between transplant centres with regard to both patient management and research. In 2006, under the direction of the International Society for Heart and Lung Transplantation (IHSHT) a multidisciplinary review of the biopsy grading system was undertaken to update the scheme, address inconsistencies of use and to consider the current knowledge of antibody-mediated rejection in the lung. This article summarises the revised consensus classification of lung allograft rejection. In brief, acute rejection is based on perivascular and interstitial mononuclear infiltrates: Grade A 0 (none), Grade A1 (minimal), Grade A2 (mild), Grade A3
(moderate), and Grade A4 (severe), as previously. The revised (R) categories of small airways inflammation, lymphocytic bronchiolitis, are as follows:

Grade B 0 (none), Grade B 1R (low grade -1995 B1 and B2), Grade B 2R (high grade-1995 B3 and B4) and B X (ungradeable). Chronic rejection, obliterative bronchiolitis, Grade C is described as present, C1, or absent, C 0 without reference to presence of inflammatory activity. Chronic vascular rejection is unchanged as Grade D. Recommendations are made for the evaluation of antibody-mediated rejection recognising that this is a controversial entity in the lung, less well developed and understood than in other solid organ grafts with no consensus reached on diagnostic features. Differential diagnoses of acute rejection, airway inflammation and chronic rejection are described and technical considerations revisited. The consensus revision of the Working Formulation was approved by the ISHLT Board of Directors on………….

Introduction

The original 1990 working formulation for the classification of pulmonary allograft rejection resulted from an International Society for Heart and Lung Transplantation (ISHLT) workshop to develop a standardised grading system for the pathological diagnosis of rejection in transplant lung biopsies.(1) A core group of pathologists developed a grading scheme for pulmonary allograft rejection which allowed data to be compared between institutions as a result of uniformity of grading. The grading system was intended to be simple, easily taught and readily reproducible and was adopted at the majority
of institutions performing lung transplantation at the time. In 1995 an expanded group of international pathologists convened to revise the original 1990 proposal in response to developments in the field and their experience of using the Working Formulation.(2) On this occasion the lung rejection study group critically assessed the merits of the initial Working Formulation and improved it on the basis of both published data and practical experience across many centres. The goal was again to maintain a uniform description and grading scheme for lung rejection, to improve communication between clinicians and investigators, to enable comparison of treatment regimes and outcomes between transplant centres, to facilitate multi-centre clinical trials and to promote further studies to determine the clinical significance of the various histological patterns. The revised classification was based on histological findings of acute and chronic lung rejection primarily using transbronchial biopsies for allograft monitoring in both adults and children. It was emphasised that all biopsy data needed to be interpreted in an integrated clinical context to allow optimum patient management and clinical decisions. It was also noted that infection/rejection often occur together and can be confused histologically and that infection needs to be rigorously excluded for the accurate and reproducible interpretation of pulmonary allograft biopsies. The 1995 revision was itself widely adopted by the lung transplant community and has served it well for over a decade.(3, 4) The revised Working Formulation represented a simplification of the original classification scheme but also highlighted some unresolved and complex issues such as the diagnosis and significance of airway inflammation. In 2004, again under the direction of ISHLT, a multi-disciplinary review of the cardiac biopsy grading
system was undertaken to address challenges and inconsistencies in its use and also to address recent advances in the knowledge of antibody-mediated rejection. The revised consensus classification was accepted by the Board of Directors and published in 2005 (5). It was clear that the success of the multidisciplinary approach could be usefully adopted for a further revision of the diagnosis of lung rejection to take into account a decade of developments in the clinical, pathological and immunological fields. To this end a multidisciplinary consensus meeting was held at the ISHLT 2006 Madrid meeting and its conclusions form the basis of this consensus report. The multidisciplinary task forces examined the histopathology of cellular rejection, humoral (antibody mediated rejection), clinical issues and future research. Comments solicited from the ISHLT membership at large and from the transplant pathology community were also taken into account. Compared with the numerous responses from ISHLT members in 2004 regarding the cardiac grading system, only a small number of responses were received about lung grading. This was interpreted as most likely reflecting an overall higher level of satisfaction with the existing scheme compared to the 1990 cardiac working formulation. This paper reflects the consensus of the revisions to the pathological classification and is accompanied by papers from the clinical and immunology task forces.

**Histological grading of pulmonary allograft rejection.**

The histopathology task force again recognised that alloreactive injury to the donor can affect both the vasculature and the airways in acute and chronic
rejection. Acute rejection is characterised by perivascular mononuclear cell infiltrate which may be accompanied by sub-endothelial infiltration, so called endothelialitis or intimitis and also by lymphocytic bronchitis and bronchiolitis. (1, 2, 6) On the other hand, chronic rejection is manifest by fibrous scarring which is often dense and eosinophilic involving the bronchioles and sometimes associated with accelerated fibrointimal changes affecting pulmonary arteries and veins. As in the original and revised classifications, the histological changes have been divided into grades based on the intensity of the cellular infiltrate and the presence or absence of fibrosis. The presence of presumed irreversible dense eosinophilic hyaline fibrosis in airways and vessels remains the key histological discriminator between acute and chronic rejection of the lung.

A. Acute rejection

A diagnosis of acute rejection is based exclusively on the presence of perivascular and interstitial mononuclear cell infiltrates. The intensity of the perivascular mononuclear cell cuffs and the distribution of the mononuclear cells including extension beyond the vascular adventitia into adjacent alveolar septa form the basis of the histological grade. Acute rejection usually affects more than one vessel (particularly true in adequate transbronchial biopsy samples) but is occasionally seen as a solitary perivascular infiltrate. These should be evaluated with the same criteria as those applied to multiple infiltrates as outlined below. In the setting of multiple foci of rejection, the grade reflects the most advanced pattern of rejection rather than the predominant pattern. The infiltrates surrounding small vessels in the sub-
mucosa of airways are again interpreted as part of the spectrum of airway inflammation rather than being diagnostic of acute rejection, Grade A.

**Grade A0 (no acute rejection)**

In grade A0 acute rejection, normal pulmonary parenchyma is present without evidence of mononuclear cell infiltration, haemorrhage or necrosis.

**Grade A1 (minimal acute rejection)**

In grade A1 acute rejection, there are scattered infrequent perivascular mononuclear infiltrates in alveolated lung parenchyma. Blood vessels particularly venules are cuffed by small round plasmacytoid and transformed lymphocytes forming a ring of two to three cells in thickness within the perivascular adventitia. This cuffing may be loose or compact and is generally circumferential. Eosinophils and endothelialitis are not present. The previous grading schemes suggested that these minimal infiltrates are not obvious at low magnification but it was felt that this criterion can be misleading. Grade A1 infiltrates can be seen at scanning magnification if the specimen is adequately alveolated and free from artefact. The consensus was that evidence of infrequent perivascular infiltrates at low power (scanning) magnification is not a reliable discriminator between A1 and A2 acute rejection.

**Grade A2 (mild acute rejection)**

In grade A2 mild rejection, more frequent perivascular mononuclear infiltrates are seen surrounding venules and arterioles and are readily recognisable at low magnification. They may be densely compacted or loose. These infiltrates
usually consist of a mixture of small round lymphocytes, activated lymphocytes, plasmacytoid lymphocytes, macrophages and eosinophils. Eosinophils are not a feature of grade A1 minimal rejection. In grade A2 rejection there is frequently subendothelial infiltration by mononuclear cells which may be associated with hyperplastic or regenerative changes in the endothelium i.e. endothelialitis. In making the distinction between A2 and higher grade acute rejection it is important to note that the perivascular interstitium can be expanded by mononuclear cells in A2 rejection but there is no obvious infiltration by mononuclear cells into the adjacent alveolar septa or air spaces. Concurrent lymphocytic bronchiolitis (see later) may be seen in association with mild acute rejection, A2, and is less common with A1 minimal acute rejection.

Mild acute rejection is therefore distinguished from minimal acute rejection by the presence of unequivocal mononuclear infiltrates which are more easily identified at scanning magnification. In addition, endothelialitis, the presence of eosinophils and co-existent airway inflammation favour mild A2 over minimal A1 acute rejection.

**Grade A3 (moderate acute rejection)**

Grade A3 acute rejection shows easily recognisable cuffing of venules and arterioles by dense perivascular mononuclear cell infiltrates which are commonly associated with endothelialitis. Eosinophils and even occasional neutrophils are common. This grade is defined by the extension of the
inflammatory cell infiltrate into perivascular and peribronchiolar alveolar septa and airspaces which may be associated with collections of intra-alveolar macrophages in the zones of septal infiltration and type 2 alveolar cell hyperplasia. The interstitial infiltration can take the form of cells percolating singly into alveolar walls or more sheet-like infiltration with corresponding expansion of the septa. There is continuity with the perivascular infiltrates. True interstitial infiltration characterizing moderate acute rejection should be distinguished from the expansion of the potential space of the perivascular adventitia in mild acute rejection.

**Grade A4 (severe acute rejection)**

In grade A4 severe rejection there are diffuse perivascular, interstitial and air space infiltrates of mononuclear cells with prominent alveolar pneumocyte damage and endothelialitis. This may be associated with intra-alveolar necrotic epithelial cells, macrophages, hyaline membranes, haemorrhage and neutrophils. There may be associated parenchymal necrosis, infarction or necrotising vasculitis although these features are more evident on surgical rather than transbronchial lung biopsies. There may be a paradoxical diminution of perivascular infiltrates as cells extend into alveolar septa and spaces where they are admixed with macrophages. Grade A4 acute rejection must be distinguished from post transplantation acute lung injury by the presence of numerous perivascular and interstitial mononuclear cells which are not a feature of re-perfusion related damage.
In summary, the diagnosis of acute rejection is based on the presence of perivascular and interstitial mononuclear cell infiltrates. After much debate about the merits or otherwise of collapsing the A1 – A4 grades into fewer grades, the consensus was to retain the existing five point system whilst recognising that in most pathologists’ experience A4 is an uncommon grade. The nature of the tissue damage in A4 however was identified as having a potential relationship with an antibody-mediated form of acute rejection (see later) and therefore potentially useful in contributing to further understanding of lung rejection in the future albeit in an infrequently diagnosed grade. The histopathology task force also recommended that perivascular infiltrates related to acute rejection should be truly circumferential and that incomplete vascular cuffing is unlikely to represent acute rejection. It is advised that further samples, deeper serials or levels into the tissue block should be obtained when the infiltrates are equivocal to discriminate both between rejection and non-rejection pathology and between the various grades of acute cellular rejection. The participants also noted that the transbronchial biopsy diagnosis of acute rejection represents but one component of an integrated approach to the assessment of lung allograft recipients. The diagnosis of acute lung rejection therefore requires integration with clinical and particularly microbiological data. (7) In relation to the treatment of acute rejection the task force noted that different clinical groups have different therapeutic algorithms and that since the 1995 working formulation the potential long-term significance of A1 minimal acute rejection has emerged. (8-10) It was decided to retain this minimal grade for further evaluation in the light of better guidance for its recognition.
B: Airway inflammation: lymphocytic bronchitis/bronchiolitis.

The 1995 working formulation allowed airway inflammation to be graded from B0 (no inflammation) to B4 (severe airway inflammation). The previous 1990 formulation had recommended airway inflammation co-existent with A grade acute rejection to be recorded as present or absent but did not reflect the intensity of the inflammatory infiltrates. The 1995 grading of airway inflammation was not accepted by all the members of the lung rejection study group for several reasons including the lack of convincing evidence that airway inflammation could be used solely to grade rejection because of its frequent co-existence with airway infection. Also there are frequent problems with adequate sampling of small airways in transbronchial biopsies and with technical issues such as tangential cutting etc. An ungradeable category was designated for those biopsies limited by sampling problems, infection, tangential cutting etc. It was accepted that the scientific and clinical usefulness and airway inflammation grades would need re-visiting over the passage of time. However, the format of A and B grades in the 1995 classification emphasised the need to retain perivascular infiltrates as the primary focus in the histological classification of acute lung rejection. At the consensus meeting, the majority of pathologists felt that the criteria for separating four grades of airway inflammation were poorly defined and difficult to discriminate on transbronchial biopsy. Previous studies of reproducibility of the 1995 working formulation both in terms of inter- and intra-observer variability had shown significant problems with the airway inflammation B
grades in comparison to the acute rejection A grades and it was recognised that new recommendations must improve reproducibility. (3, 4, 12) The revision of B grades has collapsed the four previous grades into two and retained the B0 (no airway inflammation) and Bx (ungradeable for reasons stated above). The B grade designation applies only to small airways i.e. bronchioles and the description of inflammation in cartilage-containing large airways is covered later. It is recognised that airway inflammation can be present in the absence of perivascular infiltrates and that rigorous exclusion of infection is necessary before ascribing the features to acute rejection of the airway.

Grade B0 (no airway inflammation)

In this grade there is no evidence of bronchiolar inflammation.

Grade B1R (low grade small airway inflammation) (R suffix designates Revised grade)

In this grade there are mononuclear cells within the submucosa of the bronchioles which can be infrequent and scattered or forming a circumferential band. Occasional eosinophils may be seen within the submucosa. There is no evidence however of epithelial damage or intra-epithelial lymphocytic infiltration. This grade combines and replaces the previous B1 and B2 grades.

Grade B2R (high grade small airway inflammation)
In this grade the mononuclear cells in the submucosa appear larger and activated with greater numbers of eosinophils and plasmacytoid cells. In addition there is evidence of epithelial damage in the form of necrosis and metaplasia and marked intra-epithelial lymphocytic infiltration. In its most severe form, high grade airway inflammation is associated with epithelial ulceration, fibrino-purulent exudate, cellular debris and neutrophils. A disproportionate number of neutrophils within the epithelium and submucosa in relation to the numbers of submucosal mononuclear cells is highly suggestive of infection rather than rejection. Any accompanying lavage or aspirate may also be purulent and/or show evidence of organisms.

**Grade BX (ungradeable small airway inflammation)**

In this grade the changes are ungradeable due to sampling problems, infection, tangential cutting, artefact etc.

The consensus group recommended that the diagnosis of acute rejection with co-existent airway inflammation be in the same form as the 1995 formulation i.e. acute rejection grade with airway inflammation grade. For example moderate acute cellular rejection in which there is intense small airways inflammation would be designated moderate acute rejection, grade A3 with airways inflammation grade B2R. The category of lymphocytic bronchiolitis is graded as A0, B1R or A0, B2R depending on the severity of the airway inflammation.
C: Chronic airways rejection: obliterative bronchiolitis.

Obliterative bronchiolitis describes dense eosinophilic hyaline fibrosis in the submucosa of membranous and respiratory bronchioles resulting in partial or complete luminal occlusion. This tissue can be concentric or eccentric and may be associated with fragmentation and destruction of the smooth muscle and elastica of the airway wall. It may extend into the peribronchiolar interstitium. Mucostasis and/or foamy histiocytes in the distal air spaces are commonly associated with obliterative bronchiolitis and may be observed in transbronchial biopsies in the absence of bronchiolar occlusion or any bronchiolar tissue. The 1995 working formulation concluded that the 1990 distinction between subtotal and total forms of obliterative bronchiolitis was not useful but retained the designation active versus inactive depending on the presence and degree of accompanying inflammation. The consensus in 2006 was that the distinction between active and inactive obliterative bronchiolitis is no longer useful and the condition should be designated merely as C0 indicating a biopsy with no evidence of obliterative bronchiolitis and C1 indicating that obliterative bronchiolitis is present in the biopsy. Transbronchial biopsy is an insensitive method for detecting obliterative bronchiolitis and the clinical use of bronchiolitis obliterans syndrome (BOS) with its functional grading is the preferred means of diagnosing and monitoring chronic airway rejection.

D: Chronic vascular rejection

In chronic vascular rejection/accelerated graft vascular sclerosis there is fibro-intimal thickening of arteries and veins which is similar to coronary artery
disease in transplanted hearts. In the veins, the histological appearance is usually of poorly cellular hyaline sclerosis and it is recognised that the use of older donors is associated with a higher incidence of this phlebosclerosis in biopsy material. Chronic vascular rejection is not applicable to transbronchial biopsies but may be noted on open biopsy material.

**Acute antibody-mediated (humoral) rejection.**

Acute humoral rejection is now recognised as a clinical entity in heart and renal transplants although it remains controversial with a highly varied incidence between different centres.(14-16) There is no consensus on its recognition and diagnosis either histopathologically or immunologically, nor on its significance and treatment. The 2004 ISHLT cardiac rejection meeting reviewed evidence from histopathology, immunopathology and clinical task forces and was able to suggest diagnostic criteria in specific clinical circumstances so that further assessment of this entity could be encouraged.(5) Pathologists can follow the guidance in that consensus report if they intend to investigate the possibility of antibody-mediated rejection as a cause of cardiac dysfunction. Recommendations were published to allow incorporation, as required into the revised working formulation for heart rejection. It was noted that acute antibody mediated rejection is associated with worse graft survival and is observed in allo-sensitised patients including those with previous transplantation, transfusion or pregnancy and previous ventricular assist device use.(14) The diagnosis and recognition of antibody mediated rejection in the lung is even more controversial and far less well developed than in other solid organ grafts. (15-17) However the presence of
serum anti-HLA antibodies and the deposition of complement in alveolar tissue following transplantation suggest a role for humoral immune responses in lung transplantation. A significant portion of the lung consensus meeting was devoted to reviewing evidence for antibody-mediated acute lung rejection. Pulmonary transplant recipients with evidence of sensitisation as demonstrated by elevated titres of panel reactive antibodies have significantly more ventilator days post-operatively compared with non-sensitised patients.

Humoral immune responses are also implicated in the pathogenesis of bronchiolitis obliterans possibly due to anti-HLA antibodies contributing the development of scarring fibrosis via stimulation of epithelial cells within the airway. The diagnosis of antibody-mediated rejection in the lung is very much in development. Historically acute antibody mediated rejection of the lung has been associated with “hyperacute rejection” clinically manifest by primary graft failure occurring very early after transplantation in the setting or pre-formed antibodies to donor HLA antigens or endothelial cells. Morphologically this is associated with fibrin thrombi in alveolar septa, fibrinoid necrosis of alveolar septal walls and haemorrhage. In 2006 there were no agreed histological features for antibody-mediated rejection in the lung. However there was general agreement that although pulmonary capillaritis has been described as possibly related to acute lung rejection, it is not recognised in transbronchial biopsies in the majority of institutions performing pulmonary transplants and this term should not be used to indicate the histological hallmark of antibody-mediated rejection. Pulmonary capillaritis should also be distinguished from neutrophil margination and congestion. It was agreed that the term capillary injury is more useful as it
can indicate a morphological spectrum of capillary damage although it can be a non-specific finding occurring in infection, diffuse alveolar damage and severe cellular rejection. Extrapolating from other solid organ descriptions of antibody mediated rejection, it was agreed that small vessel intimitis could raise the suspicion of humoral rejection. It was also agreed on an empirical basis that should antibody-mediated rejection be suspected clinically, immunopathologically or due to histological evidence of capillary injury, immunohistochemistry could be performed on the transbronchial biopsies for C3d, C4d, CD31 and CD68. This extrapolates from experience in heart and kidney grafts. The use of broad immunofluorescence panels and electron microscopy was not recommended. It was emphasised that antibody-mediated rejection in the lung is nowhere near as developed an entity as in the heart and kidney and more work is required for its evaluation. The use of agreed immunohistochemical markers may prove helpful in understanding the diagnosis. The use of C4d staining in particular may allow the humoral response to a lung graft to be interpreted along the lines of the NIH recommendations from the 2003 national conference (table). However a recent study of C4d staining of pulmonary allograft biopsies with immunohistochemistry showed positive staining in a variable focal non-specific pattern with no consistent staining pattern within different diagnostic groups. Specifically C4d deposition was not demonstrated in the microvasculature of lung biopsies in patients with acute or chronic rejection but it is not known if this study is representative. Another recent study demonstrated specific subendothelial C4d deposition immunohistochemically as a marker for the involvement of HLA antibodies in lung allograft rejection.
However the patchy nature, low sensitivity and specificity of the C4d staining suggested a limited clinical use in protocol biopsies but raised the possibility of specific C4d deposition serving as a marker of co-existent antibody mediated rejection in patients with refractory acute cellular rejection. No recommendations could be made on the diagnosis of concomitant acute cellular rejection and antibody-mediated rejection at this time although combined rejection of this nature is very likely to occur by extrapolation from other solid organ grafts. A more detailed analysis of the current state of knowledge regarding antibody-mediated rejection of the lung is presented in an accompanying paper from the immunology task force. Caution is urged in the diagnosis of acute antibody–mediated rejection in the lung until further evidence is forthcoming and the multidisciplinary approach is again recommended in view of the wide differential diagnosis and the potential toxicity of treatments.

**General recommendations**

**Adequacy of specimens**

Transbronchial biopsy remains the mainstay of lung allograft evaluation and it was again the uniform opinion of the consensus meeting that at least five pieces of well expanded alveolated lung parenchyma are required for an assessment of acute rejection. The bronchoscopist may need to submit more that five biopsies to provide this minimum number of adequately alveolated pieces and yet further biopsies if small bronchioles are required to be present. A strip of bronchus may be attached to the alveolated parenchyma and this should be distinguished from bronchiolar tissue. Specimens can be gently
agitated in formalin to inflate the fragments and required tender handling in the laboratory to avoid crush artefacts which can render interpretation difficult or well nigh impossible.

**Histological examination**

Histological examination should include a minimum of sections from three levels of the paraffin block for haematoxylin and eosin (H&E) staining with connective tissue stains to evaluate any submucosal fibrosis, essential for the diagnosis of bronchiolitis obliterans and arteriosclerosis. Silver stains can be performed for fungi and pneumocystis but are not absolutely mandated by the group in view of the numerous microbiological, serological and molecular techniques that are in use for the diagnosis of opportunistic infections in these patients. Beyond this minimum H & E and connective tissue stain work-up, investigators may wish to augment their evaluation with histochemical, immunohistochemical and in-situ hybridisation studies. Bronchoalveolar lavage may be performed at the time of biopsy and is useful for the exclusion of infection and for research investigations but has no clinical role in the diagnosis of acute rejection.

**Differential diagnosis of perivascular and interstitial infiltrates.**

Perivascular mononuclear infiltrates are not specific for acute rejection and many other conditions may simulate or mimic allo-reactive lung injury. Differential diagnostic considerations include cytomegalovirus pneumonitis, pneumocystis jiroveci (previously carinii) pneumonia and post transplantation lymphoproliferative disease, which can itself range from pneumonitis to active
lymphoproliferation with tumour nodules. These conditions are all described elsewhere. Cytomegalovirus pneumonitis often shows disproportionate alveolar septal cellular infiltrates in comparison with any perivascular cuffing and may include perivascular oedema. As well as diagnostic infected cells with intranuclear and intracytoplasmic viral inclusions, the presence of abundant neutrophils with the formation of micro-abscesses and marked atypia of alveolar pneumocytes may also suggest the diagnosis. The molecular and serological methods for monitoring and diagnosing CMV disease are also extremely helpful in suggesting the diagnosis. Transbronchial biopsy however remains the only standard for assessing concomitant CMV infection/pneumonitis and acute rejection. Although pneumocystis can exactly mimic acute rejection with perivascular and interstitial infiltrates, it can also manifest atypical histological reactions including granulomatous inflammation, diffuse alveolar damage and foci of necrosis. Granulomatous inflammation is not a feature of acute rejection and should always raise the possibility of mycobacterial or fungal, including pneumocystis, infection. Punctate zones of necrosis should also raise the possibility of mycobacteria, fungi or herpes virus infections rather than acute rejection. Further differential diagnoses of perivascular and interstitial infiltrates include recurrent primary disease such as sarcoidosis and, in the early post-transplant period, re-perfusion injury although the latter is more often associated with neutrophils and evidence of acute lung injury.

Other non rejection biopsy findings

Aspiration
The pulmonary allograft is not protected by cough reflex and patients are highly predisposed to recurrent aspiration. Helpful features in making this diagnosis include the identification of exogenous material with associated foreign body giant cell reaction within the airways and parenchyma. Large lipid droplets and/or macrophages with large vacuoles are helpful markers of aspiration. Distal organising pneumonia can also be seen. Since the last revision of lung rejection grading, aspiration has emerged as a significant cause of chronic allograft dysfunction which may be ameliorated by treatment.\(^{25, 26}\) It can occur early or late after transplantation and is therefore within the differential diagnosis throughout the postoperative period.

**Organising pneumonia**

Organising pneumonia with intra-alveolar fibro-myxoid tissue associated with variable interstitial inflammation is another common finding in biopsies from lung allografts.\(^{27}\) It can occur in a variety of clinical contexts and requires microbiological correlation where infection is suspected. Organising pneumonia can be seen as a subacute form of infectious lung damage. Patchy organising pneumonia may also represent reperfusion/ischaemic injury where there may have been evidence of primary graft failure. The histological pattern of organising pneumonia can also be seen in association with acute rejection of grade A3 severity and greater where there is alveolar extension of the acute inflammatory response with subsequent organisation. Idiopathic/cryptogenic organising pneumonia can also manifest identical histological features in biopsies from a lung transplant recipient but many
other causes must be excluded before the reaction is attributed to idiopathic origin.

**Large airway inflammation.**

The importance of distinguishing large and small airways inflammation was again the subject of much discussion and dissent (6, 28). No definite evidence was produced to support a separation of small and large airway inflammation as useful in the diagnosis of acute rejection. Large airway inflammation is most commonly associated with infection and aspiration (see above). Scarring can be seen in large airways in addition to the bronchiolar scarring of bronchiolitis obliterans but this feature is regarded as so non-specific as to not warrant a separate comment. However the presence of large airway scarring, like the presence of intra-alveolar macrophages, can alert the pathologist to the possibility of obliterative bronchiolitis and the need to examine further sections.

**Bronchus associated lymphoid tissue.**

Bronchus associated lymphoid tissue consists of subepithelial mucosal lymphoid follicles which are distributed along the distal bronchi and bronchioles. It is scattered throughout the lung in adults, tending to be most prominent at the bifurcation points of airways. The lymphoid follicles contain mainly B lymphocytes and normally lack true germinal centres. These follicles are associated with specialised bronchial and bronchiolar epithelium which is composed of modified cuboidal, non-ciliated, non-mucinous cells allowing for the trans-epithelial migration of antigens and cells. (29) Attention to these
histological features and recognition of the often prominent vascularity should enable distinction to be made between BALT and rejection-related airway inflammation.(29, 30) BALT is often well circumscribed and may contain macrophages with particulate matter. There should be no evidence of epithelial injury, neutrophils, or eosinophils in a BALT collection. BALT aggregates can trail off into fibrovascular septa and should not be confused with perivascular or interstitial infiltrates.

**Smokers’ type respiratory bronchiolitis.**

In respiratory (smokers’) bronchiolitis biopsies show an accumulation of tan coloured alveolar macrophages around respiratory bronchioles. Macrophages may contain flecks of brown or black material and show Prussian blue positivity. There may be associated interstitial thickening and variable accompanying chronic inflammation. There may be other features of chronic obstructive pulmonary disease with goblet cell metaplasia, mucostasis and bronchiolar metaplasia. This appearance should be distinguished from rejection related inflammation and BALT. The incidence of smokers’ type respiratory bronchiolitis in transbronchial biopsies from lung transplants has increased with the expansion of the donor pool to include older and smokers’ organs. Occasionally dust macules/nodules are seen of donor origin. The persistence of smokers’ macrophages in the donor lung should not be confused with recipient smoking.

**Alveolar septal fibrosis.**
Some members of the consensus group had observed fibrotic thickening of the alveolar septal walls in transbronchial biopsies from pulmonary allografts and noted the clinical entity of upper lobe fibrosis which has been described as a newly identified late onset complication following lung transplantation.(31, 32) However due to the lack of specificity and the difficulty in interpretation of interstitial fibrosis in transbronchial biopsy specimens it was felt to be an unhelpful observation.

Summary

This multi-disciplinary review of the classification of lung allograft rejection has taken place more than a decade since the previous revision.(2) There was continued support for retaining the previous acute rejection grades and for collapsing of the previous lymphocytic bronchiolitis (B) grades. The consensus group felt that more detailed descriptions of the various grades and differential diagnoses, mainly in the form of additional photomicrographs would enhance the usefulness of the 2006 revision and thereby improve reproducibility. The group also tackled the contentious issue of antibody-mediated rejection in the lung and reviewed the available literature. The consensus was that the available evidence supports the possibility of AMR following lung transplantation but that more studies are required to determine which of the previously described pathological lesions could be the histological counterparts of this form of acute rejection. Proposals for a standardised approach for the investigation of possible AMR have been suggested to focus research endeavours in this difficult field. The consensus meeting again emphasised the importance of amalgamating the clinical,
histological, radiological, immunological and microbiological data in a multi-disciplinary setting in order to achieve the most accurate diagnosis for a particular patient episode. As ever, the working formulation is regarded as a live document which will no doubt require further modification in the future with the advent of further molecular and other diagnostic refinements for the diagnosis and management of this complicated group of allograft recipients.

Table 1 Revised working formulation for classification and grading of pulmonary allograft rejection.

<table>
<thead>
<tr>
<th>A. Acute rejection</th>
<th>B. Airway inflammation</th>
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<tbody>
<tr>
<td>Grade 0 - None</td>
<td>Grade 0 - None</td>
</tr>
<tr>
<td>Grade 1 - Minimal</td>
<td>Grade 1R - low-grade</td>
</tr>
<tr>
<td>Grade 2 - Mild</td>
<td>Grade 2R - high grade</td>
</tr>
<tr>
<td>Grade 3 - Moderate</td>
<td>Grade X - ungradeable</td>
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<tr>
<td>Grade 4 - Severe</td>
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C. Chronic airway rejection – obliterative bronchiolitis
   0 - absent
   1 - present

D. Chronic vascular rejection – accelerated graft vascular sclerosis.
Table 2: Putative stages of humoral response to an organ graft

I: Latent humoral response
   Circulating antibody\(^1\) alone (but without biopsy findings or graft dysfunction)

II: Silent humoral reaction (accommodation vs. prerejection state)
   Circulating antibody\(^1\) + C4d deposition (but without histologic changes or graft dysfunction).

III: Subclinical humoral rejection\(^2\)
   Circulating antibody\(^1\) + C4d deposition + tissue pathology (but without graft dysfunction).

IV: Humoral rejection
   Circulating antibody\(^1\) + C4d deposition + tissue pathology + graft dysfunction.

\(^1\) Circulating antibody to HLA or other antigens expressed on donor endothelial cells.

\(^2\) May differ among organs, as the ability to detect particularly mild degrees of graft dysfunction varies among organs.

From ref. 25.

Table 3

Other pathological features to note in transbronchial biopsies.

Infection
Aspiration
Organising pneumonia
Post transplant lymphoproliferative disorder
Large airway inflammation
Bronchus-associated lymphoid tissue
Smoker’s type respiratory bronchiolitis
Diffuse alveolar damage
Recurrent native disease
Haemosiderosis
References


Appendix: Participants by Task Force

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