The Royal College of Pathologists
Pathology: the science behind the cure

Tissue pathways for non-neoplastic thoracic pathology

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NICE has accredited the process used by the Royal College of Pathologists to produce its tissue pathways. Accreditation is valid for five years from July 2017. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

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Foreword

The tissue pathways published by the Royal College of Pathologists (RCPath) are guidelines that should assist pathologists in providing a high standard of care for patients. Guidelines are systematically developed statements to assist the decisions of practitioners and patients about appropriate health care for specific clinical circumstances and are based on the best available evidence at the time the document was prepared. This guideline has been developed to cover most common circumstances. However, we recognise that guidelines cannot anticipate every pathological specimen type and clinical scenario. Occasional variation from the practice recommended in this guideline may therefore be required to report a specimen in a way that maximises benefit to the patient.

The guidelines themselves constitute the tools for implementation and dissemination of good practice.

This following stakeholders were contacted to consult on this document:

- British Thoracic Society
- Society for Cardiothoracic Surgery in Great Britain and Ireland.

The information used to develop this tissue pathway was collected from electronic searches of the medical literature and previous recommendations of the RCPath and local guidelines in the UK. Published evidence was evaluated using modified SIGN guidance (see Appendix A). Consensus of evidence in the tissue pathway was achieved by expert review. Gaps in the evidence were identified by College Fellows via feedback received from consultation. The sections of this tissue pathway that indicate compliance with each of the AGREE II standards are indicated in Appendix B.

Implementation of the tissue pathway to its full extent may have some cost implications or require some local organisational changes, as the delivery of thoracic pathology services varies widely between hospitals.

A formal revision cycle for all tissue pathways takes place on a five-yearly basis. However, each year the College will ask the author(s) of the tissue pathways, in conjunction with the relevant subspecialty advisor to the College, to consider whether or not the document needs to be updated or revised. A full consultation process will be undertaken if major revisions are required. If minor revisions are required, an abridged consultation process will be undertaken whereby a short note of the proposed changes will be placed on the College website for two weeks for members' attention. If members do not object to the changes, the changes will be incorporated into the pathways and the full revised version (incorporating the changes) will replace the existing version on the publications page of the College website.

The pathway has been reviewed by the Clinical Effectiveness department, Working Group on Cancer Services (WGCS) and Lay Governance Group. It was placed on the College website for consultation with the membership from 7 November to 5 December 2019. All comments received from the Working Group and membership were addressed by the authors to the satisfaction of the Chair of the Working Group and the Clinical Lead for Guideline Review (Cellular Pathology).

This pathway was developed without external funding to the writing group. The College requires the authors of tissue pathways to provide a list of potential conflicts of interest; these are monitored by the Clinical Effectiveness department and are available on request. Dr Irshad Soomro and Dr Andrew Robinson have declared no conflicts of interest. Professor Andrew Nicholson was a consultant for Boehringer Ingelheim, Roche and Medical Quantitative Image Analysis in relation to management of idiopathic pulmonary fibrosis. He also received fees for lectures on the diagnosis and management of idiopathic pulmonary fibrosis. Professor Nicholson gives his assurances that these potential conflicts of interest have not influenced the content of this tissue pathway.

1 Introduction

This document addresses the processing of specimens in relation to non-neoplastic thoracic pathology. Guidelines for the handling of histology specimens including biopsies for lung cancer, mesothelioma and thymic epithelial tumours can be found in the respective RCPath cancer datasets. The tissue pathways are important as they provide a consistent approach to managing histological samples in thoracic pathology and highlight ancillary techniques when appropriate. Samples from the lung are especially important in relation to potential occupational lung diseases, such as asbestosis, and the lungs are a common site for often unsuspected infections. Pulmonary manifestations of systemic disorders, such as sarcoidosis or connective tissue disorders, are also regularly seen. In addition, the lung is an organ in which reactive conditions may mimic neoplasms, e.g. granulomatosis with polyangiitis. Low-dose computed tomography screening trials, targeting high-risk individuals to detect lung cancer at an early stage, are currently active in the UK,[1] and guidelines have recently been published by NHS England.[2] This is likely to result in an increased rate of detection of non-neoplastic pulmonary pathology in the screened population. This may have an impact, in the future, on workload within thoracic pathology departments in the UK. There are many conditions that may result in lung transplantation and these include chronic obstructive pulmonary disease, alpha-1 antitrypsin deficiency, cystic fibrosis, bronchiectasis, sarcoidosis and pulmonary hypertension. In transplant centres, post-transplant biopsies are used to monitor rejection, graft-versus-host disease, drug injury and complications related to anastomosis and to detect infections. An in-depth analysis of thoracic transplant pathology is outside the scope of this document and is not directly addressed.

This document replaces previous versions that included the handling of lung samples and also reflects evidence-based recommendations that have recently been published for the management of diffuse lung diseases (referenced in relevant sections below).

1.1 Target users of this guideline

The target primary users of the tissue pathway are trainees, lead biomedical scientists and consultant cellular pathologists. It is recommended that each department should have a lead for thoracic pathology.

2 Staffing, workload and facilities

2.1 Staffing and workload

The laboratory should have sufficient capacity, with regard to pathologists, biomedical scientists and clerical staff, to cover all of its functions. In general, staffing levels should follow the workload guidelines of RCPath. Pathologists should:

- participate in auditing
- participate in RCPath's continuing professional development scheme.

Pathologists routinely reporting thoracic pathology should participate in the national pulmonary pathology external quality assessment (EQA) scheme, and the lead thoracic pathologist within a department should have appropriate expertise in thoracic pathology. It is recognised that the volume of cases in certain hospitals may be low and that thoracic cases may be limited to biopsy material. Participation in an EQA scheme is therefore expected for those in regional hospitals that deal with the breadth of pulmonary pathology, but it is only recommended for those with limited throughput on the proviso that their lung cancer service delivery has a recognised regional or national point of referral. Cover should be available at an appropriate level during periods of leave. A frozen section and out-of-hours service should be provided only by members of a thoracic pathology team who regularly report these cases.

2.2 Laboratory facilities and generic laboratory requirements

The reporting of thoracic pathology should be undertaken in an appropriate laboratory environment. Provision should be made for macroscopic and microscopic photography, especially for resection specimens that may be discussed at local multidisciplinary team (MDT) meetings.

In addition, digital technology is being increasingly adopted by histopathology laboratories in the UK and may be used for diagnostic purposes when part of an accredited service.

The laboratory should:

- be equipped to allow the recommended technical procedures to be performed safely
- be enrolled with United Kingdom Accreditation Service (UKAS)
- participate in the UK National External Quality Assessment Services (NEQAS) for cellular pathology technique
- participate in the UK NEQAS for immunocytochemistry
- participate in an EQA scheme for molecular testing (UK NEQAS consortium), if molecular testing is performed at said laboratory
- have access to light microscopy and commonly used special stains
- have access to immunohistochemistry
- have access to genetics services
- have access to microbiology and virology services
- have access to a picture archiving and communication system (PACS) radiology or the ability to discuss cases with a radiologist.

Reports should be held on an electronic database that has facilities to search and retrieve specific data items and is indexed according to SNOMED T, M and P codes. Some departments have moved to SNOMED CT. It is acknowledged that some existing laboratory information systems may not meet this standard; however, the ability to store data in this way should be considered when laboratory systems are replaced or upgraded.

It is noted, however, that SNOMED is now in a practical transition phase, as part of the intended full implementation by the NHS and Public Health England of SNOMED CT. SNOMED ceased to be licensed by the International Health Terminology Standards Development Organisation from 26 April 2017.

Workload data should be recorded in a format that makes it easier to assess what resources are required and which, if applicable, is suitable for mapping to Healthcare Resource Groups.

3 Biopsy samples (lung, pleura and mediastinum including thymus)

3.1 Specimen submission

Specimens should be immediately placed in 10% neutral buffered formalin unless there are prior arrangements for fresh samples to be submitted. If infection is suspected, separate samples should be sent to microbiology, taking precautions appropriate for the level of the potential biohazard. This may require decontamination of cryostats, as a common occurrence is an unsuspected mycobacterial infection that mimics malignancy being identified at frozen section. Laboratories should have a policy in place to manage this situation. If there is a suspicion of tuberculosis, biopsies should be thoroughly fixed for a minimum of 24 hours.

3.2 Specimen dissection and block selection

All the material should be submitted, with a brief description of the number and size of pieces.

3.3 Embedding and sectioning

All pieces are usually embedded as a group in one block. However, there is often a differential diagnosis of malignancy, and blocking into more than one block to preserve tissue for molecular analysis should be considered in relevant cases. At the discretion of the individual laboratory, dependent on the need for tissue preservation and the size of the sample, up to three step-sections (20–30 µm between each stained section) should be prepared, keeping a spare unstained section at each level for any special stains that might be required. The pathologist should aim to be around 40% of the way through the sample by the third level. If no abnormalities are identified at any of these levels and tissue remains in the block, further step-sections should be taken, since features such as granulomas may be scanty within the tissue.

3.4 Staining and evaluation of sections

A haematoxylin and eosin (H&E) stain is usually sufficient to identify common pathological changes. If infection (e.g. tuberculosis, *Aspergillus* spp. or *Pneumocystis* spp.) is suspected, appropriate stains (e.g. Ziehl-Neelsen, Grocott, periodic acid-Schiff [PAS]) should be used. Elastic van Gieson (EVG) stains are of value when a vasculitic process is suspected or when looking for evidence of interstitial fibrosis. A PAS stain is also of value in assessing cases of suspected alveolar lipoproteinosis. A Perls' stain should be undertaken if there is pigment present to assess the extent of haemosiderin deposition. Congo red staining should be considered if amyloidosis is suspected. Immunohistochemistry may sometimes be required to exclude neoplasia or assess lymphoproliferative disorders (such as in the context of lymphoid interstitial pneumonia or IgG4-related disease).[3]

3.5 Report content

The report should comment on the adequacy of the specimen and the tissues present for assessment. For example, there should be enough lung parenchyma to make an accurate assessment, especially when interstitial lung disease (ILD) is suspected. A comprehensive assessment of the biopsy should be performed referencing all relevant constituents, e.g. vasculature, bronchi, alveoli and parenchyma, with description of any morphological changes and abnormalities. The conclusion should provide a differential diagnosis of the causes of identified histological patterns. It should be noted that transbronchial biopsies are frequently uninformative in many diffuse parenchymal lung disorders. For example, the interstitial pneumonias are not diagnosable in these biopsies, although they can confirm the presence of organising pneumonia. Even then, clinical correlation is required to diagnose cryptogenic organising pneumonia. Pathologists should not be pressured into making a diagnosis on the basis of such histological patterns when it is not appropriate.[4]

For pulmonary needle biopsies, a comment that non-specific inflammatory changes may represent changes due to obstruction by hitherto unsampled pathology may also be of value, in that there may often be florid obstructive changes in relation to a neoplasm that was inadvertently sampled rather than the tumour itself.

For pleural biopsies, the presence of areas showing lamellar fibrosis characteristic of a pleural plaque should be documented, as should the type and extent of any inflammatory changes.

[Level of evidence D – The majority of data in relation to preparation of different types of biopsies is based on expert opinion.]

4 Surgical lung biopsies and cryobiopsies

4.1 Specimen submission

In the context of non-neoplastic pulmonary disease, surgical lung biopsies are taken to investigate suspected diffuse parenchymal lung disease (DPLD), most frequently to differentiate between the patterns of interstitial pneumonias. The 2% mortality rate of patients with ILD undergoing biopsies is comparable to that associated with curative resection for lung cancer.[5] Transbronchial cryobiopsies are increasingly being used as an alternative, less invasive option to surgical lung biopsies in the diagnosis of DPLD[6] (although this is not yet common practice within the UK). The ILD MDT should consider relative risks and the type of biopsy before undertaking sampling. Surgical lung biopsies may also be taken for suspected vascular disorders, most commonly vasculitis. High-resolution computed tomography (HRCT) has removed the need for surgical biopsy in a large proportion of patients who present with DPLDs. However, this type of biopsy still plays a significant role in the diagnosis of a minority of patients and will continue to since some DPLDs do not have specific HRCT features, in particular in paediatric lung disease where HRCT is less specific. Furthermore, both atypical presentations and unexpected clinical behaviour are still encountered, which may prompt the clinician to request a biopsy. Lung tissue removed after pneumothorax should be examined for bullae along with examination of cystic areas, especially in females when both lymphangioleiomyomatosis (LAM)[7] and endometriosis enter the differential diagnosis. In patients with diffuse cystic disease, genetic testing for Birt-Hogg-Dube syndrome is a further consideration.

Ideally, biopsy sites should be targeted preoperatively through HRCT correlation; at least two sites should be sampled and biopsies of at least 30 mm along the visceral pleural plane should be taken to maximise diagnostic yield.

If infection is suspected, a separate fresh specimen should be sent directly for appropriate microbiological investigations. Necessary precautions should be taken that are appropriate for the level of the potential biohazard. If tissue is in short supply and a staple line is present, then the tissue attached to the staple line, which would otherwise be redundant, can be used for microbiology. If there is a suspicion of tuberculosis, biopsies should be thoroughly fixed for a minimum of 24 hours.

Ideally, and particularly in paediatric cases, small separate pieces of tissue are snap-frozen and fixed in glutaraldehyde to facilitate additional genetic or ultrastructural investigations if required.

Under ideal circumstances, the remaining tissue from a surgical lung biopsy should be gently inflated with 10% neutral buffered formalin via a small bore needle, taking care not to over-expand the tissue as this can cause artefacts that mimic lymphangiectasia, especially in children. Over-inflation may also wash out alveolar contents, for example macrophages that are key to the diagnosis of respiratory bronchiolitis. The specimen should then be fixed overnight. Cryobiopsies should be submitted directly to the laboratory without inflation.

4.2 Specimen dissection and block selection

For surgical lung biopsies, the specimen will normally be a wedge of subpleural lung, stapled along the surgical margin. The description should include its dimensions and any parenchymal or pleural abnormalities. The row of staples is cut off (unless already used). Any bullae or lesions should be described, noting their size and location, and well sampled. If a suspicious nodule is seen, which is potentially neoplastic, then reference should be made to the appropriate RCPath dataset for guidance on how to process the specimen. The axis of slicing will depend on the volume of tissue, but ideally sections with the largest possible area should be taken. Within reason, the entire specimen should be sampled if possible. For large wedge resections, sampling should yield adequate representation of underlying pathology but this is

subject to the individual pathologist's discretion. For cryobiopsies, the sample can be placed whole in a cassette, ideally one for each biopsy. The tissue should be carefully handled and embedded to maximise the surface area available for histological assessment.

4.3 Embedding and sectioning

A single section of each slice of tissue embedded provides an adequate picture of the extent, distribution and nature of any pathology present.

4.4 Staining and evaluation of sections

An H&E stain is usually sufficient for the investigation of diffuse pulmonary disease, although an additional stain to highlight collagen and the pulmonary vasculature is recommended (e.g. EVG, haematoxylin and Movat's stain).

If asbestos is suspected as a cause of interstitial fibrosis, thick (25–30 µm) unstained sections or normal thickness sections that have been stained using the Perls' Prussian blue method should be examined for asbestos bodies. A Perls' stain is also of value in identifying haemosiderosis, both primary and secondary, and distinguishing this pigment from that of smokers' macrophages. The latter shows more varied pigment composition and less dense staining for haemosiderin.

If pulmonary infection (e.g. tuberculosis, *Aspergillus* or *Pneumocystis*) is suspected, appropriate stains (e.g. Ziehl-Neelsen, Grocott, PAS) should be used.

Immunohistochemistry is used at the discretion of the pathologist for exclusion/identification of tumours in the differential diagnosis of non-neoplastic pathology. Indeed, disorders such as Langerhans cell histiocytosis, LAM and Erdheim-Chester disease are now viewed as neoplasms rather than DPLDs.

Ultrastructural analysis is now infrequently used in a diagnostic setting, but is of value when assessing inborn errors of metabolism and some surfactant protein gene mutations. It is therefore worth ensuring assessable tissue is available, especially in children, even if the decision after reviewing the sections is that analysis is not required.

4.5 Report content

Comments on the adequacy of the specimen should be included in the report. In end-stage 'honeycombing' fibrosis, it may be impossible to determine the causative histological pattern/disease, although it may still be of value in excluding/identifying diseases such as sarcoidosis and neoplasms. This situation can be avoided in the majority of cases by preoperative targeting of multiple biopsy sites (see above).

For the idiopathic interstitial pneumonias (IIPs), the 2002 American Thoracic Society/European Respiratory Society classification is used, which was updated in 2013 to include pleuroparenchymal fibroelastosis.[8] By far the most common IIP is usual interstitial pneumonia (UIP; idiopathic pulmonary fibrosis). A comment on the degree of confidence for the diagnosis ('UIP', 'probable UIP', 'indeterminate for UIP' and 'definitely not UIP') has been recommended in two publications on management of idiopathic pulmonary fibrosis. In suspected idiopathic pulmonary fibrosis, a case that only shows end-stage (honeycomb) fibrosis is classified as 'probable UIP'.[9,10] For other IIPs, a comment on the extent and pattern of any fibrosis should also be provided. There is also a proposed classification for paediatric diffuse parenchymal lung disease.[11,12] For cases with a pattern of UIP but features suggestive of chronic hypersensitivity, these should both be commented upon in the text. If there is a combination of patterns, then these should both be documented, with 'unclassifiable' avoided whenever possible. These classification systems can be applied to cryobiopsies.

Once the pathologist has diagnosed a histological pattern, an ILD multidisciplinary review should occur, ideally through formal and regular team meetings. However, as a minimum, imaging and clinical data should be reviewed for individual cases, after which a final clinicopathological diagnosis can be given.[13,14]

For other DPLDs, the nature, pattern and severity of any pathological changes must be described, taking care to assess all anatomic compartments as, for example, collagen vascular disease can present with coexistent interstitial pneumonias and vascular disease. Again, multidisciplinary review should be part of the diagnostic algorithm. If a specific diagnosis cannot be made, a differential diagnosis for the causes of any reported changes should be given.

Care should also be taken not to miss the presence of any background precursor lesions such as neuroendocrine hyperplasia, atypical adenomatous hyperplasia and squamous metaplasia/dysplasia.

In cases for which a diagnosis of asbestosis is suspected, controversy remains over how many asbestos bodies are required for diagnosis. Identification of a single asbestos body in the setting of diffuse interstitial fibrosis raises the possibility of asbestosis, but to make a firm diagnosis two or more asbestos bodies per 1 cm^2 should be identified in a normal thickness (3–5 µm) section, in the presence of established fibrosis.[15] Multidisciplinary review of such cases is again recommended. *Guidelines on autopsy practice: industrial/occupational-related lung disease deaths including asbestos* were published in 2017 by the RCPath.[16]

In the investigation of pulmonary vascular disease, the distribution of changes such as vascular medial muscularisation and the presence of fibrinoid change and/or plexiform or angiomatoid lesions must be sought and described. Pathologists should distinguish veno-occlusive disease from those of pulmonary arterial hypertension.[17] The presence of perivascular granulomas or giant cells should also elicit a search for magnesium silicate (talc) crystals under polarised light owing to the association with intravenous drug use.

[Level of evidence B – There is consistent evidence that multidisciplinary review is the best management process for accuracy of diagnosis in ILD.]

Level of evidence D – Expert opinion is that a similar process is recommended for other non-neoplastic diseases. Evidence is reviewed in the cited classification proposals.]

5 Thoracoscopic/open pleural biopsies and non-neoplastic pleural resections

5.1 Specimen submission

If infection is suspected, submission of a second, separate specimen for microbiological study is encouraged. This must be submitted fresh. Necessary precautions should be taken that are appropriate for the level of the potential biohazard. The specimen for histopathology should be immediately placed in an adequate volume of 10% neutral buffered formalin. If there is a suspicion of tuberculosis, the tissue should be fixed for a minimum of 24 hours.

5.2 Specimen dissection and block selection

For biopsies, the whole specimen is submitted with a brief description of its size and appearance; pleura infiltrated by a neoplasm is often conspicuously nodular. For resections, a decortication (for non-neoplastic disease) is usually performed to relieve respiratory impairment caused by compression of the lung by thickened, chronically inflamed or fibrotic pleura, subsequent to inflammation, most often secondary to empyema. The specimen will consist of pieces of pleura of varying size that should be fully described, noting the consistency of the changes, the presence of any focal pathology, and the presence and nature of any exudate. Blocks should be selected as appropriate at the discretion of the pathologist. If no

conclusive diagnosis is found on initial sampling, then the remainder of the tissue should be submitted for examination.

5.3 Embedding and sectioning

It is recommended that, when possible, pieces of tissue are blocked perpendicular to the specimen surface so that there is good orientation from the surface to the deep aspect. This allows assessment of maturation, a useful feature in the distinction of desmoplastic mesothelioma from reactive pleural fibrosis. A single section from each block is usually adequate to reveal the nature of any pathology present.

5.4 Staining and evaluation of sections

An H&E stain is usually sufficient to identify basic pathological changes, with other stains performed, as required, by the pathologist.

If pulmonary infection (e.g. tuberculosis, *Aspergillus* or *Pneumocystis*) is suspected, appropriate stains (e.g. Ziehl-Neelsen, Grocott, PAS) should be undertaken. EVG stains are of value if considering a vasculitic process.

To distinguish between reactive mesothelial hyperplasia and epithelioid mesothelioma, it may be helpful to stain for desmin and epithelial membrane antigen (EMA), although the diagnosis remains primarily morphological. Since entrapped reactive mesothelial cells are nearly always limited to the pleura, and mesothelioma not infrequently extends into subpleural fat or peripheral lung, staining for cytokeratins may be of use. This may also be helpful in distinguishing reactive pleural fibrosis from sarcomatoid mesothelioma, as staining decreases towards the deep aspect of the specimen in reactive cases, while staining in mesothelioma tends to be stronger and more irregular.[18] The use of BAP1 immunohistochemistry and p16 fluorescence in situ hybridisation analysis is also helpful in distinguishing benign from malignant mesothelial proliferations.[19] (Further information can be found in the *Dataset for histopathological reporting of mesothelioma*.[20])

5.5 Report content

The report should state the nature of any pathology present, in particular the type and extent of any inflammatory changes, which should be correlated with the clinical history. As an example, if a patient has had a pneumothorax, features such as mesothelial hyperplasia and eosinophilic pleuritis are consistent with this diagnosis. The presence of areas showing lamellar fibrosis characteristic of a pleural plaque should be documented, as should the type and extent of any inflammatory changes. Cases with granulomatous pleuritis should be closely assessed for mycobacterial infection. Neoplastic entities such as desmoid-type fibromatosis, solitary fibrous tumour, inflammatory pseudotumour, mesothelioma and sarcoma should be considered in the differential diagnosis of a proliferating spindle cell lesion, and appropriate markers and stains should be employed to exclude these when indicated.

[Level of evidence D – Data in this section is based on expert opinion.]

6 Lung resections for non-neoplastic disease

6.1 Specimen submission

If infection is suspected, submission of a second, separate specimen for microbiological study is encouraged. This must be submitted fresh. Necessary precautions should be taken, appropriate for the level of the potential biohazard. The specimen for histopathology should immediately be placed in an adequate volume of 10% neutral buffered formalin. If there is a suspicion of tuberculosis, the specimen should be fixed for a minimum of 24 hours. If the

specimen is large enough, distension with formalin via an airway is desirable to aid rapid fixation. This is achieved by making an incision along the stapled bronchial margin and gently inflating the specimen using an appropriately sized syringe filled with formalin. Care should be taken to gently inflate the specimen as rapid insufflation with formalin may cause alveolar destruction, mimicking emphysema, and wash out alveolar contents. In some cases, with congenital lung disease, airways may be absent so inflation may need to be undertaken via needle and syringe.

6.2 Specimen dissection and block selection

The specimen will be a wedge, lobe or whole lung. A full description should be made, including the nature and extent of any pathological changes evident on macroscopic examination. In paediatric cases, typically with lung cysts, the bronchial anatomy must be examined closely and any abnormality must be noted. Close attention should also be paid to the pulmonary vasculature to ensure that the aberrant vessels seen in a sequestration are not missed. Photography of the specimen should be considered during cut-up, as architecture is often lost during dissection. Blocks are selected as appropriate at the discretion of the pathologist.

6.3 Embedding and sectioning

A single section from each block is sufficient to provide an adequate picture of the extent, distribution and nature of any pathology present.

6.4 Staining and evaluation of sections

An H&E stain is usually sufficient to reveal the nature of the pathology.

If pulmonary infection (e.g. persistent consolidation, cavitating masses) is suspected, further appropriate stains (e.g. Gram, Ziehl-Neelsen, Grocott, PAS) should be used. EVG staining should be undertaken if a vasculitis is suspected.

6.5 Report content

The report should describe any pathological changes that are present and their cause, if appropriate. The cause of any infective pathology is pursued as far as possible by appropriate staining, bearing in mind that microbiological examination is generally more sensitive than histopathology as a means of identifying the organism(s) responsible. Cysts are classified according to current terminology.[21] If there is a pulmonary nodule, as per British Thoracic Society guidelines, this may be solid or sub-solid (part solid or pure ground glass nodule) on radiology.[22] Histologically, this could be a neoplastic or non-neoplastic entity such as a granuloma, adenoma, primary or secondary malignancy, hamartoma, parasitic cyst, lymph node, silicotic nodule, rheumatoid nodule, meningothelial nodule, inflammatory myofibroblastic tumour, pneumocytoma, vascular tumour, lymphoma, perivascular epithelioid cell neoplasm, pneumoconiotic nodule, neural neoplasm or amyloid nodule. Appropriate special stains and immunohistochemical and molecular tests are advised as required. A separate nodule MDT could potentially reduce the workload of the lung cancer MDT.

7 Mediastinal resections for non-neoplastic disease

7.1 Specimen submission

If infection is suspected, submission of a second, separate specimen for microbiological study is encouraged. This may include cyst contents. This should be submitted fresh and necessary precautions taken that are appropriate for the level of the potential biohazard. If tuberculosis is suspected, the tissue should be fixed for a minimum of 24 hours. The specimen for histopathology should immediately be placed in an adequate volume of 10% neutral buffered

formalin. If the specimen is cystic, distension with formalin may need to be undertaken via syringe.

7.2 Specimen dissection and block selection

The specimen will usually be a thymus gland. A full description should be provided, including the dimensions and weight of the thymus gland, to assess true hyperplasia. Photography of the specimen should also be considered at this point. The nature and extent of any pathological changes evident on macroscopic examination should be described. Cysts should be characterised in terms of whether they are unilocular or multilocular. The cyst contents, wall thickness and any adherent native mediastinal elements should be documented. Blocks are selected, as appropriate, at the discretion of the pathologist. Any abnormal areas identified on macroscopic examination should be well sampled, with extensive sampling of thymic cysts to exclude neoplasia. Blocks from the background thymus should also be taken. If, on macroscopic dissection, there is a strong suspicion for neoplasia, the appropriate RCPath dataset should be referred to for guidance on how to process the specimen.

7.3 Embedding and sectioning

A single section from each block is sufficient to provide an adequate picture of the extent, distribution and nature of any pathology present.

7.4 Staining and evaluation of sections

An H&E stain is usually sufficient to reveal the nature of the pathology.

If pulmonary infection (e.g. persistent consolidation, cavitating masses) is suspected, further appropriate stains (e.g. Gram, Ziehl-Neelsen, Grocott, PAS) should be used. EVG staining is undertaken if a vasculitis is suspected.

7.5 Report content

The report must describe any pathological changes that are identified and their cause, if appropriate. The cause of any infective pathology is pursued as far as possible by appropriate staining, bearing in mind that microbiological examination is generally more sensitive than histopathology as a means of identifying the organism(s) responsible. Cysts are classified according to current terminology (e.g. pericardial, thymic, bronchogenic, enteric). Those without obvious site or differential can be classified as a congenital foregut cyst.

[Level of evidence D – Data in this section is based on expert opinion.]

8 Cytology

Thoracic organs are frequently subjected to cytological examination. Samples include bronchial brushings, washings, sputum, lavage, pleural fluids and endobronchial ultrasound (EBUS) samples from various lymph node stations.[23]

8.1 Specimen selection

These may be transported in alcohol-based fixative such as cytorich red, saline or culture medium. Cell block/clots are prepared and adequacy assessed. Along with malignancy, these samples are useful for assessing granulomatous processes and identifying infections.

8.2 Staining

For cytological preparation, Papanicolaou- and Romanowsky-stained slides are routinely practiced. H&E is used for clot and cytoblock preparation. If the EBUS sample yields microbiopsies, these should be fixed in formalin. Some tissue may be used for flow cytometric analysis to help exclude a lymphoproliferative disorder, which may require liaison with local haematophology services. Further stains can be used for granulomas and organisms. For further details, refer to *Tissue pathways for diagnostic cytopathology*.[24]

8.3 Report content

This should comment on the adequacy of the sample and the presence or absence of neoplastic cells and other cell types. Large numbers of a particular cell type such as eosinophils may indicate eosinophilic bronchitis or pneumonitis.[25] Giant cells, fungal organisms, bacterial colonies, viral inclusions, asbestos bodies, Curschmann spirals/Charcot-Leyden crystals, foreign bodies and granulomas need to be mentioned if present. Bronchoalveolar lavage samples may be used for polymerase chain reaction (PCR) to detect organisms such as cytomegalovirus, toxoplasma, tuberculosis, *Aspergillus* and chlamydia, especially in immunocompromised patients.

9 Electron microscopy

Electron microscopy is now rarely used in diagnostic thoracic pathology, since it has been superseded by immunohistochemistry. However, it may still be of value in the diagnosis of certain disorders, such as pulmonary involvement by inborn errors of metabolism and surfactant protein gene disorders. It is therefore worth considering placing two to three 1 mm^3 pieces of tissue from surgical lung biopsies into glutaraldehyde before fixing the remainder in formalin, especially in paediatric cases. These may not require processing but can be kept until a decision is made after review of glass slides. Cases with occupational lung disease may require referral to centres with the facility to count and analyse inorganic materials, e.g. asbestos fibres.

10 Molecular investigations

Several new molecular genetic studies have been carried out directed at non-neoplastic lung diseases. New genetic markers have been identified in interstitial pulmonary fibrosis ($AKAP_{13}$),[26] asthma[27] and cystic fibrosis,[28] and the list is expanding. This will change the landscape in the management of several non-neoplastic lung disorders. This will include new therapeutic regimens, gene editing and gene therapy for diseases described earlier. At present, molecular investigation of histological samples is not routinely used in a diagnostic setting within non-neoplastic thoracic pathology, although various patterns of non-neoplastic lung disease (such as alveolar proteinosis) may require correlation with results from serological and other molecular tests. Molecular investigations may be employed to diagnose or exclude neoplastic differential diagnoses, such as the use of PCR-based lymphocyte clonality studies to help exclude mucosa-associated lymphoid tissue (marginal zone) non-Hodgkin lymphoma when diagnosing nodular lymphoid hyperplasia. This should be done in consultation with experts in lymphoreticular pathology, when appropriate. PCR analysis and in situ hybridisation can be employed in the detection of viral and bacterial genomes. In particular, PCR analysis to detect tuberculosis can be performed on formalin-fixed paraffin-embedded tissue. Genetic testing is not routinely performed for non-neoplastic diseases, although histological features might prompt tests for specific diseases when there is pulmonary involvement (e.g. Birt-Hogg-Dube syndrome, inborn errors of metabolism and surfactant protein-related disorders).

11 Criteria for audit

The key performance indicators (see *Key Performance Indicators – Proposals for implementation,* July 2013, www.rcpath.org/profession/guidelines/kpis-for-laboratory-services.html) as recommended by the RCPath are:

- the inclusion of SNOMED codes within report:
  - standard: 95% of reports should have T and M codes
- the availability of pathology reports at MDT meetings:
  - standard: 90% of cases discussed at MDT meetings where biopsies or resections have been taken should have pathology reports available for discussion
- turnaround times for biopsies and resection specimens:
  - standard: 80% of diagnostic biopsies will be reported within seven calendar days of the biopsy being taken
  - standard: 90% of all histopathology will be reported within ten calendar days of the specimen being taken (except those requiring decalcification).

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Appendix A Summary table – explanation of grades of evidence

(modified from Palmer K *et al. BMJ* 2008;337:1832)

Grade (level) of evidence	Nature of evidence
Grade A	At least one high-quality meta-analysis, systematic review of randomised controlled trials or a randomised controlled trial with a very low risk of bias and directly attributable to the target cancer type or A body of evidence demonstrating consistency of results and comprising mainly well-conducted meta-analyses, systematic reviews of randomised controlled trials or randomised controlled trials with a low risk of bias, directly applicable to the target cancer type.
Grade B	A body of evidence demonstrating consistency of results and comprising mainly high-quality systematic reviews of case-control or cohort studies and high-quality case-control or cohort studies with a very low risk of confounding or bias and a high probability that the relation is causal and which are directly applicable to the target cancer type or Extrapolation evidence from studies described in A.
Grade C	A body of evidence demonstrating consistency of results and including well-conducted case-control or cohort studies and high-quality case-control or cohort studies with a low risk of confounding or bias and a moderate probability that the relation is causal and which are directly applicable to the target cancer type or Extrapolation evidence from studies described in B.
Grade D	Non-analytic studies such as case reports, case series or expert opinion or Extrapolation evidence from studies described in C.
Good practice point (GPP)	Recommended best practice based on the clinical experience of the authors of the writing group.

Appendix B AGREE II compliance monitoring sheet

The tissue pathways of the Royal College of Pathologists comply with the AGREE II standards for good quality clinical guidelines. The sections of this tissue pathways that indicate compliance with each of the AGREE II standards are indicated in the table.

AGREE II standard	Section of guideline
Scope and purpose	
1 The overall objective(s) of the guideline is (are) specifically described	Introduction
2 The health question(s) covered by the guideline is (are) specifically described	Introduction
3 The population (patients, public, etc.) to whom the guideline is meant to apply is specifically described	Foreword
Stakeholder involvement	
4 The guideline development group includes individuals from all the relevant professional groups	Foreword
5 The views and preferences of the target population (patients, public, etc.) have been sought	Foreword
6 The target users of the guideline are clearly defined	Introduction
Rigour of development	
7 Systematic methods were used to search for evidence	Foreword
8 The criteria for selecting the evidence are clearly described	Foreword
9 The strengths and limitations of the body of evidence are clearly described	Foreword
10 The methods for formulating the recommendations are clearly described	Foreword
11 The health benefits, side effects and risks have been considered in formulating the recommendations	Foreword and Introduction
12 There is an explicit link between the recommendations and the supporting evidence	2–10
13 The guideline has been externally reviewed by experts prior to its publication	Foreword
14 A procedure for updating the guideline is provided	Foreword
Clarity of presentation	
15 The recommendations are specific and unambiguous	2–10
16 The different options for management of the condition or health issue are clearly presented	2–10
17 Key recommendations are easily identifiable	2–10
Applicability	
18 The guideline describes facilitators and barriers to its application	Foreword
19 The guideline provides advice and/or tools on how the recommendations can be put into practice	2–10
20 The potential resource implications of applying the recommendations have been considered	Foreword
21 The guideline presents monitoring and/or auditing criteria	11
Editorial independence	
22 The views of the funding body have not influenced the content of the guideline	Foreword
23 Competing interest of guideline development group members have been recorded and addressed	Foreword