

11 Tissue and molecular diagnosis

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(b) Figure 11.21 (a) Low-power view of an umbilical nodule. Glands are distributed irregularly through the tissue. (b) High-power view shows benign columnar epithelium lining the glands (arrow), indicating endometriosis rather than carcinoma.

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In the past, autopsies (postmortems) allowed physicians and scientists to improve their knowledge of the human body and various diseases. The main reason for an autopsy is to confirm the cause of death, but autopsies remain very useful for medical education and audit. In the UK there are two main types. The first is the coroner's autopsy, when the coroner decides that there is a legal requirement to establish the cause of death, e.g. unexpected death or death during surgery or soon afterwards. Consent from relatives is not necessary. The second type is the hospital autopsy, which requires relatives' consent. For various reasons hospital autopsies are considerably less common than in the past. in situ

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Figure 11.29 Sanger sequencing showing wild-type BRAF (a) and a BRAF V600E mutation (b) (courtesy of Dr M Rodriguez-Justo, UCL-AD, Cancer Institute, London, UK).

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In situ hybridisation H. In situ hybridisation (ISH) uses a labelled oligonucleotide probe - that targets a specific sequence of RNA or DNA. It allows visualisation of the presence or absence and location of a particular RNA or DNA sequence in situ in tissue sections. Visualisation may depend on autoradiography, fluorescence microscopy - or bright-field microscopy. Chromogenic in situ hybridisation (CISH) combines ISH and immunohistochemistry for the detection of specific nucleic acid sequences and is a common alternative to fluorescence in situ hybridisation (FISH) for the detection of HER2 amplification. Viral genomes, e.g. EBV - (Figure 11.28), CMV and high-risk HPV types are detectable using this approach. ISH plays an important role in tissue diagnostics and the management of tumours.

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Cancer ‘precision medicine’

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This refers to the development of individualised cancer care plans, partly on the basis of molecular abnormalities in a tumour. Germline and somatic mutations may be taken into consideration, with the aim of tailoring treatments and targeting cancer cells precisely. With NGS, analysis of a single sample of tumour tissue for multiple known mutations that may predict treatment response is possible. In addition, many assays can detect mutations affecting as few as 5% of neoplastic cells. Other techniques may be used at the same time to detect abnormalities in the proteome (protein), transcriptome (mRNA), metabolome (metabolites) or epigenome, sometimes referred to as ‘omics’ assays. Such plans may, inevitably, be very complex (see Chapter 12). Cancer ‘precision medicine’

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Conventional cytogenetics is the microscopic study of chromosomal changes in individual cells. Newer techniques, including FISH, array comparative genomic hybridisation, RT-PCR and next-generation sequencing (NGS) are increasingly replacing conventional cytogenetics. Cytogenetic tests seek alterations such as gene amplification, loss of segments of chromosomal material, loss of whole chromosomes (e.g. in renal cell carcinoma) and translocations with associated fusion genes (e.g. EWSR1-FLI1 in Ewing's sarcoma). Cytogenetics and fluorescence in situ hybridisation

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Cytological assessment

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A cytological preparation consists of a sample of cells only . Assessment of architecture is not usually possible because intact tissue is absent or sparse (Figures 11.9 and 11.22) . Therefore, Sodium diphenylbisazobisnaphthylamine sulphonate is a red dye marketed in 1884 by the AGFA company of Berlin, Germany , using the name ' no longer used as a cloth dye owing to the carcinogenic risks of the benzidine moiety . assessment relies on the characteristics of the individual cells. Accordingly , diagnosis of malignancy is often difficult because the pathologist cannot assess certain features that support a diagnosis of malignancy such as invasiveness. However, cytology has several potential advantages over a biopsy . Obtaining a specimen may be easier and less traumatic. The area of sampling may be wider. Processing times are usually shorter and costs lower. Also, the ability of non-medical staff to perform a proportion of cases reduces costs. Summary box 11.7 Cytology compared with histology . It is

(b) Figure 11.22 (a) A cytology preparation of a pleural effusion. Numerous

atypical cells with atypical features are present, forming closely packed groups of overlapping cells. (b) Immunohistochemistry shows positive staining for

carcinoembryonic antigen, favouring carcinoma over mesothelioma.

Advantages Wider area of sampling in some cases Often less invasive Fast Cheap Disadvantages Cannot assess tissue architecture Less amenable to further tissue studies

Screening programmes aim to detect and treat premalignant tissue changes (dysplasia/intraepithelial neoplasia) or early-stage malignancy for which treatment is likely to be curative. The programmes may rely on clinical assessment, imaging and/or pathological assessment. The cervical cancer programme traditionally relied on cytology, with biopsy and histology follow-up if appropriate, but the alternative of HPV testing is increasingly available. The breast cancer screening programme relies on imaging and may use cytology and/or histology to assess possible lesions. The bowel cancer screening programme relies initially on a non-tissue-based test followed, if appropriate, by lower gastrointestinal endoscopy with or without biopsy of abnormal areas. Screening for neoplasia in ulcerative colitis and in Barrett's oesophagus relies on endoscopic assessment and biopsy. Cytological assessment

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Samples for cytology can be smeared immediately onto glass slides, fixed (usually in alcohol) or air dried and stained immediately or later. The process usually produces several slides, some of which are stained with a Papanicolaou (Pap) stain and some with another method such as May-Grünwald-Giemsa (MGG), H&E or Romanowsky (Figure 11.9). Liquid-based thin-layer technology is now replacing older methods. For liquid-based cytology , the sampling device is usually washed in a liquid medium and the material obtained is then processed in the laboratory using purpose-built equipment. Cytology specimen

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The term 'digital pathology' usually refers to the examination of digitised slides on a workstation (computer) or another device. Uses include education, quality assurance, surveys, research and expert consults. With the development of high-quality scanners, histopathology departments can scan all slides and store them so that pathologists can access them anywhere. Advantages include more flexible on-site and remote reporting, easy sharing, a reduction in costs and better recruitment. Disadvantages include the expense of set-up, maintenance and IT and repetitive strain injury. Additionally, diagnostic accuracy may be slightly lower than with glass slides. Some pathologists, particularly cytopathologists, dislike the loss of a three-dimensional image, and detection of very small items such as microorganisms can be difficult.

Biomarker Examples of tumours where relevant Application Mismatch repair genes CRC Gynaecological carcinomas Other digestive system carcinomas HER2 Breast carcinoma Gastric/oesophageal adenocarcinoma CRC (emerging evidence) PD-L1 Lung carcinoma Gastric carcinoma Bladder/urological carcinoma Malignant melanoma Breast carcinoma Endometrial carcinoma EGFR mutation Lung carcinoma BRAF mutation Malignant melanoma CRC KRAS mutation CRC NTRK fusions CRC ALK fusion Non-small cell lung carcinoma Renal cell carcinoma FGFR fusions Bladder carcinoma Tumour mutation burden Various CISH, chromogenic in situ hybridisation; CRC, colorectal carcinoma; FISH, fluorescence NGS, next-generation sequencing; PCR, polymerase chain reaction.

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Detection of clinically relevant abnormalities in genes

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Genomic changes in tumours

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HER2 gene amplification

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HER2 status influences the selection of therapy for breast cancer and metastatic gastric adenocarcinoma. Tumours with HER2 amplification may be treated with the monoclonal antibodies trastuzumab or pertuzumab, often in combination with other drugs. Recent data suggest that HER2 amplification may be a relevant therapeutic target in metastatic CRCs that are microsatellite stable. Summary box 11.16 Tumour types that may respond to immune checkpoint inhibitor drugs

Breast carcinoma Urothelial carcinoma Non-small cell lung cancer Small cell lung cancer
Hepatocellular carcinoma Malignant melanoma

Translocations can produce novel fusion genes that either produce a chimeric protein, e.g. BCR-ABL t(9:22) in chronic myeloid leukaemia, or may place an active promoter next to a proto-oncogene, causing its activation, e.g. t(14:18) IGH-BCL2 in follicular lymphoma. Translocations that activate tyrosine kinases can result in drug responsiveness, e.g. ALK, RET, NTRK, ROS and FGFR2. Tumour mutation burden Tumour mutation burden (TMB) is a recently recognised biomarker. A higher number of mutations within the tumour corresponds to a higher TMB. High levels of TMB can predict response to ICIs. PD-L1 immunohistochemistry and detection of MSI-H are also used for this purpose. HER2 gene amplification

HER2 status influences the selection of therapy for breast cancer and metastatic gastric adenocarcinoma. Tumours with HER2 amplification may be treated with the monoclonal antibodies trastuzumab or pertuzumab, often in combination with other drugs. Recent data suggest that HER2 amplification may be a relevant therapeutic target in metastatic CRCs that are microsatellite stable. Summary box 11.16 Tumour types that may respond to immune checkpoint inhibitor drugs

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Histological assessment

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Histological types of malignancy

Histological types of malignancy

A malignant tumour showing features of epithelial differentiation, and typically arising in an epithelial layer, is a carcinoma. Other important types of malignancy include malignant melanoma (melanocytes) (Figure 11.13b), lymphoma (lymphoid cells) and sarcoma (mesenchymal cells). Further subclassification is often appropriate and necessary. For example, categories of carcinoma include squamous cell carcinoma (with evidence of keratinisation) (Figure 11.15), adenocarcinoma (with evidence of glandular differentiation and/or mucin production) (Figure 11.16) or neuroendocrine carcinoma (Figure 11.13a) (usually requiring immunohistochemical confirmation of neuroendocrine differentiation). Some carcinomas have a pattern that raises a certain differential diagnosis, e.g. clear cell carcinoma (Figure 11.17). There are many other morphological types of carcinoma.

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Histology specimen

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On arrival in the pathology laboratory, specimens receive a unique identification number, usually with a barcode. They proceed to macroscopic assessment and sampling (colloquially - known as 'cut up'). The largest specimens require initial opening (e.g. gastrointestinal tract) or slicing (e.g. uterus, pancreas, breast) to allow further and adequate fixation in formalin, usually over 24–48 hours (Figure 11.2). When fixation is complete and the specimen is in a suitable condition for cutting and sampling, a pathologist or BMS describes the appearances and lists the method of sampling. Specimens a few millimetres in size such as endoscopic biopsies are suitable for submission in their entirety. Small resections, e.g. skin excision biopsies, may be suitable for slicing into two or more pieces and, again, submission in their entirety. For any specimen that is too large for these approaches, the prosector takes representative samples of areas of interest or relevance (Figure 11.3). This is traditionally the remit of the histopathologist, but BMSs or other non-medical staff with specific training increasingly contribute. In the UK and many other countries, there is often adherence to a regional, national or international guideline that includes a protocol for sampling. For example, samples from most types of cancer should include tumour, resection margins, lymph nodes, non-neoplastic tissue and any other abnormal areas. Inks of various colours help to identify resection margins and surfaces during microscope assessment as they remain in place after processing (Figure 11.4). The prosector places specimens, or samples from specimens, in plastic cassettes (Figure 11.5). BMSs/technical staff then embed the tissue in paraffin wax while in the cassette to produce a tissue block (Figure 11.6). BMSs then cut sections with a thickness of approximately 5 µm from the block using a microtome (Figure 11.7), place the sections on a glass slide and stain them with haematoxylin and eosin (H&E) (Figure 11.1). These steps require training and skill. A poor quality section may have various artefacts, such as lines, folds and shatter effect, which impede accurate assessment. H&E remains by far the most common initial stain for histopathology assessment, probably because it is inexpensive, safe, fast, reliable, familiar and informative. There is a wider variety of stains for cytology preparations including H&E and Giemsa. Traditionally, a pathologist examines stained sections with a microscope (Figure 11.8) and correlates the appearances with the clinical details and the macroscopic description. After special stains, completion of any additional studies such as immunohistochemistry and molecular analysis, the pathologist enters a report onto a computer system and allocates specific topography and morphology codes that will facilitate future searches. Recent improvements in technology and information technology (IT) mean that some laboratories use scanning machines to create digital images of the glass slides that pathologists and others can then access locally or remotely at any time (see Digital pathology and artificial intelligence).

Summary box 11.3 Histological processing: sequence of events

Figure 11.2 (a) A colon from a patient with familial adenomatous polyposis has been opened longitudinally, and the brown appearance reflects adequate fixation. Numerous polyps and a

carcinoma are apparent. after opening. In this example, there is less fixation, as a result of which the mucosa in the lower part of the picture remains red rather brown. (c) A uterus and an adjacent cystic lesion after slicing to allow fixation (all figures courtesy of Dr J Chin Aleong, Barts Health NHS Trust, London, UK). (b) An oesophagogastric resection containing a distal oesophageal tumour

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Figure 11.4 (a) An unopened pancreatoduodenectomy specimen (posterior view). Four inks of different colours have been painted onto separate margins and surfaces. (b) Yellow ink on the edge of a histology section (thick arrow). Tumour (thin arrow) lies

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Frozen section diagnosis is useful when a very rapid answer is necessary . Surgeons are the main users. The surgeon supplies a small representative fresh tissue sample of the area of interest. A BMS freezes the tissue quickly in the pathology laboratory and can produce sections for microscopic examination within several minutes. There are a few disadvantages in comparison with routine processing: fresh tissue carries a higher risk of infection; the quality is inferior to that of routine material, resulting in a potential reduction in diagnostic accuracy and precision; small but representative samples are necessary; certain types of tissue (e.g. fat) are di ffi cult to process; and the process is time-consuming and disruptive (Summary box 11.4). Summary box 11.4 Frozen section: advantages and disadvantages /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF

Advantages	Quick diagnosis	Disadvantages	Poorer quality sections	Potential reduction in accuracy and precision of histological diagnosis
	Labour intensive	Disruptive	Risk of infection	Small sample required
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Summary box 11.3 Histological processing: sequence of events

- Reception of specimen
- Macroscopic (gross) description
- Sampling of specimen (unless small enough to submit in its entirety)
- Specimen or samples placed in cassette(s)
- Production of paraffin wax block(s)
- Cutting of 5- μm sections with microtome
- Sections placed on glass slides
- Sections stained with H&E
- Histopathologist examines slides, taking clinical and macroscopic findings into account
- Further studies on tissue, if necessary
- Entry of report onto computer system
- Authorisation of report by pathologist

Figure 11.2 (a) A colon from a patient with familial adenomatous polyposis has been opened longitudinally, and the brown appearance reflects adequate fixation. Numerous polyps and a carcinoma are apparent. (b) An oesophagogastric resection containing a distal oesophageal tumour. (c) A uterus and an adjacent cystic lesion after slicing to allow fixation (all figures courtesy of Dr J Chin Aleong, Barts Health NHS Trust, London, UK).

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with a scalpel and forceps. (a) (b)

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Summary box 11.4 Frozen section: advantages and disadvantages

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Histology

Histology

Specimens for histology are classified as biopsies and resections, although strictly speaking all samples are biopsies. The reasons for taking small biopsies include diagnosis, further assessment and prognostic prediction. Types of small biopsy include punch biopsy, needle core biopsy and mucosal biopsy (Summary box 11.2). The purpose of a resection is usually treatment of a lesion (e.g. a tumour) by removing it. Other reasons for a resection exist, e.g. sleeve gastrectomy for obesity or creation of an ileostomy or colostomy. The pathologist's approach depends on the reason for surgery. For example, assessment of a cancer resection has multiple purposes, including confirmation of the diagnosis, classification, grading, staging, determination of further management and prediction of outcome. An excision biopsy is larger than the common types of small biopsy and serves as both a diagnostic biopsy and as a resection. For example, excision of a small skin lesion achieves its removal and also allows histological diagnosis and classification. Ultrasound-guided and computed tomography (CT)-guided biopsies of focal and less accessible lesions have become more common and may pose challenges to the pathologist because of limited sample size. Material from biopsies or resections is usually suitable for molecular analysis. Increasingly, the role of pathologists includes the identification of appropriate material for various common types of tissue sample for molecular tests and assessment of tissue suitability for molecular testing. For example, some tumour biopsies may contain insufficient tumour for molecular testing. Of course, correct diagnosis and grading are essential before molecular testing occurs, and attempts to bypass this step and take tissue for molecular testing without including the step of histological assessment run many unnecessary risks such as absence of tumour in the sample or the presence of a tumour that is different from that expected clinically. All samples for routine histology are immediately placed in a fixative, usually formalin (10% formaldehyde), by the surgical team or by other clinical staff to preserve morphology. This usually happens before delivery to the pathology laboratory.

Histology Formalin-fixed tissue Biopsy Mucosal, e.g. gastrointestinal, bronchial, oral Punch, e.g. skin Needle (core), e.g. liver Curettings, e.g. endometrium, prostate Excision biopsy Resection Fresh tissue Frozen section diagnosis Research Tissue banking Occasional special stains that require fresh tissue Cytology Cervical Washings, brushings, scrapes Fine-needle aspirate Fluids, e.g. ascites, pleural fluid Sputum

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Histology

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Immunohistochemistry

tumour pathology

Immunohistochemistry: tumour pathology

Immunohistochemistry has multiple applications in tumour pathology, including elucidation of site of origin and determination of cell type/direction of differentiation. Immunohistochemistry may also help to confirm neoplasia, determine the selection of treatment, refine prognostic predictions and screen for known underlying genetic changes. Numerous immunohistochemical stains help to determine cell type in tumours. Epithelial cells express cytokeratins. Therefore, cytokeratin positivity, though not diagnostic, favours carcinoma (Figure 11.25) over other types of malignancy. Lymphoid markers include the panlymphoid marker CD45, the T-lymphocyte marker CD3 and the B-lymphocyte marker CD20. Markers of melanocytic differentiation include S100, MelanA and HMB45. Chromogranin, synaptophysin

Summary box 11.11 Some immunohistochemical stains used for tumours

Cell type/site of origin	Epithelial (carcinoma)	Lymphoid (lymphoma)	Melanocytic (melanoma)	Neuroendocrine	Vascular	Myoid	Site of origin/cell type
Prostate	prostate-specific antigen (PSA)						
Lung	thyroid transcription factor-1 (TTF-1)						
Thyroid	thyroglobulin						
Colorectum	cytokeratin 20 (CK20), CDX2						
Liver	hepatocyte-specific antigen (HSA)						
Gastrointestinal stromal tumour (GIST)	CD117, DOG-1						

Prognosis and treatment Breast carcinoma and gastric carcinoma: HER-2 Neuroendocrine tumours: Ki67 proliferation index Screening for

Cell type/site of origin Epithelial (carcinoma): cytokeratins Lymphoid (lymphoma): CD45, CD3 (T cells), CD20 (B cells) Melanocytic (melanoma): S100, HMB45, Melan A Neuroendocrine: synaptophysin, chromogranin Vascular: CD31 Myoid: desmin, actin Site of origin/cell type Prostate: prostate-specific antigen (PSA) Lung: thyroid transcription factor-1 (TTF-1) Thyroid: thyroglobulin Colorectum: cytokeratin 20 (CK20), CDX2 Liver: hepatocyte-specific antigen (HSA) Gastrointestinal stromal tumour (GIST): CD117, DOG-1 Prognosis and treatment Breast carcinoma and gastric carcinoma: HER-2 Neuroendocrine tumours: Ki67 proliferation index Screening for

mutations Colorectal carcinoma: mismatch repair proteins (MLH1, MSH2, MSH6, PMS2) (a) (b) Figure 11.26 (a) A metastatic tumour composed of spindle cells. The clinical team suspected a diagnosis of gastrointestinal stromal tumour (GIST). (b) Positive immunohistochemistry for CD117, supporting a diagnosis of GIST.

typical immunohistochemical profiles. In all circumstances, interpretation takes place in the light of the clinical picture and imaging findings. Less often, immunohistochemistry helps to confirm malignancy. For example, kappa or lambda light chain restriction (expression of only one immunoglobulin light chain) in lymphoid proliferations suggests clonality and, in turn, neoplasia rather than a reactive process. In general, immunohistochemistry does not distinguish well between benign and malignant. Immunohistochemistry also plays a role in the selection of treatment and in predicting prognosis. For example, assessment of oestrogen receptor (ER) and human epidermal growth factor receptor-2 (HER2) status is routine for carcinomas of the breast (see Immunohistochemistry: tumour pathology while lymphomas are typically subjected to a comprehensive panel of markers that help determine treatment and prognosis. Ki67 proliferative index is an important prognostic factor for neuroendocrine neoplasms (Figure 11.27) .

Immunohistochemistry: infections and other applications There are antibodies to many infective agents, including cytomegalovirus (CMV), Epstein–Barr virus (EBV), herpes simplex virus, human herpes virus 8 (HHV8), hepatitis B virus and *Helicobacter pylori*. Some of these organisms, e.g. *pylori* and CMV, may be obvious or suspected on H&E examination, while others, e.g. EBV and HHV8, always require immunohistochemistry or other techniques for their detection.

Immunohistochemistry can also detect immunoglobulin and complement expression (e.g. in lymphomas or renal biopsies); confirm the abnormal accumulation of various proteins such as alpha-1-antitrypsin (A1AT); and help to characterise amyloid. Newer immunohistochemical markers that detect specific gene mutations are appearing and may become useful in clinical practice in the future. An important example is screening for MMR gene mutations in most gastrointestinal carcinomas Sir Michael Anthony Epstein, b.1921, Professor of Pathology, University of Bristol, Bristol, UK. Yvonne Barr, 1931–2016, Irish born virologist who emigrated to Australia. Epstein and Barr discovered this virus in 1964. BRAF V600E can replace mutational analysis in some settings. The major advantages of immunohistochemistry over other molecular tests for detecting genetic alterations are lower cost and faster turnaround. Summary box 11.12 Uses of immunohistochemistry /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF - /uni25CF /uni25CF - -

Figure 11.27 Immunohistochemistry for Ki67. The proliferative index is approximately 35% in this field. Cell type Neoplasia Direction of differentiation/phenotype Determination of anatomical site of origin Confirmation of neoplasia Grading Selection of treatment Detection of/screening for mutations Prognosis Microorganisms – detection Other Amyloid Immunoglobulins Complement

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Colorectum:	cytokeratin 20 (CK20), CDX2						
Liver:	hepatocyte-specific antigen (HSA)						
Gastrointestinal stromal tumour (GIST):	CD117, DOG-1						

Prognosis and treatment

Breast carcinoma and gastric carcinoma: HER-2

Neuroendocrine tumours: Ki67 proliferation index

Screening for mutations

Colorectal carcinoma: mismatch repair proteins (MLH1, MSH2, MSH6, PMS2)

(a) (b)

Figure 11.26 (a) A metastatic tumour composed of spindle cells. The clinical team suspected a diagnosis of gastrointestinal stromal tumour (GIST). (b) Positive immunohistochemistry for CD117, supporting a diagnosis of GIST.

typical immunohistochemical profiles. In all circumstances, interpretation takes place in the light of the clinical picture and imaging findings. Less often, immunohistochemistry helps to confirm malignancy. For example, kappa or lambda light chain restriction (expression of only one immunoglobulin light chain) in lymphoid proliferations suggests clonality and, in turn, neoplasia rather than a reactive process. In general, immunohistochemistry does not distinguish well between benign and malignant. Immunohistochemistry also plays a role in the selection of treatment and in predicting prognosis. For example, assessment of oestrogen receptor (ER) and human epidermal growth factor receptor-2 (HER2) status is routine for carcinomas of the breast (see Immunohistochemistry: tumour pathology while lymphomas are typically subjected to a comprehensive panel of markers that help determine treatment and prognosis. Ki67 proliferative index is an important prognostic factor for neuroendocrine neoplasms (Figure 11.27).

antigen, suggesting hepatocellular origin; and cytokeratin 20, typically expressed by colorectal epithelium. Several types of Carcinoembryonic antigen (CEA) is present in some carcinoma (Figure 11.22b). In practice, pathologists encountering a neoplasm of uncertain origin or uncertain phenotype - usually request a panel of markers relevant to the clinical setting and to the H&E appearances. Some malignancies especially poorly differentiated examples, do not conform to the - - -

Cell type/site of origin Epithelial (carcinoma): cytokeratins Lymphoid (lymphoma): CD45, CD3 (T cells), CD20 (B cells) Melanocytic (melanoma): S100, HMB45, Melan A Neuroendocrine: synaptophysin, chromogranin Vascular: CD31 Myoid: desmin, actin Site of origin/cell type Prostate: prostate-specific antigen (PSA) Lung: thyroid transcription factor-1 (TTF-1) Thyroid: thyroglobulin Colorectum: cytokeratin 20 (CK20), CDX2 Liver: hepatocyte-specific antigen (HSA) Gastrointestinal stromal tumour (GIST): CD117, DOG-1 Prognosis and treatment Breast carcinoma and gastric carcinoma: HER-2 Neuroendocrine tumours: Ki67 proliferation index Screening for mutations Colorectal carcinoma: mismatch repair proteins (MLH1, MSH2, MSH6, PMS2) (a) (b) Figure 11.26 (a) A metastatic tumour composed of spindle cells. The clinical team suspected a diagnosis of gastrointestinal stromal tumour (GIST). (b) Positive immunohistochemistry for CD117, supporting a diagnosis of GIST.

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Immunohistochemistry: infections and other applications There are antibodies to many infective agents, including cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus, human herpes virus 8 (HHV8), hepatitis B virus and Helicobacter pylori. Some of these organisms, e.g. pylori and CMV, may be obvious or suspected on H&E examination, while others, e.g. EBV and HHV8, always require immunohistochemistry or other techniques for their detection.

Immunohistochemistry can also detect immunoglobulin and complement expression (e.g. in lymphomas or renal biopsies); confirm the abnormal accumulation of various proteins such as alpha-1-antitrypsin (A1AT); and help to characterise amyloid. Newer immunohistochemical markers that detect specific gene mutations are appearing and may become useful in clinical practice in the future. An important example is screening for MMR gene mutations in most gastrointestinal carcinomas Sir Michael Anthony Epstein, b.1921, Professor of Pathology, University of Bristol, Bristol, UK. Yvonne Barr, 1931–2016, Irish born virologist who emigrated to Australia. Epstein and Barr discovered this virus in 1964. BRAF V600E can replace mutational analysis in some settings. The major advantages of immunohistochemistry over other molecular tests for detecting genetic alterations are lower cost and faster turnaround. Summary box 11.12 Uses of immunohistochemistry /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF

/uni25CF /uni25CF /uni25CF /uni25CF /uni25CF - /uni25CF /uni25CF - -

Figure 11.27 Immunohistochemistry for Ki67. The proliferative index is approximately 35% in this field. Cell type Neoplasia Direction of differentiation/phenotype Determination of anatomical site of origin Confirmation of neoplasia Grading Selection of treatment Detection of/screening for mutations Prognosis Microorganisms – detection Other Amyloid Immunoglobulins Complement

Immunohistochemistry

Immunohistochemistry

Immunohistochemistry emerged in the 1970s and has had a major impact on histopathological diagnosis. The technique detects a specific antigen using an antibody . The antibody is). labelled with a dye and after binding to its target antigen is visible in the tissue section as a coloured stain, often brown (Figure 11.25) . This allows the pathologist to confirm or exclude the presence of an antigen as well as determine its tissue distribution and cellular localisation. Quantification - xample, Ki67 is a cell cycle marker may also be possible. For e In situ that allows the pathologist to calculate a proliferative index, which in turn has prognostic value for neuroendocrine neoplasms and other lesions. Immunohistochemistry is appli - cable to fixed and frozen tissue and to cytological preparations (Figure 11.22b). It is safe, quick and relatively inexpensive and is often specific. However, false-positive results can result from non-specific staining or from cross-reaction with similar antigens. Excessive reliance on immunohistochemistry can lead to errors.

Figure 11.25 Diffuse immunohistochemical staining (brown) for a pancytokeratin marker in a malignancy, favouring carcinoma over other tumours.

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Introduction

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Pre-nineteenth century tissue diagnosis depended on naked eye examination of autopsy material and of a small selection of surgical specimens. The development of the light microscope allowed closer examination of tissue from autopsies and surgical procedures, with visualisation of cells, nuclei and tissue structure. Microscopic diagnosis was initially controversial, partly as a result of the 'Kaiser's cancer' (a histological diagnosis by Virchow of a non-malignant laryngeal lesion, after which Kaiser Friedrich III died of laryngeal malignancy), but the medical and surgical community eventually accepted its value. Tissue analysis is now an integral and routine element of clinical practice. It is heavily dependent on microscopic assessment, although newer methods of tissue analysis will increasingly provide additional information. Assessment of tissue is usually the responsibility of a histopathologist/cellular pathologist (a medically qualified practitioner), who depends on support from technical staff. In the UK, the staff responsible for tissue processing and the production of sections on glass slides are known as biomedical scientists (BMSs). The specialty variably known as Histopathology, Anatomic Pathology or Cellular Pathology encompasses histopathology, cytopathology, autopsy work and molecular tissue diagnosis. Developments and changes in cellular pathology are continuous. The volume of biopsies continues to increase as a result of increasing clinical demands, expectations of greater diagnostic precision, widespread flexible endoscopy and an ageing population with a higher prevalence of cancer and other illnesses. Cancer screening programmes also have an impact as they often depend heavily on cellular pathology. New techniques to refine histological assessment require additional resources. There is an increasing obligation to comply with national or international standards of reporting, e.g. for cancer, and participation by pathologists in multidisciplinary team meetings is now routine rather than occasional. Other developments may reduce activity. Newer, less invasive methods may replace tissue analysis, e.g. human papilloma virus (HPV) testing for cervical pre-neoplastic lesions is replacing cytological assessment. New methods in imaging may reduce the need for tissue analysis. - The location of a modern cellular pathology department is usually within or near a medium-sized or large hospital or in a purpose-built off-site centre. Typically, more than 80% of specimens are from the gastrointestinal tract, gynaecological tract, skin or urological system. In line with clinical services, highly specialised work such as neuropathology takes place in - major regional centres. Consolidation of clinical services may - result in reconfiguration of relevant pathology services and molecular testing facilities. -

To be aware of: The principles of microscopic diagnosis • The features of neoplasia • The importance of clinicopathological correlation • The role of additional techniques, including special stains, • immunohistochemistry and molecular pathology

Learning objectives

Learning objectives

To understand: The value and limitations of tissue diagnosis • Approaches to tissue processing •
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Microscopic features of inflammation

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Acute inflammation is characterised histologically by neutrophils (polymorphonuclear leukocytes), erosion or ulceration (Figure 11.18) and chronic inflammation by lymphocytes and plasma cells. Other inflammatory cells include eosinophils (Figure 11.19) , mast cells and histiocytes. Granulomas (collections of epithelioid histiocytes) (Figure 11.20a) raise the possibility of mycobacterial infection (Figure 11.20b), fungal 's disease or a reaction infection, parasites, sarcoidosis, Crohn to foreign material, among numerous other possible causes. s may reflect parasitic infection or Eosinophils in large number allergy . Interpretation depends heavily on the site and clinical setting.

Figure 11.18 An acute inflammatory process characterised by numerous neutrophils. Note the typical multilobated nuclei (arrows). Figure 11.19 Oesophageal mucosa infiltrated by numerous eosinophils with bright red cytoplasm, many of which are forming clusters. Eosinophils may reflect allergy, parasitic infection or a wide variety of other causes. In this example, the clinicopathological diagnosis was eosinophilic oesophagitis.

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Mismatch repair gene abnormalities in tumours

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High levels of microsatellite instability (MSI-H), also known as deficient mismatch repair (D-MMR), occur as a result of germ line mutations or acquired somatic events in the MMR genes (MLH1, MSH2, MSH6 and PMS2). The former is referred to as Lynch syndrome (previously known as hereditary non polyposis colorectal carcinoma) and is an autosomal dominant condition with predisposition to colorectal, gynaecological and other tumours (often at an early age). Summary box 11.17 Microsatellite instability and mismatch repair genes /uni25CF for MMR mutations, although some centres use PCR-based microsatellite testing for screening. Loss of immunohistochemical staining by neoplastic cells is a marker for a gene defect, an indication for further testing and may lead to genetic testing for Lynch syndrome (Figure 11.30). In most patients, a detectable MMR abnormality is sporadic and does not represent Lynch syndrome . MMR gene defects in CRC also identify sporadic tumours with different phenotypic and genetic characteristics. For example, BRAF V600E mutations are frequent in these cases and such cancers develop via the serrated polyp pathway rather than from adenomas (see Chapter 77). MMR abnormalities generally predict lower recurrence rates, better survival rates and a lack of need for 5-fluorouracil.

Microsatellite instability (MSI) Regulated by four main genes: MLH1, PMS2, MSH2, MSH6 Genetic changes responsible for MSI Sporadic hypermethylation of MLH1 (more common; 85%) Germline mutation, i.e. Lynch syndrome (less common) Microsatellite unstable (MSI-H) tumours 15% of colorectal carcinoma (CRC) 30% of endometrial carcinoma Tests Immunohistochemistry Recommended for all newly diagnosed CRCs The preferred initial test in most centres PCR-based microsatellite testing NGS Clinicopathological correlation: MSI-H CRC Typically right sided More likely to have mucinous element histologically Likely to have BRAF V600E mutation Clinical value Phenotypic classification, e.g. medullary CRC is typically MSI-H Prognosis, e.g. MSI-H better prognosis overall Selection of drug therapy, e.g. MSI-H CRC responds better to ICIs and has no response to 5-fluorouracil Screening for germline mutation, i.e. Lynch syndrome

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Molecular changes and drug therapy

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An increasingly common reason for molecular testing and related immunohistochemistry is the prediction of the response of advanced malignant tumours to specific drugs whose target is usually known ('theranostics'). For example, tumours with tyrosine kinase gene fusions that result in activation of the kinase are more likely than their counterparts to respond to tyrosine kinase inhibitors. A newer class of drugs known collectively as immune checkpoint inhibitors (ICIs) is highly successful for the treatment of a variety of advanced malignancies. Detection of any of several biomarkers may predict responsiveness to ICIs.

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Molecular profile examples of specific tumours

Molecular profile: examples of specific tumours

Colorectal carcinoma In CRC, the anti-EGFR monoclonal antibodies cetuximab and panitumumab are used in combination with chemotherapy for metastatic disease. These drugs are less likely to be effective if KRAS or NRAS mutations are present than if a tumour is 'wild type' (i.e. has no RAS mutation). Various other genetic changes assist with selection of therapy and making prognostic predictions (Summary boxes 11.17 and 11.18). Molecular analysis in colorectal carcinoma

Bronchial (lung) carcinoma In non-small cell lung cancer, specific EGFR mutations occur in a minority of lesions and identification predicts a response to the anti-EGFR tyrosine kinase inhibitor gefitinib, while ALK gene rearrangement predicts a response to the anaplastic lymphoma kinase (ALK) inhibitor crizotinib (see Chapter 60). Summary box 11.19 shows other relevant molecular changes. Summary box 11.19 Molecular and related changes in non-small cell lung carcinoma

Gynaecological carcinoma MMR status is increasingly important for the classification and management of ovarian and endometrial carcinomas. Other molecular changes that are important for prognosis and selection of therapy in endometrial cancer include polymerase ϵ (POL ϵ) and TP53 abnormalities. HER2 amplification and PD-1 expression may be relevant in some settings. Classification influences prognostic predictions and now depends not only on traditional histology but also on a number of molecular changes (many of which are detectable using immunohistochemistry) (see Chapter 87).

Breast carcinoma The most important ancillary tests for breast carcinoma remain ER immunohistochemistry and HER2 testing. As for many advanced malignancies, ICIs may be useful and accordingly PD-L1 immunostaining (with the appropriate antibody clone) may help predict outcome (see Chapter 58).

Lymphoma The distinction between benign and malignant lymphoid proliferations is sometimes difficult. Clonal immunoglobulin heavy chain (IgH) gene rearrangements in B-cell proliferations and clonal T-cell receptor gene rearrangements in T-cell proliferations favour lymphoma over reactive proliferations. Identification of characteristic cytogenetic abnormalities plays an important role in diagnosis, classification and management of several haematological neoplasms. PCR-based tests help detect minimal residual disease after therapy .

Gastrointestinal stromal tumour, soft-tissue tumours and malignant melanoma Most GISTs have either a KIT gene mutation or a PDGFRA gene mutation, more often the former. A few have defects in succinate dehydrogenase (SDH), BRAF or NF1 genes. Identification of known mutations helps confirm the diagnosis. Mutational profile also helps predict clinical outcome and response to chemotherapy . For example, imatinib, a tyrosine kinase inhibitor, is a useful drug for advanced GIST but is ineffective in those with SDH mutations. Molecular testing assists the diagnosis and classification of many types of soft-tissue tumour. Examples include Ewing's sarcoma and alveolar rhabdomyosarcoma, in which specific fusion genes

are diagnostic. FISH testing detects characteristic cytogenetic changes. In metastatic malignant melanoma, specific BRAF mutations predict response to the BRAF kinase inhibitor vemurafenib. Table 11.1 outlines the clinical applications of some biomarkers in tumours.

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Non-neoplastic and inflammatory conditions

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The diagnosis, assessment and management of non-neoplastic disease generates numerous pathology specimens. Examples include appendectomy for appendicitis, cholecystectomy for gallstone disease, hysterectomy for fibroids, skin excision for various lesions such as sebaceous cysts and prostatic chippings from glands with hyperplasia. Thorough histological examination helps to confirm or refute the provisional clinical diagnosis and also to exclude other conditions, some of which may be allbladder, malignant entirely incidental, e.g. neoplasia of the g change in uterine fibroids or carcinoma in prostatic chippings. Surgical and medical teams also generate a very large number of biopsies with the purpose of diagnosing and assessing non-neoplastic disease. In this setting, correlation with the clinical picture may be very important. For example, clinical details are essential for meaningful interpretation of inflammatory bowel disease biopsies, inflammatory skin biopsies, renal biopsies and medical liver biopsies. Non-neoplastic and inflammatory conditions

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Other terms

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(b) Figure 11.20 (a) A granuloma with necrosis, suggesting tuberculosis. Multinucleate giant cells of Langhans type are also present (arrow). (b) A Ziehl–Neelsen stain (from a different case) shows numerous

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PRINCIPLES OF MICROSCOPIC DIAGNOSIS

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(b) Figure 11.13 Cellular features of malignancy. (a) A neuroendocrine carcinoma showing nuclear pleomorphism (variation in shape) and variation in nuclear size. There are several mitotic figures (arrows). A malignant melanoma showing nuclear pleomorphism and prominent nucleoli (arrow) (courtesy of Dr E Husain, Aberdeen Royal Infirmary Aberdeen, UK). Figure 11.14 A colonic biopsy from a tubular adenoma with low-grade dysplasia. A non-dysplastic crypt is apparent at lower right. The remaining crypts mostly show features of dysplasia, including nuclear stratification (multilayering), nuclear enlargement and nuclear hyperchromaticity (dark colour). Interchanged samples Contamination Interpretative error Treatment-induced change, e.g. radiotherapy Ulceration

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Polymerase chain reaction

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The polymerase chain reaction (PCR) amplifies DNA, yielding millions of copies from a single copy of a selected target. Amplification of RNA is also possible, using the technique of reverse transcriptase PCR (RT-PCR). It is worth noting that real-time PCR (RT-PCR) is a different method, typically used for quantification, with a very similar abbreviation. PCR is fast and safe and can be performed on homogenised fresh or formalin-fixed tissue. PCR-based methods have numerous applications in oncology (see Detection of clinically relevant abnormalities in genes), including mutational analysis (Figure 11.29), testing for clonality , detection of fusion transcripts resulting from cytogenetic changes, detection of amplifications, demonstration of MSI and detection of gene hypermethylation. PCR-based methods can also detect microorganisms in tissue but this is not a common application because of the risk of false positives. Polymerase chain reaction

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Prognosis

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Tissue assessment is important for cancer prognosis. Stage is generally the most important prognostic factor for carcinomas. The internationally accepted Union for International Cancer Control (UICC)/American Joint Committee on Cancer (AJCC) staging schemes depend heavily on the histopathological TNM (Tumour Node Metastasis) category (pTNM), although the overall stage and in particular the M category are also evaluated clinically and on imaging and the final stage is derived from a combination of clinical, imaging, pathological and other assessments. The degree of differentiation may also be prognostic and is usually determined microscopically. As a rule, well-differentiated tumours have better prognoses than their non-neoplastic tissue counterparts (Figures 11.15 and 11.16), whereas poorly differentiated tumours do not (Figures 11.11 and 11.12). Other histological features associated with a worse prognosis include vascular invasion (Figure 11.11), perineural invasion (Figure 11.10) and positive resection margins. The prognostic value of these factors differs between tumour types and sites. - There is an increasing number of potential prognostic factors for a wide range of malignancies and preneoplastic lesions. These include immunohistochemical tests and molecular tests that may aim to detect underlying genetic changes such as deletions or amplifications or may help to refine grading. For example, immunohistochemistry for the proliferation marker Ki67 is now essential for grading and prediction of behaviour of well-differentiated neuroendocrine neoplasms. Screening for mismatch repair (MMR) gene abnormalities using immunohistochemistry helps to predict response to therapy, outcome and the need for genetic testing for familial disease. Although controversial, immunohistochemical staining helps to predict the behaviour of preneoplastic lesions such as Barrett's oesophagus (p53 staining) or cervical/anal intraepithelial neoplasia (p16 staining).

Figure 11.15 A well-differentiated squamous cell carcinoma. Irregular nests of squamous cells are present. They include foci of

keratinisation (arrows). Figure 11.16 A well-differentiated adenocarcinoma. Gland formation (arrow) is obvious. Figure 11.17 A metastatic clear cell carcinoma composed of sheets of cells with clear cytoplasm. A tumour with this appearance is most likely to be of renal origin but could have other sources such as liver, parathyroid gland, gynaecological tract and gastrointestinal tract.

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REASONS FOR ASSESSMENT OF TISSUE

REASONS FOR ASSESSMENT OF TISSUE

The contributions that tissue analysis makes to clinical - management include diagnosis, staging, prediction of outcome and assistance with selection of therapy . These are often - interrelated. The process of tissue assessment may make a new diagnosis or may confirm or refute a suspected or existing clinical diagnosis. There may be pointers towards a cause. Analysis may also reveal additional diagnoses that may be unsuspected. As an example, pathological assessment of an appendicec - tomy specimen most often confirms a suspected clinical diag - - nosis of acute appendicitis. However, the appendix sometimes contains an incidental neuroendocrine neoplasm, mucinous contains granulomas, raising the possibility of Crohn's disease or infection. Also, a specific cause of abdominal pain other than appendicitis, e.g. endometriosis, may be apparent in the appendiceal tissue. Absence of any histological abnormality raises the possibility of an extra-appendiceal cause. Similarly , biopsies from a patient with inflammatory bowel disease may confirm the diagnosis but may sometimes reveal or suggest an alternative cause of intestinal inflammation such as tuberculo sis, amoebiasis, ischaemia or mucosal prolapse. Summary box 11.1

Reasons for analysis of tissue /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF Tissue analysis also helps, increasingly , to determine or refine treatment and prognosis. For example, the assessment of a breast, lung, colorectal or other major cancer resection spec imen helps to confirms the diagnosis but, more importantly , provides crucial information about features such as tumour stage, vascular invasion, perineural invasion and resection mar gin involv ement, which in turn help to predict clinical outcome and determine postoperative treatment. The degree of tumour regression in a resection after neoadjuvant therapy may also have prognostic value. Additionally , pathological assessment of resections helps surgeons and radiologists to audit their accu racy and performance. Molecular pathological analysis of cancer tissue (see Diagnostic molecular pathology) increasingly contributes to management , including diagnostic categorisation, prognos tic predictions and selection of drug therapy . The molecular test or group of tests that an oncologist chooses depends on patient status, tumour location, tumour morphology and stage, among other factors. The identification of a particular bio marker may provide an indication for targeted therapy . For example, detection of high microsatellite instability (MSI) in metastatic colorectal carcinoma (CRC) may predict respon siveness to immune checkpoint inhibition. Burrill Bernard Crohn , 1884-1983, gastroenterologist, Mount Sinai Hospital, New Y ork, NY , USA. Norman Rupert Barrett , 1903-1979, surgeon, St Thomas's Hospital, London, UK. patient. Correlation with the clinical picture and the macro - scopic findings enhances the interpretation of pathological changes considerably . Ther efore, absence of relevant details may cause unnecessary delays and even er rors. For example, radiation therapy can have profound e ff ects on tissue morphol - ogy , including mimicry of other inflammatory conditions or neoplasia. Accordingly , a request form with adequate informa - - tion

should accompany all specimens. Examples of important details include site of biopsy/resection, clinical setting, reasons for the procedure, patient details, medications, relevant risk factors and past medical and surgical history, including previous chemotherapy and radiotherapy. A request form stating 'cancer' or 'Crohn's' is better than a form with no details but is clearly not sufficient. For small and large resection specimens, good quality macroscopic assessment and sampling is an important precursor to microscopic assessment (see Specimen processing).

Diagnosis Confirmation/rejection of a clinical diagnosis Additional diagnoses Classification of neoplasia Classification of non-neoplastic disease Staging of malignancy Prognosis Management Selection of therapy Assessment of response to treatment Cancer screening programmes and related programmes Cervical, bowel, breast, inflammatory bowel disease, Barrett's oesophagus Clinical trial support Audit

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RISK MANAGEMENT

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Safety and risk management are priorities in the laboratory. The use of warning labels helps to reduce the risk of contamination by transmissible infection, e.g. hepatitis B virus or human immunodeficiency virus (HIV). This is especially important when submitting and handling fresh (unfixed) tissue. Formalin kills many microorganisms, but a risk of transmissible infection still requires notification. Also, formalin itself is toxic to the eyes and skin. Accordingly, laboratory staff and indeed any staff should discard leaking or faulty specimen containers and deal immediately with formalin spillages. Patient details should be present on all specimen containers so as to avoid errors of identity (Figure 11.1). Rigorous systems are in place to avoid interchange of specimens or confusion between different patients' samples. - his - -

Figure 11.1 Sections on glass slides stained with haematoxylin and eosin. Each slide has a unique specimen identifying number (06S022081), a letter corresponding to the biopsy site (A-F) and a site label (e.g. DUOBX for duodenal biopsy).

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SPECIMEN PROCESSING

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Special stains

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A 'special stain' is a stain that is not routine, i.e. not an H&E stain. Immunohistochemical stains are conventionally separate from this category. Some special stains demonstrate normal substances in increased quantities or in abnormal locations. The periodic acid-Schiff (PAS) stain demonstrates both - glycogen and mucin, whereas a diastase PAS (D-PAS) stain demonstrates mucin, e.g. in an adenocarcinoma. Perls Prussian blue stain demonstrates iron accumulation (Figure 11.23), e.g. in haemochromatosis. A reticulin stain helps to demonstrate fibrosis (Figure 11.24). Elastic stains also show fibrosis and can highlight blood vessels by outlining their elastic laminae. Special stains can also reveal the accumulation of abnormal substances, e.g. a Congo red stain for amyloid. Summary box 11.9 Additional techniques for assessing tissue /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF Summary box 11.10 Common special stains /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF /uni25CF

Special stains Immunohistochemistry Electron microscopy In situ hybridisation, including /f_l uorescence in situ hybridisation (FISH) Molecular pathology techniques (including single biomarker polymerase chain reaction [PCR] and next-generation sequencing [NGS]) PAS: glycogen, fungi D-PAS: mucin Perls Prussian blue: iron Reticulin: reticulin /f_i bres, /f_i brosis van Gieson: collagen Congo red: amyloid Ziehl-Neelsen: mycobacteria

Special stains are also useful for the diagnosis of infection. Some microorganisms are not visible on routine H&E slides but are demonstrable with a stain. For example, a Ziehl-Neelsen stain demonstrates acid-fast bacilli, particularly mycobacteria, by staining them bright red on a blue background (Figure 11.20 Other microorganisms may be detectable on H&E but are easier to see with a special stain, e.g. fungi (PAS or Grocott stain), protozoa (Giemsa stain) and spirochaetes (Warthin- in situ hybridisation Starry stain). Immunohistochemistry and also help to detect some microorganisms (see Immunohisto chemistry: infections and other applications and hybridisation).

(b) Figure 11.23 (a) Brown pigment in a biopsy. (b) A Perls stain is posi tive, indicating that the pigment is iron.

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Specimen adequacy

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There are many reasons for an inadequate specimen. The operator may fail to sample the target organ or lesion or may take a sample that is too small to include or reveal a heterogeneous abnormality. A sample from the centre of a necrotic or ulcerated lesion might include no viable tissue. Superficial biopsies from a carcinoma may fail to distinguish dysplasia (Figure 11.14) from invasive carcinoma. Cautery and crush artefact are sometimes severe enough to impede assessment. Suboptimal laboratory processing can also cause problems with interpretation. Summary box 11.8 Reasons for an inadequate sample

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Storage

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Resection specimens are generally stored for about 4–6 weeks. Tissue blocks and slides are retained for as long as space

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permits. Fresh tissue can be frozen for future clinical review , teaching, audit or research. Many countries now have formal tissue-banking processes that allow construction of an archive of cases. In many countries, the storage, transport and subsequent use of tissue is subject to many legal constraints.

Figure 11.9 A cervical smear stained with a Papanicolaou stain. Numerous cells are present (courtesy of Professor MT Sheaff, Barts Health NHS Trust).

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