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Lung allograft standardized histological analysis (LASHA) template: A research consensus proposal

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KEYWORDS:
lung allograft;
histological template;
pathology;
lung transplantation;
transbronchial biopsies

BACKGROUND: Routine monitoring of lung-transplanted patients is crucial for the identification of immunological and non-immunological complications. Determining the etiology of acute allograft dysfunction, particularly in alloimmune-mediated disorders, relies heavily on the lung biopsy with histopathologic analysis. Standardization of the pathologic diagnosis of rejection (e.g., cellular and antibody-mediated) is based on consensus statements and guidelines, indicating the importance of a multidisciplinary approach to achieve a definitive etiological diagnosis. In addition to these statements and guidelines, refinements and standardizations are feasible through systematic analysis morphological, immunophenotypic and molecular alterations observed in transbronchial biopsies. This study is to

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Abbreviations: ACR, acute cellular rejection; AFOP, acute fibrinous ad organizing pneumonia; AMR, antibody-mediated rejection; BALT, bronchial-associated lymphoid tissue; BOS, obliterative bronchiolitis syndrome; CLAD, chronic lung allograft dysfunction; CMV, cytomegalovirus; CR, chronic rejection; ISHLT, International Society for Heart and Lung Transplantation; IF, immunofluorescence; IP, immunoperoxidase; LASHA, lung allograft standardized histological analysis; LTx, lung transplantation; OB, obliterative bronchiolitis; NSIP, non-specific interstitial pneumonia; OP, organizing pneumonia; PTLD, post-transplant lymphoproliferative disorder; RAS, restrictive allograft syndrome; TBB, transbronchial biopsy.

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identify key morphologic features to be assessed, select consistent and reproducible terminology for each histological feature, and provide standardized definitions for pathological assessment and grading.
METHODS: A template was created by experts in lung transplantation including pathologists, pulmonologists, immunologists. An initial draft was circulated, followed by discussions and multiple revisions by email and conference calls.
RESULTS: The "lung allograft standardized histological analysis − LASHA" template was created and structured as multiple-choice questions with number of fields to be filled in to allow for standardization of results and easy transfer into a future electronic spreadsheet.
CONCLUSION: This template will help facilitate multicenter studies through a uniform protocol and correlations with new diagnostic modalities. After validation in large-scale studies, an optimized template could be included in routine clinical practice to enhance graft assessment and medical decision-making.
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Lung transplantation (LTx) is the ultimate therapeutic option for many patients with end-stage lung disease. However, according to recent data from the International Society for Heart and Lung Transplantation (ISHLT), the median survival is 6.7 years[1] for adults, usually limited by immunologic and/or nonimmunologic complications.

Rejection is the main determinant of allograft failure. Although clinical monitoring by pulmonary function tests, laboratory analyses (microbiological/immunological tests) and imaging facilitate the identification of complications, currently the diagnosis of allograft rejection relies on histologic assessment of lung biopsies and the exclusion of other diagnostic entities such as infection. To date, standardization of pathologic diagnosis and grading of rejection [acute cellular rejection (ACR), antibody-mediated rejection (AMR), and chronic lung allograft dysfunction (CLAD)] has been achieved through consensus statements and guidelines supporting the importance of a multidisciplinary approach.[2−5] There is an ongoing need to standardize injurious patterns by histopathologic examination and to identify the changes associated with poor clinical outcomes.[6-13]

The proposed "lung allograft standardized histological analysis"-LASHA- template aims: (1) to identify key morphologic features to be assessed, (2) to select consistent and reproducible terminology for each histological feature, (3) to provide standardized definitions for pathological assessment and grading.

The lack of uniform histological reporting of biopsies after transplant has resulted in unreliable and nonstandardized language in histopathological reports, particularly for morphologic findings out of rejection (acute or chronic) that are not conventionally graded by the current guidelines. This has historically led to both misinterpretation of post-LTx complications, and has potentially introduced bias in clinical trials.[14]

Data reporting elements are proposed for research in a framework to facilitate multicenter collaborative studies allowing identification of currently unrecognized features of specific processes. The template is currently more comprehensive than what is currently used in clinical practice and not all elements may be required for routine diagnosis. In the future, this template, in combination with a digital platform, may be used as an educational tutorial for pathologists. After adjudication of the collected data from the research application of the template, the intention is to remove those histological features which have been proven non-relevant, and to adjust it for routine clinical practice. Correlation with clinical, radiological, and molecular features could ultimately reinforce the significance of these histological entities, allowing the refinement of ISHLT diagnostic criteria.

Methodology and description of the template

Methodology

The LASHA grid was discussed by panelists of the lung session on AMR at the 2017 14th Banff Foundation for Allograft Pathology Conference in Barcelona.[13] The panel was composed of pathologists, pulmonologists, and immunologists. The idea of such a grid was vetted through ISHLT Connect to the pathology community, selecting those colleagues involved in lung transplantation. The working group members were from different countries: Europe (8), USA (10), and Australia (1).

The grid was originally intended to focus on AMR. However, the working group agreed that the wide AMR-related range of histopathologic lesions could also be crucial in the differential diagnosis of several other post-transplant complications. Thus, the grid was planned as a comprehensive report of all pathology findings detected in a lung allograft specimen. The pathologists (all experienced in lung transplant pathology) discussed and defined the scoring system adopted for the different lesions, with the intention to evaluate its usefulness and reliability in research studies.

An initial draft of the grid was circulated by email among the panel members; subsequent discussions and multiple revisions occurred by email and conference calls. The LASHA grid was finally drafted as a Word document where it was structured as multiple-choice questions, with number fields to be filled in to allow for standardization of results and easy transfer into a spreadsheet (Figure 1). This grid will be made available in an electronic format in REDCap (Research Electronic Data Capture, https://www.project-redcap.org/) which is a secure web application for building and managing online databases. This application has several advantages, such as using a real

LUNG ALLOGRAFT STANDARDIZED HISTOLOGICAL ANALYSIS (LASHA)

Type of sample: Transbronchial biopsy ☐ Transbronchial cryobiopsy ☐ Wedge biopsy ☐ Other (_____) ☐
Stainings/techniques: H&E ☐ Connective tissue staining ☐ Silver stains ☐ Other special stains (_____) ☐
Other ancillary tools (_____) ☐
C4d evaluation: Immunohistochemistry (IP) ☐ Immunofluorescence (IF) ☐
IP: Distribution: <10% ☐ 10-50% ☐ >50% ☐ IF: Intensity (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐
Biopsy*: adequate ☐ insufficient ☐ inadequate ☐
Bronchi: YES ☐ NO ☐
Bronchioles: YES ☐ NO ☐
Artery: YES ☐ NO ☐
Lesions suggestive of acute cellular rejection:
- Perivascular mononuclear infiltrates: YES ☐ NO ☐
- Lymphocytic bronchiolitis: YES ☐ NO ☐
Lesions suggestive of chronic rejection:
- Obliterative bronchiolitis: YES ☐ NO ☐
- Vascular rejection: YES ☐ NO ☐
Alveolar septal injury pattern:
- Neutrophils in alveolar septa (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐ Ungradable** ☐
- Neutrophilic/cellular debris in alveolar septa (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐ Ungradable** ☐
- Platelet-fibrin thrombi in alveolar capillaries (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐ Ungradable** ☐
- Alveolar capillary dilatation (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐ Ungradable** ☐
- Septal wall oedema/widening (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐ Ungradable** ☐
- Mononuclear cells in alveolar septa (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐ Ungradable** ☐
- Septal fibrous thickening (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐ Ungradable** ☐
Intra-alveolar Injury pattern:
- Neutrophils in alveolar spaces (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐ Ungradable** ☐
- Hyaline membranes (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐ Ungradable** ☐
- Pneumocyte hypertrophy/reactive changes (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐ Ungradable** ☐
- Granulation tissue plugs in alveolar spaces/OP (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐ Ungradable** ☐
- Fibrin balls in alveolar spaces (suggestive of AFOP) (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐ Ungradable** ☐
- Alveolar proteinosis (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐ Ungradable** ☐
- Macrophages (score): 0 ☐ 1 ☐ 2 ☐ 3 ☐ Ungradable** ☐
Specify subtypes: normal ☐ hemosiderophages ☐ foamy ☐ cholesterol clefts ☐ giant cells ☐
Foreign body in alveolar spaces: YES ☐ NO ☐
Injury pattern in other sites (*e.g.subpleural, interlobular septa, large airways*)
- Suspected pleuroparenchymal/intralveolar fibroelastosis YES ☐ NO ☐
- Inflammation of subpleural/interlobular areas (specify the type) YES ☐ NO ☐ (specify:_____)
- Injury of the large airways (specify the type) YES ☐ NO ☐ (specify:_____)
Other:
- Arteritis/endotheliitis: YES ☐ NO ☐ (specify:_____)
- Thrombus: YES ☐ NO ☐
- Ischemic necrosis: YES ☐ NO ☐
- Viral inclusions: YES ☐ NO ☐ (specify:_____)
- Fungal organisms: YES ☐ NO ☐ (specify:_____)
- Other infectious organisms: YES ☐ NO ☐ (specify:_____)
- Granuloma: YES ☐ NO ☐
- Suspected PTLD YES ☐ NO ☐
- Suspected recurrent disease YES ☐ NO ☐ (specify:_____)
- Eosinophilia (interst/alv): YES ☐ NO ☐ (specify:_____)
- BALT***: YES ☐ NO ☐
- Previous biopsy site YES ☐ NO ☐ (specify:_____)
- Other (specify) _____

SUMMARY
Acute cellular rejection: A & B Grades
(A Grade): 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ X ☐
(B Grade): 0 ☐ 1R ☐ 2R ☐ X ☐
Infection: yes/no (specify the type)
Lesions suggestive of AMR: yes/no (specify the lesions and C4d staining)
Lesions suggestive of chronic rejection: yes/no (specify if OB, RAS-like or vascular, reporting the C and D grades for OB and vascular type)
Lesions suggestive of I/R injury: yes/no (specify the lesions)
Lesions suggestive of other diagnosis: yes/no (specify the diagnosis)

Figure 1 Lung allograft standardized histological analysis template in which all histological changes must be reported. * According the ISHLT working formulation established criteria[2] ** The features are visible but not precisely gradable or equivocal *** BALT means organized lymphoid tissue with vessels and occasional anthracotic pigmented macrophages.

DATABASE-SQL (MySQL), the availability of multiple forms connected to each other and different tools and customizations, easy management of multicenter studies, and the possibility of exporting data in an adequate format for statistical software (SAS, SPSS, R). A website will be set up with multisite access for managing the REDCap database.

The writing workgroup was divided into subgroups covering the following topics: (1) introduction about the need for standardization of pathological diagnosis reports and the potential clinical impact, (2) methodology and description of the different lesions after a comprehensive literature search and review of each topic, (3) open issues and current/future perspectives.

Description of the template

Tissue samples, staining, and scoring system

The first section of the template focuses on specimen adequacy, biopsy type and histochemical/immunohistochemical stains. The adequacy of TBB is evaluated according to the ISHLT working formulation established criteria.[2] In particular, the biopsy is considered adequate when there are at least 5 pieces of well-expanded alveolated lung parenchyma and at least 1 or 2 bronchioles. If one of the biopsy pieces does not include alveoli but only airway structures, pleura, or vessels, it is excluded from the counts. If the biopsy piece is affected by crush artifact, it will also be excluded from counting. In case of an insufficient biopsy (including at least 3 pieces of well-expanded alveolated lung parenchyma), the evaluation of pathologic changes will be reported as present/absent, skipping the scoring system.

The most commonly encountered biopsy is the fiberoptic transbronchial biopsy (TBB) and the template has been designed accordingly. However, other diagnostic techniques such as the transbronchial cryobiopsy can be accommodated.[15-18] Surgical biopsies are infrequently performed and generally attempted when other investigations fail to yield the diagnosis. In case of larger biopsies such as cryobiopsies and wedge biopsies the LASHA scoring of histologic lesions will be simply reported as present or absent, postponing the grading of changes to when the template will be widely used.

The template requires reporting of results of any ancillary testing that was done including histochemical, immunohistochemical, immunofluorescent (for C4d) stains, and molecular techniques.[19]

Some aspects of tissue processing and analysis are omitted as they are center-specific and are left to the discretion of individual centers, for example, TBB vs cryobiopsy approaches and techniques for C4d evaluation (immunohistochemistry vs immunofluorescence).

Albeit controversial (see "Remaining questions" paragraph), the working group agreed on retaining immunostaining for C4d as a supportive tool for diagnosing AMR. Currently, most centers use immunoperoxidase (IP) assays; immunofluorescence (IF) has also been included for centers that prefer this other approach. Both intensity score (from 0 to 3 for IF) and distribution of immunoreactivity in interstitial capillaries (<10%, 10%-50%, and >50% for IP) are reported in the template.

ACR, lymphocytic bronchiolitis and chronic rejection (CR) are reported according the ISHLT working formulation established criteria.[2] A key feature of this template is the application of a 4-tiered scoring system for the majority of histologic findings. The working group pathologists proposed a simple and easily applicable score for the different parameters. This score includes: 0-morphologic feature not present, morphologic feature present focally (score 1), in at least half of the samples (score 2), or in all samples (score 3).

Description of histological features

Except for ACR/CR grading, all other findings are grouped into 3 main categories based on the anatomic compartment affected: alveolar septum, intra-alveolar lumen, and other sites (e.g., subpleural, interlobular, large airways). All histological changes of the anatomical compartments are outlined briefly focusing on the description and main etiologies (Tables 1, 2, and 3).

Acute and chronic cellular rejection

ACR and CR should be reported/graded (in the summary) according to the ISHLT working formulation for lung allograft rejection.[2] Briefly, for ACR, perivascular and interstitial mononuclear cell infiltrates are graded from A0 to A4 and AX if not evaluable (A-grade). Some lesions detected in severe acute rejection (A4) are listed in the section of "intra-alveolar injury pattern" (e.g., hyaline membranes, reactive pneumocytes, granulation tissue plugs) and are morphological correlates of injury in other conditions such as infection, drug toxicity, aspiration, AMR, or ischemia/reperfusion injury. While the presence of mononuclear inflammation in a perivascular distribution increases the diagnostic confidence of severe ACR it must be emphasized that perivascular inflammation is not entirely specific for ACR. Other conditions such as infection may mimic alloreactive lung injury. Ancillary testing, often implemented as the diagnosis of acute allograft rejection (ACR and AMR), is a diagnosis of exclusion. On occasion concurrent infection and rejection are present and add diagnostic and clinical complexity.

Using the ISHLT working formulation, small airway inflammation should be graded from B0 to B2R, and BX when small airways are not visible. Infectious processes need to be excluded, especially in the presence of neutrophils or mixed airway inflammation. The literature contains contradictory data regarding inter- and intra-observer variability for A- and B- rejection grading. While some found relatively good inter-observer agreements for the A-grade (kappa values between 0.65 and 0.73), others showed only fair to moderate agreement for A- and B-grade (kappa values between −0.04 and 0.46).[6−12] In future updates of the ISHLT working formulation, an ongoing and more uniform data collection of several lesions through the present template could improve reproducibility.

Historically, TBB has shown low sensitivity in identifying CR, either of small airways (obliterative bronchiolitis, C-grade) or of vessels (vascular rejection, D-grade). Obliterative bronchiolitis (OB), the morphological correlate of obliterative bronchiolitis syndrome (BOS), is characterized by submucosal collagenous scarring producing subtotal or total airway narrowing and is currently graded as absent (ISHLT grade C0) or present (C1).[2] Vascular rejection is characterized by thickened pulmonary arteries and more often veins, due to the intimal proliferation of fibroinflammatory connective tissue. Both C and D grades are rarely identified on TBB since they usually lack bronchioles or sufficiently sized vessels. However, post-obstructive changes (e.g., foamy macrophages and cholesterol clefts), listed in the section "intra-alveolar injury pattern," are highly suggestive of airway obstruction and can be reported as suggestive of CR.

Alveolar septal injury pattern

The "Alveolar Septal Injury Pattern" section of the template focused on alterations of the normal alveolar septal structure. Septal changes may indicate the underlying cause of the observed injury pattern (e.g. immunologic or infectious disorders). For example, presence of septal neutrophils, neutrophilic/cellular debris, platelet-fibrin thrombi, septal widening/edema and/or alveolar capillary dilatation while not entirely specific are suggestive of AMR.[13,20−23] Capillary neutrophilic inflammation with varying degrees of severity (1: neutrophilic margination above baseline; 2: neutrophilic margination above baseline with at least 2 back-to-back neutrophils; and 3: neutrophilic capillaritis) has been the subject of discussion in several AMR statements.[3,20,21] However, interobserver reliability for different grades of capillary neutrophilic inflammation was low[21] and there is objective difficulty in precisely identifying the compartmentalization of neutrophils in capillaries. The working group tried to simplify the description of inflammatory changes by reporting the

Table 1 Alveolar Septal Injury Pattern

Histopathological pattern	Main etiologies	Notes[a]
Neutrophils/neutrophilic or cellular debris[13,20,21]	Immunological insults (mainly AMR but also severe ACR)	C4d staining (insensitive marker but quite specific)
	Ischemia reperfusion injury	Detected in TBB (early post-transplant within 6 weeks)
	Infection	Useful special stains [e.g., PAS, GMS, AFB, IHC (e.g., CMV)/molecular analysis]
Platelet-fibrin thrombi in alveolar capillaries[13,20,21]	Immunological insults (mainly AMR but also severe ACR)	C4d staining useful (insensitive marker but specific)
	Ischemia reperfusion injury	Detected in TBB (early post-transplant within 6 weeks)
	Infection (e.g., SARS-COV-2)	Useful special stains [e.g., PAS, GMS, AFB, IHC/molecular analysis]
	Thrombotic diathesis (hypercoagulopathy disorders such as APS, autoimmune diseases such as SLE)	
Septal edema or widening possibly due to organizing DAD, neutrophils, chronic inflammatory cells/capillary dilatation[22,23]	Immunological insults (mainly AMR but also severe ACR)	C4d staining (insensitive marker but specific)
	Ischemia reperfusion injury	Detected in TBB (early post-transplant within 6 weeks)
	Infection	Useful special stains [e.g., PAS, GMS, AFB, IHC/molecular analysis]
	Sampling artifact	Usually associated with other changes (e.g., blood extravasation)
Mononuclear cells (not perivascular cuffing)[12]	Infection (mainly viral)	Useful special stains [e.g., PAS, GMS, AFB, IHC/molecular analysis]
	PTLD (sheet like infiltration)	Useful IHC/molecular analysis for B/T lymphocytes clonality and EBV (mRNA)
	BALT	Often associated with small vessels and/or pigmented macrophages
Septal fibrous thickening[4,24,25]	Immunological insults (NSIP features in RAS)	Connective tissue staining useful (Masson trichrome or Movat stain)
	Infection (i.e., postinfectious/postinflammatory fibrosis)	
	Iatrogenic	
	Post-ischemic	

Abbreviations: ACR, acute cellular rejection; AFB, acid-fast bacillus; AMR, antibody-mediated rejection; APS, antiphospholipid syndrome; BALT, bronchus-associated lymphoid tissue; CMV, cytomegalovirus; DAD, diffuse alveolar damage; EBV, Epstein-Barr virus; GMS, Grocott-Gomori's methenamine silver; IHC, immunohistochemistry; NSIP, nonspecific interstitial pneumonia; PAS, Periodic acid—Schiff; PTLD, post-transplant lymphoproliferative disorder; RAS, restrictive allograft syndrome; SLE, systemic lupus erythematosus; TBB, transbronchial biopsy.

[a]The notes refer only to additional contributions from pathologists. Multidisciplinary discussion with transplant specialists is strongly recommended for summary and final interpretation.

presence of neutrophils/cell debris and/or mononuclear cells within the alveolar septa (Table 1).

Interstitial infiltration by mononuclear cells, not arranged in perivascular cuffing, can have different etiologic explanation, some with clinical impact and others without significance in patient outcomes. Careful attention to other concurrent morphologic changes is helpful in establishing a more definitive diagnosis for example, lymphocytic septal infiltration may occur in patients with recent infections. Viral infections may produce characteristics viral cytopathic effects and immunohistochemistry or tissue molecular analysis can be applied to confirm infection.[11] Marked mononuclear infiltrates with significant interstitial widening by "sheet-like infiltration of inflammatory cells" raises the possibility of post-transplant lymphoproliferative disorder (PTLD). An appropriate workup should be performed including assessment for Epstein-Barr virus genome (using mRNA EBER), and B-cell clonality and T-cell subsets.

However, compact interstitial infiltration by mononuclear cells may have no particular pathologic significance when accompanied by small vessels and/or pigmented macrophages. Indeed, often this feature corresponds to bronchial-associated lymphoid tissue (BALT), or donors with a smoking history. Additional details about the significance of BALT are elaborated in the "Other" section.

Septal fibrous thickening, highlighted by the Masson trichrome or Movat stains can represent a focal reparation process from interstitial injury of different etiologies for example, infection, iatrogenic/prior biopsy site, ischemic/reperfusion injury, and/or immunologic-mediated injury such as the nonspecific interstitial pneumonia (NSIP)-like pattern that has been observed

Table 2 Intra-alveolar Injury Pattern

Histopathological pattern		Main etiologies	Notes[a]
Hyaline membranes, Pneumocyte hyperplasia, Granulation tissue plugs (OP)[12,13,20,21]		Immunological insults (mainly AMR but also severe ACR)	C4d staining (insensitive marker but quite specific)
		Ischemia reperfusion injury	Detected in TBB (early post-transplant within 6 weeks)
		Infection	Useful special stains [e.g., PAS, GMS, AFB, IHC (e.g CMV)/molecular analysis]
AFOP[13,26-29]		Immunological insults (e.g. AMR, RAS)	C4d staining (insensitive marker but specific)
		Infections	Useful special stains [e.g., PAS, GMS, AFB, IHC/molecular analysis]
		Drug toxicity	
Alveolar proteinosis[30]	Infection	Useful special stains PAS and diastase PAS and special stains for microorganisms.	
	Drug toxicity		
Macrophages[31]	Normal (extensive)	Recurrent DIP-like native disease	Useful information about native disease
		If extensive, infections	Useful special stains [e.g., PAS, GMS, AFB, IHC/molecular analysis]
		Smoking/fume inhalation (rarely)	Associated other smoking lesion stigmata (e.g. antracosis)
	Hemosiderophages	Previous episodes of hemorrhage of different etiologies (infections, immunological, heart failure: in combined H-L TX; procedure)	Perls Prussian blue stain may be done in case of doubt
		Lack of pathologic significance	Especially in case of minimal infiltration (score 1)
	Foamy-w/wo cholesterol cleft/ giant cells	Indicative of bronchiolar obstruction	Useful special stain e.g Verhoeff-Van Gieson elastin stain (to highlight scar)
		Drug toxicity	Sometimes associated with OP and eosinophils

Abbreviations: ACR, acute cellular rejection; AFOP, acute fibrinous organizing pneumonia; AFB, acid-fast bacillus; AMR, antibody-mediated rejection; CMV, cytomegalovirus; DIP, desquamative interstitial pneumonia; GMS, Grocott-Gomori's methenamine silver; H-L TX, heart-lung transplantation; IHC, immunohistochemistry; OP, organizing pneumonia; PAS, Periodic acid—Schiff; RAS, restrictive allograft syndrome; TBB, transbronchial biopsy; W/WO, with/without.

[a]The notes refer only to additional contributions from pathologists. Multidisciplinary discussion with transplant specialists is strongly recommended for summary and final interpretation.

in up to 25% of restrictive allograft syndrome (RAS) patients.[24,25]

Intra-alveolar injury pattern

The "intra-alveolar injury pattern" section of the template includes a spectrum of lesions with either acute (neutrophils in alveolar spaces, hyaline membranes, pneumocyte hypertrophy/reactive changes, and acute fibrinous and organizing pneumonia -AFOP-) or ongoing reparative/chronic features (different types of macrophages and granulation tissue plugs). These lesions can be associated with several etiologies. Acute lung injury changes, especially if hyaline membranes and neutrophils are extensively present (score 3), are the morphological correlate for diffuse alveolar damage with possible etiologies that include clinically relevant infectious, ischemic or immunologic tissue injuries. Results from ancillary tools (special stains/molecular analysis for microorganisms, C4d immunostaining) may strengthen the pathological interpretation (Table 2).

Interestingly, AFOP and alveolar proteinosis can be caused by different conditions and may represent an allograft injury pattern[26–28,30]: AFOP has been reported in a few cases of AMR,[13] and can precede CLAD with the RAS phenotype.[29] Intra-alveolar macrophage infiltration should be graded independently with particular attention to the macrophage subtypes as they may provide etiologic clues. For example, foamy macrophages and/or macrophages with cholesterol clefts are often associated with aspiration or as secondary changes in patients with chronic airway rejection-OB. This aspect may be the only sign of OB in the absence of bronchiolar structures in TBB. The presence of intra-alveolar foamy macrophages with scattered, sparse eosinophils and foci of organizing pneumonia may be an indicator of drug toxicity, in some circumstances.

Table 3 Injury Patterns in Other Sites

Histopathological pattern	Main etiologies	Notes[a]
Pleuroparenchymal/intraalveolar fibroelastosis[4]	Immunological insult (RAS)	Connective tissue stains: Verhoeff-Van Gieson elastin stain. Rarely detected in post-transplant TBB (better cryobiopsy or VATS).
Inflammation/fibrosis of subpleural/interlobular septa	Often nonspecific finding (especially if mild)	Precise reporting in the template could provide new insights
Injury of large airway (lymphocytic)[32,33]	Infections Ischemic injury Immunological insults (debated)	Useful special stains [e.g., PAS, GMS, AFB, IHC/molecular analysis]

Abbreviations: AFB, acid-fast bacillus; RAS, restrictive allograft syndrome; GMS, Grocott-Gomori's methenamine silver; IHC, immunohistochemistry; PAS, Periodic acid—Schiff; TBB, transbronchial biopsies; VATS, Video-assisted thoracoscopic surgery.

[a]The notes refer only to additional contributions from pathologists. Multidisciplinary discussion with transplant specialists is strongly recommended for summary and final interpretation.

Intra-alveolar hemosiderin-laden macrophages are commonly observed in TBB. They could reflect previous episodes of inflammation, hemorrhage but, more commonly, lack of pathologic significance (e.g., residuum of bleeding from prior biopsies).[31]

Injury patterns in other sites (e.g., subpleural, interlobular septa, and large airways)

Inflammation in subpleural and/or interlobular septa are often observed in large allograft samples (i.e., cryobiopsy or surgical biopsy). The histopathologic diagnosis of pleuroparenchymal fibroelastosis, seen in RAS, requires the demonstration of intra-alveolar fibrosis and elastosis, ideally with visceral pleural fibrosis. The latter is typically absent in TBB, due to the lack of pleural tissue and/or on account of its patchy distribution. These lesions are more easily observed using Verhoeff-Van Gieson elastin stain, with alveolar parenchyma obliterated by fibrosis.[4,24,25,34–36] Isolated, mild remodeling changes (inflammatory or fibrotic) of subpleural or interlobular septum compartments has little clinical significance. However, strict evaluation in all future biopsies using the template could provide future insights into this morphological feature and its clinical significance (Table 3).

Large airway lymphocytic inflammation should be reported, specifying the exact location of the infiltrates (intraepithelial, submucosal, peribronchial). Inflammation in both in small and large airways should also be described as there is growing evidence of the association of these lesions with future development of CLAD.[32,33]

Other

Other histological findings detected in different compartments of the biopsies are listed in this section as either present or absent. Vascular injuries such as endotheliitis/arteritis and thrombi have been reported more frequently as signs of an immunological insult, such as severe ACR or AMR.[13,21] When present, these findings should always be reported and taken into account in conjunction with other lesions in the final summary report.

Even if patients receive prophylaxis for cytomegalovirus (CMV) or fungi, infections can still occur and microorganisms can be detected or confirmed by special stains (Gram, Grocott, PAS, Ziehl-Neelsen), immunohistochemistry (early/late CMV antigen), and molecular techniques (especially for viruses and mycobacteria).

PTLD and recurrent diseases are rarely diagnosed in TBB, and usually require larger specimens for diagnosis and classification. Both PTLD and recurrent disease are often unrecognized or misdiagnosed, both clinically and radiologically. Thus, lung tissue analysis is considered the gold standard for correct diagnoses.

Eosinophils are detected in lung transplant biopsies as an integral part of the inflammatory infiltrate in higher grades of ACR. When eosinophils comprise more than 50% of the inflammatory infiltrate, other etiologies should be considered in part based on localization within the lung. If detected in the airways an infectious etiology should be suspected. In particular, infection by a Pseudomonas species can be associated with a dense eosinophilic infiltrate, possibly inducing clinical symptoms of airflow obstruction indistinguishable from asthma. Another important cause of eosinophil infiltration in airways and lung parenchyma is fungal infection, most commonly, Aspergillus. Eosinophils can also be observed in pulmonary drug reactions (e.g., nitrofurantoin, sulfasalazine, penicillin[37]).

The significance of BALT, detected in TBB, is not well understood. In human lung transplantation, the presence of BALT has been associated with low-grade or no rejection (A0 or A1), leading to the speculation that BALT in human lung allografts might be involved in immunological tolerance. Only a few experimental studies have focused on this topic reporting similar data; the clinical and immunologic significance merits further studies.[38,39]

Summary

The likely pathological process(es) based on the overall interpretation of the assessed histological features is summarized at the bottom of the template.

Final interpretation of all changes requires comprehensive multidisciplinary discussion with the clinical team directing patient management

Illustrative cases (images and LASHA templates) of some important post-transplant complications are featured in Figures 2–7.

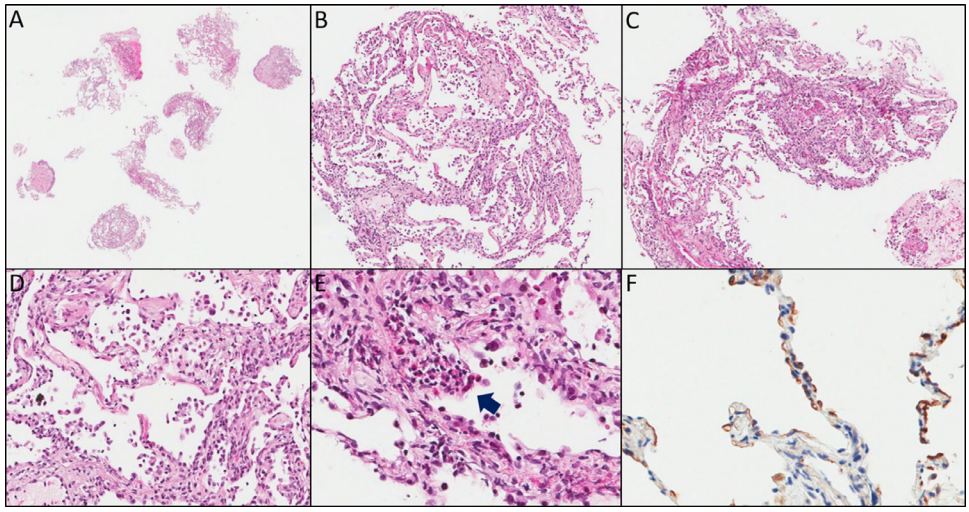

Figure 2 *Explanatory Case 1.* This 23-year-old female received a bilateral lung transplantation for cystic fibrosis. Eleven months post-transplant the patient presented with dyspnea, cough and was found to have bilateral abnormalities at chest-tomography scan (ground-glass, alveolar pattern, tree-in-bud) and a TBB was performed. Donor specific antibodies were detected at the time of biopsy (DR53: 13000 MFI). Immunosuppression levels were within range. HLA Class I and HLA Class II percent reactive antibodies were negative. Microbiologic cultures and viral polymerase chain reaction for infectious agents were negative. The biopsy was characterized by septal widening with neutrophils, cellular debris, capillary dilatation (A-E). Aggregates of neutrophils/debris are marked by arrow (E). C4d immunostaining was strongly and diffusely positive (F). See more detailed description in the template (Figure 5). The positivity for C4d immunostaining and several histological features strongly suggest antibody-mediated rejection. (A) hematoxylin and eosin, X5; (B) hematoxylin and eosin, X10; (C) hematoxylin and eosin, X10; (D) hematoxylin and eosin, X20; (E) hematoxylin and eosin, X40; (F) immunohistochemistry for C4d, X40.

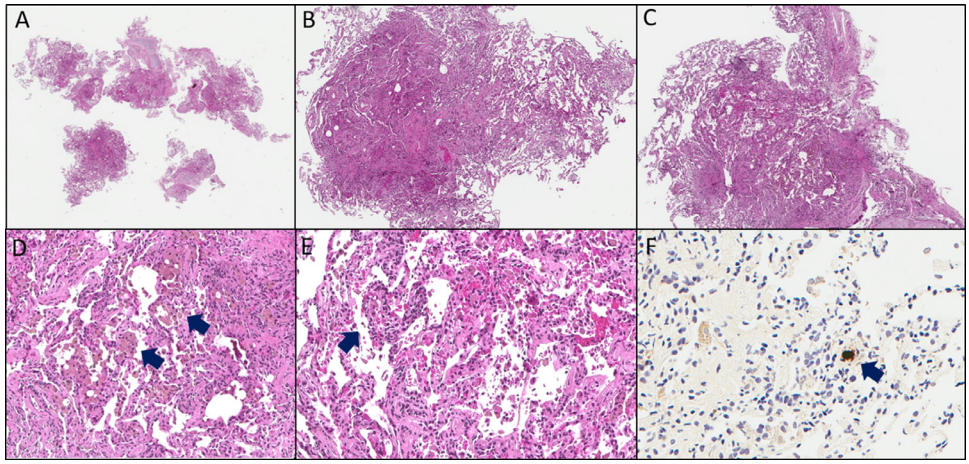

Figure 3 *Explanatory Case 2.* This 53-year old male was transplanted for chronic hypersensitivity pneumonitis. The TBB was performed at 10 months post-transplant to evaluate the mild restrictive pattern on pulmonary function tests. Immunosuppression levels were within range. HLA Class I and HLA Class II percent reactive antibodies were negative. Microbiologic cultures for bacterial infection were negative. Polymerase chain reaction for Cytomegalovirus (CMV) in blood showed high number of viral copies (1000 copie/ml). The interalveolar septa showed moderate widening with mainly lymphocytic inflammatory cell infiltrates, and diffuse macrophagic alveolitis (A-E). Hemosiderophage macrophages and lymphocytic septal infiltration are marked with arrows (D and E respectively). Immunohistochemistry for CMV showed nuclear positivity in several pneumocytes. Nuclear positive staining of pneumocyte is marked with arrow (F). See more detailed description in the template (Figure 6). The diagnosis of CMV pneumonitis was established. (A) hematoxylin and eosin, X5; (B) hematoxylin and eosin, X10; (C) hematoxylin and eosin, X10; (D) hematoxylin and eosin, X20; (E) hematoxylin and eosin, X20; (F) immunohistochemistry for CMV, X40.

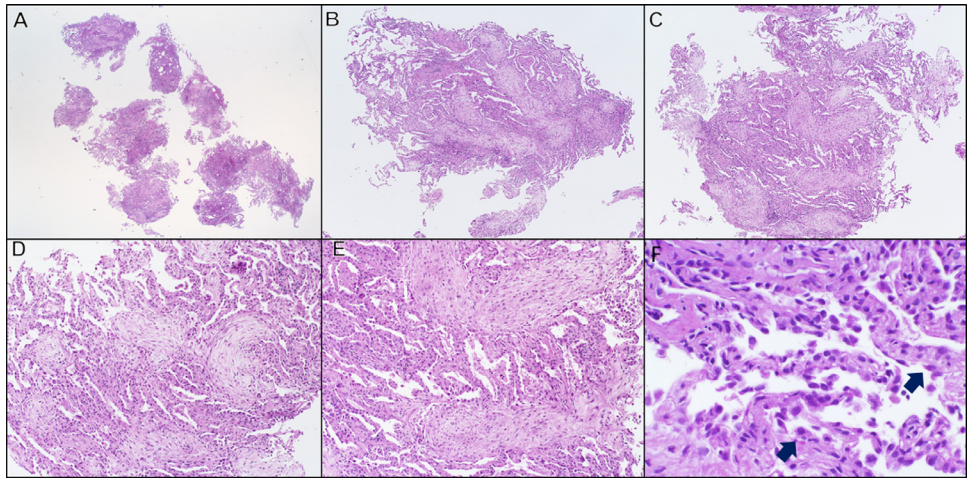

Figure 4 *Explanatory Case 3*. This 61-year-old male received a single left lung transplant for end-stage usual interstitial pneumonia/idiopathic pulmonary fibrosis. On day 15 post-transplant, a TBB was performed due to increasing shortness of breath over a 2-day period. At rest, his oxygen levels were normal, but dropped under stress to 80 percent. A new left lung infiltrate was encountered on chest x-ray. Immunosuppression levels were within range. His HLA Class I PRA was 7, and HLA Class II PRA was negative. Microbiologic cultures and viral polymerase chain reaction were negative. The biopsy showed septal widening by reactive pneumocyte change (arrows, F), and every biopsy piece showed granulation tissue plugs obstructing intra-alveolar spaces (A-F) indicating an organizing pneumonia pattern of injury. See more detailed description in the template (Figure 7). Given the time point of this biopsy (approximately 2 weeks following transplantation), the findings were consistent with ischemia-reperfusion injury. (A) hematoxylin and eosin, X5; (B) hematoxylin and eosin, X10; (C) hematoxylin and eosin, X10; (D) hematoxylin and eosin, X20; (E) hematoxylin and eosin, X20; (F) hematoxylin and eosin, X40.

Current and future applications

At present, the primary use of this template is limited to research endeavors. It is crucial that research studies designed for the identification of biomarkers of post-transplant complications include histopathologic information, in a shared manner. Several research groups are actively exploring the technical feasibility and clinical utility of molecular analysis in allograft biopsies,[32,40-42] airway brushings,[32,43,44] bronchoalveolar lavage samples[44-47] and peripheral blood.[48-50] These efforts include the use of various technologies, such as reverse transcriptase polymerase chain reaction,[40,44] cDNA microarrays,[41,42] RNA sequencing,[32,43,45,46] donor-derived cell-free DNA[47-50] and NanoString analysis, with[51] or without[32,52] laser capture microdissection. Regardless of sample type or analytical strategy, the ability to correlate molecular data with standardized histomorphologic parameters will facilitate the training and validation of new molecular diagnostic tools and prognostic parameters. It will also provide the opportunity to reevaluate the clinical significance of specific histological features based on novel phenotypes identified with these molecular platforms such as the identification of C4d-negative AMR in kidney transplants.[53] Recent advances in digital pathology and computational image analysis in the field of transplant pathology, including machine learning analysis of kidney,[54] heart,[55] and lung[56] transplant biopsies provides a rich venue for pathologic-clinical correlation. The successful translation of these technologies to lung transplant pathology will rely heavily on the availability of high quality and well-annotated histological data with which to train and optimize the artificial intelligence-based systems that power them. After validation and modification, the template or a modified version might be applied for daily clinical purposes. The template highlights lesions with proven association with clinical outcomes.

Remaining questions and unresolved issues

Despite the tremendous effort to standardize the nomenclature of morphological features and technical approaches some controversies remain unresolved.

The first concern is which lesions should be selected for reporting. This template has incorporated all histological features that may be detected in different anatomic compartments of allograft biopsies. This may be seen as a first attempt to replicate what has been successfully done in other major solid organ transplants, such as in kidney allograft biopsies.

The proposed scoring system provides a simple, reproducible and easily applicable scheme for most histopathological lesions encountered in TBB. Considering that biopsies are taken from multiple areas, diffusely detected lesions are more likely to represent the morphological correlate of diffuse organ impairment (score 3). However, a scoring system remains subjective, with uncertainties particularly encountered at the cut-off points between semi-quantitative grading schemes which result in inter- and intra-observer variability. The future application of digital algorithms on digital whole slide images will overcome this limitation providing a standardized quantification.

A crucial aspect to consider is the sensitivity and comparability of C4d staining. ISHLT guidelines consider C4d

LUNG ALLOGRAFT STANDARDIZED HISTOLOGICAL ANALYSIS (LASHA)

Type of sample: Transbronchial biopsy [X] Transbronchial cryobiopsy [ ] Wedge biopsy [ ] Other (_____) [ ]
Stainings/techniques: H&E [X] Connective tissue staining [X] Silver stains [ ] Other special stains (_____) [ ]
Other ancillary tools (_____) [ ]

C4d evaluation: Immunohistochemistry (IP) [X] Immunofluorescence (IF) [ ]
IP: Distribution: <10% __ 10-50% __ >50% [X] IF: Intensity (score): 0 __ 1 __ 2 __ 3 __

Biopsy*: adequate [X] insufficient [ ] inadequate [ ]
Bronchi: YES [X] NO [ ]
Bronchioles: YES [X] NO [ ]
Artery: YES [X] NO [ ]

Lesions suggestive of acute cellular rejection:
- Perivascular mononuclear infiltrates: YES [ ] NO [X]
- Lymphocytic bronchiolitis: YES [ ] NO [X]

Lesions suggestive of chronic rejection:
- Obliterative bronchiolitis: YES [ ] NO [X]
- Vascular rejection: YES [ ] NO [X]

Alveolar septal injury pattern:

- Neutrophils in alveolar septa (score):	0 [ ]	1 [ ]	2 [ ]	3 [X]	Ungradable** [ ]
- Neutrophilic/cellular debris in alveolar septa (score):	0 [ ]	1 [ ]	2 [ ]	3 [X]	Ungradable** [ ]
- Platelet-fibrin thrombi in alveolar capillaries (score):	0 [ ]	1 [ ]	2 [ ]	3 [X]	Ungradable** [ ]
- Septal wall oedema/widening (score):	0 [ ]	1 [ ]	2 [ ]	3 [X]	Ungradable** [ ]
- Mononuclear cells in alveolar septa (score):	0 [ ]	1 [ ]	2 [ ]	3 [X]	Ungradable** [ ]
- Alveolar capillary dilatation (score):	0 [ ]	1 [ ]	2 [ ]	3 [X]	Ungradable** [ ]
- Septal fibrous thickening (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]

Intra-alveolar Injury pattern:

- Neutrophils in alveolar spaces (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]
- Hyaline membranes (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]
- Pneumocyte hypertrophy/reactive changes (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]
- Fibrin balls in alveolar spaces (suggestive of AFOP) (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]
- Granulation tissue plugs in alveolar spaces/OP (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]
- Macrophages (score):	0 [ ]	1 [X]	2 [ ]	3 [ ]	Ungradable** [ ]

Specify subtypes: normal [X] *hemosiderophages* [ ] *foamy* [ ] *cholesterol clefts* [ ] *giant cells* [ ]
Foreign body in alveolar spaces: YES [ ] NO [X]

Injury pattern in other sites (*e.g.subpleural, interlobular septa, large airways*)

- Suspected pleuroparenchymal/intralveolar fibroelastosis	YES [ ]	NO [X]	
- Inflammation pf subpleural areas (specify the type)	YES [ ]	NO [X]	(specify:_____)
- Injury of the interlobular septa (specify the type)	YES [ ]	NO [X]	(specify:_____)
- Injury of the large airways (specify the type)	YES [ ]	NO [X]	(specify:_____)

Other:

- Arteritis/endotheliitis:	YES [X]	NO [ ]	(specify:_____)
- Thrombus:	YES [ ]	NO [X]	
- Ischemic necrosis:	YES [ ]	NO [X]	
- Viral inclusions:	YES [ ]	NO [X]	(specify:_____)
- Fungal organisms:	YES [ ]	NO [X]	(specify:_____)
- Other infectious organisms:	YES [ ]	NO [X]	(specify:_____)
- Granuloma:	YES [ ]	NO [X]	
- Suspected PTLD	YES [ ]	NO [X]	
- Suspected recurrent disease	YES [ ]	NO [X]	(specify:_____)
- Eosinophilia (interst/alv):	YES [ ]	NO [X]	(specify:_____)
- BALT***:	YES [ ]	NO [X]	
- Previous biopsy site	YES [ ]	NO [X]	(specify:_____)
- Other (specify) _____			

SUMMARY *Acute cellular rejection: A0B0; C4d staining: positive >50%; Lesions suggestive of AMR: yes (widening; neutrophilic/cellular debris in alveolar septa; endothelialitis; C4d pos)*

Figure 5 LASHA template of the explanatory case 1.

LUNG ALLOGRAFT STANDARDIZED HISTOLOGICAL ANALYSIS (LASHA)

Type of sample: Transbronchial biopsy [X] Transbronchial cryobiopsy [ ] Wedge biopsy [ ] Other (_____) [ ]
Stainings/techniques: H&E [X] Connective tissue staining [X] Silver stains [ ] Other special stains (_____) [ ]
Other ancillary tools (IHC for CMV) [X]

C4d evaluation: Immunohistochemistry (IP) [X] Immunofluorescence (IF) [ ]
IP: Distribution: <10% [X] 10-50% __ >50% __ IF: Intensity (score): 0 __ 1 __ 2 __ 3 __

Biopsy*: adequate [X] insufficient [ ] inadequate [ ]
Bronchi: YES [X] NO [ ]
Bronchioles: YES [X] NO [ ]
Artery: YES [ ] NO [X]
Lesions suggestive of acute cellular rejection:
- Perivascular mononuclear infiltrates: YES [ ] NO [X]
- Lymphocytic bronchiolitis: YES [ ] NO [X]

Lesions suggestive of chronic rejection:
- Obliterative bronchiolitis: YES [ ] NO [X]
- Vascular rejection: YES [ ] NO [X]

Alveolar septal injury pattern:

	0	1	2	3	Ungradable**
- Neutrophils in alveolar septa (score):	[ ]	[X]	[ ]	[ ]	[ ]
- Neutrophilic/cellular debris in alveolar septa (score):	[X]	[ ]	[ ]	[ ]	[ ]
- Platelet-fibrin thrombi in alveolar capillaries (score):	[X]	[ ]	[ ]	[ ]	[ ]
- Alveolar capillary dilatation (score):	[ ]	[ ]	[X]	[ ]	[ ]
- Septal wall oedema/widening (score):	[ ]	[ ]	[X]	[ ]	[ ]
- Mononuclear cells in alveolar septa (score):	[ ]	[ ]	[X]	[ ]	[ ]
- Septal fibrous thickening (score):	[X]	[ ]	[ ]	[ ]	[ ]

Intra-alveolar Injury pattern:

	0	1	2	3	Ungradable**
- Neutrophils in alveolar spaces (score):	[X]	[ ]	[ ]	[ ]	[ ]
- Hyaline membranes (score):	[X]	[ ]	[ ]	[ ]	[ ]
- Pneumocyte hypertrophy/reactive changes (score):	[ ]	[ ]	[X]	[ ]	[ ]
- Granulation tissue plugs in alveolar spaces/OP (score):	[ ]	[ ]	[X]	[ ]	[ ]
- Fibrin balls in alveolar spaces (suggestive of AFOP) (score):	[X]	[ ]	[ ]	[ ]	[ ]
- Alveolar proteinosis (score):	[X]	[ ]	[ ]	[ ]	[ ]
- Macrophages (score):	[ ]	[ ]	[ ]	[X]	[ ]

Specify subtypes: normal [ ] *hemosiderophages* [X] *foamy* [ ] *cholesterol clefts* [ ] *giant cells* [ ]
Foreign body in alveolar spaces: YES [ ] NO [X]

Injury pattern in other sites (*e.g.subpleural, interlobular septa, large airways*)
- Suspected pleuroparenchymal/intralveolar fibroelastosis YES [ ] NO [X]
- Inflammation of subpleural/interlobular areas (specify the type) YES [ ] NO [X] (specify:_____)
- Injury of the large airways (specify the type) YES [X] NO [ ] (specify: fibrosis)

Other:
- Arteritis/endotheliitis: YES [ ] NO [X] (specify:_____)
- Thrombus: YES [ ] NO [X]
- Ischemic necrosis: YES [ ] NO [X]
- Viral inclusions: YES [ ] NO [X] (specify:_____)
- Fungal organisms: YES [ ] NO [X] (specify:_____)
- Other infectious organisms: YES [ ] NO [X] (specify:_____)
- Granuloma: YES [ ] NO [X]
- Suspected PTLD YES [ ] NO [X]
- Suspected recurrent disease YES [ ] NO [X] (specify:_____)
- Eosinophilia (interst/alv): YES [ ] NO [X] (specify:_____)
- BALT***: YES [ ] NO [X]
- Previous biopsy site YES [ ] NO [X] (specify:_____)
- Other (specify) _____

SUMMARY
Acute cellular rejection: A0B0; C4d staining: <10% (negative); *Lesions suggestive of infection: yes (alveolar septal widening, lymphocytic inflammatory cell infiltrates, diffuse macrophagic alveolitis, IHC positivity for CMV)*

Figure 6 LASHA template of the explanatory case 2.

LUNG ALLOGRAFT STANDARDIZED HISTOLOGICAL ANALYSIS (LASHA)

Type of sample: Transbronchial biopsy [X] Transbronchial cryobiopsy [ ] Wedge biopsy [ ] Other (_____) [ ]
Stainings/techniques: H&E [X] Connective tissue staining [ ] Silver stains [ ] Other special stains (_____) [ ]
Other ancillary tools (GMS and PAS) [X]

C4d evaluation: Immunohistochemistry (IP) [X] Immunofluorescence (IF) [ ]
IP: Distribution: <10% [X] 10-50% __ >50% __ IF: Intensity (score): 0 __ 1 __ 2 __ 3 __

Biopsy*: adequate [X] insufficient [ ] inadequate [ ]
Bronchi: YES [ ] NO [X]
Bronchioles: YES [X] NO [ ]
Artery: YES [X] NO [ ]

Lesions suggestive of acute cellular rejection:
- Perivascular mononuclear infiltrates: YES [ ] NO [X]
- Lymphocytic bronchiolitis: YES [ ] NO [X]

Lesions suggestive of chronic rejection:
- Obliterative bronchiolitis: YES [ ] NO [X]
- Vascular rejection: YES [ ] NO [X]

Alveolar septal injury pattern:

- Neutrophils in alveolar septa (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]
- Neutrophilic/cellular debris in alveolar septa (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]
- Platelet-fibrin thrombi in alveolar capillaries (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]
- Septal wall oedema/widening (score):	0 [ ]	1 [X]	2 [ ]	3 [ ]	Ungradable** [ ]
- Mononuclear cells in alveolar septa (score):	0 [ ]	1 [X]	2 [ ]	3 [ ]	Ungradable** [ ]
- Alveolar capillary dilatation (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]
- Septal fibrous thickening (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]

Intra-alveolar Injury pattern:

- Neutrophils in alveolar spaces (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]
- Hyaline membranes (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]
- Pneumocyte hypertrophy/reactive changes (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]
- Fibrin balls in alveolar spaces (suggestive of AFOP) (score):	0 [X]	1 [ ]	2 [ ]	3 [ ]	Ungradable** [ ]
- Granulation tissue plugs in alveolar spaces/OP (score):	0 [X]	1 [ ]	2 [ ]	3 [X]	Ungradable** [ ]
- Macrophages (score):	0 [ ]	1 [X]	2 [ ]	3 [ ]	Ungradable** [ ]

Specify subtypes: normal [X] *hemosiderophages* [ ] *foamy* [ ] *cholesterol clefts* [ ] *giant cells* [ ]
Foreign body in alveolar spaces: YES [ ] NO [X]

Injury pattern in other sites (*e.g.subpleural, interlobular septa, large airways*)

- Suspected pleuroparenchymal/intralveolar fibroelastosis	YES [ ] NO [X]	
- Inflammation pf subpleural areas (specify the type)	YES [ ] NO [X]	(specify:_____)
- Injury of the interlobular septa (specify the type)	YES [ ] NO [X]	(specify:_____)
- Injury of the large airways (specify the type)	YES [ ] NO [X]	(specify:_____)

Other:

- Arteritis/endotheliitis:	YES [ ] NO [X]	(specify:_____)
- Thrombus:	YES [ ] NO [X]	
- Ischemic necrosis:	YES [ ] NO [X]	
- Viral inclusions:	YES [ ] NO [X]	(specify:_____)
- Fungal organisms:	YES [ ] NO [X]	(specify:_____)
- Other infectious organisms:	YES [ ] NO [X]	(specify:_____)
- Granuloma:	YES [ ] NO [X]	
- Suspected PTLD	YES [ ] NO [X]	
- Suspected recurrent disease	YES [ ] NO [X]	(specify:_____)
- Eosinophilia (interst/alv):	YES [ ] NO [X]	(specify:_____)
- BALT***:	YES [ ] NO [X]	
- Previous biopsy site	YES [ ] NO [X]	(specify:_____)

- Other (specify) _____

SUMMARY

Acute cellular rejection: A0B0; C4d staining: negative; Lesions suggestive of I/R injury: yes (granulation tissue plugs in alveolar spaces; pneumocyte hypertrophy/reactive change)

Figure 7 LASHA template of the explanatory case 3.

deposition in >50% of alveolar capillaries as a positive result.[20] However, there is a large body of literature that highlights different clinical and interpretative issues in C4d staining, and criteria validated in other solid organ transplants cannot be translated to the lung.

All working group members agreed that other surrogate markers, detected by new molecular approaches, could support overcoming these critical issues.

Conclusions

This template represents a crucial step forward in the standardization of pathological reporting of lung-transplanted patients. As a first step, it will serve as an important functional tool for research purposes, in particular to unify protocols and reporting for multicenter studies. Moreover, the brief description lesions present a basis for launching educational tutorials for pathologists involved in this field. It should be stressed that this is work in progress and will require implementation, updates and, eventually, modifications. Following validation of the template in multicenter studies, a refined version could serve as a reporting vehicle for routine clinical use. Standardized and clear reports are fundamental to avoid diagnostic errors which could have serious impact on the clinical management of the recipient. Ultimately, this template should be clinically useful, reproducible and easily implemented.

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Author contribution: FC, DJL, AR conceived the research consensus proposal, supervised the project, wrote and made critical revisions to the manuscript. AR, EP, FL, DN, BA, DH, MG, GJB, MI, JVT, LG, CL, KW, CG, AZ, FC, DJL and AR participate to the multidisciplinary discussions and in drafting the manuscript. FC, DJL, AR, GW, GB made critical revisions to the manuscript. All authors discussed the results and implications, commented on the manuscript at all stages, and approved the final version before submission.

Disclosure statement

The authors have no conflicts of interest to declare with respect to this research.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.healun.2022.06.021.

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