DIAGNOSTICS 4  
CELLULAR PATHOLOGY

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**Aims & Objectives:**

The aim of this lecture is to gain a broad understanding of how histopathology and cytopathology aid in diagnosis of disease.

By the end, students should be able to:

* List 3 situations where histopathology and cytopathology might commonly be used as a diagnostic method.
* Describe the nature of specimens sent for histopathology and cytopathology laboratory diagnosis.
* List 2 situations where frozen section diagnosis is required
* Summarise the main steps involved in processing a specimen for routine histopathology diagnosis and indicate the likely time needed to carry out these steps.
* Explain the additional information available from immunohistochemistry, and give an example of when this technique may be used
* Describe the benefits of the autopsy
* List 3 benefits of cytology screening

**Pathology** is the medical science and specialty practice that deals with all aspects of disease, but with special reference to the essential nature, the causes, and development of abnormal conditions, as well as the structural and functional changes that result from disease processes - more simply it is the study of disease. The literal translation of pathology from Greek (pathos, -logos) is "the words of suffering." Pathologists study: the causes of disease; how various internal and external derangements or injuries affect certain cells and tissues; the progression of disease in the human body (its pathogenesis); how disease is manifest (its clinical expression and the lesions produced); and methods for monitoring disease progression. Pathology provides a scientific foundation for clinical medicine and serves as a bridge between the basic sciences and patient care.

**Histopathology** encompasses surgical and autopsy pathology and histopathologists make diagnoses on tissue - biopsy material, surgical specimens removed at operation, make rapid diagnoses when necessary at operations by use of “frozen sections”. The autopsy (post mortem) is performed to determine the cause of death of a patient, explain why treatment was unsuccessful, to show the spread of disease and to educate doctors regarding disease processes.

**Cytopathologists** make diagnoses on cells - cellular specimens – sputum, body fluids which contain cells, cervical smears and tissue obtained by fine needle aspirate (FNA). This is a less invasive technique than obtaining tissue for a biopsy and thus has advantages to the patient.

**Biopsies are taken for numerous reasons.** Common conditions that require biopsies are skin lesions (inflammatory and suspected tumours). If a tumour is suspected clinically this may be removed intact, with a margin of normal skin to ensure excision of the tumour. However a rash may simply require a punch biopsy to determine the nature of the inflammation.

Endoscopy enables the clinician to view the gastrointestinal tract. Biopsies are commonly taken from the stomach to exclude for example cancer, the duodenum to exclude coeliac disease and the large bowel to confirm cancer or diagnose inflammatory bowel disease.

Bronchoscopy allows a view of the trachea and bronchi and biopsies of suspected tumours or inflammatory lung conditions can be taken

Liver biopsies can be performed under imaging guidance for tumours or for diagnosis of liver disease.

Renal biopsies are taken to determine the nature of glomerulonephritis and other renal disease

**Cytopathology** is very helpful in diagnosis of lung tumours and is non invasive.

Cytopathology can also aid diagnosis in endoscopy when brushings of the tumour can be taken. It is used in cervical and breast screening. Fluids such as urine and ascities can be examined for cells,

Surgical specimens removed at operation are all sent to Histopathology for diagnosis and to determine if a tumour is completely excised.

The histopathologists and cytopathogists will generate a report of the examination of the specimen, which is sent to the clinician responsible for the patient. It is important that the report gives a conclusive diagnosis and a statement of tumour excision and prognostic features – for example if surrounding lymph nodes are involved or the tumour spread on to the peritoneum.

# How long does a histopathology result take to reach the clinician?

Specimens for histopathology (biopsies and whole tissue) must be “fixed” in formalin, a preservative that stabilises protein bonds and prevents autolysis. The tissue then must be processed, sections cut and stained and the histopathologist must write the report.

For large specimens, allowing for overnight fixation or longer if the specimen is large or fatty, this process should take 2-3 days. Small biopsies can be processed in a day and if a rapid diagnosis is required then rapid processing takes 4-5 hours.

A very rapid diagnosis may be required during an operation – is this a tumour? Is the margin of excision adequate? Is this lymph node involved? Have I got the parathyroid?

Frozen section tissue is received in the laboratory without any fixative. A small sample is selected and frozen rapidly. A thin section is cut on a microtome contained in a refrigeration cabinet, fixed rapidly and then stained with haematoxylin and eosin. An answer can be given by phone within 20 minutes.

# How are sections obtained?

All specimens are allocated an identity number and logged in to the computer.

During the daily cut up of specimens the pathologist or biomedical scientist selects and describes the tissue samples to be examined. Some specimens (for example biopsies) are processed whole, while larger specimens (e.g. mastectomies, colonic specimens) have a few selected pieces removed. Each selected piece of tissue is placed in a small perforated plastic container with a lid. This plastic cassette receives the laboratory number. These tissue blocks are processed to paraffin wax. This small sample of the tissue must have the water extracted by dehydration through alcohols to be impregnated with paraffin wax that allows the block to be cut to thin sections using a microtome and the section mounted on glass slides. Sections are usually cut at around 5 microns thick - a micron is one thousandth of a millimetre!

The number of blocks cut every day varies from 50-400 and several sections may be taken from certain blocks – levels through the tissue to extract maximum information. The sections are rehydrated and stained with haematoxylin and eosin (H&E). Haematoxylin stains nuclei blue and eosin the cytoplasm pink. The sections are then viewed using the microscope.

# Additional stains

Most diagnoses can be made by simple H&E staining, however additional stains can help diagnosis. According to the dyes or chemicals used it is possible to stain certain tissue components selectively. For example, silver nitrate solutions may be used to identify melanin pigment, fungi, calcium deposits and certain types of fibres. Other dyes are used to show glycogen, mucins and types of structure within the tissues. It is also possible to demonstrate tuberculosis bacilli by the Ziehl-Neelsen methodand bacterial infections by Gram’s method. However for difficult tumours immunocytochemistry techniques are needed which use antibody systems to label antigens within tissues. Thus epithelial, mesochyme, nerve, prostate tissues can be specifically identified.

# The autopsy

Autopsies may be performed by authority of the Coroner (suspicious/ unknown cause of death) or by permission of the relatives of the decreased – the questions that can be answered include:

* What happened as a terminal event?
* Was the treatment successful but complications occurred?
* Where did the cancer originate?
* Was my diagnosis correct?

# Cytopathology

Cervical smears are prepared preserved (fixed) with alcohol, stained by a special stain called Papanicolaou and permanently preserved. Most of the preparation methods within the laboratory are automated. Preparatory work is carried out by well-trained and experienced MLSOs. Slides are read by trained screening technical staff and abnormalities found checked by consultants.

Cervical smears are performed to pick up cervical cancer as part of the screening programme - Screening only has value if these (and other) criteria can be met:

1. Test is easy and non invasive
2. High take up in the population
3. A significant number of cases can be detected.
4. Something can be done about the disease.

However there are problems with for example, cervical screening:

1. Failure to obtain a satisfactory sample (smear).
2. Failure to make a proper smear.
3. Poor staining.
4. Poor interpretation