Examination of lung specimens

A R Gibbs, R M E Seal

The lung can react to a wide variety of insults in many different ways: the range of conditions includes congenital, metabolic, infective, vasculitic, occupational and neoplastic. To a large extent the handling and examination of lung specimens should be determined by the type of pathological process affecting the lung. This can often be anticipated by close collaboration between the pathologist, radiologist, and clinicians, the benefits of which cannot be over-emphasised.

In this broadsheet we describe the types of specimens likely to be encountered, their handling, and their macroscopic examination, bearing in mind the constraints imposed on busy laboratories. The approach is intended for everyday use in district general hospitals as well as in large academic institutions. Cytological and aspiration cytological specimens will not be dealt with here.

Types of surgical lung specimen

CLOSED NEEDLE OR DRILL BIOPSY SPECIMENS

Transbronchic biopsy specimens may be of lung parenchyma or of pleura. Their size is determined by the type of instrument used to obtain them. Usually they measure 2 or 3 mm across and vary from 2 to 10 mm in length. Macroscopic examination is usually of little value; the major decision is how to handle them. This depends on the clinical information and the size and number of pieces of tissue submitted. In every case part of the material should be fixed in formalin and routinely processed. If an infective cause is suspected some of the tissue will be required for microbiological examination. Some may need to be deep frozen for histochemical or immunohistochemical procedures—for example, in lymphoproliferative processes. Special fixatives may be required for immunohistochemical or electron microscopy studies.

ENDOBRONCHIAL AND TRANSBRONCHIAL BIOPSY SPECIMENS

The advent of the flexible bronchoscope has led to widespread use of endobronchial and transbronchial biopsy in district general hospitals. Most histopathologists will therefore be expected to examine and diagnose these biopsy specimens at some time during their career. Usually they measure 2 to 3 mm in each dimension. Examination and handling is similar to that of closed biopsy specimens.

It is good practice routinely to cut multiple sections on these specimens and leave some unstained, so that small lesions such as granulomas are not missed and also that spare sections are available for special staining procedures.

OPEN LUNG BIOPSY SPECIMENS

The size of these depends on whether the tissue is obtained by limited or exploratory thoracotomy and will vary from 2 cm upwards. The pathologist should receive the tissue fresh and examine it macroscopically. Use of a hand lens helps to identify the lesions, which should be noted as solitary or multiple and their colour, demarcation, size, and if possible their relation to anatomical structures such as airways determined. The pathologist should select tissue for microbiological investigation or special procedures such as electron microscopy if appropriate. It may be useful at this point to carry out a frozen section to decide on adequacy of sampling, likely diagnosis, and what special procedures might be most appropriate. The tissue should always be handled very gently and instruments used should have sharp blades to reduce artefacts. Touch imprints may be useful; for example, with the appropriate special stain pneumocystis can be rapidly diagnosed. Whatever tissue remains should be carefully inflated with fixative using a small gauge needle. This facilitates the assessment of the distribution of lesions which is extremely important in the diagnosis of non-neoplastic conditions of the lung.

SEGMENTAL, LOBECTOMY, AND PNEUMONECTOMY SPECIMENS

Ideally these specimens should be inflated whole with formalin using a tube or catheter inserted into the lumen of the bronchial resection margin and connected to a container of fixative at a pressure of 25 to 30 mm of water. With lobectomy and pneumonectomy specimens it is often necessary to replace the tube or catheter within different segmental bronchi to obtain inflation of all parts of the
specimen. A tight seal around the catheter and a relatively intact pleura are necessary for proper inflation. The lung should be inflated until the pleura is smooth and then be left immersed in formalin for 24 hours. It is not necessary to clamp the bronchus. The need for special procedures, however, may mean that the specimen is often partly dissected before fixation and inflation may be difficult. After fixation we prefer to make one large sagittal cut perpendicular to the hilum along the whole of the specimen. For tumours we follow this by further cuts along the bronchi. Some advocate the opening of each major bronchus along its length using scissors or by cutting down on to a probe already inserted into the lumen. In non-neoplastic conditions we prefer further parallel cuts at 1 to 2 cm intervals. At necropsy the lungs should be examined in a similar manner.

MACROSCOPIC EXAMINATION AND THE SELECTION OF TISSUE BLOCKS (table 1)

This procedure applies only to the segmental, lobectomy, and pneumonectomy specimens. The type of lung specimen received should be specified and the size and weight of the specimen noted before inflation. The size and weight do not give any valuable clinical information but may sometimes be useful for identification. It is useful to consider the examination and tissue block selection under neoplastic and non-neoplastic headings as the information required from the specimens is different.

EXAMINATION OF SPECIMENS CONTAINING NEOPLASMS

The pathologist has a central role in determining the staging of a lung tumour, and the

**Table 1. Checklist for examination and blocking of surgical and necropsy lung specimens**

<table>
<thead>
<tr>
<th>Descriptive details</th>
<th>Tissue blocks</th>
</tr>
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<tbody>
<tr>
<td>Type of specimen (needle, drill, lobectomy, etc.)</td>
<td>Specimen containing neoplasms:</td>
</tr>
<tr>
<td>Size and weight (before inflation)</td>
<td>Tumour (minimum two blocks—at least one perpendicular to bronchial wall if tumour located centrally)</td>
</tr>
<tr>
<td>Characteristics of the main lesion(s)</td>
<td>Proximal bronchial resection edge</td>
</tr>
<tr>
<td>Solitary or multiple (number)</td>
<td>Non-neoplastic lung (one block from each lobe)</td>
</tr>
<tr>
<td>Shape, size, consistency, colour, distribution</td>
<td>Pleura (one nearest to the tumour and one from any focal lesion within the pleura)</td>
</tr>
<tr>
<td>For neoplasms:</td>
<td>Nodes (separately identified)</td>
</tr>
<tr>
<td>If central describe the bronchus of origin;</td>
<td>Specimen not containing neoplasms:</td>
</tr>
<tr>
<td>If peripheral describe the segment(s)</td>
<td>At least one block from each lobe to include normal, most abnormal, and intermediate areas</td>
</tr>
<tr>
<td>Distance of the tumour from the bronchial resection edge</td>
<td>At least one block of the large airways (if present)</td>
</tr>
<tr>
<td>Shortest distance of the tumour from the pleura</td>
<td>At least one block from pleura</td>
</tr>
<tr>
<td>Extent and distribution of abnormalities in the background lung—emphysema, atelectasis, fibrosis, consolidation, etc.</td>
<td>Nodes</td>
</tr>
<tr>
<td>Pleura:</td>
<td></td>
</tr>
<tr>
<td>Colour, thickness, describe any specific lesions, such as plaques, nodules, etc.</td>
<td></td>
</tr>
<tr>
<td>Nodes:</td>
<td></td>
</tr>
<tr>
<td>Size, consistency, colour for external and cut surfaces.</td>
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</table>

information required for this largely determines the selection and orientation of tissue blocks. Staging is necessary for determining the prognosis of a given tumour and may modify its treatment. Staging also allows results of different treatment regimens from different centres to be compared. The most widely used staging system for lung tumours is that developed and refined by the American Joint Committee for Cancer Staging. The system is based on the size and location of the primary tumour (T), the lymph node state (N), and the presence of metastases (M), and is often referred to as the TNM system. To stage a tumour accurately the following information is required:

1. Size of tumour (cm)
2. Distance of tumour from carina (determined by the surgeon)
3. Is the visceral pleura affected?
4. Is there a pleural effusion?
5. Does the background lung show atelectasis or obstructive pneumonitis and what is its extent?
6. Which lymph nodes are affected by tumour?

The pathologist is the only person capable of providing an accurate assessment of the size of the tumour because radiological investigations usually overestimate the size of the tumour due to surrounding obstructive or inflammatory changes. Depending on the size of the tumour, the pathologist should take a minimum of two and preferably more blocks to determine the histological type. The classification of lung tumours depends on the presence of specific differentiation—for example, glandular or squamous: the more tissue examined, the greater the likelihood of showing differentiation. Large cell undifferentiated carcinoma can only be diagnosed in the absence of any type of differentiation. Heterogeneity is not uncommon in lung tumours and more than one type of differentiation may be observed. If the tumour

**Table 2. Staging of lung cancer—modified from reference 3**

<table>
<thead>
<tr>
<th>Stage groupings</th>
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<tbody>
<tr>
<td>Stage 1 = T1 N0 M0</td>
</tr>
<tr>
<td>Stage 2 = T2 N1 M0</td>
</tr>
<tr>
<td>Stage 3 = any T or M</td>
</tr>
</tbody>
</table>

Regional lymph nodes (N)

<table>
<thead>
<tr>
<th>Metastatic disease (M)</th>
</tr>
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<tbody>
<tr>
<td>M0 No distant metastasis</td>
</tr>
<tr>
<td>M1 Disease in cervical, supraclavicular, or contralateral hilar lymph nodes or distant metastasis</td>
</tr>
</tbody>
</table>

T1 A solitary tumour 3-0 cm or less in diameter surrounded by lung or unaffected visceral pleura: no atelectasis or obstructive pneumonitis or extension proximal to a lobar bronchial orifice.

T2 Tumours greater than 3-0 cm diameter or any size if there is disease in visceral pleura, atelectasis, or obstructive pneumonitis extending to the hilum. The proximal margin of the tumour must be at least 2-0 cm from the carina. Any associated atelectasis or obstructive pneumonitis must affect less than an entire lung, and there must be no pleural effusion.

T3 Tumour of any size which directly affects the chest wall, diaphragm, or mediastinum, or extends to within 2 cm of the carina. Also if there is atelectasis or obstructive pneumonitis of an entire lung or pleural effusion.
is situated in a major bronchus at least one block should be taken perpendicular to the bronchial wall to assess the depth of invasion. Although not required for staging, the colour, shape, consistency and presence of cavitation should be noted. The bronchial mucosa should also be carefully inspected for roughness or loss of the normal bronchial ridges and blocks taken; this may show in situ malignancy, which is important clinically if it extends near to the proximal bronchial resection edge.

For central tumours, the bronchus they arise from, or for peripheral tumours, the segment, should be specified. For polypoid tumours the point of attachment of the stalk to the bronchus indicates the origin. The distance of central tumours from the proximal bronchial resection edge should be measured. A transverse block of the proximal bronchial resection edge should be taken to check for the presence of tumour.

The distance of the tumour from the pleura should be measured and if the tumour extends near to the pleura a block should be taken to include both the pleura and part of the tumour. The presence of a pleural effusion should have been noted by the surgeon at thoracotomy.

Additional blocks should be taken of the background lung to include abnormal and normal looking lung. Any abnormality of the pleura should also be blocked.

Any lymph nodes in the resection specimen such as hiliar, lobar, interlobar or segmental (all N1) should be separately identified and blocked. Usually the surgeon will submit separately identified N2 (ipsilateral, mediastinal, and subcardial lymph nodes) and M1 (contralateral, mediastinal, and hilar lymph nodes, and ipsilateral, or contralateral scaline, or supraclavicular lymph nodes) and the pathologist should examine them separately for neoplastic disease.

If a second tumour is present in the specimen it should be staged in a similar manner.

**Table 3  Macroscopic assessment of occupational lung diseases**

<table>
<thead>
<tr>
<th>Primary dust foci:</th>
<th>Average size (grade 0 to 3):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 = 1 to 3 mm; 2 = 4 to 5 mm; 3 = &gt; 5 mm</td>
</tr>
<tr>
<td>Profusion (grade 0 to 3):</td>
<td></td>
</tr>
<tr>
<td>Assessed according to the proportion of lobules affected 1 = &lt; 33% ; 2 = 33 to 66% ; 3 = &gt; 66%</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Secondary dust foci: (all N1)</th>
<th>To be recorded for each size as follows:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of stellate foci</td>
<td>a (&lt; 0.5 cm); b (&gt; 0.5 to 2 cm); c (&gt; 2 cm)</td>
</tr>
<tr>
<td>Number of round foci</td>
<td></td>
</tr>
</tbody>
</table>

**Emphysema:**

Type—centrilobular, panacinar, irregular:

**Severity (grade 0 to 3):**

1 = 1 to 3 mm; 2 = 4 to 5 mm; 3 = > 5 mm.

Profusion (grade 0 to 3):

Can be graded for upper and lower lobes separately or for the whole lung and is similar to that for the primary dust foci

**Interstitial fibrosis:**

Severity (grade 0 to 4):

A histological grading system is used

<table>
<thead>
<tr>
<th>Extent (grade 0 to 3):</th>
<th>1 = up to 10% of the lung affected; 2 = between 10 and 25%; 3 = &gt; 25%</th>
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</thead>
<tbody>
<tr>
<td>0 = absent</td>
<td></td>
</tr>
<tr>
<td>1 = slight degree of reticulin or collagen accumulation around respiratory bronchioles</td>
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</tr>
<tr>
<td>2 = fibrosis around respiratory bronchioles extending into adjacent alveolar ducts, atria and alveoli, but not extending to adjacent respiratory bronchioles</td>
<td></td>
</tr>
<tr>
<td>3 = fibrosis linking adjacent respiratory bronchioles</td>
<td></td>
</tr>
<tr>
<td>4 = widespread fibrosis with or without honeycombing</td>
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</tr>
</tbody>
</table>

**EXAMINATION OF SPECIMENS CONTAINING NON-NEOPLASTIC LESIONS**

The purpose of examining these specimens, whether necropsy or surgical, is to render a diagnosis or assess severity of the disease, or both, severity of disease being particularly important in occupational disorders. The pleura should first be examined for colour, thickness, exudate or any focal lesions such as pleural plaques. The hilar nodes can also be examined at this point and the size, colour, shape and consistency recorded. One complete sagittal slice of lung is then examined for the presence of focal or diffuse lesions. The size, colour, consistency and distribution of such lesions should be recorded. Further sagittal slices of the specimen are then take at 1 to 2 cm intervals. Tissue blocks should be taken of abnormal and normal looking lung; in some diseases the diagnostic lesions are present in the less severely affected parts of the lung.

**OCCUPATIONAL LUNG DISORDERS**

The following method is a useful way of recording dusting lesions, emphysema, and interstitial fibrosis and can be applied to ordinary lung slices or Gough-Wentworth whole lung sections (Appendix B), providing there has been good inflation.8 It is good practice to keep intact one well inflated whole lung slice for this examination and to take blocks from the other slices. The Pneumoconiosis Committee of the American College of Pathologists and the National Institute for Occupational Safety and Health have recommended that 15 tissue blocks should be taken from the lungs of cases of disease suspected of having been induced by asbestos. This is impractical for many non-specialist laboratories. As a minimum we recommend that four routine blocks should be taken: (i) apex of upper lobe; (ii) apex of lower lobe; (iii) basal segments; and (iv) major bronchus to include nodes. Other blocks should be taken from macroscopically visible lesions. Some of the blocks should include pleura.

**Dust lesions**

In general non-fibrous dusts with a low free silica content—for example, coal, kaolin, and mica—have low fibrogenicity and produce widely distributed focal, impalpable stellate lesions situated at the centres of the lobules (primary foci). These usually measure up to 5 mm in diameter. The extent of these foci can be conveniently recorded by giving the average size of the primary dust lesions on a 0 to 3 scale and the percentage of lobules affected on a 0 to 3 scale (table 3) (fig 1).

Non-fibrous dusts with a considerable free silica content produce stellate, in mixed dust pneumoconiosis, or rounded firm, palpable nodules due to a high collagen content as in classic silicosis. These can be referred to as secondary dust lesions. In some cases of coal worker's pneumoconiosis these secondary lesions may be present but are usually few in number. The number can be recorded for a range of sizes (table 3).

The definition of progressive massive
foci comprised showing primary section extent 3. Secondary dust foci comprised six stellate of <0.5 cm, four stellate of 0-5 to 2 cm, 17 rounded of < 0.5 cm and three rounded of 0-5 to 2 cm. There was centrilobular emphysema of grade 2 severity and extent 3.

Emphysema

Several different systems have been developed for quantifying emphysema macroscopically. The method we use is quick and convenient and is as accurate as point counting. It can then be used for lungs with or without pneumoconiotic lesions. The type of emphysema (panacinar, centiacinar, etc.) is recorded. The average severity in affected lobules is graded on a 0 to 3 scale as is the proportion of lobules affected, similar to assessment of dust lesions (table 3).

Appendix A

BARIUM SULPHATE IMPREGNATION METHOD

Squeeze a selected slice of fixed lung in water and place in a tray of warm barium sulphate solution (7-5%) for one minute. Press all parts gently to assist penetration of the solution. Transfer to warm sodium sulphate solution (10%) again pressing in all areas, then squeeze out after one minute. Repeat two or three times. Store reagents at 37°C. Specimens should be returned to formalin and stored as flat as possible.

Appendix B

PREPARATIONS OF WHOLE LUNG SECTIONS

Lungs for examination using the whole lung section method must be initially fixed by inflation with 10% formal saline through the main bronchus. The reservoir used to deliver the fixative should be placed four feet above the lung. Fixation will take at least seven days and if possible a longer period should be used to ensure adequate fixation. Once fixation is complete, a 2 cm thick slice is cut from the lung. The technique then proceeds as follows:

1. Wash slice in running water for 48 hours. A syphonic system is incorporated to ensure adequate washing.
2 Impregnate tissue in the following solution (A) by standing in a vacuum chamber under negative pressure for one hour at 37°C and then by leaving in an incubator for 48 hours, covering the dish to prevent drying.

**Solution A**

Gelatin (300 mg)
Ethylene glycol monoethyl ether (Cellusolve) (40 ml)
Phenoxetol (10 ml)
Glycerin (75 ml)
Water (1250 ml)

3 Place the slice in solution A in a plastic mould.

4 Place the larger microtome stage on to the lung in the gelatin and allow to set at room temperature. Remove the block and stage from the container and place in a deep freeze cabinet at −70°C for six to 18 hours.

5 Cut sections at 300–400 μm using a sledge microtome. Sections should be cut as thawing takes place.

6 Place sections into a bath of water.

7 Transfer a section to a clean water bath and mount on to strong paper.

8 Flood a perspex sheet with the following solution (B):

**Solution B**

Gelatin (75 g)
Glycerin (70 ml)
Cellulosolve (40 ml)
Water (850 ml)

Lay the section down on the perspex and then peel the paper away.

9 Lower a sheet of Whatman’s No 1 filter paper over section and gently remove excess gelatine and air bubbles with a roller.

10 Lay perspex flat until the gelatin has solidified and then dry in a radiograph drying cabinet.

11 When completely dry, peel paper from perspex. The section will remain attached to the filter paper which will now have a glazed surface.

12 Laminate the section with clear film.

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