Derek C. Allen
R. Iain Cameron *Editors*

Histopathology Specimens

Clinical, Pathological and Laboratory Aspects

Second Edition

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Editors
Derek C. Allen
Histopathology Laboratory
Belfast City Hospital
Belfast Health and Social Care Trust
UK

R. Iain Cameron
Histopathology Laboratory
Altnagelvin Hospital
Western Health and Social Care Trust
UK

ISBN 978-1-85233-740-7 1st edition
ISBN 978-1-85233-844-2 1st edition (eBook)
ISBN 978-0-85729-672-6
ISBN 978-0-85729-673-3 (eBook)
DOI 10.1007/978-0-85729-673-3
Springer London Heidelberg New York Dordrecht

Library of Congress Control Number: 2012944399

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Printed on acid-free paper

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To Alison, Katie, Rebecca and Amy.

Preface

Since the publication of the first edition of *Histopathology Specimens*: *Clinical*, *Pathological, and Laboratory Aspects*, pathology has further consolidated its position at the core of clinical multidisciplinary teams and their attendant meetings. These forums are pivotal nodal discussion points in patient investigation, treatment planning, and prognostication. Pathologists are required to produce and comment on reports that are timely, accurate, and relevant. To this end, the UK Royal College of Pathologists and other organizations (College of American Pathologists and Royal College of Pathologists of Australasia) continue to publish standards of professional practice such as the Cancer Datasets and Tissue Pathways for the handling and reporting of cancer and noncancer specimens, respectively. Indeed, the UK Royal College of Pathologists has established key performance indicators which are to be incorporated into CPA/UKAS accreditation standards aimed at ensuring laboratory processes and outcomes are beneficial to patients. These include >90% targets for attendance at multidisciplinary team meetings, coding and use of proforma histopathology reports, and diagnostic/overall report turnaround times of 7–10 days. The College has also produced a standardized user satisfaction survey in metric form that should allow assessment of measureable pathology performance and team communication. This may also potentially be considered alongside colleague and user multisource feedback as part of annual appraisal and medical revalidation.

One other standard is that laboratories should aim to have a significant minority (15–30%) of their medical and scientific staff in training grades. The structure and content of this book not only facilitates delivery of performance standards but also reflects the clinically integrated approach to the teaching of pathology as determined by the Royal College postgraduate training curriculum and the General Medical Council medical student undergraduate curriculum. Its content is also directly relevant to Biomedical Scientists in their devolved role of consultant supervised specimen dissection as evidenced by the collaborative IBMS/RCPath diploma of extended practice and advanced specialist diplomas in histological dissection.

Derek C. Allen
R. Iain Cameron

Acknowledgments

The authors gratefully acknowledge the use of illustrations from Wittekind C., Greene L., Hutter R.V.P., Klimfinger M., and Sobin L.H. *TNM Atlas*: *Illustrated Guide to the TNM/pTNM Classification of Malignant Tumors*. 5th edition. Berlin, Heidelberg: Springer-Verlag, 2005.

Grateful appreciation is expressed to Melissa Morton (medical editor), Joanna Bolesworth, Beth Schad, and the staff at Springer.

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Contributors

Derek C. Allen Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK

David S. Brooker Ear, Nose and Throat Department, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK

R. Iain Cameron Histopathology Laboratory, Altnagelvin Hospital, Western Health and Social Care Trust, Londonderry, UK

Richard I. Davis Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK

Caroline Gannon Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK

Moyra Gray Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK

Derek J. Gordon Plastic Surgery Unit, Ulster Hospital Dundonald, South Eastern Health and Social Care Trust, Belfast, UK

Oisin Houghton Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK

Richard W. Kendrick School of Dentistry, Belfast Health and Social Care Trust, Belfast, UK

Tong Fang Lioe Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK

Maurice B. Loughrey Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK

Roy W. Lyness Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK

W. Glenn McCluggage Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK

Damian T. McManus Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK

Kathleen M. Mulholland Histopathology Laboratory, Altnagelvin Hospital, Western Health and Social Care Trust, Londonderry, UK

Seamus S. Napier Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK

Declan M. O'Rourke Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK

William J. Primrose Ear, Nose and Throat Department, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK

Lakshmi Venkatraman Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK

Maureen Y. Walsh Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK

Introduction

The Role of Histopathology Specimens

Histopathology specimens are a vital cornerstone in patient care. They not only establish a tissue diagnosis but are crucial in clinical management decisions and provide important prognostic data. They are nodal events in a patient's illness shaping the choice of relevant medical and surgical therapies and determining follow-up routines. The data they provide are used to assess the efficiency of current and new investigation and treatment regimes and to monitor the impact of population screening programmes. Clinical governance has recognized their key role in auditing not only individual clinicians but the patterns and quality of overall health care provision. Biomedical research with advances in investigations and therapy would flounder without them. They are therefore a precious resource to be handled with great care by sufficient numbers of appropriately trained and experienced personnel. The data generated are of a confidential nature privy to the patient, consultant clinician or general practitioner, and the reporting pathologist. This information may be shared as appropriate with other directly involved health care professionals, for example, in the context of multidisciplinary team meetings, but laboratory practice (e.g., telephoned results and report authorization) must be geared to protect patient confidentiality at all times. The patient not only has a right to see and have explained the information in his/her specimen but must undergo a process of informed consent prior to the clinical procedure. Thus the nature, purpose, extent, and side effects of the procedure are explained in understandable terms. This process extends to the laboratory as the patients can express their wish for disposal and use of the tissue not only for diagnosis but also for educative, audit, and research purposes. Additionally research projects should be verified by an appropriate research ethics committee. Patient denial of any of these uses must then be communicated to the laboratory and incorporated into the handling and disposal procedures. The histopathology specimen report forms a permanent part of the patient's medical record and as such may be used as medico-legal evidence in negligence and compensation cases. These various factors serve to emphasize the importance of the care that should be taken with these specimens by histopathology laboratory personnel.

The Handling of Histopathology Specimens

Specimen transportation, accession, clinical prioritization, dissection, audit, and reporting are considered.

Specimen Transportation

There must be close liaison between pathology and clinical staff to ensure appropriate transportation of specimens between the operating theater and the laboratory, for example, prompt transport of fresh specimens or the provision of special fixatives. This must be reflected in shared protocols, a user information manual, and education of the portering staff.

Specimen Accession

Allocation of a unique laboratory number and accurate computer registration of patient details are fundamental to maintenance of a meaningful and practicable histopathology database. This is important not only to individual patient care (e.g., a sequence of biopsies) but also for provision of statistics, for example, download to cancer registries.

Specimen Prioritization

With ever increasing workload and limited staffing resources, pathologists may find it necessary to put in place a specimen pull-through protocol related to clinical need to ensure that diagnostic results are available within an appropriate time frame. This can be based on various criteria such as specimen type and request form information (Appendix A). Suggested turnaround times for diagnostic biopsies and resection specimens are 90% of cases reported within 5 and 10 working days respectively subject to individual case needs and in agreement with local clinical teams.

Specimen Dissection

Traditionally the role of medical pathologist specimen dissection is now also being performed by an increasing number of biomedical scientists (BMSs) as has been the situation for several decades in some laboratories in America (Pathologist Assistants) and Northern Ireland. BMSs, trainee, and consultant pathologists are all appropriate to the task provided that several principles are adhered to:

- The histopathology specimen and its report remain the overall responsibility of the reporting consultant pathologist.
- There is close proximity and ready availability of active consultant pathologist supervision before, during, and after handling of the specimen.

- There is workforce stability and staff are prepared to work together as a team. The working unit comprises a variable combination of two people (junior/senior, medic/BMS) fulfilling the roles of dissector/writer/supervisor with active consultant pathologist supervision.
- Staff recognize that acquisition of dissection skills is at the bench apprenticeship based on sufficient knowledge, time, experience, and supervision. This knowledge base requires insight into normal anatomy, clinical presentation, and investigations relevant to request form information, common pathological conditions, and their effect on specimens, surgical considerations in production of the specimen, and core report data tailored to patient management and prognostic information. Consequently the chapters in this book are structured accordingly under these headings. The cut-up supervisor plays a vital role in passing on verbal knowledge but this is supplemented by various means, for example, publications (in-house protocols, ACP broadsheets, and textbooks) or training courses. A structured training programme facilitates learning and progression.

Staff must also be familiar with the laboratory process of checking patient details, specimen labeling, and past history (cytology, biopsy, and treatment), the importance of specimen opening for adequate fixation, demonstration of resection margins, and use of orthodox or digital photography. Knife etiquette and sampling blocks of appropriate thickness and fixation are crucial. The supervising pathologist must provide active feedback as to the significance and adequacy of these blocks. Line diagrams are an invaluable communication tool between dissector and reporters. Specimens not infrequently need to be revisited prior to report authorization or following new information gained from the multidisciplinary team meeting. Retention of "wet" specimens must be sufficiently long (minimum 4 weeks) to allow this process to happen.

- Dissectors should only work to their individual level of experience and competence – this is determined by the structured training programme, audit process (see below), and categorization of specimens according to their complexity.
- Dissectors should actively seek supervisor input if a specimen is usually complex, novel, shows an unusual variation on a usual theme, or if they have any doubt.

Specimen Dissection Audit

The quality of specimen dissection must be meaningfully monitored, and the majority of this is done actively at the laboratory bench by the consultant pathologist/BMS supervisor team as part of the specimen dissection pre-/peri-/post-view and reporting feedback procedures. In addition, this team should carry out formal periodic audit and assessment of dissectors' skills. This combination of approaches forms the basis for an individual dissector's continued practice and progression between specimen categories (see Appendix D). It also identifies the areas of subspecialist expertise or in need of further training. It must be recognized that category progression cannot be proscribed by rigid time frames but rather related to the aptitude of the individual dissector and spectrum of workload that is encountered.

Specimen Reporting

Histopathology specimen reports remain the responsibility of an appropriately trained and experienced medical pathologist. Increasingly Royal College of Pathologist Cancer Datasets are mandating key audit data to assess the standards of specimen dissection and reporting, for example, colorectal cancer mean lymph node harvest and the reported percentages of serosal and extramural vascular involvement by tumor. Other key service quality indicators include pathologist participation in relevant interpretive histopathology external quality assurance (EQA) schemes and appropriate continuing professional development (CPD) activity. These issues are discussed at annual appraisal and are foundational to medical revalidation. The overall quality of a surgical pathology service depends on a number of key performance indicators summarized in a recent Royal College of Pathologists document that can be found at http://www.rcpath.org/index.asp?PageID=35. They include availability and timeliness of clinical advice, participation at multidisciplinary meetings, coding of histopathology cases, use of cancer resection report proformas, documentation of second opinions, critical results communication, reporting turnaround times, monitoring of outstanding reports, appraisal, CPD, participation in appropriate EQA schemes, user satisfaction surveys, teaching, training, supervision, and succession planning. The user survey can be accessed at http://www.rcpath.org/index.asp?PageID=1669&SearchStr=user+satisfaction+survey

The principles and practice of surgical cut-up and sample protocols for general specimen handling, categorization, and laboratory abbreviations are included in the appendices to this section.

The Core Data in Histopathology Specimens

Specimen dissection must be geared to provide information relevant to the clinician who is managing the patient. Reports must be timely, that is, prompt, but in the context of an adequate period of fixation so that acquisition of accurate data is not compromised. The report content must not only come to an interpretationally accurate diagnosis but also be qualified by assessment of various evidence-based prognostic indicators. In the field of surgical cancer pathology, this is reflected by the trend toward set format reports or datasets for the common cancers. Thus the core content should include gross specimen description, tumor histological type and grade, extent of local tumor spread, lymphovascular invasion, lymph node involvement, relationship to primary excision margins, and any associated pathology.

Gross Description

Clear distinction should be made between biopsy and resection specimens as they are handled differently and represent different nodal points in a patient's illness. This should be reflected in use of appropriate SNOMED T (topography) and P (procedure) codes – this also facilitates audit of biopsy and resection – proven cancer numbers and correlation with other techniques such as cytology, radiology, and serum markers. The site, distribution, size, edge, and appearance of a tumor within an organ greatly influence the specimen handling and creation of a diagnostic shortlist for microscopy. For example, a gastrointestinal malignant lymphoma may be multifocal, pale, and fleshy with prominent mesenteric lymphadenopathy, whereas a carcinoma is more usually ulcerated and annular, firm and irregular with more localized lymph node disease and vascular involvement.

Histological Tumor Type

There are marked prognostic and therapeutic differences between the diagnoses of carcinoma, sarcoma, germ cell tumor, and malignant lymphoma. This is further highlighted within a given anatomical site, for example, lung, where a diagnosis of carcinoma can be of various subtypes requiring either primary surgical (squamous cell carcinoma) or chemo-/radiotherapeutic (small cell carcinoma) approaches and with very different biological outcomes.

Histological Tumor Differentiation or Grade

Tumor differentiation or grade reflects the similarity to the ancestral tissue of origin and degree of cellular pleomorphism, mitoses, and necrosis. It too greatly influences choice of therapy and prognosis, for example, low-grade versus high-grade gastric lymphoma (antibiotics versus chemotherapy/surgery) or grade I (surgery alone) versus grade III (surgery and chemotherapy) breast cancer.

An accurate histological tumor type and grade cannot be ascertained unless there is appropriate specimen handling with adequate fixation.

Extent of Local Tumor Spread

Prognosis of a given cancer may be influenced by the character of its invasive margin (circumscribed/infiltrative) but is largely determined by its pathological stage, that is, the depth or extent of spread in the organ and degree of lymph node involvement. This is then updated by other information, for example, evidence of distant metastases, to formulate a clinical stage upon which management is based. The TNM (tumor nodes metastases) classification is the international gold standard for the assessment of spread of cancer and translates into hard data some of the descriptive language used in histopathology reports facilitating communication within the multidisciplinary team. The post-surgical histopathological classification is designated pTNM and is based on pre-treatment, surgical, and pathological information.

pT	Requires resection of the primary tumor or biopsy adequate for evaluation of the highest pT category or extent of local tumor spread. Due to tumor heterogeneity, this is contingent upon adequate numbers of well-orientated blocks. Where possible multiple tumors are individually staged and the highest pT category used for management decisions
pT0	No histological evidence of primary tumor
pTis	Carcinoma in situ
pT1-4	Increasing size and/or local extent of the primary tumor histologically
pN	Requires removal of nodes sufficient to evaluate the absence of regional node metastasis and also the highest pN category. Where possible all regional nodes in a resection specimen should be sought and harvested for histology
pN0	No regional lymph node metastasis histologically
pN1-3	Increasing involvement of regional lymph nodes histologically
pM	Requires microscopic examination of positive body cavity fluid cytology or distant metastases – the latter may not be available to the pathologist and therefore designated on clinical or radiological grounds

Other descriptors include unifocality (pT1a) versus multifocality (pT1b), lymphatic invasion (L), venous invasion (V), perineural invasion (Pn), classification during or after multimodality therapy (ypT), recurrent tumor (rpT), and multiple primary tumors (pTm). Subdivisions of some categories exist to allow for greater specificity, for example, pN2a and pN2b. Qualifying tumors in the TNM system are carcinoma, malignant mesothelioma, malignant melanoma, gastrointestinal neuroendocrine and stromal tumors, gestational trophoblastic tumors, germ cell tumors, and retinoblastoma. The seventh edition TNM is used throughout this book unless otherwise stated.

Lymphovascular Invasion

Usually defined histologically in blocks from the tumor edge or slightly away from it and more likely to be associated with cancers that show local recurrence, lymph node involvement, submucosal spread, and satellite lesions. This has implications for blocking of resection specimens and their margins. Some cancers (hepatocellular carcinoma and renal cell carcinoma) have a propensity for vascular involvement and care should be taken to identify this on gross specimen dissection and microscopy as it alters the tumor stage and is a marker for distant hematogenous spread.

Lymph Nodes

The pN category relates to the total node yield and the number that are involved. Nodal yields are used to audit the care of dissection by the pathologist, adequacy of resection by the surgeon, and the choice of operation, for example, axillary node sampling versus clearance in breast cancer. All regional nodes should be sampled and although ancillary techniques exist (xylene clearance and revealing solutions) there is no substitute for time spent on careful dissection. Care should be taken not to double count the same node, and those small nodes (>1 mm with an identifiable subcapsular sinus) in the histological slides immediately adjacent to the tumor should not be ignored. TNM rules state that direct extension of primary tumor into a regional node is counted as a nodal metastasis as is a tumor nodule with the form and smooth contour of a lymph node in the connective tissue of a lymph drainage area (e.g., mesorectum) even if there is no histological evidence of residual lymphoid tissue. A tumor nodule with an irregular contour is classified in the pT category, that is, as discontinuous extension. Dissection and submission of separate deposits is therefore important. When size is a criterion for pN classification, for example, vulval carcinoma, measurement is of the metastasis, not the entire node and will usually be made from the histological slides. Micrometastases (≤2 mm) are designated pN1 (mi) and isolated tumor cells (≤0.2 mm) pN0(i+) as they are not regarded as having metastatic potential. Most busy general laboratories submit small nodes (<5 mm) intact or bisected and a mid-slice of larger ones. Additional slices are processed pending microscopy. Alternatively lymph nodes are serially sliced at 2–3 mm intervals and allocated a specific cassette. Sentinel nodes are handled in this way supplemented by use of block levels and immunohistochemistry. The limit node is the nearest node to the longitudinal and/or apical resection limits and suture ties. Some specimens, for example, transverse colectomy, will have more than one and they should be identified as such. Extracapsular spread is an adverse indicator more usually recognized histologically but should be noted on gross inspection if near to or impinging upon a resection margin, for example, axillary clearance in breast carcinoma.

Excision Margins

The clearance of excision margins has important implications for patient follow up, adjuvant therapy, and local recurrence of tumor. Measurements should be made on the gross specimen and checked against the histological slide. Painting of the margins by ink supplemented by labeling of the blocks is important. Paint adheres well to fresh specimens but also works on formalin-fixed tissue. India ink or alcian blue are commonly used. Commercially available multicolored inks are helpful particularly if there are multiple margins as in breast carcinoma. If the intensity of the color on the slide is low, it can be easily checked against the paraffin block. Paint is usually applied to margins prior to dissection but can be re-applied for further emphasis after obtaining the block along its edge. The relevance of particular margins (longitudinal, quadrant, transverse, circumferential, and anatomical) varies according to specimen and cancer type and is further discussed in their respective organ systems. In general terms, involvement of longitudinal margins can be by direct, discontinuous, or multifocal spread, for example, esophageal carcinoma. Positive circumferential radial margins are an indicator of potential local recurrence and a gauge of cancer spread, local anatomy, and the extent of surgical excision. Peritoneal or pleural serosal disease allows potential trans-coelomic spread to other abdominopelvic organs or transpleural spread to the chest wall.

TNM classifies local resection as:

R0	No residual tumor
R1	Microscopic residual tumor (proven by tumor bed biopsy or cytology) and in effect if tumor involves (to within ≤1 mm) the resection margin
R2	Macroscopic residual tumor

Other Pathology

Predisposing, concurrent, and associated conditions should be noted, blocked, and documented, for example, colorectal carcinoma and adenomatous polyps, gastric carcinoma and gastric atrophy or synchronous malignant lymphoma (MALToma).

Ancillary Techniques in Histopathology Specimens

The vast majority of histopathology specimens can be adequately reported by close attention to careful gross description, dissection and block selection and microscopy of good quality formalin fixed paraffin sections stained with hematoxylin and eosin. However, key ancillary techniques are required in a proportion of cases (see Chap. 46). Some examples are

Frozen sections: confirmation of parathyroidectomy, assessment of operative resection margins in cancer surgery, and cancer versus inflammatory lesions at laparotomy.

Histochemical stains: demonstration of mucin in adenocarcinoma, congophilia in amyloid, iron in hemochromatosis, and organisms (pyogenic bacteria, tubercle and fungus) in infection.

Immunofluorescence: glomerular deposits in renal biopsies, deposition of immunoglobulin, and complement in blistering skin disorders.

Immunohistochemistry: the surgical pathologist's "second H and E" and invaluable in assessing tumor type, prognosis, and predictive factors in treatment, for example, carcinoma (cytokeratins) versus malignant lymphoma (CD45) and malignant melanoma (S100) or better prognostic and hormone responsive breast cancer (estrogen receptor positive). Tumor antigenic profile is often crucial in specifying the site of origin for a metastasis, for example, prostate carcinoma (PSA positive).

Electron microscopy: valuable in renal biopsy diagnosis, and tumors where morphology and immunohistochemistry are inconclusive, for example, malignant melanoma (pre-/melanosomes) and neuroendocrine carcinoma (neurosecretory granules).

Molecular and chromosomal studies: immunoglobulin heavy chain and T cell receptor gene rearrangements in the confirmation of malignant lymphoma and the characterization of various cancers (malignant lymphoma, sarcoma, and some carcinomas, e.g., renal) by specific chromosomal changes. Distinctive molecular findings in a wide range of solid tumors are being increasingly used with regards to diagnosis, prognosis, and predicting response to specific targeted therapies. This trend toward personalized oncological medicine also requires consideration of the pre-analytical phase with regards to optimal tissue preservation, fixation, and processing.

Quantitative methods: prognostic indicators include the Breslow depth of invasion in malignant melanoma, muscle fiber typing and diameter in myopathies, and the mitotic activity index in breast carcinoma.

Diagnostic Cytology

Fine needle aspiration, exfoliative and body cavity fluid cytology all provide valuable complementary information in diagnosis and staging (see Chap. 46). The direct smear/cytospin/liquid based preparations are supplemented by formalin fixed paraffin processed cell blocks of cell sediments and needle core fragments (mini-biopsies) which can combine good morphology and robust immunohistochemistry. Correlation between the cytology and histopathological findings is pivotal to accurate diagnosis (e.g., lung cancer) and staging (e.g., pelvic washings in gynecological cancer). Cytology may also provide a diagnosis where biopsy fails due to sampling error, inaccessibility of the lesion, or biopsy crush artifact.

Appendices

Appendix A

Histopathology Specimen Pull-Through Protocol

Specimen Type

- Urgent
- Frozen section
- Cell block – to correlate with corresponding cytology preparations
- Needle core biopsy
- Lymph node (diagnostic/sentinel)
- Bronchial/transbronchial/lung/pleural/mediastinal biopsy
- Temporal artery
- Cancer resection, wide local excision (WLE), endoscopic mucosal resection (EMR), GI polypectomy, TURBT, trachelectomy, microdochectomy, nipple biopsy
- Multidisciplinary Team Meeting (MDM/MDTM) cases
- Extras on cases pending (levels, blocks, stains)

ANY endoscopic or diagnostic biopsy specimen marked Red Flag/fast track or with the following *clinical* or *symptom terminology*:

Clinical Terminology (Abbreviations in Brackets)

* Mass	* Tumor	* Neoplasm (NG)
* Suspicious	* Malignant	* Carcinoma (Ca)
* Primary (1°)/Secondary (2°)	* Lymphoma (HD/NHL)	* Leukemia
* Melanoma (MM)	* Sarcoma	* Mesothelioma
* Myeloma	* Seminoma/teratoma	*Stricture/ulcer(ation)/ obstruction/perforation
* SCC, TCC, AdCa, GCT, NSCLC, (P)NET, Carcinoid	* Paget's disease	* Severe/high grade dysplasia/carcinoma in situ
* Vasculitis/arteritis/Wegener's	* Ectopic/molar gestation	* GvsHD/BMT
* ARF (acute renal failure)	* SLE/PAN	*Pneumonia/ consolidation
*Pyrexia		

Symptom Terminology

* Dysphagia	* Melena	* Hematemesis
* PR bleed	* PMB	* Hemoptysis
* Hematuria	* Jaundice	

Footnotes:

TURBT Transurethral resection bladder tumor
NG New growth
MM Malignant melanoma
HD Hodgkin's disease
NHL Non-Hodgkin's lymphoma
SCC Squamous cell carcinoma or small cell carcinoma
TCC Transitional cell carcinoma
AdCa Adenocarcinoma
GCT Germ cell tumor
NSCLC Non-small cell lung cancer
(P)NET Primitive neuroectodermal tumor
SLE Systemic lupus erythematosus
PAN Polyarteritis nodosa
GvsHD Graft versus host disease
BMT Bone marrow transplant

Dysphagia: difficulty in swallowing
Melena: altered blood in the feces
Hematemesis: vomiting blood
PR bleed: passage of blood per rectum
PMB: post-menopausal bleed
Hemoptysis: coughing up blood
Hematuria: blood in the urine
Jaundice: elevated bilirubin levels due to red blood cell destruction (hemolysis), hepatic damage, or bile duct obstruction

Appendix B

Surgical Cut Up – Principles and Practice

Apprenticeship	Attitude/application/accountability
	Look/listen/lifelong learning – team work
	Do – focus/organization
Patient details	Name/date of birth/healthcare number
Specimen details	Number of specimens/site/laterality
Form details	Clinical information/abbreviations
Clinical priority	Frozen/urgent/treatment decision/MDM case
Past and present	History/history/history
Knowledge base	Context/context/context
	Anatomy/clinical investigations/surgical
	procedures/pathology
Targeted dissection	*Tumor*: type/grade/stage/margins
	Fixation/sampling
	pT/pN/LVI
	Longitudinal, circumferential margins
	Diagrams and photographs
Resources	
1. Pull-through protocols	
2. Specimen dissection laboratory procedures/blocking summary sheets	
3. RCPath Tissue Pathways/Cancer Dataset documents (audit standards)	
4. TNM7	
5. Local tissue pathology cancer report protocols	
6. Texts – Lester/Westra/Rosai/Allen	
Reassess	In light of further clinical information (MDM)/audit

Appendix C

Specimen dissection – a working practice

1. Log the specimen into the day book/computer and allocate a laboratory number.
2. Point out any urgent, fresh, or inadequately fixed specimens to a supervisory BMS so that appropriate action can be taken. Record on the request form.
3. With a supervisory BMS categorize the specimens (see Appendix D) and make a provisional allocation of work.
4. Send the request form of specimen categories C, D, and E to the secretarial office for registration and attachment of any computer back history. Return the forms to the laboratory staff so that specimen dissection can proceed. Categories A and B are usually loaded into the processing cassettes before registration.
5. Preview – consult with the supervisory medical pathologist about the more complex specimens (mainly categories C, D, and E) to confirm categorization, reassign categorization or to discuss the special needs/work allocation

of particular specimens. The medical pathologist authorizes request forms at this stage.

6. Cut-up
   - Work in pairs, one to dissect and describe, the other to write, prepare cassettes, cross check data, observe and confirm findings. The second person can also have a supervisory, training role as appropriate.
   - Check and sign off request form and specimen container label details, i.e.
     - Patient name
     - Patient date of birth
     - Patient unit number
     - Specimen type, parts and numbering
     - Laboratory reference number and cassette labels.
   - Dissect to your level of experience and competence to obtain an accurate description and relevant blocks and also to allow a subsequent meaningful review process.
   - Float out the cassettes with their blocks in formalin.
   - Set the specimens (mainly categories C, D, and E) aside on and covered by appropriately numbered wet paper towels with the corresponding request form beside them.
7. Review – consult with the supervisory medical pathologist about the more complex specimens (mainly categories C, D, and E). He/she will carry out a review with direct feedback to the dissector regarding the quality of macroscopic descriptions, diagrams, and appropriateness of blocks. At this stage, the cut-up of individual specimens will either be confirmed and the request form authorized, or further description, diagnostic possibilities, or supplementary blocks indicated. The supervisory pathologist will mark certain cases for subsequent reporting because of either a special interest or unusual/complex features to the case.
8. Enclose the specimens in moist numbered paper towels in correspondingly numbered plastic bags and store in that day's plastic tray. Small specimens (mainly categories A and B) are individually wrapped in their numbered paper towels along with any spare cassette labels and enclosed in a plastic bag marked with the dissector's initials and those of his/her working partner. Some individual specimens will be retained in formalin filled containers, for example, lymph nodes and some complex cases, as indicated at the review discussion. Specimens are disposed of after 4 weeks once it has been ensured that the surgical histopathology report has been satisfactorily completed, authorized, and dispatched and the case fully discussed at the appropriate multidisciplinary team meeting.
9. For specific specimens of interest that they have cut dissectors are to note the laboratory reference number and to obtain subsequent feedback on the diagnosis, descriptions, and appropriateness of blocks. Trainee pathologists are encouraged to report and sign out cases that they have cut.

10. Knife etiquette – wipe instruments in between block selection and different specimens to avoid tissue carry-in. Use a sharp knife on well-fixed specimens.
11. Remember – if in doubt – ASK. Problems can be resolved by discussion and always be prepared to point out potential errors. Work as a team.

Appendix D

Specimen dissection – guidelines for categorization of specimens according to complexity (*RCPath.*, *recommendations*)

These are intended to be broad guideline definitions to act as a base line which departments and individual consultants may see fit to modify. The review system will permit the recognition of situations in which clinical or anatomical circumstances indicate the category as per protocol is inappropriate.

Basic Definitions

(A) Specimens only requiring transfer from container to tissue cassette.
(B) Specimens requiring transfer, but with standard sampling, counting, weighing, or slicing.
(C) Simple dissection required with sampling needing a low level of diagnostic assessment and/or preparation.
(D) Dissection and sampling required needing a moderate level of assessment.
(E) Specimens requiring complex dissection and sampling methods.

Category A

All small biopsies (endoscopic, synovial, etc.)
Uterine curettings
Simple products of conception
Bone marrow trephines
Testicular biopsies
Cervical punch biopsies
Needle biopsies (excluding those requiring special procedures, e.g., renal, muscle)
Skin curettings and skin biopsies not requiring dissection

Category B

Vasa deferentia
Fallopian tubes
Sebaceous cysts
Small lipomas
Unremarkable tonsils
Unremarkable nasal polyps
Temporal arteries
Thyroglossal cysts

- Molar pregnancy
- Transcervical endometrial resection
- Prostatic chippings
- Lymph nodes

Category C
- Appendix
- Gall bladder
- Large gastrointestinal polyps
- Meckel’s diverticulum
- Diverticular disease
- Ischaemic bowel
- Thyroid – non-tumor
- Salivary gland – non-tumor
- Placenta
- Uterus – routine hysterectomy
- Cervical cone biopsy
- Muscle and cardiac biopsy
- Small soft tissue tumors
- Femoral head
- Renal biopsy
- Skin biopsies – benign – requiring dissection
- Simple small benign breast biopsies

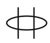
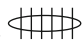
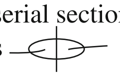
Category D
- Orchidectomy – non-neoplastic
- Simple small ovarian cysts and tumors
- Salivary gland – tumors
- Thyroid – tumors
- Pigmented skin lesions
- Gastrectomy – benign ulcer
- Complex (non-neoplastic) gastrointestinal resections

Category E
- Ovarian tumors
- Uterine carcinoma (including cervical carcinoma)
- Vulvectomy
- Gastrointestinal carcinoma
- Esophagectomy
- Renal resections
- Bladder resections
- Prostatectomy
- Penile carcinoma
- Orchidectomy – neoplastic
- Localized wide lump breast excisions
- Mastectomy
- Bone tumors
- Neck dissection
- Mandibulectomy

Appendix E

Laboratory Abbreviations

g (s)	Gram(s)
kg	Kilogram
mm	Millimeter
ml	Milliliter
cm	Centimeter
F.W.L	Fragments with levels
all processed	All tissue processed
processed intact	Tissue processed intact
fix	Undergoing further formalin fixation
retained	Tissue retained in formalin
mes	Mesentery
ser	Serosa

Bisected ∅
Mid section 
Block in three 
Multiple serial sections 
Quadrants 

Appendix F

Levels and Label Coding

1. Specimens to be cut through three levels with 100 µm between each level are:

Endoscopic biopsies	Vocal cord
	Bronchial
	Esophageal
	Gastric
	Duodenal
	Jejunal (also require examination under the dissecting microscope)
	Colonic
	Rectal
	Bladder
Needle biopsies	Liver
	Pancreas
	Breast
	Prostate
	Renal
Miscellaneous	Cervical punch biopsies
	Small skin biopsies
	Temporal artery (embed transversely)
	Cell blocks

Alternatively needle core biopsies can have an index section and a deeper with the rest of the block retained for further morphology or ancillary techniques.

2. Cassettes are identified either by a microwriter or the use of perforated paper labels placed perpendicular to and protruding from the end of the cassette. They may also be color coded to designate an urgent specimen or specimen type, for example, gastric biopsy.

Part I

Gastrointestinal Specimens

1 Gastrointestinal Specimens: General Comments

Derek C. Allen, R. Iain Cameron, and Maurice B. Loughrey

1.1 Anatomy

The type of histopathology resection specimen received is dictated by the nature of any previous operations and the current disease process, its distribution, and degree of local spread within the organ and to adjacent structures. Resection surgery must provide adequate clearance of longitudinal and deep circumferential radial margins. It must also take into account the lymphovascular supply to achieve satisfactory anastomoses and the regional lymph node drainage for an adequate radical cancer operation. Site location within any given organ may influence the nature of the pathological abnormality and surgical procedure undertaken, e.g., anterior resection for high rectal cancer versus abdominoperineal resection for low rectal cancer, or mid-esophagus (squamous carcinoma) versus distal esophagus (adenocarcinoma). Multifocal distribution may be seen in both inflammatory (Crohn's disease) and neoplastic (malignant lymphoma) disorders. Inflammatory disease can be mucosa confined (ulcerative colitis), transmural (Crohn's disease), or mixed (ischemic colitis). Tumor growth may be predominantly polypoid and intraluminal, with only a minor mural component and variable presentation depending on the organ involved, e.g., symptomatic dysphagia due to esophageal polypoid carcinoma or asymptomatic iron-deficiency anemia with a cecal carcinoma. Often cancer ulcerates and deeply invades the wall, stenosing and obstructing the proximal bowel with early access to mesenteric nodes, lymphovascular channels, and peritoneum, and potential perforation. Alternatively the tumor may be characterized by an intact mucosa and incipient thickening of the wall with a tendency for longitudinal spread and skip lesions (diffuse gastric carcinoma – linitis plastica). Thus, normal anatomy is variably distorted by differing disease processes, and this must be considered in handling the specimen to obtain appropriate management and prognostic data, e.g., depth of local tumor spread, peritoneal and regional lymph node involvement, and excision margin clearance. Allowance must also be made for variation in normal anatomy between and within individuals. For example, harvest of lymph nodes from the mesorectum is scanty compared to the sigmoid mesocolon, and in some patients

D.C. Allen (✉)
Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: derek.allen@belfasttrust.hscni.net

R.I. Cameron
Histopathology Laboratory, Altnagelvin Hospital, Western Health and Social Care Trust, Londonderry, UK
e-mail: iain.cameron@westerntrust.hscni.net

M.B. Loughrey
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: maurice.loughrey@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_1, © Springer-Verlag London 2013

few mesorectal nodes will be found. This is also made more difficult by preoperative radiotherapy, emphasizing the importance of taking into account the previous treatment history and request form information. The surgical histopathology specimen also acts as an audit tool for surgical practice and expertise, e.g., rates of anterior resection versus abdominoperineal resection or completeness of mesorectal excision in rectal cancers. Similarly it allows close correlation with preoperative clinical and radiological (e.g., MRI) assessment, and is a gauge of thoroughness of pathological examination. Thus, preoperative and operative techniques alter the specimen anatomy, resulting in differing management and prognostic implications for an equivalent degree of tumor spread in similar specimens from different patients.

1.2 Clinical Presentation and Investigations

Site-specific symptomatology and investigations are alluded to in the relevant chapters, but some general features can be noted. Clinical presentation can be nonspecific, such as weight loss or anemia, or focused on either the upper (nausea, vomiting, hematemesis) or lower (abdominal pain, bleeding per rectum, change in bowel habit) gastrointestinal tract. An iron-deficiency anemia as measured by the hemoglobin level, red blood cell indices, and serum iron/ferritin levels often means occult blood loss from ingestion of NSAIDs or from the surface of an ulcer or polypoid lesion. Serum albumin levels are decreased due to reduced food intake, protein-losing enteropathy, or liver disease. The erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are increased in neoplasia, vasculitis, and acute flare-up of chronic inflammatory bowel disease. Peripheral white blood cell counts and body temperature are often elevated in acute infection or neoplasia, e.g., leukemia. Features of malabsorption can be due to either small intestinal or pancreatic disease. Liver function (LFTs) and coagulation tests are altered in hepatic and biliary disease.

Various general radiological investigations are also helpful in diagnosing gastrointestinal disorders:

- CXR (chest X-ray) – to detect metastatic deposits in the lung fields or any enlargement of the lung hilum, heart, or aorta that might compress the esophagus; also to show air under the diaphragm following perforated duodenal ulcer
- AXR (straight erect abdominal X-ray) – to demonstrate calcification in pancreatitis or bowel loops distended by fluid levels due to intestinal obstruction
- ELUS (endoluminal ultrasound) and MRI (magnetic resonance imaging) scans – to gauge the depth of spread of a tumor through the gastrointestinal wall into adjacent structures, assess locoregional lymph node enlargement, and soft tissue margin status
- CT (computerized coaxial tomography) scan chest/abdomen/pelvis – to gauge the extent of local and metastatic tumor spread
- PET (positron emission tomography) scan – to help detect metabolically active distant metastases in tumor staging and to distinguish local tumor recurrence from post-radiotherapy fibrosis
- USS (ultrasound scan) abdomen/pelvis – to detect gallstones; biliary tract dilatation; cysts in the liver, pancreas, appendix, or retrorectal space; and mixed solid/cystic abdominopelvic tumors
- Radioisotope scan – to detect metastatic disease in gastrointestinal endocrine tumor (octreotide scan).

Serological markers of use in diagnosing and also detecting recurrence of gastrointestinal cancer are CA19-9 (pancreatic carcinoma), alphafetoprotein (AFP – hepatocellular carcinoma), and carcinoembryonic antigen (CEA – metastatic colorectal carcinoma), although sensitivities and specificities are limited.

Diagnostic laparoscopy allows inspection and biopsy of the peritoneal cavity in various disorders, e.g., tuberculous peritonitis, or, more usually, staging of tumor spread from a gastric carcinoma – a finding that would contraindicate primary surgical resection of the stomach.

The mainstay of investigation is gastrointestinal endoscopy and biopsy.

1.3 Biopsy Specimens

1.3.1 Flexible Endoscopy

Gastrointestinal mucosal biopsy specimens are obtained by flexible endoscopy due to its ease of operation and relative lack of complications. Flexible endoscopes are complex pieces of equipment consisting of a flexible shaft with a maneuverable tip and a control head which the operator holds. The control head is connected to a fiber-optic light source. Other channels such as air, water, suction, etc. pass through the light source. A channel for the passage of therapeutic or diagnostic instruments is located in the control head. The picture from the tip is transmitted to a television screen. Modern endoscopes also incorporate sophisticated magnification capacity to allow close inspection of the topography of mucosal surfaces and lesions.

Upper endoscopy involves informed consent, fasting for 6 h, intravenous sedation, and passage of the endoscope via a mouth guard with direct inspection of the esophagus, stomach, and duodenum, which can be biopsied in relevant areas. Measurements are printed on the shaft of the endoscope so that the operator knows the position of the tip relative to the incisor teeth. Lower endoscopy requires adequate bowel preparation to remove fecal debris and careful insufflation of air via the endoscope to dilate the bowel and allow navigation of the various contours. Due to the fragility of the tissues in some conditions, e.g., toxic megacolon or ischemic colitis endoscopy may be contraindicated to avoid perforation.

1.3.2 Specimen Collection

A copy of the digital endoscopy report is a great aid to the reporting pathologist, and can easily be modified to function as the histopathology request form, maximizing the clinical information provided and removing the issue of illegibility.

In diagnostic endoscopy, tissue biopsies will usually be taken sometimes supplemented by cytology specimens, and there are various accessories designed for this function.

- Forceps: These consist of a pair of sharpened cups attached by a metal cable to a control handle. The forceps are passed down the channel within the endoscope. The cups are opened and closed by an assistant pulling and pushing the plastic handle. The site for biopsy is approached perpendicularly and firm pressure applied while the cups are closed. In the esophagus the approach is tangential and so forceps with a central spike can be used to prevent them from "sliding" off the tissue to be biopsied. At least six tissue samples should be taken from a lesion. Biopsies of ulcers should include samples from the four quadrants and the base, although basal specimens may only yield necrotic slough. If malignancy is suspected, it is prudent to take several specimens from the same place as this allows the outer necrotic layer to be penetrated. With polypoid lesions the crown and base of the polyp as well as the adjacent flat mucosa should be adequately sampled. In some conditions such as Barrett's metaplasia or chronic ulcerative colitis, segments of mucosa are sequentially sampled and mapped by multiple serial biopsies to detect precancerous epithelial dysplasia. Site distribution of lesions is also helpful in differential diagnosis, e.g., ulcerative colitis versus Crohn's disease. The biopsy forceps are withdrawn through the endoscope each time and the tissue sample removed from them by an assistant. A final larger biopsy can be taken if the tissue sample is held in the cups of the forceps while the endoscope is removed.

  The tissue sample is then either put directly into fixative, or after placement onto an orientation millipore (cellulose) filter or polycarbonate strip, preferably mucosal surface upward to avoid flattening the glandular or villous architecture.
- Cytology brushings: Small-spiralled brushes on a metal cable can be used for surface cytology of a lesion. The brush is retracted into a covering plastic sleeve, which protects the

specimen during withdrawal. It is then either promptly made into direct smears or cut off and placed in a suitable transport medium for laboratory processing.

- Fine-needle aspiration cytology (FNAC): FNAC can sample submucosal, mural, and extrinsic lesions not accessible to mucosal biopsy. The syringe needle contents are gently expelled into suitable transport medium, promptly transported to the laboratory, and cytocentrifuged onto glass slides for staining and interpretation.

Mucosal biopsies are generally 2–4 mm diameter and 1 mm deep, but this varies with patient anatomy, the success of the endoscopy procedure, and the nature and configuration of the lesion. Biopsy site and technique also influence specimen size. For example, pinch biopsies obtained via the colonoscope are smaller than rectosigmoidoscopy samples using grasp or jumbo forceps or a strip technique where glucose solution or saline is injected submucosally. A wider diameter biopsy channel can accommodate jumbo forceps or a suction capsule, the latter being of use where mucosal orientation (reflux esophagitis) or deeper tissues (submucosa for the assessment of Hirschsprung's disease) are required.

Mucosal polyps vary in size and appearance. For example, in the colorectum, metaplastic polyps are often 1–2 mm diameter, while adenomas can be similar but are not infrequently larger (1–2 cm), with a distinct head and stalk or even sessile. Small polyps may be removed in toto by usual biopsy forceps, or monopolar hot biopsy forceps, which results in variable diathermy distortion of the mucosal detail. Stalked adenomas are suitable for total excision by an electrosurgical snare. This is facilitated by elevation of the mucosa after submucosal injection of adrenaline, glucose, or saline – a technique that is also used for local endoscopic mucosal resection (EMR) of sessile lesions.

Needle biopsy cores of liver and pancreas are obtained endoscopically, percutaneously, or at operation transabdominally by a variety of needles of differing lengths and caliber. They can be spring-loaded or manually operated with the cutting edge of the needle delivering a core of tissue into its lumen. The needle is then retracted and withdrawn with careful removal of its contents and placement into formalin fixative. The procedure may be done blind, under X-ray control, or at operation direct vision, depending on the individual case. A 16G needle provides a much more substantial specimen than an 18G needle and is especially recommended for "medical" liver biopsies, i.e., evaluation of diffuse liver disease processes. The larger needle is, however, associated with a slightly greater risk of bleeding. Regardless of needle size, the patient should have an adequate coagulation status confirmed beforehand and, during the procedure, vascular structures avoided to minimize any risk of bleeding. Endoscopic, percutaneous, or transabdominal FNAC can traverse abdominal viscera with no detrimental effect to sample abdominal and retroperitoneal masses not accessible to usual endoscopic procedures.

1.3.3 Specimen Handling

Fragments, *non-orientated*:

- Usually multiple fragments, free floating in fixative, non-orientated.
- Count.
- Place in cassette between foam insert pads or loosely wrap in moist filter paper.
- Insert levels label.
- Align in the block at the embedding stage as this facilitates microscopic assessment and fragments are not missed.
- Separate specimens: use separate cassettes and site identification labels appropriate to the request form information. Alternatively multiwall cassettes may be submitted by the endoscopist.
- Cut through multiple levels.

Fragments, *orientated*:

- This allows better assessment of mucosal architecture and site distribution of lesions, e.g., colonic strip biopsy in chronic inflammatory bowel disease.
- Filter paper: count the fragments and note any that have detached. Process intact between foam insert pads or covered by moist filter paper to preserve orientation for embedding and cutting through multiple levels.
- Polycarbonate strip (Fig. 1.1): the endoscopist allows a 2–4 min period of air drying prior to

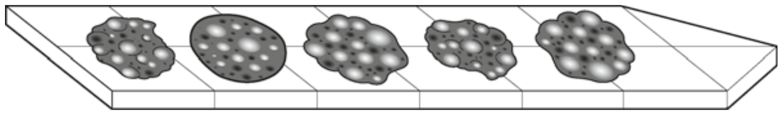


Fig. 1.1 Colonoscopic biopsies mounted on a polycarbonate or Millipore strip (Reproduced, with permission, from Allen and Cameron (2004))

formalin fixation, ensuring adherence of the mucosal fragments to the strip, which is designated according to a pre-agreed protocol, e.g., the cut pointed end is distal or anorectal. Strict alignment of the fragments on the strip by the clinician is essential as it is embedded intact and on its edge for cutting to allow representation of all the fragments at the same level in the block. Count the fragments and cut through multiple levels.

Polyps (Fig. 1.2):

- Non-orientated fragments: these are handled as indicated above.
- Snare specimens:
  ≤0.5 cm diameter – bisect vertically down through the stalk/base and embed both cut surfaces face down. Cut through multiple levels.
  >0.5 cm diameter – obtain a central, vertical mid-slice (3 mm thick) down through and to include an intact stalk/base. Embed face down in the block and the lateral trimmings in a separate block. Cut both through multiple levels. If there is a long stalk, precluding submission of a central mid-slice in one block, an initial transverse section of its resection margin may be taken.
- Local mucosal resection: endoscopic or transabdominal; this is used for stalked polyps (see above) or sessile lesions. Ideally the latter should be submitted by the surgeon to the laboratory already carefully pinned out onto a corkboard or piece of card. Remove after fixation and paint the deep and lateral mucosal resection margins. Obtain multiple vertical transverse serial slices (3 mm thick) to include the lesion and underlying base. Where the lesion edge is to within 3 mm of the mucosal margin sample at right angles to it from a 10 mm slice. Embed the slices face down in the block and cut through multiple levels.

Wedge biopsy:

- Usually derived from the edge of a perforated ulcer detected at surgical laparotomy for an acute abdomen. Its base is oversewn and a biopsy taken if the edges show any unusual features, e.g., rolled margins.
- With the mucosal surface upward, bisect or cut into multiple vertical serial slices. Embed the slices face down and cut through multiple levels.

Needle core biopsy:

- Up to 2 cm long and 1–2 mm diameter, core size is influenced by the patient's anatomy, the nature of the lesion being biopsied, the needle that is used, the route of acquisition (e.g., percutaneous or transjugular), and operator expertise. Some scirrhous carcinomas can be difficult to sample, whereas other disease processes lead to fragmentation of the core, e.g., cirrhosis of the liver. Skinny needle cores can be particularly fine, requiring careful handling and even painting or immersion in dye (e.g., alcian blue) prior to embedding so that the tissue can be seen when the block is faced at cutting.
- Count and measure the maximum core length (mm).
- Place intact in cassette between foam insert pads or loosely wrap in moist filter paper.
- Cut through multiple levels.

Fresh tissue:

- The vast majority of specimens are submitted in formalin fixative, but some cases require fresh tissue for frozen sections, e.g., acetylcholinesterase staining in Hirschsprung's disease, or an inflammatory versus malignant lesion at diagnostic laparotomy.

1.4 Resection Specimens

1.4.1 Fixation

- Ideally specimens are submitted fresh to the laboratory to facilitate sampling for research or biobanking and accurate measurement, as

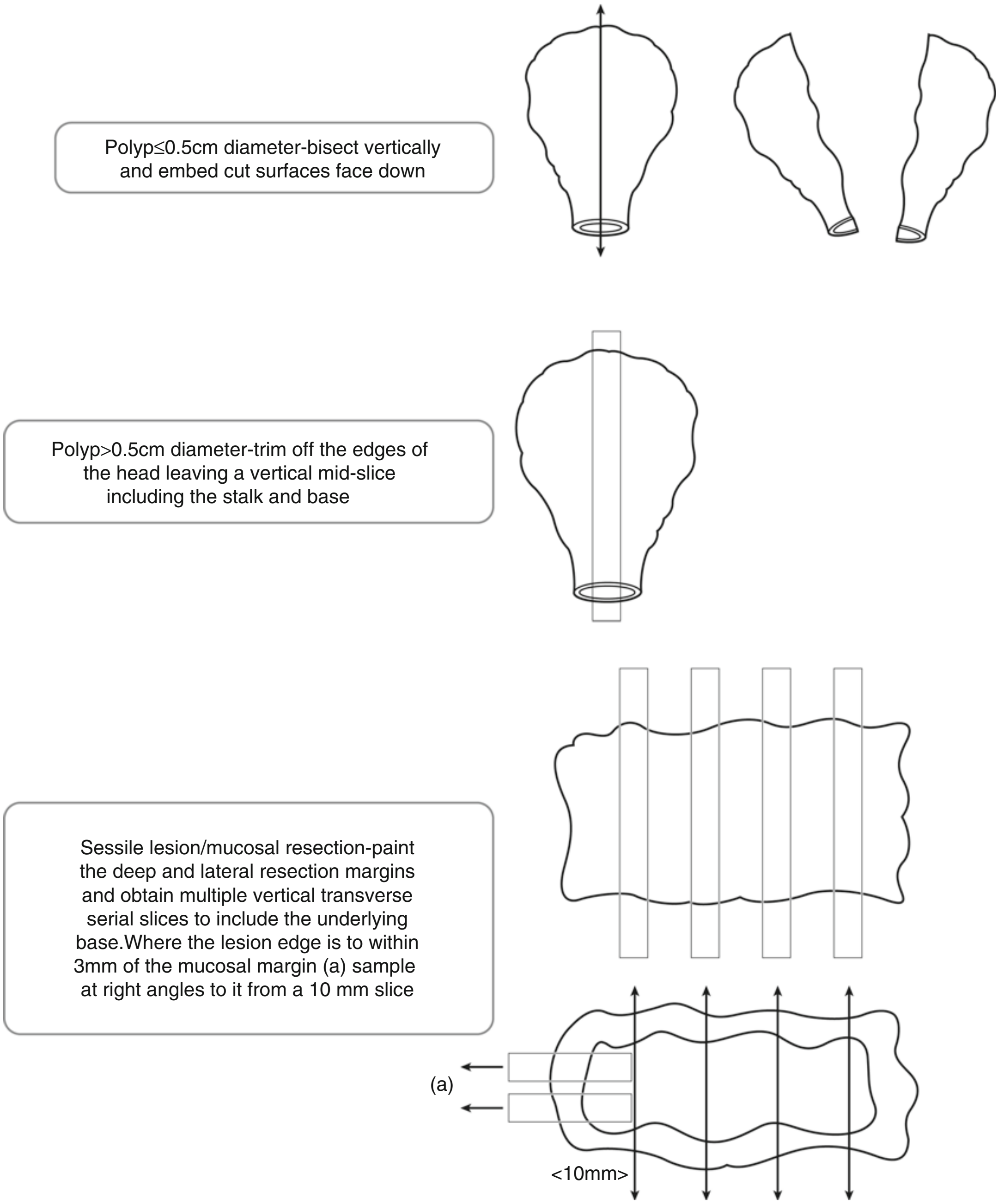


Fig. 1.2 Gastrointestinal mucosal polyps and local mucosal resections (Reproduced, with permission, from Allen and Cameron (2004))

fixation results in considerable shrinkage (15–30% on average) and discrepancy between clinical and pathological dimensions, e.g., longitudinal margins of tumor clearance. Fresh submission also permits cleaning out of the specimen and either partial or total opening for pinning out and fixation. This avoids specimen distortion and ultimately allows dissection appropriate to the specimen and tumor type, e.g., the assessment of circumferential resection margins. Adequate fixation of a cleaned, opened specimen requires 36–48 h immersion in formalin. Where it is normal practice to submit resection specimens to the laboratory already in fixative, the theater staff should be instructed on how to partially open and clean out the specimen but to avoid transecting the tumor segment, thereby compromising margin assessment.

1.4.2 Margins

Longitudinal, circumferential, and anatomical margins are considered.

- Longitudinal margins: circumferential, transverse sections are taken in non-neoplastic disorders such as ischemia or chronic inflammatory bowel disease to assess involvement. In cancer resections, separate anastomotic rings are often submitted and these constitute the longitudinal margins rather than those of the main specimen. In the absence of an anastomotic ring, the longitudinal margin should be circumferentially sampled although if the tumor is close (≤0.5–1 cm) to it a longitudinal block may be more practicable. The significance of longitudinal margin clearance varies, e.g., a macrosopic tumor clearance of 2–3 cm in an anterior resection for rectal cancer is considered satisfactory, whereas it is not for diffuse gastric or esophageal cancers where multifocal epithelial and discontinuous submucosal or mural skip lesions can occur. Longitudinal margins should be blocked first prior to dissection of the tumor to avoid knife carry-in of tumor fragments.
- Circumferential radial margin (CRM): this gives an assessment of the extent of lateral or radial spread of a tumor and its adequacy of excision, features that are strongly related to subsequent local recurrence and morbidity. Prior to dissection, the CRM should be painted and both macroscopic and microscopic measurements of tumor clearance are then made. In the mesorectum, direct tumor spread or tumor within a lymph node or lymphatic to within ≤1 mm of the CRM is considered involved. CRM involvement may indicate the need for postoperative radiotherapy. The amount and completeness of excision of circumferential tissues depend on the anatomical site and expertise of the surgeon. For example, adventitial tissues in an esophagectomy specimen may be scanty, whereas the posterior and lateral mesorectum is usually 2–3 cm deep. The success of total mesorectal excision (TME) relates to surgical training and the available time resources to carry out an adequate procedure, but TME grading by the pathologist is an important part of auditing surgical practice. The significance of tumor at the mesocolic edge or that of the gastric lesser omentum is less established but should be reported by the pathologist.
- Anatomical margins: The serosa or peritoneum is a visceral margin and breech of it allows tumor to access the abdominal and pelvic cavities with potential for transcelomic spread, e.g., diffuse gastric cancer with bilateral ovarian metastases (Krukenberg tumors). Thus, gastrointestinal cancers may present clinically with deposits at another abdominopelvic site and this should be borne in mind on assessment of tumor macroscopic and microscopic appearances. Tumor at and ulcerating the serosa represents pT4 disease and is a decision factor in selection for postoperative chemotherapy. It should be distinguished from the more common finding of carcinoma in a subserosal inflammatory fibrous reaction but not at its free surface (pT3).

1.4.3 Dissection

1.4.3.1 Cancer Resections

For optimal demonstration of the deepest point of tumor spread, its relationship to the CRM and correlation with ELUS/CT cross-sectional imaging multiple, serial, 3–4 mm thick slices of the cancer in the transverse axis are recommended. The slices can then be laid out in sequence and a digital photographic record taken. Generally four or five blocks of the tumor and wall are selected to adequately define the pT stage. Some pathologists leave the tumor segment unopened during fixation and transverse slicing to keep the CRM intact – others open it carefully avoiding suspect areas of the CRM to ensure adequate tumor fixation and ascertain tumor measurements. Either approach is justifiable as long as it is done with care and consistency. Sometimes the local anatomy or proximity of the tumor to a longitudinal margin necessitates dissection in the longitudinal plane. Such a block can be useful in a poorly differentiated carcinoma when the adjacent mucosa may show a point of origin or clue as to its histological type. Mucosal blocks away from the tumor may also demonstrate its histogenesis, e.g., metaplasia/dysplasia/cancer sequence in the stomach, or, multifocality. Multiple colonic cancers are blocked and reported individually. A clear block index within the pathology report facilitates case review, e.g., for multidisciplinary team meeting discussion, and tumor block selection for future immunohistochemical or molecular assays.

All regional lymph nodes should be sampled as size alone is not a reliable indicator of metastatic involvement and pN staging relates to total and involved numbers of nodes. Small nodes seen histologically in the tumor blocks are also counted and may only measure ≥1 mm diameter but are recognizable by their subcapsular sinus. A limit node is identified adjacent to a mesenteric pedicle suture tie – some specimens, e.g., transverse colon, may have more than one. Dukes staging for colorectal cancer varies according to whether the limit node is involved (C2) or not (C1). Techniques such as xylene clearance have been advocated to increase nodal yields, but, in general, there is no substitute for experienced, careful dissection. The TNM (tumor-node-metastasis) system recommends what is considered an appropriate regional lymphadenectomy for each type of cancer resection. Regular departmental audit of median lymph node counts for relevant pathology specimen types ensures standards are met and maintained. Preoperative radio-/chemotherapy can lead to marked tumor degeneration and fibrotic reaction compromising nodal yields and identification of residual primary tumor or nodal deposits. Most general laboratories submit small nodes (<5 mm) intact, trimmed, or bisected, and a mid-slice of larger ones. It is important that the same node is not counted twice. Alternatively nodes are serially sliced at 2–3 mm intervals and submitted in their entirety in individual cassettes.

1.4.3.2 Non-neoplastic Resections

An important descriptive feature in differential diagnosis is disease distribution, e.g., diffuse, segmental, mucosal, or transmural. Overt lesions may show only end-stage, nonspecific florid ulceration and reactive changes – the disease distribution and changes in the intervening mucosa give important diagnostic clues. For example, ulcerative colitis is mucosal and diffuse; Crohn's disease is segmental and transmural, with intervening aphthous ulcers and serosal fat wrapping; chronic ischemic stricture is preferentially located at the splenic flexure; and clostridium difficle infection shows mucosal pseudomembranes. Non-neoplastic colonic specimens therefore require sequential labeled blocks of abnormal and normal (e.g., every 10 cm) areas, with a clear block index in the report to aid case review. As the mucosa is arranged in transverse folds, long axis blocks are taken. Longitudinal limits are transverse sectioned to look for disease involvement and although mesenteric nodes are usually reactive only, they may show helpful diagnostic pointers such as granulomas in Crohn's disease. In ischemic conditions, mesenteric vessels are also sampled for signs of vasculitis or embolic thrombi. Some vascular anomalies, e.g., angiodysplasia of the colon, may require close liaison with the surgical and radiological teams necessitating preoperative injection of radio-opaque contrast medium. In some cases, e.g., gastric resections, it is not possible to tell macroscopically if the ulcer, adjacent mucosa, or regional nodes are benign or malignant or to gauge the extent of mural spread – dissection and block selection must be sufficiently comprehensive to allow for this.

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2 Esophagus

Derek C. Allen, R. Iain Cameron, and Maurice B. Loughrey

2.1 Anatomy

The esophagus is a tubular structure, approximately 25 cm long, extending from the laryngeal part of the pharynx at the level of the sixth cervical vertebra, passing through the diaphragm at the level of the tenth thoracic vertebra to join the stomach at the esophagogastric (EG) junction (Fig. 2.1). For purposes of practicality during endoscopic procedures, the site of a lesion in the esophagus is given as the distance from the upper incisor teeth. As it is approximately 16 cm from the upper incisor teeth to the proximal esophageal limit, the EG junction is at approximately 40–41 cm. The esophagus traverses the neck, thorax, and enters the abdominal cavity and so can be anatomically divided into three subsites:

D.C. Allen (✉)
Histopathology Laboratory, Belfast City Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: derek.allen@belfasttrust.hscni.net

R.I. Cameron
Histopathology Laboratory, Altnagelvin Hospital,
Western Health and Social Care Trust,
Londonderry, UK
e-mail: iain.cameron@westerntrust.hscni.net

M.B. Loughrey
Histopathology Laboratory, Institute of Pathology,
Royal Victoria Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: maurice.loughrey@belfasttrust.hscni.net

1. Cervical esophagus – 2–3 cm long and extends from the proximal esophageal limit (C6) to the thoracic inlet, which is marked by the surface landmark of the suprasternal notch of the sternum (breast bone).
2. Intrathoracic esophagus – approximately 21 cm long and extends from the thoracic inlet to the esophageal hiatus in the diaphragm. At 25 cm from the upper incisor teeth, the esophagus is constricted by the aortic arch and the left main bronchus crossing its anterior surface.
3. Abdominal esophagus – 1–1.5 cm long and extends from the esophageal hiatus in the diaphragm to the right side of the stomach. It is covered anterolaterally by the peritoneum and comes into close relationship with the left lobe of liver.

An internal landmark of relevance to determining the site of origin of an EG tumor is the EG junction where the pale esophageal squamous mucosa meets the glandular mucosa of the gastric cardia. The EG junction can be somewhat irregular in outline (the Z line) and does not necessarily correspond to the lower physiological valve or sphincter. External landmarks are distal esophagus orientated to adventitial fat while the junctional area and proximal stomach relate to a covering of serosa or peritoneum. Thus, a tumor of the distal esophagus or EG junction can spread through the wall either to adventitial fat of the mediastinum or the abdominal peritoneum. Adventitial fat is disposed laterally, but absent anteriorly and posteriorly where the esophagus is adjacent to the heart

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_2, © Springer-Verlag London 2013

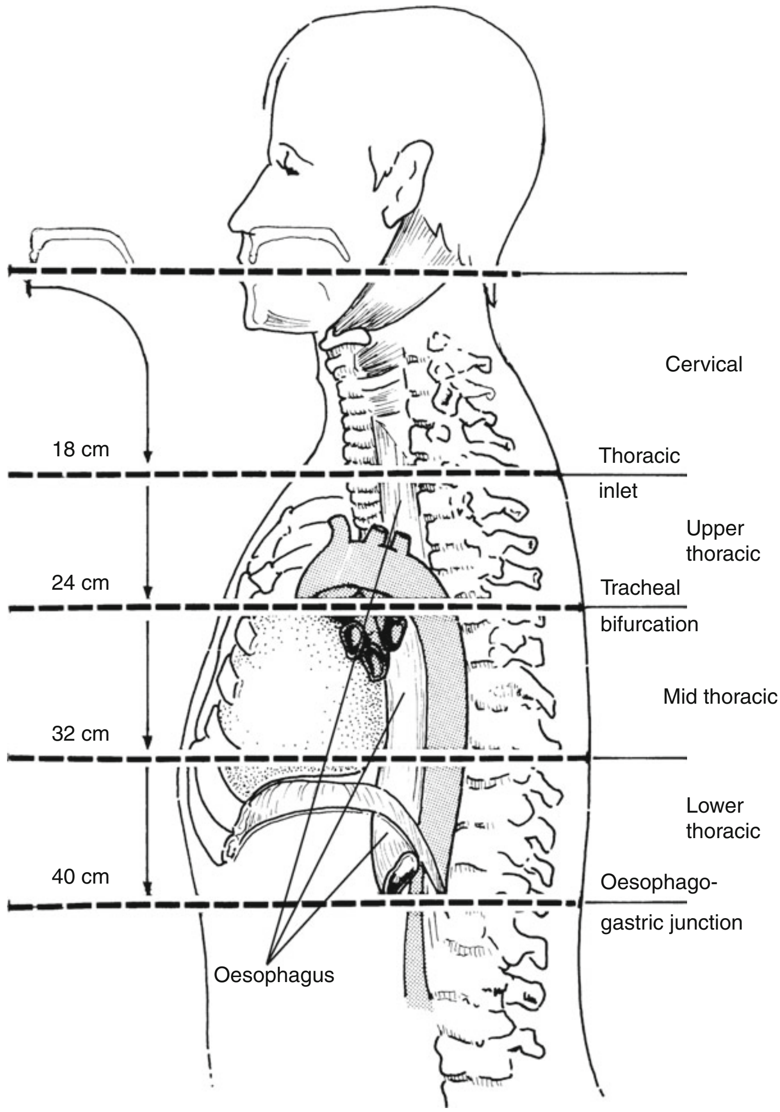

Fig. 2.1 Esophagus (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

and vertebral column, respectively. Note that the adventitia of the mid-esophagus may also relate to a serosal surface – that of resected mediastinal pleura.

As well as determining the position of the lesion within the esophagus by its anatomical site, it can also be defined by its relative position in the upper, middle, or lower third of the esophagus. This is of relevance clinically as the lymphovascular drainage is considered in these terms and is therefore important in cancer surgery.

Lymphovascular drainage (Fig. 2.2):

Upper third – deep cervical nodes

Middle third – superior and posterior mediastinal nodes

Lower third – nodes along the left gastric blood vessels and the celiac nodes

Venous drainage from the middle third (azygos vein) into the lower third (gastric vein) leads to the formation of a porto-systemic anastomosis and, with raised portal venous pressure (e.g., as in liver cirrhosis), the possibility of the

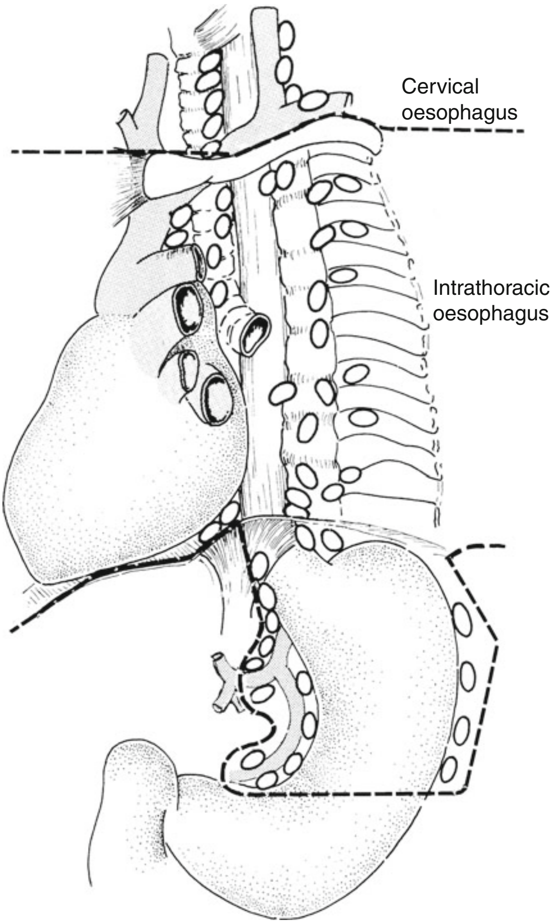

Fig. 2.2 The regional lymph nodes, irrespective of the site of the primary tumor, are those in the esophageal drainage area including celiac axis nodes and paraesophageal nodes in the neck, but not supraclavicular nodes (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

formation of esophageal varices (dilatation of esophageal veins).

2.2 Clinical Presentation

Patients with esophageal disease may be asymptomatic, but usually experience one or more of the following: chest pain, heartburn (a retrosternal burning sensation), reflux of acid/food, and dysphagia (difficulty swallowing). Dysphagia can be painful (odynophagia) and progressive due to benign or malignant strictures, i.e., initially for solid foods, e.g., meat, then soft foods, and ultimately liquids. Patient localization of the site of obstruction can be poor. Occult bleeding can lead to iron-deficiency anemia, while hemorrhage (hematemesis) can be potentially life threatening (varices) or self-limiting due to linear tears of the EG junction mucous membrane after prolonged vomiting (Mallory-Weiss syndrome).

2.3 Clinical Investigations

- Endoscopy and biopsy.
- CXR to detect any enlargement of the heart, mediastinal lymph nodes, or pulmonary hilum that might extrinsically press on the esophagus. Barium swallow to outline the contour of the esophageal lumen and wall, and assess motility/swallowing.
- For biopsy-proven cancer – ELUS and PET CT scan chest and abdomen to determine the

pretreatment tumor stage directed toward pT/pN and pM disease, respectively.

- Laparoscopy with peritoneal washings for EG junctional and select distal esophageal adenocarcinoma cases.
- Twenty-four hour pH monitoring – has a high diagnostic sensitivity for reflux esophagitis.
- Esophageal manometry can assess the effectiveness of motility, e.g., achalasia, scleroderma.

2.4 Pathological Conditions

2.4.1 Non-neoplastic Conditions

Reflux esophagitis: usually due to hiatus hernia (slippage of the EG junction into the thorax) resulting in gastro-esophageal reflux (GER) of acid and bile; there is poor correlation with symptoms, endoscopy and biopsy being normal in 20–30% of cases. Otherwise well-orientated biopsies show basal zone hyperplasia and prominent vascularized connective tissue papillae. This is superseded by inflammatory infiltrates of neutrophils and eosinophils, surface erosion, full thickness ulceration, and, ultimately, fibrous stricture formation (10% of cases). It may require operative dilatation (often repeatedly) to relieve dysphagia and, although it usually has a smooth outline, it may be difficult to distinguish endoscopically from a malignant growth. Prior to this, treatment of GER is either medical (weight loss, antacids) or occasionally surgical. This is usually done laparoscopically by wrapping the fundus of the stomach around the distal esophagus (Nissen fundoplication) to maintain lower esophageal tone and retain it in the abdominal cavity.

Infective esophagitis: may be seen in otherwise healthy individuals but is more commonly encountered where there is alteration of either local or systemic immunity (e.g., AIDS). Underlying ulceration, broad-spectrum antibiotics, diabetes, corticosteroid therapy, and immunosuppressive drugs can all alter the local gut flora resulting in superimposed infection. Causative agents are candidal fungus, herpes simplex virus (HSV 1 and 2), cytomegalovirus (CMV), and atypical mycobacteria.

Miscellaneous: Other causes of esophagitis, ulceration, and/or stricture are drugs (e.g., NSAIDs, aspirin), mediastinal radiotherapy, motility disorders (e.g., achalasia), Crohn's disease and direct injury (foreign body, prolonged nasogastric intubation, corrosive ingestion).

Incidental endoscopic findings are inflammatory or fibrovascular polyps of the EG junction.

2.4.2 Neoplastic Conditions

Benign tumors: These are rare in surgical material, e.g., squamous papilloma, leiomyoma, or granular cell tumor.

Esophageal carcinoma: Predisposing conditions to esophageal cancer include GER, obesity, diverticula, achalasia, and Plummer–Vinson syndrome (elderly females, iron-deficiency anemia, upper esophageal web). Predisposing lesions to esophageal cancer are squamous cell dysplasia and Barrett's metaplasia/dysplasia.

Squamous cell dysplasia/carcinoma in situ: Macroscopically often inapparent but seen histologically adjacent to, overlying or distant from squamous cell carcinoma.

Barrett's metaplasia or columnar epithelium lined lower esophagus (*CLO*): Seen in about 10% of patients with hiatus hernia and/or GER. It arises from erosion with differentiation of multipotential stem cells to metaplastic small intestinal or gastric glandular epithelia. The Barrett's segment appears as a velvety area proximal to the EG junction surrounded by pale squamous mucosa. It can be multifocal or continuous. The segment is either classical/long (≥3 cm) or short (<3 cm). About 10% of Barrett's cases develop mucosal dysplasia and/or adenocarcinoma, representing an increased risk of ×20–30 that of the general population. Barrett's metaplasia positive for mucosal dysplasia is classified as either low grade or high grade. The latter has a strong (20–30%) association with concurrent or subsequent adenocarcinoma, indicating the need for immediate clinicopathological reassessment and, if a tumor is identified, consideration of surgery. In the absence of tumor or in a medically unfit patient with an early lesion, local ablative therapy can be used, e.g., EMR, HALO (high-radiofrequency ablation) or laser eradication. The appearances of Barrett's metaplasia can also be altered by its treatment with antacid medication, ablative techniques, or photodynamic therapy.

Squamous cell carcinoma: Forms 30–40% of esophageal cancers and is typically seen in the mid-esophagus of elderly patients. It is usually moderately differentiated and keratinizing, ulcerates or strictures with rolled, irregular margins, involves a long segment of esophagus, and has spread through the full thickness of the wall at presentation. Palliation can be achieved by chemoradiation, ablative laser therapy, or the insertion of an expanding metal stent or tube to relieve obstruction. Primary treatment of choice in a medically fit patient with a locally confined lesion <5 cm in length is chemoradiotherapy alone or in combination with subsequent surgery. Preoperative chemoradiotherapy produces signs of tumor regression (degeneration, necrosis, fibrosis, keratin granulomas) in some 50–60% of cases, but often makes identification of tumor on gross inspection of the specimen difficult. Perforation with potentially fatal mediastinitis is a possible complication of preoperative therapy and endoscopy of malignant strictures. Depending on the CT chest findings, bronchoscopy is sometimes done to exclude the possibility of a primary lung cancer invading esophagus, which would preclude primary resection as do hematogenous and distant nodal metastases or invasion of mediastinal vessels and main structures.

Variants of squamous carcinoma are verrucous carcinoma (warty, slow growth), basaloid carcinoma (aggressive), and spindle cell/polypoid carcinoma (carcinosarcoma – intermediate prognosis).

Adenocarcinoma: Forms 50–60% of esophageal cancers and arises in the distal esophagus/EG junction, often secondary to intestinal-type Barrett's metaplasia and dysplasia. The incidence of this tumor has greatly increased in the last 20 years due in part to antibiotic eradication of helicobacter pylori with loss of its gastric acid suppressor effect, resulting in more GER disease. As well as extensive radial spread through the wall out to the CRM, it can spread upward, undermining the esophageal squamous mucosa and downward to the proximal stomach where clear distinction from a primary gastric carcinoma can be difficult. Clues as to site of origin are both anatomical and histological in the adjacent mucosa (esophagus – Barrett's metaplasia/dysplasia; stomach – gastric mucosal dysplasia). TNM 7 includes as an esophageal cancer any tumor of the proximal stomach where its epicenter is within 5 cm of the EG junction and involves the esophagus (Siewert 3). Adenocarcinoma is usually ulcerated with irregular rolled margins or polypoid, and histologically tubular or papillary with an intestinal glandular pattern but sometimes of diffuse signet ring cell type. Treatment of choice for locally confined disease is preoperative chemotherapy combined with surgical resection. This is supplemented by postoperative chemotherapy if indicated by subsequent pathological staging of the resection specimen.

Other features: Esophageal cancer tends to show multifocality (15–20%). Examination of specimen proximal and distal surgical margins is therefore important. "Early" or superficial squamous carcinoma is confined to the mucosa or submucosa with or without regional lymph node involvement and is of better prognosis than "advanced" or deep muscle invasive carcinoma. Involvement of the periesophageal CRM is partly dependent on individual patient anatomy but is also an indicator of extent of tumor spread, adequacy of surgical resection, and potential local recurrence due to residual mediastinal disease.

Other cancers: Rare but can include small cell carcinoma, malignant melanoma, leukemia/malignant lymphoma, metastatic cancer (e.g., lung or breast), leiomyosarcoma, and Kaposi's sarcoma (AIDS).

Prognosis: Prognosis of esophageal cancer is poor (5-year survival 5–15%) relating mainly to depth of spread and lymph node involvement, i.e., tumor stage, and involvement of longitudinal and circumferential excision margins. Early or superficial carcinoma does significantly better – 55% → 88% 5-year survival depending on the depth of mucous membrane invasion.

2.5 Surgical Pathology Specimens: Clinical Aspects

2.5.1 Biopsy Specimens

Two main types of esophageal endoscopy exist, namely rigid and flexible. Rigid esophagoscopy is only occasionally used to provide larger biopsies when previous flexible endoscopy (EGD – esophagogastroduodenoscopy) samples have proven non-diagnostic. Specific lesions such as polyps or ulcers necessitate multiple targeted biopsies that may be supplemented by brush cytology of the mucosal surface. Mapping and annual/biennial surveillance of flat mucosa for Barrett's metaplasia and dysplasia is achieved by multiple segmental (every 2 cm) and quadrantic biopsies. The clinical extent of the Barrett's is described according to its length of circumferential disposition (cm) and total length (cm) of the metaplastic segment respectively, e.g., C2M6. The basis of an esophageal stricture may be easier to demonstrate if malignant in nature because of carcinoma ulcerating the squamous epithelium, whereas a benign peptic stricture due to submucosal or mural fibrosis is often not accessible to mucosal biopsy. Endoscopic biopsy of achalasia or esophageal webs is often unrewarding as it provides intact surface mucosa only. EMR ("big biopsy") specimens can be both diagnostic, staging and therapeutic, e.g., in dysplastic Barrett's to diagnose and remove any "early" nodular areas of carcinoma confined to the mucosa.

2.5.2 Resection Specimens

The surgical techniques for resecting esophageal tumors fall into two broad categories – those which employ a chest incision (thoracotomy) and those which do not (transdiaphragmatic hiatal procedures). The type of procedure used depends on the general level of health of the patient, any previous operations, the preference of the operating surgeon, the size and position of the tumor in the esophagus (see Table 2.1), and the choice of esophageal substitute, i.e., stomach, jejunum, or colonic interposition. Ideally the surgeon should strive for a 5 cm longitudinal margin of clearance with adenocarcinoma and 10 cm for squamous carcinoma, with an appropriate lymphadenectomy. There is currently no evidence-based favored method of resection.

Table 2.1 Choice of surgical procedure in esophageal neoplasia

Proximal 1/3 tumors	Pharyngo-esophagectomy
Middle 1/3 tumors	Ivor Lewis technique Thoracoabdominal esophagectomy Two-field transhiatal esophagectomy
Lower 1/3 tumors	Ivor Lewis technique Thoracoabdominal esophagectomy Transhiatal esophagectomy
Barrett's	Transhiatal esophagectomy

2.5.2.1 Procedures employing a thoracotomy:

(a) *Ivor Lewis technique* – in this operation, upper abdominal and right thoracotomy incisions are made. The proximal stomach is divided and the esophagus is transected proximal to the tumor. The distal stomach is then raised into the chest and an EG anastomosis is fashioned.

(b) *Thoracoabdominal esophagectomy* – a continuous incision extending from the midline of the upper abdomen running obliquely across the rib margin and posterolateral aspect of the chest wall. The left diaphragm is divided and this gives access for potential en bloc resection of the esophagus, stomach, gastric nodes and, if required, the spleen and distal pancreas. An esophagojejunal or EG anastomosis is fashioned in the neck.

2.5.2.2 Transhiatal esophagectomy:

Depending on whether a total or distal esophagectomy is to be performed, two variations of this procedure are used.

(a) "Two-field approach" – the entire esophagus and stomach is mobilized via upper abdominal and oblique neck incisions. The cervical esophagus is divided and anastomosed to stomach which had been mobilized and raised high into the posterior mediastinum.

(b) Distal esophagectomy with proximal gastrectomy (for distal esophageal/junctional tumors) – only an upper abdominal incision is used, with the distal esophagus being mobilized and an EG anastomosis fashioned in the chest.

Although transhiatal resection for diseases of the thoracic esophagus used to be uncommon, it is now more commonly used, reducing the physiological insult experienced with a thoracotomy. Minimally invasive esophagectomy (MIE) procedures are being developed using combined laparoscopic and thoracoscopic techniques.

Whenever possible the stomach should be used in the anastomosis and with appropriate mobilization the stomach will reach the neck in virtually all patients. If the tumor is limited to the EG junction, the entire greater curvature of the gastric fundus (shaded area in Fig. 2.3), including the point which usually reaches most cephalad to the neck (*in Fig. 2.3), may be preserved while still obtaining a 4–6 cm gastric margin distal to the malignancy.

There are several benefits in performing a total thoracic esophagectomy with cervical anastomosis: maximum clearance of surgical margins is obtained while the risk of mediastinitis, sepsis, and GER that can be seen with an intrathoracic anastomosis is diminished.

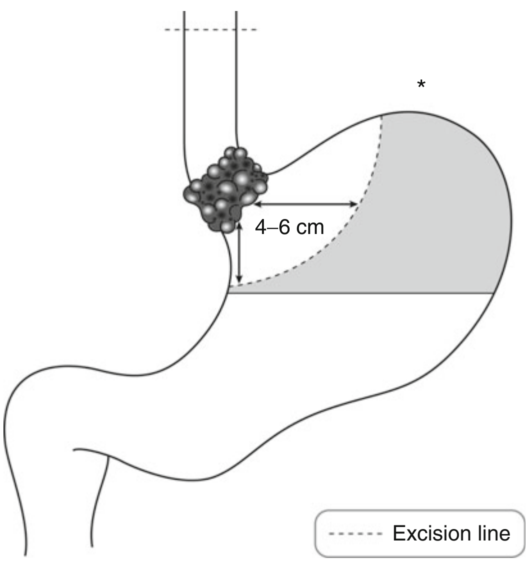

Fig. 2.3 Transhiatal esophagectomy with limited proximal gastrectomy (Reproduced, with permission, from Allen and Cameron (2004))

2.6 Surgical Pathology Specimens: Laboratory Protocols

2.6.1 Biopsy Specimens

See Chap. 1.

2.6.2 Resection Specimens

Specimen:

- Most esophageal resections are for neoplastic conditions: partial esophagectomy, total thoracic esophagectomy (TTE), esophagectomy with limited gastrectomy, esophagogastrectomy

  Initial procedure:
- By palpation and with the index finger locate the lumenal position of the tumor.
- Paint the overlying external CRM comprising adventitial fatty connective tissue and any related serosa.
- Open longitudinally with blunt-ended scissors, cutting on the opposite side of the tumor. Open proximal stomach along the greater curvature continuous with the esophageal cut. Alternatively some pathologists prefer to leave the tumor segment unopened for fixation and subsequent transverse slicing.
- Measurements:
  - Esophagus – length (cm), width (cm)
  - Proximal stomach – lengths (cm) along lesser and greater curvatures
  - Tumor – length × width × depth (cm) or maximum dimension (cm)
  - Distances (cm) to the proximal and distal limits of resection
  - Distance (cm) to the EG junction if the tumor is mid-esophageal in location
  - Relationship to the EG junction: distal esophageal tumor involving the junction (Siewert 1), tumor straddling the junction (Siewert 2), proximal gastric tumor involving the junction (Siewert 3).

  - Note that the junction may be obscured by tumor, and external landmarks (esophagus – adventitia; stomach – serosa) should also be used in determining the location.
  - Barrett's mucosa – location/length (cm).
- Photograph.
- Fixation by immersion in 10% formalin for 48 h preferably pinned out on a corkboard in the opened position but not placed under tension (to avoid splitting).

Description:

- Tumor
  - Polypoid: spindle cell carcinoma/carcinosarcoma
  - Warty/verrucous: verrucous carcinoma
  - Nodular/plaque: superficial carcinoma
  - Fungating/strictured/ulcerated/infiltrative edge: usual carcinoma
  - Multifocal
  - Regression and scarring
- Mucosa
  - Barrett's mucosa (velvety appearance)
- Wall
  - Tumor confined to mucous membrane, in the wall or through the wall
- Other
  - Achalasia, diverticulum, mucosal web, perforation.

Blocks for histology (Fig. 2.4):

- Sample the proximal and distal limits of surgical resection – complete circumferential transverse section (esophagus) or multiple circumferential blocks (proximal stomach).
- Alternatively, if separate anastomotic doughnuts are submitted, take one complete circumferential transverse section of each.
- Serially section the bulk of the tumor transversely at 3–4 mm intervals.
- Lay the slices out in sequence and photograph.
- Sample a minimum of four blocks of tumor and wall to show the deepest point of circumferential invasion.
- Sample two longitudinal blocks of tumor and adjacent mucosa, proximal and distal to the gross lesion, respectively.
- Sample one block of esophagus proximal to the tumor and one block of esophagus (or proximal stomach) distal to the tumor.
- Sample any abnormal background mucosa, e.g., multiple sequential blocks may be required to map the extent of Barrett's metaplasia.
- If tumor is not seen grossly, sequentially sample and correspondingly label unremarkable and abnormal areas of mucosa.
- Count and sample all lymph nodes.
- Sample the midpoint and proximal surgical limit (as marked by the surgeon) of any separate proximal segment of normal esophagus excised to facilitate pull-through of the EG anastomosis to the neck.

Histopathology report:

- Tumor type – adenocarcinoma/squamous carcinoma/other
- Tumor differentiation

	Adenocarcinoma	Squamous carcinoma
Well	>95% glands	Keratinization/intercellular bridges
Moderate	50–95% glands	
Poor	<50% glands	No keratinization/intercellular bridges

- Tumor edge – pushing/infiltrative/lymphoid response
- Extent of local tumor spread

pTis	Carcinoma in situ/high-grade dysplasia
pT1	Tumor invades lamina propria or submucosa
pT2	Tumor invades muscularis propria
pT3	Tumor invades adventitia
pT4	Tumor invades adjacent structures (4a: pleura/pericardium/diaphragm. 4b: aorta/vertebral body /trachea)

Note also any invasion of the proximal gastric serosa.

Siewert 1–3 tumors are staged as esophageal under TNM 7.

- Lymphovascular invasion – present/not present. Note perineural invasion.
- Regional lymph nodes
- Periesophageal, including celiac axis nodes and paraesophageal nodes in the neck, but not

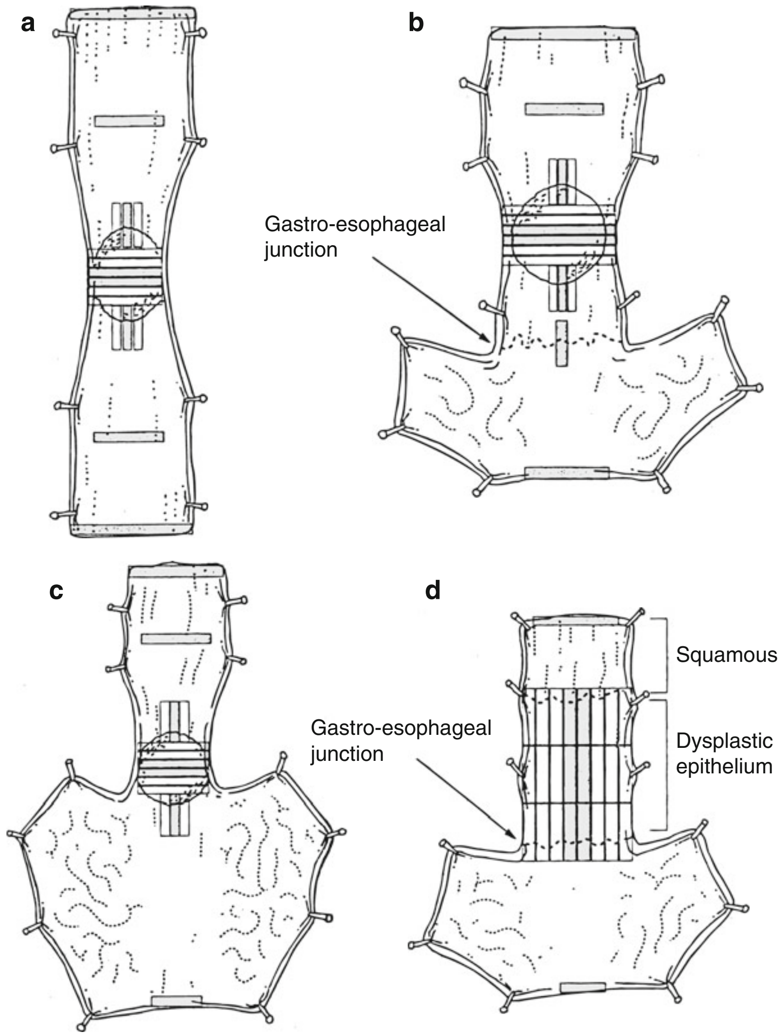

Fig. 2.4 Recommended blocks for histology in resected esophageal neoplasms (**a**) esophagectomy specimen (**b**) esophago-gastrectomy specimen containing tumor above the gastro-esophageal junction (**c**) esophago-gastrectomy specimen containing tumor at the gastro-esophageal junction (**d**) resected specimen for high-grade dysplasia/in situ carcinoma (*Shaded blocks* represent the recommended minimum number to be sampled) (Reproduced, with permission, from Ibrahim (2000))

supraclavicular nodes. A regional lymphadenectomy will ordinarily include six or more lymph nodes.

pN0	No regional lymph node metastasis
pN1	Metastasis in 1–2 regional lymph node(s)
pN2	Metastasis in 3–6 regional lymph nodes
pN3	Metastasis in 7 or more regional lymph nodes

- Excision margins
  Proximal and distal limits of tumor clearance (cm)
  Separate proximal esophageal and distal gastric anastomotic doughnuts – involved/not involved
  Deep CRM of clearance (mm)
- Other pathology:
- Squamous dysplasia, Barrett's metaplasia/dysplasia, radio-/chemotherapy necrosis and tumor regression grade (Mandard score), perforation, achalasia, esophageal web, diverticulum

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3 Stomach

Derek C. Allen, R. Iain Cameron,
and Maurice B. Loughrey

3.1 Anatomy

The stomach is a dilated portion of the gastrointestinal tract which has three main functions: storage of food, mixing food with gastric secretions, and control of the rate of release of food to the small intestine for further digestion and absorption. It is a J-shaped organ and much of it lies under the cover of the lower ribs. It has an anterior and posterior surface, two openings (the proximal cardiac and the distal pyloric orifices), and two curvatures (greater and lesser) (Fig. 3.1). Although relatively fixed at both ends, the intervening part is mobile and can undergo considerable variation in shape. The stomach is usually divided into the following parts:

Fundus – dome-shaped and projects upward and to the left of the cardiac orifice.

Body – extends from the level of the cardiac orifice to the incisura angularis (a constant notch at the junction of the lesser curve and antrum). The incisura is an important endoscopic landmark.

Antrum – extends from the incisura to the proximal part of the pylorus.

Pylorus – the most tubular part of the stomach and its thick muscular wall forms the physiological and anatomical pyloric sphincter, marked by a slight constriction on the surface of the stomach. The pylorus, which is approximately 2.5 cm long, joins the first part of the duodenum.

The cardiac orifice is where the abdominal part of the esophagus enters the stomach. Although no anatomical sphincter is present, a physiological mechanism exists, which prevents gastroesophageal regurgitation.

The lesser curvature forms the right border of the stomach, extending from the cardiac orifice to the pylorus. The greater curvature extends from the left of the cardiac orifice, over the fundus to the inferior part of the pylorus. Peritoneum completely surrounds the stomach and leaves its curvatures as double layers called omenta, which contain fat, lymph nodes, and vessels. The lesser omentum extends from the lesser curve to the liver. The gastrosplenic omentum extends from the upper part of the greater curve to the spleen, while the greater omentum runs to the transverse colon from the lower part.

The mucous membrane of the gastric body is thrown into numerous longitudinal folds or rugae. This facilitates flattening of the mucosa when the

D.C. Allen (✉)
Histopathology Laboratory, Belfast City Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: derek.allen@belfasttrust.hscni.net

R.I. Cameron
Histopathology Laboratory, Altnagelvin Hospital,
Western Health and Social Care Trust,
Londonderry, UK
e-mail: iain.cameron@westerntrust.hscni.net

M.B. Loughrey
Histopathology Laboratory, Institute of Pathology, Royal
Victoria Hospital, Belfast Health and Social Care Trust,
Belfast, UK
e-mail: maurice.loughrey@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_3, © Springer-Verlag London 2013

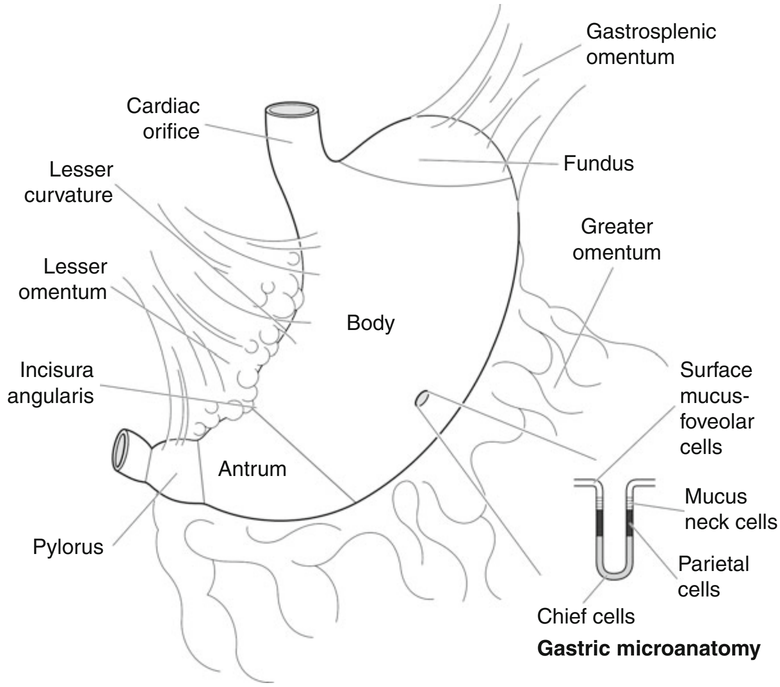

Fig. 3.1 Stomach (Reproduced, with permission, from Allen and Cameron (2004))

stomach is distended by food. The mucosal surface contains millions of gastric pits or foveolae that lead to mucosal glands. The mucosal surface is composed of columnar, mucin-secreting epithelium (surface mucus – foveolar cells), while deeper in the gastric pits are mucus neck cells. The gastric glands vary depending on their anatomic region (Fig. 3.1):

Cardia – mucin-secreting cells

Fundus/body – parietal cells (acid), chief cells (pepsin), and scattered endocrine cells

Antrum/pylorus – endocrine (mostly gastrin G cells) and mucin-secreting cells

Lymphovascular drainage:

The entire arterial supply of the stomach is derived from the celiac artery which arises from the aorta. Veins drain into the portal system. The lymphatics drain to the celiac lymph nodes. The so-called N1 and N2 node groups (12 in total) are situated along the arterial supply (Fig. 3.2). N1 nodes are within 3 cm of the primary malignancy and N2 nodes more than 3 cm from the tumor.

The main nerve supply to the stomach is from the anterior and posterior vagal trunks, with the innervation of the pylorus being mainly derived from the anterior vagus.

3.2 Clinical Presentation

Patients with gastroduodenal disease may be asymptomatic or experience one or more of the following: upper abdominal (epigastric) pain; dyspepsia (“indigestion”); vomiting, which may be projectile if there is pyloric outflow obstruction; hematemesis (vomiting blood); melena (altered blood per rectum); or dysphagia, if there is a proximal gastric lesion.

3.3 Clinical Investigations

- Endoscopy and Biopsy
- Erect CXR to detect “air under the diaphragm” in a perforation and also metastatic tumor deposits in the lungs.
- Barium swallow will outline the mucosal surface, demonstrate decreased distensibility and wall motility due to diffuse carcinoma (linitis plastica) and detect delayed emptying caused by pyloric outflow obstruction.

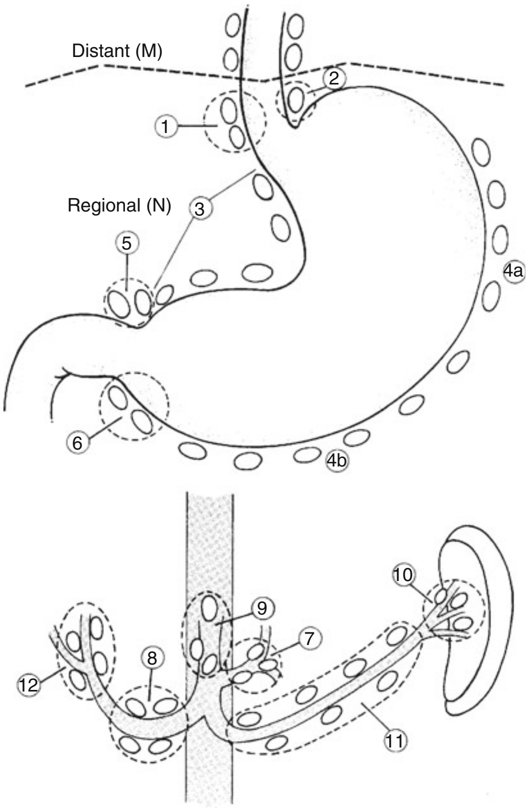

Fig. 3.2 Stomach: Regional lymph nodes. The regional lymph nodes are the perigastric nodes along the lesser (*1*, *3*, *5*) and greater (*2*, *4a*, *4b*, *6*) curvatures, the nodes located along the left gastric (*7*), common hepatic (*8*), splenic (*10*, *11*) and celiac arteries (*9*), and the hepatoduodenal nodes (*12*). Involvement of other intra-abdominal lymph nodes such as the retropancreatic, mesenteric, and para-aortic is classified as distant metastasis (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

- For biopsy-proven cancer – ELUS and CT scan chest, abdomen, and pelvis to determine the pretreatment tumor stage.
- Peritoneal aspiration of ascitic fluid for malignant cells and staging laparoscopy with peritoneal biopsy and cytology washings. Peritoneal disease is a contraindication to radical curative intent therapy.
- Gastric function tests – peak acid output is measured by the pentagastrin test and will differentiate "hypersecretors" from "non-hypersecretors" – important if surgery is to be considered for duodenal peptic ulcer disease.

3.4 Pathological Conditions

3.4.1 Non-neoplastic Conditions

Acute gastritis: Acute hemorrhagic/erosive gastritis is usually antral and drug related (aspirin, NSAIDs, alcohol) or, less commonly, in the body secondary to shock and hypoperfusion, e.g., post-trauma, sepsis or burns, and, therefore, not biopsied. Acute neutrophilic gastritis is seen in food poisoning, sepsis, and helicobacter pylorii (HP) infection.

Chronic gastritis: With poor correlation between symptoms, endoscopic appearances, and histology, it is very common in biopsy material and is *a*utoimmune, *b*acterial or *c*hemical in nature (types A, B, and C). The latter is usually antral, related to drug ingestion, or bile reflux and comprises a reactive mucosa with a lack of inflammatory cells. Autoimmune gastritis affects the corpus, resulting in a spectrum of atrophic gastritis and gastric atrophy with hypochlorhydria, pernicious anemia, and a predisposition to gastric cancer. It is associated with other autoimmune diseases, e.g., diabetes and mucosal damage is mediated by circulating antibodies to gastrin receptors on the parietal cells. The anemia is due to lack of gastric intrinsic factor with decreased vitamin B12 absorption in the terminal ileum. HP infection is the commonest form of chronic gastritis and increases in incidence with age. The Gram-negative, curved bacillus is readily identified (H and E, Cresyl Violet, Giemsa) lying under the surface mucous layer, damaging the epithelium and producing a chronic inflammatory reaction in the lamina propria with focal neutrophil polymorph cryptitis. Treatment is by antibiotic eradication.

The *Sydney System* classifies and grades chronic gastritis based on an assessment of histological (neutrophils, chronic inflammation, atrophy, intestinal metaplasia), topographical (antral/corpus predominant or pangastritis), and etiological (HP, drugs) factors.

Chronic gastritis predisposes to peptic ulceration, gastric carcinoma, and malignant lymphoma. Unusual variants such as lymphocytic, granulomatous, or eosinophilic gastritis are occasionally seen – infective gastritis occurs in immunosuppressed patients (e.g., CMV) or opportunistically overlying ulceration (e.g., candida fungus).

Peptic ulceration: there are two patient groups.

1. HP antral gastritis → loss of acid regulatory feedback → hyperchlorhydria → duodenitis → duodenal gastric metaplasia with HP colonization → further duodenitis and duodenal ulcer (DU)
2. HP pangastritis → hypoacidity → weakening of the mucosal mucous barrier → further gastritis → erosion and gastric ulceration (GU)

Further risk factors include smoking, alcohol, and drugs (NSAIDs, aspirin, steroids). DU outnumbers GU (4:1). Benign gastric ulcers are usually on the lesser curve in the vicinity of the incisura.

Complications include acute or chronic bleeding from the ulcer base, perforation with peritonitis, penetration and fistula to an adjacent organ (e.g., colon or pancreas), fibrotic repair resulting in mechanical obstruction such as pyloric stenosis, and, rarely, cancer. Surgery for peptic ulceration has decreased dramatically in the last two decades with the evolution of effective antiulcer treatments based largely on antibiotic eradication of HP infection and acid suppression (H_2 receptor antagonists, proton pump inhibitors (PPIs)). It is now reserved for those peptic ulcers refractory to medical treatment, in which complications have arisen or there is a suspicion of malignancy. Acute hemorrhage is managed conservatively by laser, electrocoagulation, or injection of sclerosant.

Hyperplastic polyps: Commonest in the antrum and up to 1.5 cm in size, they form 60% of gastric mucosal polyps and are characterized by dilated, hyperplastic glands in edematous, inflamed lamina propria. Single or multiple they probably represent healing of the mucosa after erosion – malignant change is extremely rare, although there can be cancer elsewhere in the stomach.

Other non-neoplastic polyps: rare, e.g., hamartomatous polyps (Peutz Jegher's/Cronkhite – Canada syndromes), inflammatory fibroid polyp or common, such as fundic gland cyst polyps – small, multiple, gastric body, cystic dilatation of specialized glands, incidental or associated with PPI therapy/familial adenomatous polyposis (FAP).

Note that various diseases can present as polypoidal gastric folds or hypertrophic gastropathy, e.g., Ménétrier's disease (hypochlordydria, protein loss from elongated gastric pits), Zollinger–Ellison syndrome (pancreatic/duodenal gastrinomas, hyperchlorhydria, multiple peptic ulcers), Crohn's disease, carcinoma, or malignant lymphoma.

3.4.2 Neoplastic Conditions

Predisposing conditions: Predisposition to gastric neoplasia occurs with HP gastritis, gastric atrophy, and previous partial gastrectomy with gastroenterostomy. Antecedent lesions include incomplete intestinal metaplasia (type IIb/III large intestinal variant) and epithelial dysplasia. Dysplasia occurs in flat (commonest), sessile, or polypoid mucosa and is categorized as low or high grade, corresponding to categories 3 and 4 of the Vienna Consensus Classification of Gastrointestinal Epithelial Neoplasia (Table 3.1). Low-grade dysplasia requires endoscopic follow-up, while high-grade dysplasia should be considered for surgical resection due to the strong association (30–80%) with concurrent or subsequent cancer. Polypoid adenomatous dysplasia comprises 8% of gastric polyps but has a 30–40% risk of malignancy related to size, villous architecture, and grade of dysplasia. Local resection (endoscopic or surgical) and careful background mucosal sampling are necessary for full histological assessment.

Adenocarcinoma: forms the majority of gastric malignancy and classically antral (50%) or

Table 3.1 Vienna classification of gastrointestinal epithelial neoplasia

Category	Neoplasia/dysplasia
1	Negative
2	Indefinite
3	Noninvasive low grade
4	Noninvasive high grade
5	Invasive – either intramucosal, submucosal, or beyond

lesser curve (15%) in site but with an increasing incidence in the proximal stomach and cardia, in part due to HP eradication and loss of its acid suppression effect. Histological patterns are intestinal (50%), diffuse (20%), or mixed/solid (25%), showing correlation with macroscopic appearances and behavior. Intestinal carcinomas arise from intestinal metaplasia/dysplasia, form ulcerated or polypoid lesions with expansile margins, and show lymphovascular spread to regional nodes, liver, lung, adrenal gland, and bone. Diffuse carcinomas (signet ring cells) form diffusely infiltrating linitis plastica (leather bottle stomach) undermining the mucosa with transmural spread to the peritoneum where seedlings and classical Krukenberg tumors (bilateral ovarian secondaries) occur. Gastric cancer may be multifocal – resection margins are routinely checked. Distal cancers can involve proximal duodenum, and proximal cancers, the distal esophagus. Tubule-rich, mucin-poor tumors with a circumscribed edge have a better prognosis than tubule-poor, mucin-rich tumors or an infiltrative edge. Depth of spread is defined as early gastric cancer (EGC) confined to the mucous membrane ± regional node involvement, or advanced muscle coat invasive disease which has a much worse prognosis. EGC (10% of cases) can be multifocal in distribution and raised, flat, or ulcerated in morphology.

Other carcinomas are rare (e.g., hepatoid, parietal cell, medullary) or metastatic in nature (e.g., breast, lung, kidney, malignant melanoma).

Carcinoid (*well-differentiated endocrine*) *tumor*: of gastric endocrine or enterochromaffin-like (ECL) cell origin, either related to gastric atrophy (type 1), ZE syndrome (type 2), or sporadic (type 3).

- Multiple (benign): atrophic gastritis/gastric atrophy → hypochlorhydria → hypergastrinemia → ECL hyperplasia → microcarcinoidosis (multiple, mucosal, <1.5 mm). If <1 cm endoscopic removal is sufficient: if 1–2 cm in size, treatment is by polypectomy or local resection as they have uncertain malignant potential.
- Single or sporadic (aggressive): surgical resection if >2 cm in size, invasion beyond submucosa, angioinvasion, or cellular atypia (including necrosis or mitoses). Functionally secreting tumors are also potentially malignant. Detection of metastatic disease is by CT and octreotide scintigraphy scan.

Gastrointestinal stromal tumors (GISTs): spindle or epithelioid cell in type a minority are leiomyomatous or neural and a majority stromal (CD117 (c-kit)/DOG-1 positive) in character with absent or incomplete myogenic/neural features. Malignancy cannot be accurately predicted but indicators are: size (>5 cm), cellularity and atypia, tumor necrosis and hemorrhage, infiltrative margins, and mitotic activity (>5/50 high power fields). Malignant spread is to peritoneum and liver. Endoscopic biopsy diagnosis can be problematic as GISTs are submucosal/mural lesions covered by intact mucosa except for a classical central area of "apple core" ulceration.

Malignant lymphoma: primary with disease bulk in the stomach and regional nodes, or secondary to systemic nodal disease. Single, multiple, plaque-like, ulcerated, or as thickened folds it has a rubbery, fleshy appearance. The majority are of B cell MALT (mucosa associated lymphoid tissue) type and strongly associated with HP chronic gastritis. Low or high grade, the former can be difficult to diagnose requiring an accumulation of histological, immunohistochemical, and molecular evidence over a number of biopsy episodes. Cardinal features are the density and uniformity of the lymphoid infiltrate, loss and destruction of mucosal glands, demonstration of immunoglobulin light chain restriction, and heavy chain gene rearrangements. High-grade lymphoma transforms from a low-grade lesion or presents de novo and must be distinguished immunohistochemically from poorly differentiated carcinoma. Rarely there can be an association between MALToma and concurrent or subsequent adenocarcinoma.

Prognosis: The majority of patients with gastric cancer present with advanced disease, and prognosis is poor (20–35% 5-year survival) relating to histological type, differentiation, excision margin involvement, and, crucially, stage of disease. Following a positive endoscopic biopsy, the tumor is staged radiologically and laparoscopically to determine suitability for radical surgery. Current trials indicate a beneficial role for preoperative and postoperative chemotherapy, which traditionally had been limited to palliative treatment of advanced disease. Patients with Her 2 positive recurrent or metastatic disease (20% of cases) potentially respond to trastuzumab monoclonal antibody therapy. EGC has a better prognosis (80–95% 5-year survival) and may be amenable to local mucosal resection but is converted to completion gastrectomy if the cancer shows unfavorable features such as size >3 cm, >50% surface ulceration, poor differentiation, lymphovascular invasion, or involvement of the submucosa or specimen base.

Carcinoid tumors are of low-to-intermediate-grade malignancy – 70–80% 5-year survival. Low-grade MALTomas are indolent (65–95% 5-year survival), whereas high-grade lesions are more aggressive (40–55% 5-year survival). Treatment options for gastric lymphoma after appropriate typing, grading, and staging (CT scan, bone marrow trephine) include HP eradication (low-grade disease), chemotherapy (high-grade disease), and surgery, the latter particularly if there are anatomical alterations, e.g., gastric outlet obstruction, or complications of chemotherapy, e.g., perforation.

The primary treatment of GISTs is surgical resection, with targeted therapy in the form of small molecule inhibitors such as imatinib (Glivec) reserved for tumors which are unresectable or metastatic. Neoadjuvant therapy can produce tumor regression and shrinkage, facilitating success and choice of operative technique.

3.5 Surgical Pathology Specimens: Clinical Aspects

3.5.1 Biopsy Specimens

Flexible endoscopy is the cornerstone for investigation and diagnosis of gastric-related symptoms. Biopsies for gastritis should be taken according to the Sydney protocol from antrum, body, and incisura and any abnormal areas. Specific lesions such as ulcers need multiple (at least six) biopsies from the base and margin quadrants as some 10% of endoscopically suspicious lesions need rebiopsy. A peptic ulcer has a classic endoscopic appearance in that it is round/oval and sharply "punched out" with straight walls. Heaping up of mucosal margins is rare in benign ulcers and should raise the suspicion of malignancy. Size does not reliably differentiate between benign and malignant ulcers as 10% of benign ulcers are greater than 4 cm in diameter. Tumors covered by intact mucosa such as diffuse gastric carcinoma or GISTs are often difficult to demonstrate by mucosal biopsy, and endoscopic FNA may be employed. Localized nodular or polypoid lesions, e.g., hyperplastic polyp, adenomatous polyp, carcinoid tumor, EGC can be diagnosed and successfully removed by EMR.

3.5.2 Resection Specimens

3.5.2.1 Benign Conditions

As alluded to above, surgery for chronic peptic ulceration is now unusual. It aims to remove the gastric ulcer and the gastrin-producing G cells that drive acid secretion. This is accomplished by a Bilroth I distal gastrectomy with a gastroduodenal anastomosis (Fig. 3.3). Alternatively blockage of gastric innervation is achieved by transecting the vagus nerve trunks as they emerge through the diaphragmatic hiatus (truncal vagotomy), resulting in reduced gastric secretions and motility. Because of the latter, a drainage procedure, either pyloroplasty or gastrojejunostomy, must also be done. This approach is used in elderly frail patients or for refractory DU. Highly selective vagotomy preserves pyloric innervation, negating the need for a drainage procedure. The now rare Bilroth II gastrectomy for DU comprises a distal gastrectomy with oversewing of the duodenal stump and fashioning of a gastrojejunal anastomosis of either Polya or Roux-en-Y type. The latter prevents bile reflux as the distal duodenum is joined to the jejunum some 50 cm distal to the gastrojejunal anastomosis.

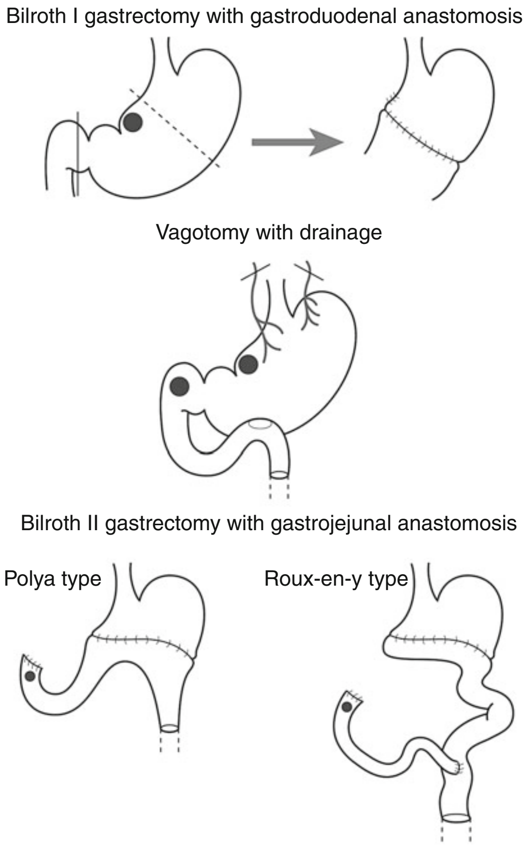

Fig. 3.3 Gastric surgery for gastroduodenal peptic ulceration (Reproduced, with permission, from Allen and Cameron (2004))

3.5.2.2 Malignant Conditions

Curative gastric surgery should involve removal of the tumor with a 5 cm rim of "normal" tissue and the related lymph nodes. The surgeon may prefer to perform a partial or total gastrectomy depending on the site and type of tumor (e.g., diffuse carcinoma) and medical fitness of the patient.

Total gastrectomy: This can be done with or without radical lymph node dissection. Both procedures employ an upper midline abdominal incision. In a total gastrectomy without radical lymph node dissection (D1 resection), the stomach is removed with the lesser and greater omenta (which contain local lymph nodes). In this resection, nodes may also be found along the greater curvature and the gastrosplenic omentum. A radical gastrectomy (D2 resection) involves removal of the stomach, lesser omentum with careful dissection of nodes along the hepatic artery and celiac plexus, greater omentum and gastrosplenic omentum. Nodes should also be removed from along the portal vein, splenic artery, and the retropancreatic area. In Japan, an even more radical procedure is popular, which involves en bloc resection of the stomach, spleen, distal pancreas, and associated lymph node groups.

Good margin clearance is crucial and so the esophagus is divided as far proximally as is needed and occasionally this may involve entering the chest. The distal margin of resection is formed by division of the first part of the duodenum. Continuity is restored by an esophagojejunostomy with a Roux-en-Y diverting limb for the duodenal stump.

Partial gastrectomy: The type of procedure employed will depend on the site of the tumor:

Proximal tumors – tumors in the vicinity of the EG junction may arise in the distal esophagus and infiltrate distally, or in the cardia/fundus and infiltrate proximally. Various procedures may be employed (see Chap. 2).

- Transhiatal distal esophagectomy with proximal gastrectomy for tumors of the distal esophagus/EG junction/cardia
- Transhiatal distal esophagectomy with total gastrectomy for tumors of the cardia with extensive distal spread
- A more extensive esophagectomy (via either a two-field approach or thoracotomy) with proximal/total gastrectomy for junctional tumors with extensive proximal spread.

Distal tumors – either a Bilroth I or Bilroth II procedure with the latter being favored as the anastomosis is wider (important if there is local recurrence) and further away from the likely site of recurrence.

3.6 Surgical Pathology Specimens – Laboratory Protocols

3.6.1 Biopsy and Local Mucosal Resection Specimens

See Chap. 1.

3.6.2 Resection Specimens

Specimen:

- The majority of gastric resections are for neoplastic conditions. However, because of the difficulty in reliably distinguishing between benign and malignant gastric ulcers on gross inspection, it is practical to use the same handling procedures. Irregular elevated mucosal margins and absence of radial mucosal folds are possible pointers to malignancy. Benign ulcers usually do not occur on the greater curvature.
- Partial gastrectomy (proximal or distal), total/radical gastrectomy, variable amounts of lesser and greater omental fat including unspecified or separately named regional lymph node groups, with or without spleen removed because of either direct involvement by gastric cancer or for technical reasons, e.g., operative access or capsular tear at surgery.

Initial procedure:

- By palpation and with the index finger locate the lumenal position of the tumor/ulcer.
- Open the specimen along the curvature opposite to and avoiding the tumor/ulcer.
- Measurements:
  Distal esophagus, greater curvature, duodenal cuff – lengths (cm)
  Tumor/ulcer
  – Length × width × depth (cm) or maximum dimension (cm)
  – Distances (cm) to the proximal and distal limits of excision
  – Relationship to the EG junction. TNM 7 includes as an esophageal cancer any tumor of the proximal stomach where its epicenter is within 5 cm of the junction and involves the esophagus (Siewert 3). External landmarks may be helpful – esophagus is orientated to adventitia, stomach to serosa.
- Photograph.
- Paint any relevant area of serosa and omental margin suspicious of tumor involvement or close to its edge.
- Fixation by immersion in 10% formalin for 48 h either gently packed with formalin-soaked lint or, if suitable, pinned out on a corkboard in the opened position.

Description:

- Tumor/ulcer site
  – Distal esophagus/cardia/fundus/corpus/antrum/pylorus/lesser curve/greater curve/anterior/posterior/multifocal/extension to duodenum or esophagus
- Tumor
  – Polypoid/ulcerated/scirrhous/mucoid/irregular margins: usual carcinoma
  – Thickened, non-expansile wall/intact granular mucosa: diffuse gastric carcinoma
  – Plaque/granular mucosa/depressed/multifocal: EGC
  – Plaque/thickened folds/ulcerated/fleshy/multifocal: malignant lymphoma
  – Nodular/ulcerated/yellow: carcinoid tumor
  – Polypoid/mural/dumb-bell shaped/apple core ulceration:GIST
- Ulcer
  – Mucosal edges: flat/punched out/elevated
  – Base: blood vessels/perforation/penetration (e.g., pancreas or fistula present)
- Mucosa
  – Edematous/atrophic/granular/thickened
- Wall
  – Tumor: confined to mucous membrane, in the wall or through the wall
  – Ulcer: perforation/penetration
- Serosa
  – Involved by tumor/coated in exudate
- Omenta
  – Involved by tumor: circumscribed/irregular margin
  – Maximum deposit size (cm)
  – Distance of tumor from the omental edge (mm)

Blocks for histology (Fig. 3.4):

- Sample the proximal and distal limits of resection – complete circumferential transverse sections (duodenum, esophagus) or multiple circumferential blocks (mid-stomach).
- Alternatively, if separate anastomotic doughnuts are submitted – one complete circumferential transverse section of each.
- Count and sample all lymph nodes (lesser/greater omenta, splenic hilum) and process separately any named lymph nodes.

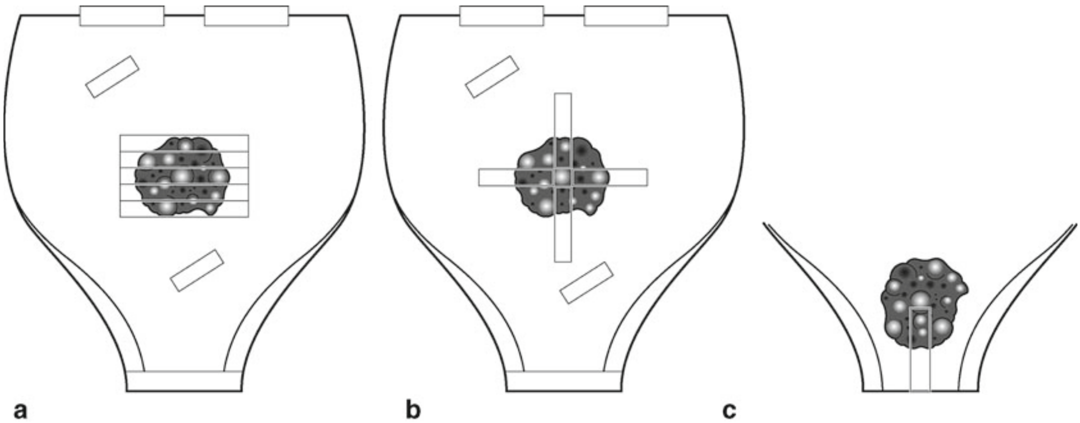

Fig. 3.4 Distal gastrectomy – serial, transverse slices (**a**) or quadrant sections (**b**) may be used according to the anatomy of the lesion and adjacent structures. A longitudinal limit block (**c**) may be taken if the tumor is close (<0.5 cm to it) (Reproduced, with permission, from Allen and Cameron (2004))

- Sample a minimum of four blocks of tumor and wall to show any serosal involvement and the deepest point of omental invasion. Serial transverse slices (3–4 mm thick) or quadrant sections may be used according to the anatomy of the lesion and adjacent structures.
  Tumor ulcer: four sections to include the ulcer base, edge, adjacent mucosa, and wall
  Tumor polyp: two sections from the body of the polyp and a minimum of two from the underlying base and wall
  Linitis plastica: six transmural blocks as a gross lesion is often not evident and the extent of local spread may vary
  GISTs: roughly one block per centimeter diameter to include mucosal, mural, and extramural components of the tumor
  Omental tumor: representative blocks in relation to the nearest omental edge/serosa
- Sample any other satellite lesions or abnormal areas of mucosa.
- Sample non-neoplastic gastric mucosa away from the tumor/ulcer (two blocks).
- Serially slice, at 1 cm intervals, spleen and pancreas (if present) and sample two blocks

Histopathology report:

- Tumor type
  – Adenocarcinoma: intestinal/diffuse/mixed/mucin-rich/mucin-poor
  – Malignant lymphoma
  – GIST
- Tumor differentiation
  – Adenocarcinoma
  Well/moderate/poor defined as tubule-rich or tubule-poor
  – Malignant lymphoma
  MALToma/mantle cell/follicle center cell or other
  Low-grade/high-grade
  – GIST
  Spindle cell/epithelioid
  Cellularity/atypia/mitoses/necrosis/margins/size
  Leiomyomatous/neural/stromal (CD117/DOG-1)
- Tumor edge – Pushing/infiltrative/lymphoid response
- Extent of local tumor spread (for carcinoma).

pTis	Carcinoma in-situ: intraepithelial tumor without invasion of the lamina propria
pT1	Tumor invades lamina propria (pT1a) or submucosa (pT1b)
pT2	Tumor invades muscularis propria
pT3	Tumor invades subserosa or lesser/greater omenta
pT4	Tumor perforates serosa (pT4a) or invades adjacent structures (spleen, transverse colon, liver, diaphragm, pancreas, abdominal wall, adrenal gland, kidney, small intestine, retroperitoneum (pT4b))

EGC = pT1 ± lymph node involvement
Advanced carcinoma = pT2/pT3/pT4 ± lymph node involvement

- Lymphovascular invasion – present/not present
- Regional lymph nodes
  Perigastric, hepatoduodenal, nodes along the left gastric, common hepatic, splenic and celiac arteries. A regional lymphadenectomy will ordinarily include 16 or more lymph nodes. Other intra-abdominal lymph nodes (retropancreatic, mesenteric, para-aortic) are distant metastases (pM1).

pN0	No regional lymph node metastasis
pN1	1–2 involved regional node(s)
pN2	3–6 involved regional nodes
pN3	More than 6 involved regional nodes (pN3a: 7–15. pN3b: >15)

- Excision margins
  Proximal and distal limits of tumor clearance (cm)
  Separate proximal esophageal/gastric and distal gastric/duodenal anastomotic doughnuts – involved/not involved/presence of mucosal dysplasia
  Deep circumferential omental margin of clearance (mm)
  Deep margin of clearance (mm) in polypectomy and endoscopic mucosal resection specimens
- Other pathology
  Satellite foci, polyps, intestinal metaplasia, dysplasia, gastric atrophy, helicobacter gastritis, MALToma, hypertrophic gastropathy (e.g., Menetrier's disease, ZE Syndrome), ECL cell hyperplasia/microcarcinoidosis, response to neoadjuvant chemotherapy

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4 Pancreas, Duodenum, Ampulla of Vater and Extrahepatic Bile Ducts

Derek C. Allen, R. Iain Cameron, and Maurice B. Loughrey

4.1 Anatomy

4.1.1 Duodenum

The small intestine is divided into three parts: duodenum, jejunum, and ileum. The duodenum is C-shaped and joins the gastric pylorus to the proximal jejunum by curving around the head of the pancreas. It is 25 cm long and receives the openings of the common bile and pancreatic ducts. The proximal 2.5 cm is covered on its anterior and posterior surfaces by peritoneum, the remainder being retroperitoneal. The duodenum, for purposes of description, is divided into four parts (D1–4). At the duodenojejunal junction, the intestine turns forward – this being called the duodenojejunal flexure.

D.C. Allen (✉)
Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: derek.allen@belfasttrust.hscni.net

R.I. Cameron
Histopathology Laboratory, Altnagelvin Hospital, Western Health and Social Care Trust, Londonderry, UK
e-mail: iain.cameron@westerntrust.hscni.net

M.B. Loughrey
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: maurice.loughrey@belfasttrust.hscni.net

The mucosa of the duodenum is thick and thrown into numerous circular folds called *plicae circulares*. The common bile duct and the major pancreatic duct pierce the medial wall of D2, approximately halfway along its length. At this point, there is a small elevation called the major duodenal papilla (see below).

Lymphovascular drainage:

The arterial supply originates from the celiac artery and the superior mesenteric artery (SMA). Venous drainage is to the portal system. The lymphatics follow the course of the arteries, i.e., those from the proximal half drain to the celiac nodes and those from the distal duodenum drain to the superior mesenteric nodes via the periduodenal nodes.

4.1.2 Pancreas, Ampulla of Vater, and Extrahepatic Bile Ducts

The pancreas is a soft lobulated retroperitoneal organ which is both an endocrine and exocrine gland. The exocrine portion produces enzymes (lipases, proteases) which are conveyed to the duodenum by the pancreatic duct and are concerned with digestion. The endocrine portion (including the islets of Langerhans) produces hormones such as insulin and glucagon. The pancreas is subdivided as follows (Fig. 4.1):

Head – that part to the right of the left border of the superior mesenteric vein (SMV). It lies within the concavity of the duodenum. The *uncinate process*, a part of the head, extends to

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_4, © Springer-Verlag London 2013

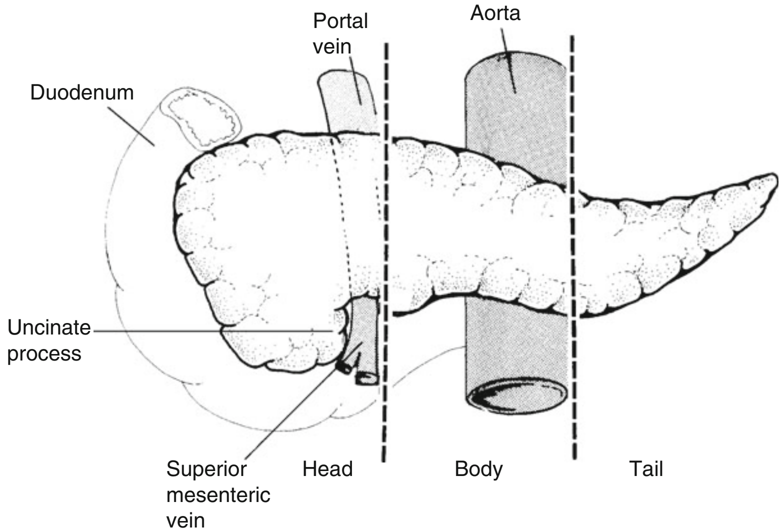


Fig. 4.1 Pancreas (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

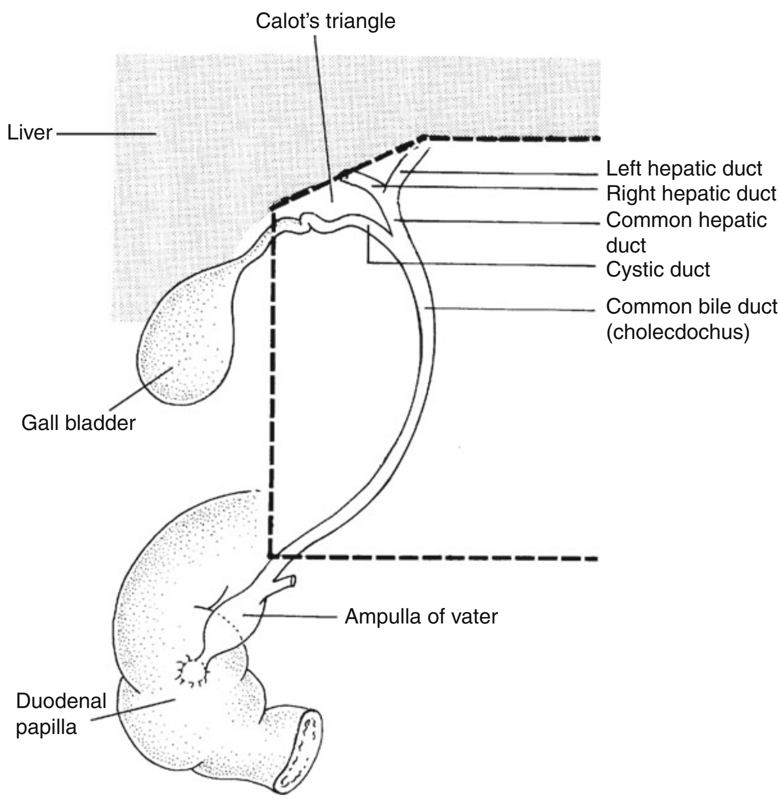


Fig. 4.2 Ampulla of Vater and extrahepatic bile ducts (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

the left posterior to the superior mesenteric vessels.

Body – lies between the left border of the SMV and the left border of the aorta.

Tail – lies to the left of the aorta and comes into contact with the hilum of the spleen. Anteriorly the pancreas has a thin covering capsule.

The extrahepatic bile ducts consist of the right and left hepatic ducts, common hepatic duct, and common bile duct (Fig. 4.2). The hepatic ducts emerge from the porta hepatis of the liver and

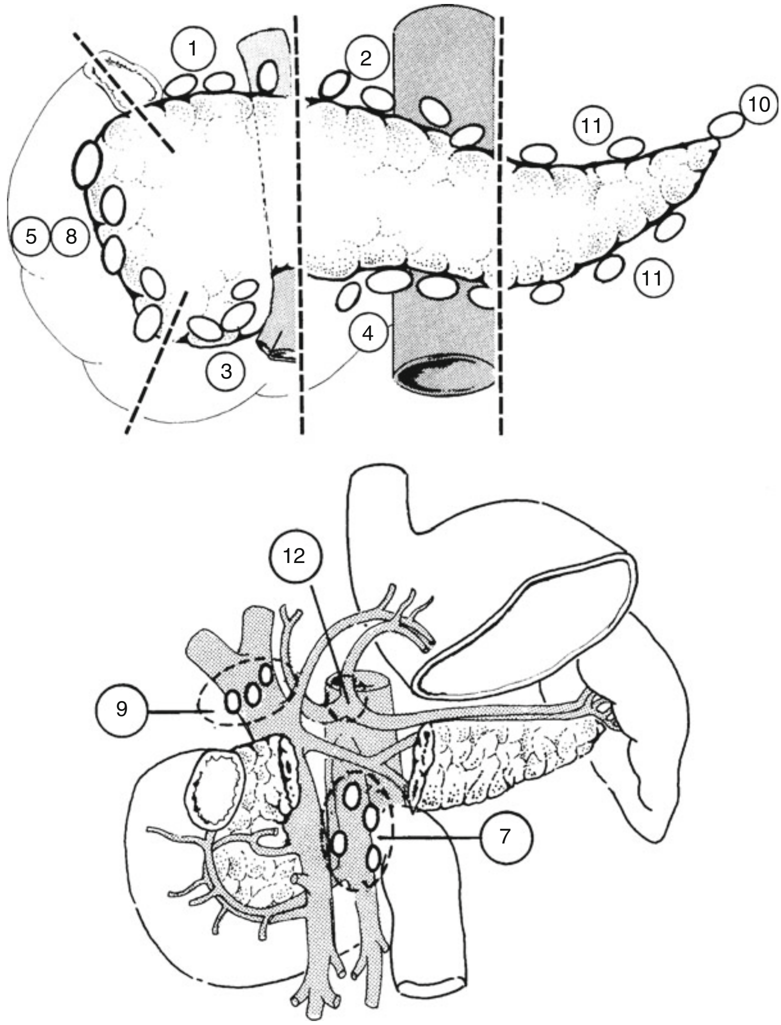


Fig. 4.3 Pancreas and ampulla of Vater: regional lymph nodes are peripancreatic (*1–4*, *11*), pancreaticoduodenal (*5–8*), splenic hilar (*10*), proximal mesenteric (*7*), common bile duct (*9*), and celiac (*12*) (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

converge to form the common hepatic duct. This descends for 4 cm until it is joined from the right side by the cystic duct when it becomes the common bile duct. This is 8 cm long and descends in the free edge of the lesser omentum, while in the distal part of its course it lies in a groove on the posterior surface of the pancreatic head. The main pancreatic duct runs the length of the gland and just before the ducts enter the duodenum they converge. Together they open into the ampulla of Vater, a small flask-shaped dilated channel situated in the duodenal wall. The ampulla then opens into the duodenal lumen by the major duodenal papilla (Fig. 4.2). The distal part of both ducts and the ampulla are surrounded by muscle fibers, this being termed the sphincter of Oddi.

The extrahepatic bile duct system may be subject to a number of variations in its anatomy between individuals.

Lymphovascular drainage:

The arterial supply of the pancreas is from the same vessels that supply the duodenum, and venous drainage is to the portal system. The lymphatics follow the arteries to the peripancreatic, pancreaticoduodenal, and pyloric nodes, and ultimately to the celiac and superior mesenteric nodes (Fig. 4.3).

The arterial supply to the bile ducts is complex, originating from both the celiac and SMAs. The lymphatics flow to the infrahepatic, peripancreatic, periduodenal, celiac, and superior mesenteric nodes.

4.2 Clinical Presentation

The symptomatology of duodenal peptic ulceration has been discussed in Chap. 3 . Duodenal neoplasms, although rare, may lead to epigastric pain, gastric outlet obstruction, and obstructive jaundice if present in the region of the ampulla.

Classically, acute pancreatitis presents with severe epigastric pain which radiates to the back. Chronic pancreatitis produces less acute, but often intractable, epigastric pain. Complications of acute pancreatitis such as shock, infection of necrotic tissue, bowel ileus, metabolic disturbance, and multiorgan failure produce characteristic clinical features.

Bacterial infection in the bile ducts is usually due to secondary infection of obstructed ducts and leads to cholangitis with pain, fever, rigors, and jaundice. If severe the cholangitis may become "ascending" and cause liver abscesses.

Neoplasms of the head of pancreas (excluding the uncinate process), ampulla of Vater, and extrahepatic bile ducts lead to obstructive jaundice. Tumors elsewhere in the pancreas do not and so will present later. Obstructive jaundice, because of a lack of absorption of fat and increased excretion of bilirubin in the urine, leads to light-colored feces and dark urine. In general, pancreatic and bile duct neoplasms result in vague, poorly localized epigastric pain, anorexia, and weight loss.

Tumors of the endocrine pancreas may produce characteristic clinical features because of the hormones they produce:

- Insulinoma – psychiatric/neurological symptoms
- Gastrinoma – Zollinger–Ellison syndrome
- Glucagonoma – diabetes mellitus and skin rash
- VIPoma – watery diarrhea, hypokalemia, and achlorhydria (WDHA) syndrome

4.3 Clinical Investigations

The investigation of duodenal peptic ulcer disease was discussed in Chap. 3.

- Urea and electrolytes (U and E) – electrolyte imbalance may occur in acute pancreatitis and certain endocrine tumors.
- Serum amylase – elevated in acute pancreatitis.
- Clotting screen – may be deranged in obstructive jaundice because of lack of absorption of fat-soluble vitamins which are required in the synthesis of certain clotting factors.
- Liver function tests – obstructive jaundice picture (elevated alk phos and α GT – see Chap. 10).
- CA19–9 – serum marker of pancreatic malignancy.
- CXR – to detect pulmonary metastases.
- AXR – 10% of gallstones are radio-opaque. Air in the biliary tree may also be seen if there has been previous surgery or a biliary-intestinal fistula.
- USS – to diagnose acute pancreatitis and may detect gallstones in the bile ducts. Tumors less than 1 cm will not be detected. It will also confirm the presence of obstructive jaundice by demonstrating dilated intrahepatic ducts.
- CT (chest, abdomen, and pelvis) and MRI scan – will detect primary tumor and any metastatic spread.
- Intravenous cholangiogram – certain contrast agents are excreted by the liver and so will opacify the bile ducts – these can then be visualized radiologically.
- Percutaneous transhepatic cholangiogram (PTC) – another method of visualizing the bile ducts is by injecting contrast into the right lobe of the liver.
- Radionucleotide scanning – good for showing obstruction and inflammation in the bile ducts.
- EGD/ERCP – may be used for both diagnostic and therapeutic purposes (see below).
- Peritoneal aspiration – for malignant cells in ascitic fluid.
- Percutaneous FNAC or needle core biopsy – may provide a preoperative diagnosis.
- Staging laparoscopy with biopsy.
- Doppler studies of the portal vein and angiography may be used to ensure that the vessels are not involved by tumor.

- Serum gut hormone levels and octreotide isotope uptake scan – pancreatic and duodenal endocrine tumors.

4.4 Pathological Conditions

4.4.1 Non-neoplastic Conditions

Duodenum: Duodenitis and DU have been previously discussed – gastric metaplasia, or nodular gastric heterotopia in D1 and Brunner's gland hyperplasia are also encountered in biopsies of the proximal duodenum. Biopsy for celiac disease is considered under small intestine.

Ampulla of Vater: Inflammatory polyps of the duodenal papilla are small, pedunculated, and often ulcerated. Partly traumatic in origin due to passage of calculi from the biliary tree. Distinction from neoplasia at EGD/ERCP can be difficult and biopsy is required.

Pancreas: The distal pancreatic duct forms a common channel with the terminal common bile duct in 50–60% of patients resulting in a strong association between pancreatitis and biliary tract disease.

Acute pancreatitis: With an overall mortality of 10–15%, it is rarely biopsied or resected. The commonest causes are gallstones, sphincter spasm, or incompetence with reflux of duodenal fluid and bile, alcohol, trauma, and hypothermia. It is due to release of pancreatic enzymes comprising pancreatic hemorrhage, necrosis, and inflammation with saponification and chalky calcification of abdominal fat. It is usually a self-limiting process, but critical complications include sepsis, shock, bowel paralysis, or perforation. Treatment is resuscitative and supportive – operative intervention can include removal of obstructing gallstones (by ERCP) or infected necrotic tissue (necrosectomy).

Chronic pancreatitis: Commonly due to excess alcohol intake, or less commonly autoimmune in etiology as a manifestation of IgG4-related systemic sclerosing disease, there is correlation between radiological calcification, pancreatic endocrine and exocrine dysfunction, and the severity of histological changes. Complications include abscess, systemic fat necrosis and pancreatic pseudocyst. Caused by disruption of the duct system due to obstruction by calculus or tumor, a pseudocyst has a thick fibrous wall lined by granulation tissue but no epithelium. It can rupture into the peritoneal cavity or splenic artery. Treatment is by endoscopic or transabdominal drainage either internally to stomach or duodenum or externally to skin. Surgical excision is used if small and localized to the body or tail, or if the pseudocyst is thick-walled and not appropriately sited for drainage.

The commonest biopsy expression of chronic pancreatitis is that seen adjacent to a pancreatic tumor or secondary to an ampullary tumor due to duct obstruction. The acinar and stromal changes can mimic pancreatic carcinoma, making interpretation difficult especially on frozen section. Chronic pancreatitis tends to retain its lobular architecture, lacks malignant cytological changes, and shows no invasion of nerve sheaths or peripancreatic fat. Prominence of plasma cells (many IgG4 immunoreactive) is highly suggestive of autoimmune pancreatitis.

Extrahepatic bile ducts: Stricture of the common bile duct may be caused by passage of a calculus with or without ascending cholangitis and secondary infection, but is more usually after surgical trauma due to inadvertent injury to or ligation of the duct. Treatment aims to reestablish free drainage of bile to the bowel either by a bypass or stenting procedure (see below).

4.4.2 Neoplastic Conditions

Ampullary adenocarcinoma: Arising from adenomatous dysplasia of either the periampullary duodenal or intra-ampullary duct mucosae, it is one of the commonest causes of death in familial adenomatous polyposis (FAP) in patients who have had a prophylactic colectomy. Adenoma may be amenable to local excision, but radical surgical resection is often required for large lesions and because a surface biopsy showing epithelial dysplasia may harbor underlying invasive adenocarcinoma. Most cases have a well-defined intestinal pattern, but in a minority it can be difficult to separate adenocarcinoma of the duodenal papilla, ampulla, distal pancreatic duct, and distal common bile duct as they can share similar well-to-moderately differentiated tubular and ductular patterns. Detailed examination of the exact anatomical location in the resection specimen is required and sometimes the only conclusion possible is adenocarcinoma of the ampullary-pancreatico-biliary (periampullary) region. Secondary involvement of the ampulla by pancreatic cancer can occasionally be specified based on the histological features and pattern of mucosal spread.

Benign pancreatic exocrine tumors: Congenital cysts (von Hippel-Lindau syndrome), acquired retention cyst, serous cystadenoma (elderly, macro-/microcystic, fluid-filled, central scar, clear cuboidal epithelium).

Pancreatic exocrine tumors of malignant potential: Intraductal papillary mucinous neoplasms (IPMN) and mucinous cystic neoplasms with a benign, borderline, and malignant spectrum of behavior related to the degree of epithelial dysplasia and extent of invasion into pancreatic parenchyma and peripancreatic fat. Mucinous cystic neoplasms arise almost exclusively in middle-aged females, typically in the pancreatic tail and unconnected to the pancreatic ductal system, whereas IPMNs are slightly more common in males, aged usually around 60 years, arise in the pancreatic head, and communicate with the ductal system. Both show indolent growth with local spread to the abdomen but prior to this are potentially resectable.

Solid pseudopapillary neoplasm – Young females, pseudopapillae of uniform cells, cystic with necrosis, of low malignant potential, 90% cured by surgical resection.

Pancreatic exocrine carcinoma: Arising from dysplastic pancreatic duct epithelium and forming the vast majority of pancreatic tumors, 80–90% are adenocarcinomas which are graded according to the degree of gland formation. Most (70–80%) arise in the pancreatic head with a minority in the body or tail and occasionally multifocal. Perineural invasion is characteristic and diagnostically helpful in biopsies. There is limited suitability for resection (10–20% of cases only). Node-negative tumors of the pancreatic head <3 cm in size may be resected by a Whipple's procedure with an average increase in survival of 12 to 18 months, but a majority presents with locally advanced disease into regional nodes and retroperitoneal tissues. Treatment is mainly palliative – pain control, nutritional support, and relief of jaundice by open or laparoscopic bypass, or endoscopic stent insertion to combat biliary obstruction.

Other cancers: Unusual but include undifferentiated carcinoma, acinar cell carcinoma, small cell neuroendocrine carcinoma, malignant lymphoma (usually from an adjacent nodal lymphoma), and sarcoma, which often represents spread from a primary sarcoma of gut or retroperitoneum.

Pancreatic endocrine tumors: Single or multiple and forming a minority (3%) of pancreatic tumors, they can be small (<1–2 cm), well circumscribed, and pale or yellow in color. They are positive for general neuroendocrine markers (chromogranin, synaptophysin) and occasionally specific peptides, e.g., insulin, glucagon, gastrin. Many (60–85%) are associated with a functional hormonal syndrome, e.g., Zollinger–Ellison syndrome due to pancreaticoduodenal gastrinomas. The pancreas is also involved in 80–100% of type I multiple endocrine neoplasia (MEN) syndrome comprising hyperplasia or tumors of parathyroid, pituitary, adrenal glands, and pancreas (usually gastrinoma). Histology does not reliably predict behavior and better indicators of potential malignancy are functionality and established metastases – insulinoma (85% benign), gastrinoma (60–85% malignant), size >3 cm, site (e.g., duodenal), invasion of vessels, nodes, adjacent organs, and liver. Detection of metastases is by CT and octreotide scans. Determination of tumor grade on needle core biopsy (mitotic count and Ki-67 index) indicates likely behavior and influences their oncological management.

Extrahepatic bile duct carcinoma: There is an increased incidence in various disorders including ulcerative colitis, sclerosing cholangitis, gallstones, and congenital bile duct anomalies. The majority (50–75%) arise in the upper third (including the hilum) with lesser numbers in the middle and distal thirds (10–25% each) or even diffuse and multifocal. Sometimes polypoid but often nodular, ulcerated, sclerotic, or strictured, prognosis relates to the stage of disease, location, and histological grade. There is characteristic perineural invasion often with involvement of regional lymph nodes, peritoneum, or the liver (upper third tumors) at presentation. Other rare cancers are carcinoid tumor, malignant melanoma, lymphoma/leukemia, and in childhood embryonal rhabdomyosarcoma.

Prognosis: Prognosis of pancreatic ductal adenocarcinoma is poor with an overall 10–20% 5 year survival rate. Chemotherapy has an adjunctive and palliative role for select patients. Cystadenocarcinomas are relatively rare but potentially resectable. Pancreatic endocrine tumors have an indolent time course with a 50% 10-year survival and potential chemoresponsiveness even in the presence of metastases. Ampullary carcinoma has a 5 year survival of 25–50%, improving to 80–85% if early stage (pT1) disease confined to the sphincter of Oddi. Distal bile duct cancers may be potentially resected with 25% 5-year survival. Sclerosing bile duct carcinoma at the hilum (Klatskin tumor) can have an indolent course, but the majority of bile duct cancers present late with very limited survival and only palliative biliary drainage (open bypass or laparoscopic/endoscopic stent insertion) is justified.

4.5 Surgical Pathology Specimens: Clinical Aspects

4.5.1 Biopsy Specimens

The endoscopic technique for the diagnosis of benign lesions of the duodenum has been discussed previously. Endoscopic biopsy of duodenal and ampullary tumors is by EGD or ERCP (*endoscopic retrograde cholangiopancreatography*). Demarcation of ampullary tumors is important as they have a better prognosis and are more amenable to resection than those in the pancreas proper.

ERCP has many applications in both the investigation and, in certain cases, the treatment of biliary and pancreatic disease. The ERCP scope differs in that the camera views from the side (lateral view) and not from the end (forward view) as in the gastroscope. This allows the major duodenal papilla to be viewed relatively easily when the tip of the scope is 60–70 cm from the incisor teeth.

Diagnostic techniques: The papilla is cannulated by a catheter passed through the scope. Contrast is then injected at intervals to outline the duct system and radiographs, which appear on a monitor, are taken in real time to check the position of the catheter. The bile duct (cholangiography) and the pancreatic duct (pancreatography) are cannulated in turn and radiographs taken, which may provide clues to the etiology of the condition, e.g., stones, stricture, etc. The following specimens can be taken by ERCP:

- Bile and pancreatic juice for cytology.
- Brushings from the ducts for cytology.
- FNAC specimens from the duct systems (or duodenum) are useful in submucosal lesions.
- Biopsies can be taken from the ampulla or relevant duct system.

ERCP can also be used for *therapeutic procedures*:

- Sphincterotomy (division of the sphincter of Oddi) can be performed in patients with a history of common bile duct stones to allow free drainage
- Stone extraction can be performed using a balloon catheter or basket
- Dilatation of stricture using a balloon catheter
- Both benign and malignant biliary strictures may be stented (a palliative procedure in the latter) to reduce jaundice.

ERCP is not without its complications, two of the most common being acute pancreatitis (1–3% of cases) and cholangitis.

4.5.2 Resection Specimens

4.5.2.1 Neoplastic Lesions – Duodenum, Ampulla, Distal Common Bile Duct, and Exocrine Pancreas

At the time of presentation, pancreatic carcinoma is beyond resection in more than 80% of patients. Also, given the advanced age of presentation in the vast majority of patients, over 95% of cases are treated palliatively. However, if the patient is fit,

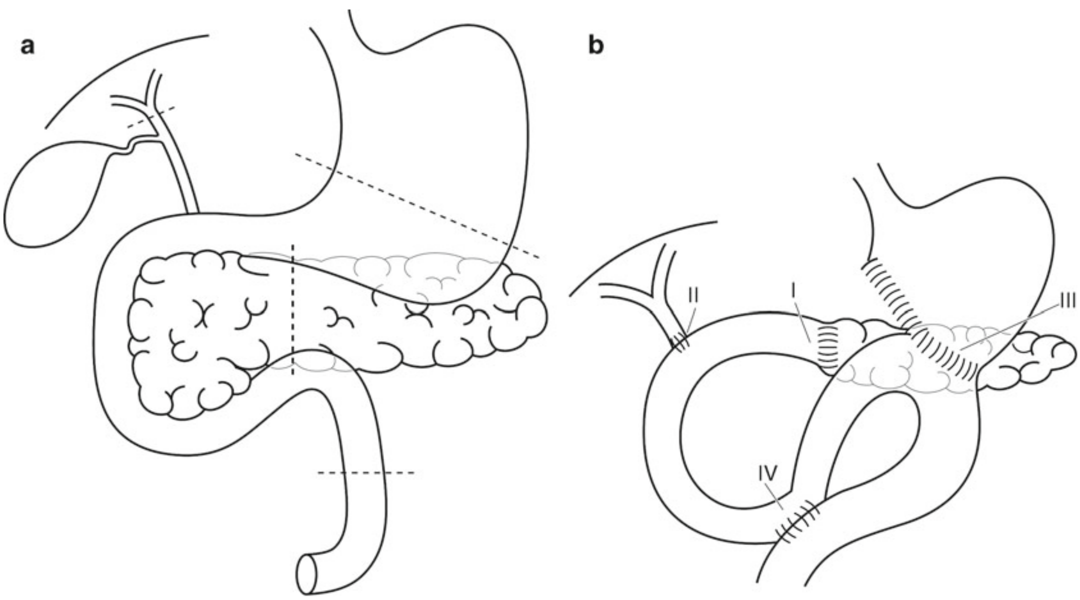


Fig. 4.4 Whipple's procedure: (**a**) limits of resection and (**b**) reconstruction anastomoses (Reproduced, with permission, from Allen and Cameron (2004))

the tumor is less than 3 cm, does not involve major vessels, and has not metastasized on imaging, then a curative procedure may be considered.

Although the type of operation will depend on the site and size of tumor, the curative procedure of choice for duodenal, ampullary, distal common bile duct, and pancreatic head tumors is a standard Kausch–Whipple pancreaticoduodenectomy (PD) –*Whipple's procedure*.

This procedure involves a transverse subcostal incision and initial exploration to assess operability. It then involves the en bloc resection of the pancreatic head (with a variable amount of body depending on the location and size of the tumor), distal two-thirds of the stomach, duodenum (and proximal 10 cm of jejunum), gallbladder, and common bile duct (Fig. 4.4a), which may be extended proximally for distal common bile duct tumors. There are many methods (up to 70!) of reconstruction after PD. One of the most popular is the formation of the following (Fig. 4.4b):

1. Pancreaticojejunostomy (end to end)
2. Hepaticojejunostomy (end to side) – anastomosis of the hepatic duct to the jejunum
3. Gastrojejunostomy (end to side)
4. Jejunojejunostomy (side to side) – this decompresses the proximal jejunal loop and reduces jejuno-gastric reflux

If there is involvement by tumor of the body and tail, the procedure can be modified to a *total* PD which includes resection of the body and tail of the pancreas ± the spleen.

For some small ampullary and periampullary (i.e., head of pancreas, distal common bile duct, and duodenum) tumors, a *pylorus-preserving PD* is performed. This is essentially identical to a standard PD except that the distal stomach and proximal 3 cm of duodenum are left in situ, thus retaining the food storage and release functions of the stomach.

A *distal pancreatectomy* consists of resection of the body and tail of the pancreas, usually including the spleen. This procedure may be used for tumors – many benign – which are located in the distal pancreas.

In all the above procedures, the pancreatic resection margin may be sent for frozen section examination to ensure adequate excision.

4.5.2.2 Neoplastic Lesions of the Endocrine Pancreas

The goals of surgery for tumors of the endocrine pancreas are twofold:

- To locate and excise all abnormal tissue
- To differentiate between benign and malignant tumors by conducting a search for metastatic deposits

Localization of tumours may be carried out by a combination of preoperative imaging (MRI, octreotide scanning) and intraoperative palpation and USS. Once the tumor has been localized, there are two main methods of excision:

- Enucleation – Excision of the tumor and a surrounding segment of normal pancreas can be carried out if the tumor is small (<1.5 cm) and superficial.
- Resection – For larger tumours which are deep-seated, a formal pancreatic resection is required, i.e., a distal pancreatectomy for distal tumors or a proximal pancreatectomy (duodenum-preserving resection of the pancreatic head) for proximal tumors. A PD procedure is only rarely required for large tumors in the head of pancreas or duodenum.

4.5.2.3 Neoplastic Lesions of the Extrahepatic Bile Ducts

Cancer of the bile ducts (cholangiocarcinoma) is treated palliatively in 80–90% of cases and resection should only be considered in localized tumors without metastatic spread. When surgical resection is considered, the type of procedure will depend on the site of tumor:

- Tumors in the distal common bile duct (i.e., lying behind the duodenum and pancreas) – Whipple's procedure.
- Tumors proximal to this and distal to the confluence of the right and left hepatic ducts – wide excision of the supraduodenal biliary tree, gallbladder, and related nodes. A length of jejunum is isolated in a Roux-en-Y loop and an end-to-side hepaticojejunal anastomosis allows biliary drainage.
- Tumors proximal to the hepatic confluence require the above plus a relevant liver resection (see Chap. 10).

Palliation for distal common bile duct tumors is most commonly done by ERCP stenting. Other methods of operative palliation (i.e., "by-pass" techniques) are:

- Choledochoduodenostomy – proximal common bile duct is anastomosed to D1.
- Hepaticojejunostomy – can be used in more proximal biliary tumors (i.e., common hepatic duct/proximal common bile duct).

For proximal biliary (hilar) tumors, a segment III hepaticojejunostomy can be used. In this the liver is divided to the left of the falciform ligament until the segment III duct is visualized. An anastomosis is then fashioned between this and a Roux-en-Y loop of jejunum.

4.5.2.4 Non-neoplastic Lesions

Two of the most common complications of *acute pancreatitis* requiring surgical intervention are:

- Necrotizing pancreatitis – Surgical intervention has a mortality rate of 60% and involves removal of necrotic tissue from the pancreas and retroperitoneal spaces, and drainage of fluid collections.
- Pancreatic pseudocyst – cystogastrostomy – a pseudocyst in the lesser sac is drained into the stomach via an opening in the posterior wall of the stomach.
- In *chronic pancreatitis*, the following procedures may be employed:
- The pain associated with chronic pancreatitis is caused by obstruction of the pancreatic duct leading to duct hypertension. The *Frey operation* (localized resection of the pancreatic head and side to side pancreaticojejunostomy) is designed to decompress the duct.
- When there is disruption of the duct distal to the head, a distal pancreatectomy is indicated.
- Occasionally, when the disease is maximal in the head, a Whipple's procedure may be employed, possibly when investigations are equivocal regarding malignancy. Another

option would be a duodenum-preserving resection of the pancreatic head or total pancreatectomy.

Bile duct stones may be removed laparoscopically or by an open procedure if they cannot be removed by ERCP. Strictures may be stented or bypassed using one of the techniques described above.

4.6 Surgical Laboratory Specimens: Laboratory Protocols

4.6.1 Biopsy and Local Mucosal Resection Specimens

See Chap. 1.

4.6.2 Resection Specimens

Specimen

- Most pancreatic resections are for neoplastic conditions, although operative intervention may be indicated for debridement of necrotic tissue in acute pancreatitis, trauma to a major duct, or removal of the pancreatic head or pseudocyst in chronic pancreatitis. Resections are either local for pseudocyst or cystic neoplasms, or radical for ampullary, pancreatic head or bile duct cancers. Carcinoma of the body and tail usually presents late and is typically irresectable.
- To demonstrate the lesion and its relationship to the surgical margins, the pancreas is cut into multiple parallel slices in either vertical, coronal, or horizontal planes, the latter allowing correlation with CT scan cross-sectional images.
- Specimens may be opened and partially incised to aid fixation prior to complete dissection.
- The presence of any stent or surgically labeled structures, e.g., portal vein, SMV, should be noted.

4.6.2.1 Local Resection of Cystic Lesions

- Weight (g) and maximum dimension (cm).
- Capsule: intact/deficient/smooth/nodular/adhesions/circumscribed/lobulated.
- Cut surface: uni-/multilocular/septate/solid areas (cm)/contents – fibrin, mucoid, serous fluid.
- Photograph.
- Paint the external surface.
- Fixation by immersion in 10% formalin for 48 h.
- Sample one block per centimeter diameter of the tumor/cyst to include thin, nodular, and solid areas of its wall and internal aspect.
- Sample adjacent tissues to include the resection margins and any other structures.

4.6.2.2 Local Resection of Pancreatic Head in Chronic Pancreatitis

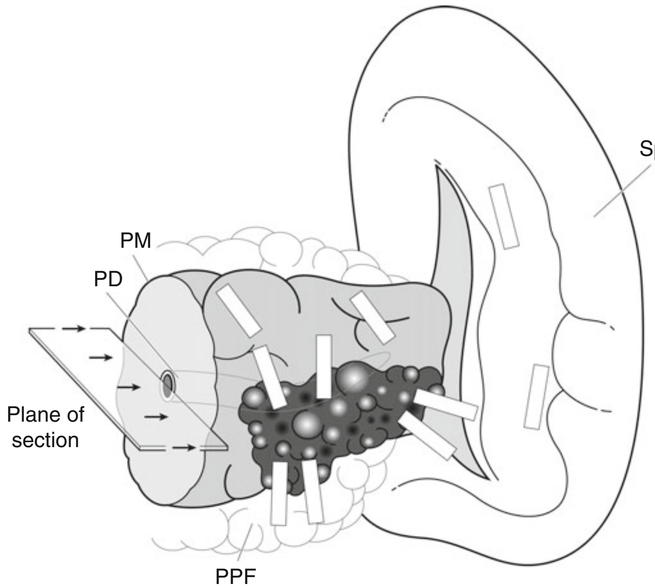
- Weight (g) and dimensions (cm): then fix in 10% formalin for 48 h.
- Serially slice perpendicular to the pancreatic duct.
- Inspect and describe, e.g., hemorrhage, abscess, necrosis, calculi, calcification.
- Select five representative blocks, assuming no suspicion of malignancy on macroscopic examination.

4.6.2.3 Necrosectomy Specimen

- Number of pieces, total weight (g), and maximum dimension (cm).
- Fix in 10% formalin for 48 h.
- Serially slice, inspect, and describe, e.g. hemorrhage, abscess, necrosis, calculi, calcification, tissues present.
- Select five representative blocks.

4.6.2.4 Distal Pancreatectomy (Fig. 4.5)

- Orientate – cut end is proximal, distal end is uncut ± spleen.
- Weight (g) and measurements – length × width × depth (cm).
- Paint the proximal cut margin and the external surfaces using different colors of ink for the various anatomical and surgical aspects – superior, inferior, anterior capsule, posterior retroperitoneal.
- Fixation by immersion in 10% formalin for 48 h.
- Transverse section the proximal margin to include the duct.



1. Sample resection margins: PD pancreatic duct
   PM proximal pancreatic margin
2. Sample tumor in relation to pancreas, pancreatic duct, peripancreatic fat (PPF) and its margins and spleen.
3. Sample all regional lymph nodes, non-tumorous pancreas and spleen.

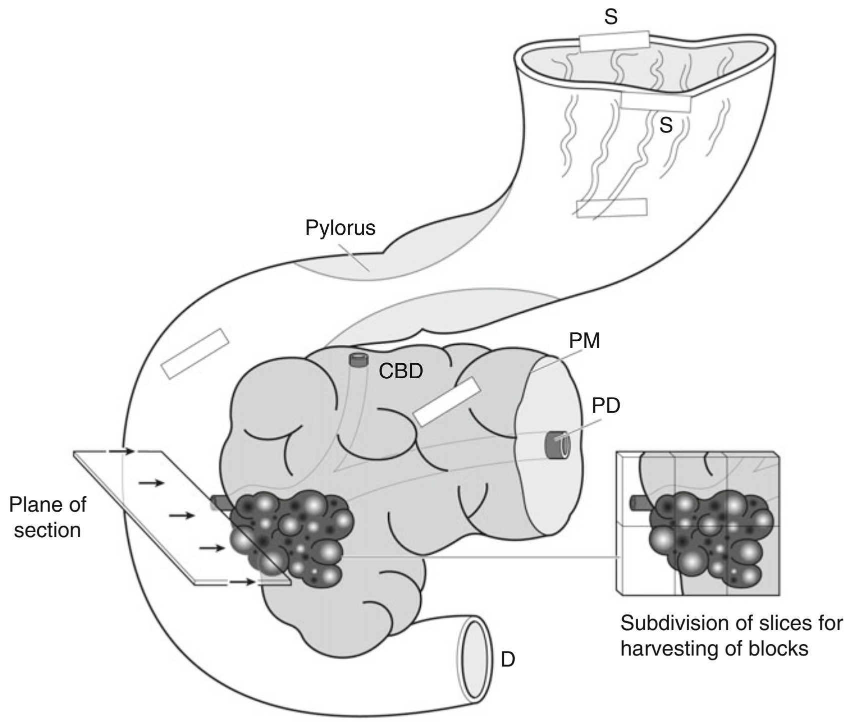
Fig. 4.5 Distal pancreatectomy (Reproduced, with permission, from Allen and Cameron (2004))

- Serially section the pancreas at 3–4 mm intervals in a horizontal plane parallel to its long axis.
- Lay the slices out in sequence and photograph.
- Tumor: size (cm), edge (circumscribed/irregular), appearances, consistency, relationship to the pancreatic duct, distances (mm) to the specimen edges.
- Sample a minimum of five blocks of tumor in relation to pancreas, pancreatic duct, peripancreatic fat and its margins, and spleen.
- Sample all regional lymph nodes, non-neoplastic pancreas and spleen (if present).

4.6.2.5 Whipple's Procedure (Fig. 4.6)

Initial procedure:

- Open with scissors by cutting along the lesser curvature of the stomach and the free border of the duodenum.
- Measurements:
  Lengths (cm) of distal sleeve of stomach and duodenum, and parts present (D1–4).
  Dimensions (cm) of pancreas, and parts present (head, body, attached named vessel, e.g., SMV).
  Lengths of gallbladder (if attached) and bile duct (cm).
- Fixation by immersion in 10% formalin for 24 h.
- Sample the following surgical margins: proximal gastric, distal duodenal, distal pancreatic face to include the pancreatic duct, and, proximal common bile duct.
- Paint the external anatomical and surgical margins using different colors of ink and labeled appropriately – superior, inferior, anterior capsule, posterior retroperitoneal, medial (uncinate SMV).
- Carefully insert a fine probe into the distal end of the pancreatic duct and gently advance, if possible, to the ampulla.
- Place the specimen flat on the bench and with a long, sharp knife use the probe as a guide to horizontally hemisect it cutting through the peripancreatic duodenum and pancreas. If cannot cannulate the duct, serial 3–4 mm horizontal slices may be performed, from inferior to superior (trimming off excess duodenum first facilitates this slicing).



1. Sample resection margins: S stomach
   D distal duodenum
   CBD common bile duct
   PD pancreatic duct
   PM pancreatic margin
2. Sample tumor in relation to the ampulla, pancreas, duct structures, duodenum and peripancreatic tissues including the painted anatomical and surgical margins.
3. Sample duodenum and stomach.
4. Sample all regional lymph nodes and non-tumorous pancreas.

Fig. 4.6 Whipple's procedure for carcinoma of ampulla of Vater, head of pancreas or distal common bile duct (Reproduced, with permission, from Allen and Cameron (2004))

- Further 24 h fixation may be required.
- Photograph.

Description:

- Tumor
  - Site: duodenal mucosa or papilla/ampulla/pancreas (duct/parenchyma)/bile duct
  - Size: length × width × depth (cm) or maximum dimension (cm)
  - Appearance:
    Polypoid/diffuse/ulcerated – ampullary/bile duct tumors
    Cystic/papillary/mucoid/scirrhous/thickening – pancreatic exocrine tumors
    Circumscribed/pale/homogeneous – pancreatic endocrine tumors
  - Edge: circumscribed/irregular
- Pancreatic and bile ducts: dilatation/stenosis/extrinsic or intraduct tumor/stent
- Pancreas: indurated/edematous/fat necrosis
- Peripancreatic lymph nodes: location/number/size
- Other organs: involvement of SMV, duodenum or stomach etc.

Blocks for histology (Fig. 4.6):

- Resection margins (see above).

- Serial 3–4 mm cuts parallel to the plane of hemisection provide suitable slices for subdividing and harvesting of blocks.
- Sample the tumor (a minimum of six blocks) in relation to the ampulla, pancreas, duct structures, duodenum, and peripancreatic tissues including the painted and labeled anatomical/surgical margins.
- Sample non-neoplastic pancreas, stomach, and duodenum.
- Count and sample all lymph nodes.
- If other organs are present (total or regional pancreatectomy – PD, gastrectomy, splenectomy, portal vein, transverse colectomy, mesocolon, omentum, and regional nodes): describe, weigh, measure, paint, and block according to the macroscopic degree of tumor spread. Label the blocks as to their site of origin.

4.6.2.6 Proximal Extrahepatic Bile Duct Cancer Resection

- Bile duct segment
  – Site: common bile duct/common hepatic duct/right or left hepatic duct/cystic duct
  – Length × diameter (cm)
  – Dilated/ulcerated/strictured/cyst and maximum dimension (cm) of lesion
  – Calculi
- Tumor
  – Maximum dimension (cm)
  – Site: common bile duct/common hepatic duct/right or left hepatic duct/cystic duct/intraduct/mural/extramural/involvement of liver
  – Appearance: strictured/ulcerated/nodular/polypoid/multifocal
- Hepatic resection
  – Segment(s)
  – Dimensions (cm) and maximum dimension (cm) of tumor present
- Biliary stent
  – Not present/present/placement (within or outside the lumen).
- Gallbladder
  – Present/not present/involved by tumor.
- Sample the distal bile duct limit (circumferential transverse section) and the proximal bile duct limit or the hepatic resection margin (two or three blocks).
- Paint the external adventitial CRM.
- Serially section the specimen transversely at 3–4 mm intervals.
- Sample five or six blocks to demonstrate the worst point of tumor invasion in relation to the CRM, liver, gallbladder, proximal and distal surgical limits.
- Sample all lymph nodes.

Histopathology report:

- Tumor type
  – Ampulla/bile duct: adenocarcinoma.
  – Pancreas: benign/of low malignant potential/adenocarcinoma/endocrine tumor/other.
- Tumor differentiation/grade:
  – Ampullary/bile duct adenocarcinoma: well/moderate/poor.
  – Pancreatic carcinoma:

Well/grade 1	>95% glands
Moderate/grade 2	50–95% glands
Poor/grade 3	5–50% glands
Undifferentiated/grade 4	<5% glands

- Tumor edge
  – Pushing/infiltrative/lymphoid response.
- Extent of local tumor spread.

Ampulla

pTis	Carcinoma in situ
pT1	Tumor limited to the ampulla or sphincter of Oddi
pT2	Tumor invades duodenal wall
pT3	Tumor invades pancreas
pT4	Tumor invades peripancreatic soft tissues, or other adjacent organs or structures

Pancreas

pTis	Carcinoma in situ
pT1	Tumor limited to the pancreas, ≤2 cm maximum dimension
pT2	Tumor limited to the pancreas, >2 cm dimension
pT3	Tumor extends beyond pancreas, but without involvement of celiac axis or SMA
pT4	Tumor involves celiac axis or SMA

Distal extrahepatic bile ducts – perihilar tumors are classified separately

pT1	Tumor confined to the bile duct
pT2	Tumor invades beyond the wall of the bile duct
pT3	Tumor invades the liver, gallbladder, pancreas, duodenum, or other organs
pT4	Tumor involves the celiac axis or the SMA

- Lymphovascular invasion – present/not present. Perineural space or lymphovascular invasion is present in up to 50% of pancreaticobiliary carcinomas with spread to regional nodes at diagnosis. Involvement of large named vessels, e.g., SMV, portal vein, is a major determinant of postoperative survival.
- Regional lymph nodes:
  Peripancreatic, pancreaticoduodenal, pyloric and proximal mesenteric. Also celiac (for head of pancreas tumor) and tail of pancreas/splenic hilum nodes (for body/tail of pancreas tumors). Also pericholedochal and SMV (for distal extrahepatic bile duct tumors). A regional lymphadenectomy will usually include ten or more lymph nodes.

pN0	No regional lymph node metastasis
pN1	Metastasis in regional lymph node(s)

- Excision margins
  Proximal gastric and distal duodenal limits of tumor clearance (cm).
  Distal pancreatic surgical margin/common bile duct margin of tumor clearance (mm) – also comment on pancreatic intraepithelial neoplasia (PanIN).
  Peripancreatic edge tumor clearance (mm) – superior/inferior/anterior capsule/posterior retroperitoneal/medial (SMV). The peripancreatic circumferential margin is the most commonly involved.
  For extrahepatic bile duct cancer – tumor clearance (mm) of the distal and proximal bile duct, hepatic and radial resection margins.
- Other pathology
  Duodenal adenoma (s), secondary pancreatitis, fat necrosis, calculi, ulcerative colitis, sclerosing cholangitis, choledochal cysts.

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Small Intestine

5

Derek C. Allen, R. Iain Cameron,
and Maurice B. Loughrey

5.1 Anatomy

The small intestine is the longest part of the gastrointestinal tract and is divided into the duodenum (discussed previously), jejunum, and ileum (Fig. 5.1). It is primarily concerned with digestion and absorption of food. Together the jejunum and ileum measure approximately 6 m (jejunum 2.5 m/ileum 3.5 m) in the adult. The jejunum commences at the duodenojejunal junction (flexure) and the ileum ends at the ileo-cecal valve (two horizontal folds of mucosa that project around the orifice of the ileum as it joins the cecum). A fan-shaped fold of peritoneum (the small intestinal mesentery) attaches the small intestine to the posterior abdominal wall. The long edge of the mesentery completely encloses the intestine, allowing it to be mobile, while the short "root," which is attached to the posterior abdominal wall, admits blood vessels, lymphatics, and nerves which supply the intestine by traversing the mesentery.

Although there is a gradual change from jejunum to ileum, in general, the jejunum tends to be located in the upper part of the abdominal cavity, is thicker walled with more prominent *plicae circulares* (permanent mucosal folds), and has more numerous *Peyer's patches* (aggregations of lymphoid tissue).

Histologically the mucosa of the small intestine projects into the lumen in the form of finger-like structures covered by absorptive columnar

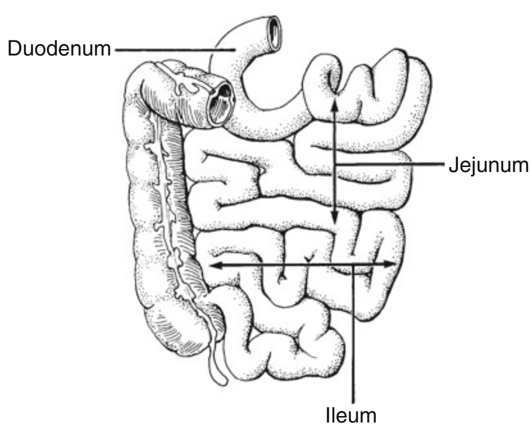


Fig. 5.1 Small intestine (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

D.C. Allen (✉)
Histopathology Laboratory, Belfast City Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: derek.allen@belfasttrust.hscni.net

R.I. Cameron
Histopathology Laboratory, Altnagelvin Hospital,
Western Health and Social Care Trust,
Londonderry, UK
e-mail: iain.cameron@westerntrust.hscni.net

M.B. Loughrey
Histopathology Laboratory, Institute of Pathology,
Royal Victoria Hospital, Belfast Health and
Social Care Trust,
Belfast, UK
e-mail: maurice.loughrey@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_5, © Springer-Verlag London 2013

epithelium. These projections are called *villi* and increase the surface area for absorption. The circular and longitudinal muscle layers are continuous.

Lymphovascular drainage:

The arterial supply of the jejunum and ileum is from the superior mesenteric artery. Numerous intestinal branches run in the mesentery and anastomose with one another to form “arterial arcades,” which in turn supply the intestine. Venous drainage is via the superior mesenteric vein to the portal system. Lymphatics traverse through a series of mesenteric nodes and ultimately drain to the superior mesenteric nodes situated at the origin of the superior mesenteric artery.

5.2 Clinical Presentation

Patients with small intestinal disease may present with vague symptoms and signs such as poorly localized dull central (periumbilical) abdominal pain. If there is full thickness inflammation, the peritoneal somatic pain receptors are stimulated and the pain becomes more severe and localized. A patient with an obstructing lesion will classically present with vomiting, colicky abdominal pain (cramps), absolute constipation (i.e., neither flatulence nor feces passed per rectum), and abdominal distension.

Bleeding into the lumen of the small intestine may lead to hypovolemic shock and altered blood (melena) per rectum. Intussusception produces a mixture of blood and mucus – “redcurrant jelly stool,” particularly in infants. Perforation, although rare (e.g., trauma, Meckel’s diverticulum), will lead to a generalized peritonitis. A heart murmur or irregular pulse may provide a diagnostic clue in embolic small intestinal infarction.

The presentation of Crohn’s disease may be insidious or acute, with symptoms including diarrhoea, anorexia, and weight loss. Various forms of fistulae (abnormal connection between two epithelial-lined structures) may occur including an enterovesical fistula (between small intestine and urinary bladder) which leads to gas in the urine (pneumaturia) and repeated urinary tract infections. Enterocolic and enteroenteric (between adjacent small bowel loops) fistulae may also occur. Enterocutaneous fistulae usually only happen after previous surgery. One of the differential diagnoses of a right iliac fossa mass is Crohn’s disease with a peri-intestinal abscess around the distal ileum.

Several conditions, including Crohn’s disease, may lead to a protein-losing enteropathy, resulting in generalized edema. Celiac disease presents in young children as a failure to thrive and in middle-aged adults with unexplained weight loss or iron-deficiency anemia. Melanin spots may be seen in the buccal mucosa and lips of those with Peutz–Jegher’s syndrome.

5.3 Clinical Investigations

- U&E – electrolyte imbalance due to malabsorption.
- LFTs/albumin – liver enzymes may be deranged in Crohn’s disease and hypoalbuminemia will occur in protein-losing enteropathy.
- Folate, B12, and iron studies – pernicious anemia in Crohn’s disease.
- Erect CXR – air under the diaphragm in a perforation.
- Erect and supine AXR – will detect gas shadows and fluid levels in distended loops of small intestine in obstruction.
- Small bowel series – radiological contrast is drunk and abdominal images are taken at regular intervals to outline the mucosal surface of the small intestine and to measure the transit time. This is particularly useful for obstructing lesions and Crohn’s disease, and may also detect a Meckel’s diverticulum.
- Barium enema – will demonstrate an ileocolic intussusception and may be used as a therapeutic procedure (see below).
- Sinogram/fistulogram – useful to delineate the extent of the complications of Crohn’s disease.
- CT scan – useful in delineating an abdominal (e.g., right iliac fossa) mass.
- Radioisotope scanning – can be used in cases of repeated gastrointestinal hemorrhage of

unknown etiology and will localize heterotopic gastric mucosa in a Meckel's diverticulum. Radiolabeled red blood cells may show a site of active bleeding.

- Selective arteriography (superior mesenteric) – will aid identification of a site of small intestinal bleeding. This may detect angiodysplasia of the small intestine providing the bleeding rate is greater than 2 ml/min.
- Enteroscopy – allows direct visualization of small intestinal mucosa.
- Distal duodenal biopsy and serology (anti-endomysial (EMA) and tissue transglutaminase (tTG) antibodies) – in celiac disease.

5.4 Pathological Conditions

5.4.1 Non-neoplastic Conditions

Duodenitis and duodenal ulcer (DU): HP distal gastritis leads to hyperchlorhydria, duodenitis with surface gastric metaplasia, colonization by HP, and further duodenitis and ulceration. Occurring mainly in the cap and first part of the duodenum, DU is invariably benign and only rarely biopsied at laparotomy for perforation if its mucosal edges are irregular. DU is successfully treated by HP eradication – occasionally it is due to other causes, e.g., NSAIDs, Crohn's disease, or Zollinger–Ellison syndrome.

Celiac disease: Traditionally investigated by Crosby capsule biopsy of the proximal jejunum, it is now assessed by a combination of celiac serology and distal duodenal biopsies taken at flexible esophagogastroduodenoscopy (EGD). Cardinal features are an excess of surface intraepithelial lymphocytes, lamina propria inflammation, villous atrophy, and crypt hyperplasia. Diagnostic proof is by clinical improvement on a gluten-free diet and deterioration on subsequent rechallenge. Celiac disease involves the entire small intestine and can be complicated by malignant lymphoma (usually enteropathy-associated T-cell lymphoma, EATCL) or adenocarcinoma. Other conditions can produce similar histological changes, e.g., giardia lamblia infestation, lactose intolerance, or postinfective enteritis, but are not gluten sensitive. Giardia is a kite-shaped flagellate protozoon present in the intervillous mucus, causing diarrhoea with or without mucosal inflammation – it typically affects children or the elderly.

Crohn's disease (*regional enteritis*): a pan-gastrointestinal inflammatory condition of uncertain etiology, lesions can occur anywhere from the mouth to the anus. It is characterized by segmental, transmural chronic inflammation associated with linear and fissuring ulceration, and, in 40% of cases, non-caseating epithelioid and giant cell granulomas present either in the mucous membrane, bowel wall, or regional lymph nodes. The terminal ileum, ileum, and colon, or colon alone, are affected in decreasing order of frequency. Macroscopically the classical features are skip lesions comprising stenotic ring strictures and hosepipe segments, serosal fat encroachment (fat wrapping), fissure ulcers with fistulae to other organs, and abscess formation, and, ulceration that can be pinpoint (aphthous), linear, or contiguous. Perianal fissures or fistulae are also often present. Due to its segmental distribution, subsequent recurrence elsewhere in the gut is not infrequent. Complications can be gastrointestinal, e.g., adenocarcinoma or malignant lymphoma, or extraintestinal, such as liver disease, amyloidosis, or arthritis.

Other causes of ileitis include backwash ileitis in ulcerative colitis, ileo-cecal tuberculosis, or yersinial infection. Viral adenitis of the mesenteric lymph nodes can also mimic ileitis or appendicitis. Relatively common viral or bacterial gastroenteritis rarely provide histopathology material. Immunodeficency, e.g., AIDS predisposes to various opportunistic infections (giardia lamblia, mycobacterium avium intracellulare, CMV, etc.) and malignancies (malignant lymphoma, Kaposi's sarcoma) that need to be considered on duodenal or terminal ileal biopsy.

Meckel's diverticulum: In 2% of people, 2 in long, 2 ft from the ileo-cecal valve, and, "too" important to forget, this is a remnant of the fetal vitellointestinal duct comprising an outpouching of the ileal wall on its antemesenteric border with or without a fibrous cord attaching it to the umbilicus. Its wall is continuous with the ileal muscle coat and the small intestinal lining not infrequently shows heterotopic gastric or pancreatic tissue. Complications (4% of cases) include peptic diverticulitis with ulceration, hemorrhage or perforation, intussusception, or rarely malignancy.

Obstruction: Broadly, small intestinal obstruction is either due to loss of peristaltic bowel movement (paralytic ileus), or mechanical in nature. Ileus is commonly seen in the postoperative period of abdominal surgery and is self-limiting, although it is also encountered in various metabolic disturbances and can be difficult to manage – histopathology specimens are rarely provided. Mechanical obstruction is due to blockage of the bowel lumen or distortion of its wall. Common causes are primary or secondary malignancy, ulceration with ring stricture/diaphragm formation (Crohn's disease, NSAIDs), incarceration within a hernia, or extrinsic compression by postoperative adhesions or fibrous bands. The proximal bowel dilates, fills with fluid, and ultimately becomes atonic – sepsis or ischemia are possible complications. Particular forms of enteric obstruction are volvulus, where a loop of bowel twists around its mesenteric pedicle and, intussusception, where a lumenal or mural abnormality (e.g., tumor) acts as a nidus for peristalsis to propel a proximal segment (the intussusceptum) forward and inside a receiving distal segment (the intussuscipiens). The intussusception can be benign or malignant in nature and variable in site, e.g., ileal–ileal or ileal–cecal. Handling of all these specimens is targeted at determining the nature of the obstructing abnormality, its distribution and completeness of excision, and the presence and extent of secondary changes such as inflammation or ischemia.

Inflammatory fibroid polyp: An inflammatory mucosal pseudotumor of unknown etiology that can form the apex of an intussusception, it comprises edematous and inflamed fibrovascular granulation tissue with an infiltrate of eosinophils.

Diaphragm disease: Due to chronic ingestion of NSAIDs, it comprises multiple diaphragm-like mucosal ring strictures with variable lumen stenosis and intervening compartmentalization and sacculation. The strictures have a triangular cross-sectional profile of fibrovascular connective tissue and are probably partly ischemic in nature. Presentation is with subacute obstruction.

Ischemia: Acute, subacute, or chronic, depending on the nature, severity, and rapidity of onset of the cause. Acute ischemia is characterized by hemorrhagic necrosis of bowel wall that becomes paper-thin and gangrenous with subsequent electrolyte imbalance, sepsis, and shock. Chronic ischemia comprises ulcerated segments or strictures with replacement of bowel wall by fibrovascular connective tissue, evidence of secondary vascular thickening, and hemosiderin deposition. Examination of these specimens must include assessment of resection limit viability and any abnormality of the mesenteric vessels. Common causes are arterial, such as mesenteric artery embolism or thrombosis (particularly if superimposed on a low flow state due to mesenteric atheroma or cardiogenic hypotension), or venous thrombosis. The latter is usually due to obstruction of venous flow by bowel entrapment within a hernia or kinking of its mesentery by a fibrous band or adhesion resulting from previous surgery. Less usual causes of ischemia are systemic vasculitis (e.g., polyarteritis) or amyloid deposition which thickens and occludes mesenteric and mural vessels. Drugs must always be considered as a cause of isolated ulcers or chronic ischemic segments, especially NSAIDs.

Hernia: Herniation of the bowel can be either internal or external. Internal hernias are into anatomical spaces, e.g., the lesser omental sac or across fibrous bands, which can be acquired (postoperative) or congenital (e.g., persistent vitellointestinal duct). External hernias involve protrusion of the peritoneum ± bowel into the layers of the abdominal wall (particularly at the site of a previous surgical incision), groin, or femoral canal. They can be intermittent and reducible or irreducible with the risk of secondary ischemic changes. The surgical specimens are dealt with elsewhere (see Chap. 11).

Non-neoplastic polyps: In the duodenum these include gastric heterotopia, Brunner's gland hyperplasia/hamartoma, and pancreatic heterotopia. Small intestine is the commonest site for Peutz–Jegher's syndrome, which is autosomal dominantly inherited, comprising oral pigmentation and pan-gastrointestinal polyposis – the polyps have a branching smooth muscle core and twisting of the polyp can produce glandular herniation into the submucosa and mimicry of adenocarcinoma. The terminal ileum can show mucosal nodular lymphoid hyperplasia which is usually of unknown etiology but occasionally linked to immunodeficiency. A protruberant ileocecal valve or fatty hyperplasia of its submucosa can simulate a tumor on radiological investigation, and, if not adequately investigated by colonoscopy and biopsy, can lead to unnecessary right hemicolectomy.

5.4.2 Neoplastic Conditions

Forming less than 10% of all bowel tumors, duodenal/jejunal lesions tend to be adenomas or adenocarcinoma, whereas carcinoid tumor and malignant lymphoma have a predilection for the ileum.

Adenoma: Relatively unusual in the small bowel but commoner in D2, particularly in FAP where there is a strong association with periampullary adenocarcinoma. Surgical removal is either by endoscopy or duodenotomy with thorough assessment of the ampullary region to exclude underlying tumor that would necessitate radical resection. Adenomas can also occur sporadically in the jejunum or ileum giving rise to adenocarcinoma.

Adenocarcinoma: Duodenal cancers (70% of cases) are often polypoid, while distal lesions are ulcerated and napkin-ring-like. Presentation is late, with regional lymph node metastases and serosal involvement due to the fluid content of the small bowel and consequent lack of symptoms. Prognosis is poor and incidence is increased in Crohn's disease and celiac disease.

Carcinoid (*well-differentiated neuroendocrine*) *tumor*: Single or multiple, carcinoid tumor is of intermediate grade malignancy metastatic potential relating to size (>1–2 cm), angioinvasion, invasion beyond the submucosa, and functionality. It produces vasoactive peptides, e.g., serotonin, that cause vascular thickening and elastotic stromal fibrosis which distorts the bowel wall and mesentery with characteristic spiculate CT appearances leading to subacute obstruction or intussusception. Metastatic deposits in the liver result in the peptides accessing the systemic venous circulation and carcinoid syndrome – facial flushing, asthma, and thickening of cardiac valves. Carcinoid tumors can be ulcerated or nodular, and are usually yellow. Other neuroendocrine lesions occur in the duodenum and include gastrinoma as part of Zollinger–Ellison syndrome, somatostatinoma, and gangliocytic paraganglioma, both of which may be associated with von Recklinghausen's syndrome (neurofibromatosis).

Malignant lymphoma: Solitary or multifocal, primary or secondary to systemic nodal disease, the vast majority are non-Hodgkin's in type. Established disease is ulcerated, segmental, and rubbery or fleshy in appearance. Many are MALT-derived of B cell character and variably low or high grade, prognosis relating to the grade and stage of disease. Unusual variants of malignant lymphoma include multiple lymphomatous polyposis (ileo-colonic nodular polyps of mantle cell lymphoma), ileo-cecal Burkitt's lymphoma in children and immunosuppressed patients, and EATCL. EATCL is strongly associated with celiac disease, either occult or clinically established of short or long duration. Presentation can be with perforated ulcerative jejunitis, a change in response to the gluten-free diet or with abdominal pain/mass.

Gastrointestinal mesenchymal or stromal tumors (*GISTs*): Spindle or epithelioid cell in type, a minority are leiomyomatous or neural, and a majority stromal (CD117 (ckit)/DOG-1 positive) in character derived from interstitial cells of Cajal, which regulate peristalsis. Malignancy cannot be accurately predicted but indicators are size (>2–5 cm), cellularity and atypia, tumor necrosis and hemorrhage, and infiltrative margins and mitotic activity (>5/50 high power fields). Small intestinal GISTs tend to behave more aggressively than their equivalent gastric counterparts with spread to the abdominal peritoneum and liver. The tumor can be polypoid, mural, or dumbbell-shaped with an extramural component. Occasionally they arise primarily in the small bowel mesentery or retroperitoneum with no attachment to gastrointestinal wall.

Metastases: The small intestine is particularly prone to involvement by metastatic adenocarcinoma either from other abdominopelvic sites, e.g., stomach, pancreas, colorectum, and ovary, or due to distant spread, e.g., lung, breast, malignant melanoma. Deposits can be nodular, ulcerate or stricture the bowel wall mimicking a primary lesion – designation as a primary small intestinal adenocarcinoma therefore necessitates exclusion of spread from a more common site or evidence of a point of origin, e.g., an adjacent mucosal adenoma. Alternatively the deposits may be as diffuse peritoneal seedlings detected at CT scan or at laparoscopy. Malignant melanoma can be pigmented.

Prognosis: Small bowel adenocarcinoma is unusual being 50 times less common than colorectal cancers. Presentation is late, with poor prognosis (10–30% 5-year survival). Carcinoid tumor has an overall 5-year survival rate of 50–65% with smaller (<1–2 cm), early lesions, confined to the bowel wall being more favorable. Prognosis is better for low-grade B cell lymphomas (44–75% 5-year survival) than high-grade B or T cell lymphomas (25–35% 5-year survival) and is strongly grade and stage dependent. GISTs are of intermediate behavior, and unresectable/metastatic lesions respond to targeted therapy with the tyrosine kinase inhibitor imatinib (Glivec) leading to tumor shrinkage, cystic degeneration, and hyalinization. Neoadjuvant imatinib treatment may be considered to downstage a surgically unresectable GIST and permit resection.

5.5 Surgical Pathology Specimens: Clinical Aspects

5.5.1 Biopsy Specimens

Biopsy specimens can be obtained from the ileocecal valve and terminal ileum by colonoscopy, and from the duodenum by flexible EGD. They can also be obtained during laparotomy by either enteroscopy or wedge resection of a serosal lesion. In enteroscopy, an incision is made in the wall of the small intestine and an endoscope is passed along the small intestinal lumen to view the region of interest. The endoscope can also be introduced orally and the surgeon can guide it through the stomach and small intestine during a laparotomy.

5.5.2 Resection Specimens

Although small intestine may be resected as part of another procedure, e.g., right hemicolectomy, in primary small bowel resection, the goals of surgery are removal of the lesion and restoration of intestinal continuity. However, the exact procedure will depend on the type of lesion to be dealt with:

5.5.2.1 Neoplastic Conditions

Tumors: The type of resection will depend on the site of the tumor, e.g., a distal tumor will require a right hemicolectomy. For more proximal lesions, the operation of choice is a local resection with en bloc resection of a wedge of mesentery (Fig. 5.2). At least 5 cm of intestine on either side of the tumor should be removed with an

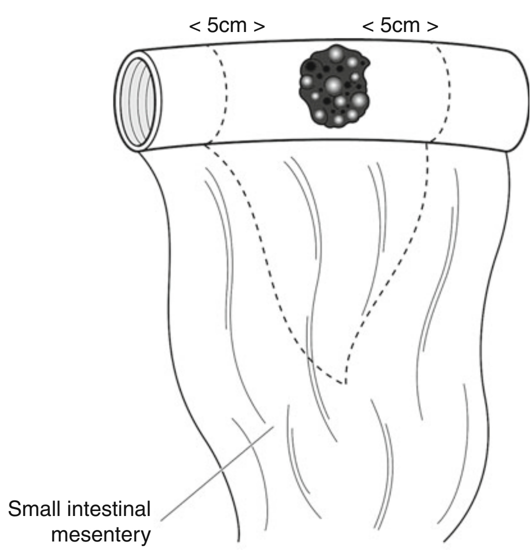


Fig. 5.2 Resection of tumor in small intestine (Reproduced, with permission, from Allen and Cameron (2004))

end-to-end anastomosis to reestablish continuity. Occasionally a hamartomatous polyp may be removed by making a longitudinal elliptical incision in the intestinal wall to include the base of the polyp. Closure should be done transversely to avoid luminal narrowing.

5.5.2.2 Non-neoplastic Conditions

Small intestinal resection in non-neoplastic conditions is essentially similar to that for neoplastic disease in that the affected length of intestine is resected with continuity being restored by a hand-sewn end-to-end anastomosis. Some specific conditions are discussed below:

Crohn's disease: Small bowel resection is usually reserved for those individuals for whom medical treatment has failed or who are suffering complications, e.g., obstruction (due to strictures), peri-intestinal abscess, fistula formation, or perforation. Essentially the extent of resection is limited to the macroscopically involved intestine as extensive resection does not reduce the risk of recurrent lesions and may lead to short bowel syndrome if subsequent resections are necessary.

If there are multiple areas of stricturing, these need not be resected in order to preserve intestinal length. Instead, a "widening procedure" called a stricturoplasty may be employed. In this procedure, the strictured region is incised longitudinally, the walls retracted, and the incision then sutured transversely (Fig. 5.3).

Infarction: At laparotomy, the infarcted intestine will appear dusky and should be resected until there is active bleeding from the ends that are going to form the anastomosis. A primary anastomosis may be fashioned or in cases of extensive intraperitoneal leakage or uncertain intestinal viability, an ileostomy (or jejunostomy) and distal mucus fistula can be fashioned. Essentially an ileostomy (or jejunostomy) is produced by bringing the cut opened end of the intestine out through an opening in the abdominal wall where it is sutured in place. A special ileostomy bag is then fitted to collect the effluent.

Meckel's diverticulum: They are usually only resected if symptomatic or found incidentally during another procedure. Essentially the diverticulum is excised with the opening in the intestinal wall closed in a transverse fashion to avoid luminal narrowing. If the diverticulum is large or broad based, a limited ileal resection may be required.

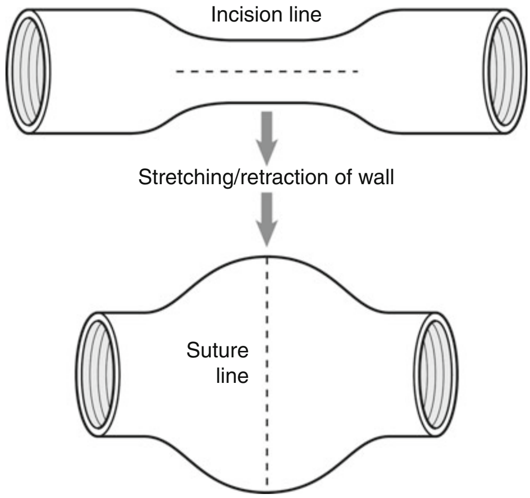


Fig. 5.3 Small-intestinal stricturoplasty (Reproduced, with permission, from Allen and Cameron (2004))

Intussusception: Barium enema can be used both as a diagnostic procedure, and if the reservoir of barium is elevated 1 m above the abdomen, hydrostatic reduction under radiological screening can be attempted as a therapeutic procedure. Reduction is signified when barium flows freely to the proximal loops of ileum. If hydrostatic reduction fails, or there is evidence of perforation/peritonitis, operative management is indicated. In this reduction may be facilitated by squeezing the distal colon and pushing the intussuscepted intestine proximally. If this is unsuccessful, then resection of the affected segment should be carried out.

5.6 Surgical Pathology Specimens: Laboratory Protocols

5.6.1 Biopsy Specimens

See Chap. 1. Formal Crosby capsule jejunal biopsies are larger than flexible EGD distal duodenal samples. They are usually submitted on filter

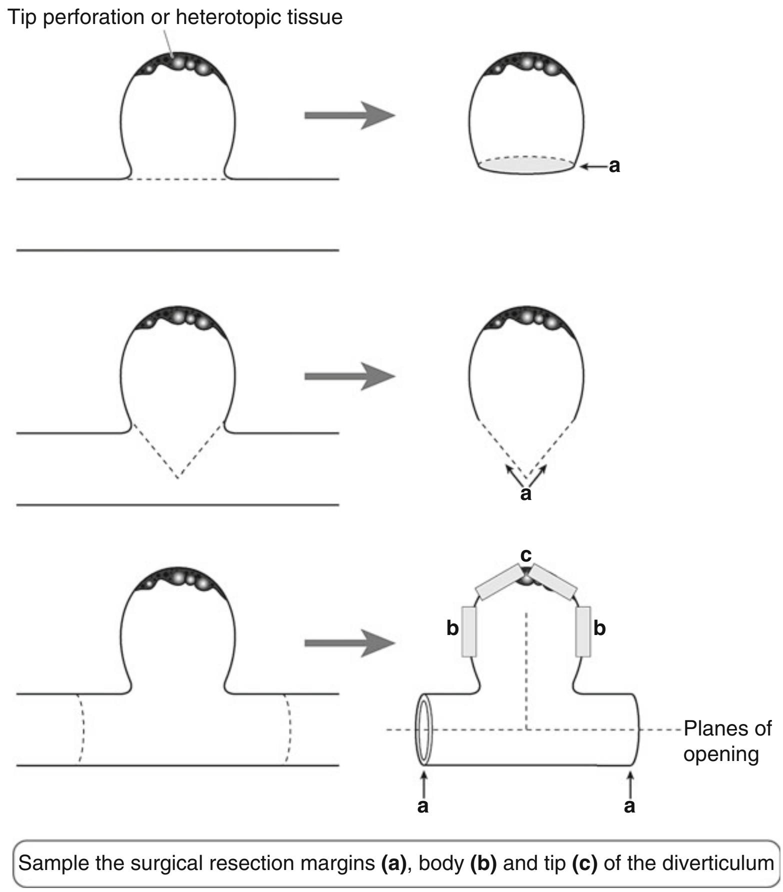


Fig. 5.4 Meckel's diverticulum – specimens (Reproduced, with permission, from Allen and Cameron (2004))

paper to allow orientation and inspection of the mucosal surface under a dissecting microscope and correlation with histology. Finger-like, cerebriform, and mosaic patterns correspond to normal, partially atrophic, and flat mucosae, respectively.

5.6.2 Resection Specimens

Specimen:

- Resection of small intestine can be for specific conditions such as Meckel's diverticulum or ischemia or, for obstruction due to various inflammatory, mechanical and neoplastic disorders.

5.6.2.1 Meckel's diverticulum:

- Measurements: Ileal base or segment – length × diameter (cm). Diverticulum – length × diameter (cm).
- Open the ileum longitudinally with blunt-ended scissors along its mesenteric border opposite the diverticulum, and then cut at right angles to this along the diverticulum toward its tip (Fig. 5.4). Photograph before and after dissection.
- Paint the external aspect of the diverticulum and fix by immersion in 10% formalin for 36–48 h.
- Inspect and describe the diverticulum (especially its tip), e.g., heterotopic mucosa, ulceration, perforation, abscess, fibrous bands, or tumor.
- Inspect and describe the ileal segment, e.g., inflammation, ischemia, or signs of intussusception.
- Transverse section the proximal and distal ileal limits of resection or ileal/diverticulum base.
- Sample normal appearing ileum, diverticulum, and its tip.

- Sample additional blocks as indicated by any macroscopic abnormalities present.

5.6.2.2 Ischemia:

- Measurements: Small bowel segment – length and maximum diameter (cm). Mesentery – length × depth (cm).
- Inspect and describe: hyperemia/duskiness of the serosa, perforation, constriction bands across the bowel or mesentery.
- Open longitudinally with blunt-ended scissors along the mesenteric border – inspect for mucosal thinning, ulceration, hemorrhage, necrosis, perforation, stricture formation, or any underlying tumor that might have precipitated volvulus or intussusception.
- Fix by immersion in 10% formalin for 36–48 h.
- Transverse section the proximal and distal limits of resection.
- Sample (two blocks minimum) representative macroscopically normal and abnormal areas as indicated (Fig. 5.5).
- Sample mesentery with constituent vessels.
- Sample mesenteric lymph nodes.

5.6.2.3 Obstructive enteropathy:

- The resection specimen is dictated by the site and nature of the abnormality and extent of any complications that are present. For example, jejunal ring diaphragm disease results in resection of the radiologically and macroscopically involved segment, whereas Crohn's terminal ileitis produces a limited right hemicolectomy. Intussusception complicated by ischemia needs a more extensive resection than would be otherwise necessary. A cancer operation will necessitate more radical dissection of mesentery and regional lymph nodes. In some instances, it is not possible clinically or macroscopically to distinguish between inflammatory and neoplastic ulcers or strictures – handling of the specimen must therefore cover these various options pending histopathological assessment.

  Initial procedure:
- Open longitudinally with blunt-ended scissors along the mesenteric border, avoiding any obvious areas of tumor or perforation.
- Measurements:

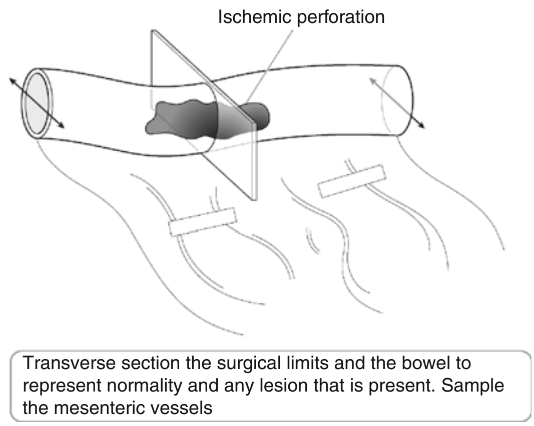
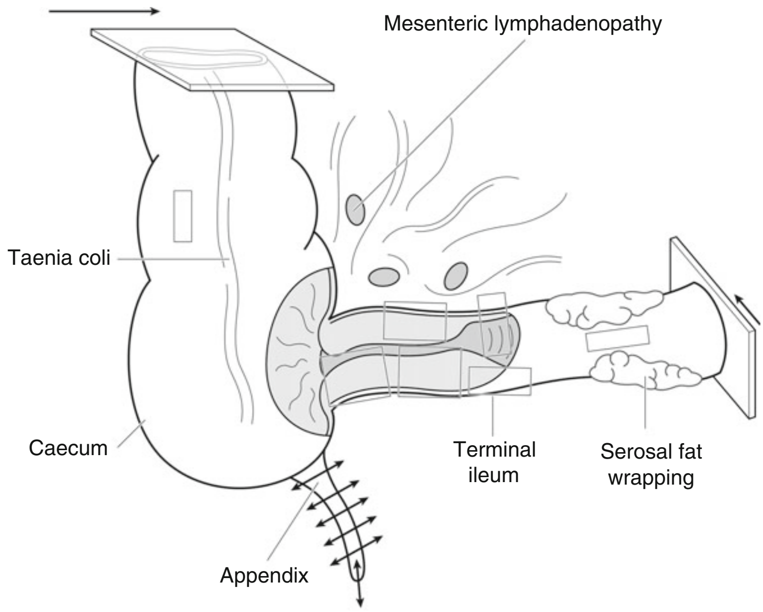


Fig. 5.5 Small bowel ischemia (Reproduced, with permission, from Allen and Cameron (2004))

  Lengths and maximum diameter (cm) of the parts present – duodenum, jejunum, ileum, cecum, ascending colon, and appendix.
  Lengths (cm) of ischemic, strictured, or hosepipe segments, intussusception.
  Maximum dimensions (cm) of any perforation(s), ulcer(s), polyp(s), and tumor(s).
  Distances (cm) of the abnormality from the proximal and distal resection limits.
- Photograph.
- Gently pack the bowel lumen with formalin-soaked lint and fix by immersion in 10% formalin for 48 h.

  Description:
- Tumor
  - Site: duodenal/jejunal/ileal/ileo-cecal valve. Lumenal/mural/extramural/mesenteric. Size: length × width × depth (cm) or maximum dimension (cm).
  - Appearance: Polypoid/nodular–inflammatory fibroid polyp, carcinoid, malignant melanoma, adenoma, carcinoma, multiple lymphomatous polyposis, GIST.
    Ulcerated/stricture – carcinoma, carcinoid, malignant lymphoma, metastatic carcinoma.
    Fleshy/rubbery – GIST, malignant lymphoma.
    Multifocal – metastases (carcinoma, melanoma), carcinoid, malignant lymphoma.
    Adjacent atrophic mucosa – EATCL
  - Edge: circumscribed/irregular.
- Crohn's disease: cobblestone mucosa/ulceration (aphthous, linear, confluent)/ring strictures/



1. Transverse section the surgical limits
2. Process the appendix as usual
3. Sample normal ileum and colon
4. Sample representative blocks of the hose pipe segment, any ulceration and adjacent mucosa.
5. Sample mesenteric lymph nodes

Fig. 5.6 Right hemicolectomy for Crohn's disease (Reproduced, with permission, from Allen and Cameron (2004))

hosepipe segments/fat wrapping/fistula/polyps or tumor/lymphadenopathy/sharp demarcation at the ileo-cecal valve/cecal or colonic disease/adhesions/abscess formation.

- Diaphragm disease: ring strictures – number, width, lumen aperture, intervening sacculation, mucosal ulceration.
- Extrinsic compression: constriction band/extrinsic tumor/lumen stenosis/mucosal ulceration/proximal dilatation.
- Intussusception: apex (inflammatory fibroid polyp, tumor, Meckel's, mesenteric lymphadenopathy)/ischemia/perforation/ileo-ileal/ileo-cecal.

Blocks for histology (Fig. 5.6):

Non-neoplastic conditions

- Sample by circumferential transverse sections the proximal and distal limits of resection.
- Sample macroscopically normal bowel.
- Sample representative blocks (a minimum of five) of any abnormality that is present to include its edge and junction with the adjacent mucosa, e.g., ulceration, stricture, fistula, perforation, serosal adhesions or constriction band, intussusception apex. These can be taken transversely or longitudinally depending on the anatomy and the abnormality present.
- Sample mesenteric lymph nodes and any adjacent structures, e.g., cecum, appendix, or ileocecal valve.

Neoplastic conditions

- Sample the nearest longitudinal resection margin if tumor is present to within <2 cm of it.
- Sample macroscopically normal bowel – usually one section but several if a multifocal condition, e.g., FAP or EATCL, is suspected.

- Serially section the bulk of the tumor transversely at 3–4 mm intervals.
- Lay the slices out in sequence and photograph.
- Sample (four blocks minimum) tumor and wall to show the deepest point of circumferential invasion. With tumors <1 cm diameter, fewer blocks will be possible. Include adjacent mucosa where feasible.
- Count and sample all lymph nodes – identify a suture tie limit node.
- Sample multifocal serosal tumor seedlings as indicated by inspection and palpation.

Histopathology report:

- Ischemia
  Necrosis – mucosal/transmural/gangrenous
  Resection limits – ischemic/viable
  Mesenteric vessels – thrombosis/embolism/vasculitis
  Miscellaneous – constriction band/volvulus/intussusception/stricture

- Crohn's disease
  Chronic transmural inflammation/granulomas/fissures/fistulae/abscess formation/ileal confined/ileo-cecal/appendiceal or resection limit disease/malignancy

- Intussusception
  Apex/secondary ulceration, stricture, ischemia or perforation/site (ileo-ileal/ileo-cecal).

- Neoplastic conditions
- Tumor type – adenocarcinoma/malignant lymphoma/GIST
- Tumor differentiation
  – Adenocarcinoma – well/moderate/poor
  – Malignant lymphoma – MALToma/mantle cell/follicular/Burkitt's/other, low grade/high grade
  – GIST – spindle cell/epithelioid cellularity/atypia/necrosis/mitoses/margins/size
- Tumor edge – pushing/infiltrative/lymphoid response.
- Extent of local tumor spread (for carcinoma).

pTis	Carcinoma in situ
pT1	Tumor invades lamina propria (pT1a) or submucosa (pT1b)
pT2	Tumor invades muscularis propria
pT3	Tumor invades through the wall into subserosa or perimuscular connective tissues (mesentery or retroperitoneum) with extension ≤2 cm
pT4	Tumor perforates the serosa or invades other organs/structures, e.g., mesentery >2 cm, small bowel loops, abdominal wall

- Lymphovascular invasion – present/not present.
- Regional lymph nodes: A regional lymphadenectomy will ordinarily include 6 or more lymph nodes.
  Duodenum: pancreatioduodenal, pyloric, hepatic, superior mesenteric nodes.
  Ileum/jejunum: mesenteric.
  Terminal ileum: ileo-colic, posterior cecal.

pN0	No regional lymph node metastasis
pN1	Metastasis in 1–3 regional lymph node(s)
pN2	Metastasis in 4 or more regional lymph nodes

- Excision margins
  Proximal, distal, and mesenteric limits of tumor clearance (cm).
- Other pathology
  FAP, Peutz-Jegher's, Crohn's disease, celiac disease, EATCL.

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6 Colorectum

Derek C. Allen, R. Iain Cameron, and Maurice B. Loughrey

6.1 Anatomy

The colon and rectum together measure between 125 and 140 cm in the adult. The colon is divided into the cecum (10 cm) and ascending (15 cm), transverse (40 cm), descending (25 cm), and sigmoid (25–40 cm) colons. The rectum measures approximately 13–15 cm (Fig. 6.1). The main function of the colon is absorption of water and electrolytes and the storage of fecal material until it can be excreted. The cecum is that part that lies below the ileocecal valve and receives the opening of the appendix. It is mostly surrounded by peritoneum, allowing it to be mobile in the right iliac fossa. The base of the appendix is attached to the posteromedial surface of the cecum. The ascending colon extends upward from the cecum to the inferior surface of the right lobe of liver. Here it becomes continuous with the transverse colon by turning sharply to the left, forming the right colic or hepatic flexure. The ascending colon is bound to the posterior abdominal wall by peritoneum covering its front and sides. The transverse colon extends from the hepatic flexure to the left, hanging downward and then ascending to the inferior surface of the spleen, where it turns sharply downward to form the left colic or splenic flexure. The transverse colon is completely surrounded by peritoneum with the transverse mesocolon being attached to its superior border (the length of the transverse mesocolon accounts for the variability in the position of the transverse colon) and the greater omentum to its lower border. The descending colon extends downward from the splenic flexure to the left side of the pelvic brim. It is bound to the posterior abdominal wall by peritoneum covering its sides and front. The sigmoid colon is continuous with the descending colon and hangs as a loop into the pelvic cavity. It is completely surrounded by peritoneum and a fan-shaped piece of mesentery attaches it to the posterior abdominal wall, thus allowing mobility. The rectum begins as a continuation of the sigmoid colon in front of the third sacral vertebra and follows the curvature of the sacrum and coccyx to where it pierces the pelvic floor to become continuous with the anal canal. Peritoneum covers the anterior and lateral surfaces of the upper third and the anterior surface of the middle third, the lower third being devoid of

D.C. Allen (✉)
Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: derek.allen@belfasttrust.hscni.net

R.I. Cameron
Histopathology Laboratory, Altnagelvin Hospital, Western Health and Social Care Trust, Londonderry, UK
e-mail: iain.cameron@westerntrust.hscni.net

M.B. Loughrey
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: maurice.loughrey@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_6, © Springer-Verlag London 2013

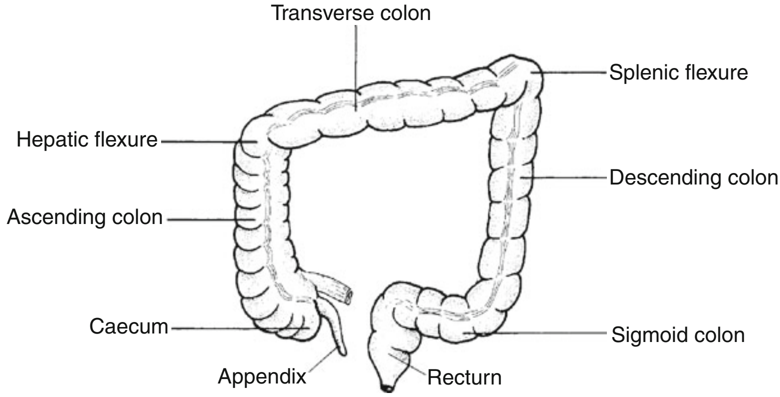

Fig. 6.1 Colorectum (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

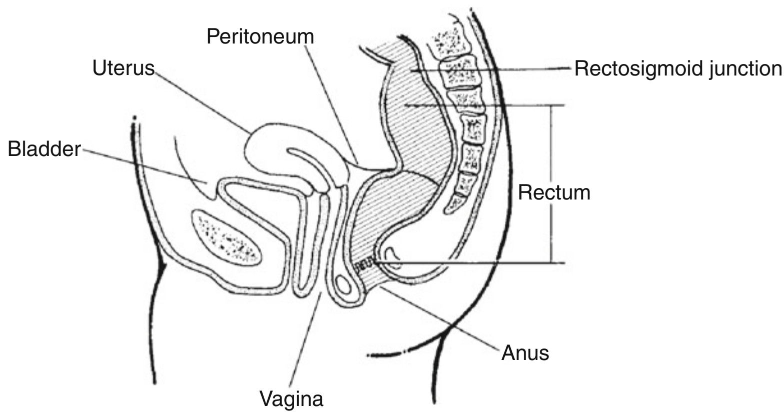

Fig. 6.2 Rectosigmoid and peritoneal reflection (lateral view) (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

a peritoneal covering. At the junction of the middle and lower third, the peritoneum is reflected onto the posterior surface of the upper vagina in the female to form the rectovaginal pouch (pouch of Douglas) and onto the upper part of the posterior bladder in the male, forming the rectovesical pouch (Fig. 6.2). The extent of serosal covering in the colorectum is illustrated in Fig. 6.3. The rectum is surrounded by a bilobed encapsulated fatty structure which is bulkier posterolaterally than anteriorly – the mesorectum.

The small and large intestines differ in their appearance in a number of ways:

- The longitudinal muscle in the small intestine forms a continuous layer, whereas in the colon it comprises three bands called *taeniae coli*. However, in the rectum, the taeniae coli come together to form a broad band on the anterior and posterior surfaces.
- The wall of the colon is sacculated, whereas the small intestine is smooth.
- The colon has "fatty tags" called *appendices epiploicae*.
- The permanent mucous membrane folds (*plicae circulares*) in the small intestine are not present in the colon.

Microscopically the colonic mucosa is made up of tubular crypts lined by columnar epithelium with mucin-secreting goblet cells and endocrine cells also being present.

Lymphovascular drainage:

Embryologically the gastrointestinal tract is divided into three segments (fore, mid, and hindgut) with each region being supplied by its own artery:

- Celiac artery supplies the foregut (distal esophagus to the mid-portion of the second part of the duodenum).
- Superior mesenteric artery supplies the midgut (mid-portion of the second part of the duodenum to the junction of the proximal two-thirds and distal third of the transverse colon).

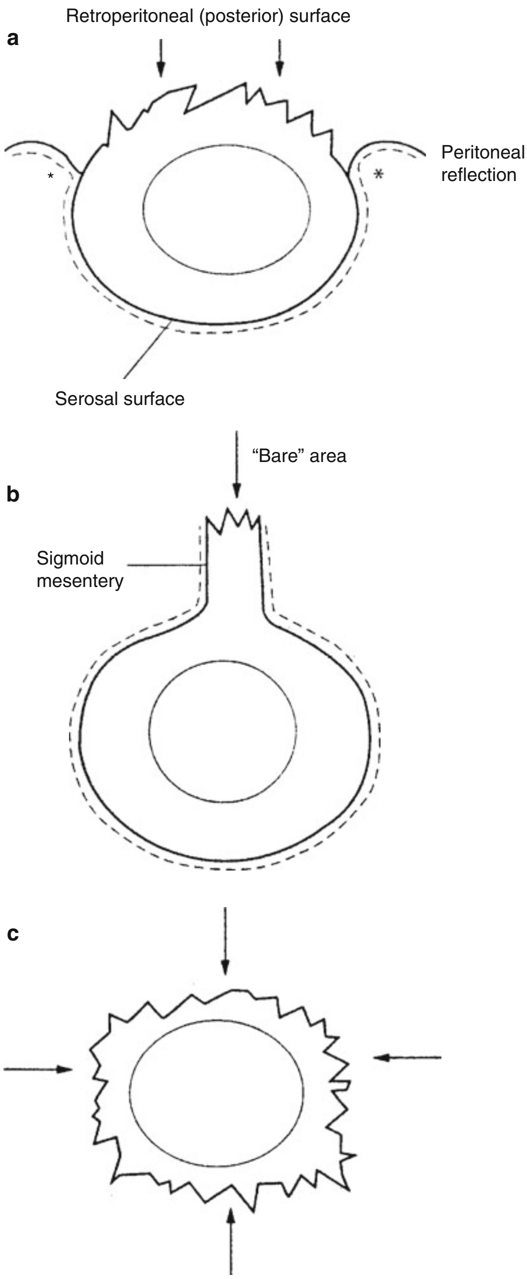

Fig. 6.3 Extent of serosal covering of the large intestine. *Arrows* indicate the "bare" non-peritonealized areas of different levels. (**a**) The ascending and descending colon are devoid of peritoneum on their posterior surface. (**b**) The sigmoid colon is completely covered with peritoneum, which extends over the mesentery. (**c**) The lower rectum lies beneath the pelvic peritoneal reflection. The *asterisks* in (**a**) indicate the sites where serosal involvement by tumor is likely to occur (Reprinted, with permission, from Burroughs and Williams (2000))

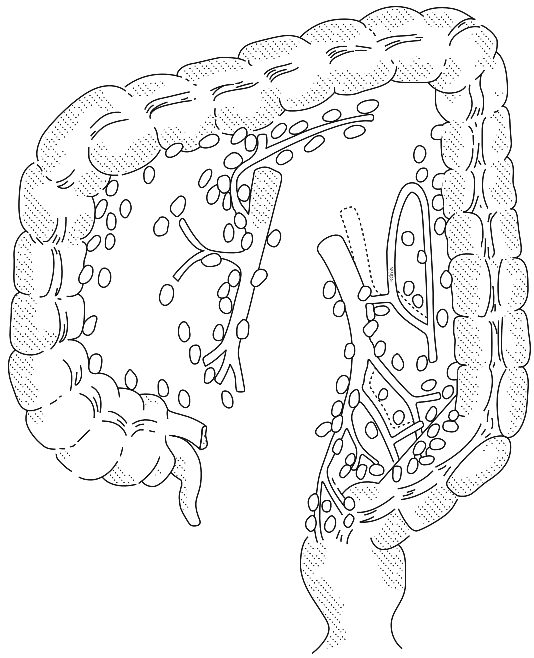

Fig. 6.4 Colorectum: regional lymph nodes are the pericolic, perirectal and those along the ileocolic, right colic, middle colic, left colic, inferior mesenteric, superior rectal (hemorrhoidal) and internal iliac arteries. (Used with permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

- Inferior mesenteric artery supplies the hindgut (distal third of the transverse colon to the junction of the superior and inferior half of the anal canal).

The rectum is also supplied by branches of the internal iliac artery. The anastomosis of the colic arteries around the concavity of the colon forms the marginal artery. The venous drainage of the colon is to the portal venous system and the rectum to the inferior mesenteric and internal iliac veins.

The lymphatics accompany the colic vessels draining to the superior and inferior mesenteric nodes. Those from the rectum drain into nodes (pararectal nodes) situated in the perirectal connective tissue (mesorectum) and thence to the superior mesenteric and internal iliac nodes (Fig. 6.4).

6.2 Clinical Presentation

There is considerable variability in the clinical presentation of colorectal disease.

Angiodysplasia usually presents with persistent occult bleeding or repeated small bleeds. In colonic ischemia/infarction there may be a history of arrhythmia or cardiac failure, and it may present acutely with abdominal pain and bloody diarrhea or less acutely with stricturing and symptoms of obstruction. Infective conditions usually lead to diarrhea, crampy abdominal pain, and fever. A careful antibiotic drug history should be obtained if pseudomembranous colitis is suspected. Inflammatory bowel disease may have an indolent presentation with lethargy, anorexia, and weight loss. However, more characteristic symptoms of ulcerative colitis include bloody diarrhea (>10 stools/day), urgency, and abdominal pain. Peritonitis and systemic sepsis may occur with toxic megacolon and perforation. Colorectal Crohn's disease characteristically presents with diarrhea. Obstruction due to stricturing may occur and fistulae leading to specific symptoms (e.g., colovesical – pneumaturia and recurrent urinary infection; rectovaginal – fecal discharge per vagina). Perianal fissures/fistulae and anorectal sepsis are relatively common in Crohn's disease. Extragastrointestinal manifestations of inflammatory bowel disease include finger-clubbing and erythema nodosum. Diverticular disease may present insidiously with lower abdominal pain or fistula formation (e.g., colovesical), or acutely as acute diverticulitis (abdominal pain, diarrhea, and localized peritonitis), pericolic abscess, obstruction (due to stricturing), perforation (generalized peritonitis), or hemorrhage (relatively rare).

Adenomatous polyps are usually asymptomatic, but large villous adenomas in the rectum may elicit an alteration in bowel habit, mucus per rectum (may cause pruritis ani), tenesmus (a sensation of incomplete evacuation), and electrolyte loss (particularly potassium). Colorectal carcinoma is usually asymptomatic early in its existence and later may present with nonspecific symptomatology such as an alteration in bowel habit, mucus PR, abdominal mass or discomfort, and PR bleeding (may be occult and can lead to iron-deficiency anemia). As a rule, the more proximal the tumor, the darker the blood. Tumors in the right colon are more likely to be ulcerated and so tend to present with PR bleeding, whereas tumors of the left colon are often constrictive and present with obstruction – this is compounded by the fact that the fecal material is more solid in the distal colon. Perforation may occur either through the tumor itself or distant and proximal to it due to obstruction and back pressure, e.g., in the cecal pouch. Rectal tumors can lead to tenesmus and local invasion may produce back pain and sciatica (involvement of the sacral plexus), rectovaginal fistula, etc. Liver metastases may cause clinical jaundice.

6.3 Clinical Investigations

- FBP – iron-deficiency anemia as a result of PR bleeding.
- U&E – electrolyte disturbance in diarrhea/mucus PR.
- LFTs – deranged in liver metastases or in the hepatobiliary manifestations of Crohn's disease.
- C-reactive protein/ESR – allows the activity of inflammatory bowel disease to be monitored.
- Stool culture – rule out infective colitis.
- Fecal occult blood – will detect occult bleeding.
- CXR – will detect pulmonary metastases.
- AXR – will show signs of colonic obstruction. Any dilatation of the colon >6 cm in diameter heralds the onset of toxic megacolon. In ischemic colitis, there will be dilated colon with characteristic "thumbprinting." In colovesical fistula, gas is present in the bladder. Free intraperitoneal gas will be seen in colonic perforation. In patients being investigated for chronic constipation, radio-opaque markers are ingested and an AXR is taken 5 days later with passage of <80% of the markers considered abnormal.
- Barium enema – widely used investigation in colorectal disease. There will be characteristic "thumbprint" filling defects caused by edematous mucosa in ischemia/infarction. The extent of ulcerative colitis can be assessed and in Crohn's disease it will show skip lesions, areas of stricturing, and any fistulae. It will reveal the presence of diverticula. Barium enema is useful in the detection of large polyps and carcinomas

with constricting tumors producing a characteristic "apple core" lesion. However, it will not reliably define rectal lesions.

- CT scan – will detect a pericolic abscess (can be drained under CT guidance) and is useful in showing the site of a tumor and any metastatic spread. CT colonogram and barium enema can be of use in a medically unfit patient or where there is a distal stricture not passable by the colonoscope.
- MRI scan and ELUS – allow assessment of local pelvic tumor spread in rectal carcinoma for staging purposes and selection for neoadjuvant therapy.
- PET CT scan – helps to distinguish recurrent carcinoma from post-radiotherapy fibrosis in the pelvis and to detect occult distant metastases.
- Angiography – will demonstrate a bleeding point, e.g., in angiodysplasia if there is active bleeding >2 ml/min.
- Cytology – examination of ascitic fluid or peritoneal washings.
- Endoscopy and biopsy – inspection and biopsy of the mucosa, determination of disease distribution, solitary or multiple lesions.
- Laparoscopy – staging laparoscopy may be undertaken and any peritoneal deposits biopsied.
- CEA serum levels – elevated in colorectal neoplasia particularly in metastatic or recurrent disease.

6.4 Pathological Conditions

6.4.1 Non-neoplastic Conditions

These comprise inflammatory (acute or chronic), mechanical, ischemic, and iatrogenic disorders.

6.4.1.1 Inflammatory Disorders

Acute proctocolitis: Infective or drug-induced, e.g., antibiotics, there is preservation of the mucosal architecture and acute inflammation with biopsies only being submitted if symptoms persist beyond several weeks. Infective cases (campylobacter, shigella, salmonella) are usually self-limited and culture positive in only 40% of cases. Drug-induced inflammation often responds to its withdrawal.

Chronic proctocolitis: Characterized by disturbance of the mucosal architecture and a chronic inflammatory cell infiltrate ± foci of active inflammation. Commonly due to idiopathic chronic inflammatory bowel disease (CIBD) but also seen overlying diverticulosis and pneumatosis coli, in infection (shigella, amoebiasis, schistosomiasis), obstructive enterocolitis, and with drugs. Microbiological culture and travel and drug history should always be ascertained in patients with chronic diarrhea.

CIBD – ulcerative colitis and Crohn's disease: The latter has been discussed previously (see Chap. 5) but can present either as isolated colonic disease or associated with ileitis. It is a segmental, transmural chronic inflammatory condition and there is often rectal sparing but anal disease (fissure, fistula, abscess) present. The segmental distribution, focality of inflammation, presence of granulomas, and ileal component are all useful diagnostic pointers in colonoscopic biopsy or resection specimens. Recurrence elsewhere in the gut is not uncommon despite surgical resection, and, because of this, Crohn's disease is a contraindication to pouch formation in restorative proctocolectomy. Occasionally it presents isolated to the appendix or sigmoid colon coexisting with diverticulitis.

In contrast to this ulcerative colitis is a diffuse, chronic active mucosal inflammatory condition involving the rectum and a contiguous length of large intestine, e.g., left-sided proctocolitis or pancolitis. It is of variable severity with episodic exacerbations and remissions – acute fulminant colitis may be complicated by severe hemorrhage, toxic dilatation or megacolon, perforation, and peritonitis. Other complications include mucosal dysplasia and malignancy (usually adenocarcinoma) in extensive disease of long-standing duration (pancolitis >10 years). Villiform or polypoid DALMs (dysplasia-associated lesions or masses) can be difficult to distinguish from the much more common inflammatory mucosal polyps and may harbor underlying adenocarcinoma. Alternatively dysplasia may occur in flat mucosa, and colonoscopic surveillance of chronic colitis involves sequential mucosal sampling as well as target biopsy of any macroscopic abnormality.

Biopsy orientation onto a polycarbonate strip aids subsequent localization of any histological abnormalities. Macroscopically ulcerative colitis shows mucosal granularity, linear or confluent ulceration, and polyps of varying size. The terminal ileum is only involved in severe pancolitis over a length of 1–2 cm (backwash ileitis) and although there is usually proctitis, the rectum may be spared due to treatment effects, e.g., predsol enemas. Extraintestinal effects include arthritis, iritis, and, in the liver, primary sclerosing cholangitis which can lead to cirrhosis and cholangiocarcinoma.

In a minority of cases, clear distinction cannot be made between ulcerative colitis and Crohn's disease on macroscopic/colonoscopic and microscopic examination – so-called indeterminate colitis (in a resection specimen) or CIBD, unclassified (in biopsy material).

Diversion proctocolitis: Follows fecal stream diversion, e.g., after ileostomy or colostomy for tumor, trauma, or CIBD. The defunctioned segment develops florid reactive lymphoid hyperplasia which can be mucosal or transmural, mimicking or superimposed on an underlying inflammatory disorder such as CIBD. Persistent severe symptoms may necessitate surgical excision of the segment, e.g., the rectal stump following colectomy for ulcerative colitis.

Microscopic colitis: Minimal inflammation may be apparent grossly or histologically for various reasons, e.g., treated CIBD, postinfection, drug ingestion, uremia, stercoral trauma, etc. However, microscopic colitis which causes chronic, voluminous watery diarrhea is radiologically and colonoscopically normal. It occurs in middle-aged to elderly women and has variable associations with HLA type, autoimmune diseases, and NSAID ingestion. Diagnosis is by histology with a normal architecture and transmucosal infiltrate of chronic inflammatory cells. Its main variants, collagenous and lymphocytic colitis, show a thickened subepithelial collagen band and excess surface intraepithelial lymphocytes, respectively. Not infrequently there is spontaneous resolution or response to anti-inflammatory therapy.

Infective proctocolitis: Investigation includes microbiological culture with microscopy for cysts (amoebiasis) and ova (schistosomiasis). Infection should be considered particularly where there is a history of travel or immunosuppression, e.g., AIDS, chemotherapy or post-transplant. In immunosuppression, infection with unusual opportunistic organisms can occur, e.g., cryptosporidiosis, atypical mycobacteria.

6.4.1.2 Mechanical Disorders

Melanosis coli: Characterized by pigmented macrophages in the lamina propria that impart a dusky mucosal appearance mimicking ischemia. The pigment is lipofuscin and degenerative in nature thought to relate to cellular apoptosis. There is an association with use of laxatives and bowel dysmotility.

Volvulus: Usually comprises a markedly dilated atonic sigmoid colon in either Africans (due to a high-fiber diet with bulky stools) or constipation-related-acquired megacolon in the elderly. The sigmoid loop twists on its mesentery, obstructs, and may become secondarily ischemic. Resection specimens are often dilated, thinned, and featureless. Melanosis coli may be present. Congenital megacolon and Hirschsprung's disease are discussed elsewhere (see Chap. 21).

Pneumatosis coli: Submucosal gas cysts lined by macrophages and giant cells with overlying mucosal chronic inflammation or pseudolipomatosis. There is an association with volvulus, constipation, diverticulosis, and chronic obstructive airways disease. Pathogenesis relates to retroperitoneal tracking of air into the bowel mesentery, abnormal luminal gas production linked to the increased intraluminal pressure seen in the above disorders, and introduction of gas during endoscopy. About 50% of cases resolve, but recurrent or severe lesions may require colectomy of the involved segment.

Obstructive enterocolitis: Continuous or segmental areas of inflammation or ulceration adjacent to or distant from an obstructing distal lesion, e.g., annular carcinoma or diverticulosis. Small bowel may also be involved with mimicry of Crohn's disease. A dilated, thinned cecal pouch can become ischemic and perforate.

Diverticulosis: Very common in Western society due to a low-fiber diet, high intraluminal pressure, and subsequent transmural mucosal herniation in the sigmoid colon through points of vessel entry from the mesentery. Presentation is with altered bowel habit, per rectum bleeding, left iliac fossa pain or a mass. The latter implies diverticulitis with possible perforation and pericolonic reaction/abscess formation. Portal pyemia, liver abscesses, and peritonitis can ensue. The diverticular segment is thickened and contracted with muscle coat hypertrophy and visible diverticular pouches in the muscularis and mesenteric fat. They may be filled and obstructed with fecal or vegetable debris, and ulcerated with a coating of pericolonic exudate and abscess. The concertina-like redundant mucosal folds can show crescentic colitis due to abrasion of their tips by the passing fecal stream. Occasionally the chronic inflammation may be transmural and granulomatous mimicking or coexisting with Crohn's disease. Treatment is often conservative, e.g., by diet alteration, but severe or complicated cases require colectomy. Copresentation with an occult carcinoma within the strictured segment must be excluded by careful pathological examination.

Mucosal prolapse: A mechanism producing reactive mucosal changes of crypt hyperplasia, smooth muscle thickening of the lamina propria, and variable surface erosion. It is common to a number of situations including solitary rectal ulcer syndrome (SRUS), inflammatory cloacogenic polyp, diverticular-related crescentic colitis, mucosa adjacent to a polyp, stricture or tumor, stercoral trauma, and the mucocutaneous junction of stomas. In SRUS, there is a history of abnormal anterior rectal wall descent due to straining at defecation. This results in induration of the wall that can mimic a plaque of tumor on palpation and rectoscopy. Biopsy is diagnostic and treatment is usually conservative, related to better stool habit – occasional cases require resection of the involved sleeve of mucous membrane with apposition and plication of the intervening muscle (Delorme's procedure).

6.4.1.3 Ischemic Disorders

The pathogenesis of intestinal ischemia has been previously discussed and in the large intestine is often due to mesenteric vascular insufficiency because of systemic hypotension (myocardial infarction, cardiac arrhythmia, blood loss) or mesenteric atheroma/thrombosis/embolism. Acute lesions may resolve if mucosa-confined but are potentially fatal if transmural. Late or chronic ischemia has a predilection for the splenic flexure and rectosigmoid watershed areas of vascular supply. This can result in nonspecific ulceroinflammatory and stricturing lesions – end-stage changes that can be produced by various other conditions, e.g., CIBD, infection (*E. coli* 0157:H7 bacterium), pseudomembranous colitis due to Clostridium difficle overgrowth, obstructive enterocolitis, and stercoral trauma. Occasional cases are due to vasculitis or amyloid infiltration. Assessment of resection limit viability and mural/mesenteric vessels is necessary in ischemia.

A vascular abnormality that can present with iron-deficiency anemia in elderly patients is colonic angiodysplasia. Thought to be degenerative in nature due to increased intraluminal pressure compressing mural vessels, the commonest site is the cecum. Operative injection of radio-opaque contrast may be needed to demonstrate areas of vascular ectasia so that targeted blocks can be sampled. The ectatic vessels involve the submucosa and lamina propria.

6.4.1.4 Iatrogenic Disorders

These include drugs, radiation therapy, and graft versus host disease.

Drugs: NSAIDs should always be considered in the presence of any unusual colitis, localized ulceration, stricture, perforation, or mucosal diaphragm formation. Antibiotics can commonly cause dysfunctional diarrhea, an acute proctocolitis, or, particularly in the elderly, pseudomembranous colitis. The latter is due to the production of Clostridium difficile toxin leading to ischemic-type lesions with yellow surface plaques of acute inflammatory and fibrinous pseudomembrane. Severe cases result in end-stage ulceration and colectomy may be indicated although initial treatment is with appropriate antibiotics.

Radiation therapy: Acute and chronic phases with the potential for mucosal healing and usually produced by radiotherapy for pelvic (uterine cervix, rectum, prostate) or retroperitoneal cancer. Acute radiation proctocolitis is normally self-limited and seldom biopsied. Chronic changes result in mucosal atrophy, hyaline fibrosis, vascular thickening, and strictures.

Graft versus host disease: Immunosuppressed bone marrow transplant patients risk developing a range of acute and chronic changes similar to those seen in radiation damage.

6.4.2 Neoplastic Conditions

Serrated polyps: Simple hyperplastic or metaplastic polyps are benign and more prevalent in the left colon/rectum with increasing age. Sessile serrated polyps/adenomas are recently recognized lesions thought to be precursors of right-sided serrated pathway carcinomas, typically in elderly females. Traditional serrated adenomas are more commonly distal and share some morphological features and cancer risk of conventional adenomas.

Adenoma (*conventional*): Designated as tubular, tubulovillous, or villous, depending on the relative proportions of glands and fronds present and composed of low- or high-grade dysplastic epithelium. Increasing in frequency with age, and in the left colon, the risk of malignancy relates to the size (>2 cm = 40–50% risk), degree of villous morphology, and grade of dysplasia. Tubular adenomas are nodular and tend to develop a distinct stalk, whereas villous lesions are sessile. Stalked adenomas can twist and prolapse (typically in the sigmoid colon) resulting in glandular herniation into the submucosa that mimics invasive carcinoma – the low power lobular configuration, the presence of lamina propria hemosiderin, and lack of stromal fibrous desmoplasia are useful histological clues to benignity. Invasive carcinoma is defined by the presence of neoplastic epithelium infiltrating submucosa, and in stalked adenomas, polypectomy may be considered therapeutic if the tumor is well or moderately differentiated and does not show lymphovascular invasion or involvement (<1 mm) of the diathermied polyp base. Otherwise colonic resection is required and, therefore, good orientation of the adenoma to its stalk and assessment of the base are crucial. In contrast, invasion in a sessile adenoma accesses true mural submucosa, and colonic resection is usually considered more appropriate based on greater risk of lymph node metastases, unless the patient is very elderly or medically unfit. Local mucosal resection is an option, but in such cases further radical surgery is required if the cancer involves muscle coat, the base of the specimen, lymphovascular channels, or is poorly differentiated.

It is not unusual for patients to have several sporadic adenomas, but in FAP, there are hundreds or thousands with progression to colorectal cancer 20–30 years earlier than average, indicating a need for prophylactic colectomy. There is also a strong association of FAP with duodenal adenomas and periampullary carcinoma.

Flat adenomas are less common and difficult to identify macroscopically without the use of magnification or dye spray technique. They have proportionately higher grades of dysplasia and frequency of carcinoma and may account for a proportion of the 30% of carcinomas without an identifiable adenoma at their edge.

In the UK, the National Bowel Cancer Screening Programme targets the detection of adenomas in asymptomatic patients in an attempt to prevent cancer formation. It invites the population aged 60–69 years to participate in 2-yearly fecal occult blood testing. About 2% have a positive result and are referred for colonoscopy (or CT colonogram, depending on fitness and availability of local resources) and 50% of these will have a detectable abnormality (80% adenoma or tumor: 20% others, e.g., CIBD). Initial results have shown a significant yield of precancerous adenomas and a shift toward a higher frequency of early stage (Dukes' A) cancers. Future plans for the screening program include age extension to 75 years and one-off flexible sigmoidoscopy at age 55 years.

Adenocarcinoma: Comprising the vast majority of colorectal malignancies, 80–85% are moderately differentiated adenocarcinoma of no special type. A minority are mucinous, signet ring cell, or poorly differentiated. Distribution is throughout the colorectum although rectosigmoid is the commonest site (50% of cases), 10–15% of sporadic cases are multiple, occurring either synchronously or subsequently/metachronously. Predisposing conditions are chronic ulcerative colitis, FAP, and hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome. In HNPCC, there is a tendency for right-sided cancers, which may be multiple, mucinous, or poorly differentiated and with a family history of cancer at a younger age (<50 years), also involving other sites, e.g., uterus, stomach, ovary, ureter, and small intestine. Its genetic basis is different from that of sporadic colorectal cancer, due to a deficiency in one of the DNA mismatch repair proteins caused by a heritable germline mutation.

As previously noted, the cancer site and its macroscopic growth pattern influence clinical presentation. Important prognostic indicators are the extent of local tumor spread, a circumscribed or infiltrative margin, involvement of the serosa, longitudinal or mesocolic/mesorectal resection margins, and tumor perforation. Tumor present within ≤1 mm defines involvement of the mesenteric margin irrespective of whether it is nodal, lymphovascular or direct spread. Generally a macroscopic clearance of 2–3 cm from a longitudinal margin is satisfactory unless histology shows the cancer to be unusually infiltrative or poorly differentiated. All mesenteric lymph nodes should be identified, counted, and sampled (aiming for a departmental median count of at least 12 nodes per specimen) and a suture tie limit node identified – in some colectomy specimens this may mean more than one. Involvement of adjacent organs or structures (e.g., abdominal wall) is documented and predisposing lesions such as adenoma(s) or colitis represented. Multiple tumors are dissected and staged individually with respect to mural and nodal spread.

Other cancers: Carcinoid tumors are usually small incidental mucosal rectal polyps, GISTs are rare, and malignant lymphoma can complicate ulcerative colitis or AIDS.

Prognosis: Relates mainly to the depth of tumor spread, lymph node involvement, and adequacy of local excision with overall 5-year survival 35–40%. Cancers confined to the mucous membrane or wall do much better than those that invade beyond this or show nodal disease. Adverse prognostic indicators also include a mucinous character, poor differentiation, tumor perforation, obstruction, and resection margin involvement. It is estimated that about 50% of patients are cured, 10% die from local recurrence and 40% from lymphatic and vascular spread. Treatment is typically surgical excision with adjuvant chemotherapy considered for cancers showing poor differentiation, nodal, peritoneal, and/or extramural vascular spread, tumor perforation, or resection margin involvement. Rectal cancers often receive 5-day short-course preoperative radiotherapy in an attempt to downstage the lesion or facilitate resection. This usually does not produce the marked macroscopic and histological features of regression that can be seen with the alternative 6-week-long course of neoadjuvant chemoradiotherapy. The latter is given to patients with clinically fixed tumors that show significant spread on MRI scan into the mesorectum, its nodes, or near its investing fascia (circumferential radial margin: CRM).

6.5 Surgical Pathology Specimens: Clinical Aspects

6.5.1 Biopsy Specimens

A number of procedures can be undertaken to obtain biopsy specimens from the colorectal mucosa:

Proctoscopy is used to inspect the distal rectum and anal canal.

Sigmoidoscopy can be carried out by using either a rigid or flexible sigmoidoscope. Rigid sigmoidoscopy is usually done without bowel preparation at the bedside or in the outpatient clinic. A hollow rigid plastic tube measuring 25 cm in length with an attached light and air supply is inserted into the rectum up to the distal sigmoid colon. Forceps can be passed through the tube to biopsy any lesion visualized. The scope is also used to assess tumor fixation and its distance from the anus. Flexible sigmoidoscopy (and colonoscopy) involves formal bowel preparation. A flexible fiber-optic endoscope is inserted and works in the same way as an upper GI endoscope, with a controllable tip and ports for inserting instruments, e.g., forceps, snare, etc. This should visualize up to the proximal sigmoid colon.

Colonoscopy is carried out using a colonoscope which is essentially a longer sigmoidoscope, with scopes of different lengths available (ranging from 140 to 185 cm). An experienced endoscopist should be able to pass the endoscope through the ileocecal valve to visualize the terminal ileum. Intraoperative endoscopy can be used during a laparotomy to, for instance, locate lesions, e.g., polyps found by barium enema that require localized resection and which cannot be palpated by the surgeon.

Biopsy specimens can be taken from the colonic mucosa by forceps passed through the endoscope in much the same way as that used in upper GI endoscopy. The colonoscopic management of polyps is important and depends on the size and type of polyp:

- Large pedunculated polyps can be removed by "snaring." A circular wire is passed over the polyp onto its stalk. An electrical current is passed along the wire to coagulate the vessels in the base of the stalk, which is then transected by closing the wire. If the stalk is large, adrenaline can be injected into the base to minimize bleeding. The polyp is retrieved by using the snare, a Dormia basket or suction.
- Smaller polyps (5–7 mm) can also be snared and removed by suction.
- Polyps <5 mm can be removed by "hot biopsy." Biopsy forceps grasp the polyp and a current is applied to electrocoagulate the base, and then the head of the polyp is pulled off by the forceps.
- Broad-based sessile polyps can either be removed piecemeal using the snare or by injection polypectomy. This involves injecting adrenaline solution into the submucosa around the polyp, raising it, and allowing it to be snared completely. This method can be used for polyps up to 5 cm in diameter.
- In patients with multiple small polyps, these can be highlighted by spraying dye onto the mucosa. This will reveal polyps 0.5 mm and larger as pale areas on a blue background.
- If the endoscopist is concerned that a polyp may be malignant, the site of polypectomy can be marked by tattooing the bowel mucosa with India ink. This allows the site to be revisited at a later date.

Submucosal lesions can be sampled by endoscopic FNA. Colonoscopy may also be used as a therapeutic tool, e.g., foci of angiodysplasia may be coagulated using hot biopsy forceps.

6.5.2 Resection Specimens

Resection of the colon and rectum is performed for a wide variety of both non-neoplastic and neoplastic conditions (Table 6.1), the type of procedure depending on the site and nature of the lesion, e.g., a malignant tumor, will require a more extensive resection than that for a large adenomatous polyp. Likewise the extent of mesenteric resection will depend on the type of lesion, i.e., wide mesenteric resection for neoplastic lesions and limited resection for non-neoplastic conditions. It also depends on the "intention" of the surgery for a malignant condition, i.e., a wide mesenteric resection with proximal ligation of vessels and, hence, removal of lymph node groups if the intention is curative, or limited, if the disease is advanced and the intention is palliative. The variety of terms used to describe the different types of colonic resection (colectomy) is depicted in Fig. 6.5. Choice is also determined by the distribution or multiplicity of lesions detected at preoperative colonoscopy. Planned elective laparoscopic sur-

Table 6.1 Colorectal resections

Specific	Diverticular disease
	Volvulus
	Pneumatosis coli
	Colonic angiodysplasia
	Rectal stump (CIBD, diversion proctitis)
	Rectal mucosa (prolapse)
Ulceroinflammatory	Ulcerative colitis
	Crohn's disease
	Pseudomembranous colitis
	Ischemia
Neoplasia	Large or multiple adenomas
	Carcinoma
	Malignant lymphoma

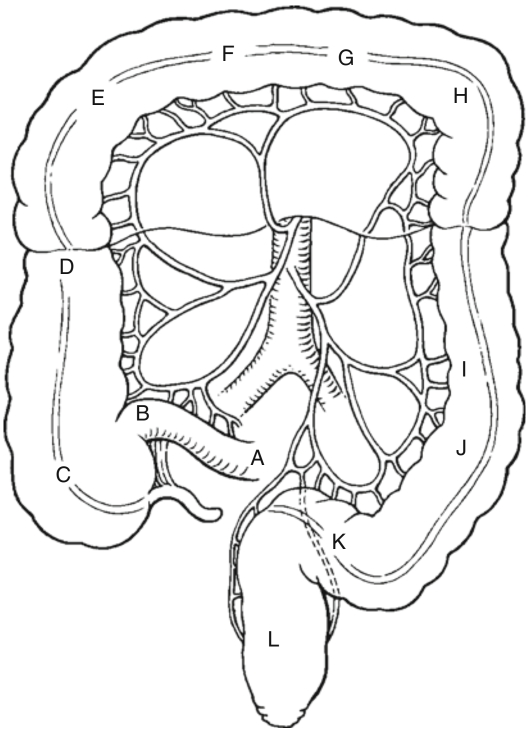

Fig. 6.5 Types of colonic resection. A → C Ileocecectomy; ±A + B → D Ascending colectomy; ±A + B → F Right hemicolectomy; ±A + B → G Extended right hemicolectomy; ±E + F → G ± H Transverse colectomy; G → I Left hemicolectomy; F → I Extended left hemicolectomy; J + K Sigmoid colectomy; ±A + B → J Subtotal colectomy; ±A + B → L Total colectomy; ±A + B → L Total proctocolectomy; L Proctectomy (Reprinted, with permission, from Fielding and Goldberg (2002))

gery is the preferred option – open abdominal surgery (laparotomy) may be necessary for extensive disease, or if the patient presents as an acute emergency.

6.5.2.1 Resection in Neoplastic Conditions

Adenomatous polyps – As discussed above, the majority of adenomatous lesions can be removed by endoscopic techniques. However, large sessile polyps >5 cm in diameter and occupying more than one-third of the colon circumference should be removed by a localized resection. Sessile adenomas in the rectum can be removed by *transanal submucosal resection*. In this procedure, adrenaline solution is infiltrated into the submucosa around the lesion and the mucosa is incised by scissors 1 cm from the lesion. This can then be easily lifted off the circular muscle in a single piece and the mucosal defect is closed by sutures. This "advanced" polypectomy with submucosal infiltration is termed endoscopic mucosal resection (EMR). Extensions of this are endoscopic submucosal dissection (ESD) and transanal endoscopic microsurgery (TEMS) providing complete mucosectomy to full-wall-thickness specimens. These "big biopsy" specimens are both diagnostic (benign vs. malignant) and potentially therapeutic, allowing assessment of risk factors that might necessitate subsequent radical surgery, e.g., deep margin status, substaging of submucosal invasion, and lymphovascular involvement in carcinoma. Occasionally large rectal polyps may require formal proctectomy or anterior resection.

Malignant lesions – The type of resection for colonic tumors will depend on the site of the lesion and the intent of the surgery. As previously stated, the colonic lymphatics accompany the main blood vessels and the extent of resection depends on the lymphatic clearance required. In cancer operations of curative intent, the affected colon with its lymphovascular mesenteric pedicle is resected. Continuity is restored by either an ileocolic or colocolic end-to-end anastomosis. However, on occasion, an end ileostomy/colostomy may be required if the surgeon thinks that primary anastomosis would be compromised (e.g., if there is extensive intraperitoneal contamination).

The curative resection of rectal tumors may be carried out by one of two methods:

- *Anterior resection of rectum* – In this procedure, the rectum is mobilized by entering the fascial plane around the mesorectum. This allows the rectum to be removed en bloc with the mesorectum which contains the initial draining lymphovascular channels and nodes (low anterior resection and total mesorectal excision – TME) (Fig. 6.6a). Continuity is reestablished by a stapling device forming an end-to-end colorectal anastomosis. Occasionally, in low anastomoses, a protective loop colostomy/ileostomy may be fashioned to divert the fecal stream. This can be closed at a later date. To obtain an adequate length of colon to form a safe anastomosis, the splenic flexure will usually need to be mobilized. On occasion, the

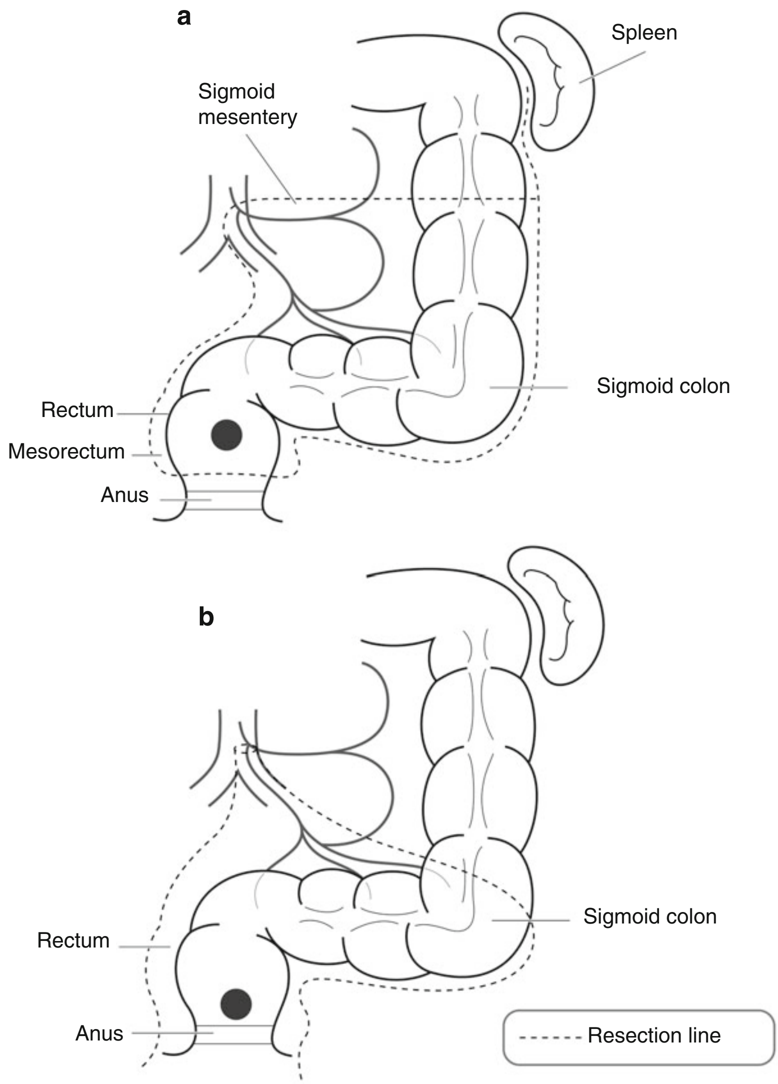

Fig. 6.6 (**a**) Resection in low anterior resection and total mesorectal excision; (**b**) resection in abdominoperineal excision (Reproduced, with permission, from Allen and Cameron (2004))

spleen may be damaged during this mobilization and a splenectomy would then have to be performed. In cases where the tumor is in the proximal rectum, a high anterior resection and mesorectal division can be employed. This entails division of the rectum and mesorectum 5 cm distal to the tumor and allows a larger rectal stump for anastomosis.

- *Abdominoperineal* (*AP*) *excision of rectum* (*APER*) – In this procedure, the rectum is mobilized as above and the colon is divided at the apex of the sigmoid. The anal canal and distal rectum are then resected from below via the perineal route (Fig. 6.6b). The entire rectum (and mesorectum) and anus are then removed en bloc. The perineal wound is closed and a permanent end colostomy is fashioned in the left iliac fossa using the transected end of the sigmoid colon.

Until the early 1980s, anterior resection was used in less than 50% of patients with rectal tumors, i.e., those in the proximal rectum. However, it is now used for approximately 90% of tumors in the rectum. Initially it was feared that because less tissue is excised and the clearance of the distal margin is not as great during anterior resection, there would be increased local recurrence rates if anterior resection was used for low rectal tumors. However, it appears that the degree of lateral clearance is similar in the two procedures and that a distal clearance of 2 cm is adequate to prevent local recurrence. Given the physical and psychological problems associated with a permanent colostomy, and the higher incidence of bladder and sexual problems in patients undergoing AP resection, it is felt that a sphincter-saving procedure (i.e., anterior resection) should be employed whenever possible. However, tumors extending to less than 2 cm from the anorectal junction (i.e., less than 6 cm from the anal verge) should be treated by AP resection.

Occasionally, in a medically unfit patient, localized resection is used for a well-differentiated, pT1 rectal cancer that is <3 cm in diameter. Accurate preoperative staging is crucial in selection of these patients and some may then need to proceed to salvage resection if adverse pathological features are identified in the pathological specimen, e.g., poor differentiation, lymphovascular involvement, or invasion of the deep margin or muscle coat. Sometimes patients with obstructing cancers undergo piecemeal resection (essentially palliative and non-curative), partial laser ablation, or stenting to restore intestinal continuity and avoid the risk of perforation. This may even allow resection to be carried out more safely at a later date.

6.5.2.2 Resection in Non-neoplastic Conditions

Hartmann's procedure – This is one of the most commonly used emergency operations for colorectal disease. Although this was initially devised for the elective treatment of proximal rectal tumors, it is now usually used in the emergency setting to treat conditions such as perforated diverticular disease (most commonly), perforated tumor, etc. The procedure itself is defined as resection of the sigmoid colon (and a variable length of proximal rectum if required) with the fashioning of a terminal-end colostomy and closure of the rectal stump. The colostomy may be reversed at a later date by forming an end-to-end colorectal anastomosis.

Nonacute-presenting diverticular disease is usually treated surgically by either sigmoid colectomy or left hemicolectomy depending on the extent of the disease.

Surgery in colorectal inflammatory bowel disease – The surgical management of colorectal Crohn's disease is similar to that in the small intestine (see Chap. 5). Namely surgical intervention is reserved for those in whom medical management has failed (i.e., minimal resection of the diseased segment) or who are suffering complications, e.g., obstruction, pericolic abscess, fistula, etc.

As in Crohn's disease, close liaison between surgeons and physicians is required in the management of ulcerative colitis. Emergency surgery is needed in cases of acute severe colitis and/or toxic megacolon. The procedure of choice is a subtotal colectomy and end ileostomy with the proximal end of the rectum brought to the surface in the form of a mucus fistula. This spares an already sick patient the added trauma of pelvic surgery and, if ulcerative colitis is confirmed by histological examination, allows an ileoanal pouch procedure to be considered in the future. Prior to the mid-1970s, patients with refractory ulcerative colitis underwent a panproctocolectomy (removal of the colon, rectum, and anus) with a permanent end ileostomy. However, in 1976, the procedure of *restorative proctocolectomy* was introduced and removed the need for a permanent ileostomy in suitable patients. In this procedure, the entire colon and rectum are removed and the mucosa may be stripped from the upper anus above the dentate line (some surgeons prefer to leave this mucosa intact as it is thought to improve future continence). An ileal reservoir (pouch) is formed (Fig. 6.7) and an ileoanal anastomosis is fashioned. A protective loop ileostomy is formed as close to the ileal pouch as possible and this can be closed at a later date (usually 2–3 months) after healing has been completed. A proportion of these patients (approximately 10%) may develop "pouchitis" – increased frequency of stool and feeling generally unwell. The exact etiology of this is unknown but some feel it may be due to bacterial overgrowth in the pouch.

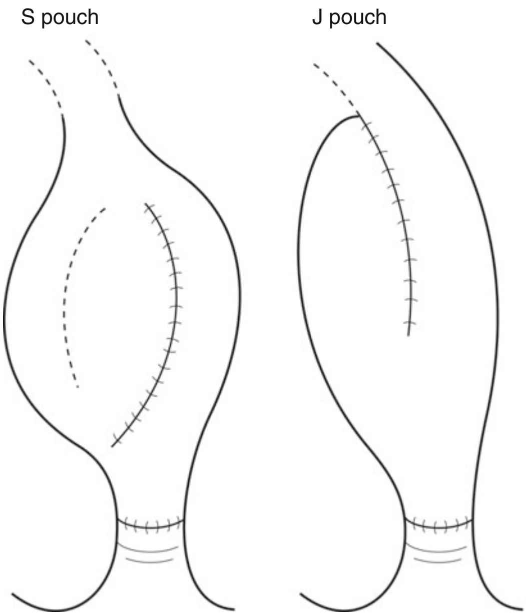

Fig. 6.7 Two popular designs of ileal pouch (Reproduced, with permission, from Allen and Cameron (2004))

Angiodysplasia – If bleeding is severe enough to require surgical intervention, and if conservative treatment such as endoscopic coagulation has been unsuccessful, the procedure of choice will be dictated by the site of the bleeding point(s). However, if the site of bleeding cannot be discovered, a total colectomy with ileorectal anastomosis (or end ileostomy, rectal mucus fistula, and reversal at a later date) may be required.

6.6 Surgical Pathology Specimens: Laboratory Protocols

6.6.1 Biopsy Specimens

For biopsy specimens and local mucosal resections see Chap. 1.

6.6.2 Resection Specimens

Specimen:

- Colorectal specimens are for a range of either specific, ulceroinflammatory, or neoplastic conditions (Table 6.1). These can be complicated by obstruction with or without associated enterocolitis or perforation or show evidence of background disease such as CIBD or FAP. The resection specimen is dictated by the site and nature of the abnormality and extent of any complications or predisposing lesions that are present.

Initial procedure:

- In general, specimens are measured, opened with blunt-ended scissors along the antimesenteric border, and then blocked longitudinally (but see diverticular disease and tumor) following gentle washing out of fecal debris, pinning out with avoidance of unnecessary traction, and immersion in 10% formalin fixative for 48 h. Photographs may be taken before and after dissection.
- When opening avoid areas of perforation or tumor. Tumor segments may either be left unopened for fixation and subsequent transverse slicing or carefully opened – the latter gives better fixation, but the cut should be guided by palpation with the index finger to avoid disturbing the relationship of the tumor to the circumferential margin.

6.6.2.1 Diverticular disease

- Measurements: length × diameter (cm) of the thickened colonic segment
- Inspect and describe: perforation, fistula, pericolonic exudate, or abscess
- Open and fix
- Serially transverse section at 5 mm intervals
- Sample (four blocks minimum) the diverticula, any associated inflammation, or thickened mucosa that might represent crescentic colitis, mucosal prolapse, or tumor
- Sample mesenteric lymph nodes

6.6.2.2 Volvulus, pneumatosis coli, rectal stump, rectal mucosa in prolapse

- Measurements: length × maximum diameter (cm)
- Open and fix
- Inspect and describe

  Volvulus – dilatation, thinning, melanosis, stercoral ulceration, ischemia, perforation

Pneumatosis – mucosal cobbling, blebs or gas cysts, inflammation, ulceration, perforation
Rectal stump – mucosal granularity, ulceration, polyps, fistulae, tumor
Rectal mucosal prolapse – mucosal granularity, thickening, induration, ulceration

- Sample (four blocks minimum) macroscopically normal and abnormal areas as indicated
- Sample mesenteric lymph nodes

6.6.2.3 Ulceroinflammatory and neoplastic conditions

- Open and inspect
- Measurements:
  Lengths and maximum diameter (cm) of the parts present – terminal ileum, appendix, colon, rectum, anus
  Lengths (cm) of ischemic, inflamed, or strictured segments
  Maximum dimensions (cm) of any perforation(s), ulcer(s), polyp(s) or tumor(s)
  Distances (cm) of the abnormality from the proximal and distal resection limits
  Distances (cm) of the polyp/tumor/ulcer from the anorectal dentate line and relationship to the peritoneal reflection (above/straddling/below) and colorectal circumference (anterior/posterior/right or left lateral)
  Distances (mm) of tumor from the nearest aspect of the mesocolic/mesorectal CRM
- Grade the plane of mesorectal excision (mesorectal fascia, intramesorectal, muscularis propria)
- Photograph
- Paint any aspect of the mesocolic/mesorectal margin adjacent to or overlying tumor
  Gently pin out and fix for 48 h
  Description:
- Tumor
  - Site
    Ileocecal valve/cecum/colon (which segment, flexure)/rectum (above, straddling, or below the peritoneal reflection and upper, mid, or lower, anterior, posterior, or lateral)/anus
    Lumenal/mural/extramural/mesenteric
  - Size
    Length × width × depth (cm) or maximum dimension (cm)
  - Appearance
    Polypoid/nodular – adenoma, carcinoma, carcinoid, multiple lymphomatous polyposis, GIST
    Ulcerated/stricture – carcinoma, malignant lymphoma, metastatic carcinoma
    Fleshy/rubbery – malignant lymphoma, GIST
    Multiple – adenomas, carcinoma (primary or metastatic), malignant lymphoma
  - Edge: Circumscribed/irregular
    Perforation
    Adjuvant therapy changes: necrosis, ulceration, fibrosis
- CIBD – ulcerative colitis: contiguous/diffuse mucosal distribution, granularity, ulceration (linear/confluent), inflammatory polyps, synechiae, nodular or sessile DALMs, tumor, mucosal reversion with healing and atrophy, backwash ileiitis, treatment-related rectal sparing
  - Crohn's disease: segmental/transmural distribution, cobblestone mucosa, ulceration (aphthous/linear/confluent), stricture, fat wrapping, fistula, polyps or tumor, lymphadenopathy, adhesions, abscess formation, ileal/anal disease
- Ischemia – serosal hyperemia/constriction band, mucosal hyperemia/hemorrhage/ulceration/necrosis, wall thinning/perforation/stricture
- Pseudomembranous colitis – pseudomembranes (adherent/yellow), mucosal granularity/erosion/ulceration, stricture
- Obstructive enterocolitis – ulceration or stricture (contiguous or distant, diffuse or segmental), dilatation, wall thinning, perforation, ileal component

Blocks for histology:

Ulceroinflammatory conditions (Fig. 6.8)

- Sample by circumferential transverse sections the proximal and distal limits of resection
- Sample macroscopically normal bowel
- Sample representative longitudinal blocks (a minimum of four) of any focal abnormality that is present to include its edge and junction with the adjacent mucosa, e.g., ulceration, stricture, fistula, perforation, pseudomembranes,

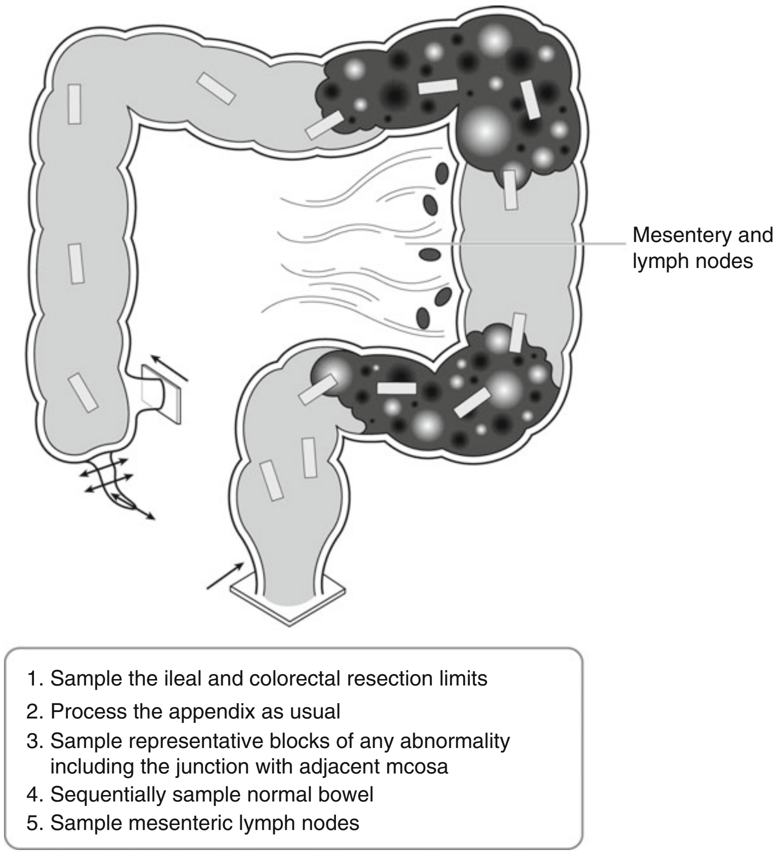

Fig. 6.8 Ulceroinflammatory colorectal conditions (Reproduced, with permission, from Allen and Cameron (2004))

inflammatory polyps, serosal adhesions or constriction bands. Also
  – CIBD: Sequential labeled samples at 10 cm intervals from cecum to anus and additional blocks from any unusual nodular or sessile abnormality (DALM)
  – Ischemia: Sample the mesenteric vessels
- Sample mesenteric lymph nodes and any other structures, e.g., appendix or terminal ileum.

Neoplastic conditions (Fig. 6.9)
- Sample the nearest longitudinal resection margin if tumor is present to within <3 cm of it.
- Sample macroscopically normal bowel and representative blocks of other mucosal lesions that are present, e.g., adenomatous polyps (if multiple particularly those >1 cm diameter).
- Serially section the bulk of the tumor transversely at 3–4 mm intervals.
- Lay the slices out in sequence and photograph.
- Note and measure the relationship of the deep aspect of the tumor to the nearest site-orientated point of the serosa and the CRM. Note serosal tumor perforation or CRM involvement (≤1 mm).
- Sample (four blocks minimum) tumor and wall to demonstrate these relationships. With bulky mesentery/mesorectum, the block may have to be split and appropriately labeled for loading in the cassettes.
- Count and sample all lymph nodes and identify a suture tie limit node. Take care to count the nodes in the tumor slices and also those in the mesentery away from the tumor, e.g., sigmoid mesocolon in a rectal cancer.
- Sample multifocal serosal seedlings and omental deposits (pM disease) as indicated by inspection and palpation.

Histopathology report:
- Ulcerative colitis – site-related disease activity (healed/quiescent/mild/moderate/severe), rectal sparing, appendiceal and cecal skip lesions, backwash ileiitis, toxic dilatation,

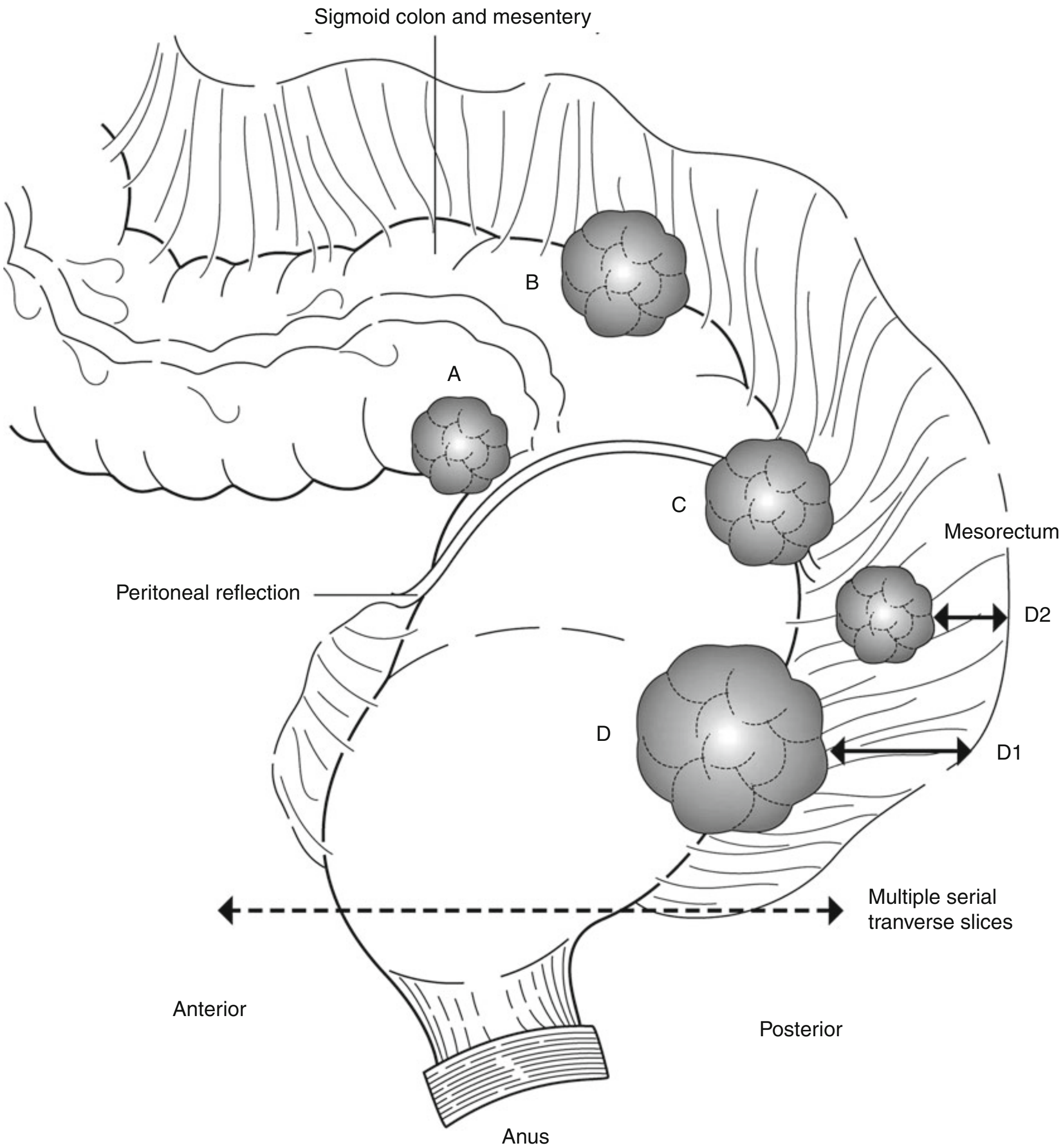

Fig. 6.9 Rectal carcinoma. The upper anterior rectum is invested in peritoneum. The anterior mesorectum is thinner (0.75–1 cm) than the posterior mesorectum (1.5–3 cm). Cut the resection specimen into multiple serial transverse slices about 3–4 mm thick. Blocks for histology are: Above the reflection: *A* tumor, rectal wall, and serosa; *B* tumor, rectal wall, and serosa; tumor, rectal wall, and mesentery; At the reflection: C tumor, rectal wall, and serosa; tumor, rectal wall, and mesorectum; Below the reflection: *D* tumor, rectal wall, and mesorectum; *D1* distance (mm) of the deepest point of continuous tumor extension to the nearest point of the painted CRM; *D2* distance (mm) of the deepest point of discontinuous tumor extension (or in a lymphatic, node, or vessel) to the nearest point of the painted CRM (Reproduced, with permission, from Allen (2000))

superimposed infection (e.g., CMV), DALMs, carcinoma, or lymphoma
- Crohn's disease – chronic transmural inflammation, granulomas, fissures/fistulae, abscess formation, segmental distribution/appendiceal/ileal disease, malignancy
- Ischemia – necrosis (mucosal/transmural/gangrenous), resection limits (ischemic/viable), mesenteric vessels (thrombosis/embolism/vasculitis), miscellaneous (constriction band/volvulus/stricture)
- Pseudomembranous colitis – pseudomembranes, ulceration, necrosis, perforation, strictures
- Obstructive enterocolitis – note ulceration/perforation/stricture/distribution and features specific to the etiological abnormality

Neoplastic conditions
- Tumor type – adenocarcinoma/malignant lymphoma/other
- Tumor differentiation
  - Adenocarcinoma
    Well or moderate/poor
  - Malignant lymphoma
    MALT/mantle cell/follicular/Burkitt lymphoma/other
    Low grade/high grade
- Tumor edge – pushing/infiltrative/lymphoid response
- Extent of local tumor spread (for carcinoma)

pTis	Carcinoma in situ: intraepithelial (within basement membrane) or invasion of lamina propria (intramucosal) with no extension through muscularis mucosae into submucosa
pT1	Tumor invades submucosa
pT2	Tumor invades muscularis propria
pT3	Tumor invades through the wall into subserosa or non-peritonealized pericolic/perirectal tissues
pT4	Tumor invades other organs or structures and/or perforates visceral peritoneum

- Lymphovascular invasion – present/not present
- Regional lymph nodes
  - Pericolic, perirectal, those located along the ileocolic, colic, inferior mesenteric, superior rectal and internal iliac arteries; a regional lymphadenectomy will ordinarily include 12 or more lymph nodes

pN0	No regional lymph node metastasis
pN1	1–3 involved regional lymph node(s)
pN2	4 or more involved regional lymph nodes.

  - In the UK, the Royal College of Pathologists currently recommend continuing using TNM 5 due to concerns over data comparison in ongoing clinical trials, the observer reproducibility in applying TNM 6 and TNM 7 rules (the subcategory details of which show some differences to TNM 5) and the lack of an evidence base for the proposed changes.

Dukes' stage	
A	tumor limited to the wall, node negative
B	tumor beyond the wall, node negative
C_1	nodes positive, apical node negative
C_2	apical node positive
D	distant metastases

- Excision margins
  Proximal and distal longitudinal (cm) and mesocolic/mesorectal
  Circumferential (mm) limits of tumor clearance
- Other pathology
  Tumor regression grade in response to neoadjuvant therapy, adenoma (s), FAP, ulcerative colitis, Crohn's disease, diverticular disease

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7 Appendix

Derek C. Allen, R. Iain Cameron,
and Maurice B. Loughrey

7.1 Anatomy

The vermiform ("worm-like") appendix is a vestigial organ in the right iliac fossa. Although there is considerable variability in its length and position, the base of the appendix is always found attached to the posteromedial surface of the cecum, approximately 2 cm below the ileocecal valve. The base is the only part of the appendix which is fixed, the remainder being free, thus accounting for the great variability in the position of the body and tip (Fig. 7.1). It is completely surrounded by peritoneum which is continuous with the mesentery of the small intestine, this connection being termed the mesoappendix. Again the size of the mesoappendix is variable, and the distal appendix may occasionally be devoid of a mesenteric covering. As was stated, the position of the appendiceal base is constant, the surface landmark of this being one-third the way along a line drawn from the right anterior superior iliac spine to the umbilicus – *McBurney's point*. Internally the base can be found by following the taeniae coli of the cecum to the base of the appendix where they converge to form a continuous appendiceal longitudinal muscle coat.

Histologically the appendiceal lumen is lined by colonic-type columnar epithelium with abundant lymphoid follicles (which decrease with age) in the submucosa. There are continuous circular and longitudinal muscle coats.

Lymphovascular drainage:

The appendix is supplied by terminal branches of the superior mesenteric artery and vein. Lymphatics drain to nodes in the mesoappendix, ileocolic nodes, and subsequently to superior mesenteric nodes.

7.2 Clinical Presentation

Acute appendicitis (and its complications) is among the most common surgical emergencies encountered. Classically it presents initially with vague, colicky, central abdominal (periumbilical) pain, which is associated with vomiting and anorexia. When the inflammation becomes transmural, a localized peritonitis is elicited and the pain becomes sharp in nature, localized in the right iliac fossa, and associated with pyrexia. Palpation reveals signs of localized peritonitis in the vicinity of *McBurney's point*.

D.C. Allen (✉)
Histopathology Laboratory, Belfast City Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: derek.allen@belfasttrust.hscni.net

R.I. Cameron
Histopathology Laboratory, Altnagelvin Hospital,
Western Health and Social Care Trust,
Londonderry, UK
e-mail: iain.cameron@westerntrust.hscni.net

M.B. Loughrey
Histopathology Laboratory, Institute of Pathology,
Royal Victoria Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: maurice.loughrey@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_7, © Springer-Verlag London 2013

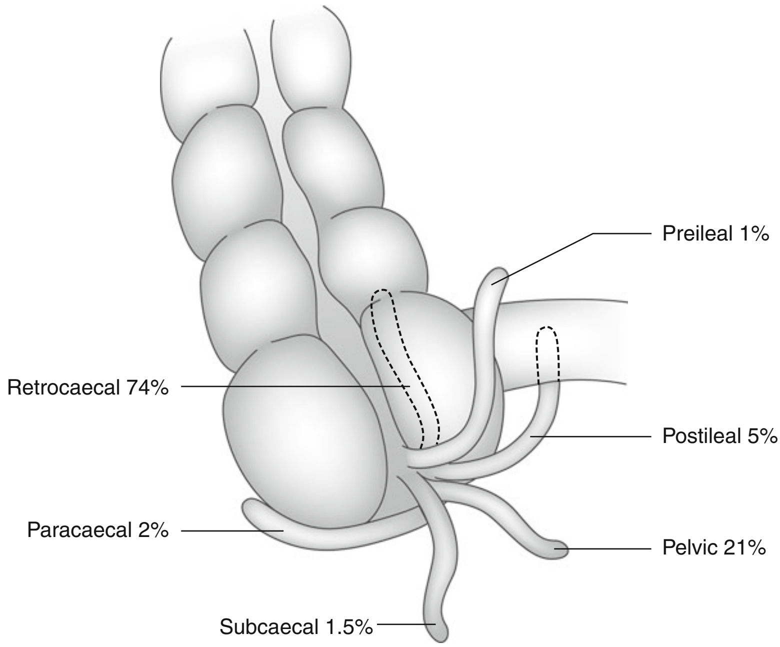

Fig. 7.1 The various positions of the appendix

As was stated above, the position of the body and tip of the appendix is variable and so the nature of the symptoms and signs will vary accordingly, e.g., flank pain and tenderness in retrocecal appendicitis. Although perforation of the appendix usually remains localized (due to "walling off" by the greater omentum), occasionally it may lead to a generalized peritonitis.

The list of differential diagnoses for acute appendicitis is myriad and includes ectopic pregnancy, torsion of an ovarian cyst, Meckel's diverticulitis, urinary tract infection, terminal ileitis, endometriosis, etc. An appendiceal abscess (which usually develops 3 days after a bout of acute appendicitis) can usually be palpated by a combination of abdominal and rectal examination. Differential diagnoses of an appendiceal mass also include carcinoma of the cecum, Crohn's terminal ileitis, and ovarian carcinoma.

7.3 Clinical Investigations

- FBP – white cell count will be elevated in >75 % of cases of acute appendicitis.
- Pregnancy test – will be positive in an ectopic pregnancy.
- Urinalysis – to test for a urinary tract infection.
- USS – can demonstrate a swollen appendix and will detect a pelvic mass.
- CT scan – can delineate the nature of an "appendiceal mass."
- Laparoscopy – can be used to differentiate acute appendicitis from gynecological conditions. It will also detect a small mass in the appendix, such as a mucocele, and may be used to biopsy suspected deposits of pseudomyxoma peritonei.

7.4 Pathological Conditions

The appendix may be resected incidentally as part of a radical cancer operation, e.g., right hemicolectomy for cecal carcinoma, or, opportunistically, at laparotomy for other reasons, e.g., Meckel's diverticulectomy. However, the vast majority of appendices are removed because of clinically significant primary inflammation and a small minority for neoplasia.

Table 7.1 Obstructive causes of appendicitis

Fecolith	Hardened, impacted fecal debris
Foreign body	Vegetable matter, fruit pips
Tumor	Carcinoid, adenocarcinoma appendix or cecal base
Mucosal lymphoid hyperplasia Endometriosis	Mesenteric adenitis, infectious monocleosis, yersinia enterocolitica infection.

7.4.1 Non-neoplastic Conditions

Appendicitis: Caused by epithelial ulceration, then infection by bowel bacteria, it may be precipitated by an underlying structural abnormality such as a diverticulum, or, more commonly, by luminal obstruction for one of various reasons (Table 7.1). It is characterized by transmural acute neutrophilic inflammation with the serosal component eliciting signs of peritonism. There is usually close correlation between the macroscopic and histological findings with acute appendicitis, resulting in serosal congestion, inflammatory exudate, and adherence of fat. Serious complications can arise from the resultant mural necrosis with wall thinning, gangrene, and perforation, potentially leading to generalized peritonitis, periappendicular abscess formation, portal vein pyemia, and hepatic abscesses. In general, the high risk of morbidity and mortality serves to emphasize the crucial importance of early diagnosis and therapeutic appendicectomy. Chronic appendicitis is a more controversial entity, but in a minority of cases the inflammation may resolve, leaving only residual thickening of the tissues.

Other unusual causes of subacute appendicitis are: granulomatous appendicitis (Crohn's disease, sarcoidosis, TB, schistosomiasis, but usually isolated and idiopathic), measles, CMV, or secondary to ulcerative colitis. Periappendicitis or serosal inflammation without a mucosal or mural component should be noted as this may indicate inflammation emanating from another abdominopelvic organ, e.g., pelvic inflammatory disease (salpingitis) or colonic diverticulitis. In the older patient, such an exudate must also be closely scrutinized for evidence of peritoneal spread of carcinoma cells.

Fibroneural obliteration of the appendiceal tip and body is now regarded as an age-related physiological phenomenon rather than representing evidence of previous inflammation.

7.4.2 Neoplastic Conditions

Carcinoid (*well-differentiated neuroendocrine*) *tumor*: Forming over 80% of appendiceal tumors, carcinoid tumor of classical type is usually small (<1 cm diameter) and found as an incidental finding at the appendiceal tip with or without associated appendicitis. It can be a histological finding only amidst the inflammation, or macroscopically discernible as a firm, pale-yellow mass replacing the lumen and wall. It has a variable nested and tubular pattern of uniform neuroendocrine cells that are positive with chromogranin A and synaptophysin antibodies. Despite showing transmural, serosal, and lymphovascular spread, appendicectomy is usually totally therapeutic and recurrence is only seen in a very small number of cases where the lesion is greater than 2 cm diameter or there is involvement of the appendiceal base, cecal wall, mesoappendix, or local lymph nodes. Conversely the much less common mucin-rich, goblet cell carcinoid (adenocarcinoid/crypt cell carcinoma) more frequently involves the appendiceal base with potential for nodal metastases, local invasion of the cecal pouch, and transcoelomic peritoneal spread with ovarian metastases. Because of this, goblet cell carcinoid requires consideration for right hemicolectomy. Due to the difficulties in distinguishing between carcinoid tumor and inflammatory fibrotic reaction, the appendiceal tip and base are sampled and separately identified as part of the routine blocking procedure to assess adequacy of tumor excision if present.

Polyps: Hyperplastic polyps; sessile serrated polyps/adenomas; tubular, tubulo-villous or villous adenomas; adenomas are more often sessile than polypoid comprising low- or high-grade dysplastic epithelium. All these lesions may be associated with synchronous/metachronous polyps or tumors elsewhere in the colorectum, adenomas with FAP, mucocele (see below) or adenocarcinoma of the appendix or adjacent cecal pouch.

Adenocarcinoma: A relatively unusual lesion that may be mucinous and cystic, secondary involvement of the proximal appendix from the cecal pouch is more common than a primary appendiceal lesion. Very occasionally mixed adenocarcinoma–neuroendocrine tumors occur. Other cancers metastatic to the appendix are from ovary, stomach, breast, and lung.

Mucocele: Macroscopic distension of the appendiceal lumen by abundant mucus often with marked thinning of the wall. Obstructed or non-obstructed in character the former represents a retention cyst lined by attenuated and atrophic but non-dysplastic mucosa. Non-obstructed mucocele is due to oversecretion of mucus by an abnormal mucosal lining that can be either hyperplastic, adenomatous (LAMN – low-grade appendiceal mucinous neoplasm) or adenocarcinomatous in nature. Extrusion of mucus through the wall to the serosa results in pseudomyxoma peritonei which is localized to the periappendiceal tissues or generalized in the peritoneal cavity. The latter can be refractory to surgical debridement with reaccumulation over a prolonged time course of months to years resulting in bowel obstruction and death. It is due to spillage of either atypical or frankly malignant appendiceal epithelium into the peritoneal cavity, whereas mucocele due to benign hyperplastic or adenomatous epithelium that is limited to the appendix more often results in a self-limited localized reaction.

It is now recognized that there is a strong association between generalized pseudomyxoma peritonei, appendiceal mucinous tumors, and bilateral ovarian mucinous borderline tumors with the latter regarded as either implantation deposits or metastases from the appendiceal lesion.

Prognosis: Carcinoid tumors less than 2 cm diameter are generally adequately treated by local appendicectomy. Those that are larger, involve the base, or are of goblet cell type may require right hemicolectomy. Prognosis of mucocele depends on the nature of the underlying mucosal epithelium and degree of spillage of epithelium into the peritoneal cavity. Adenocarcinoma treated by appendicectomy alone does worse (20% 5-year survival) than when right hemicolectomy is performed (60–65% 5-year survival) – outlook is tumor grade and stage dependent.

7.5 Surgical Pathology Specimens: Clinical Aspects

7.5.1 Biopsy Specimens

Not applicable.

7.5.2 Resection Specimens

7.5.2.1 Appendicectomy

Although the appendix may be removed laparoscopically or in the course of other procedures for diagnostic and/or staging purposes (e.g., suspected ovarian malignancy), the operation of choice in acute appendicitis is open appendicectomy. In the case of an "uncomplicated appendicitis," a muscle-splitting *Gridiron* oblique incision centered over McBurney's point is used. The cecum is delivered into the wound and the taeniae coli are followed to the base of the appendix. The appendicular vessels in the mesoappendix are divided and ligated. The appendiceal base is crushed and ligated, and the appendix is divided distal to the ligation.

If appendiceal perforation with generalized peritonitis is present preoperatively, a midline incision may be employed to facilitate better access to the abdominal cavity. This will allow an adequate laparotomy examination and peritoneal lavage to be carried out and so will lessen the risk of postoperative abscess formation.

In the case of an appendiceal abscess, the patient may be initially treated conservatively with antibiotics and close clinical supervision, followed by an interval appendicectomy at a later date. However, if there is diagnostic doubt or worsening symptomatology (e.g., increasing pyrexia), early operative intervention is indicated. Although a simple appendicectomy may suffice, a right hemicolectomy may be needed if a large mass is present.

7.5.2.2 Right Hemicolectomy

The technique of right hemicolectomy (removal of the terminal ileum, cecum, and proximal ascending colon) is described in detail elsewhere (see Chap. 6).

As well as for a large appendiceal mass, other lesions of the appendix requiring a right hemicolectomy include primary adenocarcinoma and, as previously discussed, a minority of carcinoid tumors.

7.6 Surgical Pathology Specimens: Laboratory Protocols

7.6.1 Resection Specimens

Specimen:

- Handle similarly whether as part of a radical cancer resection specimen or a simple appendicectomy. The former will require sampling of adjacent structures and locoregional lymph nodes (see Colorectal specimen Chap. 6).
- Some appendicectomies are submitted in several pieces due to difficulties in surgical excision. This precludes assessment of the base unless a surgical clamp mark is visible.

Initial procedure:

- Orientate the tip (rounded end) and the base (clamp marked).
- Measurements:
  Appendix – length (cm) × maximum diameter (cm).
  Mesoappendix – maximum dimension (cm).
  Exudate (serofibrinous/mucin)/perforation/mucocele/tumor – maximum dimension (cm) and distances (cm) from the tip and base.
- Photograph before and after blocking as appropriate.
- Fix in 10% formalin for 24–36 h.

Description:

- Tumor
  - Nodular/yellow: carcinoid
  - Cystic: cystadenoma/adenocarcinoma
  - Ulcerated/stricture/polypoid: adenocarcinoma/goblet cell carcinoid
- Wall
  - Tumor confined to mucous membrane, in the wall or through the wall
- Mesoappendix
  - Maximum dimension (cm) of abscess/tumor/mucin deposits
- Mucocele
  - Maximum diameter (cm)/intact or ruptured/mucin coating (location and extent)
- Diverticulum
  - Maximum diameter (cm) and location
- Appendicitis
  - Exudate/perforation/gangrene: location and extent

Blocks for histology (Fig. 7.2):

- Trim off any excess mesenteric fat and only process that which appears abnormal.
- Process in one cassette a 1–1.5 cm longitudinal slice from the tip along with a transverse section from the base.
- Serially section the rest of the appendix transversely at 3 mm intervals with a sharp scalpel.
- Sample five to six slices, approximately one slice per 1–1.5 cm length and process in a separate cassette from that of the tip/base.
- Sample any area of mural thinning or focal lesion as indicated by gross inspection.
- If part of a formal cancer resection specimen, e.g., right hemicolectomy, dissect and sample as previously described (see Colorectal specimens – Chap. 6).

Histopathology report:

- Appendicitis
  - Cause: fecolith, tumor, diverticulum, endometriosis
  - Type: acute (transmural/gangrenous/perforation/abscess), granulomatous, periappendicular
- Mucocele
  - Obstructed/non-obstructed
  - Intact/ruptured
  - Mucosal hyperplasia/adenoma(LAMN)/adenocarcinoma
  - Pseudomyxoma: localized/generalized/nature of the epithelium present

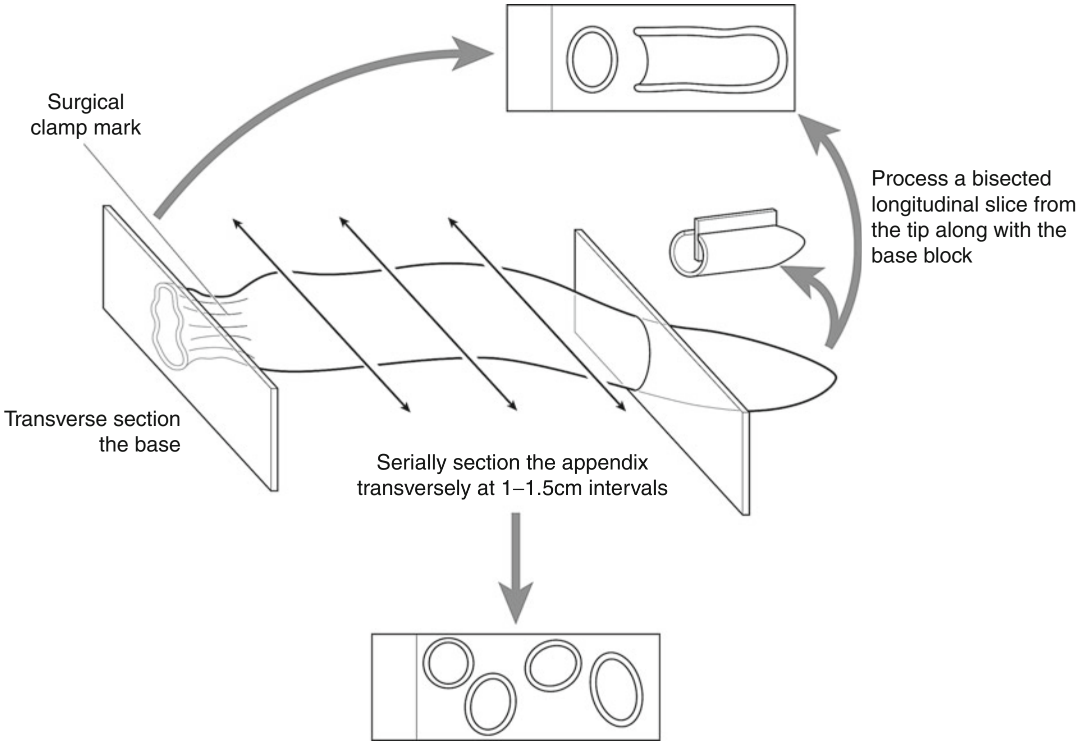

Fig. 7.2 Appendicectomy specimen (Reproduced, with permission, from Allen and Cameron (2004))

- Carcinoid tumor
  - Type: classical/goblet cell
  - Size: ≤ or >2 cm
  - Spread: mesoappendix, appendiceal base
- Adenocarcinoma
  - See Colorectal carcinoma Chap. 6. In the TNM 5 system, appendix is an anatomical subsite of colorectum.

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Anus

8

Derek C. Allen, R. Iain Cameron, and Maurice B. Loughrey

8.1 Anatomy

The anal canal (anus) is 4 cm long and is continuous with the rectum above the pelvic floor. The mucous membrane of the upper half of the anal canal is lined by columnar epithelium and supplied by autonomic nerves, being sensitive only to stretch. The lower half is lined by stratified squamous epithelium and has a somatic nerve supply, being sensitive to pain, touch, etc. There is a transition zone with a sharp demarcation between the two types of mucosa, termed the *dentate line*. Distally the canal terminates at the anal verge merging with the appendage-bearing perianal skin. The circular muscle layer is thickened around the upper anal canal to form the internal (involuntary) sphincter. A sheath of striated muscle encloses this – the external (voluntary) sphincter. The longitudinal muscle coat descends between the internal and external sphincters. The ischiorectal fossa is a fat-filled space on either side of the anal canal between it and the bony pelvis (Fig. 8.1).

Lymphovascular drainage:

The upper half is supplied by the superior rectal artery (a branch of the inferior mesenteric artery) and the lower half by the inferior rectal artery (a branch of the internal iliac artery). The veins correspond to the arteries and the lymphatics from the upper and lower halves drain to the perirectal and inferior mesenteric, and internal inguinal and superficial inguinal nodes, respectively (Fig. 8.2).

8.2 Clinical Presentation

Anorectal conditions are relatively common in surgical practice and present in a number of ways including pain, itch, bleeding, discharge, pyrexia, a mass or inguinal lymphadenopathy. An accurate diagnosis is crucial to successful treatment of the condition.

8.3 Clinical Investigations

- FBP – occasionally chronic bleeding can lead to iron-deficiency anemia.
- Serology – if a syphilitic ulcer is suspected.

D.C. Allen (✉)
Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: derek.allen@belfasttrust.hscni.net

R.I. Cameron
Histopathology Laboratory, Altnagelvin Hospital, Western Health and Social Care Trust, Londonderry, UK
e-mail: iain.cameron@westerntrust.hscni.net

M.B. Loughrey
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: maurice.loughrey@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_8, © Springer-Verlag London 2013

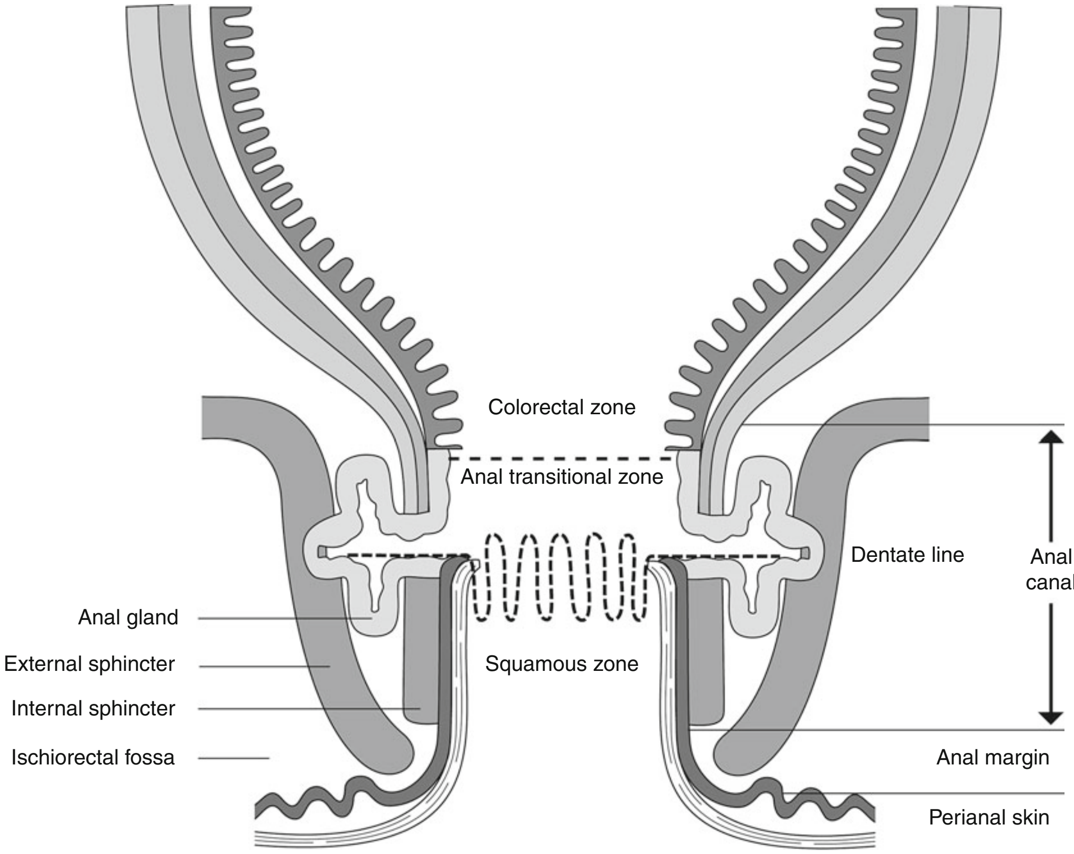

Fig. 8.1 The anatomy of the anal canal (Reproduced, with permission, from Williams and Talbot (1994))

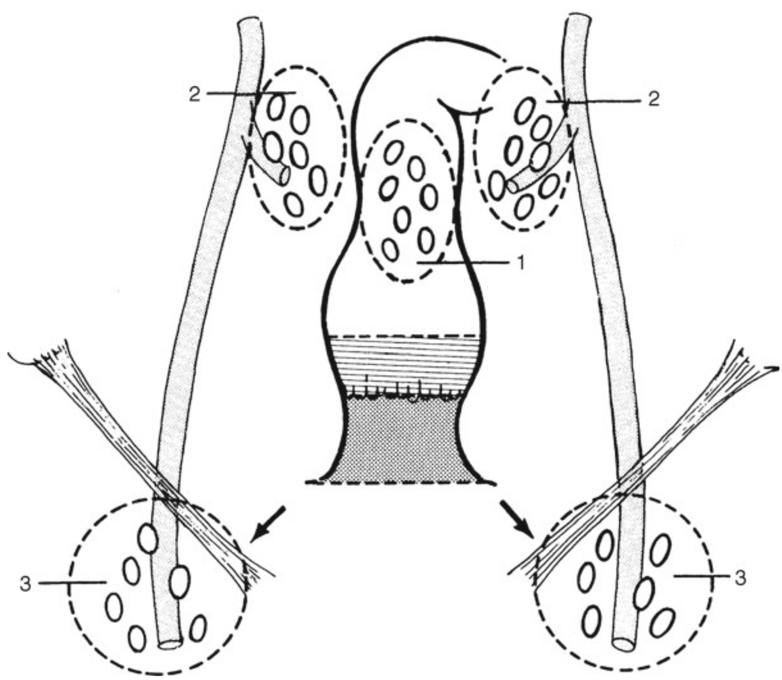

Fig. 8.2 Anus: regional lymph nodes. Perirectal (*1*), internal iliac (*2*), and inguinal (*3*) (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

- Blood glucose – in those with recurrent anorectal sepsis to rule out diabetes mellitus.
- Microbiology – pus from an abscess should be cultured and antibiotic sensitivities obtained.
- Proctoscopy – used to inspect the anus and anorectal ring. Biopsy of lesions above the dentate line can be taken without anesthesia.
- Sigmoidoscopy/colonoscopy – should be undertaken when an anorectal condition is thought to be secondary to inflammatory bowel disease.
- MRI scan – useful in delineating the course of complicated fistulae and with ELUS, the extent of local tumor spread. CT scan (chest, abdomen, and pelvis) will demonstrate local and distant metastases.
- Trucut needle biopsy – used when there is suspicion of recurrent or residual tumor in the ischiorectal fossa.

8.4 Pathological Conditions

8.4.1 Non-neoplastic Conditions

Hemorrhoids (*piles*): Comprising engorgement of submucosal veins, they are common and predisposed to by increased pelvic pressure, e.g., constipation, pregnancy, obesity, pelvic tumor. They bulge into the anal canal and are traumatized by straining at stool and hard feces. Complications include bleeding, reversible prolapse into the anal canal or persistent prolapse outside the anal margin – these so-called external hemorrhoids, which are located below the dentate line and covered by anal skin, are particularly prone to painful strangulation and thrombosis, which is an indication for surgical excision.

Skin tags: Fibrous skin tags at the anal margin can indicate various abnormalities, e.g., a previous thrombosed external hemorrhoid, Crohn’s disease, fissure, or fistula.

Fissure-in-ano: A tear at the anal margin that often follows the passage of a constipated stool, it is usually posterior and midline in location. It is painful and may be marked by a skin tag at its distal aspect. Multiple fissures can complicate Crohn’s disease.

Anorectal abscesses: Resulting from infection of anal submucosal glands, they are perianal, ischiorectal, submucous, or pelvirectal in location. Underlying Crohn’s disease or diabetes must always be excluded in anorectal sepsis.

Fistula-in-ano: An abnormal communication between two epithelial surfaces; commonly the anal canal and perineal skin. The majority arise from infection of anal glands resulting in an anorectal abscess that tracks and opens discharging onto the perineum externally and the anal canal internally. Associated conditions such as Crohn’s disease, ulcerative colitis, and mucinous carcinoma must be excluded histologically.

Prolapse: A consequence of mucosal prolapse at this site is inflammatory cloacogenic polyp. It arises at the internal margin comprising a mixture of thickened low rectal and high anal glandular and squamous mucosae associated with hypertrophic muscularis mucosae. These polyps are often excised to exclude the possibility of adenoma or carcinoma which can share similar clinical appearances.

8.4.2 Neoplastic Conditions

Benign tumors: These are rare, e.g., granular cell tumor.

Human papilloma virus (*HPV*): A common etiological agent associated with a spectrum of anal viral lesions, preneoplasia (anal intraepithelial neoplasia – AIN) and carcinoma, as well as concurrent lesions of the uterine cervix. HPV subtypes 16/18 are particularly neoplasia progressive in this viral–AIN–carcinoma sequence.

Anal margin/perianal skin carcinoma: Commonly well-differentiated keratinizing squamous carcinoma with predisposing conditions being viral warts (condyloma accuminatum) and perianal squamous intraepithelial neoplasia (PSIN – previously known as Bowen’s disease or squamous cell carcinoma in situ of perianal skin). Variants include the exophytic, indolent verrucous carcinoma. Treatment is primarily by local surgical excision as for skin carcinoma.

Anal canal carcinoma: A squamous cell carcinoma with variable degrees of squamous, basaloid (synonym: cloacogenic/non-keratinizing small cell squamous carcinoma) and ductular differentiation. Proximal canal cancers are poorly differentiated and basaloid, whereas distal anal cancers are well differentiated and more overtly squamous in character. There is an increased incidence in Crohn's disease, smoking, immunosuppression, and sexually transmitted diseases. At diagnosis 15–25% have spread through sphincteric muscle into adjacent soft tissues (vagina, urethra, prostate, bladder, etc.) and 5–10% have hematogenous metastases to liver, lung, and skin. Small "early" lesions may be locally excised but otherwise primary therapy is concurrent radio-/chemotherapy with good preservation of anal sphincter function and tumor response. Abdominoperineal or exenterative resection is rare and reserved for extensive (e.g., vaginal involvement), recurrent or nonresponsive tumors. Inguinal node disease may require block dissection of the groin and can be determined on fine-needle aspiration cytology. Many arise in the vicinity of the dentate line from the transitional/cloacal zone with upward submucosal spread, presenting as an ulcerated tumor of the lower rectum from which it must be distinguished by biopsy as rectal cancer requires surgical resection following neoadjuvant treatment.

Other cancers: A not uncommon differential diagnosis is a low rectal carcinoma with distal spread into the anal canal. Relatively rare cancers are mucinous adenocarcinoma in an anal fistula, anal gland adenocarcinoma, extra-mammary Paget's disease (associated with low rectal adenocarcinoma, anal gland adenocarcinoma, or isolated), malignant melanoma, and leiomyosarcoma.

Prognosis: Perianal carcinoma 85% 5-year survival, anal canal carcinoma 65–80% 5-year survival. Adverse indicators are advanced stage or depth of spread, inguinal node involvement, and posttreatment pelvic and perineal recurrence. Malignant melanoma is aggressive with poor outlook; the prognosis of leiomyosarcoma is related to tumor grade.

8.5 Surgical Pathology Specimens: Clinical Aspects

8.5.1 Biopsy Specimens

The anal canal is best inspected by proctoscopy. The proctoscope is a rigid disposable tube with a light source attached, which is inserted with the patient in the left lateral position. Forceps can be passed through the tube to biopsy any visible lesion. Biopsy specimens may also be received from the walls/roof of areas of anorectal sepsis to rule out granulomatous inflammation.

8.5.2 Resection Specimens

8.5.2.1 Resection of Neoplastic Disease

Anal carcinoma – Small lesions (<2 cm) present at the anal verge are usually treated by local excision with a 2 cm margin of skin around the tumor. The resection should extend down to the perianal fat. For larger tumors, or extensive tumors of the anal canal that are unresponsive to radio-/chemotherapy, abdominoperineal resection is the procedure of choice. A 2 cm margin of perineal skin should be excised around the tumor and there should be a radical ischiorectal resection. If there is metastatic spread to superficial inguinal nodes, then a radical groin dissection may be considered.

8.5.2.2 Resection of Non-neoplastic Lesions

Fissure-in-ano – Acute fissures can usually be treated conservatively by introducing stool-softening measures. However, a chronic anal fissure can be treated by either anal dilatation or lateral internal sphincterotomy.

Hemorrhoids – If hemorrhoids are small and asymptomatic, then no treatment is necessary except for measures to avoid constipation. Non-prolapsing piles are probably best treated by injection sclerotherapy. Larger prolapsing piles above the dentate line are treated by rubber band ligation. Both the above procedures can be performed during routine proctoscopy without anesthesia. Piles too large to band and/or which extend below the dentate line can be treated by formal hemorrhoidectomy. The procedure most commonly used involves excision of the three main piles, with preservation of the intervening anal mucosa. The wounds are left open to heal by secondary intention.

Anorectal abscess/fistula – An abscess is drained under general anesthetic, after thorough proctoscopic/sigmoidoscopic examination, by incision and laying open the abscess cavity. The surgical treatment of fistula-in-ano depends on the position of the tracts and is often complicated. Essentially the general principle of anorectal fistula surgery is to lay open the primary tract and drain any secondary tracts while maintaining sphincter function. It is crucial to continence to preserve the function of the upper part of the external sphincter and so laying open "high" fistulae is not advised. Instead, a permanent *seton* suture is passed through the tract and allows drainage while the secondary tract heals. The primary tract should then heal after removal of the suture.

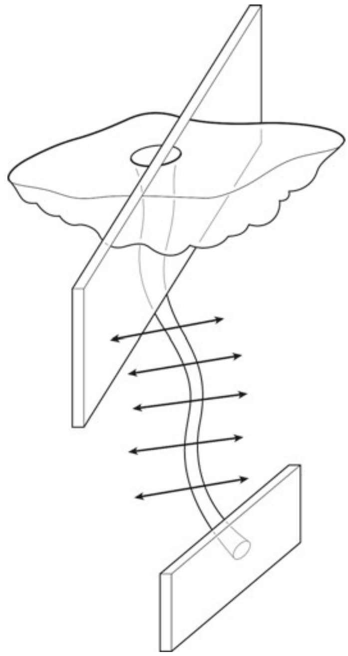

Fig. 8.3 Fistula-in-ano (Reproduced, with permission, from Allen and Cameron (2004))

8.6 Surgical Pathology Specimen: Laboratory Protocols

8.6.1 Biopsy Specimens

Elliptical incisional and excisional biopsies of the perineal skin and anal margin are handled similarly to skin biopsies (see Chap. 38). Anal canal biopsy fragments are processed as previously described (see Chap. 1). Specific points of note are as follows:

Hemorrhoids: Typically nodular and 1–2 cm in diameter with a smooth covering mucosa and ectatic submucosal vessels. Bisect vertically down through the epithelial surface and process both halves. With larger or multiple specimens, a mid-slice of each is taken.

Skin tags: Count and measure, process intact, vertically bisect or take a representative mid-slice according to size.

Fissure-in-ano: Not usually excised, although biopsy fragments of granulation tissue from its edge may be submitted.

Anorectal abscess: Usually heavily inflamed ellipses of tissue from the covering skin, lateral or deep aspects of the abscess wall. Measure, process intact, vertically bisect or take a representative mid-slice according to size.

Fistula-in-ano (Fig. 8.3): Rarely resected but typically a small skin ellipse often with a punctate opening on the surface, minimal subcutaneous tissue, and a stringy attachment which may be up to several centimeters long – the fistulous tract. It may also be submitted in fragments if excision was difficult. Measure the skin ellipse, its opening, and the tract. Take a block vertically through the skin to include the punctum and represent any subcutaneous abscess. Sample multiple transverse sections of the fistulous tract and label a transverse deep resection limit block.

Cloacogenic polyp: Measure, vertically bisect or take representative slices. Prior to this, paint the deep and lateral margins in case it turns out to be polypoid tumor.

8.6.2 Neoplastic Conditions

Anal margin/perianal skin lesions such as condyloma, PSIN, or carcinoma are handled as for skin specimens.

Anal canal carcinoma if resected will either be because of recurrent or extensive disease in the context of abdominoperineal resection or pelvic exenteration specimens where the tumor spread may be partially masked by fibrotic radio-/chemotherapy changes. For general comments see Chaps. 6 and 35.

Specific points of note in abdominoperineal resection for anal canal carcinoma are:

- Open the canal longitudinally with blunt-ended scissors on the opposite side of the tumor having previously painted the external CRM (circumferential radial margin).

- The tumor is frequently submucosal ± overlying mucosal ulceration. Pale and variably fleshy to scirrhous in character; pigmentation and rubbery/fleshy qualities should raise the possibility of malignant melanoma or leiomyosarcoma, respectively. Mucinous carcinoma may occur in a fistula while anal gland carcinoma is also submucosal and sclerotic.
- The relationships and distances (mm) to the anal margin/perianal skin and anorectal dentate line.
- Upward or downward spread to the lower rectum and perianal skin, respectively.
- The extent of mucosal/mural/extramural spread and distances (mm) to the nearest longitudinal and radial margins (perianal skin, site-orientated aspect of the CRM). Note that the CRM comprises a tube of perianorectal levator musculature which also forms a tight neck or constriction at its junction with the lower edge of the mesorectum.
- Serially section the tumor transversely at 3–4 mm intervals. Sample a minimum of four blocks of tumor and wall to show the deepest point of invasion in relation to the painted CRM. Sample a longitudinal block of tumor and proximal/distal limit if close (<0.5–1 cm) to it.

Histopathology report:

- Tumor type – anal canal squamous carcinoma/other
- Tumor differentiation – basaloid/keratinizing/non-keratinizing/ductular component
- Tumor edge – pushing/infiltrative/lymphoid response
- Extent of local tumor spread

pTis	Carcinoma in situ
pT1	Tumor ≤2 cm in greatest dimension
pT2	2 cm < tumor ≤5 cm in greatest dimension
pT3	Tumor >5 cm in greatest dimension
pT4	Tumor of any size invading adjacent organ(s), e.g., vagina, urethra, bladder

- Lymphovascular invasion – present/not present.

  Anal margin/perianal skin lesions: inguinal nodes – regional lymphadenectomy will ordinarily include 6 or more lymph nodes.

  Anal canal lesions: perirectal, internal iliac, inguinal nodes in that order – a regional lymphadenectomy will ordinarily include 12 or more lymph nodes.

pN0	No regional lymph node mestastasis
pN1	Metastasis in perirectal lymph node(s)
pN2	Metastasis in unilateral internal iliac and/or unilateral inguinal lymph node(s)
pN3	Metastasis in perirectal and inguinal lymph nodes and/or bilateral internal iliac and/or bilateral inguinal lymph nodes

- Excision margins

  Proximal rectal and distal perianal/perineal limits of tumor clearance (cm)

  Deep circumferential radial margin of clearance (mm)
- Other pathology

  Condylomatous warts, Bowen's disease (PSIN), anal fistula, Crohn's disease, AIN, radio-/chemotherapy necrosis and tumor regression

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9 Gallbladder

Derek C. Allen, R. Iain Cameron, and Maurice B. Loughrey

9.1 Anatomy

The gallbladder is a sac that lies on the inferoposterior surface of the liver. It is divided into the fundus (rounded portion that projects below the liver), body (lies in contact with the liver), and neck (becomes continuous with the cystic duct). Stones may cause a dilatation at the junction of the neck and cystic duct known as Hartmann's pouch. The gallbladder is two thirds surrounded by peritoneum which binds the non-peritonealized adventitial aspect of the body and neck to the under surface of the liver. The cystic duct is 4 cm long and joins the neck of the gallbladder to the right side of the common hepatic duct to form the common bile duct. The course of the cystic duct shows great variation between individuals. The gallbladder is concerned with the concentration, storage, and delivery of bile. To aid the concentration process the mucous membrane is thrown into permanent folds. The bile salts emulsify fats in the duodenum and so facilitate their digestion and absorption. When fatty food enters the duodenum, endocrine cells release hormones, which lead to contraction of the gallbladder and relaxation of the sphincter of Oddi, thus allowing bile to be delivered to the duodenum. The mucous membrane of the cystic duct is raised in the form of a spiral fold. This is thought to assist in keeping the lumen patent. An important surgical landmark (where the cystic artery can be found) is *Calot's triangle* which is formed by the common hepatic duct, the cystic duct, and the liver (see Fig. 4.2).

Lymphovascular drainage:

The main arterial supply to the gallbladder is from the right hepatic artery via the cystic artery that runs through Calot's triangle. The cystic vein drains directly to the portal system. Lymphatics from the gallbladder and bile ducts pass to the cystic node (situated near the gallbladder neck) and then through the infrahepatic nodes. At the distal end of the common bile duct, they pass into the peripancreatic and periduodenal nodes, and ultimately drain to the celiac and superior mesenteric nodes (Fig. 9.1).

9.2 Clinical Presentation

There is considerable overlap in the clinical features of gallbladder and extrahepatic bile duct disease. Gallstones are often asymptomatic.

D.C. Allen (✉)
Histopathology Laboratory, Belfast City Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: derek.allen@belfasttrust.hscni.net

R.I. Cameron
Histopathology Laboratory, Altnagelvin Hospital,
Western Health and Social Care Trust,
Londonderry, UK
e-mail: iain.cameron@westerntrust.hscni.net

M.B. Loughrey
Histopathology Laboratory, Institute of Pathology,
Royal Victoria Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: maurice.loughrey@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_9, © Springer-Verlag London 2013

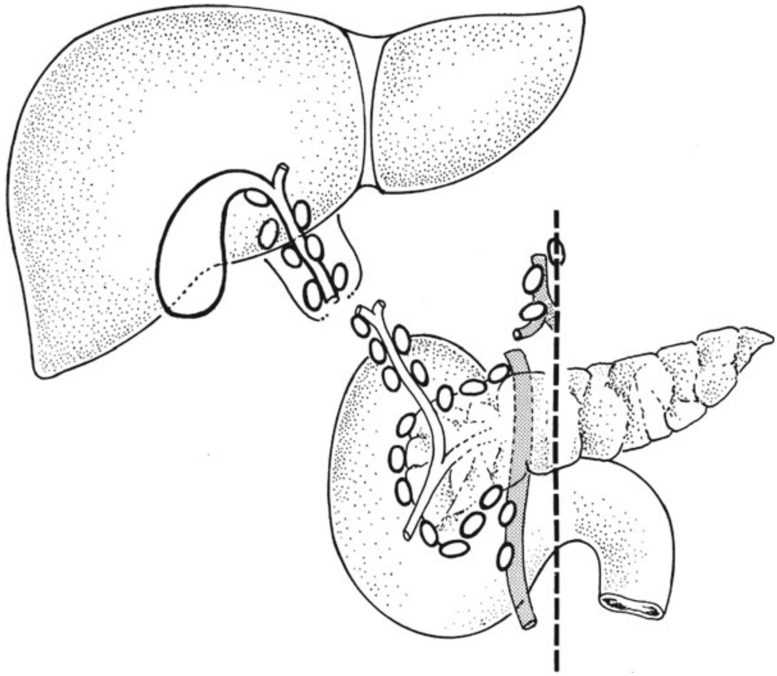

Fig. 9.1 The regional lymph nodes are the hepatic hilus nodes (including nodes along the common bile duct, common hepatic artery, portal vein, and cystic duct). Celiac, periduodenal, peripancreatic, and superior mesenteric artery node involvement is considered distant metastasis (M1) (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

However, if there is gallbladder outlet obstruction by a stone, then progressively severe right upper quadrant "colicky" pain (biliary colic), associated with nausea and vomiting, may be felt. If the stone remains impacted, the gallbladder may become infected and acutely inflamed (acute cholecystitis) – this leads to severe constant right upper quadrant pain, pyrexia, and signs of localized peritonitis. This can progress to an empyema (pus-filled gallbladder). Stone impactation may also lead to a mucocele, i.e., a dilated gallbladder in which the bile has been resorbed but mucus secretion continues. A mucocele is heralded by a palpable gallbladder and dull right upper quadrant pain. Occasionally in the elderly the gallbladder may perforate, leading to generalized peritonitis. Gallstones localized to the cystic duct will occasionally cause obstructive jaundice, especially if the duct is short. The inflammation and edema around the cystic duct impedes the flow of bile through the adjacent common bile duct (Mirizzi syndrome). Gallstone ileus (small bowel obstruction due to impactation of a stone at the ileocecal valve after the formation of a fistula between the gallbladder and duodenum) is a rare complication of cholecystitis.

Gallbladder carcinoma may present in a similar manner to gallstone disease, although weight loss and jaundice are additional features, obstructive jaundice being caused by metastatic spread to nodes which compress the bile ducts.

9.3 Clinical Investigations

There is considerable overlap in the investigation of gallbladder and extrahepatic bile duct disease.

- FBC – elevated WCC in cholecystitis.
- AXR – 10% of stones are radio-opaque; gas in the gallbladder wall (emphysematous cholecystitis) is a serious complication of cholecystitis seen most commonly in diabetics; in gallstone ileus, it will show the classic triad of small intestinal obstruction, gallstone in the right iliac fossa, and gas in the biliary tree.
- USS – sensitive for stones >4 mm.
- Oral cholecystogram – oral contrast is taken and this is absorbed from the gut, bound to albumin in the portal vein, and subsequently

secreted in bile. Radiological imaging of the gallbladder is then carried out 10 h after ingestion. Although largely replaced by USS, this investigation is still indicated when the clinical symptoms are strongly suggestive of gallstones and the USS is negative.
- Radionucleotide scanning – high sensitivity in acute cholecystitis.
- Percutaneous drainage – under radiological guidance can be used to drain the gallbladder in, e.g., empyema.
- CT scan (chest, abdomen, and pelvis), cholangiography (percutaneous or at ERCP) – to demonstrate a tumor mass, invasion of the liver, and compression of bile ducts.

9.4 Pathological Conditions

9.4.1 Non-neoplastic Conditions

Cholelithiasis (*gallstones*): The commonest etiological agent in gallbladder pathology and classically occurring in fair, fat, fertile, females in their 40s. Mixed stones are the most frequent (80%) formed from an amalgam of bile, cholesterol, and calcium, and comprising biliary sludge, calculous gravel or multiple, faceted, laminated stones. Occasionally stones can be pure such as dark bilirubinate pigment stones in a congenital hemolytic disorder, e.g., spherocytosis, or, solitary, large, yellow, and cholesterol-rich.

Acute cholecystitis: 95% of cases are due to impaction of a stone in the cystic duct resulting in stasis, a bile-induced chemical reaction, and then secondary infection. The acute inflammation often subsides with conservative medical treatment but can persist producing an empyema – perforation and bile peritonitis are unusual. In a mucocele, the wall may calcify and form a "porcelain" gallbladder.

Chronic cholecystitis: Invariably associated with calculi, there are varying degrees of mucosal and transmural chronic inflammation, thickening of the muscularis, perimuscular fibrosis, and adherence to the liver bed. Indicators of chronicity are mucosal pseudopyloric metaplasia and transmural mucosal herniation to form Rokitansky–Aschoff sinuses. These mucosal pouches can inspissate with bile and mucus, becoming inflamed and forming extramural abscesses which may only partly resolve leaving a marked xanthogranulomatous histiocytic inflammatory reaction that encases the gallbladder. Prominent sinus formation at the fundus can similarly mimic a mucosal polyp or tumor, so-called cholecystitis glandularis proliferans. Unusual variants of chronic cholecysitis are follicular (reactive lymphoid aggregates), eosinophilic (often acalculous and chemical in nature), and malakoplakia. Due to the strong association with pancreatitis, fat necrosis and calcification may be seen.

Cholesterolosis: A relatively common finding of yellow mucosal flecks ("strawberry" gallbladder) due to accumulation of cholesterol-laden macrophages in the lamina propria. It is usually incidental and not associated with hypercholesterolemia.

Oleogranulomas: The cystic duct lymph node is not infrequently enlarged and submitted along with the cholecystectomy specimen. It often contains oleogranulomas comprising fat spaces surrounded by histiocytes, presumably representing a gallbladder drainage phenomenon.

9.4.2 Neoplastic Conditions

Adenoma: Relatively uncommon in surgical material, adenomas can be polypoid or sessile, tubular, tubulovillous or villous, and comprise variably dysplastic biliary-, pyloric-, or enteric-type epithelium. The dysplasia may be multifocal and the proximal cystic duct margin should be checked for this.

Carcinoma: Usually occurs in late middle-aged females and many (50–75%) present already with regional lymph node metastasis and involvement of the gallbladder bed, liver, or other direct spread to duodenum, stomach, colon, and peritoneum. Calculi (80–90% of cases), chronic inflammatory bowel disease, and primary sclerosing cholangitis are risk factors. Often clinically inapparent and found incidentally as diffuse thickening of the wall at cholecystectomy for gallstones, 10–20% are initially diagnosed by histology of routine blocks, there having been no macroscopic suspicion of tumor. Fundal in location (60%) and grossly diffuse (70%) or polypoid (30%), the vast majority (95%) are adenocarcinomas of tubular or papillary patterns arising from a sequence of intestinal metaplasia – dysplasia – carcinoma. Assessment of the depth of invasion can be difficult and extension of carcinoma in situ into Rokitansky–Aschoff sinuses must be distinguished from true invasion of the wall. Perineural involvement is characteristic. Gallbladder cancer may, therefore, be encountered either in the context of an incidental finding in a simple cholecystectomy, or infrequently, as an electively planned extended cholecystectomy (+/− segmental resection of liver) with radical lymph node dissection.

Other cancers: Rare but can include embryonal rhabdomyosarcoma (children), leiomyosarcoma and malignant lymphoma, or metastatic carcinoma, especially transcoelomic – stomach, pancreas, ovary, bile ducts, colon, and breast.

Prognosis: Better if lesions are of papillary type, low histological grade, and confined to the mucous membrane when resection is potentially curative (90% 5-year survival). However, many cases present with disease beyond the gallbladder, involvement of liver (25% 5-year survival), and overall 5–10% 5-year survival figures.

9.5 Surgical Pathology Specimens: Clinical Aspects

9.5.1 Resection Specimens

9.5.1.1 Benign Conditions

In benign disease, the gallbladder may be removed either laparoscopically or by an open procedure.

Laparoscopic cholecystectomy is now by far the most popular method. There are no absolute contraindications except for those that apply to other operative procedures, e.g., poor anesthetic risk. However, previous abdominal surgery with resultant fibrous adhesions and obesity may make a laparoscopic approach difficult. It may have to be abandoned and converted to an open cholecystectomy. In a laparoscopic cholecystectomy, an initial small infraumbilical stab wound is made and a spring-loaded Veress needle is passed through the abdominal wall into the peritoneal cavity. A CO_2 supply is then connected to the needle and gas is insufflated into the abdomen to produce a pneumoperitoneum. The needle is then removed and the incision extended and deepened. A sheath is then inserted and the laparoscope is passed through this to make the optic port. The image from the laparoscope is transferred to a monitor and can be viewed by the surgeon. Three other incisions (ports) are made under direct visualization: for retraction and irrigation, for tools such as an electrosurgical hook, scissors, etc., and for grasping forceps. An initial examination of all areas of the abdomen is performed, including the pelvis. The fundus of the gallbladder is then grasped, the cystic artery and duct in Calot's triangle both clipped and divided. If cholangiography ± exploration of the common bile duct is required, a cannula is passed into the common bile duct via an opening in the cystic duct before clipping. The gallbladder is then dissected from the liver bed and the contents removed by suction. It is then placed in a bag and removed through the optic port.

Open cholecystectomy is used in the few cases deemed inappropriate for laparoscopic cholecystectomy via a Kocher's incision parallel to the right subcostal margin.

9.5.1.2 Gallbladder Cancer

Careful patient selection for surgery of gallbladder cancer is essential and only relatively fit patients with localized tumors and no evidence of metastatic spread should be considered. However, despite this, the results of surgery remain poor, with 90% of patients dying within 12 months.

A right subcostal incision is used and a complete examination of the abdomen undertaken. If there is no invasion of the bile ducts and no or only superficial liver invasion, an *extended cholecystectomy* is performed. In this, after determination of the depth of liver invasion by intraoperative USS, the gallbladder and the hepatic gallbladder bed are removed in the form of a wedge resection. A regional lymph node dissection is carried out by removing the lymph nodes draining the gallbladder as far as the celiac nodes.

If the tumor extends more deeply into the liver, then a *segmental liver resection* (usually IV and V or IV, V, and VI) will be required. Very occasionally, if the tumor has spread to the extrahepatic bile ducts, a segmental liver resection and extrahepatic duct resection are required. Rarely a liver resection and an extended Whipple's procedure may be used. Palliation can involve bypass surgery or stenting to relieve gastric outlet obstruction or jaundice.

9.6 Surgical Pathology Specimens: Laboratory Protocols

9.6.1 Biopsy Specimens

Not applicable.

9.6.2 Resection Specimens

Specimen:

- Most cholecystectomy specimens are now done laparoscopically rather than by open surgery and submitted opened or unopened containing 5–10 ml of bile fluid. Occasionally, specimens are received in several pieces if operative access has been technically difficult. The proximal end comprises a variable length of cystic duct adjacent to which an enlarged lymph node may be present.
  Initial procedure:
- Measurements:
  - Gallbladder – length × maximum diameter (cm)
  - Cystic duct – length × maximum diameter (cm)
  - Lymph node – number and maximum diameter (cm)
- Open longitudinally from the fundus toward the cystic duct with blunt-ended scissors draining off the bile and noting any contents.
- Photograph if appropriate.
- Paint the external serosal and adventitial aspects if there is any suspicion of tumor.
- Fixation by immersion in 10% formalin for 36–48 h.

Description:

- Received
  - Opened/unopened/intact/deficient/perforated/fragments
- Adventitia
  - Adhesions/rim of liver/tumor
- Serosa
  - Adhesions/exudate/perforation/tumor
- Wall
  - Thickness (cm)/fibrosis/tumor/thinning/necrosis/perforation/sinuses/abscess/calcification
- Mucosa
  - Tumor: polypoid/nodular/ulcerated/diffuse/mucinous
    Length × width × depth (cm) or maximum dimension (cm)
    Location (fundus/body/neck/cystic duct) and distance (mm) to the cystic duct limit
    Confined to mucous membrane, in the wall or through the wall
  - Cholesterolosis/ulceration/hemorrhage/polyps
- Contents
  - Bile/mucus/stones (size, number, shape, mixed, pigment, cholesterol)/fibrin/pus
- Cystic duct
  - Stone impaction/dilatation/lymph node

Blocks for histology (Fig. 9.2):

- Sample by circumferential transverse section the proximal cystic duct limit.
- Sample the cystic duct lymph node and any other separately submitted named nodes.
- Serially transverse section the gallbladder at 3–4 mm intervals with either a sharp knife or scissors.
- Usually one broken transverse ring will suffice for histology in the absence of any macroscopic abnormality.
- Sample gross lesions with multiple transverse blocks as indicated, e.g., ulceration, perforation, tumor, abscess, polyps, wall thickening. Demonstrate tumor in relation to the serosa and adventitia including its resection margin.
- If part of a radical cancer resection – describe and measure the attached segments of liver and bile ducts, and the relationship of any tumor to them and their resection limits. Sample multiple blocks to demonstrate these

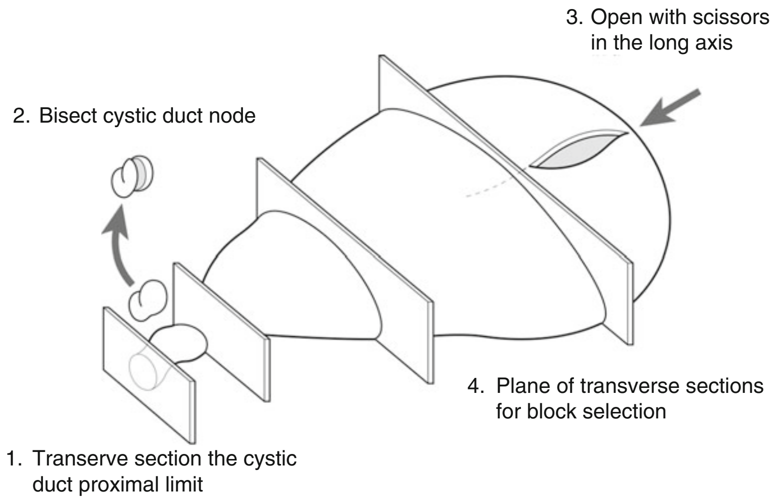

Fig. 9.2 Opening and transverse sectioning of the gallbladder (Reproduced, with permission, from Allen and Cameron (2004))

relationships. Sample all regional lymph nodes.

Histopathology report:

- Non-neoplastic
  - Inflammation: acute/chronic/xanthogranu lomatous
  - Necrosis/perforation/abscess/empyema/fistula
  - Mucocele
- Tumor type
  - Adenocarcinoma/other
- Tumor differentiation
  - Well/moderate/poor
- Tumor edge
  - Pushing/infiltrative/lymphoid response
- Extent of local tumor spread

pTis	Carcinoma in situ
pT1	Tumor limited to gallbladder wall
a	Lamina propria
b	Muscularis
pT2	Tumor invades perimuscular connective tissue; no extension beyond serosa or into liver
pT3	Tumor perforates serosa (visceral peritoneum) and/or directly invades the liver and/or one other adjacent organ or structure, e.g., stomach, duodenum, colon, pancreas, omentum, extrahepatic bile ducts
pT4	Tumor invades main portal vein or hepatic artery, or invades two or more extrahepatic organs or structures

- Lymphovascular invasion
  - Present/not present. Note perineural invasion
- Regional lymph nodes

  Cystic duct, common bile duct, common hepatic artery, portal vein; a regional lymphadenectomy will ordinarily include three or more lymph nodes

pN0	No regional lymph node mestastasis
pN1	Regional lymph node metastasis

- Excision margins

  Cystic duct limit of tumor and mucosal dysplasia clearance (mm)

  Adventitial margin of tumor clearance (mm)

  Hepatic and common bile duct margins of tumor clearance (mm)
- Other pathology

  Calculi, primary sclerosing cholangitis

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10 Liver

Derek C. Allen, R. Iain Cameron, and Maurice B. Loughrey

10.1 Anatomy

The liver, the largest gland in the body, is concerned with the production and secretion of bile and many metabolic functions crucial to normal homeostasis. The majority of it is surrounded by a peritonealized fibrous capsule and it is situated in the right upper quadrant of the abdomen for the most part under the cover of the ribs. It is divided into a large right and smaller left lobe by the attachment of the falciform ligament. The right lobe is further subdivided into the quadrate and caudate lobes by the gallbladder and the ligamentum teres (Fig. 10.1). However, this is a purely anatomical subdivison as it has been found that the quadrate and caudate lobes are actually a functional part of the left lobe, i.e., they are supplied by the left hepatic artery and left hepatic duct. This has led to a different division of the liver into surgical lobes and segments (see below).

The hilum of the liver, or *porta hepatis*, is found on the infero-posterior surface with the lesser omentum attached to its margin. Emerging from and entering the porta hepatis (from posterior to anterior) are the portal vein, right and left branches of the hepatic artery, the right and left hepatic ducts, and autonomic nerves.

Histologically the liver is composed of lobules (Fig. 10.1). Each lobule comprises a central vein (a tributary of the hepatic veins) with the portal tracts situated at the periphery. The portal tracts contain a branch of the hepatic artery, portal vein, and bile duct. Each lobule is divided into triangular-shaped *acini* with terminal branches of the hepatic artery and portal vein at their bases and the central vein at the apex. The acinus is divided into three zones (zone 3 being the most remote from the blood supply). The liver cells (hepatocytes) are arranged in anastomosing cords, with those adjacent to the portal tract forming the limiting plate.

Between the cords of liver cells are vascular channels (sinusoids) lined by a discontinuous layer of endothelial cells. These sinusoids carry blood (both arterial and portal) from the portal tract to the central vein. Channels (canaliculi) formed between adjacent hepatocytes conduct bile to the ducts in the portal tracts and then to the extrahepatic bile ducts and gallbladder.

Lymphovascular drainage:

The liver receives 30% of its blood from the hepatic artery (oxygenated blood), the remaining 70% being supplied by the portal vein (venous

D.C. Allen (✉)
Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: derek.allen@belfasttrust.hscni.net

R.I. Cameron
Histopathology Laboratory, Altnagelvin Hospital, Western Health and Social Care Trust, Londonderry, UK
e-mail: iain.cameron@westerntrust.hscni.net

M.B. Loughrey
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: maurice.loughrey@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_10, © Springer-Verlag London 2013

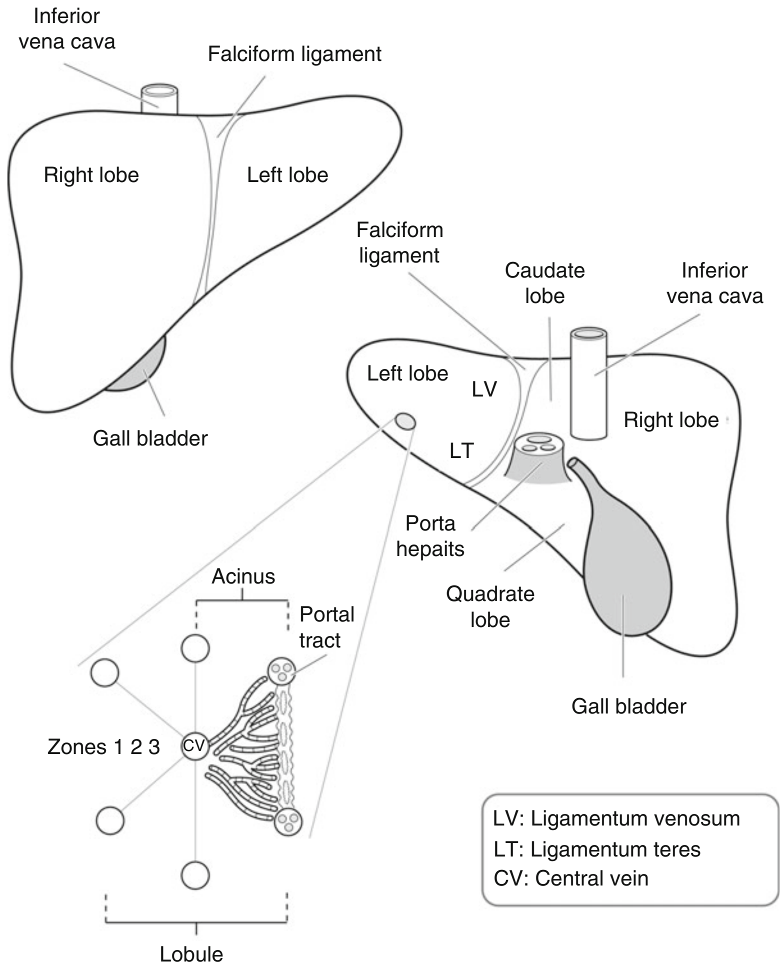

Fig. 10.1 Liver anatomy and histology (Reproduced, with permission, from Allen and Cameron (2004))

blood rich in nutrients absorbed from the gut). The blood is conducted through the sinusoids from the portal tracts to the central veins, which in turn drain into the hepatic veins and ultimately the inferior vena cava. Most of the lymphatics drain to nodes in the porta hepatis (hepatoduodenal ligament) and then pass to the celiac nodes. A small number pass through the diaphragm into the posterior mediastinum.

10.2 Clinical Presentation

Patients with liver disease may be asymptomatic. The most common clinical sign is jaundice, although other stigmata of chronic liver disease such as spider nevi, finger-clubbing, gynecomastia, etc. may also be present. If the jaundice is obstructive, then the patient will have dark urine and pale feces. Weight loss, anorexia, anemia, and ascites may suggest cirrhosis and/or an underlying malignancy. Fever and rigors may be seen if an abscess is present. Hepatomegaly may be encountered in numerous conditions including cirrhosis and malignancy.

A careful clinical history including medication (prescription and otherwise), alcohol intake, foreign travel, and sexual practice is diagnostically invaluable to the pathologist.

10.3 Clinical Investigations

- U&E – electrolyte imbalance may occur and hyponatremia (low sodium) is a poor prognostic sign in liver failure.

- Liver function tests (LFTs) – these should include tests for liver secretory capacity (bilirubin, alkaline phosphatase, and gamma glutamyl transferase (αGT)); synthetic capacity (albumin) and inflammation (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)).
- Coagulation screen – measures clotting potential and as such is a test of hepatic function (synthesis of clotting factors).
- Serology – viral titres of hepatitis A–E.
- Autoimmune screen – including anti-mitochondrial, anti-smooth muscle, and antinuclear antibodies.
- Serum immunoglobulins.
- Specific tests – serum iron, ferritin, total iron-binding capacity (TIBC), and HFE gene analysis (genetic hemochromatosis); serum and urinary copper (Wilson's disease); serum alpha-1 antitrypsin (alpha-1 antitrypsin deficiency).
- Tumor markers – alpha fetoprotein (AFP: hepatocellular carcinoma); CEA (metastatic colorectal carcinoma).
- CXR – may detect primary lung tumor (which has metastasized to the liver).
- AXR – may show calcification around a hydatid cyst.
- USS – shows dimensions of intra- and extra-hepatic bile ducts; useful in demonstrating both primary and metastatic tumors (colorectal metastases have a characteristic echogenic picture).
- CT scan – used to further define the nature and anatomical relationships of a lesion diagnosed by USS. CT PET can distinguish metabolically active metastatic tumor from benign or necrotic lesions.
- Radioisotope scanning – this provides information on the liver texture and is useful in diagnosing cirrhosis or multiple tumors (if >2 cm). Tumors will have decreased uptake.
- Angiography – hepatic artery angiography can be used to delineate the vascular anatomy of a tumor prior to resection. It is the most sensitive method of diagnosing hepatocellular carcinoma and is useful in ensuring that a metastasis, which is considered for resection, is indeed a single deposit. It will also allow the extent of a vascular tumor to be mapped and, if indicated, embolized. An inferior venogram will establish if the inferior vena cava is involved by tumor prior to any resection.
- Peritoneal aspiration – may detect malignant cells in ascitic fluid.
- FNAC – percutaneous under USS/CT guidance.
- Needle core biopsy – Tru-cut needle biopsy under USS/CT guidance can be carried out on focal lesions which have given a poor yield on FNAC. A biopsy of "normal" parenchyma adjacent to the lesion will show the state of background liver disease which may provide a clue to diagnosis (e.g., association of cirrhosis and hepatocellular carcinoma) and may rule out any attempt at resection. Diagnosis of hepatocellular carcinoma is often based on a combination of serum AFP and appropriate radiological features avoiding the need for biopsy. A needle core biopsy may also be performed on a suspicious lesion during laparotomy. Alternatively diffuse medical liver conditions can be sampled percutaneously and blind by a needle (16–18 G).
- Staging laparoscopy with biopsy.

10.4 Pathological Conditions

Patients with liver disease may present with signs of liver failure or complications of it, e.g., esophageal varices or because of biochemically detected abnormal LFTs. The latter can indicate whether the pattern of damage is hepatic (parenchymal), extrahepatic (obstructive) or mixed in nature. Hepatic assault is typified by viral hepatitis, alcohol or drug damage, and extrahepatic disease by duct obstruction due to stones or tumor, e.g., head of pancreas. Mixed biochemical profiles are not infrequently seen in these various disorders. Needle core biopsy is interpreted in close correlation with full clinical information that includes a detailed history and wide range of investigations (see above). Its aims are to distinguish between a surgical and medical cause for the damage, and, in non-neoplastic conditions, to assess the degree of necroinflammatory activity that is present and the reparative response of the liver to it. It also establishes a baseline against which subsequent treatment can be assessed or indicated, e.g., interferon therapy in chronic viral hepatitis.

Liver damage has potential to resolve, but if it is unresponsive to treatment or ongoing, a nonspecific, end-stage or cirrhotic pattern may be reached with few histological clues as to its etiology. It is due to lobular damage and collapse of its framework with fibrous repair expanding and linking portal tracts with each other and the central veins. This micronodular (<0.3 cm diameter) or macronodular pattern disturbs liver function and also its internal vascular relationships. As a consequence, liver failure (jaundice, anemia, generalized edema, and ascites due to hypoalbuminemia, hepatic encephalopathy) and portal venous hypertension with the risk of catastrophic hemorrhage from esophagogastric varices can ensue. In neoplasia or hepatic mass lesions, the biopsy may be for diagnostic purposes to distinguish between hepatocellular carcinoma, metastatic carcinoma, malignant lymphoma and abscess, or, for staging of known primary tumor elsewhere, e.g., colorectal carcinoma. The information accrued is then factored into future management decisions.

10.4.1 Non-neoplastic Conditions

Viral hepatitis: Commonly hepatitis A, B, C, D, or E (hepatotropic viruses). Hepatitis A (feco-oral transmission) is usually of short duration, self-limiting without sequelae, and not biopsied. Hepatitis B and C (transmission by blood, serum, secretions – hepatitis D is often a cofactor) are strongly associated with blood transfusion, sharps injuries, and shared needles in drug abusers. Occasionally there is acute fulminant hepatitis, but a significant minority go on to chronic carriage of viral antigen that can lead to chronic active hepatitis (>6 months clinical duration) with eventually cirrhosis and hepatocellular carcinoma. Diagnosis is by positive serology matched to distinctive histological features (e.g., portal tract lymphoid follicles and bile duct damage in hepatitis C) which are also graded (the degree of necroinflammation) and staged (the absence or presence of fibrosis/cirrhosis) as a gauge of need for treatment, treatment response, and/or evolution of disease. Tissue localization of viral antigens can be demonstrated immunohistochemically or by in situ hybridization. Hepatitis E (feco-oral transmission, epidemic, or sporadic) can cause a mild, cholestatic hepatitis (resembling hepatitis A) or fulminant disease with decompensation, particularly in those with preexisting liver disease.

Alcohol (*C_2H_5OH*): Chronic excess alcohol intake is a common etiological factor in liver disease and is noted for variable individual susceptibility to it. Its hepatotoxic effect causes a spectrum of change from simple steatosis (fatty change), alcoholic steatohepatitis (lobular necroinflammation with ballooning and Mallory's hyaline – tufts of intracytoplasmic intermediate filaments) to perivenular fibrosis, cirrhosis, and hepatocellular carcinoma. Abstinence short of the stage of cirrhosis leads to potential reversibility of even severe damage. Similar morphological features are seen in NASH (non-alcoholic steatohepatitis) commonly associated with hypertension, diabetes mellitus, and obesity (metabolic syndrome), and also gut bypass procedures and some drugs.

Drugs: The vast majority of drugs are metabolized in the liver and cause damage either due to excess dosage (actual or apparent due to preexisting decreased liver function) or individual idiosyncratic reaction to them. Various effects are seen with different agents: steatosis, cholestasis (commonest), granulomas, necrosis, hepatitis, veno-occlusion, and peliosis (dilated blood channels). Location of damage varies within the acinar zones related to the blood supply and the particular agent involved. Diagnosis is strongly dependent on an appropriate clinical history and chronology of drug usage correlating with the liver dysfunction. Common agents are – tricyclic antidepressants (chlorpromazine), methotrexate, NSAIDs, anesthetic agents (halothane), antibiotics (tetracyclines, erythromycin), and paracetamol.

Autoimmune and cholangiodestructive diseases: Characteristically in late middle-aged females, autoimmune hepatitis is associated with a range of autoantibodies, including antinuclear and anti-smooth muscle antibodies, and is steroid responsive. In this respect, it is of paramount importance to separate it from an infective hepatitis in which steroids are contraindicated. Primary biliary cirrhosis, some cases of which overlap with autoimmune hepatitis, affects a similar patient demographic and is a non-suppurative, destructive, granulomatous disorder of bile ducts that leads to their disappearance (ductopenia), fibrosis and ultimately cirrhosis. Serum IgM antimitochondrial antibody is typically elevated and progress can be gradual over a long time period, treatment being with ursodeoxycholic acid to reduce bile acid accumulation and symptomatic to relieve related itch. Primary sclerosing cholangitis can affect intra- or extrahepatic bile ducts with a chronic inflammatory infiltrate and surrounding fibrosis, leading to obstructive tapering of the ducts and their eventual disappearance. Diagnosis is often by endoscopic retrograde cholangiopancreatography (ERCP). There is a strong association with ulcerative colitis and predisposition to cholangiocarcinoma.

Systemic diseases: The liver can be involved in many other generalized conditions, e.g., diabetes, celiac disease, Crohn's disease, systemic vasculitis, amyloid (primary or secondary, e.g., due to rheumatoid arthritis) and hereditary disorders such as glycogen storage diseases, alpha-1 antitrypsin deficiency, cystic fibrosis, Wilson's disease (defect of copper metabolism) and hemochromatosis (defect of iron metabolism).

Focal mass lesions: These need to be distinguished radiologically and histocytologically from neoplastic conditions (see below) and include simple sporadic cysts (often biliary in origin), multiple simple cysts (polycystic disease of liver and kidneys), infective cysts, abscess, hemangioma, and focal nodular hyperplasia. Abscess may arise from septicemia, acute cholecystitis, or portal pyemia after perforated appendicitis or diverticulitis. Focal nodular hyperplasia is usually solitary in young-to-middle-aged women and has a central stellate fibrous scar containing proliferating bile ducts. It is considered to be a localized vascular abnormality, its main differential diagnosis being hepatocellular adenoma and well-differentiated hepatocellular carcinoma. Bile duct adenoma and hamartoma (von Meyenberg complex) are usually encountered as small, pale, subcapsular nodules at laparotomy, e.g., at staging of gastric carcinoma and submitted as a wedge biopsy for frozen section to exclude metastatic cancer deposits.

10.4.2 Neoplastic Conditions

Adenoma: Rare, causing acute abdominal presentation due to lesional hemorrhage in a middle-aged female with a history of oral contraception. Devoid of portal tracts or central veins within the nodule but lack of cellular atypia and preservation of the pericellular reticulin pattern and liver cell plates – these features help distinguish it from well-differentiated hepatocellular carcinoma.

Macroregenerative or dysplastic nodules: Irregular nodules in background cirrhosis, 1–3 cm diameter with cytoarchitectural atypia and potentially premalignant.

Hepatocellular carcinoma: Often in background cirrhosis, and serum AFP is elevated in 25–40% of cases. Single, diffuse or multifocal, bile stained, and prone to venous invasion with metastases to lung, adrenal gland, and bone. The commonest patterns are trabecular, plate-like, or sinusoidal comprising variably differentiated hepatoid cells.

A minority are encapsulated, pedunculated, or, in a younger patient, fibrolamellar in type, these variants having a better prognosis than usual hepatocellular carcinoma.

Cholangiocarcinoma (*intrahepatic*): Scirrhous, solitary, or multifocal adenocarcinoma with a ductuloacinar pattern and predisposed to by primary sclerosing cholangitis, ulcerative colitis, liver fluke, and biliary tree anomalies.

Metastatic carcinoma: Commonly from gastrointestinal tract, lung, and breast, there are some characteristic clues as to origin.

Colorectum – multiple, large nodules with central necrosis/umbilication, ± mucin ± calcification

Gallbladder – bulk of disease centered on the gallbladder bed

Lung – medium-sized nodules

Stomach, breast – medium-sized nodules or diffuse cirrhotic-like pattern

Note that carcinoma rarely metastasizes to a cirrhotic liver.

Other cancers: Carcinoid (well-differentiated endocrine) tumor metastatic from gastrointestinal tract (particularly ileum), pancreas or lung, malignant lymphoma (portal infiltrates or tumor nodules), leukemia (sinusoidal infiltrate), angiosarcoma, epithelioid hemangioendothelioma.

Prognosis: In hepatocellular carcinoma, this relates to size (>5 cm), differentiation, encapsulation, multifocality, high serum AFP levels, vascular invasion, and the presence of background cirrhosis (adverse). The majority die within several months of presentation and 5-year survival is at most 5–10%. Chemotherapy, chemoembolization, or radiofrequency ablation are used palliatively. Small tumors, encapsulated, pedunculated, and fibrolamellar variants are potentially curable by resection or local ablation. Few patients with cholangiocarcinoma survive longer than 2–3 years due to late presentation and limited resectability. Solitary metastases, e.g., colorectal carcinoma or carcinoid tumor can be resected to good effect. Metastatic carcinoid tumor can show good chemoresponsiveness.

10.5 Surgical Pathology Specimens: Clinical Aspects

10.5.1 Biopsy Specimens

FNAC and needle core biopsy can be carried out percutaneously either blind or preferably under radiological guidance, during laparoscopy, laparotomy, or as a radiologically guided transvascular (vena cava) procedure. Coagulation status is checked prior to core biopsy to avoid risk of hemorrhage.

10.5.2 Resection Specimens

10.5.2.1 Neoplastic Lesions

The key to successful hepatic resection of malignant disease is careful patient selection. In general:

- A primary liver tumor may be considered for resection if it involves a single lobe and there is no invasion of the portal vein or inferior vena cava. There should be no evidence of cirrhosis in the surrounding liver.
- A solitary metastatic deposit (the vast majority of which will be from a primary colorectal carcinoma) localized to a single lobe may be considered for resection. There should be no evidence of metastatic spread elsewhere and the primary tumor should have been adequately excised. More recently, this criterion has been extended to include multiple hepatic metastases provided resection is technically feasible leaving sufficient functioning hepatic remnant. Use of neoadjuvant chemotherapy, intravascular embolization, or radiofrequency ablation facilitates operative resection by downsizing the tumor deposits.

Obviously the background physiological state of the patient has to be taken into account before surgery is considered, i.e., resection is only justified in relatively young and medically fit individuals.

As was stated above, the liver is divided into right and left "surgical lobes," which are different to the anatomical lobes. The surgical lobes are separated along a plane which extends from the gallbladder bed to the inferior vena cava – the main portal plane. The surgical lobes are then subdivided into eight segments – each segment is supplied by its own portal venous and hepatic arterial pedicle (Fig. 10.2).

Major Liver Resection

An S-shaped right subcostal incision is used in all cases and once the abdomen is opened an initial laparotomy examination is done to ensure no other metastatic deposits are present. The definitive type of resection will depend on the site and extent of the tumor.

- *Right hepatectomy* – In this the right surgical lobe is resected by transecting the liver through the main portal plane (main portal scissura). The cut surface of the residual liver is sprayed with thrombin glue to reduce postoperative blood loss and especially bile leakage.

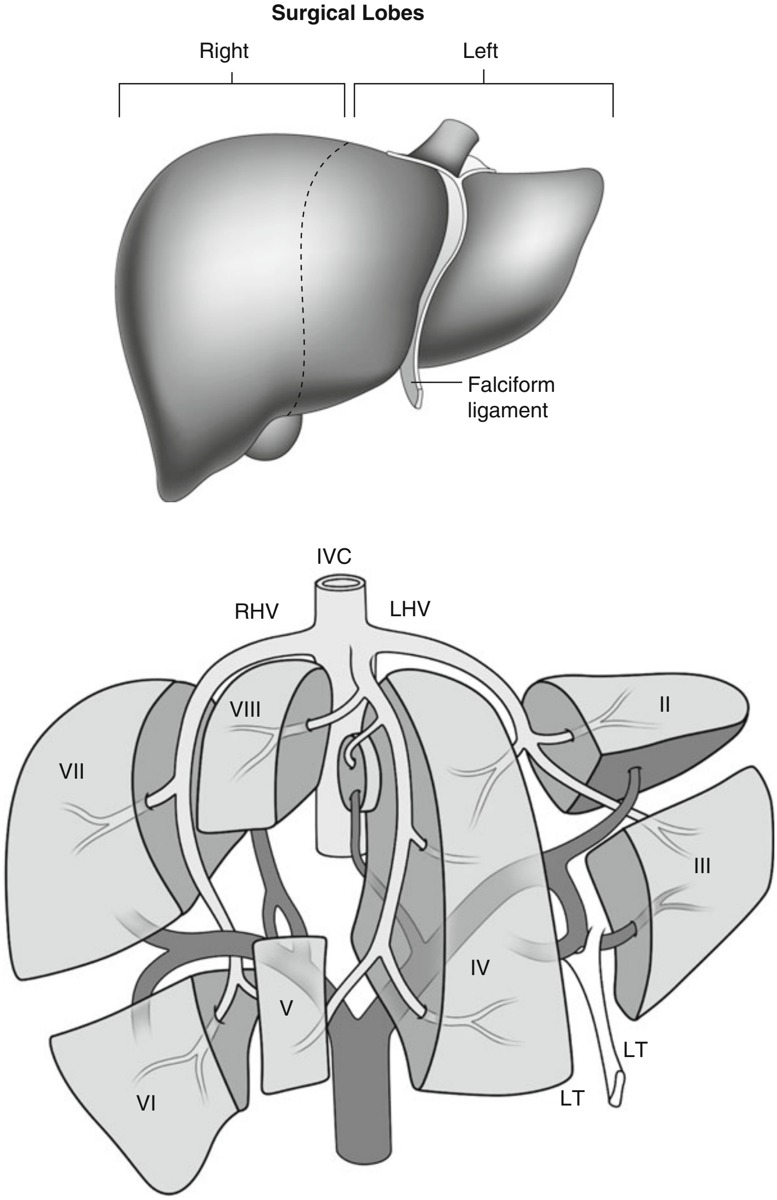

Fig. 10.2 (**a**) Surgical lobes of the liver. The surgical lobes of the liver compared with the usual anatomical division into left and right lobes by the falciform ligament. (**b**) Segments of the liver (after Couinard). *IVC* inferior vena cava, *RHV* right hepatic vein, *LHV* left hepatic vein, *LT* ligamentum teres

- *Left hepatectomy* – This usually involves resection of the anatomical left lobe, the quadrate and caudate lobes (i.e., the left surgical lobe, although the caudate lobe may be left in-situ). Again the line of resection is the main portal plane.
- *Left lobectomy* – In this the anatomical left lobe is resected by dividing the liver just to the left of the falciform ligament.
- *Extended right hepatectomy* – This involves the resection of the anatomical right lobe, i.e., the surgical right lobe plus the caudate and quadrate lobes. Again the line of resection is just to the left of the falciform ligament.
- *Extended left hepatectomy* – This is essentially a left hepatectomy which has been extended to also resect segments I, V, and VIII.

As well as neoplastic conditions, major liver resection may also be used for other conditions such as trauma.

Segmental Liver Resection

Although major hepatic resection may be employed for large tumors, when a small tumor (either primary or secondary) occupies one or two segments, a segmental resection can be carried out. This removes a segment(s) of liver, which is supplied by its own vascular pedicle, and is, therefore, an anatomically based procedure. Whatever the segment to be resected, its vascular anatomy is delineated by intraoperative USS before dissection.

Segmental resection has several advantages over major resection; namely as much functioning parenchyma is left as possible and the vascular supply to this is less likely to be compromised, there is reduced blood loss, and the procedure is less likely to leave residual tumor.

If a metastatic deposit is single, small, and superficial, a simple *wedge resection* using diathermy can be employed. This procedure may be performed during resection of the primary tumor, e.g., colorectal carcinoma, and sent for frozen section.

It is known that most metastatic tumors reach the liver by the portal circulation. However, the deposit itself gains its blood supply almost exclusively from hepatic arterial flow. Therefore, in inoperable metastatic disease, numerous techniques have been used to deliver chemotherapy directly into the hepatic arterial circulation:

- Infusion therapy – a catheter is passed percutaneously via the femoral artery.
- Implantable device – this can be placed at laparotomy and allows long-term infusion. An example of this technique is by using a *portacath*, which employs a self-sealing port which is placed subcutaneously and drugs can be injected into this at regular intervals. A catheter runs from the port to the hepatic artery.

10.5.2.2 Non-neoplastic Lesions

Liver Cysts

Liver cysts may be congenital or acquired (e.g., neoplastic, inflammatory/infective, traumatic, etc.). When surgery is to be carried out for a liver cyst, an extensive preoperative clinical and radiological workup is required to ascertain, as closely as possible, its etiology. An initial thorough laparotomy examination is undertaken. For noninfective cysts, the cyst is opened and the contents aspirated and sent for cytological and microbiological examination. The cyst wall can then be excised using cautery if a neoplastic lesion is suspected. However, in non-neoplastic lesions (e.g., simple cyst) complete excision is not necessary and a large opening is made in the cyst to allow free drainage into the peritoneal cavity.

Hydatid cysts (echinococcus tapeworm) may vary in size and situation within the liver. They may be excised without removing adjacent liver parenchyma (*pericystectomy*) or if the cysts are large or multiple, a segmental or major resection may be needed. When a pericystectomy is carried out and the cyst is opened, pads soaked in saline are packed around the cyst to prevent spillage of its contents into the peritoneal cavity.

For pyogenic abscess/cyst there are three main forms of treatment: long-term antibiotics, percutaneous drainage under radiological guidance, and open surgical drainage. Percutaneous drainage is now by far the most popular method. However, if surgical drainage is employed, the abscess is identified and separated from the peritoneal cavity by pads. The abscess contents are then aspirated and the cavity washed out. The cyst wall is then de-roofed to facilitate resolution. Pyogenic abscesses may also be treated by laparoscopic drainage.

Transplantation

The first successful human liver transplant was carried out in 1967 and today over 80% of recipients survive 1 year. Not only can adult livers be transplanted to adult recipients, but the shortage of donor organs has led to adult donor organs being transplanted to children. This is facilitated by resecting and transplanting only part of the donor liver, e.g., left liver (segments I–IV). General indications for transplantation are acute liver failure, end-stage chronic liver disease, and neoplasms. Conditions encountered in the explant specimen can, therefore, be diverse including viral, autoimmune and alcoholic hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, end-stage cirrhosis, and primary hepatocellular or cholangiocarcinoma. The liver transplant can be subject to various pathologies including rejection, effects of immunosuppression, and recurrence of the original disease.

10.6 Surgical Pathology: Laboratory Protocols

10.6.1 Biopsy Specimens

For needle core and wedge biopsy specimens see Chap. 1.

Note that viral hepatitis is a category III pathogen – it should be submitted to the laboratory with an attached "hazard of infection" sticker and handled appropriately after 24–48 h of thorough formalin fixation.

Routine histochemical stains that should be provided to help assess the degree of hepatic parenchymal loss, reticulin collapse/elaboration, and fibrous distortion/replacement, respectively, are, PAS (± diastase), silver reticulin, and Masson Trichrome. Elastin stains such as Shikata's orcein or elastic-van Gieson can help distinguish recent collapse (elastin negative) from old fibrosis (elastin positive). Hemochromatosis is diagnosed using biochemical and genetic investigations and the degree of iron deposition on biopsy is graded by Perl's Prussian Blue or the dry weight iron concentration. Other stains are: rhodanine/Shikata's orcein for copper or copper-associated protein deposition in Wilson's disease, primary biliary cirrhosis, or other chronic cholestatic disorders; PAS + diastase (positive globules in alpha-1 antitrypsin deficiency); and Congo Red (amyloid).

Needle cores may have an adherent fragment of skin if obtained percutaneously. They can also fragment in diseased liver with cirrhosis or tumor. Fatty liver is pale; hemochromatosis rust-colored. One aspect of a wedge biopsy is covered by peritonealized capsule and its cut margin is often frayed by diathermy. This margin should be painted and the wedge then cut into multiple perpendicular serial slices.

10.6.2 Resection Specimens

Specimen:

- Liver resection is more commonly performed for a focal mass lesion such as a cyst, adenoma, focal nodular hyperplasia, or metastatic colorectal carcinoma and is, therefore, limited in extent, e.g., segmentectomy, lobectomy, or partial hepatectomy. Other indications are major trauma and a small minority of resectable primary liver cancers. Specimen handling and reporting should document the nature of the abnormality, its extent, completeness of excision, vascular invasion, and status of the background parenchyma. Total hepatectomy is encountered in transplantation surgery – aims are to identify the cause of hepatic failure, and for tumor to determine the stage and assess porta hepatis margins.

  Initial procedure:

  For partial resection
- Weight (g) and measurements (cm) in each dimension.
- Identify the capsular and cut parenchymal surfaces – the latter constitutes the surgical margin. Further orientation can only be given if marked appropriately by the submitting surgeon.
- Paint the surgical margin and any areas of capsular bulging, retraction, or reaction that might be related to an underlying mass lesion.
- Serially section the liver perpendicular to the parenchymal resection margin at 0.5 cm intervals (Fig. 10.3).
- Photograph.
- Fixation by laying flat and immersion in 10% formalin for 36–48 h.

  Description:
- Note the number, size, and distances (mm) to the capsule and surgical margin for each lesion.
- Specific points are:

  Abscess
  - Contents (pus: pyogenic/"anchovy sauce": amoebiasis), walled-off, capsular reaction

  Cyst
  - Contents (fibrin, fluid (serous/mucoid)), wall (chitinous-hydatid)

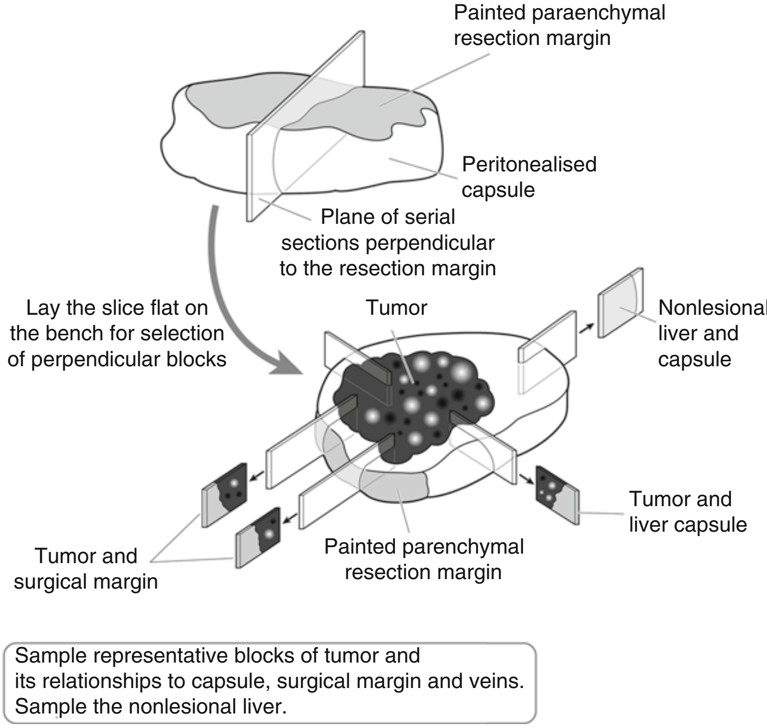

Fig. 10.3 Partial hepatectomy specimen (Reproduced, with permission, from Allen and Cameron (2004))

Trauma
- Capsular tear, subcapsular hemorrhage, parenchymal laceration

Tumor mass
- Edges: circumscribed/irregular/nodular/elevated
- Central scar: focal nodular hyperplasia
- Hemorrhage: hematoma, adenoma
- Bile stained: hepatocellular carcinoma
- Central necrosis/umbilication/mucinous/peripheral calcification: metastatic carcinoma

- Look for vascular invasion, e.g., thrombi in intrahepatic veins and/or any attached length of vena cava.
- Non-lesional liver
  Fatty change/cholestasis/necrosis/cirrhosis/hemochromatosis.

Blocks for histology (Fig. 10.3):

- For abscess, cyst, or trauma, four or five representative blocks of the wall, any capsular tear or hemorrhage, and adjacent hepatic parenchyma are usually sufficient.
- For a tumor mass, also sample a minimum of four or five representative blocks to demonstrate the lesion in relation to the capsule, surgical margin, uninvolved liver, and any other relevant structures, e.g., veins. Additional blocks are taken as required, e.g., if in close proximity to the surgical margin. Sections from the periphery of a tumor are often more informative than from the center as there is less necrosis with preservation of tumor tissue, and its interface with the parenchyma can be demonstrated.
- Sample non-lesional liver.

For total hepatectomy specimens (Fig. 10.4):

- Weight (g) and measure (cm) in three dimensions.
- If there is a previous diagnosis of hepatitis, incise deeply at several points to ensure an adequate period (48–72 h) of fixation prior to further handling.
- Identify the porta hepatis and transverse section its surgical margin to include the distal limit of the bile duct, hepatic artery, and portal vein. Further transverse sections at mid-duct and hilar levels can be submitted.
- Count and sample all lymph nodes.
- Dissect off the gallbladder and routinely process if macroscopically normal.
- Section the liver in its long axis either side of the hilum.

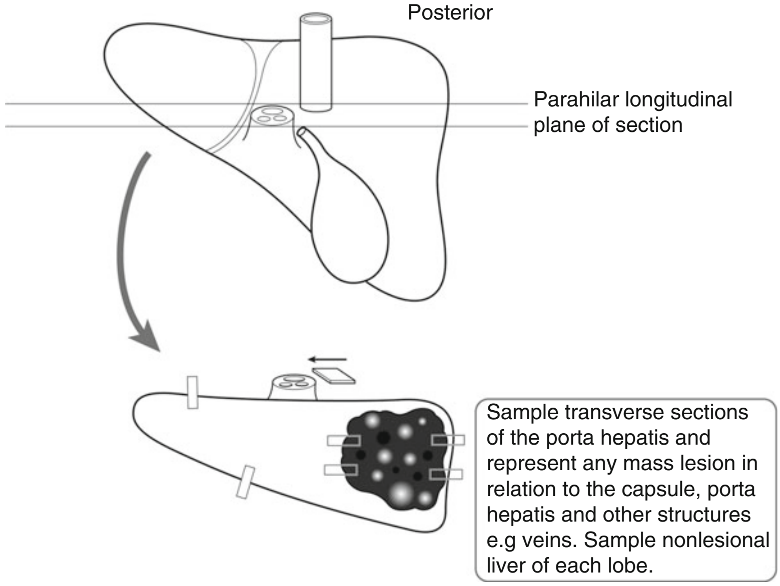

Fig. 10.4 Total hepatectomy specimen (Reproduced, with permission, from Allen and Cameron (2004))

- Sample representative blocks from the anatomical lobes and additionally as indicated by any mass lesion to demonstrate its relationship to the capsule, vessels, and porta hepatis.
- Serially slice the rest of the liver to detect any further lesions and sample accordingly.

Histopathology report:

- Tumor type – hepatocellular carcinoma/cholangiocarcinoma/metastatic carcinoma
- Tumor differentiation – well/moderate/poor
- Extent of local tumor spread (hepatocellular carcinoma)

pT1	Solitary tumor without vascular invasion
pT2	Solitary tumor with vascular invasion or multiple tumors, none more than 5 cm in greatest dimension
pT3	Multiple tumors more than 5 cm (pT3a) or tumor involving a major branch of the portal vein or hepatic vein (pT3b)
pT4	Tumor(s) with direct invasion of adjacent organs other than the gallbladder or with perforation of visceral peritoneum

- Lymphovascular invasion – present/not present. Note the propensity for hepatocellular carcinoma to invade portal tract veins, major branches of portal and hepatic veins, and inferior vena cava. Cholangiocarcinoma typically shows perineural space invasion with spread to lymph nodes, lungs, and peritoneum
- Regional lymph nodes: hilar (hepatoduodenal ligament), hepatic (along the proper hepatic artery), periportal (along the portal vein), and those along the abdominal inferior vena cava above the renal veins (except the inferior phrenic nodes). A regional lymphadenectomy will ordinarily include three or more lymph nodes

pN0	No regional lymph node metastasis
pN1	Metastasis in regional lymph node(s)

- Excision margins
  Distances (mm) to the capsule and limits of excision of the hepatic parenchyma, bile ducts, and major veins
- Other pathology
  Hepatocellular carcinoma – hepatitis, cirrhosis (hepatitis/alcohol/hemochromatosis, etc.), dysplastic nodules, liver cell dysplasia.
  Cholangiocarcinoma – primary sclerosing cholangitis, ulcerative colitis, liver fluke, biliary tree anomaly.

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Abdominal Wall, Umbilicus, Hernias, Omentum, and Peritoneum

11

Derek C. Allen, R. Iain Cameron, and Maurice B. Loughrey

11.1 Anatomy

The anterior abdominal wall is formed by skin, fascia, and striated muscles including rectus abdominus and the oblique muscles. The inguinal canal is an oblique passage through the groin area of the lower abdominal wall, and although present in both sexes, it is more prominent in the male, allowing structures to pass to and from the testis.

The umbilicus is present in the midline of the anterior abdominal wall and is a scar caused by the attachment of the umbilical cord, allowing blood vessels to pass to and from the fetus.

The greater omentum is a two-layered fold of visceral peritoneum that is attached to the greater curvature of the stomach and transverse colon. It hangs down between the coils of small intestine and anterior abdominal wall. The omentum contains lymphoid aggregates which are thought to have an immunological function locally in the peritoneal cavity. The omentum provides a large area for electrolyte/fluid absorption and will also adhere to sites of inflammation/bleeding.

11.2 Clinical Presentation

Abdominal wall lesions present with a lump that may be associated with discomfort or discharge.

A hernia occurs when part or all of a viscus protrudes through the confines of the body cavity in which it is normally situated. It presents with a dull "dragging" sensation or palpable lump usually evident on coughing. Severe constant pain is a sign of impending strangulation and ischemia of any omentum or small bowel contents which may also become obstructed.

Umbilical disease can present with a lump, discharge, or inflammation (omphalitis) and infection leading to pain and abscess formation. Caput medusa results from dilatation of periumbilical veins secondary to increased portal venous pressure usually due to liver cirrhosis.

Omental disease is usually due to inflammation or malignancy in adjacent organs but primary disease can also lead to adhesions and small bowel obstruction; torsion produces nausea and vomiting, and tumor, an abdominal mass.

D.C. Allen (✉)
Histopathology Laboratory, Belfast City Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: derek.allen@belfasttrust.hscni.net

R.I. Cameron
Histopathology Laboratory, Altnagelvin Hospital,
Western Health and Social Care Trust,
Londonderry, UK
e-mail: iain.cameron@westerntrust.hscni.net

M.B. Loughrey
Histopathology Laboratory, Institute of Pathology,
Royal Victoria Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: maurice.loughrey@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_11, © Springer-Verlag London 2013

11.3 Clinical Investigations

- FBP – elevated WCC in omphalitis
- LFTs/liver biopsy – in patients with caput medusa
- Pus swab – umbilical abscess/suppuration
- AXR – dilated small bowel loops in obstructed hernia
- Fistulogram – contrast may be passed into an umbilical fistula to show its origins and course
- Herniography – this rarely used investigation involves injecting contrast into the peritoneal cavity. A plain AXR will then reveal the presence and position of a hernial sac
- USS – useful in diagnosing a hernia and in distinguishing it from other groin conditions and in the investigation of tumors of the abdominal wall
- CT/MRI scan – will outline tumors of the abdominal wall, and pelvic and abdominal tumors involving the omentum
- FNA/needle core biopsy – used in the diagnosis of abdominal wall tumors
- Laparoscopy and biopsy – useful in the investigation of omental lesions, particularly secondary tumors

11.4 Pathological Conditions

11.4.1 Abdominal Wall and Umbilicus

Various conditions can affect the abdominal wall and result in both FNA and histopathology specimens.

Secondary carcinoma: Commonly due to either gastrointestinal or gynecological cancer involvement can be by direct spread at presentation or because of a subsequent metastatic recurrence. The former is not infrequently seen with a perforated bowel cancer and the inner layers of the abdominal wall may be dissected off separately or in continuity with it. The latter tends to be encountered as an intramural nodule or deposit with a previous history of bowel resection and is often amenable to diagnosis by clinical FNA. Classically secondary carcinoma (colon, ovary, breast) can present as an umbilical deposit (Sister Mary Joseph's nodule) which is also a site for hernias, endometriosis, or fistula due to persistence of an embryonic structure, e.g., the vitellointestinal duct or urachus. These result in umbilical protrusion, cyclical menstrual hemorrhage, or serous discharge, respectively. A persistent urachal remnant may be attached to the dome of the urinary bladder potentially acting as a source for internal hernia with bowel entrapment and ischemia.

Abdominal fibromatosis (*"desmoid tumor"*): A locally infiltrative and recurrent form of fibromatosis typically in the anterior abdominal wall of a woman of child-bearing age with a previous history of caesarean section. It has no potential to metastasize but causes problematic recurrent intestinal obstruction and may occasionally be associated with intestinal polyposis (Gardner's syndrome), a variant of FAP.

Amyloidosis: Involvement of anterior abdominal wall subcutaneous fat in systemic amyloidosis allows a diagnosis to be made by needle aspiration or biopsy with smearing of fat onto glass slides for Congo Red staining.

*Stoma*s: Such as ileostomy or colostomy fashioned during a gastrointestinal surgical operation may subsequently be taken down as part of the planned procedure, or revised due to dysfunction. The latter can be due to ischemia or mucosal prolapse with obstruction. Occasionally secondary carcinoma involves the stomal site.

11.4.2 Hernias

External hernias involve protrusion of peritoneum ± omentum and bowel into the layers of the abdominal wall (particularly at the site of a previous surgical incision), inguinal or femoral canals. The hernial sac is usually thin walled, comprising fibrous connective tissue lined by peritoneum. It may become irreducible and undergo secondary ischemia of the contents and with ulceration and infection of the overlying skin. Internal hernias into anatomical spaces (e.g., the lesser omental sac) or across fibrous bands (congenital or acquired) may also obstruct and become ischemic.

11.4.3 Omentum and Peritoneum

The omental fat and peritoneal serosa may be involved by various inflammatory and neoplastic disorders.

Inflammation: Acute due to appendicitis or a perforated viscus (gastric ulcer, diverticulitis), or, granulomatous, e.g., tuberculosis, fungal peritonitis (chronic ambulatory peritoneal dialysis – CAPD) or after previous surgery. CAPD can also be associated with the rare condition of sclerosing peritonitis.

Infarction: Spontaneous, idiopathic omental infarction in the right iliac fossa mimicking acute appendicitis (rare), or, more commonly, infarction of an appendix epiploica (pericolonic fat tag), which may then undergo saponification and calcification. The latter are also seen as a consequence of acute pancreatitis or abdominal trauma. Omentum incarcerated within a hernial sac may also undergo ischemia.

Keratin granulomas: An unusual finding most often related to treatment and follow-up of a previous gynecological cancer, e.g., endometrioid adenocarcinoma of the uterus or vulval squamous carcinoma.

Peritoneal inclusion cysts: Relatively common, solitary, or multiple, and should be distinguished from lymphangitic cysts (cytokeratin negative endothelial lining) and well-differentiated multicystic peritoneal mesothelioma. The latter is rare, occurring on the surfaces of the uterus, ovary, bladder, rectum, and pouch of Douglas, with potential for recurrence and invasion locally into retroperitoneum, bowel mesentery, and wall. Some have a previous history of surgery, endometriosis, or pelvic inflammatory disease.

Mesothelial proliferation: Other mesothelial proliferations include

Mesothelial hyperplasia – commonly seen as a reactive phenomenon in omentum adherent to an inflammatory or neoplastic abdominopelvic lesion and within a hernia. Sometimes it is florid, and distinction from mesothelioma can be difficult.

Well-differentiated papillary peritoneal mesothelioma – rare, with most being an incidental finding at hysterectomy, usually localized and benign but occasionally diffuse.

Diffuse malignant mesothelioma – epithelioid/sarcomatoid or mixed in pattern and a strong association with occupational asbestos exposure and spread from a primary pleural mesothelioma. Prognosis is poor, with the majority of patients dying from their disease within months or 1–3 years. Of very limited suitability for resection.

Peritoneal serous epithelial proliferation: Strongly associated with ovarian serous borderline tumors and either regarded as benign (endosalpingiosis) or potentially progressive (invasive proliferating implants). Also frankly malignant – primary peritoneal carcinoma. The former two conditions are microscopic findings, while the latter is an ovarian serous-type adenocarcinoma with extensive peritoneal disease but minimal ovarian involvement.

Pseudomyxoma peritonei: Characterized by filling of the peritoneal cavity with abundant mucin, which may contain variably bland, atypical, or frankly malignant epithelium. Prognosis is poor as it is refractory to treatment, slowly progressive, and leads to bowel obstruction. There is a strong association with appendiceal and ovarian mucinous tumors and occasionally secondary colorectal or pancreaticobiliary neoplasms. Appendicectomy should be considered in the presence of bilateral cystic ovarian tumors associated with peritoneal disease to rule out a primary appendiceal malignancy.

Secondary adenocarcinoma: Staging and therapy are considered:

Staging – diagnosed either by peritoneal aspiration cytology, laparoscopic biopsy, or open biopsy with frozen section at exploratory laparotomy as a prequel to consideration of suitability for operative resection of an abdominopelvic cancer. Postoperative pathological staging of ovarian carcinoma also partly relates to the size

(<or>2 cm) of the peritoneal deposits – it requires removal of the primary ovarian lesion, biopsy of the contralateral ovary, omentum, and peritoneum, and peritoneal washings for cytology if ascitic fluid is not present.

Therapy – tumor debulking or cytoreductive surgery of extensive omental disease is an important initial step prior to adjuvant chemotherapy in ovarian and other abdominopelvic cancers.

Other cancers: These are rare, e.g., intra-abdominal desmoplastic small round-cell tumor – divergent cellular differentiation, aggressive, pelvis and abdomen of young people.

11.5 Surgical Pathology Specimens: Clinical Aspects

11.5.1 Biopsy Specimens

Needle core biopsies of abdominal wall and umbilical tumors provide a diagnosis and allow future management to be planned. Laparoscopy and omental biopsy can be used as a staging investigation, and is particularly useful in gastric tumors and ovarian tumors/pseudomyxoma peritoneii.

11.5.2 Resection Specimens

Groin hernias – In uncomplicated groin hernias, the principle of surgery is to reduce the hernia sac and repair the defect in the abdominal wall. This can be done by either suturing or introducing a prosthetic mesh. In complicated hernias in which the small bowel may be incarcerated, the hernia sac needs to be opened and the viability of the intestine assessed. If it is in question, then a small bowel resection may be required.

Abdominal wall tumors – Primary abdominal wall tumors such as desmoid tumors are treated by wide excision. The excision usually entails excising the skin and rectus sheath, and may even extend down to the parietal peritoneum.

Umbilical lesions – Primary tumors of the umbilicus, e.g., squamous carcinoma, are treated by excision of the umbilicus (omphalectomy), surrounding skin, and full thickness of the periumbilical wall. If abscess formation occurs following umbilical infection, this should be treated by incision and drainage. Omphalectomy may be required if infection is recurrent.

Omentectomy – This is a relatively straightforward procedure usually undertaken as part of more extensive surgery, e.g., during a gynecological cancer operation for therapeutic cytoreduction and staging purposes. It involves ligation of the vessels along the greater curvature of the stomach and transverse colon with division of the omentum in this area.

11.6 Surgical Pathology Specimens: Laboratory Protocols

11.6.1 Abdominal Wall

Ranging from biopsy fragments taken at laparotomy to formal excision of an abnormal segment of tissue. Specimens from the inner aspect of the wall comprise rectus sheath muscle orientated along one edge to peritoneum and with distortion by the relevant pathological condition. External specimens are composed of skin, subcutaneous fat ± abdominal wall muscle and may also contain the umbilicus, a stoma, or incisional hernia. Biopsy fragments are processed in the usual manner, but larger specimens need to be individually described as to their constituent parts, their respective dimensions, and the abnormalities that are present. These specimens are usually submitted already fixed in formalin.

Mass lesion (tumor, fibromatosis, endometriosis, abscess):

- Maximum dimension (cm), edges (circumscribed/irregular), cut surface (mucinous/scirrhous/fibrotic/hemorrhage/pus), distances (mm) to the skin and nearest resection margin, involvement of skin (ulceration/tethering), subcutis, muscle, or peritoneum.
- Paint the deep and lateral resection margins.
- Serially section transversely at 3–4 mm intervals perpendicular to the skin or peritoneal surfaces.

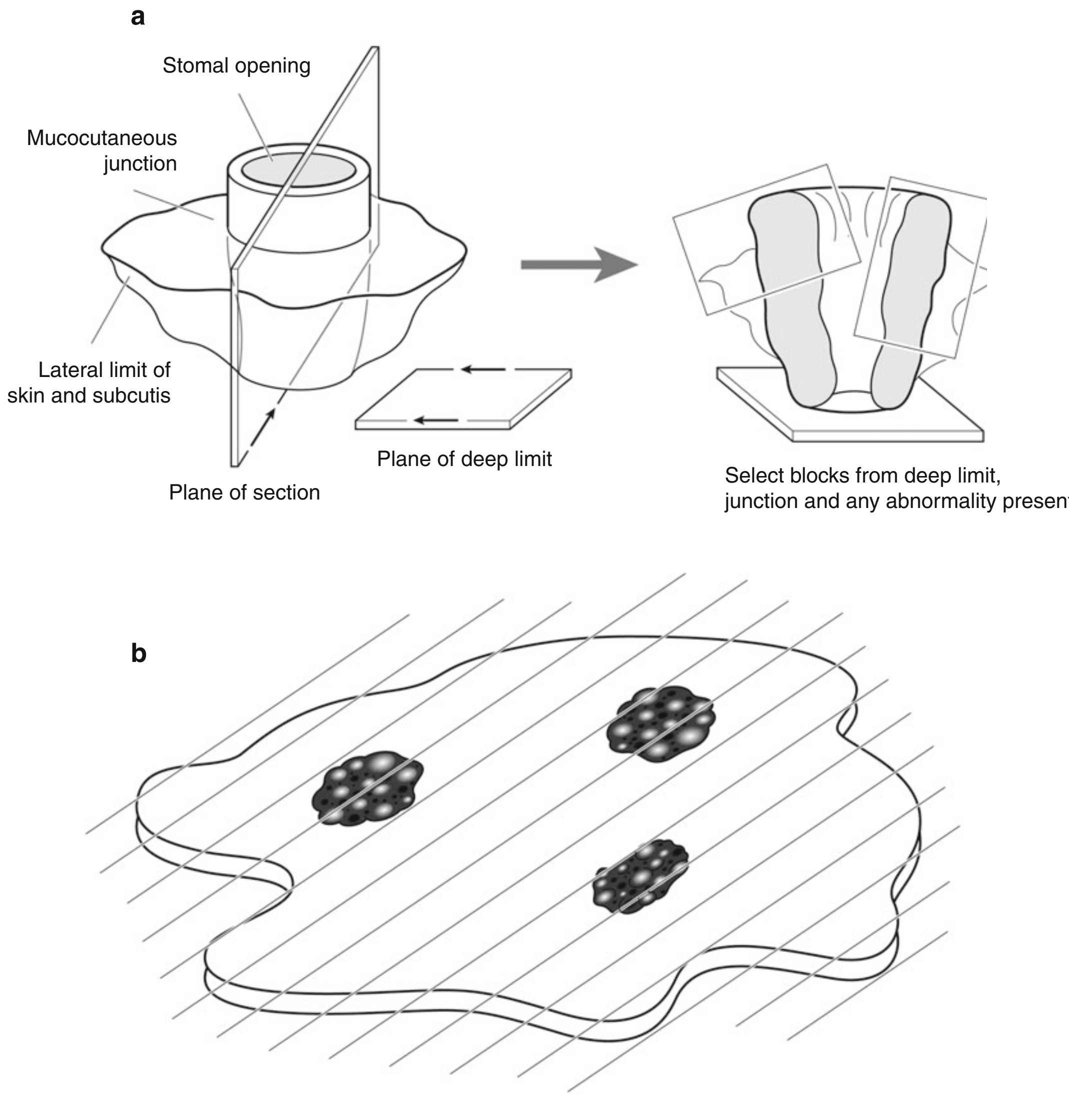

Fig. 11.1 (**a**) Sectioning of an abdominal wall mass, stoma (illustrated), or hernia; (**b**) sectioning of omentum (Reproduced, with permission, from Allen and Cameron (2004))

- Sample four or five representative blocks of the lesion showing its relationship to the various anatomical layers and resection margins. If close (≤0.5 cm) to a long axis margin, obtain a longitudinal block to demonstrate this.

Stomas:

- Note any mucosal prolapse, ulceration, ischemia, or tumor at the mucocutaneous junction, or bowel stricture – record their maximum dimensions (cm) and distances (cm) to the cutaneous, subcutaneous, and proximal bowel resection limits.
- Paint the deep and lateral resection margins.
- Transverse section the proximal bowel resection limit.
- Serially section the specimen transversely at 3–4 mm intervals perpendicular to the skin surface (Fig. 11.1a).
- Sample four or five representative blocks of the stomal junction/opening and any other relevant macroscopic abnormality.

11.6.2 Hernias

- Note any surgical scars or ulceration of the skin, necrosis in the skin, subcutis, abdominal muscle, wall of the hernial sac or its contents.
- Hernial sac – dimensions and wall thickness (cm).
- Contents – omentum, bowel, and their dimensions (cm).
- Paint the deep and lateral resection margins.
- Transverse section bowel resection limits, if present.
- Sample four or five representative blocks of the hernial sac and its contents to demonstrate its relationship to the various anatomical layers and any abnormality that is present.

11.6.3 Omentum and Peritoneum

- Laparoscopic biopsy fragments are processed in the usual manner and cut through multiple levels.
- Omental specimens vary in size, depending on whether the investigation is for diagnostic, staging, or therapeutic purposes. Typically comprising lobulated fat or the omental curtain with a shaggy, lace-like appearance weights vary from a few to several hundred grams. Record the weight (g) and dimensions (cm).
- Serially slice at 0.5 cm intervals and closely inspect (Fig. 11.1b).
- Note any macroscopic abnormalities – nature (abscess/fat necrosis/cysts/tumor), edge (circumscribed/irregular), consistency (cystic/fibrotic/mucoid/scirrhous), contents (serous fluid/lymph/mucin), number, and the maximum dimension (cm).
- Sample three representative blocks of macroscopically abnormal or unremarkable omental specimens.

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Part II

Breast Specimens

12 Breast

Tong Fang Lioe and R. Iain Cameron

12.1 Anatomy

The mature adult breast is composed of fatty tissue and parenchyma in which terminal ductulo-lobular units of epithelium are surrounded by fibrous connective tissue stroma. There are about 15–25 lobes of parenchymatous elements associated with each of the lactiferous ducts which drain into the nipple. The presence of this functional lobar arrangement provides an anatomical framework for some surgical procedures such as major duct excision and quadrantectomy for cancer. The anatomical boundaries of the breast are not well defined except at the deep surface where the gland overlies the pectoralis fascia. The general topographical anatomy of the breast is illustrated in Fig. 12.1

Lymphovascular drainage:

There are three routes of lymphatic drainage in the breast. The most important is to the axilla which receives 75% or more of the lymphatic flow to the axillary lymph nodes. These are located at various anatomical levels – Levels I, II, and III (Fig. 12.2). Drainage via the internal lymphatics into the internal thoracic nodes comprises 25% or less of the lymph flow and the third and least important is to the posterior intercostal nodes where the ribs and vertebrae articulate.

12.2 Clinical Presentation

Symptomatic: Patients commonly present with a palpable lump, breast pain, nipple discharge, or rash. Breast pain is usually not associated with any significant pathology. Nipple discharge if bloody can be due to an intraduct proliferation which may require surgical excision (microdochectomy). Nipple rash may need to be biopsied to exclude Paget's disease. A palpable discrete lump can be cystic or solid. The majority of cystic lumps are benign simple cysts which can be drained by needle aspiration. Occasionally cancerous lumps may have a cystic component which is usually bloody. Solid lumps are investigated by the triple assessment approach (see below) to provide a nonoperative diagnosis of benignity or malignancy.

Screening: At present, all women aged between 50 and 70 are invited to the National Health Service Breast Screening Programme, which initially involves mammography to screen out lesions that require further evaluation by the triple assessment approach such as a spiculate density or areas of microcalcification. Microcalcifications with no associated soft tissue abnormality may be

T.F. Lioe (✉)
Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: tong.lioe@belfasttrust.hscni.net

R.I. Cameron
Histopathology Laboratory, Altnagelvin Hospital, Western Health and Social Care Trust, Londonderry, UK
e-mail: iain.cameron@westerntrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_12, © Springer-Verlag London 2013

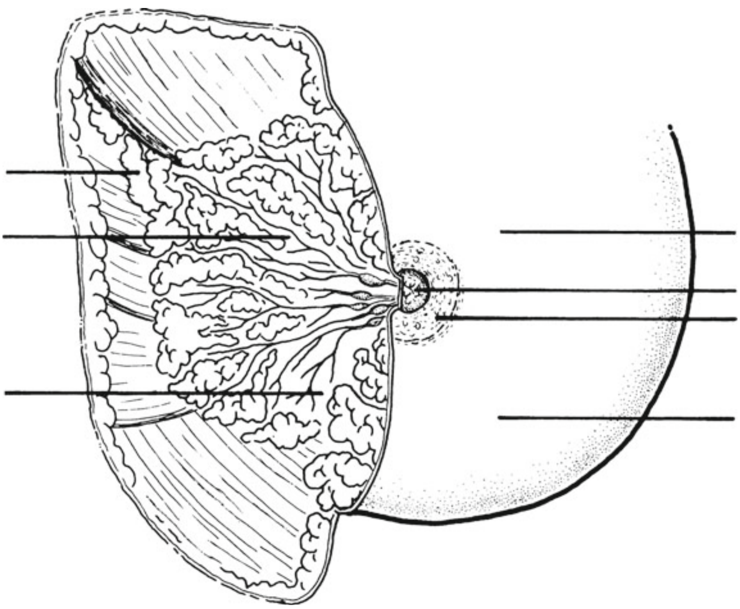

Fig. 12.1 Topographical anatomy of the right breast (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

the only sign of malignancy and is the mode of presentation of up to one third of cancers found at screening. Linear branching microcalcifications are usually associated with comedo ductal carcinoma in situ (DCIS) and have a higher predictive value for malignancy than nonlinear irregular microcalcifications. Overall the sensitivity of mammography is 85–90%.

12.3 Clinical Investigations

Clinical examination: Symptomatic patients are referred to a dedicated Breast Clinic, where they are assessed by a multidisciplinary team of specialists. The patient is usually first seen and examined by a breast surgeon who instigates further investigations where appropriate.

Radiological imaging: The patient normally has standard two-view mammography, i.e., craniocaudal and medio-lateral oblique views performed. Additional magnification views may be required to focus on a suspicious area and facilitate more detailed examination. The radiologist then decides if the lesion warrants further investigation by ultrasonography. Younger women with dense breast tissue are usually investigated by ultrasonography. Suspicious areas of microcalcification and parenchymal deformity are then aspirated and/or core biopsied. In cases of suspected malignancy, ultrasound scanning of the ipsilateral axilla is undertaken, and if an enlarged or abnormal node is found this can be aspirated or biopsied.

Fine needle aspiration cytology (*FNAC*): This involves the insertion of a 23 G needle into the lump. The needle is moved about within the lump and negative pressure is applied with the attached syringe on a holder. The procedure ideally is performed by the radiologist under ultrasound guidance. The aspirated material is then smeared on glass slides, air dried, and stained for immediate microscopic examination by the cytopathologist who will then indicate if the sample is adequate for diagnosis and if so whether the lesion is benign or malignant.

Needle core biopsy (*NCB*): This is performed with a wide-bore spring-loaded device which requires local anesthesia prior to the procedure. It can be done either with radiological guidance or freehand. In some centers, NCB is only carried out when the aspirate is non-diagnostic. However, in other centers NCB is carried out on all lesions

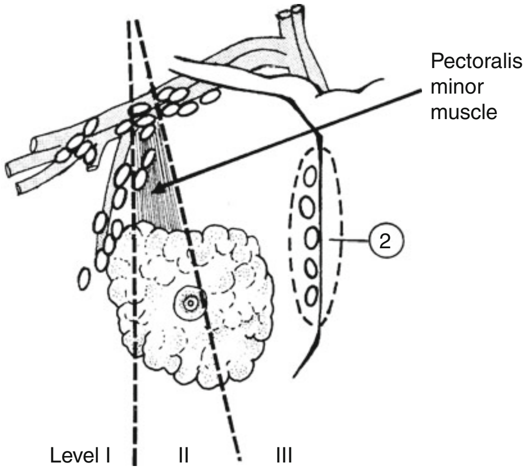

Fig. 12.2 Breast: regional lymph nodes (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))
1. Axillary (ipsilateral): interpectoral (Rotter) nodes and lymph nodes along the axillary vein and its tributaries, which may be divided into the following levels:
(i) *Level I* (low axilla): lymph nodes lateral to the lateral border of pectoralis minor muscle.
(ii) *Level II* (mid-axilla): lymph nodes between the medial and lateral borders of the pectoralis minor muscle and the interpectoral (Rotter) lymph nodes.
(iii) *Level III* (apical axilla): lymph nodes medial to the medial margin of the pectoralis minor muscle including those designated as subclavicular or apical. *Note*: Intramammary lymph nodes are coded as axillary lymph nodes.
2. Internal mammary (ipsilateral): lymph nodes in the intercostal spaces along the edge of the sternum in the endothoracic fascia (2). Any other lymph node metastasis is coded as a distant metastasis (M1), including supraclavicular, cervical, or contralateral internal mammary lymph nodes

and may be the only preoperative sample. In malignant lesions, in contrast to FNAC, NCB allows the distinction between in situ and invasive carcinoma to be made. In contrast to aspirate cytology, which allows immediate reporting, needle cores require overnight processing before a result is obtainable by histology.

Triple assessment approach: The above triple approach, utilizing the combination of clinical (surgical) examination, radiological imaging by X-ray and/or ultrasound, and cytological assessment of aspirated material along with NCB, has been shown to be highly accurate in the preoperative diagnosis of breast cancer. This has superseded "frozen-section" examination of suspected breast cancers. Patients proven to have breast cancer by the triple assessment can then go on to a one-stage therapeutic procedure which is excision of the tumor together with an axillary node procedure (see below).

Open excision biopsy: In a small minority of cases, a nonoperative diagnosis is not conclusive or malignancy cannot be excluded; hence, an open biopsy is required for histological diagnosis. Lesions like radial scars or papillary growths need formal histological assessment to exclude associated in situ or invasive malignancy. Impalpable lesions and areas of microcalcification require radiological needle localization to guide the surgeon to the area in question for adequate excision.

12.4 Pathological Conditions

12.4.1 Non-neoplastic Conditions

Fibroadenosis/fibrocystic changes: These are common in the breast and present as ill-defined masses or plaques. There is a varying degree of epithelial proliferation and hyperplasia, with or without cyst formation, and associated apocrine metaplasia or sclerosing adenosis. Excision of these lesions sometimes occurs at the request of the patient despite a nonoperative diagnosis of benignity by the triple assessment.

Cysts: Simple cyst formation is very common and presents as a firm but fluctuant lump. Needle aspiration to dryness is usually all that is required.

Breast abscess: Most commonly encountered in non-lactating premenopausal women in a subareolar location as a result of duct obstruction. It is usually diagnosed by FNAC and seldom requires surgical intervention unless there is failure to resolve.

Fat necrosis: This is most commonly seen in overweight women and those with pendulous breasts usually following a history of trauma. Clinically and radiologically, fat necrosis can mimic a carcinoma but can be distinguished by FNAC. However, an excision biopsy may be required if the lesion persists.

Duct ectasia: This is due to duct dilatation with filling of the duct lumen by amorphous material and accompanying chronic inflammation in the duct wall and periductal stroma. Nipple discharge is usually the first symptom, but a worm-like palpable mass may form in the subaerolar region in more advanced cases where there is periductal fibrosis. Excision of the area may be necessary to exclude DCIS.

Gynecomastia: This is the most common clinical and pathological abnormality in the male breast. It is encountered in adolescent or adult males and is usually unilateral. In older men, it may be due to certain drug usage such as digitalis, spironolactone, and cimetidine. It forms a firm to rubbery plaque deep to the nipple. Patients with bilateral involvement tend to have diffuse lesions as compared to unilateral gynecomastia which is more discrete. FNAC usually produces a low to moderately cellular specimen which may show a mild degree of nuclear atypia, but the presence of bare nuclei should be reassuring. Excision of the lesion is most likely performed for cosmetic reasons. A small minority of cases is due to an underlying malignancy.

Reduction mammoplasty: Bilateral reduction mammoplasty surgery may be performed on large pendulous breasts for physical, psychosexual, or cosmetic reasons. Symmetrical volumes of fatty breast tissue are removed with overlying non-nipple-bearing skin. There is normally no significant pathology in the tissues.

Leakage from silicone implants: A fibrous capsule usually forms around a silicone implant, but silicone may migrate into and through it. Rupture of the capsule can occur by accident, mammography, or closed capsulotomy. Rupture of the silicone rubber envelope may occur asymptomatically and once outside the envelope, silicone can disperse through soft tissue, lymph nodes, or the vasculature. Silicones are detected in the tissue as small round-to-irregular translucent droplets of amorphous refractile nonpolarizing material. Silicone leakage into the capsule is characterized by a typical microscopical appearance of oval-to-round holes partly filled with silicones. Giant cells of foreign body type may be found and granulomas as a reaction to silicone ("siliconomas") are seen after extracapsular rupture of an implant and after injection with silicone. Calcification of the capsule is common around implants which have been in situ for many years.

12.4.2 Neoplastic Conditions

12.4.2.1 Benign Tumors

Fibroadenoma: This is the commonest benign tumor of the breast most often encountered in premenopausal women who present with a palpable painless and mobile discrete lump. Nonoperative diagnosis can be confidently made by the triple approach except in large lesions where excision may be advised to exclude a low-grade phyllodes tumor.

Proliferative lesions (*radial scar/complex sclerosing lesion, intraduct papilloma, nipple adenoma, myoepithelioma*): These lesions are due to epithelial proliferations of various complexities which can present as firm palpable masses. Mammography may show parenchymal deformity and foci of microcalcification, thus necessitating cytological assessment. The latter usually shows a highly cellular sample with some degree of nuclear atypia, indicating either a core biopsy or local excision. Some of these lesions may harbor DCIS, which can only be confirmed or excluded following histological examination.

Miscellaneous: Rarely benign lesions such as adenomas, hamartomas, fibromatosis, or pseudo-angiomatous stromal hyperplasia are encountered.

12.4.2.2 Malignant Tumors

Carcinoma in situ: Carcinoma in situ is a proliferation of malignant epithelial cells within the ductulo-lobular system of the breast, which on light microscopy shows no evidence of breaching the basement membrane to invade the adjacent stroma. There are two forms – ductal (DCIS) and lobular (LCIS) carcinoma in situ. LCIS cannot be diagnosed preoperatively and is seen incidentally on excision specimens as a marker for increased risk of developing malignancy. DCIS, on the other hand, is a heterogeneous group of premalignant lesions, which are usually asymptomatic and impalpable but may be identifiable on mammography as foci of microcalcification. It can sometimes present as a mass lesion. Nonoperative diagnosis of DCIS is based on FNAC and core biopsy. DCIS is categorized by the degree of nuclear pleomorphism as low, intermediate, or high grade, and by its architectural patterns – cribriform, solid, or micropapillary with or without comedo necrosis. DCIS with comedo necrosis is usually associated with dystrophic calcification and has a high nuclear grade. This subtype has the highest risk of stromal invasion. The treatment of DCIS depends on the size and distribution of the lesion. Localized DCIS may be amenable to wide local excision, while extensive disease requires a total mastectomy. Sentinel node biopsy is carried out in those patients with DCIS associated with a mass or in those with widespread DCIS requiring mastectomy. In cases of wide local excision, the specimen resection margins are carefully identified and labeled by an agreed protocol for close histological examination to assess completeness of surgical removal. Width of the excision margins around the tumor remains the most important factor in terms of risk of local recurrence, and a minimum clearance of 10 mm should be achieved. All cases treated by breast conserving surgery should have adjuvant radiotherapy.

Invasive carcinoma: Breast carcinoma is a heterogeneous group of tumors with different morphological growth patterns which reflect the clinical behavior and, hence, the prognosis. The most common form of invasive breast cancer is the ductal type, not otherwise specified, accounting for 75–80% of all breast cancers. This tumor type is diagnosed by exclusion of other specific types, viz., lobular, colloid or mucoid, tubular, medullary, and cribriform. Invasive lobular carcinoma is the next most common tumor type forming about 10–15% of cases. Tumors of mixed ductal and specific types are also encountered. Some of the specific tumor types such as colloid, tubular, and medullary have a better prognosis as they are less aggressive. Not infrequently, breast cancer may be multifocal (within the same quadrant) or multicentric (involving other quadrants) and, in some cases, bilateral. Breast cancers are graded 1, 2, or 3, depending on the degree of diffferentiation (see below) which has been shown to correlate with biological behavior. The tumor size, type, grade, presence, or absence of lymphovascular invasion and nodal status are pathological prognostic factors determining adjuvant therapy and outcome for the patient. Certain biological markers such as estrogen and Her2/neu receptor status can predict tumor response to hormonal or cytotoxic therapy, respectively.

Paget's disease of the nipple: Paget's disease of the nipple is characterized by infiltration of malignant ductal epithelial cells into the epidermis of the nipple-areolar complex. Clinically, it presents as an itchy and scaly rash which may be mistaken for eczema but gradually gives rise to ulceration, crusting, and bloody nipple discharge in advanced cases; 1–2% of breast cancers have associated Paget's disease. In patients presenting with features of Paget's disease without a clinically palpable mass, high nuclear grade DCIS is nearly always detected in the large subareolar lactiferous ducts and up to 40% will have an occult invasive tumor within the breast. An excision biopsy of the nipple is performed to confirm Paget's disease and treatment is usually by mastectomy.

Phyllodes tumor (*cystosarcoma phyllodes*): This is a tumor of fibroepithelial origin usually seen in older women compared to those with a fibroadenoma. These tumors average 4–5 cm in size and have a history of rapid growth. Radiology reveals a lobulated or rounded solid mass. FNAC usually produces a highly cellular aspirate composed of epithelium and stroma, features which overlap with a fibroadenoma hence making distinction between the two difficult. However, stromal fragments that are densely cellular may suggest a phyllodes tumor and, taking the patient's age and size of lesion into account, an excision biopsy would be indicated in these circumstances. The biological behavior of these neoplasms is unpredictable. Toward the benign end of the spectrum, they may locally recur if incompletely excised (a 10 mm margin should be achieved), but tumors with sarcomatous transformation will metastasize by the hematogenous route.

Mesenchymal tumors: Malignant mesenchymal tumors such as angiosarcoma, malignant fibrous histiocytoma, leiomyosarcoma, liposarcoma are all rare and have to be distinguished from a metaplastic carcinoma or sarcomatous transformation in a phyllodes tumor.

Metastatic tumors: Occasionally metastases from other primary sites may present as breast lumps such as melanoma, lymphoma, small cell lung carcinoma, ovarian and gastrointestinal adenocarcinoma. Some of these cases may be diagnosable preoperatively by FNAC/NCB.

Treatment and prognosis: The mainstay of treatment for breast cancer is surgery (with a 5 mm minimum tumor clearance of excision margins) followed by hormonal or chemotherapy where appropriate. Radiotherapy may be indicated to prevent local recurrence. In a small number of cases, neo-adjuvant therapy is instituted if the cancer is large and advanced or if surgery is contraindicated due to poor general health. Hormonal treatment is determined by estrogen and progestogen receptor status as assayed by immunohistochemistry. Chemotherapy is usually indicated in high-grade and node-positive cancers, particularly in the younger patient. Her2/neu receptor status is assessed either by immunohistochemistry or in situ hybridization techniques (fluorescent/chromogenic) as a predictive factor for patients with distant disease relapse who may respond to Herceptin (trastuzumab monoclonal antibody) therapy.

The single most important prognostic factor in breast cancer is nodal involvement at time of diagnosis. However, the 5-year survival rate has also been shown to correlate with histological tumor type and the Nottingham Prognostic Index (NPI) (see below).

Excellent prognosis tumor types with a 5-year survival of greater than 80% include tubular, mucinous, cribriform, and tubulolobular carcinoma. Good prognosis types with 60–80% 5-year survival include mixed ductal NST/special type, tubular mixed, and alveolar lobular variant. Intermediate prognosis (50–60% 5-year survival) types include classical lobular, medullary, and invasive papillary. Those with poor prognosis (<50% 5-year survival) are high-grade ductal NST, ductal and lobular mixed, pleomorphic lobular, and metaplastic carcinoma.

12.5 Surgical Pathology Specimens: Clinical Aspects

12.5.1 Biopsy Specimens

Needle core biopsy: This is performed in cases of equivocal cytology for carcinoma, to assess stromal invasion in cases of DCIS and suspicious microcalcifications, and, for grading and receptor status immunohistochemical studies in cancer, and advanced cancer requiring neo-adjuvant therapy. Usually up to six cores of tissue, particularly for DCIS and microcalcifications, are taken with a 19 G needle mounted on a spring-loaded gun under radiological guidance to yield 2–3 cm long worm-like samples. They are X-rayed prior to fixing in formalin in cases of microcalcification to ascertain that the right area has been sampled.

Needle localization biopsy: In cases where the nonoperative diagnosis by FNAC and/or core biopsy is inconclusive, a diagnostic biopsy is required for histological assessment. The majority of these cases are from screening and involve foci of microcalcification of radiological concern. Some cases of stromal or parenchymal deformity, even though they have been proven to be malignant by FNAC or core biopsy, also require needle localization (by stereotaxis or ultrasound) because they are impalpable and this assists the surgeon in removing the appropriate area.

Nipple biopsy: This is done either as a small wedge or punch biopsy of the nipple skin to confirm or exclude Paget's disease.

Microdochectomy: Also known as main duct excision for cases of persistent nipple discharge or an intraductal epithelial growth which may have been suggested on smear cytology of the discharge material.

12.5.2 Resection Specimens and the Types of Surgery

Surgical treatment for localized breast cancer: Most patients with breast cancer will have a combination of local treatment to control local disease and systemic treatment to manage metastatic disease. Local treatment consists of surgery and radiotherapy. Surgery can be an excision of the cancer with surrounding normal breast tissue (breast-conserving surgery) or a mastectomy. Certain clinicopathological factors influence the selection for breast-conserving surgery or mastectomy, depending on the likelihood of local recurrence after the former. These include the site and size of tumor, extent of DCIS, multifocal or multicentric disease, incomplete initial excision, and the age of the patient. All patients treated with breast-conserving surgery should have adjuvant radiotherapy and also those with tumor involvement of the deep margin following mastectomy.

Breast-conserving surgery (*BCS*): BCS may consist of removal of the tumor with a 1 cm margin of normal tissue (wide local excision) or a more extensive excision of a whole quadrant of the breast (quadrantectomy). This comprises a cylinder of tissue taken from the skin superficially to the pectoralis fascia at the deep aspect. A partial mastectomy involves removal of the tumor with surrounding breast tissue and an ellipse of non-nipple-bearing skin. Small and impalpable cancers, often detected by screening, are usually localized radiologically by a guide wire prior to surgery to assist the surgeon in excising the appropriate area. The single most important factor predicting local recurrence following BCS is completeness of excision. Invasive or in situ disease at the resection margins increases local recurrence by a factor of 3.4. An extensive in situ component increases local recurrence only when margins are involved. The presence of tumor lymphovascular invasion (LVI) doubles the local recurrence rate and Grade I tumors are less likely to recur locally compared to higher-grade tumors. Some of these factors are only fully appreciated after detailed histological assessment and subsequent conversion to mastectomy as a second procedure is not an infrequent occurrence. Clinically breast cancers that are suitable for treatment by breast conservation include a single clinical and mammographic lesion, tumor <3 cm in diameter or >3 cm in large breasts and with no sign of local advancement (i.e., no extensive nodal involvement or metastases).

Cavity shavings: These are additional portions of breast tissue submitted separately as shavings from the cavity after excision of the tumor when the surgeon assesses that he/she does not have clear margins following the initial excision. They are labeled accordingly as to site, viz., medial, lateral, superior, inferior, deep, or superficial.

Mastectomy: About a third of localized breast cancers are unsuitable for BCS and will require a total mastectomy. However, some patients who are suitable for BCS may also choose to have a mastectomy. Mastectomy removes the breast tissue with overlying skin including the nipple, while the chest wall muscles are left intact. Patients who are best treated by mastectomy include those with multifocal disease, extensive in situ component, centrally situated cancers, tumors >4 cm in diameter or for whom BCS would produce an unacceptable cosmetic result. A subcutaneous mastectomy involves the removal of all breast tissue including the nipple–areolar complex but retaining the skin usually as part of a breast reconstruction procedure.

Axillary node surgery: Axillary node dissection is performed in conjunction with BCS or mastectomy for prognostic/staging and therapeutic purposes. This can take the form of either sentinel node biopsy or axillary node clearance.

Sentinel node biopsy (*SNB*): Approximately two thirds of symptomatic and the majority of screening breast cancers are node-negative. Therefore, axillary node clearance (ANC) in these cases is not indicated if it could be predicted that the nodes are negative and to avoid any risk of morbidity such as arm pain and lymphedema. Lymphatic mapping and SNB is a new minimally invasive technique to identify patients with axillary node involvement. The sentinel node is defined as the first node to receive drainage from the tumor and is identified by injecting a vital blue dye, a radiocolloid, or both around the area of the tumor just prior to surgery. A small incision is made in the axilla and a handheld gamma probe or direct visual inspection used to identify the radioactive node or blue-stained lymphatic channels leading to a blue lymph node. The average number of sentinel nodes identified is two. These are excised and immediately sent to the laboratory where intraoperative examination in the form of imprint cytology and/or frozen section may be employed (see below). During this procedure, the surgeon performs the breast operation. If the node is found to be involved by metastatic tumor, then immediate ANC can be undertaken. Occasionally small metastatic deposits are not seen by intraoperative examination and are only seen after histological examination of the sections of node. In these cases, the patient will require a second stage ANC at a later date. The metastatic deposit in a sentinel node is classified according to size: macrometastasis >2 mm; micrometastasis 0.2–2 mm; isolated tumor cells <0.2 mm. How to manage sentinel nodes with isolated tumor cells is controversial, but currently it is felt that ANC is not indicated. An axillary sampling of 3–4 nodes may be provided if operative identification of the sentinel nodes is uncertain.

Axillary node clearance (*ANC*): The axilla is formally dissected out and nodes from Levels I, II, and III are retrieved for histological assessment and this usually totals between 20 and 40 nodes.

12.6 Surgical Pathology Specimens: Laboratory Protocols

12.6.1 Biopsy Specimens

Needle core and nipple biopsies: These are counted, measured (mm), processed whole, and cut through multiple levels. Nipple ellipses may need initial bisection depending on size.

Main duct excision specimens: These are weighed, measured, and externally painted, and then serially and transversely sliced to look for any intraluminal papillary growths. Multiple slices are processed for histology.

Benign biopsies: Excisions of preoperatively proven benign lesions such as fibroadenoma are routinely weighed, measured, and painted and representative tissue blocks taken for histology.

Needle localization biopsy specimens: These are treated as resection specimens (see below).

12.6.2 Resection Specimens

Specimen types:

1. Total mastectomy, SNB ± ANC
2. Breast conserving surgery – wide local excision, quadrantectomy, partial mastectomy, SNB ± ANC
3. Needle localization biopsy
   (a) For microcalcification
   (b) For parenchymal deformity

Specimens submitted fresh to the laboratory that have a clearly palpable lesion can be initially incised following painting of excision margins and prior to thorough formalin fixation (24–48 h). This allows sampling for research and optimal tumor fixation. Impalpable and localization specimens are not incised prior to fixation as this may distort the lesion precluding accurate assessment of histological appearances and relationship to the margins compounded by leaching of the paint onto the cut surface.

Initial procedure:

12.6.2.1 Mastectomy (total/partial) and quadrantectomy specimens

- Obtain all relevant histories of preoperative investigative procedures, e.g. FNAC, NCB, etc., especially in cases of multifocal disease.
- Weigh (g) and measure (cm) the specimen. Measure any ellipse of skin and note the presence or absence of the nipple–areolar complex and orientation sutures. Note the laterality (right or left).
- Differentially paint all margins using artists' pigments or similar dyes according to an agreed protocol.
- Serially slice transversely at 0.5–1 cm intervals (Fig. 12.3a) from the deep aspect to the

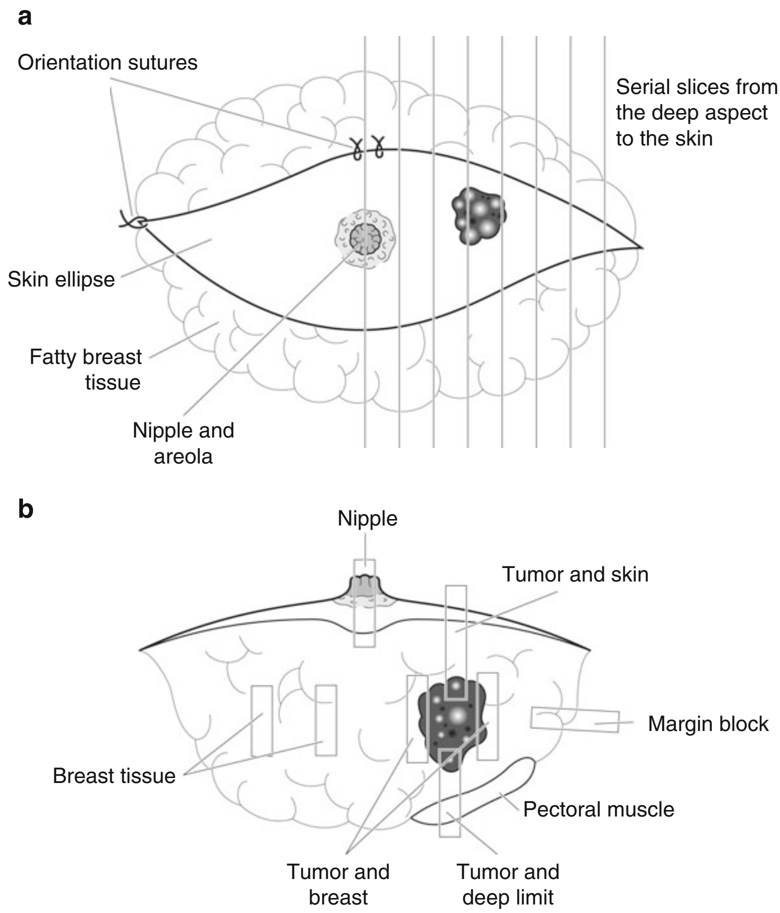

Fig. 12.3 Blocking mastectomy/quadrantectomy specimens (**a**). surface view (**b**). lateral view of a serial slice showing tumor, nipple, skin and specimen margins (Reproduced, with permission, from Allen and Cameron (2004))

skin using it as a spine to hold the specimen together.

- Identify invasive tumor or DCIS areas (oozes toothpaste-like material in comedo-type) and measure the largest diameter (mm).
- Measure distances (mm) of the tumor edge to the excision margins.

12.6.2.2 Needle localization and wide local excision specimens

- Obtain all relevant histories of preoperative investigative procedures, e.g. FNAC, NCB, etc.
- Make sure that laterality is stated (left or right) and orientation sutures are correctly placed before commencing. Note any accompanying specimen radiograph (obligatory for localization specimens) and the presence and location of any guide wire(s).
- Orientate the specimen with sutures or surgical clips as per protocol agreed with the surgeon (e.g., long suture for lateral margin, dark suture for deep).
- Differentially paint all margins using artists' pigments according to an agreed protocol.
- Serially slice the specimen at 0.3 cm intervals (Fig. 12.4a), lay out, and number the slices in sequence. Inspect with reference to the radiograph and guide wire tip (if applicable) and note any macroscopic lesion(s).
- Needle localization specimens for microcalcification may show no obvious abnormality grossly. The laid out and numbered tissue slices should be X-rayed by a Faxitron machine to help locate the area(s) in question for block selection. If an X-ray facility is not available, block especially fibrous parenchyma rather than

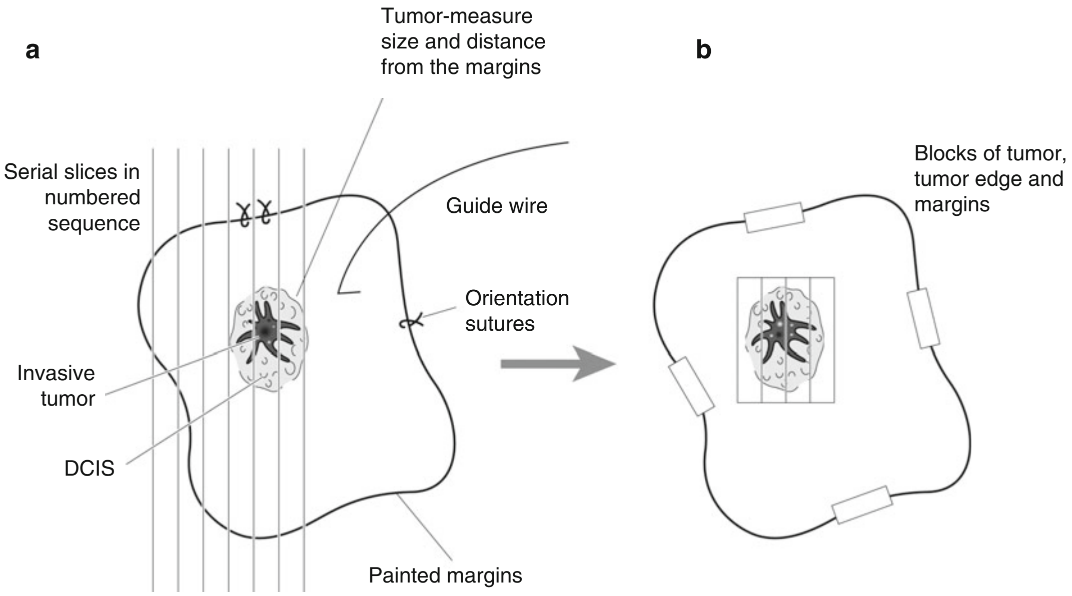

Fig. 12.4 Blocking a breast localization/wide local excision specimen (**a**). surface view (**b**). lateral view of a serial slice showing tumor and specimen margins (Reproduced, with permission, from Allen and Cameron (2004))

fatty tissue. Small specimens less than 20 g, however, may be processed in their entirety.

- Stromal deformity and mass lesions are identified grossly; the size and distances to the excision margins are measured.
- Cavity shavings from various margins are weighed, painted, and labeled accordingly before submitting for processing.

Description:

- Measure tumor size (mm) and note multifocal disease.
- Measure distances (mm) of tumor to the margins.
- Note the tissue block where the guide wire tip is in localization specimens, that orientation sutures are in place correctly, or if some sutures have fallen out.
- Note the character of tumor (scirrhous, mucoid), edge (circumscribed, irregular), etc.
- Note the character of surrounding breast tissue, and presence of skin, nipple, and skeletal muscle at the deep aspect.

Sentinel node biopsy:

- Cut the node into 2 mm serial sections perpendicular to the long axis.
- The cut surface of each section is imprinted several times on a glass slide.
- Air dry and stain with Rapi-Diff II stain (MGG).
- If suspicious on imprint cytology, standard frozen section procedure of section of node can be employed.
- Sections of node are fixed in formalin for 24 h.

Blocks for histology (Figs. 12.3b and 12.4b)

- Sample tumor, tumor edge, and, tumor and nearest margin(s) (minimum three blocks).
- Sample areas suspicious of DCIS.
- Sample surrounding breast tissue (1–2 blocks).
- Sample nipple skin in mastectomy specimens and tumor-involved skin or muscle.
- In completion mastectomy specimens, sample cavity wall (four blocks).
- Cavity-shave margins (1–2 blocks, selecting fibrous tissue).
- One H&E-stained section of each section of sentinel node.
- If sentinel node is negative on H&E examination, immunohistochemistry with a cytokeratin stain such as MNF 116 (*Dako*) can be used to reveal small metastatic deposits.

Table 12.1 Histological grading of invasive breast carcinoma

Three parameters are assessed and scored as follows:				
1.	Tubule formation			Score
	Majority of tumor (>75%)			1
	Moderate (10–75%)			2
	Little or none (<10%)			3
2.	Nuclear pleomorphism			
	Regular, uniform nuclei			1
	Larger irregular nuclei			2
	Marked variation in size and shape (±multiple nucleoli)			3
3.	Mitotic count (per 10 high-power fields – related to the objective field diameter)			
	Leitz Diaplan	Leitz Ortholux	Nikon Labophot	
	×40 obj.	×25 obj.	×40 obj.	
	0–11	0–9	0–5	1
	12–22	10–19	6–10	2
	>22	>19	>10	3
Total score	Grade	Differentiation		
3–5	I	Well		
6–7	II	Moderate		
8–9	III	Poor		

- Count and sample all axillary lymph nodes and label separately where indicated (Levels I, II, and III).
- A representative complete section of any grossly involved lymph node is adequate.
- Lymph nodes over 5 mm in maximum size should be sliced at approximately 3-mm intervals perpendicular to the long axis.

Histopathology report:

- Type and side of specimen
- Specimen size: dimensions, weight
- Tumor type
- Tumor grade: I, II, III (see Table 12.1 for grading system)
- DCIS present: no, yes (within/around/away from tumor)
- DCIS type
- DCIS nuclear grade (low, intermediate, high)
- Size of invasive component (cm)
- Size of DCIS (cm)
- Size of invasive + DCIS (cm)
- Nearest margin: medial, lateral, inferior, superior, deep, superficial (skin)
- Distance from margin: invasive/DCIS (cm)
- Lymphovascular invasion: not seen/present within or outside tumor
- Axillary nodal status: sentinel, Levels I, II, and III number of nodes and number involved by metastases
- Extranodal tumor deposit: yes/no
- Paget's disease of nipple: yes/no
- Skin involvement by tumor: yes/no
- TNM staging

pTis	Carcinoma in situ, Paget's with no tumor	
pT1	Tumor	≤20 mm
	T1 mic	≤1 mm
	T1 a	1 mm<tumor≤5 mm
	T1 b	5 mm<tumor≤10 mm
	T1 c	10 mm<tumor≤20 mm
pT2	20 mm <tumor ≤50 mm	
pT3	Tumor> 50 mm	
pT4	Tumor of any size with direct extension to chest wall (ribs, intercostal muscles, serratus anterior but not pectoral muscle) or skin (a) Chest wall (b) Edema including peau d'orange, skin ulceration, or satellite nodules in the same breast (c) a and b (d) Inflammatory carcinoma – sore red breast due to tumor involvement of dermal lymphatics	

pNx	Nodes cannot be assessed (not removed/previously removed)
pN0	No regional lymph node metastasis
pN1 mi	Micrometastasis (>0.2 mm but ≤2 mm in greatest dimension)
pN1a	Metastasis in 1–3 ipsilateral axillary lymph node(s) (movable)
pN1b	Internal mammary nodes with micrometastasis detected by sentinel node dissection but not clinically apparent
pN1c	a + b
pN2a	Metastasis in 4–9 ipsilateral axillary nodes (fixed)
pN2b	Metastasis in clinically apparent internal mammary nodes in the absence of axillary node involvement
pN3a	Metastasis in ten or more ipsilateral axillary nodes, or ipsilateral infraclavicular nodes
pN3b	Internal mammary with axillary node involvement
pN3c	Supraclavicular node metastasis

- Prognosis

Nottingham Prognostic Index(NPI)

<3.4	Good prognosis,	85% 5-year survival
3.4–5.4	Intermediate prognosis	68% 5-year survival
>5.4	Poor prognosis	21% 5-year survival

NPI = 0.2 × tumor size (cm) + tumor grade + nodal score

Nodal score	
1	Node negative
2	One to three low axillary nodes involved
3	Four or more nodes/apical node involved

- Predictive factors

Hormone receptor status – estrogen (ER) and progesterone (PR) receptors

Oncogene receptor status (Her 2/neu)/C-erb B2

For scoring methods, see Tables 12.2 and 12.3.

Table 12.2 Quick score method for immunohistochemical detection of ER status. Score for proportion of cells staining and score for staining intensity

0 – No nuclear staining	0 – No staining
1–<1% nuclei staining	1 – Weak staining
2–1–10% nuclei staining	2 – Moderate staining
3–10–33% nuclei staining	3 – Strong staining
4–33–66% nuclei staining	
5–66–100% nuclei staining	

Adding the two scores together gives a maximum score of eight. Data so far suggest that with this scoring system, response to hormonal therapy correlates with the following cutoff values:

Score 0 indicates hormonal therapy will definitely not work

Score 2–3 indicates a small (20%) chance of treatment response

Score 4–6 indicates an even (50%) chance of response

Score 7–8 indicates a good (75%) chance of response

Where PR content has also been determined, hormonal therapy is thought worthwhile in patients with low ER but high PR scores

Table 12.3 Scoring method for Her2/neu oncogene overexpression by immunohistochemistry

0–	No membrane staining	Her2 negative
1+	Faint/partial membrane staining in >10% of the tumor cells	Her2 negative
2+	Weak-to-moderate complete membrane staining in >10% of the tumor cells	Her2 status equivocal, for [a]FISH/CISH testing
3+	Strong complete membrane staining in >30% of the tumor cells	Her2 positive

Gene amplification studies with [a]fluorescent or chromogenic in situ hybridization

Her2 positive cases for anti-Her2 therapy

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Part III

Head and Neck Specimens

13 Nasal Cavities and Paranasal Sinuses

Seamus S. Napier with clinical comments
by David S. Brooker

13.1 Anatomy

The external nose contains the right and left *nostrils* (or nares), each communicating with the nasal cavities via a slight dilation just inside the nostril called the *nasal vestibule*. Bone from the frontal, maxillary and nasal bones supports the upper one third of the external nose, while cartilage supports the lower two thirds.

Each nasal cavity extends posteriorly from just behind the nasal vestibule, through the opening called the *anterior choana*, to communicate with the nasopharynx via the *posterior choana*. They are separated by the *nasal septum*, which is composed of bone posteriorly and cartilage anteriorly. Each nasal cavity has a roof, a floor, a medial (or septal) wall, and a lateral wall (Fig. 13.1). The *roof* of the nose is closely related to the frontal sinuses, the anterior cranial fossa, the ethmoidal sinuses, and the sphenoidal sinus. The *floor* of the nose is closely related to the anterior maxillary teeth and the vault of the palate, while the *medial wall* represents the nasal septum. The *lateral wall* of the nose is complex and bears three (occasionally four) horizontal projections called *turbinates* or *conchae*, the superior turbinate being the smallest and the inferior turbinate the largest. The passageway of the nasal cavity below and lateral to each of the turbinates is called the *superior*, *middle*, and *inferior meatus*, respectively; above and behind the superior turbinate lies the sphenoethmoidal recess. The paranasal sinuses open onto the lateral wall of the nasal cavity, as does the nasolacrimal duct, so that disease affecting this region of the nose can obstruct the drainage of secretions and present as sinusitis.

Each nasal cavity is divided into functional areas, reflected in the nature of the epithelial lining. The nasal vestibule is lined by skin and contains many short hairs that help to filter particles from the inspired air. The olfactory area, concerned with the sense of smell, is restricted to the upper part of the nasal cavity and is centered on the cribriform plate of the ethmoid bone, the adjacent part of the nasal septum and the superior turbinate. The rest of the nasal cavity is lined by respiratory mucosa, the function of which is to warm and humidify the air and to trap particulate material. The complex architecture of the lateral wall of the nasal cavities facilitates this process by increasing the surface area and the turbulence of the airflow.

The *paranasal sinuses* are extensions of the nasal cavities and represent air-filled spaces in the

S.S. Napier (✉)
Histopathology Laboratory, Institute of Pathology,
Royal Victoria Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: seamus.napier@belfasttrust.hscni.net

D.S. Brooker
Ear, Nose and Throat Department,
Royal Victoria Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: dave.brooker@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_13, © Springer-Verlag London 2013

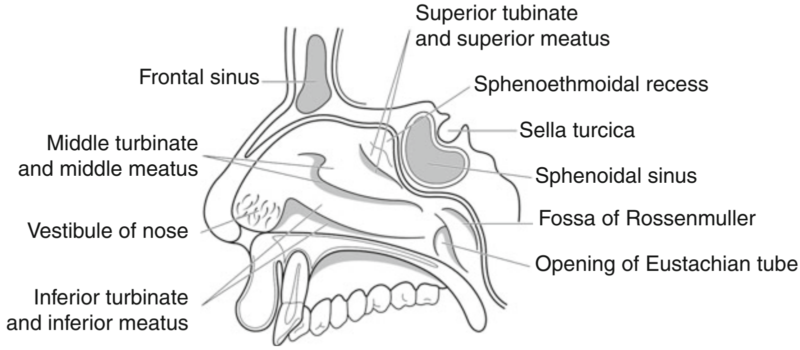

Fig. 13.1 Right lateral wall of nasal cavity (Reproduced, with permission, from Allen and Cameron (2004))

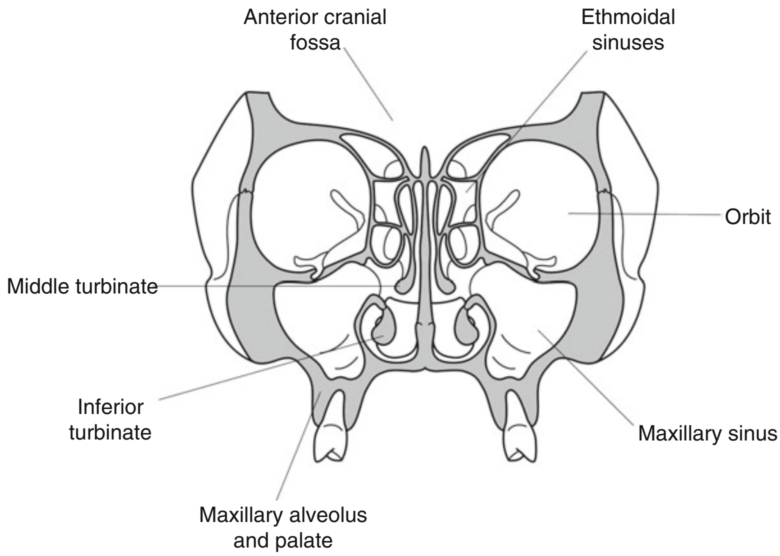

Fig. 13.2 Ethmoidal sinuses and maxillary sinuses. Coronal view of nasal cavities at level of first molar tooth showing maxillary and ethmoidal sinuses (Reproduced, with permission, from Allen and Cameron (2004))

skull bones lined by respiratory mucosa (Fig. 13.2). They are usually absent or poorly developed at birth but enlarge most during the eruption of the permanent teeth and after puberty. They are located in the frontal, ethmoidal, sphenoidal, and maxillary bones as paired structures about the midline but tend to be considered from a pathophysiological perspective into anterior and posterior groups. The *anterior group* comprises the frontal sinus, the anterior and middle ethmoidal sinuses, and the maxillary sinus, all opening into the middle meatus, while the posterior ethmoidal sinuses and the sphenoidal sinus represent the *posterior group* and drain into the superior meatus and the sphenoethmoidal recess, respectively. The nasolacrimal duct opens into the inferior meatus anteriorly.

The *frontal sinuses* lie between the outer and inner tables of the frontal bone and are closely related to the anterior cranial fossa. Disease in the frontal sinus is often associated with intracranial complications. The *ethmoidal sinuses* number between 3 and 18, and consist of a labyrinth of thin-walled bony cavities between the upper part of the nasal cavity and the orbits. The pathway for drainage of the frontal sinus passes through the anterior ethmoidal sinus group and may be impeded by disease in this area. The *sphenoidal sinuses* lie within the body of the sphenoid bone posterior to the upper part of the nasal cavity. Adjacent structures such as the optic chiasma, pituitary gland, internal carotid artery, and cavernous sinus may be affected by disease of the sphenoidal sinuses. The *maxillary sinus* is the largest of the paranasal sinuses and is closely related to the posterior maxillary teeth, the floor of the orbit, the inferior portion of the lateral wall of the nose, and the pterygoid plates of the sphenoid bone.

Lymphovascular Drainage:

Lymphatics from the external nose and anterior nasal cavity, together with those from the skin of the mid-portion of the face, drain to Level I lymph nodes in the submandibular region. The rest of the nasal cavity and the paranasal sinuses drain to Level II lymph nodes in the upper part of the deep cervical chain (Fig. 20.1), sometimes via the retropharyngeal nodes.

13.2 Clinical Presentation

Disease affecting the nose presents with unilateral or bilateral nasal obstruction, rhinorrhea (watering of the nose), epistaxis (bleeding), facial pain, facial swelling, epiphora (watering of the eye), or proptosis (bulging outward of the eye). Deafness or otitis media may be due to obstruction of the opening of the Eustachian tube in the nasopharynx by extension of a nasal tumor.

13.3 Clinical Investigations

- Direct visualization of the nasal cavities is performed using a speculum for the anterior aspect or a postnasal mirror for the posterior portion. Endonasal endoscopy is the preferred method of sampling tissue from the nose and nasal sinuses.
- Plain radiographs of the nose and sinuses may demonstrate bone destruction, soft tissue mass, or fluid levels, although these are more accurately determined by CT and MRI scanning.
- Markedly elevated erythrocyte sedimentation rates (ESR) and titers of "cytoplasmic" antineutrophil cytoplasmic antibodies (cANCA) are helpful adjuncts to diagnosis in the so-called Lethal Midline Granuloma syndrome, a collection of diseases characterized by progressive destruction of the nose and sinuses. These tests help distinguish principally between Wegener's granulomatosis and T-cell/natural killer cell lymphoma.

13.4 Pathological Conditions

13.4.1 Non-neoplastic Conditions

Sinusitis: Acute infections are usually bacterial and often follow the common cold. Empyema or mucocele may result if the draining of the secretions is obstructed. Chronic sinusitis follows acute sinusitis and may be associated with obstruction (e.g., by polyp or tumor) or immune compromise. Maxillary sinusitis may occur alone or may be associated with involvement of frontal and/or ethmoidal sinuses. Most cases respond to antibiotics and topical medications to improve drainage. Functional endoscopic sinus surgery (FESS) is the commonest surgical management of recurrent sinusitis; opening of the osteo-meatal complex under the middle turbinate or removal of pneumatized middle turbinates (concha bullosa) or nasal polyps will improve physiological drainage and allow biopsy sampling.

Pain of dental origin can mimic maxillary sinusitis and vice versa. Extraction of upper premolar or molar teeth may damage the floor of the maxillary sinus and result in an oroantral fistula through the socket.

Inflammatory polyps: A frequent complication of long-standing rhinitis, often but not exclusively allergic in origin. Often multiple and bilateral, they are a cause of sinusitis and nasal obstruction. Histologically, there is abundant myxoid or edematous stroma covered by respiratory epithelium; ulceration and/or squamous metaplasia are common in larger polyps, where they contact the nasal walls. The antrochoanal polyp is an uncommon large single inflammatory polyp that arises in the maxillary sinus and extends into the nasal cavity, presenting at the posterior choana. Nasal polyps in children are often associated with cystic fibrosis.

Wegener's granulomatosis: An uncommon systemic disorder characterized by necrotizing granulomatous inflammation and vasculitis that usually presents in the upper respiratory tract, lungs, and/or kidneys. Symptoms can be nonspecific (malaise, pyrexia) or related to the anatomical sites involved; in the nose it may manifest as sinusitis, rhinorrhea, epistaxis, or nasal obstruction. Rarely are the classical features present in nasal biopsies; diagnosis requires a high index of suspicion and careful clinicopathological correlation. ESR and cANCA titers are useful at confirming the diagnosis, although a negative cANCA does not exclude. A distinctive form of small multinucleate giant cell with clumped smudged nuclei, foci of granular collagen necrosis, and neutrophil microabscesses are characteristic if under-recognized features.

Other non-neoplastic conditions that may affect the nose and sinuses include fungal infections (chronic noninvasive colonization by *Aspergillus*, acute fulminant or angioinvasive aspergillosis, allergic fungal sinusitis), pyogenic granuloma, hemangioma and other vascular malformations, lymphoid hyperplasia, glial heterotopia, and hairy polyp.

13.4.2 Neoplastic Conditions

Benign tumors: Sinonasal papillomas are uncommon but are the most frequent benign neoplasms, subdivided into fungiform, inverted, and cylindrical cell types. Occur twice as often in males as in females and affect adults aged between 30 years and 60 years. They are usually unilateral lesions but may be multiple or multifocal. *Inverted papillomas* are the commonest form, found on the lateral nasal wall and sinuses. They have an endophytic growth pattern and are composed of thick non-keratinizing, "transitional" epithelium within edematous stroma. *Fungiform papillomas* are exophytic lesions composed of transitional epithelium supported by fibrovascular stroma, found exclusively on the nasal septum. *Cylindrical cell papillomas* are rare. They are similar in distribution and appearance to inverted papillomas but are composed of tall columnar (cylindrical) oncocytic cells.

Other benign neoplasms include pleomorphic adenoma, solitary fibrous tumor, hemangiopericytoma, nasopharyngeal (juvenile) angiofibroma, sinus osteoma, meningioma, teratoma, and paraganglioma.

Sinonasal cancer: The maxillary sinus is the commonest site for sinonasal malignancy and is usually either squamous cell carcinoma or adenocarcinoma in type. The nasal cavity is the second commonest site and is affected by a broad spectrum of lesions, but tumors of the sphenoidal and frontal sinuses are rare. Risk factors include tobacco use, exposure to hard and soft wood dusts, nickel, and irradiation.

Squamous cell carcinoma: The vast majority of malignant tumors of the mucosal lining of the nasal cavities and sinuses are classified as squamous cell carcinoma. The maxillary or ethmoid sinuses are the commonest sites but the nasal vestibule or septum can be affected. Many tumors have a "transitional cell" pattern, similar to that seen in inverted papillomas but exhibiting pleomorphism, necrosis, and a broad pushing invasive front. The term "non-keratinizing squamous cell carcinoma" can be used, but a spectrum of changes including the presence of single cell infiltration and/or abundant keratinization may be seen, sometimes making distinction from the usual type of squamous cell carcinoma impossible.

Salivary gland-type adenocarcinoma: The second commonest type of malignant tumor with adenoid cystic carcinoma is the pattern most often encountered.

Intestinal-type sinonasal adenocarcinoma: Adenocarcinoma exhibiting the differentiation pattern of large or small intestinal mucosa, with or without cytological atypia. Strongly associated with hardwood dusts (males, ethmoidal sinuses) but may occur sporadically (females, maxillary sinus). Commonest pattern mimics colonic adenocarcinoma – metastasis needs to be excluded. Mucinous tumors with signet ring cells are rare.

Malignant lymphoma: All types of non-Hodgkin's lymphoma may affect the sinonasal region either as a site of origin or as part of disseminated disease; diffuse large B-cell lymphoma is the commonest. T-cell and natural killer cell lymphomas often demonstrate a striking tendency for vascular involvement, sometimes with bizarre acute ischemic changes, such as tooth exfoliation and bone necrosis. The tumor cells may be small, large or intermediate in size; the admixture of other inflammatory cells masks the neoplastic component by mimicking an inflammatory condition such as infection or Wegener's granulomatosis.

Others: Low-grade sinonasal adenocarcinoma, olfactory neuroblastoma, malignant melanoma, small cell neuroendocrine carcinoma, sinonasal undifferentiated carcinoma, rhabdomyosarcoma, chondrosarcoma and chordoma are all uncommon.

Prognosis: Outcome depends on the histological type of tumor as well as the extent of spread. Most lesions are advanced at presentation although lymph node metastasis with carcinomas is relatively infrequent. Local recurrence is a common problem in spite of radical surgery and radiotherapy. Melanomas, small cell neuroendocrine carcinomas, and sinonasal undifferentiated carcinomas are particularly aggressive but 5-year survival is the norm with adenoid cystic carcinomas. In certain subtypes, such as intestinal type sinonasal adenocarcinoma and olfactory neuroblastoma, grading based on the degree of differentiation is important in that low-grade lesions do well while high-grade lesions do badly. Around 20% 5-year survival is customary.

13.5 Surgical Pathology Specimens: Clinical Aspects

13.5.1 Biopsy Specimens

Rigid or fiber-optic endoscopy is the usual method of sampling lesions in the nose and paranasal sinuses. When malignant disease is suspected, detailed examination and large biopsy samples are best obtained with this technique under general anesthesia, as it avoids contamination with tumor and compromising later definitive surgical procedures. Benign tumors such as nasal papillomas may be resected using endoscopic laser surgery but tend to be delivered as small fragments.

13.5.2 Resection Specimens

Excision specimens of nasal septum are easily delivered intact via a lateral rhinotomy incision. *Medial maxillectomy* is the commonest surgical procedure for low-grade tumors of the lateral aspect of the nasal cavity and/or maxillary, ethmoid, and frontal sinuses. Resection specimens tend to be fragmented because of the fragile nature of the bone; in these cases, precise interpretation of surgical margins requires orientation of the tissue samples by the surgeon. Alternatively, separate biopsy samples of critical or suspicious areas may be taken after clearance of tumor and submitted separately. *Palatal fenestration* is recommended for low maxillary sinus tumors involving the oral cavity; definite or possible involvement of the posterior wall of the maxillary sinus requires *maxillectomy*. Prosthetic rehabilitation with an obturator constructed around an upper denture provides optimal functional and aesthetic results and allows good visualization of the wound postoperatively facilitating re-biopsy of suspicious areas.

Craniofacial resection describes a surgical approach through both the anterior skull and the mid-face performed for tumors of the frontal or ethmoid sinus that extend into the anterior cranial fossa. Total ethmoidectomy, nasal exenteration, maxillectomy, and orbital exenteration can be performed if necessary.

Involvement of the orbital floor or medial wall is an important nodal point in the management of sinonasal tumors. Breach of the bony wall or involvement of periosteum by tumor may necessitate clearance or exenteration of the orbit.

Concomitant neck dissections are usually not indicated unless there is proven metastatic disease.

13.6 Surgical Pathology Specimens: Laboratory Aspects

13.6.1 Biopsy Specimens

Usually as small samples from open biopsies or core needle specimens, free-floating in formalin. Measure in three dimensions or length of core and submit in total. Specimens containing bone require decalcification and can be recognized by their tendency to sink rapidly in the fixative.

13.6.2 Resection Specimens

13.6.2.1 Septal Excision, Medial Maxillectomy, and Craniofacial Resection Specimens

Most septal excision and medial maxillectomy specimens are for the less extensive or less locally aggressive neoplastic diseases, such as inverted nasal papilloma, olfactory neuroblastoma, and even malignant melanoma. They are usually received as multiple fragments of mucosa with underlying bone and/or cartilage. In medial maxillectomy specimens, at least the inferior turbinate is included, but, depending on tumor location, all turbinates may be represented.

Most craniofacial resection specimens are for extensive or locally aggressive neoplastic diseases of the frontal or ethmoid sinuses, where a curative outcome is expected. As such, they will represent composites of septal excision, medial maxillectomy, maxillectomy, and skull-base excisions. They are usually received intact or as two or three large fragments. They are handled as if they represented an extended medial maxillectomy specimen. Invasion into dura is an ominous finding.

Samples of critical or clinically suspicious margins taken at clearance of tumor should be submitted separately and handled as biopsy specimens.

Initial procedure:

If intact, orientate the specimen and ink its margins.

Otherwise, if a larger specimen is submitted, ink its margins. A line of ink drawn across medium-sized fragments can aid orientation after microscopic examination and assist assessment of margins.

Slice the larger pieces into 0.4 cm thick slices transversely, using a band saw or equivalent.

Measurements:

If intact, dimensions (cm)

If fragmented, number of fragments, total weight (g), and dimensions of largest specimen (cm)

- Tumor
  Size (cm)
  Number of fragments consisting of tumor
- Distance to closest surgical margins (cm)

Description:

- Tumor
  Size, shape, and color
  Presence of necrosis
  If fragmented, number of fragments containing tumor
- Adjacent mucosa
  Color and consistency
  Presence of other lesions
  Other
- Lymph nodes, neck dissection

Maxillectomy specimens

See Maxilla, Mandible, and Teeth (Chap. 15).

Blocks for histology:

In cases of neoplastic disease, the histology should represent the tumor, its relationship to the adjacent mucosa, and underlying bone or cartilage. Focal abnormalities of mucosa need to be sampled.

Three blocks of tumor to illustrate the interface with adjacent normal tissues.

Three blocks of adjacent mucosa.

Closest deep surgical margin. Samples of other lesions, e.g., nodules or polyps.

In intact specimens, sample the mucosal margins before sawing the bone and submit separately (reduces contamination of the margins). Cut with a sharp blade firmly down to bone and use a flat blunt instrument to dissect mucosa free from the bone.

In intact specimens, saw the bone into 0.5 cm slices in the transverse plane (vertical plane in vivo).

If fragmented, bread-slice larger specimens and submit as labeled blocks. Microscopic analysis may allow reconstruction and useful assessment of margins.

Histopathology Report

Final reports of sinonasal specimens should include details on:

- The specimen type and side
  If fragmented, the number of fragments and the size of the largest
- The type, subtype, and grade of tumor present
  Sinonasal papilloma variants
  Squamous cell carcinoma and variants
  Low-grade adenocarcinoma
  Intestinal-type adenocarcinoma
  Lymphoma
- The macroscopic size of tumor
- The presence or absence of invasion of bone
- The distance of tumor from the nearest margin
- The presence or absence of vascular invasion
- The presence or absence of dural invasion (if craniofacial resection)
- Other pathology such as radiation injury
- TNM: Classification of Tumor Spread

Maxillary sinus

pT1	Mucosa
pT2	Bone erosion/destruction, hard palate, middle nasal meatus
pT3	Posterior bony wall maxillary sinus, subcutaneous tissues, floor/medial wall of orbit, pterygoid fossa, ethmoid sinus
pT4a	Anterior orbit, cheek skin, pterygoid plates, infratemporal fossa, cribriform plate, sphenoid/frontal sinus
pT4b	Orbital apex, dura, brain, middle cranial fossa, cranial nerves other than V2, nasopharynx, clivus

Nasal cavity and ethmoid sinus

pT1	One subsite
pT2	Two subsites or adjacent nasoethmoidal site
pT3	Medial wall/floor orbit, maxillary sinus, palate, cribriform plate
pT4a	Anterior orbit, skin of nose/cheek, anterior cranial fossa (minimal), infratemporal fossa, pterygoid plates, sphenoid/frontal sinus
pT4b	Orbital apex, dura, brain, middle cranial fossa, cranial nerves other than V2, nasopharynx, clivus

Malignant melanoma

pT3	Involvement of mucosa only
pT4a	Deep soft tissue/cartilage/bone or overlying skin
pT4b	Brain/dura/skull base/lower cranial nerves, masticator space, prevertebral space, carotid artery, mediastinal structures

As malignant melanomas of the upper aerodigestive tract are generally aggressive lesions, Stage I and II disease (i.e., pT1 N0 and pT2 N0) have been omitted.

All sites except nasopharynx: regional lymph nodes

pN0	No regional node metastasis
pN1	Metastasis in an ipsilateral single ≤3 cm
pN2	Metastasis in: (a) Ipsilateral single > 3–6 cm (b) Ipsilateral multiple ≤6 cm (c) Bilateral or contralateral ≤6 cm
pN3	Metastasis in a lymph node >6 cm.

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14 Lips, Mouth, and Tongue

Seamus S. Napier with clinical comments by Derek J. Gordon

14.1 Anatomy

The mouth extends from the lips and cheeks to the oropharyngeal isthmus at the palatoglossal fold. It comprises a number of subsites, which can be divided into three functional types, although the microscopic structure of each varies subtly from one region to the next. "Masticatory mucosa" is found on the maxillary and mandibular gingivae, the hard palate, and on the dorsum of the tongue. It is bound tightly to underlying tissue and covered by keratotic relatively thick stratified squamous epithelium to withstand the trauma of chewing. In contrast, "lining mucosa" is elastic and is present on the inner aspect of the lips, on the buccal mucosae and their respective upper and lower sulci, the ventral surface of the tongue, and the floor of mouth. It is covered by relatively thin stratified squamous epithelium supported by loosely textured fibrovascular connective tissue (Figs. 14.1 and 14.2). "Specialized mucosa" refers to the taste buds.

The lips are composed of skin and mucosa around the opening to the mouth. They contain the orbicularis oris, fibrofatty tissue, and many minor salivary glands. Upper and lower lips join at the buccal commissure (angle of the mouth). The mucosa of the lips begins at the vermilion border with skin and extends across the free surface into the oral cavity proper. The cheeks are continuations of the lips; the skin forms most of the facial skin, while the buccal mucosa is continuous through the upper and lower sulci with the gingivae and with the soft palate/oropharynx. Buccinator is the principal muscle of the cheek; it is perforated opposite the upper second molar tooth by the parotid duct. Many minor salivary glands lie between the muscle layer and the mucosa, while the facial (or buccal) lymph node lies external to buccinator below the level of the occlusal plane.

The gingival margin has a scalloped outline as it encircles the teeth, forming the interdental papilla between adjacent teeth. Behind the last molar tooth on each side of the mandible, the gingiva forms a flat triangular region known as the retromolar trigone (or retromolar pad). The hard palate is formed mostly by the palatine processes of the maxillary bones and is covered by mucosa continuous with the upper gingivae. In the midline of the palate anteriorly just behind the incisor teeth, there is a small mucosal elevation called the incisive papilla. In the anterior palate, the mucosa forms four or five transverse ridges called rugae while posteriorly it is smooth. Small numbers of minor salivary glands are present in the hard palate posteriorly and laterally close to the alveolar processes of the maxilla. The hard palate is continuous posteriorly with the soft palate, a

S.S. Napier (✉)
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: seamus.napier@belfasttrust.hscni.net

D.J. Gordon
Plastics Surgery Unit, Ulster Hospital Dundonald, South Eastern Health and Social Care Trust, Belfast, UK
e-mail: derek.gordon@setrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_14, © Springer-Verlag London 2013

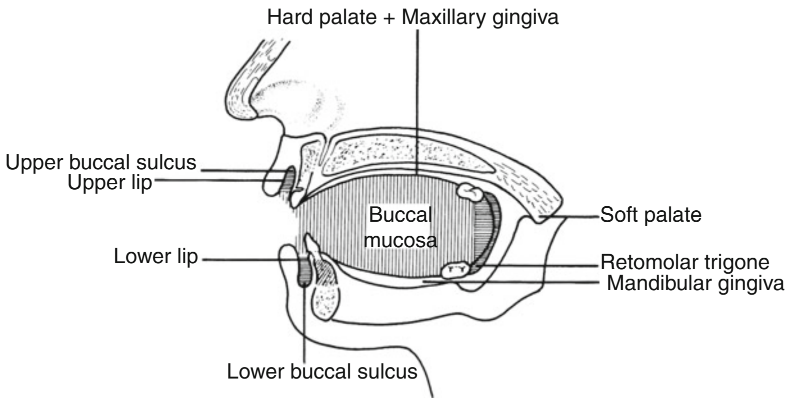

Fig. 14.1 Mucosal subsites of lips and oral cavity (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

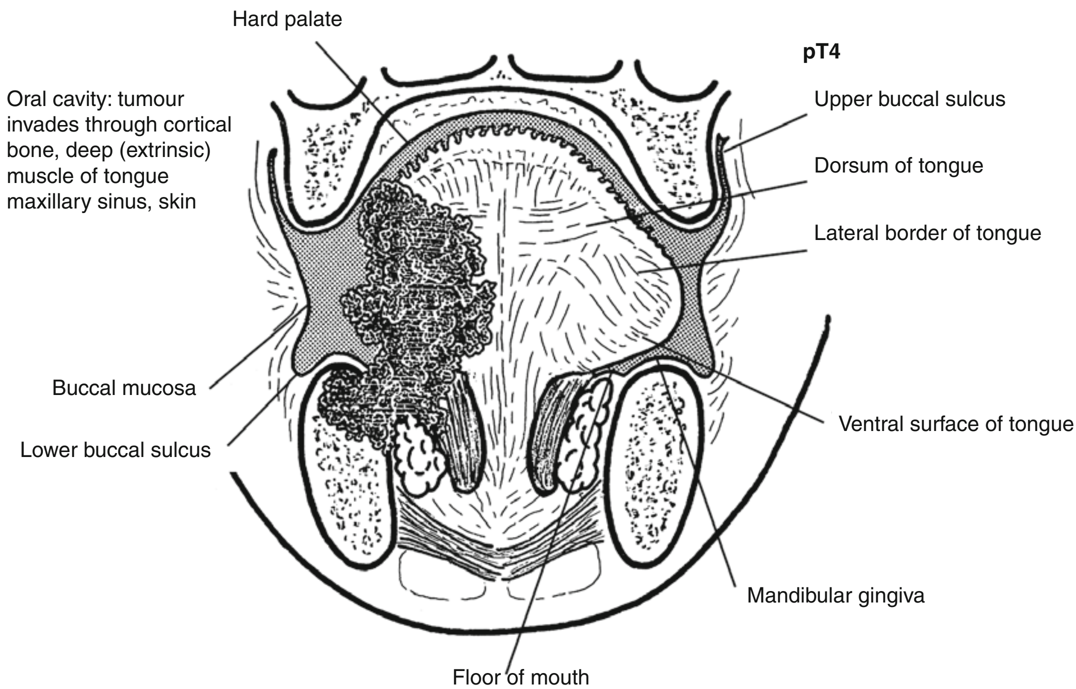

Fig. 14.2 Mucosal subsites of tongue and floor of mouth demonstrating *pT4* tumor of tongue (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

mobile flap of mucosa, striated muscle, and fibrofatty tissue separating the nasopharynx from the oropharynx.

The floor of mouth is a horseshoe-shaped region between the tongue, the mandible, and mylohyoid. It contains the sublingual salivary glands, the submandibular ducts, the lingual nerves, and some of the extrinsic muscles of the tongue. Right and left submandibular ducts converge on the lingual frenulum, a midline fold running from the ventral surface of the tongue to the gingiva behind the lower central incisor teeth, forming a mucosal papilla in the floor of mouth approximately 1 cm posterior to the lingual gingiva.

The tongue is divided into two parts by a V-shaped groove called the sulcus terminalis; the anterior two-thirds lies within the oral cavity and the posterior one- third (the base) lies within the oropharynx. The anterior two-thirds is divided into:

- Dorsal surface – the superior surface. It is covered by innumerable filiform papillae; between 30 and 50 dispersed fungiform papillae measuring approximately 1 mm in diameter and between 8 and 12 circumvallate papillae, measuring between 3 mm and 4 mm in diameter located in a line just anterior to the sulcus terminalis. The fungiform and circumvallate papillae bear taste buds.
- Ventral surface – the inferior aspect. It has a smooth surface, merging with the floor of mouth.
- Lateral border – the side of the tongue. It extends from the tip to the palatoglossal arch (anterior pillar of the fauces).

The posterior one-third of the tongue has a cobblestone surface due to the accumulation of lymphoid tissue from the lingual tonsil. No papillae are present, although taste buds may be numerous. The mucosa is contiguous with the palatine tonsils laterally and the vallecula of the epiglottis posteriorly.

The tongue is divided into right and left halves by a median fibrous septum, which is attached inferiorly to the hyoid bone. The muscles of the tongue are divided into the intrinsic group and the extrinsic group. The extrinsic group represents genioglossus, hyoglossus, stylogossus, and palatoglossus, having attachments outside the tongue and considered important in the staging of malignant tumors; involvement of the extrinsic muscles by tumor signifies pT4 staging (Fig. 14.2).

Lymphovascular drainage:

The face, oral tissues, and tongue possess many lymphatic channels and display a variable pattern of lymphatic drainage. In general, the tissues of the anterior face and lips drain to lymph nodes in the submental and submandibular regions. The tissues of the lateral face, eyelids, and anterior portion of the scalp and external ear drain to lymph nodes around the parotid region. The tissues of the posterior scalp and behind the ear tend to drain to retroauricular and suboccipital lymph nodes. These superficial lymph node groups ultimately drain to lymph nodes in the deep cervical chain situated around the internal jugular vein (see Fig. 20.1).

Within the oral cavity, the tissues of the palatal gingiva, hard palate, and soft palate drain to retropharyngeal lymph nodes or directly to lymph nodes in the deep cervical chain. The tissues of the floor of the mouth and those of the lingual gingiva drain to nodes in the submental and submandibular regions, and ultimately to lymph nodes in the deep cervical chain.

There is much subregional variability in the lymphatic drainage of the tongue influenced by the presence of the median septum. Malignant tumor drains to ipsilateral lymph nodes in the deep cervical chain but contralateral node involvement should be considered with lesions at the tip of the tongue, lesions that cross the midline or involve the median fibrous septum and lesions in the posterior one-third.

14.2 Clinical Presentation

Disease affecting the mouth and tongue may be clinically silent or may present as a mass lesion, an ulcer, a white/red patch, or with a painful/burning sensation often on eating hot or spicy foods. Pain is a rare presenting feature of malignancy, which is usually asymptomatic until advanced.

14.3 Clinical Investigations

- The ease with which the oral cavity can be examined by direct visualization facilitates preoperative diagnosis and reduces the need for complex investigative procedures. Nevertheless, hematological investigations are often useful to determine hemoglobin levels, red blood cell indices, serum ferritin, Vitamin B_{12}, and folate levels.
- Identification of pathological forms of *Candida* species can be achieved by direct visualization of periodic acid/Schiff-stained smears sampled directly from affected mucosa. Precise subclassification can be performed following culture of swabs or an oral saline rinse, the latter also providing a quantitative measure of oral fungal load.
- Biopsy techniques are used frequently to sample mucosal abnormalities. The value of direct

and indirect immunofluorescence should not be underestimated in blistering and/or ulcerating conditions. Endoscopy of the upper aerodigestive tract is performed prior to surgery for malignant disease to identify occult second primary neoplasms.

- Fine-needle aspiration cytology (FNAC) can provide additional information to facilitate the resolution of a differential diagnosis of submucosal masses; cytology brushes can be used but interpretation of atypia in mucosal samples can be problematic.
- Plain radiographs may detect a concurrent odontogenic or bony lesion and will often identify gross bone destruction by mucosal cancers. CT and MRI scanning are essential in planning surgery by indicating the depth of the tumor and detecting other changes in the neck. CT has less motion artifact and is good for bone detail, while MRI gives superior soft tissue contrast without dental amalgam artifact or exposure to ionizing radiation.

14.4 Pathological Conditions

14.4.1 Non-neoplastic Conditions

The oral mucosa is affected by a bewildering number of non-neoplastic conditions that are the subject of many textbooks. These are divided according to their clinical presentation as either lumps, ulcers, or white/red patches.

Lumps: Most discrete mass lesions of the oral mucosa represent forms of fibrous tissue overgrowth (*fibroepithelial polyp)* as a consequence of low-grade chronic trauma. The term *fibrous epulis* is reserved for lesions on the gums, while those associated with dentures can be described as *denture-induced hyperplasia*. *Mucous extravasation cysts* and *mucous retention cysts* arise from small salivary glands within the submucosal tissues. Vascular anomalies, such as hemangioma and lymphangioma, can affect any oral site. *Giant cell epulis* (or *peripheral giant cell granuloma*) usually arises from the gum anterior to the premolar region, presumably as a response to irritational stimuli, but such lesions in older patients may be a manifestation of hyperparathyroidism.

Persistent diffuse swellings of the oral mucosa are much rarer and most represent vascular anomalies (such as *hemangioma* or *lymphangioma*) present since birth. Causes of intermittent diffuse swelling of the oral mucosa are *orofacial granulomatosis* (sometimes a manifestation of *Crohn's disease*) or *angioedema*, a selective deficiency of components of the complement system.

Ulcers: Common on the lining mucosa and tongue and are often due to trauma from teeth, dentures, or foodstuffs. *Recurrent aphthous ulceration* is characterized by crops of ulcers on the lining mucosa of young patients that heal spontaneously over a 2-week period but recur. Three clinical subtypes are recognized: *minor* (ulcers 2–4 mm in diameter), *major* (single ulcer at least 10 mm in diameter, located posteriorly in the mouth that heals slowly), and *herpetiform* (a very rare type, usually close to the front of the mouth, composed of minute coalescing ulcers). Around 20% of patients with recurrent aphthous ulcers may suffer from a hematological deficiency due to a systemic disorder but most patients are otherwise healthy. Drugs can produce ulcers through either topical or systemic effects. Vesiculobullous disorders, such as *erythema multiforme*, *pemphigus vulgaris*, and *mucous membrane pemphigoid*, are more likely to present with ulcers than with intact blisters because of the relative fragility of the oral mucosa compared to skin. Squamous cell carcinoma may often present as a non-healing ulcer.

White/red patches: The oral mucosa may become white due to accumulation of keratin or epithelial hyperplasia and may become red because of epithelial atrophy, increased vascularity, or inflammation. Physical stimuli such as friction from teeth or dentures or through the use of tobacco can produce an irritational keratosis on any part of the oral mucosa, most often lining mucosa. "Chevron" parakeratosis and melanin incontinence point to tobacco-related lesions. Lichen planus/lichenoid reaction occurs commonly on the lining mucosa and dorsum of the tongue as white striae or papules against a red background. Erosive forms are characterized by ulceration. Some lesions are a consequence of systemic drug therapy or as a response to amalgam restorations in adjacent teeth. Geographic tongue is characterized by irregular areas of mucosal erosion affecting the dorsal surface. Central areas of atrophy are outlined by a narrow peripheral zone of white mucosa and may be accompanied by deep fissuring of the tongue. The pattern of atrophic and white areas changes gradually, affected areas returning to normal and new lesions developing. *Candida* may affect the oral mucosa and may present as red or white lesions. Candidal infection is often a marker of underlying disease ("disease of the diseased"), although a number of local factors can precipitate candidal infection, particularly smoking, xerostomia, high carbohydrate diet, and topical steroid application. Furthermore, any mucosal lesion can be secondarily infected by *Candida*. A small proportion of white/red lesions of oral mucosa may ultimately develop squamous cell carcinoma, although it is not possible to predict which lesions will develop malignancy and when such an event might occur. Most authorities consider the presence of dysplasia in these potentially malignant lesions to be a worrying sign, although there are significant problems with inter- and intra-observer variability in the assessment of dysplasia. Furthermore, approximately 50% of cases will never develop a tumor within the lifetime of the patient. Careful correlation of clinical, histopathological, and other laboratory data is required to establish a precise diagnosis of oral white/red lesions.

14.4.2 Neoplastic Conditions

Benign tumors: *Squamous cell papilloma* commonly occurs on the lips, cheeks, and tongue, and is often associated with viral warts on the hands. *Neurilemmoma*, *neurofibroma*, and the *granular cell tumor* are not infrequently encountered. *Lipoma* presents as a mucosal polyp, clinically similar to a fibroepithelial polyp. Benign tumors of salivary gland origin arise in the upper lip and in the palate, usually at the junction between the hard and soft palates, the commonest of which is the *pleomorphic adenoma*. Benign salivary tumors are rare in the tongue and floor of mouth; most salivary tumors in the lower parts of the oral cavity are adenocarcinomas.

Malignant tumors: As at other sites in the upper aerodigestive tract, tobacco and alcohol use are the major risk factors for oral cancers. Their effects are related to dose and duration of use; together they have a multiplicative rather than additive effect. Recent interest has focused on the role of viruses in oral malignancy. Certain forms of Human Papillomavirus have been detected in a proportion of tumors, but their precise role in oral oncogenesis is unclear.

Squamous epithelial dysplasia: A rare finding; most lesions of the oral mucosa are not dysplastic. As with invasive tumors, epithelial dysplasia is strongly associated with tobacco smoking and alcohol use, but, paradoxically, lesions arising in patients who do not use tobacco are most likely to develop carcinoma. In contrast to the cervix with which it has often – probably erroneously – been compared, oral dysplastic lesions are frequently hyperkeratotic with varying degrees of epithelial hyperplasia and/or atrophy. The grade of dysplasia can vary from mild to severe. Development of invasive squamous cell carcinoma seems to occur more frequently with increasing degrees of cytological disturbance (less than 5% for non-dysplastic lesions and low-grade dysplasia; around 50% for high-grade dysplasia) but there are no agreed criteria for grading or recognizable features of prognosis. Identifying high-grade dysplasia highlights the considerable risk of synchronous or metachronous squamous cell carcinoma but other factors such as site and the clinical appearance of the lesions need to be considered. Conservative surgery or ablative therapy (e.g., by laser or photodynamic therapy) will often be attempted, but the effects of treatment are difficult to evaluate. The area of abnormal mucosa is almost certainly much greater than the white or red area detected clinically. Most authorities accept that no form of active treatment can predictably ensure that cancer will not develop within the patient's mouth and that careful clinical follow-up is essential to detect newly formed tumors at the earliest opportunity.

Intraoral squamous cell carcinoma: Accounts for over 85% of primary malignant tumors in the mouth. Males are affected at least twice as often as females and most patients are aged between 40 and 60 years. Smoking and alcohol use are the main risk factors. The commonest intraoral sites are the lateral border/ventral surface of the anterior two-thirds of tongue (35%) and the floor of mouth (20%), followed by the mandibular gingiva/retromolar trigone, soft palate, buccal mucosa/buccal commissure, and hard palate/ maxillary gingiva. Tumors of the tongue and floor of mouth tend to metastasize frequently to neck nodes – up to 30% of patients with carcinoma of the tongue and floor of mouth who have clinically negative necks will have metastatic disease. Tumors of the hard palate rarely involve nodes.

Histological and reportedly prognostic variants of squamous cell carcinoma include verrucous carcinoma, papillary squamous cell carcinoma (better than usual type), spindle cell squamous cell carcinoma, adenoid squamous cell carcinoma (same prognosis), basaloid squamous cell carcinoma, and adenosquamous cell carcinoma (worse prognosis).

Prognosis: Outcome for patients with intraoral squamous cell carcinoma depends on the precise anatomical site within the mouth (the further back in the mouth, the worse the outcome), the size of the tumor and the presence of regional nodal metastasis. Lymph node metastasis is the most significant factor in determining prognosis; extracapsular spread from affected nodes is also an indicator of limited prognosis, with increased risk of recurrence in the neck and of distant spread. The size and anatomical site of the tumor affects the ability to achieve surgical clearance with the risk of local recurrence but the pattern of tumor invasion is probably the most significant factor in determining lymph node metastasis. As with all upper aerodigestive tract malignancies, comorbidity from cardiovascular and respiratory disease due to the effects of age, tobacco, and alcohol use is a major adverse factor in survival.

Five-year survival with node-negative tongue carcinomas is approximately 50%, falling to around 20% for patients with large tumors and positive nodes.

Squamous cell carcinoma of the lip: Arising on the vermilion border of the lower lip, although a few are seen on the upper lip. Probably represents a cutaneous rather than intraoral malignancy, as it is associated with long solar exposure. Less than 20% involve lymph nodes. Easily amenable to early detection and surgical excision, the 5-year survival is in excess of 80%.

Squamous cell carcinoma involving either upper or lower lip but arising within the oral cavity (e.g., from the buccal commissure) is a true intraoral cancer, strongly associated with tobacco use. There is a greater likelihood of nodal metastasis, but the prognosis is still reasonably good in comparison with similar lesions at other intraoral sites.

Other malignant tumors in the oral cavity include malignant lymphoma (usually a deposit of disseminated nodal disease), salivary gland types of adenocarcinoma, malignant melanoma (palate and maxillary gingiva), rhabdomyosarcoma (around the soft palate), and Kaposi's sarcoma (junction of hard and soft palate). The oral mucosa may be involved by direct spread from a malignant tumor in the minor salivary glands or from the nasal cavity/maxillary sinus.

14.5 Surgical Pathology Specimens: Clinical Aspects

14.5.1 Biopsy Specimens

Most oral biopsy specimens represent cold knife samples of an incisional or excisional nature. These are usually taken under direct vision and are repaired with either resorbable or non-resorbable sutures. Punch biopsies are being employed increasingly for mucosal disease, but they tend to yield less diagnostic tissue than the traditional "scalpel biopsy" and, in inexperienced hands, carry an increased risk of iatrogenic injury to deep structures, such as nerve trunks.

14.5.1.1 Biopsy Technique

An ellipse of mucosa is removed with the scalpel blade of ideal minimal dimensions of 10×6 mm and tissue to a depth of 3 mm. The area sampled is selected to represent the most significant area of the lesion and to include the interface with adjacent normal tissues. It is a common misconception that the normal tissue is required to allow comparison with the diseased areas; rather the presence of normal tissue facilitates biopsy handling in that a stabilizing suture may be placed through the normal tissue without distorting the abnormal tissue. The tissue is placed in a fixative or onto orientation filter paper. The wound is repaired with sutures.

14.5.2 Resection Specimens

The type of surgical procedure for tumors of the lips and oral cavity depends on the precise location of the tumor, its T-stage, the presence of nodal disease, concurrent second primary lesions, and the health of the patient.

Adequate local clearance with preservation of function is the aim with surgical treatment for intraoral cancers. Very large defects are repaired with microvascularized free-tissue flaps, although, in the tongue in particular, allowing the wound to granulate naturally often results in a complete return to normal function and speech. A margin of 10 mm is the ideal but anatomical constraints and the large size of some of the tumors mean that surgical clearance is often restricted to 2 or 3 mm. In addition, extensive areas of abnormal mucosa are often present around the tumors.

Small superficial tumors of the tip or lateral border of the tongue can be treated by local “wedge” excision, although formal hemiglossectomy is preferable for deeply infiltrative lesions. Subtotal or total glossectomy is a very rare procedure, usually reserved for very large tumors invading widely across the midline fibrous septum, involving the extrinsic muscles or affecting the posterior one-third that have recurred following first-line combined chemotherapy and radiotherapy.

The ipsilateral sublingual gland is usually included with resections of anterior floor of mouth mucosa; both sublingual glands are included for midline lesions. Partial glossectomy will be included with resections for tumors of the anterior floor of mouth that spread into the tongue. Likewise with tumors encroaching on the gingiva, compromised mucosa from the lower alveolus will be resected.

Resection may be restricted to the alveolar mucosa for superficial tumors of the upper and lower gingiva, but larger tumors often require extensive resection. For example, a widely infiltrative carcinoma of the retromolar trigone may require removal of a portion of lower alveolar mucosa and bone, lingual sulcus, posterior buccal mucosa with lower and upper sulci, the posterior portion of the upper alveolar mucosa, the tonsillar bed, and part of the soft palate. Part of the posterior tongue may also be included.

A full-thickness wedge excision of lip (V-shaped or W-shaped) is the commonest treatment for squamous cell carcinoma of the lip. To limit the development of new lesions, in-continuity mucosal “shave” excision of adjacent mucosal changes on the vermilion border is more often than not carried out at the same time.

When tumor encroaches on the periosteum at any intraoral site, the decision to resect bone depends on whether or not there has been previous radiotherapy to the jaw, the precise anatomical relationship of tumor and bone, and how easily the periosteum dissects from the bone. The clinical extent of disease is almost always greater than that detected radiographically but the periosteum offers a considerable barrier to bone invasion and the usual pathway for direct spread into the jawbone is from the alveolar crest rather than through the cortical plate. In the nonirradiated jawbone with no bone erosion, there is no need to resect bone if tumor-bearing periosteum elevates easily. With radiographic bone destruction, marginal mandibulectomy (rim resection) or segmental mandibulectomy (hemimandibulectomy) is performed. Where there is radiation injury to the bone, this periosteal barrier is lost and direct spread through the cortical bone is more likely, warranting bone removal.

Ideally, where there is proven or a high likelihood of regional lymph node metastasis, an in-continuity neck dissection is performed.

14.6 Surgical Pathology Specimens: Laboratory Protocols

14.6.1 Biopsy Specimens

Usually one fragment is present free-floating in formalin, although several specimens may be taken simultaneously. Portions of stabilizing sutures may be present, usually located anteriorly. The surgeon only includes them to reduce artifacts of tissue handling when transferred to the container; rarely are they of significance.

- Measure.
- Place in cassette; if very small wrap in moist filter paper.
- Mark for levels and D/PAS particularly if the sample represents a white/red patch or where a candidal infection is suspected.
- Orientate the specimen at the embedding stage to facilitate microscopic assessment.

14.6.2 Resection Specimens

14.6.2.1 Major Resection Specimens

The vast majority of lip and oral mucosal resections are for neoplastic disease, although some smaller specimens will represent local excisions of non-healing traumatic ulcers where there is a low index of suspicion. Resections of larger lesions may include mucosa from adjacent sites as well as bone from the mandible or maxillary alveolus. Orientation of large resection specimens is generally easy because specific anatomical landmarks are discernible, but smaller local excisions may be difficult.

Procedure:

- Orientate the specimen(s) using the anatomical or surgeon's landmarks.
- Ink only critical mucosal and deep resection margins. For glossectomy specimens and resections of retromolar trigone, the critical margins are usually the posterior limits with the lingual sulcus/tonsillar bed. In the tongue, tumor can unexpectedly involve the deep medial margin inferiorly and posteriorly in the floor of mouth/oropharynx by sarcolemmal spread of tumor along intrinsic muscle bundles in the tongue. To facilitate assessment of possible bone involvement, the periosteal limits of alveolar mucosa not in continuity with the underlying bone should be inked.
- With large or complex specimens or those with in-continuity resections of bone, e.g., resections of the retromolar trigone, sample the mucosal limits first by taking radial blocks (Fig. 14.3).
- Cut the specimen into 4mm thick slices transversely (in the anatomical vertical plane).
- Measurements:
  - Dimensions of mucosa, deeper tissue, and other components, e.g., bone (cm).
    - Tumor
      Anteroposterior length × width (cm).
      Maximum depth (cm) from reconstructed mucosal surface.
      Distances to closest mucosal and deep surgical margins (cm).
    - Mucosal abnormalities (cm).

Description:

- Tumor
  Plaque-like/ulcerated/fungating: usual type SCC
  Warty: well-differentiated SCC, verrucous carcinoma
  Polypoid: spindle cell SCC
- Mucosa
  White/thickened: in situ lesions
- Extent
  Document local spread, e.g., to salivary gland, tonsil, bone
- Other
  Neck dissection, mandibular bone

Blocks for histology:

The histology should represent the deepest extent of the tumor, the relationship to the surface, mucosal and deep soft tissue margins, and changes in adjacent oral mucosa (Figs. 14.3 and 14.4).

- At least one block of tumor per centimeter of maximum dimension
- Mucosal surgical margins, particularly the floor of mouth, retromolar region, and soft palate where the incidence of dysplasia is highest
- Deep surgical margins, particularly posterior and inferomedial margins
- Proximal lingual nerve, if present
- Adjacent uninvolved mucosa and associated tissue, e.g., sublingual gland

Histopathology report:

Final reports of oral mucosal resection specimens should include details on:

- The specimen type, side, and tissues present
- The type of tumor present
  Squamous cell carcinoma NOS
  SCC variants include basaloid, adenosquamous, spindle cell, verrucous

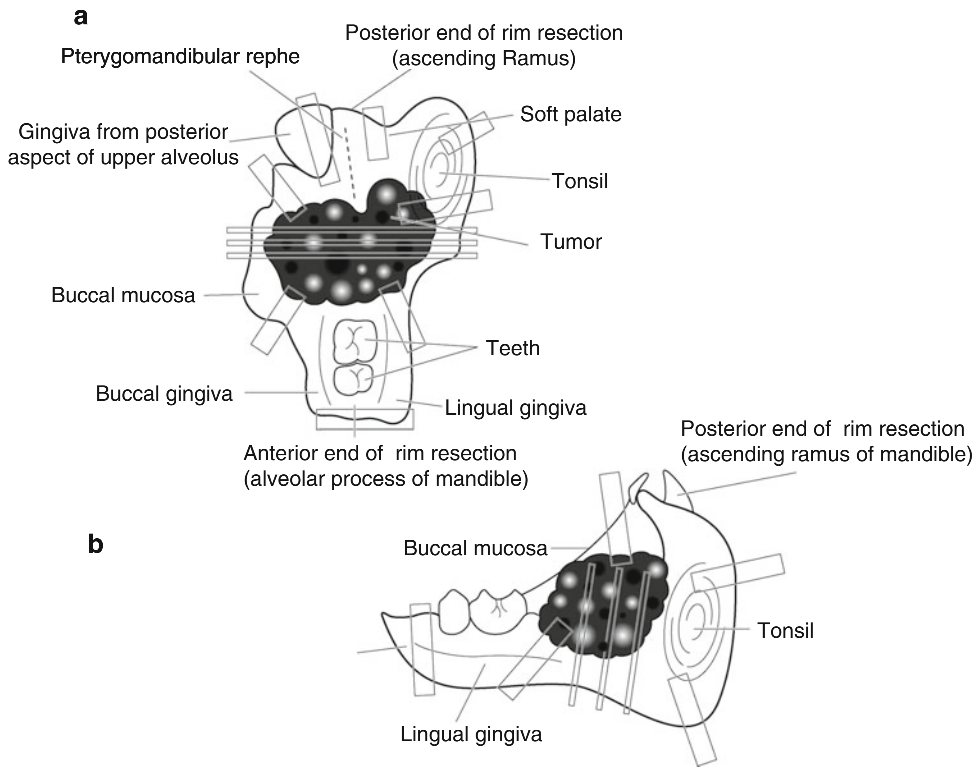

Fig. 14.3 Resection of right retromolar trigone with rim resection of mandible. Suggested siting and orientation of tissue blocks for resection of retromolar trigone. (**a**) View from above; (**b**) view from lingual aspect (Reproduced, with permission, from Allen and Cameron (2004))

Adenocarcinoma (salivary gland types)

- The grade of tumor assessed at invasive front

  Cohesive or non-cohesive patterns (more metastasis with non-cohesive)
- The extent of local spread
- The distance of tumor from the nearest mucosal margin
- The distance of the tumor from the nearest deep margin
- Intravascular and/or perineural spread

If other specimens are attached as an in-continuity dissection (e.g., oropharyngeal mucosa, neck dissection, bone), these can be cut separately in the usual fashion.

14.6.2.2 Wedge Resection Specimens of Lip

Procedure:

- Orientate the specimen.
- Ink the deep and lateral resection margins.
- Slice the specimen parasagitally so that the blocks contain both skin and intraoral mucosa, including the lateral shave excisions of vermilion border if present.
- If the tumor lies within 2 mm of a lateral limit, take a vertical block through the lateral limit block to illustrate the relationship of tumor to the surgical margin.
- Measurements:
  - Length of the lip along the vermilion border and height (cm)
  - Tumor Length × width (cm)

    Maximum depth from reconstructed mucosal surface (cm)

    Distances to closest mucosal and deep surgical margins (cm)
  - Mucosal abnormalities

Description:

- Tumor

  Plaque-like/ulcerated/fungating: usual type SCC

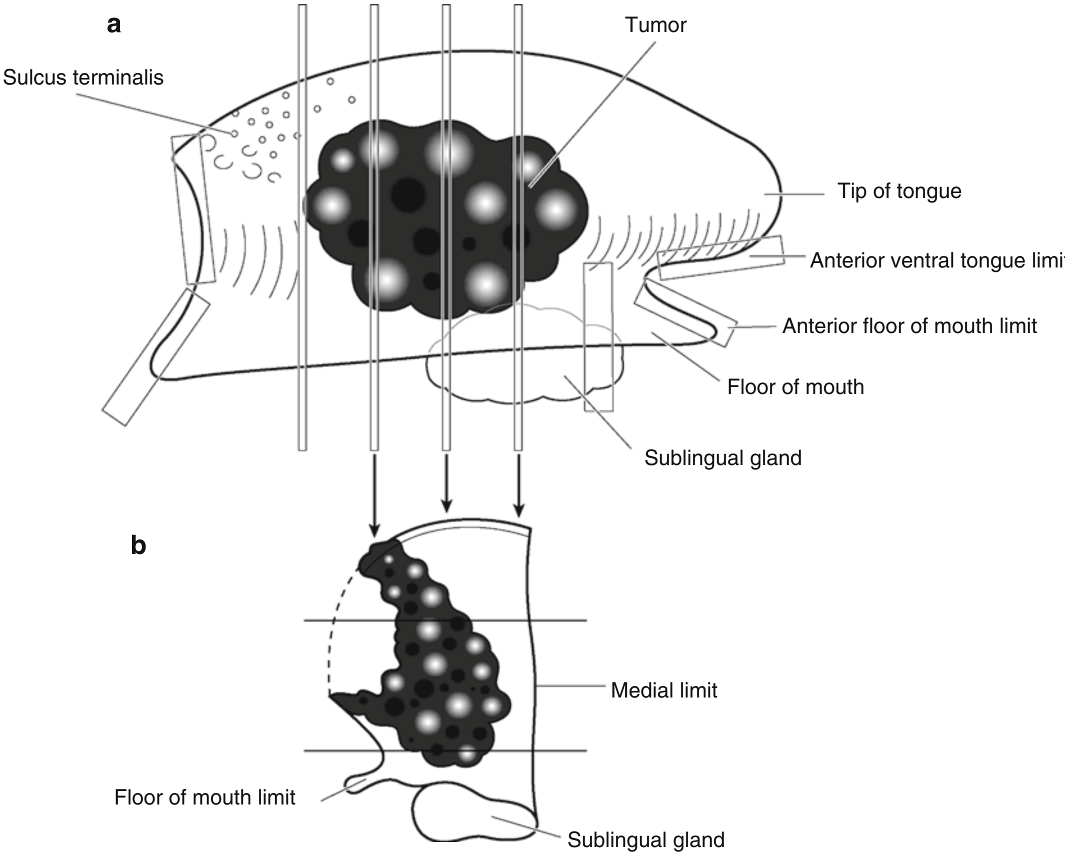

Fig. 14.4 Right hemiglossectomy specimen. Suggested siting of blocks for hemiglossectomy specimen: (**a**) Lateral view; (**b**) view of transverse slice (Reproduced, with permission, from Allen and Cameron (2004))

Warty: well-differentiated SCC

- Mucosa

  White/thickened/ulcerated: in situ lesions

- Extent

  Spread into muscle

Blocks for histology:

The histology should represent the deepest extent of the tumor, the relationship to the cutaneous, mucosal and deep soft tissue margins, and changes in adjacent vermilion border mucosa and skin (Fig. 14.5).

- At least one block of tumor per centimeter of maximum dimension.
- If lateral shave excisions of vermilion border are present, take one block from the cutaneous to mucosal aspects at the junction with the wedge and one transverse block of the lateral limit of each shave. If greater than 2 cm in length, take one additional block for every centimeter.
- Cutaneous, mucosal, and deep surgical margins
- Samples of other lesions not already represented, e.g., mucosal white areas or ulcers

Histopathology report:

Final reports of lip resection specimens should include details on:

- The specimen type, size, and side
- The type of tumor present

  Squamous cell carcinoma NOS

  Adenocarcinoma (salivary gland types)

- The grade of tumor assessed at invasive front
- The extent of local spread
- The distance of tumor from the nearest lateral mucosal margin
- The distance of the tumor from the nearest deep margin
- Intravascular and/or perineural spread
- Other pathology such as solar damage, dysplasia, or radiation injury

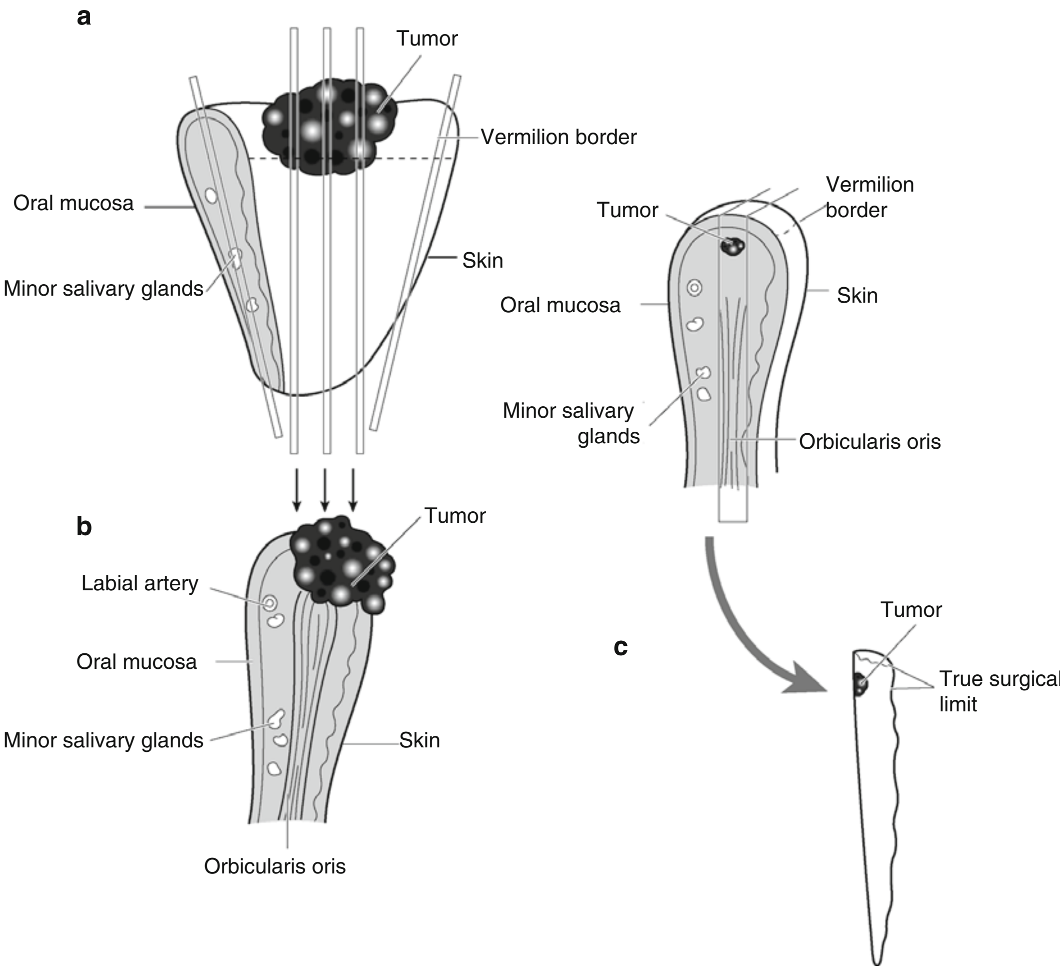

Fig. 14.5 Wedge resection of lower lip. Suggested siting and orientation of blocks for wedge resection of lip: (**a**) View from in front; (**b**) outline of central block(s); (**c**) selection of transverse limits if original limit blocks contain tumor (Reproduced, with permission, from Allen and Cameron (2004))

- TNM classification of tumor spread lip and oral cavity

pTis	Carcinoma in situ
pT1	Tumor ≤2 cm in greatest dimension
pT2	Tumor >2 cm but ≤4 cm in greatest dimension
pT3	Tumor >4 cm in greatest dimension
pT4	Lip: Tumor invades adjacent structures, e.g., through cortical bone, inferior alveolar nerve, floor of mouth, skin of face Oral cavity: Tumor invades adjacent structures, e.g., through cortical bone, into deep (extrinsic) muscle of tongue, maxillary sinus, skin of face
	pT4b (lip and oral cavity): Tumor invades masticator space, pterygoid plates, skull base, or encases carotid artery

Regional lymph nodes

pN0	No regional node metastasis
pN1	Metastasis in an ipsilateral single ≤3 cm
pN2	Metastasis in: (a) Ipsilateral single >3–6 cm (b) Ipsilateral multiple ≤6 cm (c) Bilateral or contralateral ≤6 cm
pN3	Metastasis in a lymph node >6 cm

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15 Maxilla, Mandible, and Teeth

Seamus S. Napier with clinical comments
by Richard W. Kendrick

15.1 Anatomy

Detailed consideration of all craniofacial bones is impossible in a text of this sort, but by focusing on the maxilla and mandible alone, this chapter offers a view of the processes affecting facial bones as a whole and how specimens derived from them might be handled.

The maxilla is the largest bone of the upper facial skeleton and houses the maxillary sinus. It articulates with a large number of other bones, relating to a number of clinically important anatomical areas, including the nasal cavity, the pterygomaxillary space, the infratemporal fossa, and the orbit. It is composed of a body and four processes, namely alveolar, frontal, nasal, and zygomatic processes. The *body of the maxilla* is pyramidal in shape with four surfaces, namely anterior, nasal, orbital, and posterior (or infratemporal) surfaces. The *anterior surface* extends from the alveolar process of the upper anterior teeth below to the infraorbital margin, while the *orbital surface* forms the floor of the orbit. The *nasal surface* articulates with the ethmoid, lacrimal, and palatine bones and the inferior turbinate to complete the lateral nasal and medial orbital walls. The *infratemporal surface* is convex and projects posteriorly and laterally.

The *upper alveolar process* projects from the inferior aspect of the maxilla and contains the sockets of the maxillary teeth. The roots of teeth posterior to the first premolar may be intimately related to the maxillary sinus; teeth and sinus may each become involved in diseases originating in the other. The slightly thickened posterior end of the alveolar process is called the maxillary tuberosity. The *frontal process* projects superiorly between the nasal and lacrimal bones, articulating with the frontal bone and contributing to the medial wall of the nasal cavity. The *palatine process* projects medially from the inferior aspect of the maxilla and forms most of the palatal vault and the nasal floor. The *zygomatic process* is a pyramidal projection from the lateral aspect of the maxilla where anterior, infratemporal, and orbital surfaces converge, articulating with the zygomatic bone.

The mandible is composed of an arched *body*, which runs posteriorly on each side to attach to the flat *ramus*. The body of the mandible has an external surface, an internal surface, an upper border, and a lower border. The *lower border* is rounded and well defined, outlining the profile of the lower jaw. The *external surface* bears the mental foramen between the premolar roots. The *internal surface* of the body is indented by the sublingual and submandibular glands. The superior border, more usually referred to as the *lower alveolar ridge*, contains the sockets of the mandibular teeth.

S.S. Napier (✉)
Histopathology Laboratory, Institute of Pathology,
Royal Victoria Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: seamus.napier@belfasttrust.hscni.net

R.W. Kendrick
School of Dentistry, Belfast Health and Social Care Trust, Belfast, UK
e-mail: kendrick.deramore@ntlworld.com

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_15, © Springer-Verlag London 2013

The posterior aspect of the body joins the ramus behind the last molar tooth. The anterior surface of the ramus extending from the abrupt change in angulation of the bone is called the *ascending ramus*, while the area around the junction of the body and ramus is called the *angle*. The coronoid process extends upward and slightly forward from the anterosuperior aspect of the ramus and bears the attachment of temporalis. The condylar process extends upward and posteriorly from the posterior aspect of the ramus and bears the knuckle-shaped articulating *condylar head* on the narrow *condylar neck*. The *sigmoid notch* lies between these two processes. Near the center of the medial surface of the ramus lies the mandibular foramen, where the inferior alveolar branch of the mandibular nerve and its accompanying vessels enter the mandible. The lateral and medial surfaces of the mandible bear several shallow fossae and roughened elevations corresponding to the attachments of muscles both of facial expression and of mastication.

Each fully developed tooth is composed predominantly of dentine. The *crown*, the portion visible within the mouth, is covered by a layer of hard translucent enamel, while a thin layer of bone-like substance called cementum covers the conical *root*, which may be single or multiple. The crown and root join at a slight narrowing called the *cervical margin*. The dentine encloses a central cavity called the *pulp canal*; the portion toward the crown is dilated to form the *pulp chamber*, while the pulp canal narrows at the end of the root, the *apex*, into an *apical foramen*. The pulp chamber and canal contain the neurovascular supply to the tooth, passing through the apical foramen. The tooth is suspended in the alveolar bone by the periodontal membrane, composed of thick bundles of collagenous tissue running between the cementum and the bone. At the cervical margin, the periodontal ligament merges with the gingival mucosa; a narrow sleeve of epithelium continuous with the gingiva called the *epithelial attachment* surrounds the cervical margin.

The crown of each tooth has five surfaces. The biting surface is called the *occlusal* surface; on incisor teeth, this is termed the incisal edge. The surface closest to the tooth in front is called the *mesial* surface; that closest to the tooth behind is called the *distal* surface. The surface lying closest to the cheek is called the *buccal* surface (or the *labial* surface on anterior teeth); the surface lying closest to the tongue is called the *lingual* surface (or the *palatal* surface on upper teeth). Incisor teeth have a relatively broad crown with flattened edge for cutting food, while canine teeth have a single point or *cusp*. Premolar teeth have two cusps on the occlusal surface, one buccal and one lingual, while molars have four or five cusps.

There are 20 deciduous (or milk) teeth in the primary dentition – two incisors, a canine, and two molars in each quadrant of the jaw. The teeth of the primary dentition begin their development in the first trimester of pregnancy as epithelial ingrowths from the lining of the oral cavity. The epithelial component of the tooth bud forms the enamel while the mesenchymal element gives rise to the remaining parts of the tooth. The crown of the tooth forms first but the root only forms after the crown is complete; root development is closely linked with eruption into the mouth. The deciduous incisors begin to appear in the mouth at around 6 months of age, usually the central before the lateral, followed by the first deciduous molar at around 12 months. The deciduous canine appears around 18 months and finally the second deciduous molar at around 24 months of age. The precise timing of eruption into the mouth is variable, although the sequence is relatively unchanging and lower teeth tend to appear before the uppers.

There are 32 teeth in the permanent or secondary dentition – two incisors, a canine, two premolars, and three molars in each quadrant of the jaw. There is an ordered pattern of replacement of the deciduous dentition by the permanent dentition. Beginning around 6 years of age, the first permanent molar erupts distal to the second deciduous molar, soon followed by shedding and replacement of the incisors. The deciduous molars are replaced by the premolars, while the eruption of the permanent canines straddles the eruption of the second permanent molar with the upper canine appearing latest, usually around 13 years of age. The process ends with the eruption of the third permanent molar or *wisdom tooth* around 18 years.

Lymphovascular drainage:

Lymph drainage of the teeth and jawbones corresponds to that of the regional cutaneous and mucosal sites, leading ultimately to the deep cervical chain (see Fig. 20.1).

15.2 Clinical Presentation

The maxilla and mandible are more usually affected by disease arising from closely related structures such as the teeth, oral mucosa, and salivary glands, and, in the case of the maxilla, the maxillary sinus, the orbit or the infratemporal fossa, rather than from primary bone disease. Many of these conditions are described elsewhere; primary diseases of the odontogenic apparatus and jawbones will be considered here.

Disease of the teeth presents as pain, mobility, or swelling, although some conditions are detected as incidental findings at radiographic examination. Caries and periodontal disease are painless until advanced destruction of tissue has occurred. A draining sinus opening onto mucosa or facial skin may accompany dental abscesses but others may present with soft tissue swelling of the face and other signs of spreading infection. Gingival bleeding is often the only sign of chronic marginal periodontal disease until increased tooth mobility or the drainage of pus from between gingiva and tooth occur. Developmental cysts, odontogenic hamartomas, and neoplasms are often painless but may present with bony swelling, facial asymmetry, or a failure of teeth to erupt. Discoloration of teeth, rapid wear, or abnormal morphology are features of the hereditary developmental tooth disorders such as dentinogenesis imperfecta or amelogenesis imperfecta; usually all the teeth will be affected.

Primary disease of the jawbones presents as bony swellings that may or may not involve overlying mucosa or adjacent teeth.

15.3 Clinical Investigations

- Vitality testing, using the cooling effect of evaporation of ethyl chloride or small electric currents, can assess the health of the pulpal tissues. Tenderness to percussion (TTP) indicates involvement of periodontal tissues. Probing the junction between tooth and gum can assess the depth and extent of periodontal destruction, the presence of bleeding signifying active inflammation. Mobility is assessed in terms of buccolingual and vertical movement and is due to destruction of periodontal support, perhaps as a result of periodontal disease or because of an adjacent cyst or tumor.
- Plane radiographs are essential for the assessment of intrabony cystic lesions, particularly to determine the size, outline (unilocular or multilocular), and bone–lesion interface (sclerotic and well-defined implies a slow-growing lesion; ill-defined suggests a rapidly growing destructive lesion). CT scanning particularly with 3D reconstruction is useful for large lesions and assessing relationships with adjacent anatomical structures. MR images are not good at penetrating bone.

15.4 Pathological Conditions

15.4.1 Non-neoplastic Conditions

Radicular cyst (also known as *apical periodontal cyst, dental cyst)*, *apical granuloma*, and *chronic dental abscess*: These inflammatory lesions form a spectrum of changes related to the apical region of a non-vital tooth (usually a consequence of dental caries), with considerable overlap in clinical, radiological, and pathological findings. Granulomas tend to be smaller (<10 mm), have a sparser inflammatory cell infiltrate, and show less active inflammation than radicular cysts. Very large radiolucencies tend to be cysts rather than abscesses, although they may be infected. Very common, 70%+of jaw cysts; 60% occur in the maxilla; all ages but rare in children and with deciduous teeth. Arise when the contents of the necrotic pulp canal leak out of the apical foramina and set up an inflammatory reaction at the apex. The persistent inflammatory stimulus induces granulation tissue formation to help wall off the necrotic debris. Epithelial rests around the root ("cell rests of Malassez") proliferate, initially as complex strands and arcades, then as a well-defined lining; when present, the epithelium allows the term radicular cyst to be used. Cysts enlarge by a hydrostatic mechanism – the high protein content of the inflammatory exudate in the lumen draws water into the cyst, while the lack of lymphatics in the wall prevents it draining away – producing a rounded radiolucency usually with a sclerotic border. May resorb the apical portion of the tooth. Most are located apically but 10% are seen in lateral relationship (accessory apical foramina). Treatment usually involves endodontic therapy (root canal treatment), apicectomy (removing the apical 2 mm of the tooth root via a surgical approach and sealing off the pulp canal), or removal of the tooth. Recurrence is uncommon but relates to a failure to control the contents of the pulp canal.

Dentigerous cyst (follicular cyst): A developmental cyst that surrounds the crown of an unerupted tooth and is attached at the cervical region. Common, 15% of jaw cysts; often in younger patients but not exclusively; usually seen in the upper canine, lower second premolar and third molar regions. Well-defined radiolucency, unilocular in form with a sclerotic border surrounding the crown of an unerupted tooth (so-called dentigerous relationship). May resorb roots of adjacent teeth. Develops from the dental follicle surrounding the crown of the unerupted tooth but through an unknown mechanism. Enlargement is by hydrostatic mechanisms but what generates the forces is not clear. Has a thin fibrous wall, minimal inflammatory cell infiltrate (if any), and a thin lining of stratified squamous epithelium. Treatment requires removal of the unerupted tooth, the cyst being delivered at the same time. Recurrence is rare.

Odontogenic keratocyst: A developmental cyst characterized by a distinctive lining of keratinizing stratified squamous epithelium and a marked tendency for recurrence. Common, about 10% of jaw cysts; all ages, any site (but especially near angle of mandible) – "any cyst in the jaw can be a keratocyst." Well-defined radiolucency, often multilocular in form with a sclerotic border, which may be in dentigerous relationship. May resorb roots of adjacent teeth. Histology shows a thin lining of highly organized keratinizing stratified squamous epithelium, which has a prominent palisaded basal layer. Daughter cysts within the wall are common. Derived from primordial dental structures, the epithelium has an active growth potential of its own, unlike that of radicular cysts and dentigerous cysts. Probably enlarges by epithelial growth; epithelium proliferates between trabeculae of bone where it accumulates fluid and keratin in the center. This infiltrative growth pattern produces a multilocular radiolucency, in contrast to the ovoid or circular unilocular lesion of expansile cysts like the radicular cyst. Recurrences (20%) are due to small pieces of lining and/or daughter cysts that remain following curettage. Large cysts are treated by marsupialization and packing; over time the cyst shrinks in size and may disappear completely. A small proportion of patients with keratocysts, particularly those aged under 18 years, have Gorlin's syndrome (many stigmata, including multiple synchronous and metachronous keratocysts, skeletal abnormalities especially of skull form, ribs and vertebrae, multiple basal cell carcinomas).

Other cysts: A large variety of cysts can occur in the jaws. Some will be developmental cysts unrelated to teeth (nasopalatine duct cyst, nasolabial cyst, dermoid cyst), others will be associated intimately with the odontogenic apparatus and will be developmental (lateral periodontal cyst, gingival cyst of adults, glandular odontogenic cyst) or inflammatory in nature (paradental cyst). In addition, samples from a periodontal pocket or inflamed dental follicle can mimic cystic lesions. Of these only the glandular odontogenic cyst is likely to recur because of the presence of daughter cysts in its wall.

15.4.2 Neoplastic Conditions

Odontogenic neoplasms and hamartomas provide a bewildering array of complex histological patterns although they are relatively uncommon clinical problems. Classification is based on resemblance to normal tooth formation. Most are benign or self-limiting and can be managed in a similar semiconservative fashion. Of the many different types, only ameloblastoma and odontome are common.

Ameloblastoma: The commonest odontogenic neoplasm, accounting for 1% of all jaw tumors. Usually found in the mandible, especially near the angle (60%), although up to 20% arise in the maxilla. Peak in fourth and fifth decades, but all ages affected. X-rays usually show multilocular radiolucency, often in dentigerous relationship; erosion of the lingual cortex or lower border is a characteristic sign; roots can be resorbed. The tumor may be solid, cystic, or microcystic; in solid areas the histology is characteristic but can be very subtle in more cystic areas. Peripheral tall columnar ameloblast-like cells with polarized hyperchromatic oval nuclei and clear cytoplasm ("piano keyboard") surrounding more centrally placed cells resembling stellate reticulum. The tumor grows by epithelial proliferation and infiltrates along the soft tissues between bone trabeculae, usually extending far beyond the radiographic margins. Recurrence is inevitable if not resected completely. Multiple recurrences run the risk of soft tissue involvement (especially into the parapharyngeal spaces) and dissemination of tumor into lungs and lymph nodes.

There are many different histological subtypes: follicular, plexiform, acanthomatous, desmoplastic, granular cell, etc., which probably have no real clinical significance. Two variants have a better prognosis – the unicystic ameloblastoma and the extraosseous ameloblastoma.

Unicystic ameloblastoma: Younger patients (teens/early twenties), predominantly in the lower third molar region: associated with unerupted tooth in dentigerous relationship. A single large cystic cavity is lined by epithelium that is not always typical of ameloblastoma; sometimes, there is epithelial proliferation into the wall or as a luminal polyp. On account of the subtle character of the epithelium, diagnosis is easily missed. Fortunately, this type of ameloblastoma usually responds to thorough curettage and does not always require resection.

Extraosseous ameloblastoma: Less than 5% of ameloblastomas arise in gingival soft tissue alone without bone involvement where they may resemble a fibrous epulis. Histologically fairly typical of ameloblastoma, less radical surgery is required than their intraosseous cousins; nevertheless, cortical bone may have to be removed from the deep aspect of the tumor to ensure clearance.

Odontome: Hamartomatous malformation forming distinct tooth-like structures (*compound*), disorganized masses of dentine, enamel, cementum (*complex*), or any combination of the two forms. Commonly identified in teenagers or young adults. Most are small, are related to the permanent dentition, and are discovered accidentally when an unerupted tooth is being investigated; larger ones may produce bony expansion. X-rays show dense radiopaque masses surrounded by a well-defined radiolucent zone; lesions in younger patients may have large radiolucent portions. Complex odontomes are seen most often in the posterior segments; compound odontomes in the anterior segments (especially maxilla). Multiple odontomes suggest Gardner's syndrome. Histologically they are composed predominantly of dentine with varying amounts of enamel, cementum, and other soft tissue components typical of the odontogenic apparatus. Less well-developed forms have abundant pulpal and ameloblastic areas and can resemble other types of odontogenic tumor (e.g., ameloblastic fibroma, ameloblastic fibro-odontoma), while odontomes may be associated with other odontogenic tumors such as the calcifying odontogenic cyst.

Other rarer benign tumors or hamartomatous lesions include calcifying epithelial odontogenic tumor of Pindborg, adenomatoid odontogenic tumor, calcifying odontogenic cyst, ameloblastic fibroma, odontogenic fibromyxoma, cementoblastoma. Many will display speckled calcification on X-ray, differentiating them from cysts.

Malignant tumors: Involvement of the jawbones by malignant tumor is usually a consequence of direct spread into the bone from mucosal or salivary lesions, although a number of primary bone and soft tissue sarcomas can arise in the jaws. Malignant tumors of the odontogenic apparatus are rare and are usually only diagnosed histologically, although pain, paresthesia, rapid growth, mucosal fixation, or ulceration may be present. Radiographs may show irregular bone destruction.

They include malignant ameloblastoma/ameloblastic carcinoma, clear cell odontogenic carcinoma, carcinoma arising in an odontogenic cyst (any type of odontogenic cyst and usually squamous cell carcinoma), primary intra-osseous carcinoma, and odontogenic sarcomas, the latter being very rare. Overall they tend to be low-grade malignancies, although uncontrolled local tumor growth, recurrences, and metastasis complicate some cases.

15.5 Surgical Pathology Specimens: Clinical Aspects

15.5.1 Biopsy Specimens

The vast majority of teeth are removed because of dental caries or periodontal disease and are not submitted for histological examination unless there are unusual clinical or radiological findings. Teeth adjacent to cystic lesions are removed either as part of the treatment for the lesion (e.g., the unerupted tooth associated with a dentigerous cyst) or because they cannot be restored to useful function (e.g., a tooth whose roots have been extensively resorbed by a keratocyst). Where a primary neoplastic lesion is suspected, teeth may be removed to provide access to underlying lesional tissue via the socket. Teeth may be submitted whole or as fragments; deeply buried unerupted teeth are most likely to be divided by the surgeon prior to removal.

Apicectomy is the removal of a short portion of the tooth root apex to control persistent periapical infection not responsive to nonsurgical endodontic procedures. A flap of mucosa and associated periosteum is reflected to expose the area, the apical portion of the tooth is removed with a drill, and the pulp canal opening sealed usually with amalgam. Soft tissues associated with periapical infection are removed en passant; most will represent a radicular cyst, apical granuloma, or chronic dental abscess. Other benign-looking odontogenic lesions, such as small cysts or odontomes, will be accessed in a similar fashion, shelled out, and the cavity curetted. The resulting specimens are usually submitted in total. Very large cystic lesions tend to be marsupialized rather than removed in total because of the risk of fracture or iatrogenic injury to nerves. A portion of the lining will be sampled, primarily to detect ameloblastoma, which requires more radical surgery than a keratocyst.

The close proximity of important anatomical structures in the jaws means that biopsy samples of primary bone lesions tend to be small. Benign-looking lesions will be removed in total, often as fragments, while suspected malignancies will be sampled to avoid compromising later definitive surgery. Accurate histological assessment often requires demonstration of the interface with normal bone so, in the mandible in particular, it is important to avoid sampling only the cortical bone. Access to lesional tissue is achieved either by reflecting a mucoperiosteal flap or extracting teeth in the region and using the sockets to expose the lesion. Biopsies are taken either as curettings or intact pieces removed with a drill or chisel.

15.5.2 Resection Specimens

Resection specimens of maxilla for neoplastic processes include maxillary alveolectomy, palatal fenestration (also known as partial maxillectomy), maxillectomy (also known as hemimaxillectomy), and radical maxillectomy (also known as extended maxillectomy). *Maxillary alveolectomy* is indicated when a small tumor of the alveolar mucosa encroaches on or invades for a short distance into the bone. The resection lies within the alveolar process and does not involve the maxillary sinus. *Palatal fenestration* is performed for relatively localized tumors of the upper alveolar mucosa or floor of the maxillary sinus. The specimen comprises a portion of unilateral maxillary alveolar bone and alveolar mucosa, the opposing mucosa on the floor of maxillary sinus with a minimum of the medial and lateral sinus walls. Tissue from the upper buccal sulcus and a portion of the palatal vault may be included. *Maxillectomy* is indicated for larger tumors of the maxillary sinus and mouth that involve all or part of the maxillary sinus. There are a number of modifications, but the specimen includes all of the maxillary alveolar bone from the midline to the tuberosity; bone from the lateral and medial walls of the maxillary sinus are included at least to the level of the zygomatic buttress. The orbital floor may be included or left intact. *Radical maxillectomy* is indicated for tumors extending beyond the confines of the maxillary sinus into adjacent sites. The specimen includes the orbital floor, orbital contents, or pterygoid plates and muscles with the maxillectomy.

Resection specimens of mandible for neoplastic processes include rim resection (also known as marginal mandibulectomy) and hemimandibulectomy (also known as segmental mandibulectomy). *Rim resection* is performed for tumors of the lower alveolus or floor of mouth mucosa where there is minimal invasion of bone. If teeth are present, the line of excision passes below their apices, often including the inferior alveolar canal. If the ascending ramus is involved, the excision line may include the coronoid process.

Hemimandibulectomy is indicated for extension of mucosal tumor into the cancellous bone of the body of the mandible either from the alveolar aspect or from the buccal or lingual cortical plates such that preservation of sufficient bone at the lower border to prevent stress fracture cannot be achieved. Reconstruction is facilitated by preserving as much bone as possible, consistent with clearance. However, if there is a risk of perineural spread of tumor within the mandible, a block of bone containing the entire inferior alveolar canal is excised from lingula to mental foramen. Ameloblastomas and other locally aggressive odontogenic tumors in the mandible usually require hemimandibulectomy; there is little risk of perineural spread, so the excision can be less radical.

15.6 Surgical Pathology Specimens: Laboratory Aspects

15.6.1 Biopsy Specimens

15.6.1.1 Teeth

Should be received in formalin. Identify tooth (e.g., upper left second premolar or lower right second deciduous molar). Note the presence of caries or restoration, root resorption, or attached soft tissue. Sample the soft tissue and process in the usual manner.

For an intrinsic developmental disorder of dental hard tissue (e.g., dentinogenesis imperfecta), submit for preparation an undemineralized 50-micron slice through the buccolingual plane of the tooth.

If no such intrinsic abnormality is suspected, decalcify in 5% formic acid. Endpoint can be tested radiographically or with ammonia water. When negative, bisect molars in the mesio-distal plane; others in the buccolingual plane. Demineralize further briefly (2 or 3 days) ,then process and embed as normal. Sections should demonstrate pulpal tissue in pulp chamber and root canal as well as the interface between pulp and dentine.

15.6.1.2 Jaw Cysts

Usually as fragments free-floating in formalin; record number of pieces and dimensions of largest. Submit small specimens in total; if large, submit representative slices.

NB: Small pieces of tooth root and/or bone are frequently included. Test carefully; specimens with hard tissue tend to sink quickly in the fixative.

15.6.1.3 Jaw Bone Biopsies

Usually as fragments in formalin; record number of pieces and dimensions of largest. If small, submit in total for decalcification; otherwise submit representative samples.

15.6.2 Resection Specimens

Most maxillary and mandibular resections are for tumor arising in adjacent structures, although some will be for bone or odontogenic lesions or reactive conditions, such as osteoradionecrosis. Rim resections of alveolar bone will usually be accompanied by definitive resection of a mucosal tumor.

15.6.2.1 Hemimandibulectomy and Maxillectomy Specimens

Procedure:

Radiograph the specimen.

Ink only the critical external periosteal limit and associated soft tissue limits around the tumor, usually posteriorly and superiorly.

Measurements:

- Anteroposterior length (cm) along lower border (hemimandibulectomy) or along alveolar process to tuberosity (maxillectomy)
- Maximum bone height (cm) of ramus (hemimandibulectomy) or of nasal aspect (maxillectomy)
- Associated soft tissue elements (e.g., oral mucosa, pterygoid muscles, orbital contents)
- Tumor maximum dimensions (cm)
- Distance to closest mucosal and deep soft tissue limits (cm)
- Distance to nearest anterior or posterior bone limit (cm)

Sample the mucosal and deep surgical margins as "radial" sections before sawing the bone and submit separately (reduces contamination of the margins). Cut with a sharp blade firmly down to bone and use a flat blunt instrument to dissect mucoperiosteum free from the bone in the way one might peel an orange (see . Fig. 14.4).

Sample soft tissue elements of mucosal tumor prior to sawing the bone unless the tumor is very small (see next section).

Saw the bone into 0.5 cm slices in buccolingual plane (vertical plane passing between crowns of adjacent teeth).

15.6.2.2 Rim Resections of Alveolus

Procedure:

Ink the external periosteal limit along one aspect to aid orientation of subsequent histological sections.

Measurements:

- Anteroposterior length (cm) along alveolus
- Maximum bone height (cm)
- Associated soft tissue elements (e.g., mucosa) in the usual fashion

Saw the bone into 0.5 cm slices in the buccolingual plane (vertical plane passing between crowns of adjacent teeth). If the attached mucosal tumor is larger than 1 cm diameter, sample tumor and margins in the usual fashion prior to sawing the bone.

If the attached mucosal tumor is smaller than 1 cm diameter, saw the bone into 0.5 cm slices in the buccolingual plane (vertical plane passing between crowns of adjacent teeth) without disturbing the soft tissue.

Description:

Tumor – solid, cystic, or both solid and cystic

- Circumscribed or infiltrative

Arising in bone or extension from adjacent structures

Adjacent bone

- Periosteal reaction? Osteomyelitis?

Mucosa

- Origin of tumor or secondarily involved?

Other

- Associated soft tissue elements (e.g., oral mucosa) in the usual fashion

Blocks for Histology:

The histology should represent the tumor, its deepest extent, the relationship to the bony, mucosal and deep soft tissue margins, and changes in adjacent tissues (Figs. 14.4, 15.1, and 15.2).

At least one block of tumor per centimeter diameter.

Abnormal areas of distant bone or mucosa.

Anterior and posterior surgical bone margins as transverse sections.

Mucosal and deep soft tissue and neurovascular surgical margins.

If other specimens are attached as an "in-continuity dissection" (e.g., mucosa, skin, lymph nodes), these can be cut separately in the usual fashion.

Histopathology Report:

Final jawbone resection reports should include details on:

- Specimen type
- Type of tumor present (and grade, if relevant)
- Extent of spread
- Distance of tumor from the nearest cutaneous/mucosal margin
- Distance of tumor from the nearest deep soft tissue margin
- Distance of tumor from the nearest bone margin

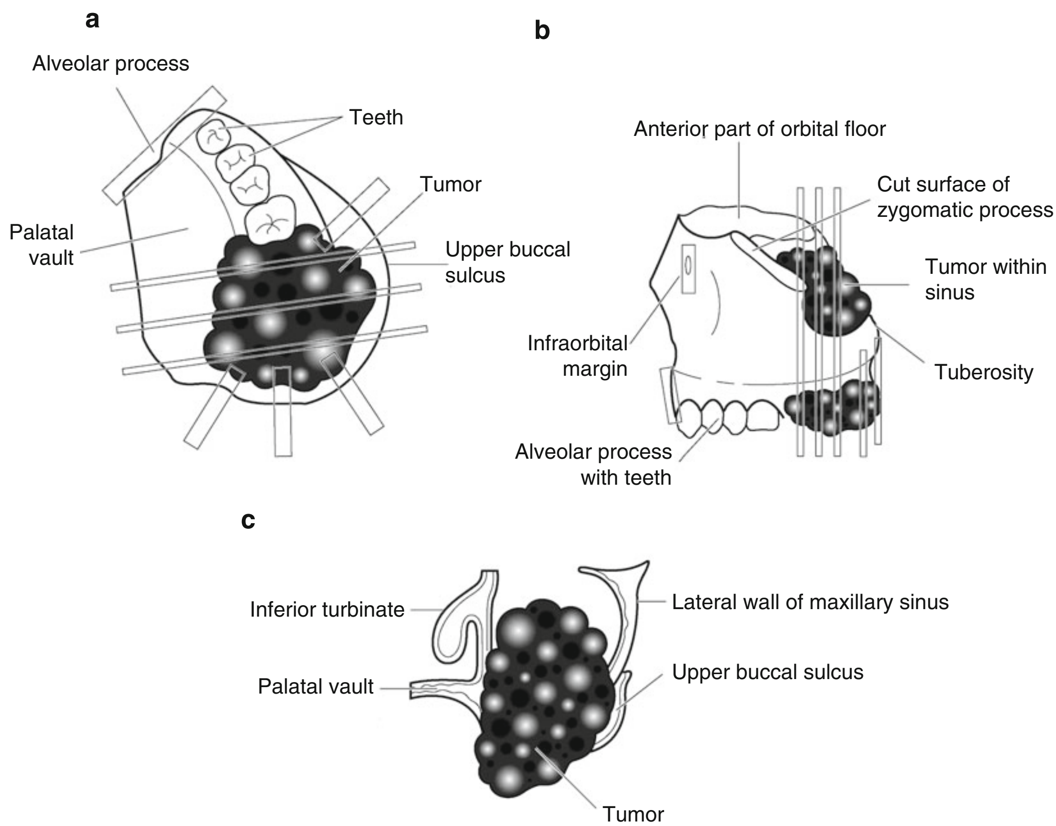

Fig. 15.1 Left maxillectomy specimen for carcinoma. Suggested siting and orientation of tissue blocks for maxillectomy specimens. (**a**) View of palatal aspect; (**b**) view from lateral aspect; (**c**) view of transverse cut surface (Reproduced, with permission, from Allen and Cameron (2004))

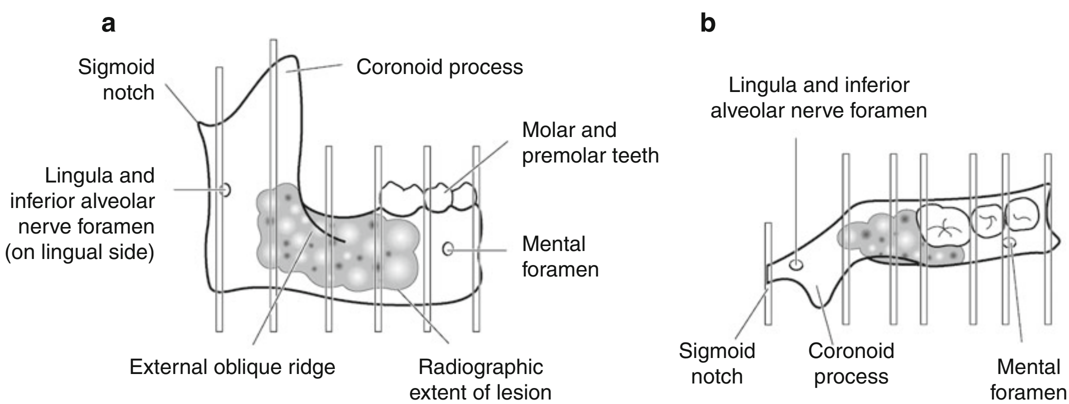

Fig. 15.2 Right hemimandibulectomy for ameloblastoma. Suggested siting and orientation of tissue blocks for hemimandibulectomy for ameloblastoma or other intrabony tumor. (**a**) View from lateral aspect; (**b**) view from above (Reproduced, with permission, from Allen and Cameron (2004))

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16 Pharynx and Larynx

Seamus S. Napier with clinical comments by
William J. Primrose

16.1 Anatomy

The pharynx connects the nasal cavities and mouth with the larynx and esophagus. It is divided into three functional parts, namely the nasopharynx, the oropharynx, and the hypopharynx (Fig. 16.1).

The *nasopharynx* lies behind the nasal cavities and above the level of the soft palate. The roof and posterior wall relate closely to the skull base and the first cervical vertebra. The lateral wall is an extension of fascia from the skull base called the *pharyngobasilar fascia*. The *Eustachian tube* opens into the lateral wall of the nasopharynx just behind and at approximately the same level as the inferior turbinate. It is lined by respiratory mucosa with accessory mucous glands, particularly numerous around the opening of the Eustachian tube. The slight depression posterior to the opening of the Eustachian tube is called the *fossa of Rosenmüller* (or *pharyngeal recess*). The *oropharynx* extends from the soft palate into the depth of the *vallecula*, the gutter between the posterior tongue and the epiglottis. The *tonsillar fossa* lies in the lateral aspect, between the palatoglossal and palatopharyngeal folds. The *hypopharynx* extends from the upper border of the epiglottis to the lower border of the cricoid cartilage. A narrow recess termed the *piriform fossa* lies on each side of the larynx between the aryepiglottic fold and the thyroid cartilage. Together with the oropharynx, it is lined by stratified squamous epithelium and contains accessory mucous glands (Fig. 16.1).

A ring of lymphoid tissue surrounds the opening of the pharynx, comprising the pharyngeal tonsil (or adenoid), the palatine tonsils, and the lingual tonsil. The adenoid lies on the posterior wall of the nasopharynx in the midline between the posterior edge of the nasal septum and the openings of right and left Eustachian tubes. The palatine tonsils each lie in their tonsillar fossa. This group of lymphoid aggregates is collectively described as *Waldeyer's ring*.

The larynx lies between the posterior one-third of the tongue superiorly and the trachea inferiorly. It is composed of three large midline cartilages – the epiglottis, the thyroid, and the cricoid – with the smaller paired arytenoid cartilages. Other smaller paired cartilages are present, the corniculate and cuneiform cartilages, that are of lesser importance in surgical practice.

The *cricoid cartilage* is the most inferior of the laryngeal cartilages but is the cornerstone of the larynx. It is shaped like a signet ring, with the broadest part, the lamina, located posteriorly and the narrower arch continuous anteriorly encircling the

S.S. Napier (✉)
Histopathology Laboratory, Institute of Pathology,
Royal Victoria Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: seamus.napier@belfasttrust.hscni.net

W.J. Primrose
Ear, Nose and Throat Department,
Royal Victoria Hospital,
Belfast Health and Social Care Trust,
Belfast UK
e-mail: william.primrose@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_16, © Springer-Verlag London 2013

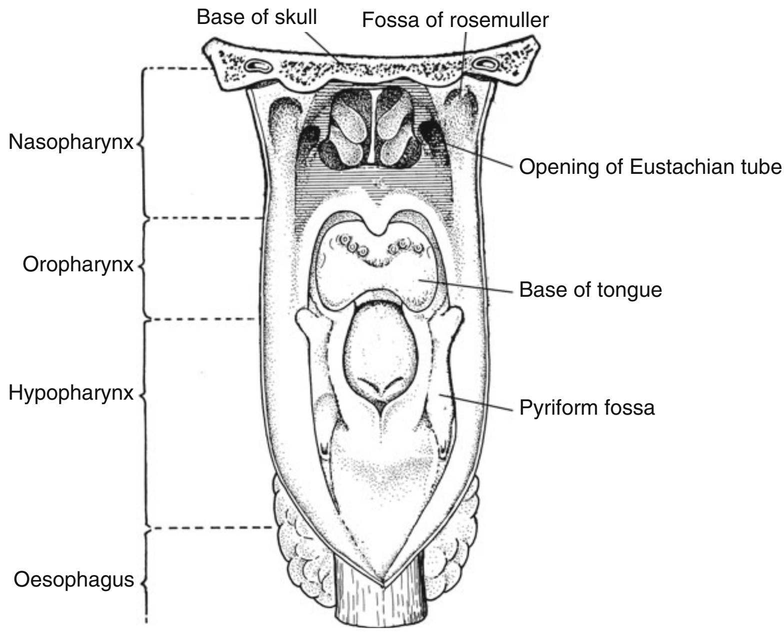

Fig. 16.1 Anatomy of the pharynx. View of pharynx opened from behind to reveal major subdivisions and anatomical landmarks of the pharynx (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

opening to the trachea. It is connected to the highest tracheal cartilage by the cricotracheal ligament. The *thyroid cartilage* overlaps outside the cricoid cartilage. It is composed of two quadrangular laminae that join in the midline anteriorly (forming the laryngeal prominence or "Adam's apple") and diverge posteriorly, ending as two slender processes, a larger superior cornu and a smaller inferior cornu. It is attached to the cricoid by the cricothyroid membrane and to the hyoid bone above by the thyrohyoid membrane. The *epiglottis* is a thin leaf-shaped cartilage, attached at its inferior aspect to the inner surface of the thyroid cartilage just below where the thyroid laminae join anteriorly and extending superiorly and posteriorly to overhang the inlet to the larynx. The whole assembly is suspended from the hyoid bone by the thyrohyoid and hyoepiglottic membranes (Fig. 16.2).

Right and left *arytenoid cartilages* are smaller than the epiglottis, thyroid, and cricoid cartilages, and are pyramidal in shape. They sit on top of the cricoid lamina, just lateral to the midline and are overlapped outside by the thyroid laminae. They each possess a muscular process (posteriorly and laterally), a vocal process (anteriorly), and an apex (superiorly and posteriorly). Extending anteriorly from the vocal process of each arytenoid to the inner surface of the thyroid cartilage is the vocal ligament. Each vocal ligament forms the basis of the *vocal cord*. A complex arrangement of extrinsic and intrinsic muscles coordinates the movements of the larynx and its constituent cartilages.

The surface anatomy of the endolarynx is defined by three sets of prominent mucosal folds – the aryepiglottic folds, the vestibular folds, and the vocal cords. The *aryepiglottic folds* sweep upward and laterally from the arytenoid cartilages posteriorly to the tip of the epiglottis, encircling the inlet to the larynx and representing the border between larynx and hypopharynx. The *vestibular folds* (or *false cords*) lie just above the vocal cords and run in the horizontal plane parallel to the vocal cords, separated from them by a shallow pouch called the *vestibule* (or *ventricle*). The larynx is divided into three regions – supraglottic, glottic, and subglottic – according to their relationship with the vocal folds. The glottic region corresponds to the region of the vocal cords, while supraglottic and subglottic regions lie above and below, respectively (Fig. 16.3). A number of compartments are present within the larynx that can influence the spread of tumors. The *pre-epiglottic space*

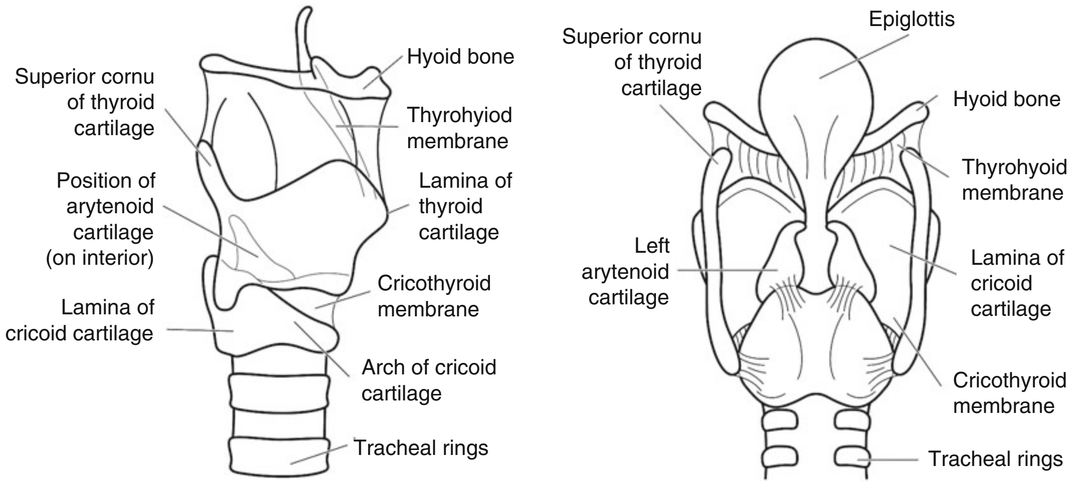

Fig. 16.2 The laryngeal cartilages. (**a**) View from the right lateral aspect; (**b**) view from the posterior aspect (Reproduced, with permission, from Allen and Cameron (2004))

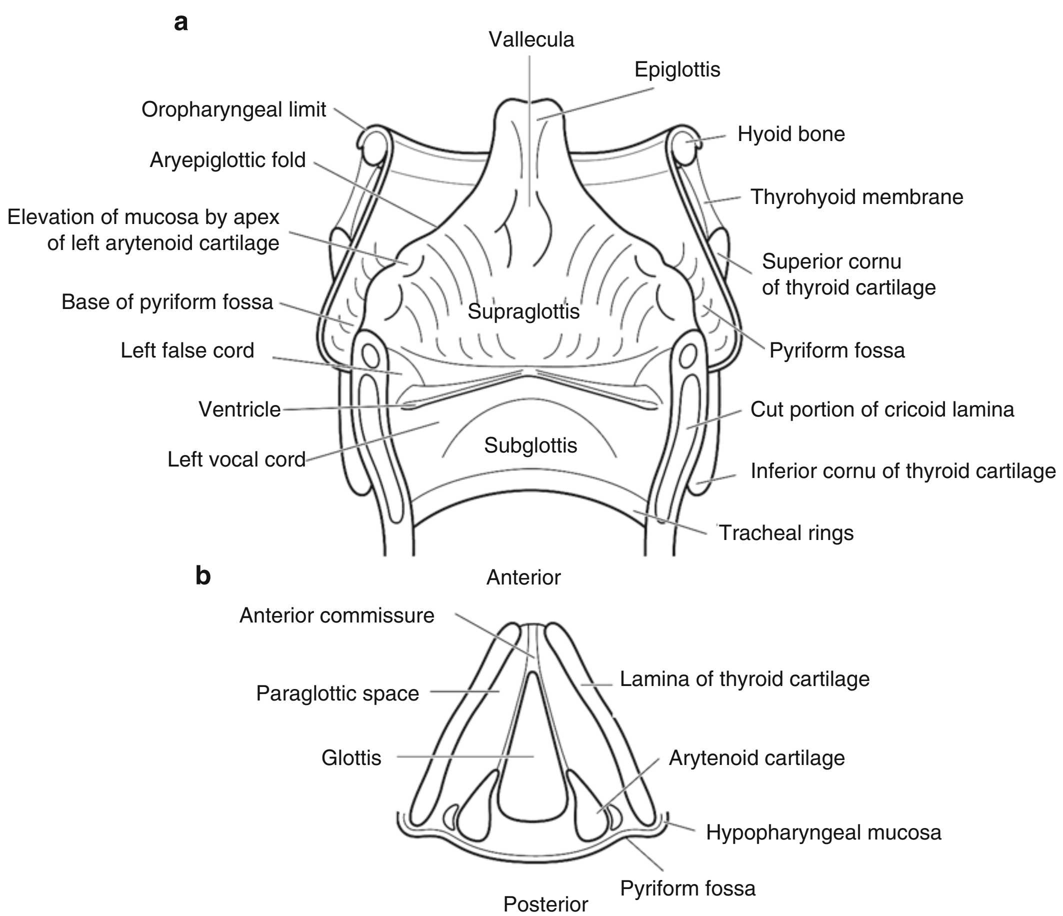

Fig. 16.3 Laryngectomy specimen. (**a**) Idealized view of laryngectomy specimen opened from posterior; (**b**) coronal section through larynx at level of vocal cords (Reproduced, with permission, from Allen and Cameron (2004))

lies outside the larynx between the tongue, the hyoid bone, and the epiglottis, while the *supraglottic space* lies just below the mucosa of the supraglottic larynx from epiglottis to the false cords, these spaces communicating through fenestrations in the epiglottis. The *paraglottic space* lies between the vocal ligament and the lamina of the thyroid cartilage and communicates superiorly with the pre-epiglottic and supraglottic spaces. *Reinke*'*s space* is restricted to the submucosa of the vocal cord, communicating with the paraglottic space and the *subglottic space*, the latter extending submucosally from the vocal cord into the trachea.

The larynx is covered almost entirely by respiratory mucosa with many seromucinous accessory glands in the submucosal tissues, particularly around the epiglottis and the vestibule. In contrast, the vocal cords are covered instead by stratified squamous epithelium with only a minimum of connective tissue around the vocal ligaments in which few (if any) lymphatic channels are found.

Lymphovascular drainage:

Lymph vessels from the nasopharynx, oropharynx, hypopharynx, and the tonsils drain to Level II nodes in the upper deep cervical chain either directly or via the retropharyngeal groups (see Fig. 20.1). Bilateral drainage is common with nasopharyngeal, hypopharyngeal, and laryngeal lesions.

16.2 Clinical Presentation

Disease at any site in the pharynx can present with *dysphagia* (difficulty swallowing), *dysphonia* (change in voice quality), *otalgia* (earache), cranial nerve palsies, or cervical lymphadenopathy. In the nasopharynx, tumors may evoke deafness, *otitis media*, *epistaxi*s (nose bleeds), nasal obstruction, or palsy of cranial nerves (esp. II – VI, IX, X, XII), while those in the oropharynx usually present with sore throat or dysphagia. Hypopharyngeal masses may cause dysphagia or signs of laryngeal involvement, such as hoarseness or a whistling sound during inspiration (*stridor*).

Patients with laryngeal disease may present with alterations in the voice, particularly hoarseness or stridor.

16.3 Clinical Investigations

- The nasopharynx can be inspected using a post-nasal mirror placed behind the soft palate but biopsy specimens are usually obtained with either rigid or flexible endoscopes. The hypopharynx and larynx can be inspected by indirect visualization using a laryngeal mirror held against the soft palate or a fibre-optic endoscope passed through an anesthetized nose. Biopsies can be readily obtained under general anesthesia using a laryngoscope and operating microscope. Endoscopy of the upper aerodigestive tract is performed prior to surgery for malignant disease to identify occult second primary neoplasms.
- Serological studies for Epstein–Barr virus antigens are useful in nasopharyngeal carcinoma both in assessing the effects of therapy and in detecting recurrence. Baseline function of the thyroid gland should be determined prior to radical surgery or radiotherapy to the neck.
- Ultrasonography has proved useful in evaluation of lymphadenopathy and in guidance of needles for FNA and core needle biopsy.
- Barium studies are performed in cases of dysphagia and to assess swallowing function prior to treatment for malignant disease. Chest radiographs may identify a concurrent bronchial or lung lesion. CT and MRI scanning are essential in planning surgery by indicating the depth of the tumor and detecting other changes in the neck. CT has less motion artifact and is good for bone detail, while MRI gives superior soft tissue contrast without dental amalgam artifact or exposure to ionizing radiation.
- FNA is essential in assessing patients presenting with cervical lymphadenopathy, particularly when there is a high probability of malignant disease.

16.4 Pathological Conditions

16.4.1 Non-neoplastic Conditions

Polyps and nodules: Mucosal polyps are uncommonly detected in the nasopharynx and oropharynx and are likely to represent florid lymphoid proliferations in association with adenoid or tonsillar enlargement.

Localized thickenings of the vocal cords usually arise at the junction of the anterior and middle one-thirds and may be unilateral or bilateral. They are due to trauma or voice abuse (hence the alternative term *singer's node*) but are also associated with smoking. Nodules are broadly based sessile lesions that are usually bilateral and arise in females, while the pedunculated polyps are unilateral and predominate in males. Myxoid degeneration of Reinke's space (*Reinke's edema*) arises in older females, affects both cords along their length and is associated with smoking and not voice abuse. All are characterized by mild hyperplasia of the stratified squamous epithelium, accumulation of myxoid matrix in the lamina propria, increased vascularity, and fibrin deposition. Similar lesions may arise in the myxedema of hypothyroidism. *Contact granuloma* or *contact ulcer* occurs posteriorly between the vocal processes of the arytenoid cartilages and consists of granulation tissue and ulcer slough; voice abuse and recent laryngeal intubation are common causes. *Amyloid* may present with laryngeal nodules or diffuse submucosal thickening but usually affects the ventricle or false cords; only rarely is there associated systemic amyloidosis. *Post-radiation spindle cell nodules* can mimic spindle cell carcinoma.

Cysts: Tonsillar cysts arise in the oropharynx and hypopharynx. They represent accessory tonsillar tissue and are composed of a crypt of stratified squamous epithelium distended by squames and abundant lymphoid tissue in the wall. Laryngeal cysts may contain mucus or air. Mucus-filled cysts are the commonest and usually represent mucous retention cysts of the accessory glands in the supraglottic larynx, usually the ventricle or false cords. They are lined by ductal epithelium. Laryngoceles and saccular cysts are both due to obstruction of the saccule in the laryngeal ventricle, the former containing air and the latter mucus. Both are lined by respiratory epithelium.

Tonsillar enlargement: Tonsillitis is a common disorder of childhood characterized by frequent episodes of sore throat, dysphagia, and otitis media. Although it tends to resolve with age, persistent exacerbations may be treated by tonsillectomy with or without concomitant adenoidectomy. Tonsils may be removed in adults for chronic tonsillitis or if a neoplasm is suspected, particularly if there is asymmetrical or unilateral enlargement. Lymphoid follicles with well-formed germinal centers are seen; there may be fibrosis. Actinomyces colonies (*sulphur granules*) may be present within the crypts. Florid tonsillar follicular hyperplasia may occur bilaterally in HIV infection.

16.4.2 Neoplastic Conditions

Benign tumors: Human Papillomavirus–associated squamous papillomas arise in the larynx either in children under 5 years of age with equal gender mix (*juvenile-onset laryngeal papillomatosis*) or in adults over 20 years of age, mostly in males (*adult-onset laryngeal papillomatosis*). The lesions in juvenile-onset laryngeal papillomatosis are multiple and affect the entire laryngeal mucosa. They may require repeated endoscopic laser or microdebrider de-bulking for airway obstruction; resolution usually occurs in adolescence, but a small proportion of cases persists and may even spread into the trachea and bronchi. Adult-onset laryngeal papillomas are fewer in number and relatively easily excised although multiple lesions are more likely to recur. Histologically, hyperplastic stratified squamous epithelium covers well-formed fibrovascular papillary cores, sometimes with abundant koilocyte-like cells. Cytological atypia is absent or minimal although the epithelium often portrays florid basal cell hyperplasia. Development of malignancy is a rare event; these patients usually have been exposed to other factors known to be associated with laryngeal squamous cell carcinoma (radiation, tobacco use).

Nasopharyngeal (*juvenile*) *angiofibroma*: An uncommon lesion found only in teenage and young adult males. Arises in the lateral wall of the nasal cavity posteriorly and grows into the nasopharynx; presents with unilateral nasal obstruction and epistaxis. A well-circumscribed mass, it has a fibrous cut surface and is characterized by irregular branching dilated vascular channels with partially muscularized walls. Plump spindle cells and mast cells are present in the stroma.

Other benign tumors that arise uncommonly in the pharynx and larynx include salivary gland adenomas (e.g., pleomorphic adenoma), neural tumors (e.g., neurilemmoma, neurofibroma, granular cell tumor), carcinoid tumors, and paraganglioma (from paraganglia in the supraglottic or less often the subglottic larynx).

Malignant tumors: Tobacco and alcohol use are the major risk factors for oropharyngeal, hypopharyngeal, and laryngeal cancers. Their effects are related to dose and duration of use; together they have a multiplicative rather than additive effect. Glottic carcinomas are strongly linked to tobacco use and less associated with alcohol. Post-cricoid carcinoma is associated with Patterson Brown-Kelly syndrome (Plummer–Vinson syndrome – Northern European females, iron-deficiency anemia, achlorhydria, and upper esophageal web) and with alcohol. Approximately 10% of patients with Patterson Brown-Kelly syndrome will develop post-cricoid carcinoma. Recent interest has focused on the role of viruses in pharyngeal and laryngeal malignancy. Human Papillomaviruses are detected in an increasing proportion of tumors in patients who are "never-smokers," especially tonsillar squamous cell carcinoma and laryngeal carcinoma arising against a background of papillomatosis. Epstein–Barr virus is so strongly associated with nasopharyngeal carcinoma that it is almost a *sine qua non*, although the consumption of dietary nitrosamines and smoking play a role.

Squamous epithelial dysplasia: An uncommon clinical problem on its own – most often seen adjacent to established tumors – although the more hyperplastic and/or keratotic lesions can present because of alterations in voice quality. Strongly associated with tobacco smoking. Characterized histologically by hyperkeratosis, epithelial hyperplasia, and/or atrophy with varying grades of dysplasia. Development of invasive squamous cell carcinoma occurs more frequently with increasing degrees of cytological disturbance (less than 5% for non-dysplastic lesions and mild/low-grade dysplasia; around 15% for high-grade dysplasia) but the effects of treatment are difficult to evaluate. A number of classification systems have been proposed each with slightly differing terminology but all suffer problems of reliability. Identifying high-grade dysplasia highlights the considerable risk of synchronous or metachronous squamous cell carcinoma and should trigger further conservative surgery or ablative therapy (e.g., by laser) to the lesion.

Squamous cell carcinoma: Accounts for approximately 90% of primary malignant tumors in the larynx, oropharynx, and hypopharynx. Males are affected at least five times more often than females and most patients are aged between 40 and 60 years. In the oropharynx, squamous cell carcinoma most commonly arises in the posterior one-third of the tongue and tonsil. Tumors of the posterior tongue tend to be very large at presentation; tonsillar tumors are often occult, presenting with nodal metastasis. Most cases of hypopharyngeal squamous cell carcinoma arise in the pyriform fossa (75%) or the posterior pharyngeal wall (20%).

The commonest site of laryngeal squamous cell carcinoma is the glottis (75%), followed by the supraglottic larynx (15–20%), while subglottic tumors account for less than 5% of cases. Glottic tumors tend to be small and localized, while supraglottic and subglottic tumors tend to be large with nodal metastasis in over 50% of cases.

Histological and reportedly prognostic variants of squamous cell carcinoma include verrucous carcinoma, papillary squamous cell carcinoma (better than usual type), spindle cell squamous cell carcinoma, adenoid squamous cell carcinoma (same prognosis), basaloid squamous cell carcinoma, and adenosquamous cell carcinoma (worse prognosis).

Nasopharyngeal carcinoma: Has a striking geographic distribution, being commonest in Southern China. Males are affected more often than females but the disproportion is not as marked as at other sites in the head and neck. Incidence peaks between 40 and 60 years, although occasionally adolescents and young adults may be affected. The fossa of Rosenmüller is the commonest site, although there may be no obvious mucosal abnormality on inspection. There are a number of histological subtypes – keratinizing squamous cell carcinoma and non-keratinizing carcinoma, the latter being subdivided into differentiated and undifferentiated patterns. Two thirds of cases will have involved regional lymph nodes at presentation. Similar tumors can arise at other sites in the pharynx but tend not to be associated with Epstein–Barr virus.

Other malignant tumors in the pharynx and larynx include sinonasal transitional cell carcinoma, salivary gland-type adenocarcinoma (especially adenoid cystic carcinoma, mucoepidermoid carcinoma), lymphoma (particularly in the tonsil; diffuse large B-cell type), malignant melanoma, neuroendocrine carcinomas (larynx; moderately and poorly differentiated), chondrosarcoma (larynx), and metastatic tumors.

Prognosis: The precise site within the pharynx and larynx has a major impact on prognosis, probably because of the mass effect and the density of lymphatic channels in the submucosal tissues. Tumor biology is certain to have some influence as well as the likely response to therapy. Glottic tumors usually affect the anterior portion of the vocal cords, presenting with hoarseness while still small. In contrast, supraglottic and hypopharyngeal tumors are often very large fungating masses with extensive submucosal spread at presentation. Lymph node metastasis is rare with glottic cancers – there are few lymphatics in the vocal cords – but up to two-thirds of hypopharyngeal tumors have bilateral nodal disease at presentation. The mucosal/submucosal spread of the tumor affects the ability to achieve surgical clearance but the depth of invasion is probably the most significant factor in determining lymph node metastasis. Lymph node metastasis at presentation halves the chances of survival and doubles the risk of distant metastasis. Extracapsular spread from affected nodes is also an indicator of limited prognosis, with increased risk of recurrence in the neck and of distant spread. The effects of age, tobacco, and alcohol use influence patient's general health; comorbidity from cardiovascular and respiratory disease is a major adverse factor in survival.

Five-year survival with small glottic carcinomas is in excess of 80%, falling to less than 20% for patients with large tumors.

With nasopharyngeal carcinoma, female patients, those aged less than 40 years at presentation and those with undifferentiated carcinoma have improved survival, while patients with cranial nerve involvement, keratinizing squamous cell carcinoma, and positive nodes in the lower neck do less well. Five-year survival with nasopharyngeal carcinoma is approximately 60%, dependent on the response to radiotherapy and chemotherapy.

Small oropharyngeal squamous cell carcinomas, in particular when arising in the tonsil, respond well to surgical excision (80% 5-year survival) but larger lesions, especially when associated with Human Papillomavirus, respond well to cisplatin-based chemoradiotherapy, surgery being reserved for locoregional recurrence (up to 30% of cases). The development of a second primary tumor is an ominous event.

16.5 Surgical Pathology Specimens: Clinical Aspects

16.5.1 Biopsy Specimens

Incisional biopsies in the upper aerodigestive tract are usually directed at a specific lesion located either by visualization or by CT or MR imaging. "Blind" biopsies may be taken, particularly from the fossa of Rosenmüller, base of the tongue, pyriform fossa, and palatine tonsil, in the search for an occult primary carcinoma. Superficial biopsies of tonsil may miss a small submucosal tumor; tonsillectomy is preferred. Biopsies of pharyngeal and laryngeal lesions are usually taken at endoscopy with punch or cup forceps. While usually sufficient, it is sometimes difficult to make a histological diagnosis of malignancy as the specimens tend to be superficial and submucosal tumors or the invasive components of well-differentiated squamous carcinoma may not be represented.

16.5.2 Resection Specimens

In general, tonsillectomy specimens are only submitted in cases of unilateral enlargement or where malignancy is suspected; specimens from children for repeated infective episodes or airway obstruction rarely require histological evaluation. In cases of metastatic squamous cell carcinoma to a cervical lymph node, the ipsilateral tonsil is removed when clinical and radiological evaluation fails to locate a primary lesion.

The type of surgical procedure for tumors of oropharynx, hypopharynx, and larynx depends on the precise location of the tumor, its T-stage, the presence of nodal disease, concurrent second primary lesions, and the health of the patient. The surgical clearance possible is limited by the anatomy and is in the region of a few millimeters at best.

In general, T1 and T2 glottic and supraglottic tumors without neck node metastasis are best managed either with radiotherapy or conservative surgery in the first instance. Laser resection using the operating microscope is becoming more widely used for glottic and supraglottic lesions. T3 glottic tumors with stridor are often managed with total laryngectomy but radiotherapy is an option if disease is limited and there is no stridor.

Total laryngectomy is the operation of choice in cases of radiotherapy failure, bulky T3 and T4 lesions, subglottic tumors, and where cord immobility and post-radiation perichondritis result in the so-called "crippled larynx". The ipsilateral lobe of thyroid is included when there is a likelihood of extralaryngeal spread in the subglottic region. The larynx will be included in major resections of hypopharynx.

Partial laryngectomy procedures can be divided into supraglottic laryngectomy and vertical hemilaryngectomy. Supraglottic laryngectomy removes the upper part of the larynx to the level of the ventricle, preserving the glottis while vertical hemilaryngectomy removes the vocal cord and false cord on one side. These operations are rarely performed nowadays but may be indicated for small volume T2 and T3 tumors. They can be combined with neck dissection procedures. Intraoperative frozen section analysis is essential to ensure clear margins in these conservative procedures.

Pharyngectomy with laryngectomy or pharyngolaryngoesophagectomy are the commonest operations for T2-T4 hypopharyngeal tumors, the defects being repaired by free jejunal transfer and gastric transposition, respectively. T1 hypopharyngeal tumors can be resected endoscopically, especially lesions on the posterior wall. Lesions of the pyriform fossa may require partial pharyngectomy with laryngectomy.

16.6 Surgical Pathology Specimens: Laboratory Aspects

16.6.1 Biopsy Specimens

Usually one fragment is present free-floating in formalin, although several specimens may be taken simultaneously.

Measure:

- Place in cassette; if very small wrap in moist filter paper.
- Mark for levels.
- Orientate the specimen at the embedding stage to facilitate microscopic assessment.

16.6.2 Resection specimens

16.6.2.1 Tonsillectomy specimens

Specimen:

Most tonsillectomy specimens are submitted for exclusion of neoplastic disease in cases of tonsillar asymmetry or cervical lymphadenopathy. Specimens of oropharyngeal mucosa, posterior tongue, or neck dissection may be attached.

Procedure:

Orientate the tonsil(s).

Ink the deep resection margins.

Cut the tonsil into 4-mm-thick slices transversely.

Measurements:

- Dimensions of tonsil (cm) and weight (g)
- Dimensions of oropharyngeal mucosa, if present
- Tumor

  Length × width (cm)

  Maximum depth (cm)

  Distances to closest mucosal and deep surgical margins (cm)
- Mucosal abnormalities

Description:
- Tumor
  Infiltrative/occult: usual type SCC
  Bulky/fleshy: lymphoma
- Mucosa
  White/thickened: in situ lesions
- Extent
  Confined to tonsil or spread into adjacent soft tissues

Blocks for histology:

The histology should represent the deepest extent of the tumor, the relationship to the surface, mucosal and deep soft tissue margins, and changes in adjacent tonsillar tissue.

NB: If tumor is not seen macroscopically in cases of proven nodal metastasis, submit in total.

At least one block of tumor per centimeter of maximum dimension

- Mucosal and deep surgical margins
- Adjacent uninvolved tonsil

Histopathology report:

Final reports of tonsillectomy specimens should include details on:
- Specimen side
- Type of tumor present
  Squamous cell carcinoma NOS
  SCC variants include basaloid, adenosquamous, spindle cell, verrucous
  Adenocarcinoma (salivary gland types)
  Neuroendocrine carcinomas
  Lymphoma
- Grade of tumor assessed at the invasive front
- Cohesive or non-cohesive patterns (more metastasis with non-cohesive)
- Extent of local spread
- Distance of tumor from the nearest mucosal margin
- Distance of the tumor from the nearest deep margin
- Presence of intravascular and perineural spread

If other specimens are attached as an in-continuity dissection (e.g., oropharyngeal or lingual mucosa, neck dissection), these can be cut separately in the usual fashion.

16.6.2.2 Laryngectomy specimens

Specimen:

Most laryngectomy procedures are for neoplastic disease in the larynx, although some will be required for hypopharyngeal tumors or because of post-radiation dysfunction. Specimens of neck dissection, hypopharyngeal resection, thyroidectomy, tracheostomy site, or skin from neck may be attached.

Partial laryngectomy specimens are handled in a similar fashion but require orientation by the surgeon.

Procedure:

Paint a vertical line of ink along one side of the larynx from epiglottis to tracheal limit to aid orientation and reconstruction after slicing.

Open the larynx vertically from behind with scissors and identify site of tumor.

Ink only the critical resection margins. This depends on the location and spread of the tumor, e.g., base of tongue and perihyoid soft tissues for anterior supraglottic lesions, lateral pharyngeal wall for lateral supraglottic and pyriform fossa tumors, post-cricoid region for large glottic or post-cricoid tumors, lateral perithyroid region for subglottic tumors.

Dissect off the hyoid bone, strap muscles, thyroid, neck dissection, etc. Look out for extralaryngeal spread of tumor. Supraglottic tumors often spread out of the larynx via the thryohyoid membrane and subglottic tumors via the cricothyroid membrane. Tumor will permeate directly through ossified cartilages more readily than through cartilage that is not ossified.

Cut the larynx into 4-mm-thick slices in the coronal plane (i.e., in the plane of the vocal cords) to provide “rings” of tissue, working from the lowermost aspect to the base of the epiglottis. This is easiest with a band saw or other heavy-duty slicing device.

Slice the remaining supraglottic portion parasagitally with a knife to define precisely the upper aspect of supraglottic lesions.

Measurements:
- Length of the larynx superiorly from to the inferior border of the cricoid (cm)

- Length of trachea (cm)
- Dimensions (cm) of mucosal defects and other specimens
- Tumor
  Length × width (cm)
  Maximum depth (from reconstructed mucosal surface (cm))
  Distances to closest mucosal and deep surgical margins (cm)
- Mucosal abnormalities

Description:

- Tumor
  Plaque-like/ulcerated/fungating: usual type SCC
  Warty: well-differentiated SCC, verrucous carcinoma
  Polypoid: spindle cell SCC
- Mucosa
  White/thickened: in situ lesions
- Extent
  Confined to larynx or spread through/between cartilages
- Other
  Tracheostomy, neck dissection, thyroid gland

Blocks for histology:

The histology should represent the deepest extent of the tumor, the relationship to the laryngeal cartilages, mucosal and deep soft tissue margins, and changes in adjacent tissues (Fig. 16.4).

- At least one block of tumor per centimeter of maximum dimension
- Mucosal and deep surgical margins
- Both vocal cords (individually identified) even if normal
- Samples of other lesions, e.g., mucosal white areas
- Tracheostomy site (if present)
- Perilaryngeal lymph nodes not part of the neck dissection

Histopathology report:

Final reports of laryngectomy specimens should include details on:

- Specimen type
- Type of tumor present
  Squamous cell carcinoma NOS
  SCC variants include basaloid, adenosquamous, spindle cell, verrucous
  Adenocarcinoma (salivary gland types)
  Neuroendocrine carcinomas
- Grade of tumor assessed at the invasive front
- Cohesive or non-cohesive patterns – more metastasis with the latter
- Extent of local spread
- Distance of tumor from the nearest mucosal margin
- Distance of the tumor from the nearest deep margin
- Intravascular and/or perineural spread
- Involvement of perilaryngeal lymph nodes
- Other pathology such as dysplasia or radiation injury.

If other specimens are attached as an in-continuity dissection (e.g., neck dissection, thyroid gland, esophagus, skin), these can be cut separately in the usual fashion.

16.6.2.3 Pharyngectomy specimens

Specimen:

Most pharyngectomy procedures are for neoplastic disease in the pharynx, although some will be required for large laryngeal tumors. Specimens of neck dissection, laryngectomy, esophagectomy, thyroidectomy, tracheostomy site or skin from neck may be attached. Smaller tumors removed by laser will already have been transected by the surgeon – cutting through tumor allows precise assessment of the depth – and will arrive in at least two pieces.

Procedure:

Open the pharynx longitudinally with scissors and identify site of tumor.
Ink the external and mucosal resection margins.
Slice into 4-mm-thick slices transversely.
Measurements:

- Length and width of specimen (cm)
- Maximum thickness (cm)
- Dimensions (cm) of mucosal defects and other specimens
- Tumor
  Length × width (cm)
  Maximum depth (from reconstructed mucosal surface (cm))

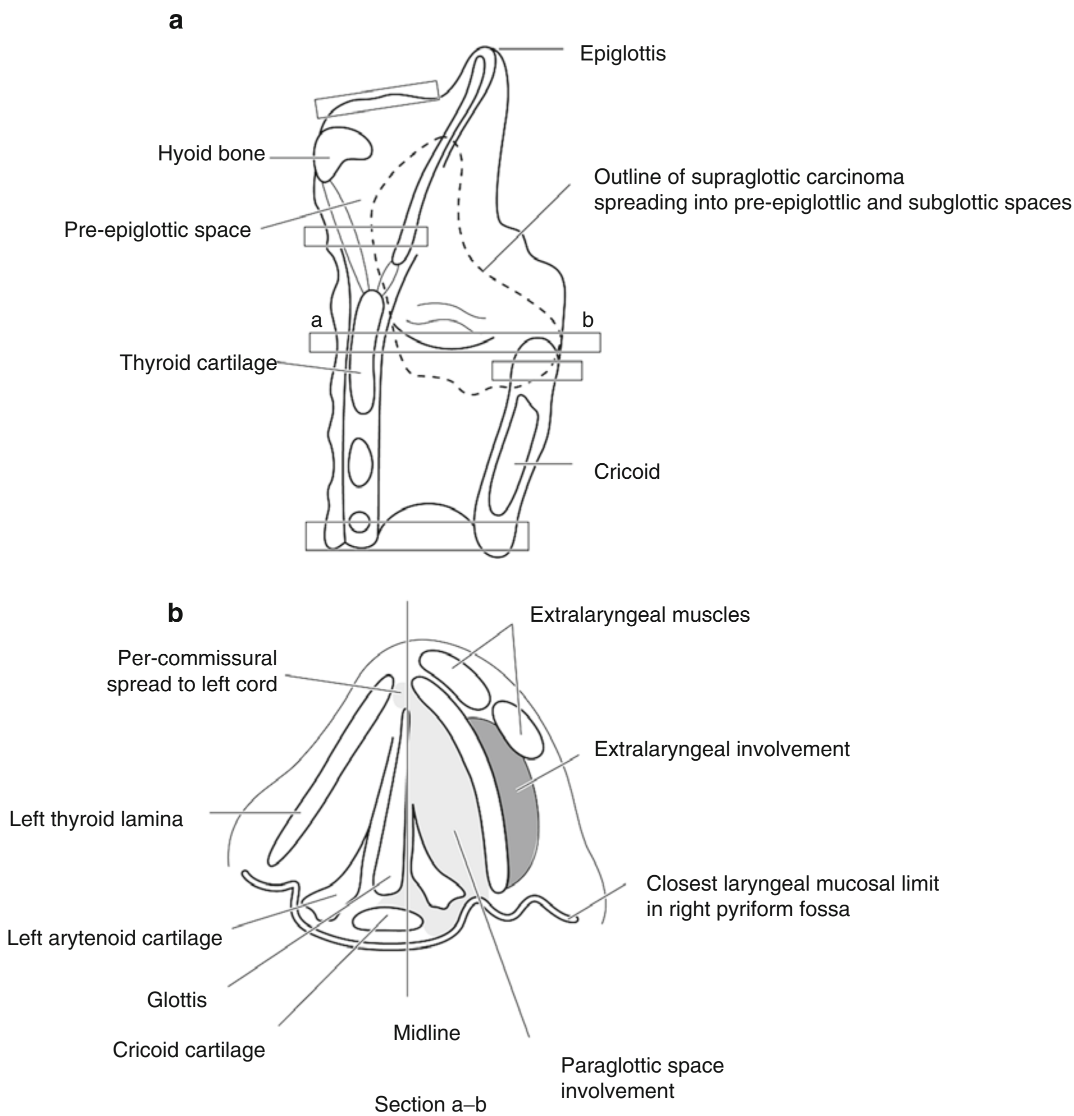

Fig. 16.4 Laryngectomy for supraglottic carcinoma with transglottic and extralaryngeal spread. Suggested siting and orientation of tissue blocks for laryngectomy specimen. (**a**) View from right lateral aspect; (**b**) slice through vocal cords viewed from above (Reproduced, with permission, from Allen and Cameron (2004))

Distances to closest mucosal and deep surgical margins (cm)

- Mucosal abnormalities

Description:

- Tumor
  Plaque-like/ulcerated/fungating: usual type SCC
  Warty: well-differentiated SC, verrucous carcinoma
  Polypoid: spindle cell SCC
- Mucosa
  White/thickened: in situ lesions
- Extent
  Confined to pharynx or spread to adjacent structures
- Other
  Neck dissection, esophagectomy, thyroid gland

Blocks for Histology:

The histology should represent the deepest extent of the tumor, the relationship to the adjacent structures or organs, mucosal and deep soft tissue margins, and changes in adjacent tissues.

At least one block of tumor per centimeter of maximum dimension

Mucosal and deep surgical margins

Samples of other lesions, e.g., mucosal white areas

Histopathology report:

Final reports of pharyngectomy specimens should include details on:

- Specimen type
- Type of tumor present
  Squamous cell carcinoma NOS
  SCC variants include basaloid, adenosquamous, spindle cell, verrucous
  Adenocarcinoma (salivary gland types)
- Grade of tumor assessed at the invasive front
- Cohesive or non-cohesive patterns (more metastasis with non-cohesive)
- Extent of local spread
- Distance of tumor from the nearest mucosal margin
- Distance of the tumor from the nearest deep margin
- Intravascular and/or perineural spread
- Involvement of peripharyngeal lymph nodes
- Other pathology such as dysplasia or radiation injury

If other specimens are attached as an in-continuity dissection (e.g., neck dissection, thyroid gland, esophagus, skin), these can be cut separately in the usual fashion.

TNM classification of tumor spread larynx

pTis	Carcinoma in situ
pT1[a]	Tumor confined to one subsite[a], normal cord mobility
pT2[a]	Tumor invades more than one subsite[a], impaired cord mobility
pT3	Tumor confined to larynx, fixation of one or two cords
pT4	Tumor through thyroid cartilage and/or extends beyond larynx to, e.g., trachea, soft tissues of neck, thyroid, esophagus

[a]Exact details depend on whether tumor site is supraglottic, glottic, or subglottic.

Regional lymph nodes

pN0	No regional lymph nodes involved
pN1	Metastasis in a ipsilateral single node ≤3 cm diameter
pN2	Metastasis in:
	(a) Ipsilateral single >3–6 cm
	(b) Ipsilateral multiple ≤6 cm
	(c) Bilateral or contralateral ≤6 cm
pN3	Metastasis in a lymph node >6 cm diameter

TNM classification of tumor spread pharynx Oro-(hypopharynx)

pT1	Tumor ≤2 cm in greatest dimension (hypopharynx – and limited to one subsite)
pT2	2 cm < tumor ≤4 cm in greatest dimension (hypopharynx – and more than one subsite)
pT3	Tumor >4 cm in greatest dimension (hypopharynx – or with fixation of hemilarynx)
pT4	Tumor invades adjacent structures, e.g., pterygoid muscles, mandible, hard palate, deep muscle of tongue, larynx (hypopharynx – thyroid/cricoid cartilage, carotid artery, soft tissues of neck, pre-vertebral fascia/muscles, thyroid and/or esophagus)

Nasopharynx (rarely used)

pT1	Tumor confined to nasopharynx, oropharynx, and nasal cavity
pT2	Tumor into parapharyngeal space
pT3	Tumor into bone of skull base and/or nasal sinuses
pT4	Intracranial extension, cranial nerves, hypopharynx, orbit, infratemporal fossa/masticator space

Regional lymph nodes
Oro- and hypopharynx

pN0	No regional lymph nodes involved
pN1	Metastasis in a ipsilateral single node ≤3 cm diameter
pN2	Metastasis in:
	(a) Ipsilateral single >3–6 cm
	(b) Ipsilateral multiple ≤6 cm
	(c) Bilateral or contralateral ≤6 cm
pN3	Metastasis in a lymph node >6 cm diameter

Nasopharynx

pN1	Unilateral cervical or retropharyngeal nodal metastasis ≤6 cm, above supraclavicular fossa
pN2	Bilateral nodal metastasis ≤6 cm
pN3a	Metastasis in nodes >6 cm
pN3b	Metastasis in nodes in supraclavicular fossa

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17 Salivary Glands

Seamus S. Napier with clinical comments by Richard W. Kendrick

17.1 Anatomy

There are three paired major salivary glands – the parotid gland, the submandibular gland, and the sublingual gland – as well as a multitude of minor salivary glands (see Fig. 20.1).

The *parotid gland* is the largest, weighing between 15 and 30 g. It is roughly pyramidal in shape, lying in front of and below the ear in the space between anterior aspect of sternocleidomastoid and the ramus of the mandible, projecting anteriorly onto the external surface of the masseter muscle for a variable distance. The lateral surface lies just below the skin and is roughly triangular in shape with its base superiorly close to the zygomatic arch and its apex (or *tail*) in the upper part of the neck. Medially, the gland extends into the infratemporal fossa, where it is closely related to the pharynx, the carotid sheath, and the styloid complex. It is traversed by the facial nerve, entering on the posteromedial surface at the stylomastoid foramen and running forward to the anterior surface on the outer aspect of the mandible, dividing into five terminal branches within the gland. The "Stenson's duct" runs anteriorly across masseter to pierce buccinator and open opposite the upper second molar tooth. Accessory parotid gland tissue may be present on the masseter between the duct and the zygomatic arch. The gland contains serous acinar components divided into lobules and varying amounts of fat with a few mucinous or sebaceous elements.

The *submandibular gland* weighs between 7 and 15 g and occupies most of the submandibular triangle. It straddles the posterior border of mylohyoid; the larger superficial lobe lies just deep to the skin near the angle of the mandible, while the smaller deep lobe lies inside the mouth between the tongue, mandible, and the sublingual gland. Posteromedially it is separated from the pharynx by the styloid complex, glossopharyngeal nerve, and hypoglossal nerve. The "Wharton's duct" begins as tiny branches in the superficial lobe and runs posteriorly, emerging from the anterior aspect of the deep lobe. It runs through the sublingual space to open into the anterior floor of mouth at the sublingual papilla. The lobules of the submandibular gland are populated mostly by serous cells with lesser mucinous elements.

The *sublingual gland* is the smallest of the major salivary glands and weighs between 1.5 and 4 g. It lies in the floor of mouth between the mucosa, mylohyoid, the mandible, and its fellow on the other side. Numerous tiny ducts ("Rivinus' ducts") drain directly into the floor of the mouth but there may also be a larger common sublingual duct ("Bartholin's duct") that drains into the submandibular duct near its

S.S. Napier (✉)
Histopathology Laboratory, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: seamus.napier@belfasttrust.hscni.net

R.W. Kendrick
School of Dentistry, Belfast Health and Social Care Trust, Belfast, UK
e-mail: kendrick.deramore@ntlworld.com

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_17, © Springer-Verlag London 2013

opening. It is predominantly mucinous in type with lesser serous elements.

Hundreds of *minor salivary glands* are dispersed in the submucosal tissues throughout the mouth and oropharynx. They are not found in the anterior hard palate or the attached gingiva except in the retromolar regions of the mandible. Each minor gland measures between 1 and 5 mm in diameter and they are usually not palpable clinically except in the lips. Most are innominate but for two sets of glands in the tongue, namely the glands of von Ebner around the circumvallate papillae and the glands of Blandin and Nunn in the ventral surface near the tip. Most minor glands are mucinous in type, although the glands of von Ebner are serous.

Similar glands are located in the nasal mucosa, nasopharynx, larynx, and hypopharynx but are referred to as "accessory glands" rather than salivary glands.

Lymphovascular drainage:

Lymph nodes are found in intraglandular, intracapsular, and extraglandular locations in the parotid region. They drain the tissues of the scalp and lateral face and ultimately empty into the lymph nodes of the deep cervical chain. Small numbers of lymph nodes are located close to the submandibular gland but intraglandular and intracapsular nodes are rare. Tissues of the lower lip, tongue, and anterior floor of mouth (including the sublingual gland) drain through these nodes, communicating with those elsewhere in the deep cervical chain (see Fig. 20.1).

17.2 Clinical Presentation

Disease affecting the salivary glands will present with enlargement (that may or may not be painful) or as a consequence of dysfunction, usually hypofunction.

Enlargement can affect both major and minor glands and may be episodic or persistent. In the major glands, episodic swellings, worse at mealtimes and accompanied by pain, are due to obstruction of outflow by intraluminal sialoliths or mucous plugs. Symptoms and signs of ascending infection by pyogenic bacteria may also be present. Extrinsic pressure on the duct system, e.g., by tumors, can present with obstruction. The submandibular gland is most often affected by obstruction, the parotid gland less so.

Persistent localized swellings in the major glands are likely to represent a primary salivary gland neoplasm or lymph node disease. Most major gland tumors arise in the parotid gland and over 80% are benign lesions. From a surgical standpoint, the parotid gland is divided into superficial and deep lobes by the plane of the facial nerve as it traverses the gland. Most tumors arise in the superficial lobe and will present as facial swellings, while deep lobe lesions may present medially as parapharyngeal swellings or as a diffuse enlargement of the parotid region. Around 10% of salivary gland neoplasms arise in the submandibular gland around half of which will be malignant; sublingual gland tumors are very rare and almost always malignant. Motor or sensory nerve dysfunction or pain are sinister findings with a localized swelling in a major salivary gland and often signify malignancy.

Bilateral or multi-gland diffuse swellings point to a systemic process such as sarcoidosis, sialosis, or autoimmune phenomena (myoepithelial sialadenitis or HIV).

Minor salivary gland swellings present as submucosal masses. Cystic lesions in young patients located toward the front of the mouth, particularly in the lower lip, will be mucous extravasation cysts; those cystic lesions toward the back of the mouth in older patients are likely to represent retention cysts, although some will turn out to be cystic tumors. Neoplasms of the minor salivary glands have an overall benign-to-malignant ratio of 1:1 but tumors of the palate and upper lip are much more likely to be benign than malignant, while the converse is true of tumors in the tongue, floor of mouth, and retromolar pad.

Hypofunction usually manifests as xerostomia, although some of those who complain of a dry mouth will have perceived rather than real salivary dysfunction. Common causes of xerostomia include drug effects, post-radiotherapy changes, and autoimmune disease, and, less often, endocrine disturbances.

17.3 Clinical Investigations

- Ultrasonography can provide useful information about swellings in the major salivary glands, particularly with cystic lesions. Plain radiography may identify sialoliths while sialography with injection of contrast media will outline the duct system. The relationship of tumor and the adjacent tissues can be evaluated with CT scanning and MR imaging, the differential weighting of MR images often allowing better visualization of the tumor–tissue interface. Assessment of the status of the neck nodes can be performed at the same time.
- Stimulated parotid salivary flow rates are assessed in cases of xerostomia and can indicate the presence of organic disease. Serological investigations can assist with the diagnosis of viral infections and with diseases of autoimmune pathogenesis.
- Fine needle aspiration cytology (FNAC) has greatly facilitated the management of patients with major salivary gland swellings. Definitive diagnosis of primary salivary gland disease can be difficult, but FNAC can be used to identify cases where open biopsy might not be advantageous, e.g., if a metastatic deposit of squamous cell carcinoma in a juxtasalivary lymph node were suspected.
- Open biopsies of major glands carry risks of nerve damage and salivary fistula formation. Core needle biopsies can provide a preoperative diagnosis but suffer the same sampling problems as FNA. Incisional biopsies of minor salivary gland lesions are rarely performed because most of the lesions are relatively small and are either entirely innocent lesions or of low-grade malignancy. In either case, optimum results are provided by complete but non-mutilating excision at the first attempt. Diagnostic biopsies of large malignant minor gland lesions can assist the planning of a more radical excision by providing an estimate of tumor type and/or grade.

17.4 Pathological Conditions

17.4.1 Non-neoplastic Conditions

Cysts: Common in minor glands, particularly in the lower lip. "*Mucocele*" is a nonspecific clinical term used to mean "a localized collection of mucin" and may represent a mucous extravasation cyst, mucous retention cyst, or cystic tumor. *Mucous extravasation cyst* arises when an excretory duct is ruptured; saliva escapes into the tissues, where it evokes a low-grade inflammatory reaction and is walled off by granulation tissue. May arise as a larger lesion from the sublingual gland when the term "ranula" refers to its resemblance to the belly of a frog. *Mucous retention cyst* occurs when an excretory duct becomes obstructed but is not ruptured. The duct becomes distended and forms the wall of the cyst so an epithelial lining is present.

Salivary duct obstruction: Due to calculus formation, duct stricture, mucous plugs, or external pressure on the duct system, and is often accompanied by ascending bacterial sialadenitis. Calculi represent foci of dystrophic calcification occurring in the duct system. Occurring in the submandibular gland in 80% of cases, they may be intraglandular or extraglandular but lie in the duct system. The rest are found in the parotid, usually outside the main body of the gland, and in the minor glands. Duct stricture, mucous plugs, and external pressure on the duct can be difficult to locate without sialography. The changes are often mild; the severity of the obstructive symptoms is inversely proportional to the degree of tissue destruction. If the gland is smaller than normal, it will be fibrotic and contain a stone. Histologically, there is ductal dilation, marked periductal and interlobular fibrosis, focal acinar atrophy, and a focal moderate chronic inflammatory cell infiltrate. Respiratory metaplasia is seen in the duct system; there may be squamous metaplasia of the epithelium in contact with the stone. If the gland is relatively unremarkable macroscopically, there will be no stone and the microscopic changes will be subtle.

Sjögren's syndrome: A clinical condition characterized by xerostomia and xerophthalmia. Secondary Sjögren's syndrome refers to the changes arising in association with a systemic connective tissue disease (such as rheumatoid arthritis), while in primary Sjögren's syndrome there is no connective tissue disease. Mostly females (9 F:M), peak 30–40 years. Histologically there is marked lymphocytic infiltration of the major glands (T-cells mostly with occasional B-cells) causing acinar destruction. Proliferation of the ductal cells gives the ducts a solid appearance (known as epithelial–myoepithelial islands). There is little fibrosis. These appearances are called "myoepithelial sialadenitis" (MESA) or "benign lymphoepithelial lesion." Seen best in the parotid, but submandibular gland also affected. Minor glands show similar features, but usually to a lesser degree – myoepithelial islands are not encountered in minor glands. There may be blastic transformation of the B-cells beginning around the ducts and gradually extending outward into the parenchyma, giving rise to a slowly progressive low-grade non-Hodgkin's lymphoma (MALToma). Some authorities believe that the so-called MESA should be regarded as a low-grade lymphoma from the outset. Overall 10% develop lymphoma of major salivary glands; the risk is greater with primary Sjögren's syndrome.

17.4.2 Neoplastic Conditions

Benign tumors are common. *Pleomorphic salivary adenoma* is the commonest tumor with peak prevalence in the second and third decades but occurs in all ages even in the new-born. Common in the parotid, palate, and upper lip. Histologically, "pleomorphic" describes the architecture, not the nuclear morphology. There is an incomplete capsule, a mixture of ducts, sheets of epithelium, and myxoid matrix that may in areas resemble cartilage. Locally, recurrence may follow incomplete excision, especially if "shelled out" or if the capsule ruptures. Malignancy can occur in a pleomorphic adenoma, but usually only after many years. Rare examples can metastasize to lymph nodes, lung, liver, or bone. This so-called (*benign*) *metastasizing pleomorphic adenoma* resembles the usual variant, although there is usually a history of previous surgery suggesting vascular implantation as a major factor.

Warthin's tumor: Occurs at the lower pole of parotid and may be bilateral. More often seen in males and usually older patients. Never in a minor gland and rare in submandibular gland. Probably represents a form of epithelial proliferation of entrapped epithelial elements in a lymph node. Histologically, very distinctive with multiple papillary projections of altered ductal epithelium into cystic spaces containing debris. Many lymphocytes in stroma with germinal centers, hence the older term "papillary cystadenoma lymphomatosum." Benign; behaves like a lymph node in that other tumors may metastasize to a Warthin's tumor.

A number of other benign tumors can arise in the salivary glands. The term "monomorphic adenoma" has been abandoned, since any salivary adenoma that is not a pleomorphic adenoma is a monomorphic adenoma. All are uncommon and include basal cell adenoma, canalicular adenoma, myoepithelioma (a variant of pleomorphic adenoma), oncocytoma, and a variety of ductal papillomas and sebaceous adenomas.

Malignant tumors: Are relatively uncommon. There are many different types; most are low-grade, although some are aggressive malignancies that metastasize widely. Males and females are affected equally. There is a wide age range, peak prevalence in 40–50 years, but tumors in elderly patients are often high-grade cancers. Most patients have no known risk factors, although increased incidence of salivary gland malignancy can follow head and neck irradiation or a long-standing untreated/recurrent benign tumor (e.g., pleomorphic adenoma). Infection by Epstein–Barr virus is linked with lymphoepithelial carcinoma in the Inuit.

Adenoid cystic carcinoma: Common in the parotid gland and in minor glands, especially in the palate. Clinically, it is often very subtle. Minor gland lesions are soft diffuse and purple, mimicking a dental abscess, while parotid lesions produce unusual signs like pain, facial paralysis, or trismus before there is a palpable mass. Histologically, it has a classical "Swiss cheese" appearance with cribriform clusters of small darkly staining epithelial cells without much nuclear pleomorphism, mitotic activity, or necrosis. Up to half display perineural invasion, but this is only significant if it extends beyond the invasive front. Local spread is often extensive. Because the tumor evokes no response from the tissues through which it infiltrates, it extends for considerable distances beyond what is identified clinically as the edge. Survival for 10 years is common, but patients are often troubled by persistent local disease. Metastasis is via hematogenous routes (e.g., to lung) rather than to nodes.

Mucoepidermoid carcinoma: Common in minor glands, especially in the palate, and the parotid gland. It is the commonest salivary tumor in children. Histologically, a mixture of goblet cells, squamous cells, and other populations such as intermediate cells or clear cells; may be solid, cystic, or both. Histology is little guide to prognosis, although tumors with a poor prognosis tend to be large, solid rather than cystic, predominantly epidermoid in type, are infiltrative and cytologically pleomorphic with necrosis and many mitotic figures. Only 10% metastasize, usually after multiple recurrences or if cytologically aggressive and usually to nodes.

Acinic cell carcinoma: Uncommon in minor glands; 95% occur in the parotid gland. Histologically, lobulated masses of benign-looking epithelial cells with abundant cytoplasm resembling the serous cells of salivary gland. Populations of other cells are present in varying proportions, e.g., clear cells, cells with vacuolated cytoplasm, ductal cells, and/or cells of nonspecific glandular type. Low-grade malignancy with nodal metastasis late (especially after recurrences rather than at presentation) in 10%.

Polymorphous low-grade adenocarcinoma: Only found in minor salivary glands. Characterized by variable morphological patterns (papillae, cysts, solid areas, cribriform areas, tubular/ductal areas), cytological uniformity (cells often have clear nuclei) and indolent behavior. Perineural and perivascular whorling is a characteristic feature. Can recur locally if incompletely excised; spread to regional nodes in 10%.

Carcinoma ex-pleomorphic adenoma: Malignant change may occur in pleomorphic adenoma but usually only in long-standing lesions in major glands. However, it is increasingly recognized in minor glands, sometimes without the long history. Rapid enlargement of a long-standing lump, pain, or VII nerve palsy and fixation to skin or deeper structures are common findings. Histologically, the commonest finding is a poorly differentiated adenocarcinoma overrunning an old pleomorphic adenoma. Often, there is a mixture of different salivary type carcinomas. Survival is related to the type of the carcinomatous component and the extent of invasion beyond the capsule of the pleomorphic adenoma, so that the prognosis for some patients with minimally invasive and/or histologically low-grade lesions might not be so bad.

Other salivary gland malignancies include epithelial–myoepithelial carcinoma, basal cell adenocarcinoma, hyalinizing clear cell carcinoma and MALT lymphoma (low-grade), myoepithelial carcinoma (intermediate grade), salivary duct carcinoma, adenocarcinoma NOS, small cell undifferentiated (neuroendocrine) carcinoma, lymphoepithelial carcinoma, and primary squamous cell carcinoma (high-grade). Metastatic tumors are not rare in the parotid region; nodal deposits usually derive from squamous cell carcinoma or malignant melanoma in the scalp or facial skin, while intraparenchymal deposits usually represent hematogenous dissemination of tumor from sites outside the head and neck.

17.5 Surgical Pathology Specimens: Clinical Aspects

17.5.1 Biopsy Specimens

Salivary cysts are generally dissected intact from the surrounding tissues together with adjacent minor salivary glands that may have been damaged by the procedure. Cyst rupture is only problematical if the lesion is a cystic tumor. Ranulas are usually marsupialized, although recurrent or "plunging" types are treated by excision in-continuity with the sublingual gland.

Open biopsies of minor salivary gland tumors are uncommon because precise interpretation of limited samples of large neoplasms is difficult and most tumor types can be managed with clearance by local excision. Core needle biopsies of parotid gland or, less frequently, submandibular gland tumors may be necessary for deep lobe tumors where malignancy is suspected but FNA inconclusive.

Where Sjögren's syndrome is suspected, sampling of minor salivary glands is preferable to open biopsy of the parotid or submandibular glands, although, given the positive and negative predictive values of serological tests and the often nonspecific nature of the changes in the minor salivary glands, the value of this procedure is generally to exclude other abnormalities, such as hemochromatosis, amyloidosis, or a granulomatous disorder.

17.5.2 Resection Specimens

Surgical resections of parotid gland are classified according to their relationship with the facial nerve. *Superficial parotidectomy* is performed for tumors lying lateral to the plane of the facial nerve as it courses through the gland. If the tumor lies in contact with but does not infiltrate branches of the facial nerve, it is dissected clear with preservation of the nerve. If infiltrated by malignant tumor, the nerve may be sacrificed and repaired with a graft from the nearby greater auricular nerve if a curative (rather than palliative) procedure is anticipated. *Extracapsular resection* can be performed when the tumor is small or lies distant to the nerve. The surgeon dissects external to the tumor capsule with preservation of the nerve; although conservative of glandular tissue, there is considerable risk of capsular rupture. Total parotidectomy procedures are divided into nerve-preserving and nerve-sacrificing types. *Total parotidectomy with nerve preservation* is performed for deep lobe tumors that can be dissected clear of the nerve. The superficial lobe is removed initially to identify, dissect, and preserve the nerve before removing the deep lobe. *Total parotidectomy with nerve sacrifice* (radical parotidectomy) is warranted for curative excision of clinically malignant tumors of either lobe that infiltrate the nerve. Nerve grafting may be performed if the proximal and distal stumps are free of tumor. Occasionally access to a deep lobe tumor is obtained via a median mandibulotomy procedure (splitting the mandible and reflecting the ipsilateral hemimandible laterally to expose the parapharyngeal space); the superficial lobe is conserved.

The submandibular and sublingual glands are removed in their entirety. If tumor is encountered in the deep lobe of the submandibular gland, the dissection can be continued anteriorly into the floor of mouth, including the sublingual gland and all of Wharton's duct, if required. En bloc resection of either gland with adjacent tissues as required is performed for widely infiltrating malignant tumors.

17.6 Surgical Pathology Specimens: Laboratory Aspects

17.6.1 Small Biopsy Specimens of Minor Glands

Excisions of mucoceles:

A single tissue nodule, free-floating in fixative, non-orientated. Usually less than 20 mm in diameter and may include overlying mucosa. Adjacent minor glands may be present. Measure. Bisect; submit in total.

Specimens from marsupialized ranulas represent floor of mouth mucosa. Measure as a mucosal specimen, bisect, and submit in total.

Sampling of Minor Glands for Xerostomia

Several small tissue nodules free-floating in fixative, non-orientated. Count number of glands (minimum of six glands recommended for useful assessment of focal lymphocytic sialadenitis). Measure largest in three dimensions; submit in total.

17.6.2 Resection Specimens

17.6.2.1 Parotidectomy and Submandibulectomy Specimens

Most superficial and total parotidectomy specimens are submitted for neoplastic conditions, although less often the gland may be removed because of persistent infections. In total parotidectomy specimens where the facial nerve has been preserved, the superficial and deep lobes will be separate.

Submandibulectomy

Most submandibulectomy specimens are removed for calculus/obstructive sialadenitis.

Procedure:

Orientate the specimen. The medial aspect is usually the most critical margin. The surgeon should give some indications on the request form, but it can still be difficult, particularly with fragmented specimens. Superficial parotidectomy specimens usually resemble an isosceles triangle; the smoothest surface will represent the superficial aspect and the shortest side, the superior aspect of the gland. The markings of the mandibular ramus and/or mastoid process may be present. If separate from the superficial portion, the deep lobe may be impossible to orientate. The superficial lobe of the submandibular gland has a smooth capsular surface, while the irregular edge and the indentation of mylohyoid identify the deep aspect.

Ink sparingly and allow to dry fully – gelatinous pleomorphic adenomas often separate easily from the thin capsule and overrun of ink will overestimate involvement of the surgical limits.

Measurements:

- Dimensions of specimen(s) (cm)
- Weight(s) (g)
- Dimensions of tumor (cm)
- Distance (cm) to closest margin

Description:

Tumor

- Location (deep lobe or superficial lobe)
- Consistency of tumor (solid/cystic; gelatinous, fleshy, firm)
- Interface with adjacent parenchyma (encapsulated, circumscribed, or infiltrative margin)
- If encapsulated, proportion of capsule exposed on outer surface of specimen

Gland

- Parenchyma – normal, fibrotic, or fatty

Other

- Note the presence of stones and lymph nodes

Blocks for histology:

- One block per centimeter diameter of tumor
- Closest margin
- Adjacent uninvolved parenchyma
- Lymph nodes

17.6.2.2 Minor gland excisions for tumor

50% of minor gland lesions will be malignant, although most of these will be of low histological grade. Usually received as free-floating specimens in formalin.

Procedure:

Orientate the specimen. The deep aspect is usually the most critical margin. The surgeon should give some indications on the request form as to laterality and anterior–posterior orientation but it can still be difficult particularly with specimens from lip or buccal mucosa. The color and texture of the mucosa from the hard palate may help orientate palatal resections.

Ink sparingly and allow to dry fully.

Measurements:

- Dimensions of mucosa and depth of specimen (cm)
- Dimensions of tumor (cm)
- Distance to closest mucosal and deep margins (cm)

Description:

Tumor

- Location
- Consistency of tumor (solid/cystic; gelatinous, fleshy, firm)
- Interface with adjacent parenchyma (encapsulated, circumscribed, or infiltrative margin)

Mucosa

- Intact or ulcerated?

Other

- Appearances of adjacent minor glands and neurovascular bundles

Blocks for histology:

The histology should represent the deepest extent of the tumor, the relationship to the adjacent structures or organs, mucosal and deep soft

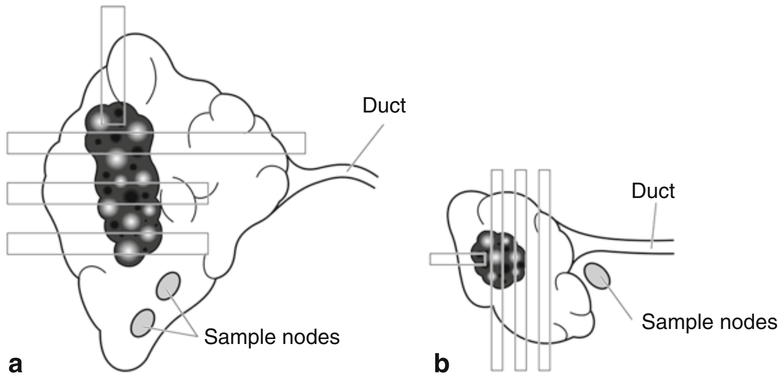

Fig. 17.1 Resection of parotid and submandibular salivary glands Recommended siting and orientation of blocks for resection of parotid (**a**) and submandibular (**b**) glands (Reproduced, with permission, from Allen and Cameron (2004))

tissue margins, and changes in adjacent tissues (Fig. 17.1).

- One block per centimeter diameter of tumor
- Closest mucosal margin (if appropriate)
- Closest deep margin
- Adjacent uninvolved mucosa and glands
- Proximal (and distal, if relevant) nerve limits

Histopathology report:

Final reports of resection specimens of tumor should include details on:

- Specimen type
- Type of tumor present (and grade if relevant)
- Distance of the tumor from the nearest cutaneous/mucosal margin (if appropriate)
- Distance of the tumor from the nearest deep margin
- Presence or absence of perineural and vascular invasion
- Presence or absence of lymph node metastasis (if appropriate)
- TNM classification of tumor spread of salivary glands

pT1	Tumor <2 cm, without extraparenchymal extension[a]
pT2	Tumor >2–4 cm, without extraparenchymal extension[a]
pT3	Tumor >4 cm, and/or with extraparenchymal extension[a]
pT4	Tumor invades skin, mandible, ear canal, facial nerve, base of skull, pterygoid Plates or encases carotid artery

[a]Extraparenchymal extension is clinical or macroscopic evidence of invasion of soft tissues or nerve, except those listed under pT4. Microscopic evidence alone is not sufficient

Regional lymph nodes

pN0	No regional lymph nodes involved
pN1	Metastasis in a ipsilateral single node <3 cm diameter
pN2	Metastasis in: (a) Ipsilateral single >3–6 cm (b) Ipsilateral multiple <6 cm (c) Bilateral or contralateral <6 cm
pN3	Metastasis in a lymph node >6 cm diameter

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18 Thyroid Gland

Moyra Gray and Seamus S. Napier

18.1 Anatomy

The thyroid gland lies in the lower part of the anterior neck, partly enveloping the larynx and upper trachea (Fig. 18.1). It is composed of right and left *lobes*, interconnected by a narrower *isthmus*, from which may occasionally arise the *pyramidal lobe*. The entire gland normally weighs 15–30 g, each roughly conical lobe measuring approximately 4 × 3 × 2 cm. The lower pole of each lobe is usually located at the level of the third or fourth tracheal cartilage with the upper pole ascending and diverging laterally to lie close to the superior aspect of the lamina of the thyroid cartilage. Medially, each thyroid lobe is related to the thyroid cartilage, the cricoid cartilage, and the upper tracheal cartilages, anteriorly to the strap muscles of the neck and posterolaterally to the carotid sheath. The isthmus is variable in size, usually measuring about 1 cm in length and width and lies over the trachea inferior to the cricoid cartilage. The pyramidal lobe, when present, ascends from the isthmus along the line of the thyroglossal duct and probably represents colonization of that embryonic structure by thyroid cells during the descent of the developing organ in early life from the foramen cecum in the tongue.

Histologically, the thyroid gland is composed of follicles of cuboidal epithelial cells surrounding eosinophilic colloid, arranged in small pear-shaped lobules supported by delicate fibrovascular stroma. C-cells that secrete calcitonin are dispersed throughout the gland singly or in small clusters but are most numerous around the junction of the upper and middle one-thirds of the lateral lobes. They are usually inconspicuous on routine stains but can be identified with immunohistochemical stains lying within the follicles. Other lesser components of the thyroid gland include solid cell nests (remnants of the ultimobranchial body), thymic tissue, and paraganglia; occasionally parathyroid glands may become incorporated within the thyroid.

Lymphovascular drainage:

The rich lymphatic supply of the thyroid gland drains to lymph nodes in the central and lateral compartments of the neck, located in pretracheal, paratracheal, prelaryngeal, retropharyngeal, and retro-esophageal sites and in the deep cervical chain (see Fig. 20.1).

18.2 Clinical Presentation

Disease affecting the thyroid gland can present as enlargement (called *goitre*) that may be diffuse or nodular or as a consequence of hormonal imbalance; rarely there may be pain. Hypothyroidism is characterized by lethargy, mental slowness or

M. Gray (✉) • S.S. Napier
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: moyra.gray@belfasttrust.hscni.net; seamus.napier@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_18, © Springer-Verlag London 2013

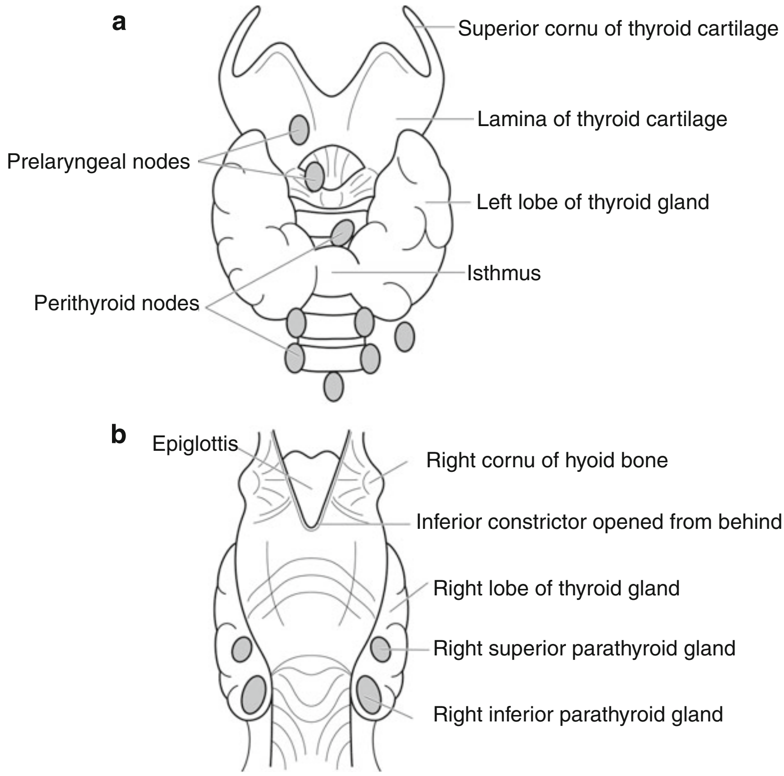

Fig. 18.1 Thyroid gland and parathyroid glands. (**a**) View of thyroid gland from anterior aspect to show relation to larynx and regional lymph nodes; (**b**) view of thyroid gland from posterior aspect to show location of parathyroid glands (Reproduced, with permission, from Allen and Cameron (2004))

depression, intolerance of cold, or weight gain, while thyrotoxicosis manifests as intolerance of heat, excessive sweating, weight loss in spite of increased appetite, anxiety, tiredness, and, occasionally, cardiac arrhythmias.

Tumors of the thyroid gland usually present with a solitary nodule with normal thyroid function, although some tumors can secrete hormones. Occasionally metastasis to cervical lymph nodes or bone may represent the initial symptom of differentiated thyroid cancer. High-grade cancers can present with hoarseness, dysphagia, or difficulty breathing.

18.3 Clinical Investigations

- Thyroid function is routinely assessed by measuring blood levels of thyroid stimulating hormone (TSH) and, if appropriate, the levels of thyroxine and triiodothyronine in patients with thyroid gland disease, including those with neoplastic conditions. Occasionally, calcitonin levels are measured. Elevated plasma thyroglobulin (and calcitonin in medullary thyroid carcinoma) following ablative therapy for malignant disease can indicate recurrence or metastasis. Autoantibodies to thyroglobulin, microsomal antigen, and the TSH receptor may also be evaluated.
- Ultrasonography is helpful in distinguishing a solitary nodule from a multinodular goitre with a so-called dominant nodule but cannot differentiate benign from malignant disease. Plain radiographs of the neck and chest may demonstrate deviation of the trachea, mediastinal expansion, or lymphadenopathy, although they are more accurately determined by CT and MRI scanning. Scintiscanning, particularly with Iodine-123 rather than Technetium-99m, can determine the functional status of the tissue. Functioning or “hot” nodules are very unlikely to be malignant.
- FNA is the investigation of choice for thyroid enlargement, particularly for solitary nodules in euthyroid patients or when there is a history

of a rapidly growing mass with airway obstruction. A definitive diagnosis is often possible with FNA, although the distinction between a cellular colloid nodule, follicular adenoma, and a follicular carcinoma is generally impossible.

- Assessment of vocal cord function is important in the clinical assessment of patients with goitre; vocal cord paralysis is a sinister finding.

18.4 Pathological Conditions

18.4.1 Non-neoplastic Conditions

Graves' disease: The commonest cause of hyperthyroidism, and is characterized by thyrotoxicosis, a diffuse goitre, ocular signs, and pretibial myxoedema. It affects females much more often than males and usually presents between the ages of 20 and 40 years. It is an autoimmune disease; the thyrotoxicosis is due to activation of the TSH receptor when the autoantibodies bind to it. Typically, the gland is symmetrically and diffusely enlarged, weighing between 50 and 150 g. Histologically the lobular architecture is preserved. There is reduced colloid in the small irregular follicles, which are lined by tall active-looking columnar cells. Simple non-branching papillary infoldings are often present.

Hashimoto's thyroiditis: A common cause of hypothyroidism and characterized by hypothyroidism and firm diffuse goitre. It affects females much more often than males and usually presents between the ages of 30 and 50 years. It is an autoimmune disease; the hypothyroidism is probably due to a combination of inactivation of the TSH receptor by autoantibodies and destruction of functioning gland. A proportion of patients may be euthyroid or hyperthyroid at presentation, but hypothyroidism is inevitable. The gland is symmetrically and diffusely enlarged, weighing between 50 and 100 g. Histologically there is diffuse lymphoplasmacytic infiltration with germinal center formation. The follicles are small and sparse with reduced or absent colloid. Hürthle cell change is widespread. A rare variant shows much fibrosis, affecting older patients and often males.

Multinodular goitre: A common cause of thyroid gland enlargement. Probably the end result of persistent stimulation of the glandular epithelium to proliferate and synthesize colloid through the TSH-negative feedback loop, due to dietary deficiency of iodine, ingested *goitrogens* that interfere with the availability of iodine or an enzymatic defect, alone or in combination. Patients are generally euthyroid but, when severe, hypothyroidism will result; occasionally late-stage disease will be associated with a mild degree of hyperthyroidism. Congenital enzymatic deficiencies (*dyshormonogenic goitre*) present in early childhood, marked dietary iodine deficiency or goitrogen ingestion is seen in certain geographical regions (*endemic goitre*) affecting adolescents, while sporadic (or *non-endemic*) goitre is rarely detected until much later in life. Initially in all forms there is a simple diffuse goitre, but gradually the gland becomes nodular as a consequence of follicle rupture, inflammation, and fibrosis with re-epithelialization due to the persistent stimulation. Macroscopically, multinodular goitres are characterized by massive enlargement of the gland with heterogeneous nodularity, histology revealing follicles distended by colloid, old and recent hemorrhage, and irregular areas of scarring with calcification.

Others: Ectopic or accessory thyroid gland tissue, multifocal granulomatous thyroiditis (due to vigorous palpation of the gland), tuberculosis, sarcoidosis, de Quervain's thyroiditis, radiation changes, Reidel's thyroiditis (a form of systemic fibrosing disorder).

18.4.2 Neoplastic Conditions

Follicular adenoma: Expansile round lesion 1–3 cm in diameter with thin complete capsule. Soft and fleshy, pale or brown in color; hemorrhage, fibrosis, cyst formation, or calcification may be present. Uniform pattern of growth; follicles of similar sizes (in contrast to hyperplastic nodules). Embryonal, microfollicular, normofollicular, or macrofollicular subtypes. No invasion of capsule. Variants include Hürthle cell adenoma and hyalinizing trabecular adenoma.

Thyroid cancer: Risk factors for thyroid carcinoma include irradiation (particularly in first two decades of life), underlying thyroid disease (especially Hashimoto's thyroiditis), family history of thyroid cancer including rare inherited syndromes such as Multiple Endocrine Neoplasia (MEN) syndromes 2A and 2B, or non-MEN familial medullary thyroid carcinoma.

Thyroid carcinomas are classified into three broad types "differentiated thyroid cancer," medullary thyroid carcinoma, and anaplastic carcinoma. *Differentiated thyroid cancer* encompasses papillary carcinoma, follicular carcinoma, and their variants.

Papillary carcinoma: Commonest thyroid malignancy, F:M = 3:1, 20–50 years. A few cases arise against a background of familial adenomatous polyposis syndromes. Very variable macroscopic appearances from tiny grey-white foci to tumors replacing the entire gland, but many are 2–3 cm diameter, white, firm, granular, infiltrative masses. Cystic degeneration is common, especially in nodal metastases. It has characteristic nuclear features of enlargement, optical clarity, grooving, and cytoplasmic pseudoinclusions. True papillary processes with fibrovascular cores are common but not required for the diagnosis. Psammoma bodies are seen in 50%. Multiple foci of papillary carcinoma are found in both lobes in 20% of cases, probably representing intraglandular lymphatic metastases.

A papillary microcarcinoma (≤10 mm in diameter) discovered incidentally is not thought to have a significant risk of recurrence or metastasis and is not an indication for further surgery.

The follicular variant of papillary carcinoma, especially if encapsulated, has a very low metastatic potential. Tall cell and columnar variants should be recognized as these are thought to show more aggressive behavior. Oncocytic and diffuse sclerosing variants are also recognized but the prognostic significance is not clear.

Follicular carcinoma: Second commonest thyroid malignancy accounting for 15% of primary tumors, 30–60 years, F:M = 3:1. Similar macroscopic features to follicular adenomas but the capsule is thicker. Key diagnostic features are full-thickness capsular penetration and vascular invasion. Tumors with only capsular invasion have minimal risk of metastasis.

Vascular invasion indicates a higher risk, increasing with the frequency of invasion. Those with multiple areas of capsular and vascular invasion are termed "widely invasive follicular carcinoma," may metastasise to bone and lung and have a variable prognosis. Oncocytic (or Hürthle) cell carcinoma, a variant of follicular carcinoma, tends to have a poorer prognosis.

Both of these tumors may have a dedifferentiated or poorly differentiated component. It is important to identify this as it will have an adverse effect on outcome. The dedifferentiated component may have features of a papillary or a follicular carcinoma and can be classified as such. Others will have a purely insular pattern and are referred to as poorly differentiated carcinoma. These are part of a spectrum between differentiated and anaplastic tumors.

Patients with differentiated thyroid cancer are stratified according to a number of factors related to the risk of recurrence or metastasis, summarized by the acronym GASH (gender age stage histology) (see Table 18.1).

Medullary carcinoma: A neuroendocrine malignancy with a pattern of C-cell differentiation accounting for 5–10% of thyroid neoplasms. About 25% of cases of medullary thyroid carcinoma are familial and arise against a background of MEN syndromes 2A and 2B or as a pure

Table 18.1 GASH risk assessment of patients with differentiated thyroid cancers

Risk category	Criteria
Low-risk patients	Females less than 45 years of age
High-risk patients	All patients under 16 years of age All males Females over 45 years of age
Low-risk tumors	Papillary carcinoma less than 1 cm in diameter Minimally invasive follicular carcinoma less than 1 cm in diameter
High-risk tumors	Papillary or follicular carcinoma greater than 1 cm in diameter Multifocal neoplasms. Metastasis to regional nodes or beyond

familial form but most are sporadic. New cases should undergo testing for RET mutations. Macroscopically, it is tan-colored or pink, may feel soft, and is usually well circumscribed but not encapsulated. There are a number of histological patterns (solid, nested, trabecular) and cell types (spindle, clear, granular); amyloid is present in 80%. C cell hyperplasia may be present in the background. Metastasis to regional lymph nodes occurs particularly with larger tumors, and spread to lung, liver, or bone may occur. The prognosis is linked to stage; involvement of soft tissues in the neck and regional nodes usually indicate reduced survival.

Anaplastic carcinoma: A rare thyroid malignancy that arises in older patients. Presents as a rapidly enlarging mass (may be history of goitre or a preexisting nodule) with hoarseness, dyspnea, or dysphagia. Very large pale fleshy tumor that infiltrates widely in the neck; may have foci of hemorrhage and necrosis. Histological patterns include osteoclastic, carcinosarcoma, lymphoepithelial, and paucicellular types. There is marked nuclear pleomorphism and a high mitotic count; vascular invasion is usually present. The prognosis is poor, most patients die from local tumor growth in spite of external beam radiotherapy. Metastasis occurs frequently in regional lymph nodes and beyond.

Primary malignant lymphoma: A rare neoplasm in the thyroid gland (1–2%), presenting in elderly patients with a rapidly enlarging mass, stridor, and dysphagia. There is a strong association with preexisting Hashimoto's thyroiditis (MALToma). The tumor is large and replaces much of the thyroid gland. Diffuse large B-cell lymphoma may arise without a previous low-grade lesion. The tumor cells infiltrate between follicles, produce lymphoepithelial lesions, and extend into the perithyroid soft tissues. In contrast to anaplastic carcinoma, it responds well to radiotherapy and chemotherapy, although the long-term prognosis depends on the stage.

Others: "Pure" squamous cell carcinoma, paraganglioma, small cell neuroendocrine carcinoma, sarcoma, thymic tumors, carcinomas showing thymus-like differentiation (CASTLE), mucoepidermoid carcinoma, metastatic carcinoma.

18.5 Surgical Pathology Specimens: Clinical Aspects

18.5.1 Biopsy Specimens

Open incisional biopsy is rarely performed on the thyroid gland. Core needle biopsy can provide a tissue diagnosis where the differential diagnosis rests between anaplastic carcinoma and malignant lymphoma. Very occasionally, incisional biopsy and intraoperative frozen section assessment may be required when an inoperable thyroid mass is encountered.

18.5.2 Resection Specimens

Total thyroidectomy is carried out for treatment of Grave's disease when surgery is required. On the few occasions when operation is required for aesthetics and/or obstructive symptoms in Hashimoto's thyroiditis, total thyroidectomy is the operation of choice. Likewise, total thyroidectomy may be required for patients with multinodular goitre because of goitre size and/or compressive symptoms; if unilateral, lobectomy may suffice.

Lobectomy with resection of the isthmus in continuity represents the minimum appropriate surgical procedure in any patient with a solitary thyroid nodule, particularly when there is suspicion of a follicular neoplasm on FNA. When a firm preoperative diagnosis of differentiated thyroid malignancy is made, total thyroidectomy is performed. Subtotal resection represents adequate surgery for follicular adenomas, the low-risk papillary carcinomas, and minimally invasive follicular carcinomas. Total thyroidectomy is preferred for high-risk tumors in high-risk patients. The intermediate group of low-risk patients with high-risk tumors or high-risk patients with low-risk tumors can be managed by either total thyroidectomy or lobectomy. Extensive differentiated thyroid carcinomas involving adjacent viscera may require laryngectomy, tracheal resection. and pharyngectomy. Medullary thyroid carcinoma is treated by total thyroidectomy.

Neck dissection is performed in differentiated and medullary tumors if there is clinically palpable nodal metastasis. Occasionally nodes from Level VI are removed even if there is no suspicion of metastasis.

18.6 Surgical Pathology Specimens: Laboratory Aspects

18.6.1 Biopsy Specimens

Usually as small samples from open biopsies or core needle specimens, free-floating in formalin. Measure in three dimensions or length of core and submit in total.

18.6.2 Resection Specimens

Specimen:

Most thyroid resections are performed for neoplastic disease, to prevent recurrence in Graves' disease or to relieve compressive symptoms from multinodular goitre or Hashimoto's thyroiditis. Some lobectomy specimens will represent diagnostic procedures for suspicious lesions with equivocal FNA findings. Only occasionally will specimens of neck dissection be included.

Initial procedure:

- Orientate the specimen, if possible
- Search for parathyroid glands
- Ink the external resection margins
- Slice into 4-mm-thick slices transversely in the coronal plane
- Measurements:
  - Weight of specimen (g)
  - Dimensions of specimen (cm)
  - Tumor size (cm)
  - Distance to closest surgical margins (cm)

Description:

- Tumor
  Number of tumor deposits
  Size, shape, and color
  Solid or cystic or both
  Encapsulated or infiltrative
- Capsule
  Present or absent
  Thick or thin
  Regular or irregular
  Intact or breached by tumor
- Adjacent gland
  Outer surface: smooth or roughened/breached by tumor
  Color and consistency
  Presence or absence of nodules
- Others
  Lymph nodes, neck dissection, parathyroid gland

Blocks for histology:

In cases of neoplastic disease, the histology should represent the tumor, its relationship to its own capsule (if any), the capsule of the thyroid gland, and adjacent structures (Fig. 18.2). Focal abnormalities of the thyroid parenchyma need to be sampled as do adjacent lymph nodes and the parathyroid glands.

In inflammatory or diffuse reactive disease, representative samples of gland and capsule are required.

Macroscopically encapsulated neoplastic disease:

- For tumors up to 5 cm in diameter, submit total circumference, the blocks illustrating the interface of tumor, capsule, and adjacent gland.
- For tumors greater than 5 cm in diameter, submit one additional block of tumor per centimeter diameter, blocks illustrating the interface of tumor, capsule, and adjacent gland.
- Closest surgical margin.
- Samples of other lesions, e.g., nodules or fibrous areas.
- Parathyroid gland(s).
- Perithyroid lymph node(s).

Macroscopically invasive neoplastic disease:

- Three blocks of tumor to illustrate interface with adjacent normal tissues
- Three blocks of adjacent gland
- Closest surgical margin
- Samples of other lesions, e.g., nodules or fibrous areas
- Parathyroid gland(s)
- Perithyroid lymph node(s)

Multinodular disease:

- One block from each nodule up to a maximum of five blocks.

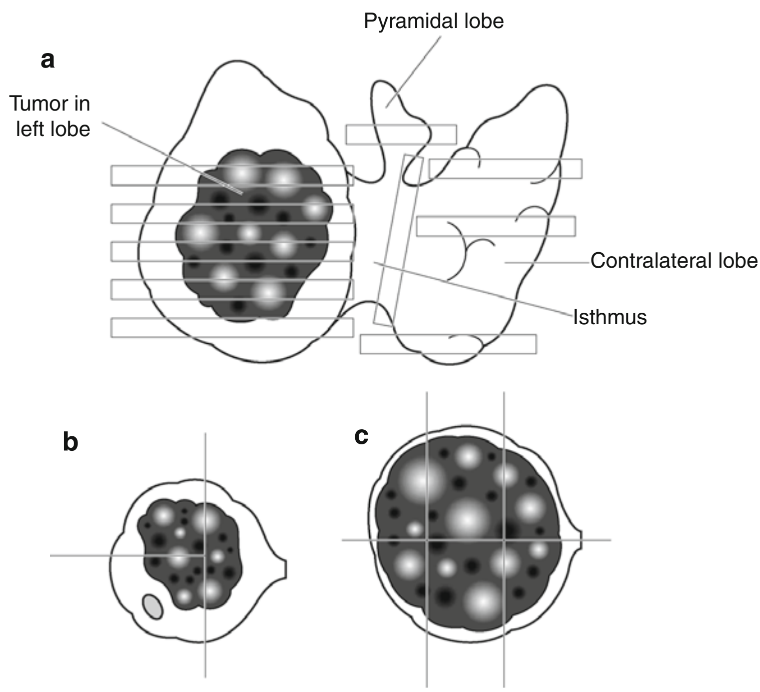

Fig. 18.2 Total thyroidectomy specimen. (**a**) Recommended block selection to include isthmus, pyramidal lobe, and contralateral lobe if present. Tissue block composites depend on the size of the tumor; (**b**) small; (**c**) large. Include other nodules (Reproduced, with permission, from Allen and Cameron (2004))

- For dominant nodules, submit one additional block of tumor per centimeter diameter, the blocks illustrating the interface of nodule and adjacent gland.

Inflammatory disease:

- Submit three representative blocks from each lobe and one block from the isthmus if present.

Histopathology report:

Final reports of thyroid specimens should include details on:

- Specimen type, side, size (cm), and weight (g)
- Type and subtype of tumor present, if any
  - Follicular adenoma
  - Papillary carcinoma and variants
  - Follicular carcinoma and variants
  - Medullary carcinoma
  - Anaplastic carcinoma
  - Lymphoma
- Macroscopic size of tumor and degree of encapsulation
- Presence or absence of invasion of the capsule and surrounding tissues
- Distance of tumor from the nearest margin
- Presence or absence of vascular invasion
- Involvement of perithyroid lymph nodes
- Other pathology such as Hashimoto's thyroiditis or radiation injury

  If other specimens are attached as an in-continuity dissection (e.g., neck dissection), these can be handled separately in the usual fashion.
- TNM 7 indicates that papillary microcarcinoma is classified separately as pT1a, emphasizing its clinically benign behavior.

pT1a	≤10 mm, limited to thyroid
pT1b	≤20 mm but >10 mm, limited to thyroid
pT2	>20 mm, ≤40 mm, limited to thyroid
pT3	>40 mm, limited to thyroid or any tumor with minimal extrathyroidal extension, e.g., extension to sternothyroid muscles or perithyroid soft tissues
pT4a	Tumor invades beyond thyroid capsule and invades any of: subcutaneous soft tissues, larynx, trachea, esophagus, recurrent laryngeal nerve
pT4b	Tumor invades prevertebral fascia, mediastinal vessels, or encases carotid artery

All anaplastic carcinomas are considered pT4 tumors

pT4a	Anaplastic carcinoma limited to thyroid
pT4b	Anaplastic carcinoma extends beyond thyroid capsule
pN0	No regional nodes involved
pN1a	Metastasis in level VI (pretracheal, paratracheal, and prelaryngeal/Delphian) Lymph nodes
pN1b	Metastasis in other unilateral, bilateral or contralateral cervical (levels I, II, III, IV, or V) or retropharyngeal or superior mediastinal lymph nodes

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Parathyroid Glands

19

Moyra Gray and Seamus S. Napier

19.1 Anatomy

There are usually four parathyroid glands, arranged as superior and inferior pairs on either side of the midline closely related to the thyroid gland (see Fig. 18.1). About 5% of people will have more than four glands. These tan-colored oval structures each normally measure 4–6 mm in maximum dimension and weigh around 50 mg. The combined weight should be between 120 and 140 mg. The superior parathyroid gland (derived from the fourth pharyngeal pouch) is fairly constant in position and lies on the posteromedial aspect of the superior thyroid pole. A few superior parathyroid glands are located medial to the upper pole or in the retropharyngeal or retroesophageal space. The inferior parathyroid glands, derived from the third pharyngeal pouch are less constant in location, although they tend to be symmetrical bilaterally. Over half are found around the inferior pole of the thyroid lobe, although other common locations include the thymus or high up on the anterior aspects of the thyroid lobe. Occasionally they may be located in the mediastinum or rarely in association with the roots of the great vessels.

Lymphovascular drainage:

The rich lymphatic supply of the parathyroid glands drains with that of the thyroid gland to lymph nodes in the anterior and lateral neck, located in the deep cervical chain as well as in pretracheal, paratracheal, prelaryngeal, retropharyngeal, and retro-esophageal sites (see Fig. 20.1).

19.2 Clinical Presentation

Disease affecting the parathyroid glands usually presents as a consequence of altered function. Hyperparathyroidism describes an altered metabolic state due to increased secretion of parathyroid hormone (parathormone) which usually manifests as disordered calcium metabolism. Rarely seen nowadays is the full spectrum of "bones, stones, groans, and moans"; biochemical investigation of nonspecific complaints such as profound tiredness, nausea, or thirst is the usual method of diagnosis, although a small proportion of cases are detected during investigation of patients with organ-specific complaints. *Primary hyperparathyroidism* is due to an increased secretion of parathormone from one or more of the parathyroid glands, usually caused by an adenoma. *Secondary hyperparathyroidism* is due to the physiological response of the four parathyroid glands to persistent hypocalcemia, usually renal failure, malabsorption syndromes, or Vitamin D deficiency. *Tertiary hyperparathyroidism* is a result of persistent autonomous hypersecretion of parathormone in long-standing

M. Gray (✉) • S.S. Napier
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: moyra.gray@belfasttrust.hscni.net; seamus.napier@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_19, © Springer-Verlag London 2013

secondary hyperparathyroidism following correction of the hypocalcemia.

Hypoparathyroidism is a result of reduced secretion of parathormone and is characterized by neuromuscular excitability. Rapid onset of hypoparathyroidism, e.g., following surgery to the neck, results in muscular tetany and paresthesia, while an insidious onset, such as with autoimmune disease, may induce mucocutaneous candidosis, cataracts, and basal ganglia changes as well.

Pseudohypoparathyroidism refers to a rare inherited defect of parathyroid hormone receptor function in peripheral tissue characterized by insensitivity to circulating parathormone; the glands are hyperplastic.

19.3 Clinical Investigations

- Biochemical tests are the mainstay of the diagnosis of parathyroid gland disease. In conjunction with the clinical history and physical examination, relative plasma concentrations of calcium, inorganic phosphate levels, and parathormone allow classification of hyperparathyroidism as primary, secondary, or tertiary. Alkaline phosphatase levels are elevated when there is increased osteoblastic activity as a result of bone resorption and stimulation of osteoblastic activity. Evaluation of autoantibodies is useful when autoimmune-associated hypoparathyroidism is suspected.
- Chest radiographs are helpful in excluding a paraneoplastic effect of bronchogenic carcinoma as a cause for the hyperparathyroidism. CT and MRI scanning may locate enlarged parathyroid glands in the neck, but currently the most reliable method is technetium labeled isotope scintigraphy (Technetium 99 sestamibi).

19.4 Pathological Conditions

19.4.1 Non-neoplastic Conditions

Primary chief cell hyperplasia: Usually accounts for 15% of cases of primary hyperparathyroidism, but is also seen against a background of secondary hyperparathyroidism. About 25% of cases of primary chief cell hyperplasia are familial and arise in multiple endocrine neoplasia (MEN) syndromes 1 and 2 or as an isolated familial form, but most are sporadic. The four parathyroid glands are symmetrically enlarged in around 50% of patients, while the asymmetric enlargement of the remainder mimics parathyroid adenoma (*pseudoadenomatous hyperplasia*). Microscopically, the glands contain numerous chief cells in diffuse sheets or as nodules with oncocytes and transitional forms also present. Mitotic figures may be present. Intraglandular adipocyte numbers are usually much reduced, although rarely the fat cells may be abundant (*lipohyperplasia*). Cystic change may occur in very large glands. Distinction from adenoma formation can be difficult, but the enlargement of multiple glands is usually diagnostic.

Primary clear cell hyperplasia: This is seen mostly in men in the fifth decade. The four parathyroid glands are markedly enlarged, particularly the superior glands. The chief cells are arranged in small nests and have profoundly clear cytoplasm ("water-clear" cells). There is usually marked hypercalcemia but water-clear cell hyperplasia is not MEN-associated.

Secondary parathyroid hyperplasia: Very similar appearances to primary chief cell hyperplasia, but there tends to be symmetrical enlargement, particularly in the early stages. Early changes include loss of the intraglandular adipocytes with conspicuous nests of chief cells but in long-standing cases, the glands usually have a marked nodular pattern. Oncocytes may be prominent in established cases; cystic change and fibrosis may develop.

Cysts: Usually arise due to degenerative changes in an adenoma or hyperplastic parathyroid gland but some are developmental anomalies of the third and fourth branchial arches. Cystic degeneration in an adenomatous or hyperplastic gland is usually associated with hyperparathyroidism; typical chief cells line the fibrous wall of the cyst. Developmental cysts tend not to be functional and are usually associated with the inferior parathyroid glands; these cysts are lined by respiratory or cuboidal epithelium with parathyroid cells in the fibrous wall.

19.4.2 Neoplastic Conditions

Adenoma: This accounts for 80% of cases of primary hyperparathyroidism. It is a benign tumor of the parathyroid glands with a F:M ratio of 3:1, mostly affecting patients aged 40–60 years. Very rarely may arise in MEN syndromes 1 and 2. Usually only one gland is affected and it may be located either in the neck or at an ectopic site. Usually a tan-colored circumscribed nodule; large tumors may be cystic. Microscopically, it is composed of chief cells in cords and nests with occasional gland-like structures; neoplastic chief cells are larger than their normal counterparts. Variable numbers of oncocytic cells are present in clusters. Nuclear pleomorphism is common and is probably a degenerative phenomenon. Fibrosis is not common but may be present if there has been previous hemorrhage. Correlation of surgical and pathological findings is required to distinguish adenoma from hyperplasia; the presence of one enlarged gland usually signifies an adenoma. A rim of compressed normal or atrophic parathyroid tissue may be seen in around 50% but is less commonly seen in larger lesions.

Double adenoma: Very rare and requires the presence of two enlarged glands (each weighing more than 70 mg) and two normal sized glands; MEN 1 syndrome; may be impossible to distinguish from hyperplasia.

Variants include microadenoma (<6 mm diameter), oncocytic adenoma, lipoadenoma.

Carcinoma: A rare cause of primary hyperparathyroidism (0.5–5.2%). Patients are usually older than those with adenomas and often have very high levels of parathormone secretion. Usually pale solid tumor; may be encapsulated but often infiltrates adjacent soft tissues. Microscopically the lesion is composed of chief cells arranged in a solid or trabecular pattern with thick fibrous bands, numerous mitotic figures, and capsular invasion, but these changes may also be present in a proportion of adenomas. Invasion of nerves, blood vessels, and adjacent soft tissues are more reliable features of malignancy. Local recurrence and hypercalcemia are the main problems; metastasis to lymph nodes or to lung and liver occur in a third of cases.

19.5 Surgical Pathology Specimens: Clinical Aspects

19.5.1 Biopsy Specimens

Incisional biopsy is rarely performed for parathyroid disease, although intraoperative frozen section analysis may be required to establish that parathyroid tissue has been removed rather than a thyroid nodule or small lymph node.

19.5.2 Resection Specimens

Surgical exploration of the neck and parathyroidectomy is curative in most cases of primary and tertiary hyperparathyroidism. In primary hyperparathyroidism, it is usual for both parathyroid glands from the affected side to be removed to facilitate distinction between hyperplasia and adenoma. Subtotal parathyroidectomy is the treatment of choice for hyperplasia and for tertiary hyperparathyroidism. Approximately 100 mg of parathyroid tissue is left in the neck or transplanted into the patient's forearm.

Parathyroid carcinoma is usually diagnosed after excision of the affected gland. Recurrent disease is treated by en bloc resection and removal of the ipsilateral lobe of thyroid gland. Neck dissection is usually performed only if there is clinically palpable nodal metastasis.

19.6 Surgical Pathology Specimens: Laboratory Aspects

19.6.1 Resection Specimens

Specimen:

Most parathyroid resection procedures are performed for neoplastic disease or for primary or tertiary hyperplasia. Only when recurrent primary carcinomas are being resected will specimens of neck dissection be included.

Procedure:

- In cases of known or suspected parathyroid carcinoma, ink the external resection margins.

- Remove the surrounding fat.
- Slice into 4-mm-thick slices transversely in the coronal plane. If less than 5 mm in maximum dimension, submit in total.
- Measurements (after the removal of the periglandular fat):
  - Weight of specimen to three decimal places (g).
  - Dimensions of specimen (cm).
  - Tumor size (cm).

Description:
- Tumor
  Size and color
  Solid or cystic
  Encapsulated or infiltrative
- Adjacent gland
  Compressed rim of normal or suppressed parathyroid gland
- Other
  Neck dissection, thyroid gland resection; presence of other parathyroid glands

Blocks for histology:
- The histology should represent the abnormal parathyroid tissue, its relationship to its capsule (if any), and adjacent parathyroid gland parenchyma.
- Submit each parathyroid gland in total (maximum of three blocks for markedly enlarged glands).

Histopathology report:

Final reports of parathyroid specimens should include details on:
- Specimen location (right/left, superior/inferior), size and weight
- Type of tumor present, if any
- Macroscopic size of tumor and degree of encapsulation
- Presence or absence of invasion of capsule and surrounding tissues
- Distance of tumor from the nearest margin
- Presence or absence of vascular invasion
  If other specimens are attached as an in-continuity dissection (e.g., neck dissection), these can be handled separately in the usual fashion.

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20 Neck: Cysts, Tumors, and Dissections

Seamus S. Napier with Clinical Comments by Derek J. Gordon

20.1 Anatomy

The neck extends from the lower border of the mandible and the base of the skull superiorly to the thoracic inlet at the level of the clavicles inferiorly. Within this area are contained pharynx, larynx and esophagus, submandibular and the tail of the parotid salivary glands, bones, skeletal muscles, nerves, blood vessels, lymph nodes, and the thyroid and parathyroid glands. The side of the neck is divided by the sternocleidomastoid muscle, which passes obliquely across the neck from the sternum and clavicle below to the mastoid process and occipital bone above. The area in front of this muscle is called the *anterior triangle* and extends to the anterior midline of the neck. The area behind the muscle is called the *posterior triangle* and extends to the anterior margin of trapezius muscle behind (Fig. 20.1).

S.S. Napier (✉)
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital,
Belfast Health and Social Care Trust, Belfast, UK
e-mail: seamus.napier@belfasttrust.hscni.net

D.J. Gordon
Unit of Plastic Surgery, Ulster Hospital Dundonald, South Eastern Health and Social Care Trust,
Belfast, UK
e-mail: derek.gordon@setrust.hscni.net

The anterior triangle is divided into:

- The submental triangle, which lies between the anterior belly of digastric, the mandible, and the body of the hyoid bone
- The digastric triangle, which lies between the anterior and posterior bellies of digastric below and the lower border of the mandible above
- The carotid triangle, which lies between the superior belly of the omohyoid, the anterior border of sternocleidomastoid, and the stylohyoid and posterior belly of digastric muscle superiorly
- The muscular triangle, which extends from the hyoid bone to the sternum and is limited posteriorly by the superior belly of omohyoid

The posterior triangle of the neck is divided into:

- The occipital triangle lying between the anterior border of trapezius, the posterior border of sternocleidomastoid, and the inferior belly of omohyoid below
- The supraclavicular triangle lies between the inferior belly of omohyoid, the clavicle, and the lower part of the posterior border of sternocleidomastoid

Lymphovascular Drainage

The neck contains many lymph nodes subdivided into groups and located both superficially and deep within the neck. While individual nodes can be described with reference to adjacent anatomic structures, it is common practice, particularly in oncology, to divide the node groupings in the neck into six levels (Fig. 20.2).

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_20, © Springer-Verlag London 2013

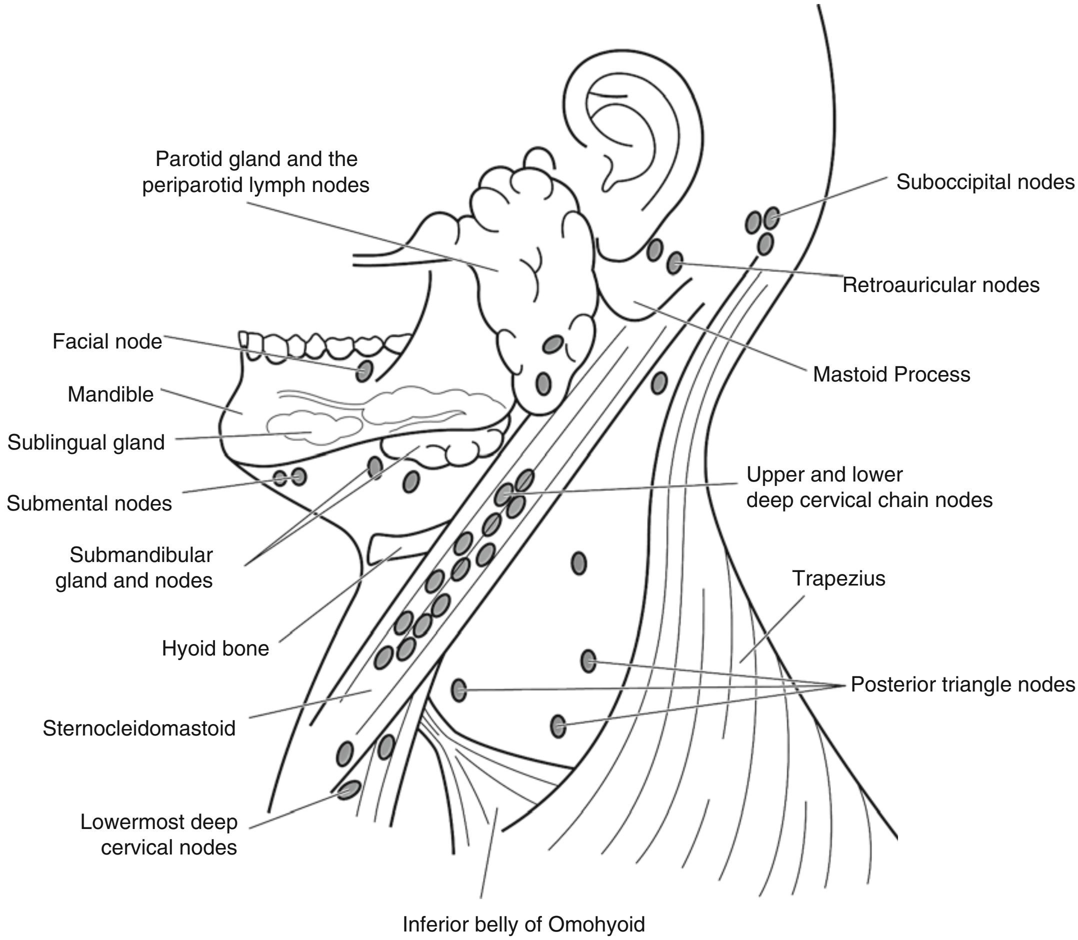

Fig. 20.1 Lymph node distribution in the lateral neck and the major salivary glands (Reproduced, with permission, from Allen and Cameron (2004))

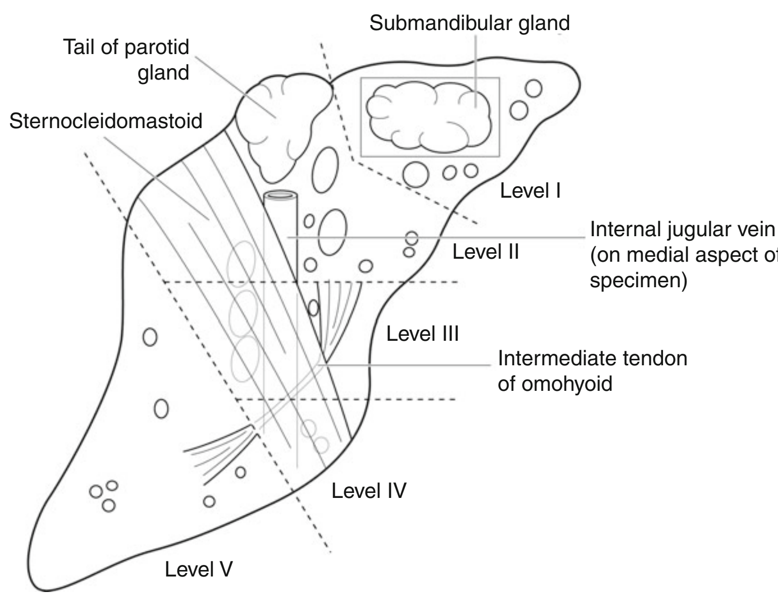

Fig. 20.2 Right radical neck dissection. *Dashed lines* indicate the boundaries of the cervical lymph node groups (Reproduced, with permission, from Allen and Cameron (2004))

Level I
This group consists of the nodes within the submental and digastric triangles and is also known as the submandibular group. In practice, the submandibular salivary gland is included in the specimen when lymph nodes in this level are resected.
Level II
Level II nodes represent the upper jugular group and consist of the nodes around the upper third of the internal jugular vein (IJV) and the adjacent spinal accessory (XIth) nerve. They extend from the level of the carotid bifurcation (approximating to the superior border of the thyroid cartilage) to the base of the skull. The posterior boundary of this group is the posterior border of the sternocleidomastoid muscle and the anterior boundary is the lateral border of the sternohyoid muscle. The tail of the parotid gland is often included when nodes from this group are resected.
Level III
This group of lymph nodes corresponds to the middle jugular group and consists of lymph nodes located around the middle third of the IJV. They extend from the carotid bifurcation to the intermediate tendon of omohyoid, where it crosses the IJV. The posterior boundary is the posterior border of the sternocleidomastoid muscle and the anterior boundary is the lateral border of the sternohyoid muscle.
Level IV
This group of lymph nodes, also known as the lower jugular group, consists of nodes located around the lower third of the IJV extending from the intermediate tendon of omohyoid where it crosses the IJV to the clavicle below. The posterior boundary is the posterior border of the sternocleidomastoid muscle, while the anterior boundary is the lateral border of the sternohyoid muscle. Lymph nodes within Levels II, III, and IV correspond to the jugular group or deep cervical chain of lymph nodes. They tend to be regarded as subdivisions of a functional unit rather than as distinct groups in their own right.
Level V
Lymph nodes in this group, also known as the posterior triangle group, comprise the lymph nodes located along the lower half of the spinal accessory (XIth) nerve and represent the lymph nodes in the occipital triangle. The anterior boundary is the posterior border of the sternocleidomastoid muscle; the posterior boundary is the anterior border of trapezius with the clavicle below.
Level VI
Lymph nodes in this group, also known as the anterior compartment group, comprise the nodes surrounding the midline structures of the neck extending from the level of the hyoid bone above to the suprasternal notch below. On each side, the lateral boundary is the medial border of the carotid sheath. Individual groups of lymph nodes within this compartment are the perithyroid nodes, the paratracheal nodes, and the precricoid nodes.

Other groups of lymph nodes within the neck are also recognized and include the suboccipital, periparotid, retropharyngeal groups, and the buccal lymph node.

20.2 Clinical Presentation

Lesions in the neck usually present as swellings and may be associated with any of the major anatomical structures in the region, in particular lymph nodes, thyroid gland, and salivary glands, or from other tissues such as skin, blood or lymphatic vessels, nerves or fat. Disease affecting lymph nodes usually presents as enlargement, affecting either a single lymph node or several nodes, unilaterally or bilaterally. The nodes may be tender or painless and may vary in consistency from soft to firm, rubbery or hard. In young patients, cervical lymphadenopathy is usually due to a reactive process but a neoplasm is more likely in older patients. Malignant lymphoma commonly presents as cervical lymphadenopathy; metastatic deposits in cervical lymph nodes may be the presenting feature of tumors in the posterior tongue, nasopharynx, tonsil, larynx, or thyroid gland. Metastases in cervical lymph nodes usually derive from primary lesions above the level of the clavicles, although 10% will arise from distant sites such as lung, stomach, or testis; the neck mass will be present on the left side (*Virchow's node*). Cystic lesions in young patients are usually due to developmental abnormalities, such as thyroglossal duct cysts or branchial cysts, while those in adults are most likely to represent metastasis to a lymph node in which there is cystic degeneration. Sinuses opening onto the skin surface may arise from thyroglossal duct cyst or branchial cyst lesions or infective lesions, e.g., related to the mandibular teeth.

20.3 Clinical Investigations

Swellings in the neck require thorough clinical evaluation to determine the tissue or organ affected, whether the enlargement is solid or cystic, and whether or not adjacent tissues are involved. Movement on swallowing tends to point to an intimate relationship with the hyoid bone or thyroid gland while pulsation or the detection of a *bruit* indicates association with or origin from major vascular structures. Tumors may involve adjacent nerves producing characteristic patterns of paralysis or altered sensation, such as Horner's syndrome or Trotter's syndrome. Ultrasound investigation is helpful in identification of cystic lesions and can provide information on the presence of other lesions in adjacent organs without the risks of ionizing radiation. Plain radiographs of the facial bones or sinuses may reveal clinically undetected lesions, while barium studies are useful in visualizing pharyngeal diverticula or in tracking developmental sinuses or fistulae. CT scanning and MR imaging can determine the consistency of the lesion, identify enlarged lymph nodes, and detect the presence of occult primary tumors in clinically silent anatomical sites. Scintiscans may be required if neoplastic or developmental lesions of the thyroid gland are suspected. Angiography will demonstrate the presence of a significant vascular component within a lesion and its relationship to adjacent vascular structures. Fine needle aspiration (FNA) cytology has greatly facilitated the investigation of neck lumps in recent years. Even if characteristic features allowing diagnosis are not present, FNA can distinguish cases where open biopsy can be performed effectively; ultrasound guidance allows precise sampling of deeply placed lesions. Endoscopic examination of the nasal cavities, pharynx, larynx, esophagus, and bronchi under general anesthesia with biopsy is commonly performed prior to definitive surgery for squamous cell carcinoma of the upper aerodigestive tract; an occult second primary tumor can occur in up to 10% of cases.

20.4 Pathological Conditions

A wide variety of diseases can account for enlargement of cervical lymph nodes. These may be reactive or neoplastic; they may be a consequence of local or systemic conditions and may or may not have a known cause. Conditions not related primarily to reactive disorders of lymph nodes or malignant lymphomas are discussed below.

20.4.1 Non-neoplastic Conditions

Thyroglossal duct cyst: Probably the commonest developmental neck cyst, due to failure of the embryonic thyroglossal duct (extending from the posterior tongue into the neck) to atrophy. Midline in 90%, below hyoid in 70%; associated with a sinus in 40% of cases. Lined by squamous and/or respiratory epithelium; less than half contain thyroid follicles and may represent the patient's only functioning thyroid tissue.

Branchial cleft cyst: Derived from remnants of the embryonic branchial apparatus following incomplete obliteration of the branchial pouches; the most common form is believed to derive from the second branchial pouch. Cyst lies in the lateral neck near the angle of the jaw at the anterior border of sternocleidomastoid; the sinus may open onto the skin at the junction of the middle one-third and lower one-third, while the tract follows the carotid sheath and may fistulate into the tonsillar fossa. Lined by squamous epithelium with reactive lymphoid tissue in the wall; 10% contain respiratory epithelium. Beware the cystic metastasis from an occult head and neck primary squamous cell carcinoma masquerading as a branchial cyst in the patient over 40 years of age.

Miscellaneous lesions: Other developmental cysts in the neck include dermoid cyst (often extending into the neck from the sublingual region), cervical thymic cyst, and cervical bronchial cyst. The "plunging ranula" is a mucous extravasation cyst from the sublingual gland that extends into the neck through mylohyoid. Cutaneous and subcutaneous hemangiomas are relatively common but do not differ from their counterparts elsewhere. Lymphangiomas are uncommon in the neck but usually arise low in the posterior triangle. Lesions composed of very dilated vessels can be termed cystic hygroma, although all forms are more usually described as "lymphatic malformations."

20.4.2 Neoplastic Conditions

Metastatic malignant tumor in cervical lymph nodes: Metastasis to regional lymph nodes from primary tumors elsewhere in the head and neck is common, particularly with mucosal squamous cell carcinoma, cutaneous malignant melanoma, and thyroid gland carcinoma. The frequency of lymph node involvement and the distribution of metastatic deposits vary with the site and type of the primary tumor. For example, nasopharyngeal carcinoma often involves multiple nodes throughout the neck, while squamous cell carcinoma of the lower lip rarely spreads to nodes and then usually only as a single deposit in Level I. Tumors of the anterior two-thirds of the tongue generally metastasize to one or two ipsilateral Level II/III nodes, while carcinomas from the posterior one-third of the tongue, hypopharynx, or larynx can involve several nodes on both sides of the neck. Cervical lymph node metastasis is not just reserved for head and neck primaries; tumors of lung or upper gastrointestinal tract can occasionally spread to nodes in the supraclavicular region. Lymph node metastasis may be detected either at the time of presentation with the primary lesion or after initial therapy, although in about one-third of patients, the cervical lymph node deposit is the presenting feature. Usually the primary tumor is located following clinical/endoscopic examination or imaging but may not be found in 10% of cases. Over 80% of occult primary tumors presenting with cervical metastasis are located in the head and neck, especially nasopharynx, posterior one-third of tongue, tonsil, hypopharynx, and thyroid gland. Adverse prognostic features can vary with the type of tumor but include the presence of multiple tumor deposits particularly in Levels IV and V, extracapsular spread into adjacent tissues, involvement of extranodal lymphatic channels, and tumor involvement of surgical margins.

Paraganglioma: An uncommon neuroendocrine tumor arising at sites of autonomic paraganglia within the head and neck. Commonest in the bifurcation of the common carotid artery (carotid body paraganglioma or *chemodactoma*); others include the superior bulb of the jugular vein (*glomus jugulare*), the promontory of middle ear (*glomus tympanicum*), the ganglion nodosum of the vagus nerve, and the larynx. Carotid body paraganglioma affects males and females equally, although the others are more common in females. Usually adults; 10% bilateral or multicentric, 10% familial or associated with syndromes such as neurofibromatosis or Multiple Endocrine Neoplasia syndrome; 10% recur; 10% malignant. Slowly growing painless mass; may evoke neural symptoms such as hoarseness, conductive deafness, or an intracranial mass effect. Characteristic histology of discrete cell nests (*Zellballen*) of polygonal endocrine chief cells and spindle-shaped neural sustentacular cells. Neither nuclear pleomorphism nor the presence of mitotic figures signifies malignancy; rather markedly infiltrative growth pattern and/or metastasis required. The intimate relationship to adjacent vital structures makes wide excision impossible with recurrence likely.

20.5 Surgical Pathology Specimens: Clinical Aspects

20.5.1 Biopsy Specimens

A lymph node in the neck may be excised for histopathological assessment when persistently enlarged and when there is no clear reactive cause. In most cases, FNA has indicated the presence of a lymphoproliferative disorder that might represent lymphoma. Exclusion of metastatic squamous cell carcinoma is important; definitive treatment of the neck following such inadvertent open biopsy with possible skin contamination is more extensive than might otherwise be required.

20.5.2 Resection Specimens

Excision of a thyroglossal duct cyst requires clearance of the entire thyroglossal tract from the base of tongue to the isthmus of the thyroid and perhaps beyond; the central portion of the hyoid bone may be included. The risk of recurrence of the cyst is much reduced by this procedure.

Treatment of a branchial cyst requires removal of the entire lesion; fibrosis following infection, and intimate relationships to carotid sheath and a number of large nerves in the neck makes the procedure difficult.

Neck dissection is either elective (clinically negative neck) or therapeutic (known metastasis). Justification for an *elective neck dissection* rests on three observations: occult disease will develop into clinically evident disease, sometimes inoperable when eventually detected; there is a risk of distant metastasis with untreated occult neck metastasis; and additional histological information of prognostic value may be gained. Arguments against elective neck dissection include unnecessary treatment when there is a low risk of metastasis and significant morbidity and a risk of mortality in elective surgery. The decision to perform an elective neck dissection is based on a risk of metastasis of more than 20%, whether or not the neck nodes can be easily assessed clinically, the availability of the patient for close follow-up, and the fitness of the patient for surgery. Sentinel node sampling is not used widely as a technique to identify occult metastasis in head and neck cancers given the vagaries of lymphatic drainage in the neck and the requirement for considerable pathological expertise for accurate interpretation. Elective irradiation of the neck may be an acceptable alternative to a “watch and wait” policy.

Usually, the less extensive neck dissection procedures are the operations of choice in the clinically negative neck. The choice depends on the nature and site of the primary tumor and the expected pattern of nodal spread. Nodes in Levels I–IV are removed for oral and oropharyngeal tumors, Levels II and III for laryngeal and hypopharyngeal tumors, but elective dissection is rare for thyroid carcinomas. Bilateral dissection may be indicated if the primary tumor crosses the midline.

The rationale of *therapeutic neck dissection* is the clearance of disease with preservation of function. Classical radical neck dissection is performed for nodal metastasis >6 cm in diameter, multiple large metastatic deposits in several levels, recurrent disease following neck irradiation, gross extranodal spread involving the skull base, and skin involvement. Other so-called functional dissections may be performed to preserve the spinal accessory nerve, sternocleidomastoid, and internal jugular vein, consistent with achieving clearance.

Two or more positive nodes following histological examination of the specimen and/or the presence of extracapsular spread merit postoperative radiotherapy.

20.6 Surgical Pathology Specimens: Laboratory Aspects

20.6.1 Biopsy Specimens

20.6.1.1 Thyroglossal Duct Cysts

Usually as a single strip of fibrous tissue with or without a nodule surrounded by fat free-floating in fixative, non-orientated; the body of the hyoid may be present. Measure in three dimensions. If 10 mm in length or less, submit in total. If larger, block serially and submit representative samples.

20.6.1.2 Branchial Cleft Cysts

Usually as a single cystic nodule surrounded by fat free-floating in fixative, non-orientated. A sample of adjacent lymph nodes may be present. Measure in three dimensions. If 10 mm in diameter or less, submit in total. If larger, block serially and submit representative samples. If the patient is aged over 40 years, block serially and submit in total.

Table 20.1 Categories and components of neck dissection specimens

Neck dissection specimens	Lymph node groups	Other structures present
Comprehensive		
Radical	Levels I–V	SCM, IJV and XIth nerve
Modified radical	Levels I–V	Variable; one or all will be lacking
Extended radical	Levels I–V	Other lymph node groups or non-lymphatic structures
Selective		
Suprahyoid	Levels I–II	Variable, one or all will be lacking
Supraomohyoid	Levels I–III	Variable, one or all will be lacking
Anterolateral neck	Levels II–IV	Variable, one or all will be lacking
Posterolateral neck	Levels II–V and suboccipital nodes	Variable, one or all will be lacking
Anterior compartment	Level VI	Variable, one or all will be lacking

20.6.2 Resection Specimens

20.6.2.1 Resections of Carotid Body Paraganglioma

Usually received as non-orientated soft tissue mass in formalin. Larger specimens may be fragmented.

Procedure:

- Ink the external limits of the specimen.
- Measurements:
  - Dimensions of specimen (cm)
  - Weight (g)
  - Tumor maximum dimensions (cm)
  - Distance (cm) to the closest soft tissue limits

Description:

- Tumor
  Color; areas of hemorrhage or necrosis (preoperative embolization?)
  Circumscribed, encapsulated, or infiltrative margin
- Other
  Vessels or nerves identifiable

Blocks for histology:

- The histology should represent the tumor and the relationship to the margins, vessels and/or nerves.
- Section the specimen into 4 mm thick slices and sample the tumor and margins.
- Select at least one block of tumor per cm diameter to represent the various morphological patterns present.

Histopathology report:

Final paraganglioma reports should include details on:

- The type of tumor present
- The nature of the tumor–tissue interface
- The relationship to major vessels and nerves
- The distance of tumor from the nearest margin

20.6.2.2 Neck Dissection Specimen

The vast majority of neck dissection specimens are submitted for neoplastic conditions, although occasionally nodes from Level I may be removed to facilitate access to vessels for microvascular anastomosis during reconstructive jaw surgery. Neck dissection specimens may be classified as "comprehensive" neck dissection specimens (radical or modified radical neck dissections) or selective neck dissection specimens (e.g., suprahyoid or supraomohyoid neck dissection specimens). These are listed in Table 20.1.

Procedure:

- Orientate the specimen. The surgeon should give some indications on the request form but it can still be difficult particularly with selective dissections, for example, of Levels II and III only. Figure 20.2 and the following anatomical landmarks may assist:
  - The superior aspect: Submandibular gland, mandibular periosteum, tail of parotid gland, posterior belly of digastric

  - The inferior aspect: Intermediate tendon of omohyoid where it crosses the IJV; the fatty tissues from the posterior triangle region (Level V) tend to be bulkier inferiorly
  - The medial aspect: The IJV running inferiorly and slightly anteriorly
  - The lateral aspect: Skin, platysma, sternocleidomastoid
- Open the IJV longitudinally, if present, to view its luminal aspect. Extracapsular spread from a tumor deposit is easily identified at this time as a white area in the wall of the IJV and may indicate the location of the closest medial deep surgical margin. The involvement of the lumen of the vein by tumor is not considered an independent risk factor in survival.
- Dissect off the muscles. Start with platysma, then sternocleidomastoid. The presence and extent of extracapsular spread approaching the lateral deep surgical margin can be easily detected at this stage. Then remove mylohyoid, digastric, etc. Leave omohyoid attached (if present) to mark the junction of Levels III and IV. Reducing the bulk of the specimen improves the detection of smaller nodes.
- Divide the specimen into the respective node levels (Levels I–V) as detailed above. The precise anatomical boundaries of the various node groups are lost during the operation, particularly if the specimen is distorted during removal, but precise distinction is usually not critical.
- Separate the submandibular gland from Level I nodes, by dissecting along the plane of the capsule. Separate the tail of the parotid gland from the Level II nodes.
- Harvest the nodes from each of the tissue portions. Remove palpable nodes intact with scissors. These should be bisected along the long axis or sliced serially, depending on size. Slice the remaining tissue into 4-mm slices. Palpate slices for smaller nodes as usual.

Measurements:

- Length of IJV, if present (cm).
- Dimensions (cm) of tumor deposits (NB: not the size of the node).
- If a tumor mass of several matted nodes is present, record its maximum dimensions.
- Micrometastasis (<0.2 cm) is recorded as nodal involvement, but its precise significance is unclear.
- Distance (cm) to the nearest deep surgical margin (usually medial).

Blocks for histology:

- For large nodes, submit one representative slice; for smaller nodes, submit in total. Remember to keep the nodes from each level separate.
- One block of each of the following is recommended unless involved by tumor:
  - The submandibular and parotid glands.
  - The closest medial and lateral deep surgical margins.
- If other specimens are attached to the neck as an "in-continuity dissection" (e.g., skin, mandible, oral mucosa, thyroid gland, larynx or pharynx), these can be cut separately in the usual fashion.

Histopathology report:

Final neck dissection reports should include details on:

- The specimen type
- The type and grade of tumor present
- The number of lymph nodes recovered from each level
- The number of positive lymph nodes in each level
- The location of the largest tumor metastasis and its dimensions
- The presence or absence of extracapsular spread and the levels involved
- The distance of the tumor from the nearest deep margin

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Part IV

Eye, Muscle and Nerve Specimens

21 Eye, Muscle, and Nerve Specimens (Including Rectal Biopsy for Hirschprung's Disease)

Roy W. Lyness

21.1 Anatomy

The adult eye measures approximately 23 × 23 × 24 mm (Fig. 21.1). The anterior aspect (*cornea*) is transparent, allowing light to enter and be focused by the crystalline *lens* before being picked up by the photosensitive *retina* and converted into electrical impulses which are transmitted to the brain by the *optic nerve*.

The surface of the cornea is protected by the *eyelids* and lubricated by the *lacrimal gland* which produces tears. The tears flow over the eye keeping the surface moist and are collected at the *caruncle* where they drain via the *naso-lacrimal duct*.

The amount of light admitted to the eye is controlled by the *pupil* rooted in the *ciliary body* which controls the focusing of the *lens* and the production of aqueous humor that fills the *anterior chamber* draining to the systemic circulation (conjunctival veins) via the *trabecular meshwork* in the *filtration angle* and *Schlemm's canal*.

The *posterior chamber* contains gelatinous material known as *vitreous humor*.

The eye is "inflated" by systemic blood pressure within the capillary meshwork that is the *choroid* which also contains the nerve supply to the ciliary body and pupil. Blood pressure is responsible for the tension or firmness of the eye. Take away the blood pressure and the eye will become soft. The choroid is covered on the external surface by the *sclera* to which the *extraocular muscles* are attached in order to move the eye.

21.2 Eyelids

The ophthalmic surgeon's most common biopsy is the eyelid. To most intents and purposes the laboratory management is that of a biopsy of skin. However, the problem for the ophthalmologist is gaining adequate clearance for malignant lesions as a wide excision of a lid lesion may deprive the patient of sufficient lid cover for the eye, resulting in a lack of lubrication and protection from abrasion and infection. To this end, marking the orientation of the specimen and the limits of excision is of great importance.

It is best practice that the surgeon draws a diagram of the area and marks the lateral or medial, superior or inferior margins of the biopsy specimen with sutures and records the marks made on the diagram. The deep limit can be marked with dye in the laboratory.

The most common tumors of the eyelid are basal cell carcinoma and squamous cell carcinoma. Other tumors to be considered are malignant melanoma, sebaceous gland tumor, and Merkel cell carcinoma. In some oculo-plastic operations, a series of biopsies from the margins of excision may be sent to the laboratory for frozen section and report, in order to ascertain whether or not

R.W. Lyness
Histopathology Laboratory, Belfast City Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: roy.lyness@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_21, © Springer-Verlag London 2013

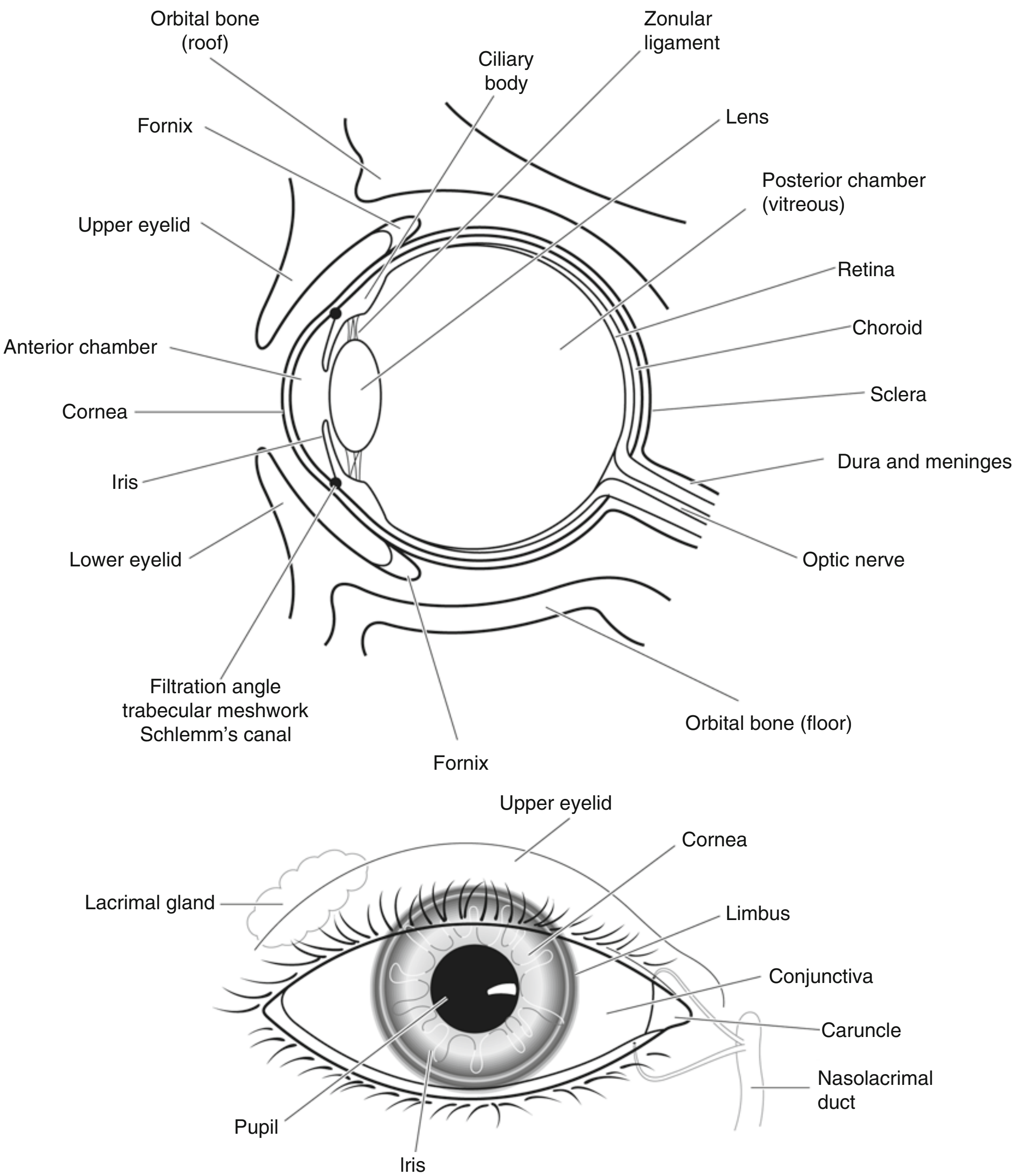

Fig. 21.1 Anatomy of the eye (Reproduced, with permission, from Allen and Cameron (2004))

excision of a malignant lesion is complete. This is difficult work relying on cooperation between surgeon and the laboratory to identify correctly the orientation of the specimens.

The gamut of benign tumors includes squamous papillomas, keratoses, nevi, inclusion cysts, chalazion, and molluscum contagiosum.

The eyelid biopsy is often a wedge resection of the lid. A central section through the eyelid to ascertain the deep limit of excision and a superior or inferior limit of excision is taken. The lateral blocks are cut through 2–3 mm serial slices from the lateral or medial aspect toward the center. This allows all six limits of excision to be judged (Fig. 21.2). Eyelid

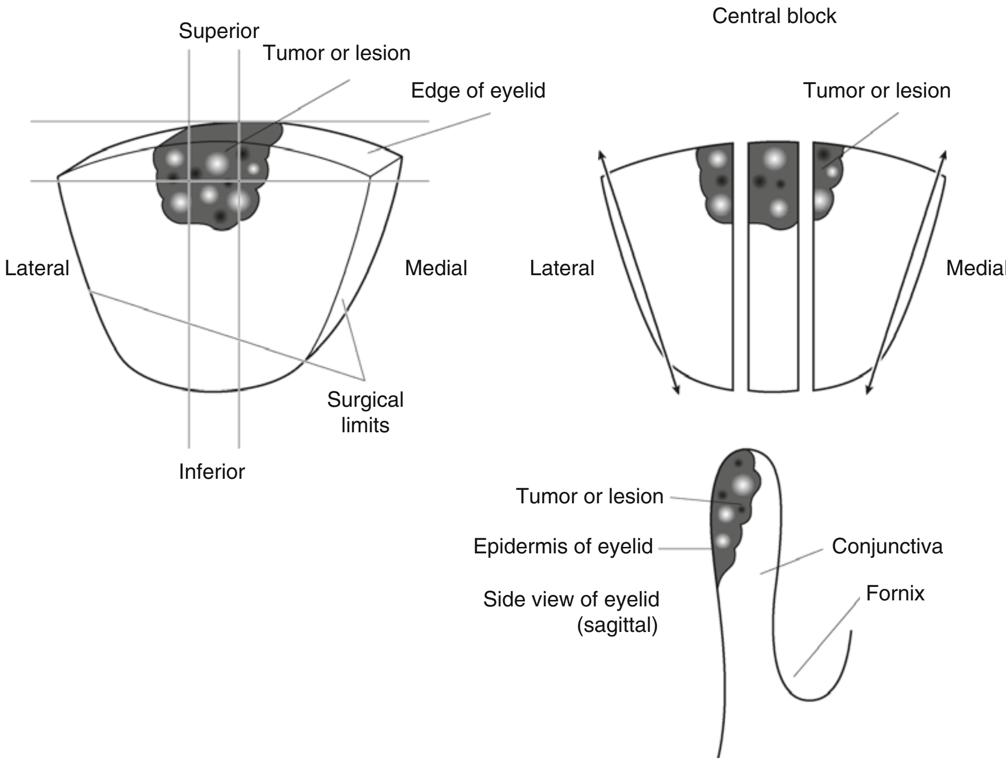

Fig. 21.2 Wedge resection of eyelid (Reproduced, with permission, from Allen and Cameron (2004))

resections can be partial (cutaneous aspect only) or full thickness (anterior cutaneous, eyelid margin, and posterior conjunctival aspects).

21.3 Conjunctiva

The conjunctiva is covered by specialized squamous epithelium containing mucus-secreting cells. The specialized epithelium is on the posterior aspect of the eyelids and covers the eye to the limbus, where it becomes entirely squamous epithelium to cover the cornea.

The common lesions of the conjunctiva are

1. *Degenerative*
   – Pinguecula
   – Pterygium
2. *Pigmented*
   – Nevi
   – Malignant melanoma
   – Extrinsic, e.g., injury
3. *Inflammatory*
   – Not usually biopsied but may be scraped for diagnosis of trachoma or other parasite infection
4. *Malignancy*
   – Basal cell carcinoma
   – Squamous cell carcinoma
   – Malignant melanoma

The purpose of conjunctival biopsies is usually diagnostic ± cosmesis. As with eyelid biopsies, an assessment of the limits of excision may be of importance, but usually the presence of infiltrating tumor in a biopsy from the fornix of the conjunctiva is sufficient justification for more radical treatment. Incisional biopsies may be used to "map" the full extent of a neoplastic process prior to definitive excision. Classic blistering disorders affecting the conjunctiva (e.g., pemphigoid, linear IgA disease) require a fresh specimen and direct immunofluoresence techniques with specialist interpretation.

21.4 Cornea

Corneal "scrapes" of ulcerating lesions may be sent to microbiologists in order to identify organisms by direct microscopy and culture. Bacterial infections, fungi, and Acanthamoeba may be identified this way. Occasionally the edge of a corneal ulcer may be submitted to the laboratory for histology as the lesion is resisting antibiotic therapy. These small biopsies are submitted whole and examined through levels. Using special stains, fungi (usually Aspergillus but occasionally Fusarium or Penicillium) or Acanthamoeba may be identified. It is unusual to find bacteria due to the therapeutic measures taken.

Corneal "buttons," as they are known in the trade, produced at full- or partial-thickness keratoplasty, are submitted to the laboratory for evaluation of and corroboration of clinical diagnoses. They should be measured for maximum diameter and described grossly for evidence of ulceration, scarring, transparency/opacity, and abnormal pigmentation.

Using a long sharp blade (e.g., disposable microtome blade) the cornea is cut across the ulcer, the pigmentation, or maximum opacity so as to leave roughly three-fifths of the lesion for processing and a reserve of two-fifths of the lesion for other investigations, e.g., electron microscopy. Care has to be taken to avoid artifacted abrasion or removal of the squamous epithelium covering the cornea or the posterior layer of endothelial cells.

Histological sections are cut and stained with H+E. In essence, there are two main types of histology:

1. *Inflamed/scarred* – Indicating an ongoing or previous infection, ulcer, or failed corneal graft
2. *Noninflamed* – Indicating a congenital dystrophy or abnormality, e.g., keratoconus, Fuch's dystrophy

It may be that a congenital abnormality such as keratoconus will result in ulceration and scarring but most dystrophies have little evidence of inflammation or vascularization.

Electron microscopy may have to be used to differentiate between some of the more obscure lesions affecting the cornea.

21.5 Iris

Small biopsies or localized resections of the iris may be taken to confirm and/or treat a clinical diagnosis of *malignant melanoma*.

Pigmented lesions of the iris are most often *benign nevi*. However, serial clinical observations may identify lesions that are growing rapidly, particularly when they involve the filtration angle, have an irregular pattern of growth and show changes in pigmentation.

In the laboratory, one uses a dissecting microscope to attempt to orientate the small specimen especially if the operative procedure has been a sector iridectomy where the root of the iris (filtration angle, adjacent cornea/sclera, and ciliary body) is included. This is especially important as the presence of infiltration of the trabecular meshwork and *Schlemm's canal* by tumor is an indicator of a poor prognosis.

21.6 Orbit

Biopsies of the orbit are of two types:

1. Where the clinicians believe they can gain access to the pathology via Tenon's capsule and take a pinch from the subjacent tissue. These are often unsatisfactory.
2. Where after clinical and radiological evaluation, a formal biopsy for diagnosis and/or treatment is made, often involving a lateral orbitotomy (cutting bone at the side of the orbit) to gain access to the lesion deep within the orbit or in the cone formed by the extraocular muscles.

Lesions of the orbit present clinically as proptosis (eye coming forward) with varying degrees of squint, double-vision, and discomfort.

As this is a tricky site for surgery, patients are often referred to specialist ophthalmic surgeons and centers with their attendant pathological facilities.

Patients require a complete clinical examination and evaluation as often clues to the cause of the proptosis may be found as a result of detecting the presence of tumor elsewhere, e.g., lobular breast carcinoma, prostate carcinoma, lymphoma, von

Recklinghausen's syndrome. Equally, blood biochemistry (thyrotoxicosis, tumor markers), serology (systemic lupus erythematosus, Wegener's granulomatosis), and radiology (e.g., bony sclerosis versus bony erosion, presence or absence of calcification, e.g., meningioma, varix within a tumor mass), CT scanning, and MRI are valuable.

The process of clinical evaluation is important as ideally surgeons attempt to remove only small resectable primary tumors, e.g., cavernous hemangioma, pleomorphic adenoma of the lacrimal gland while avoiding irresectable malignancies, and metastatic tumors.

In the laboratory, the investigation of biopsies is the same as from anywhere else in the body with the following caution. Biopsies of inflammatory lesions should be investigated thoroughly as missing an infective lesion (fungus, tuberculosis) may result in a patient being treated inappropriately with steroids exacerbating the condition. Similarly, confusion between chronic inflammatory lesions and the usual low-grade lymphomas seen in the orbit is made more likely by the often miserly and inadequate biopsies of the orbital fat taken via Tenon's capsule.

21.7 Lacrimal Apparatus

This consists of a lacrimal gland situated in the superolateral aspect of the orbit, making tears which are drained via the punctum to the naso-lacrimal duct in the nasopharynx. The gland measures approximately 1–1.5 cm in diameter, has a histological appearance similar to the salivary glands and is subject to a similar spectrum of pathological lesions.

The two main tumors of the lacrimal gland are *pleomorphic adenoma* and *adenoid cystic carcinoma*. Pleomorphic adenoma is benign occurring in middle age with a tendency to recur if inadequately excised. It causes painless proptosis and has bony sclerosis on x-ray. Adenoid cystic carcinoma occurs in a younger age group, infiltrates local structures, causing painful proptosis as it has an affinity for nerves and erodes the surrounding orbital bone.

Both require complete excision for adequate therapy, pleomorphic adenoma being usually amenable to lateral orbitotomy in the first instance, but may require more drastic action as the recurring tumor infiltrates between the two bony tables of the skull, causing serious problems of tumor control. Adenoid cystic tumor may require oculo-plastic surgery to gain adequate control of the disease.

For the laboratory, besides diagnosing the lesion, it is important to be able to identify the limits of excision and comment on their involvement or otherwise by the tumor.

Tumors of the naso-lacrimal ducts include benign polyps and malignancies common to the nasal sinuses and upper respiratory tract. The clinical presentation is epiphoria as the tears are unable to drain via the duct overflowing the eyelid. Laboratory management is as always in ENT cases, which is to diagnose the nature of the lesion, and, if malignant, detail the adequacy of the excision (see Sect. 21.8.3).

21.8 Eyes: Evisceration, Enucleation and Exenteration

There are three types of biopsy involving the eye or "globe" as it is called in ophthalmic pathology:

1. *Evisceration*
   – This is where the contents of the eye are removed via an incision around the limbus, taking iris, ciliary body, lens, vitreous, choroid, and retina but leaving the sclera.
2. *Enucleation*
   – This is where the eye or globe is removed with a short piece of optic nerve.
3. *Exenteration*
   – This is where the eye, surrounding orbital contents, eyelids, naso-lacrimal apparatus ± orbital bone are removed.

There are three main reasons for removing an eye or its contents

1. *Life-threatening conditions*
   – Either pus or tumor
2. *Pain*
   – Usually absolute glaucoma with loss of vision

3. *Cosmesis*
   – To remove an unsightly eye or to prevent facial asymmetry in young people around a collapsed micro-ophthalmic or expanding buphthalmic eye.

21.8.1 Evisceration

The dishevelled and disorganized contents of the eye are submitted in formalin fixative. If possible they should be examined grossly and, if recognizable, sorted into component parts. Calcified material (lens or remnants of osseous metaplasia in a phthisical eye) should be identified and processed via acid to avoid damage to microtome blades.

When examining the contents of the eye, it is difficult to give a comprehensive account to validate the clinical history of the eye and one is often reduced to noting the degree and type of the inflammatory process and, in the case of acute inflammation and pus, the presence of and type of organisms. Rarely is this procedure used to treat a tumor.

21.8.2 Enucleation

21.8.2.1 External Anatomy

It is necessary to confirm that the specimen is indeed the right or left eye as given on the histopathology request form. This is done by orientating the eye using the positions of the rectus muscle, tendons, and the superior and inferior oblique extraocular muscles. The superior oblique muscle has a long string-like tendon inserting into the sclera lateral and slightly posterior to the superior rectus muscle and superior to the optic nerve. The inferior oblique muscle is fleshy and brown right up to the insertion into the globe and is situated lateral and inferior to the optic nerve. So the eyes should have the superior oblique muscle, optic nerve, and inferior oblique muscles forming a triangle when viewed posteriorly (Fig. 21.3).

Having orientated the eye a systematic list of the external features is made. The normal diameter is 23–24 mm. Conditions such as glaucoma may thin the sclera causing herniations or staphylomata in areas of weakness. Otherwise there may be a slight increase in diameter as may also be seen in myopia. Examination of the cornea includes noting any surgical or traumatic incisions, sutures, opacities, or pigmentation. Is the iris visible? Is it symmetrical, abnormally pigmented, or deficient? If it is deficient, where on a clock-face (12 o'clock = superior) and what size? Is there pus or proteinaceous fluid in the anterior chamber?

The conjunctiva and sclera are examined for abnormal pigment, incisions, or evidence of radiotherapy plaques or bands for the treatment of detached retina.

The optic nerve and scleral vessels are examined for evidence of tumor. After making these gross observations, it is advisable to photograph the external surfaces of the eye.

21.8.2.2 Laboratory Details

Fixation of the eye: Globes are usually fixed in formalin fixative. Prior to cutting, they are transferred to 95% alcohol as this "hardens" the sclera making it slightly easier to cut and improves the color and presentation of the eye prior to photography.

Tools required: The best instrument to cut a globe is a long, very sharp, thin blade. The idea is to get one long smooth cut that cuts the whole of the eye, rather than a series of sawing movements that disrupt the internal anatomy and make processing and sectioning difficult. A disposable microtome blade (10–15 cm) may be used, but extreme care has to be taken to avoid spilling an innocent person's blood over the specimen.

Eyes opened and found to contain bone or calcified debris, are gently decalcified in acid before finishing off the cutting. Eyes containing foreign bodies should have them removed carefully after noting and photographing their location.

Transillumination in eyes containing tumor: It may be possible to transilluminate the eye using a strong narrow beam of light (usually fiber-optic) in order to locate the mass. This is helpful in deciding how to cut and open the eye in order to have the classical section of central cornea, center of pupil, lens, lesion, and optic nerve.

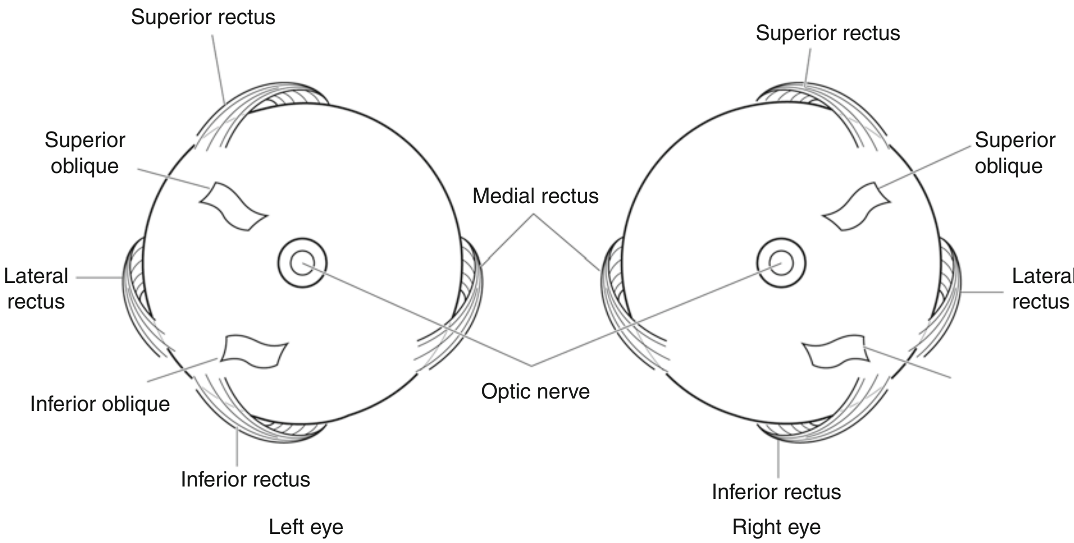

Fig. 21.3 Anatomical orientation of the eye. Landmarks on the eyes as viewed from the posterior aspect (Reproduced, with permission, from Allen and Cameron (2004)).

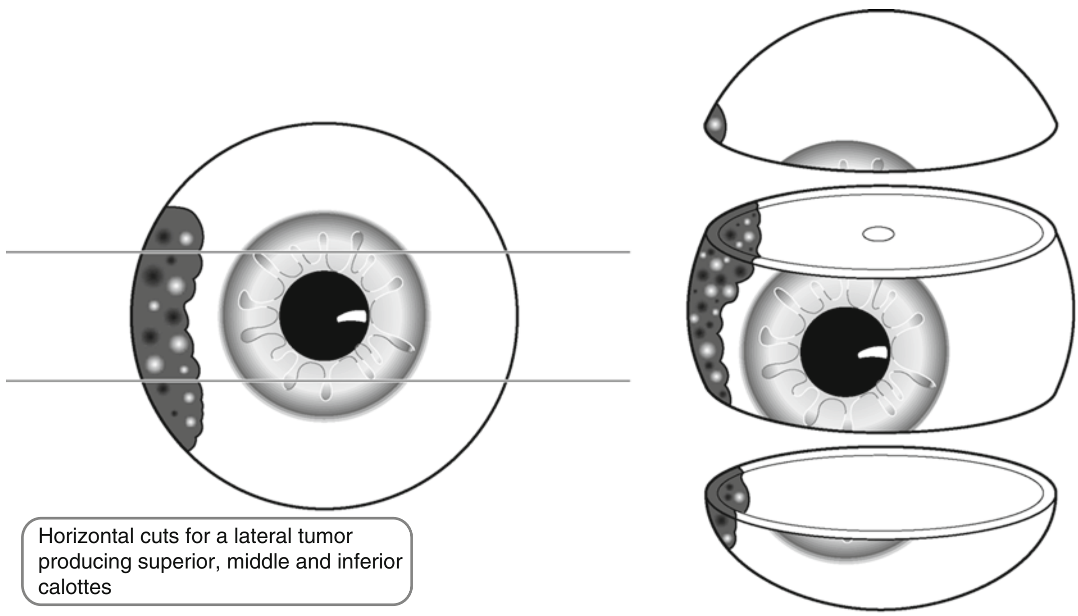

Fig. 21.4 Blocking an eye specimen (Reproduced, with permission, from Allen and Cameron (2004))

Similarly, X-ray examination for bone or foreign bodies may be undertaken using needles on the external surface to help locate the lesion.

Techniques (Fig. 21.4): A thin section of the optic nerve is taken first and submitted for histology.

Vertical cuts to give calottes is the technique used when studying surgical lens extractions and the surgical site following a failure of or complications of cataract surgery. An implanted plastic lens may interfere with the smooth cutting of the blade and therefore should be anticipated.

Horizontal cuts give calottes which include cornea, center of pupil, lens, lesion, macula, and optic nerve. The inferior oblique helps one identify the posterior temporal sclera.

The calottes should be cut first 1 mm from the optic nerve proceeding anteriorly to cut the cornea about 2 mm medial to the limbus. The globe is placed on the cutting block and the procedure repeated on the other side of the optic nerve. Vertical cutting results in a nasal calotte, a central wedge-shaped block, and a temporal calotte. These can be further examined under liquid to conserve the anatomy, keeping the retina in situ.

All blocks are examined, carefully noting:

- The depth and contents of the anterior chamber
- The presence or absence of the lens
- The presence or absence of cataract, the vitreous (clear, gelatinous or turbid, the presence or absence of membranes)
- The retina whether it is in-situ or detached (a subretinal exudate indicates a true as opposed to artifactual retinal detachment)
- The presence or absence of recent/old hemorrhage in the vitreous or choroid
- The thickness of the sclera

21.8.2.3 Pathological Conditions

Tumors: There are two main tumors affecting the eye, *malignant melanoma* (presents from second decade to very elderly) and *retinoblastoma* (which presents in childhood). However, many other tumors have been described within the eye, including adenomas and adenocarcinomas of the ciliary body epithelium, schwannomas, lymphomas, hemangiomas, and metastatic tumors from lung, breast, and stomach. Prior to treatment, fine needle aspiration cytology is undertaken in some specialist centers to distinguish between primary and metastatic tumors.

Malignant melanomas may occur anywhere in the uveal tract but are most commonly found in the choroid. They arise in the choroid pushing Bruch's membrane over them until they perforate it, causing the overlying retina to detach with consequent formation of a subretinal exudate. The tumor gains access to the venous side of the systemic circulation via the perforations of the sclera by the artery, vein, and nerve bundles, and may be seen causing black pigmentation in the region of the vortex veins. Alternatively, malignant melanomas may infiltrate the filtration angle of the anterior chamber en route to Schlemm's canal and the conjunctival veins. This causes abnormal pigmentation of the conjunctiva at the limbus. Uveal malignant melanoma classically metastasizes to the liver.

Prognosis for these tumors depends on three major factors:

1. Age at presentation: Older is worse than young.
2. Site within the eye : Posterior is better than equatorial, which is better than anterior where it quickly gains access to the venous side of the systemic circulation via Schlemm's canal. Iris melanoma has a much lower mortality due to earlier clinical presentation.
3. Size of tumor (maximum diameter):
   >15 mm poor prognosis
   >10 mm guarded prognosis
   5 mm interesting, but not immediately lethal
4. Presence and extent of extraocular invasion.

The size of tumor covers factors such as cell type, as small tumors tend to be spindle B cell type and the larger tumors have increasing numbers of epithelioid cells. Similarly, larger tumors tend to exit the eye via the sclera or Schlemm's canal. Size is most accurately determined by preoperative ultrasound. Treatment is generally by enucleation, but sight sparing localized resection of the iris, ciliary body, or choroid is performed by some specialist centers for tumors of limited extent.

TNM 7 classification of tumor spread of ciliary body and choroid malignant melanoma is based on the tumor maximum thickness, sclera basal diameter, and the presence and extent of extraocular invasion.

Retinoblastoma – two types:

1. Congenital: Where both eyes ± the pineal gland are affected
2. Sporadic: Where one eye is affected and the patient carries a genetic risk for the next generation

   The tumor arises in one, two, or all three of the layers of the retina, forming retinoblasts which may infiltrate the overlying vitreous (endophytic) or the underlying subretinal space (exophytic). The tumor exits the eye via the optic nerve to the brain. It infiltrates the choroid if there has been damage to Bruch's membrane (usually following x-radiation treatment). From there it may metastasize systemically.

Treatment of congenital retinoblastoma usually involves excising the worse eye and treating the better eye with collumated irradiation, hoping to conserve some function. For sporadic cases, the affected eye is removed, hoping to avoid spread to the brain via the optic nerve. In general, enucleation for retinoblastoma is carried out in patients with advanced intraocular disease and if there has been failure of conservative management.

TNM 7 classification and prognosis of retinoblastoma are based on whether the tumor is localized to the eye, and the presence and extent of involvement of the choroid, the optic nerve and its resection limit, and extraocular tissues. These unfavorable high-risk factors warrant consideration of postoperative adjuvant chemotherapy and radiotherapy.

Glaucoma: The main cause for enucleation is the painful blind eye. Such eyes usually have a long history of attending an ophthalmologist with episodes of therapy (medical and surgical) before opting for pain relief and preemptively preventing the eye from rupturing due to the increased intra-ocular pressure causing thinning and anesthesia of the cornea.

Such eyes are cut and processed with attention to the clinical history in order to corroborate the clinical findings and demonstrate the cause of the open- or closed-angle glaucoma.

Inflammation: The eye is subject to endophthalmitis secondary to penetrating injuries or surgical procedures. This may be treated by steroids provided it is not infected. Infections, bacterial, fungal, helminthic (toxocariasis), and protozoal (toxoplasmosis), cause a spectrum of acute to chronic inflammation. The presence of pus and the potential of infection to track to the CNS may necessitate evisceration or enucleation. Often the inflammation subsides, but the resultant healing process precipitates detachment of the retina and glaucoma requiring enucleation of a painful blind eye.

Granulomatous inflammation affecting the choroid or sclera may be the result of sympathetic endophthalmitis, sarcoidosis, rheumatoid arthritis, Wegener's granulomatosis and the terminal stages of miliary TB.

21.8.3 Exenteration

Exenteration is carried out to gain control of a malignant tumor affecting the tissues around the eye, e.g., eyelids, orbital contents, nasal sinuses, palate. Often there is no direct extension of the tumor into the eye and no evidence of tumor metastasis within the choroid. The object of the laboratory investigation is to determine whether or not the radical surgical excision of tissue from around the eye has removed the tumor with clear surgical margins and if the tumor is in the microvasculature leading to cervical lymph nodes.

21.8.3.1 Procedures

Radical dissections may come with many fragments including a dissection of the eye, eyelids, and orbital contents. It is necessary to identify and orientate the components of the overall dissection in order to determine the surgical limits of excision.

For the central block of eye, eyelids, and orbit, it is useful to mark the cut edges of the block of tissue with different dyes to ensure that the superior/inferior and medial/lateral limits are easily identified. The eye is orientated using the lids and caruncle to confirm the side from which it was taken according to the request form and the superior/inferior, medial/lateral limits. Any gross evidence of tumor should be accurately located and described.

Using the cornea and optic nerve as landmarks, as described in the procedures for the enucleated globe, antero-posterior incisions are made on either side of the cornea and optic nerve to obtain a central block that should have lids, cornea, lens, vitreous, retina, choroid, sclera, and optic nerve within surrounding tissues as a central block leaving medial and lateral calottes of eye and surrounding soft tissues. Further blocks may be cut to obtain medial and lateral limits of excision as required. These are often in a horizontal plane.

21.9 Muscle Biopsies

This is specialized work and should only be undertaken by laboratories and pathologists with experience of enzyme histochemistry and the evaluation of the results. Small muscle biopsies (other than for tumors) are taken from the belly of viable muscles in order to diagnose conditions causing muscle weakness. Biopsies should not be taken from extremely wasted muscles as only end-stage pathology rather than ongoing pathological changes will be demonstrated.

Conditions causing muscle weakness fall into three main categories:

1. Congenital disorders of muscle, i.e., biochemical abnormalities resulting in a dystrophy or myopathy
2. Acquired disorders of muscle secondary to pathology of the nervous system
3. Acquired disorders due to inflammation of muscle or the microvasculature

As with all biopsies, a full history of the condition and clinical workup is of importance to the pathologist.

21.9.1 Laboratory Techniques

Muscle biopsies are processed using frozen section techniques in order to enable histochemical methods of staining and morphometric evaluation of muscle fiber types, innervation, and enzyme biochemistry. Muscle biopsies in formalin fixative and paraffin wax embedding are of limited use, allowing only evaluation of inflammatory conditions and confirming muscle wasting.

Orientation of the small muscle biopsy is of great importance. The classical histological section is a cross section of muscle fibers and fascicles. Longitudinal sections of muscle fibers are of limited use except when looking for neuromuscular junctions.

It is necessary for the biopsy to be taken to the laboratory immediately following removal from the patient, so as to conserve the potential for enzyme histochemistry. If this is not possible, then ammonium sulphate fixative solution may be used, but this requires practice and familiarity by technicians and pathologists to ensure that the histochemical techniques work.

The biopsy is orientated to give a transverse section using a dissecting microscope. The biopsy is placed in O.C.T. on a small piece of cork and snap frozen in iso-pentane cooled in liquid nitrogen. The process of snap freezing is important as crystal artifact will result if the freezing process is too slow. Transverse sections are cut and stored using a cryostat.

The standard histochemical stains besides H+E, are ATPase and NADH to differentiate between muscle fibers, a modified Gomori to study mitochondria, and a battery of other enzyme histochemical stains to study the biochemistry of the muscle including acid phosphatase, succinic dehydrogenase, cytochrome oxidase, and phosphorylase. Immunocytochemical staining (e.g., Dystrophins 1–3, Merosin, Adhalin, Utrophin, and Spectrin) is also being used to examine for and subtype muscle dystrophies.

21.10 Peripheral Nerves

A peripheral nerve biopsy may be taken to evaluate disorders causing peripheral weakness and/or loss of sensation when no central nervous system cause is implicated. Over the age of 45 years, there is a gradual loss of peripheral nerve axons, but the process may be accelerated by disease. Ordinarily, peripheral nerves have a process of degeneration and regeneration with wear and tear due to the vicissitudes of life. The process may be accelerated by disease. Most often the pathologist is seeking to determine the condition of the nerve, i.e., the number of axons relative to the patient's age, the condition of the myelin sheath, and the presence or absence of any abnormal cellular or noncellular infiltrate.

Peripheral nerve biopsies are placed in formalin fixative. In the laboratory, they are orientated using a dissecting microscope as it is important to obtain the classical transverse section of the nerve, which allows a relative count of the axons as well as staining for and evaluation of the myelin nerve sheaths. The nature of infiltrates may be determined using routine staining methods. Only very rarely is one asked to tease out the longitudinal axons in a biopsy for clinical purposes.

21.11 Colorectal Mucosal Biopsies for Hirschsprung's Disease

Hirschsprung's disease is characterized by an absence of ganglion cells and the demonstration of abnormal nerve trunks passing through the muscularis mucosae.

Pinch biopsies of colonic and rectal mucosa may be taken for the evaluation and determination of the severity and extent of Hirschsprung's disease in children. If paraffin-embedded material is the only method available, then looking for ganglion cells in multiple sections (50 on average) is advised. This can be supplemented by immunohistochemistry, e.g., synaptophysin or calretinin. However, the innervation and motility of the gut can be determined using enzyme histochemistry for acetyl-cholinesterase activity and this is the preferred option. If there is no staining, the bowel is innervated. Positive staining indicates an aganglionic or hypoganglionic area, which results in a bowel stricture and constipation. Using a series of biopsies, the distribution of the hypoganglionic segments can be mapped out prior to surgical resection.

The deep mucosal pinch biopsies are taken by the clinician from at least 2 cm above the dentate line and sent immediately to the laboratory in order to conserve the enzyme activity. The biopsy is orientated in the laboratory using a dissecting microscope so as to give a classical section of mucosa with epithelium, lamina propria, muscularis mucosae, and submucosal connective tissue from top to bottom. It is not necessary to see a full thickness of bowel wall as the enzyme technique evaluates the presence of autonomic innervation by testing for the presence of acetyl-cholinesterase which breaks down the presence of acetyl-choline and by inference proves the presence of a nerve supply.

The orientated biopsy is placed in O.C.T. on a piece of cork and snap frozen in iso-pentane cooled in liquid nitrogen, so as to avoid ice crystal artifact distorting the specimen if cooled too slowly.

Frozen sections are cut and conserved using a cryostat. The sections are stained for H+E in order to examine for ganglion cells from Meissner's plexus in the submucosa and acetyl-cholinesterase for the presence or absence of positive staining.

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Part V

Gynaecological Specimens

22 Ovary

Oisin Houghton and W. Glenn McCluggage

22.1 Anatomy

The ovaries are paired structures lying in the right and left iliac fossae, on either side of the uterus attached to the posterior aspect of the broad ligaments (Fig. 22.1). In the reproductive age group, each ovary is typically approximately 3 cm in maximum dimension, but the size may vary considerably. In the prepubertal and postmenopausal periods, the ovaries are usually smaller. Typically the ovary is ovoid in shape. The external surface may be smooth or convoluted, especially in the late reproductive period. The ovary contains an outer cortex and an inner medulla. Follicular structures, including cystic follicles, corpora lutea, and corpora albicantia are usually visible on sectioning. A hilar region is also apparent. The ovary is covered by a layer of peritoneum, the mesovarium.

O. Houghton (✉) • W.G. McCluggage
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: oisin.houghton@belfasttrust.hscni.net; glenn.mccluggage@belfasttrust.hscni.net

Lymphovascular drainage:

The blood supply to the ovary is from the ovarian artery, a branch of the aorta. This courses along the surface of the ovary and anastomoses with the ovarian branch of the uterine artery. Subsidiary branches enter the ovarian hilus and then travel through the medulla and cortex, anastomosing to form capillary channels. Veins accompany the arteries and in the hilum form a plexus which drains into the ovarian vein. The ovarian veins course along the surface of the ovaries, the right draining into the inferior vena cava and the left into the left renal vein.

The ovarian lymphatics originate with the theca layers of the follicles and course through the ovarian stroma to form a hilar plexus. Eventually they accompany the ovarian vessels to drain into the upper para-aortic lymph nodes at the level of the lower pole of the kidney.

22.2 Clinical Presentation

Clinical features related to ovarian pathology are often nonspecific and, in general, with ovarian neoplasia symptoms occur late in the course of the disease when the tumor has often spread beyond the ovary. Symptoms related to ovarian tumors include swelling or a feeling of fullness in the abdomen or pelvis, the presence of an abdominal mass, irregular uterine bleeding, abdominal or pelvic pain, and gastrointestinal symptoms. There may be associated ascites, especially with ovarian

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_22, © Springer-Verlag London 2013

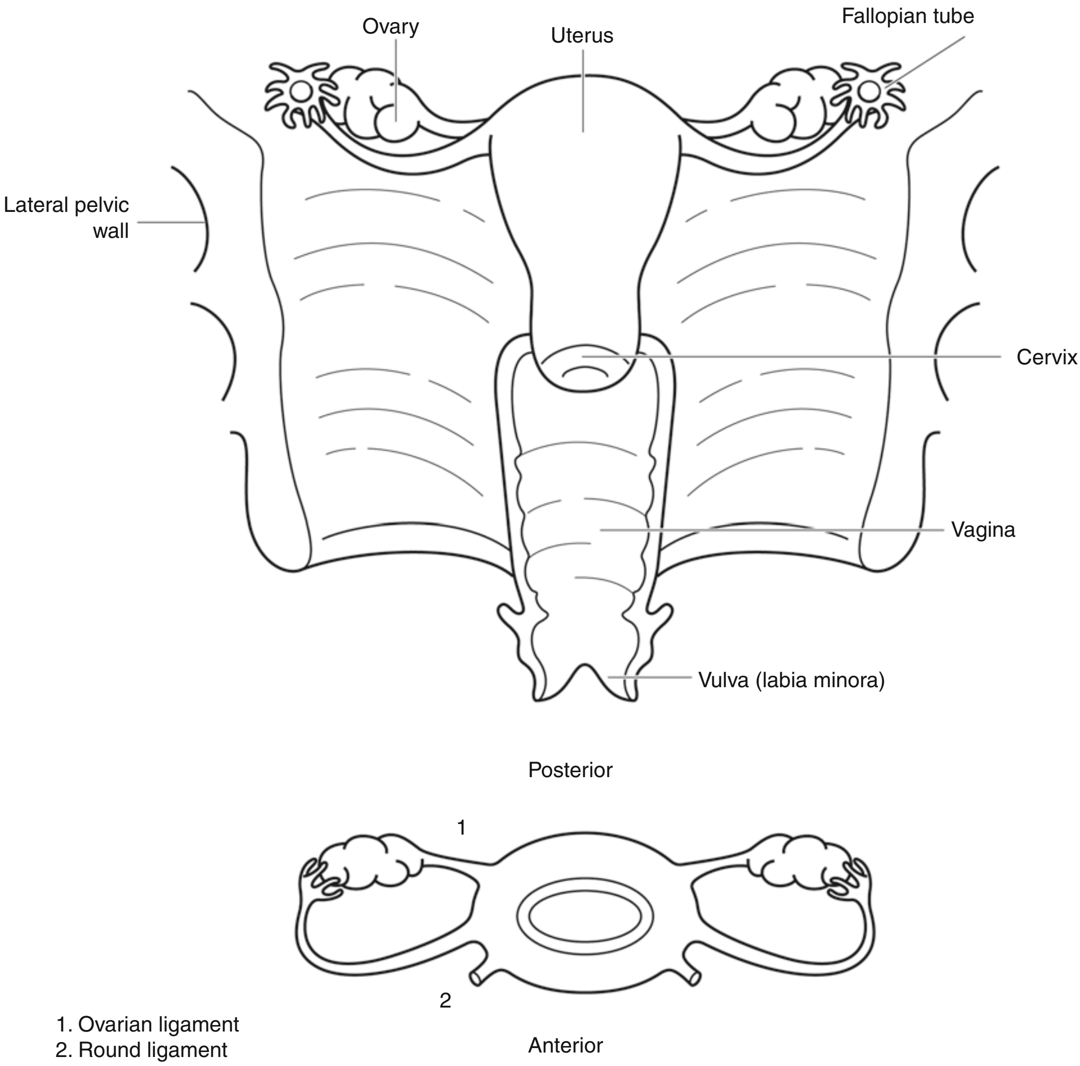

Fig. 22.1 Overview of gynecological anatomy (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

malignancies, but also with some benign neoplasms such as fibromas. With ovarian endometriosis, pain and swelling may fluctuate depending on the phase of the menstrual cycle. In younger patients, ovarian pathology may be discovered during the course of investigations for infertility. Ovarian pathology may also be discovered incidentally during abdominal or pelvic imaging or as a result of an increased serum CA-125. Serum CA-125 measurements and abdominal ultrasound are currently being evaluated in screening programs for ovarian cancer.

22.3 Clinical Investigations

- Serum CA-125 measurements: An increase in serum CA-125 may be an indicator of ovarian malignancy. However, modest or even marked elevation of serum CA-125 may occur in many non-neoplastic diseases or non-ovarian neoplastic diseases, this serum marker being relatively nonspecific. CA-125 is produced by mesothelial cells, and conditions which involve the peritoneal cavity with its lining of mesothelial cells are especially liable to result in an elevated serum CA-125. These conditions include ascites, endometriosis, peritoneal tuberculosis, and disseminated non-ovarian neoplasms.

- Abdominal USS and CT scan: In cases of ovarian neoplasia abdominal USS or CT scan often shows a complex ovarian mass with alternating solid and cystic areas. There may be coexistent ascites and an omental cake, indicating omental involvement by tumor. Unilocular or multilocular thin-walled cystic lesions often indicate benign neoplasms. CT scanning is often performed to stage ovarian neoplasia.
- Peritoneal aspiration: Aspiration of ascitic fluid and cytological examination may be performed in the investigation of ovarian neoplasia.
- Core biopsies: Radiologically guided core biopsies, especially of the omental disease, may be undertaken for definitive diagnosis of an ovarian malignancy in patients who are not likely to be amenable to optimal surgical debulking.
- Laparoscopy: This may be indicated in certain conditions, e.g., suspected endometriosis. Biopsy can be performed at laparoscopy.

In patients with suspected ovarian neoplasia, a *risk of malignancy index* is calculated. This is a means of assessing the likelihood that an ovarian mass is malignant and takes into account the menopausal status (pre- or postmenopausal) of the patient, the ultrasound findings, and the serum CA-125 measurement.

22.4 Pathological Conditions

22.4.1 Non-neoplastic Conditions

Follicular or functional cysts: These are extremely common and are secondary to an absence of the normal preovulatory luteinizing hormone surge that triggers ovulation. They are usually found in the reproductive age group and are generally asymptomatic, although acute abdominal pain and bleeding into the peritoneal cavity may occur secondary to rupture. Follicular cysts may be multiple. In children they can be associated with sexual pseudoprecocity.

Grossly follicular cysts are usually thin walled and contain watery fluid. Histologically they are lined by granulosa or theca cells or a combination of both. These cell types are often luteinized.

Corpus luteum cyst: During the ovulatory cycle, a corpus luteum is formed which, when fertilization does not occur, involutes. At the time of menstruation, the center of the corpus luteum is cystic and filled with blood. This is known as a cystic corpus luteum. When this cystic mass becomes greater than 3 cm in diameter, it is designated a corpus luteum cyst. These lesions are often asymptomatic but can be associated with menstrual irregularities. Rupture may result in pain and intraabdominal hemorrhage.

Grossly a corpus luteum cyst is lobulated or well circumscribed. The wall is composed of luteinized granulosa cells and the lumen contains fresh or altered blood.

Polycystic ovarian disease: This disease is characterized by anovulation and the development of multiple follicular cysts within both ovaries. Often patients are infertile and have menstrual irregularities. Typically the ovaries are enlarged with multiple small cysts located just below the cortex. The outer cortex of the ovary is typically thickened. Histologically the cysts are usually lined by a thin layer of granulosa cells and a more prominent layer of lipid-laden theca interna cells.

Endometriosis: The ovary is the most common site of endometriosis which is defined as the presence of endometrial tissue, usually, but not invariably, both glands and stroma, outside the uterus. Most common in the reproductive age group, but occasionally encountered in postmenopausal women, the symptoms are protean and varied. Patients may present with a palpable abdominal mass, abdominal or pelvic pain, dysmenorrhea, dyspareunia, irregular uterine bleeding, or infertility.

Endometriosis within the ovary may take the form of an endometriotic cyst. These can be single or multiple and are often bilateral. They generally have a thick fibrous wall which is yellow to brown in color with a ragged internal surface. The cyst contents are typically dark brown fluid which may be inspissated, giving rise to the term "chocolate cyst" of the ovary. Rarely tumors can arise within ovarian endometriotic cysts and these usually take the form of a thickened area within the wall. Endometriosis within the ovary may also be non-cystic appearing as small red, blue, or brown spots. Often endometriotic foci are not apparent to the naked eye.

Histologically endometriosis is typically composed of endometrial glands and stroma. In some cases, one or both of these components may be absent, or obscured by a superimposed hemorrhagic, inflammatory, or fibrotic process. Occasionally, all that remains is a fibrotic area containing hemosiderin or ceroid-laden macrophages. In such cases, a presumptive diagnosis of endometriosis may be made.

Simple cysts: Simple benign cysts are common within the ovary. They cannot be classified into any specific type since the lining is attenuated or lost. Most are probably of epithelial origin being lined by attenuated epithelial cells or of follicular origin lined by atrophic granulosa or theca cells. Immunohistochemistry for EMA (epithelial cells positive) or α-inhibin (granulosa cells positive) assists in determining the origin of the cyst.

Stromal hyperplasia: This is relatively common in the perimenopausal or early postmenopausal age group. Both ovaries are enlarged, often only mildly so, by a nodular stromal proliferation. Usually the nodules are yellow to white in color and they may be confluent. Histology confirms a nodular proliferation of stromal cells with scant cytoplasm. There may be androgenic or estrogenic manifestations and, on occasions, associated endometrial hyperplasia or adenocarcinoma.

Stromal hyperthecosis: This uncommon condition is often associated with signs of hyperandrogenism. Estrogenic manifestations and coexistent endometrial hyperplasia or adenocarcinoma may also occur. Typically both ovaries are enlarged and yellow/white in color with a vague nodular pattern. Histologically there is usually accompanying stromal hyperplasia, but in addition luteinized stromal cells with clear or eosinophilic cytoplasm are present singly or in small groups.

Massive edema: This is a rare cause of unilateral ovarian enlargement and is probably secondary to partial torsion of the ovary. Presentation is often with abdominal pain and a palpable ovarian mass. There may be evidence of androgen excess. On sectioning the ovary it is typically edematous and pale in color and watery fluid exudes from the cut surface. Histologically there is separation of the stromal cells by edema fluid that surrounds residual ovarian structures. Luteinized stromal cells may be present. The outer cortex is typically not edematous but rather is composed of dense fibrous tissue.

22.4.2 Neoplastic Conditions

The ovary is unique in that an extremely wide range of neoplasms, both benign and malignant, may arise here. Primary tumors may be of surface epithelial, germ cell, or sex cord-stromal derivation.

Benign tumors: May be of surface epithelial type (serous, mucinous, or endometrioid cystadenoma/cystadenofibroma and Brenner tumor), germ cell type (e.g., benign cystic teratoma), or sex cord-stromal type (e.g., fibroma). They can be solid or cystic or contain a mixture of solid and cystic components.

Malignant tumors: Primary malignant ovarian neoplasms are of *surface epithelial*, *germ cell*, *or sex cord-stromal type*. Surface epithelial tumors are most common and these comprise serous, mucinous, endometrioid, clear cell, transitional, or undifferentiated carcinomas. Mixed carcinomas can occur but are rare. Borderline neoplasms (tumors of low malignant potential) also occur and these may be one of any of the morphological subtypes described, most commonly serous or mucinous. These are neoplasms which exhibit epithelial proliferation but in which there is no evidence of stromal invasion. Omental involvement by serous borderline tumor may be seen in the form of noninvasive or invasive implants.

Ovarian surface epithelial carcinomas are most common in middle-aged and elderly women, in nulliparous women, and those with an early menarche and late menopause. The oral contraceptive pill is protective. It has been suggested that women who are exposed to ovulation-inducing drugs are at increased risk of the development of ovarian carcinoma. Women with BRCA1 or BRCA2 gene mutations are at increased risk of the development of both ovarian and breast cancer. Outside BRCA1 and BRCA2 groups, there is a familial predisposition to the development of ovarian cancer. The risk of ovarian cancer is also increased in patients with Lynch syndrome (hereditary non-polyposis colorectal cancer syndrome). The risk of developing ovarian adenocarcinoma increases with the number of ovulations over a lifetime (incessant ovulation theory). Repeated ovulation with disruption of the ovarian surface mesothelium is thought to result in the development of cortical inclusion cysts. It is thought that high-grade serous carcinomas may arise from these cortical inclusion cysts or ovarian surface epithelium. There is now increasing and compelling evidence that many high-grade serous carcinomas may arise from the tubal fimbria from a precursor lesion known as serous tubal intraepithelial carcinoma (serous TIC). Two distinct types of ovarian serous carcinoma occur, low grade and high grade. Low-grade serous carcinoma arises in many cases from a preexisting benign and borderline serous tumor, while there is no relationship between borderline serous tumor and high-grade serous carcinoma.

Mucinous, endometrioid, and clear cell adenocarcinomas have an alternative pathogenesis. For example, K-ras mutations are found in mucinous adenocarcinomas and these develop from preexisting borderline mucinous neoplasms, unlike high-grade serous adenocarcinomas. Endometrioid and clear cell carcinomas can be associated with endometriosis in the ipsilateral or contralateral ovary or elsewhere in the pelvis and it is clear that many of these neoplasms arise from endometriosis; molecular abnormalities are similar to those seen in uterine endometrioid adenocarcinomas. Since the preferred theory for the development of endometriosis is retrograde menstruation, it is interesting that tubal ligation is protective for the development of ovarian endometrioid and clear cell carcinomas but not for other morphological subtypes. Endometrioid neoplasms may coexist with similar tumors in the endometrium in up to 25% of cases and most, but not all, of these represent synchronous independent neoplasms. In such cases, the finding of endometriosis in close association with the ovarian endometrioid or clear cell carcinoma or even remote from this is a helpful clue as to their primary origin. The ovary is a common site for metastatic carcinomas, and metastatic tumor may closely simulate an ovarian primary histologically. The most common primary sites include colon, appendix, pancreas, biliary tree, stomach, breast, and endometrium. Features favoring metastasis, but by no means specific for this, include bilaterality, the presence of nodular tumor deposits especially on the cortical surface of the ovary, extensive necrosis and vascular invasion, extracellular mucin, and signet ring cells.

Etiological factors in the development of ovarian germ cell (e.g., immature teratoma, yolk sac tumor, dysgerminoma) and sex cord-stromal (e.g., granulosa and Sertoli-Leydig tumors) neoplasms are not well known. Gonadoblastomas often occur in patients with underlying gonadal dysgenesis, while sex cord-stromal tumor with annular tubules can be associated with Peutz–Jeghers syndrome. Sex cord-stromal neoplasms may cause estrogenic or androgenic excess due to hormone elaboration. Occasionally this may result in endometrial hyperplasia or adenocarcinoma.

A wide variety of other neoplasms, both benign and malignant, also arise within the ovary.

Treatment: Treatment of malignant ovarian neoplasms is usually total abdominal hysterectomy and bilateral salpingo-oophorectomy. Omentectomy and peritoneal washings are usually performed as part of the staging procedure. Lymphadenectomy may also be undertaken. Unilateral salpingo-oophorectomy and limited staging, such as omentectomy and lymphadenectomy, may be undertaken for suspected malignant neoplasms in young women who wish to preserve their fertility. Postoperative chemotherapy is often necessary, especially for tumors which have spread beyond the ovary or for tumors which are confined to the ovary but where the neoplasm is high grade, the capsule is deficient and, or, there is ascites or positive peritoneal washings. The FIGO staging system for ovarian cancer is used. In the UK, the British Association of Gynaecological Pathologists, British Gynaecological Cancer Society, and gynecological clinical reference group of the National Cancer Intelligence Network recommend that FIGO staging rather than TNM be used for gynecological cancers. Borderline tumors should be staged in the same way as invasive carcinomas.

Prognosis: The prognosis of ovarian adenocarcinoma is generally poor, overall 5-year survival being in the region of 30–40%. This is largely due to the fact that at presentation many carcinomas have spread beyond the ovary, and are FIGO stage III or IV. The prognosis for stage I tumors is generally good. Borderline epithelial neoplasms have an excellent prognosis, if adequately staged, and if there are no invasive peritoneal or omental implants.

Some ovarian sex cord-stromal neoplasms, e.g., granulosa cell tumors exhibit a low-grade malignant behavior with late recurrence or metastasis being a common feature. Many malignant germ cell tumors, especially those occurring in children or young adults, are highly aggressive but often respond well to modern chemotherapeutic regimens.

22.5 Surgical Pathology Specimens: Clinical Aspects

22.5.1 Biopsy Specimens

Fine needle aspiration (FNA) specimens of ovarian cystic lesions may be performed under ultrasound guidance (transvaginal or transabdominal) or at laparoscopy or laparotomy. Ovarian wedge biopsies are occasionally performed at diagnostic laparotomy for lower abdominal pain and core biopsies may be carried out when it is unclear whether an ovarian mass is benign or malignant. Radiologically guided core biopsies, usually of the omental metastasis, may be performed for ovarian neoplasms which are being treated primarily with chemotherapy rather than surgery. Cystectomy with preservation and reconstruction of the residual ovary may be performed in young patients in whom benign cystic lesions are suspected clinically.

22.5.2 Resection Specimens

In general, with the exception of young women, when a malignant ovarian tumor is suspected, total abdominal hysterectomy, bilateral salpingo-oophorectomy, and omentectomy are performed. This is generally via an abdominal approach. Any ascitic or free peritoneal fluid is sent for cytological examination and if none is present peritoneal washings are performed. The appendix may be removed in cases of a suspected ovarian mucinous neoplasm. In young women with a clinically and/or radiologically malignant ovarian lesion, in whom preservation of fertility is desirable, unilateral salpingo-oophorectomy (usually with omentectomy) may be performed. This should be followed by discussion of the case and assessment of the need for further surgery at a multidisciplinary gynecological oncology meeting. In occasional cases, where the presence of widespread disease precludes total tumor debulking, only small fragments or a proportion of the tumor will be removed. Unilateral salpingo-oophorectomy may be performed when a benign ovarian neoplasm or a benign cyst is suspected. Prophylactic salpingo-oophorectomies may be performed in those with a hereditary predisposition to developing ovarian cancer (e.g., BRCA1 or BRCA2 mutation) or where is a strong family history of ovarian cancer.

22.6 Surgical Pathology Specimens: Laboratory Protocols

22.6.1 Biopsy Specimens

FNA specimens are centrifuged and the specimens examined cytologically, usually with both Giemsa and Papanicolaou stains. Ovarian wedge biopsies are weighed and measured, sectioned thinly, and examined intact. Core biopsies are examined intact, usually at multiple levels.

22.6.2 Resection Specimens

Initial procedures and description:

- Abrading the cortical surface should be avoided in order to preserve the mesothelial lining.
- Each ovary is weighed and measured in three dimensions (cm) and if necessary photographed. The presence of fallopian tubes is confirmed and they are measured.

- The cortical surfaces of the ovaries may be inked. In general, this is not necessary but some pathologists find this helps identify the capsular blocks and facilitates assessment of capsule integrity. It may also help determine whether the block is fully faced when examining tissue sections histologically.
- The cortical surface of each ovary is closely inspected around the whole circumference. The presence of obvious tumor deposits on the capsular surface or of papillary areas or capsular breech is noted. This is important since if a malignant tumor breeches the capsular surface it is at least stage IC. In many instances, this is the cutoff for adjuvant chemotherapy. The percentage of the surface involved by papillary areas should be documented.
- The ovaries may be sliced to aid fixation prior to sampling. This should only be done after careful examination of the capsular surface and evaluation for capsular breech. If the uterus is present, it may also be opened to ensure fixation of the endometrium.
- Abnormal ovaries are serially sectioned at approximately 1-cm intervals. Note that large cystic lesions may contain abundant fluid which can exude under pressure. The characteristics of the fluid should be noted, e.g., serous, mucinous, or bloody. Scissors can also be of use in opening and blocking cystic lesions.
- If the ovary is predominantly solid, the color and consistency of the lesion is noted as is the presence or absence of areas of hemorrhage or necrosis.
- If the lesion is both solid and cystic, record the proportion of each.
- If the lesion is cystic, note whether the cyst is unilocular, multilocular, or whether a main cyst is present together with multiple smaller daughter cysts. The presence of residual ovary should be documented.
- With a cystic lesion, describe whether the internal surface of the cysts is smooth or whether they contain papillary projections or nodular thickenings. The percentage of the internal surface involved by papillary areas should be documented.
- The presence of other elements within the lesion is recorded, e.g., hair and teeth in dermoid cysts.
- Ovaries removed prophylactically in those with a hereditary predisposition to develop ovarian cancer are serially sectioned parallel to the short axis at 2–3 mm intervals. The presence of any gross abnormality is noted. The entire ovaries (and fallopian tubes) should be submitted for histological examination since very small neoplasms, which are not recognizable grossly, may be present, especially in the fallopian tube.
- Grossly normal ovaries removed during a hysterectomy for benign disease or for uterine or cervical neoplasms are bisected longitudinally and inspected.
- Any paraovarian cystic or solid lesions should be treated in a similar way.
- Omentum is weighed, measured in three dimensions (cm), and sectioned thinly. The presence of obvious tumor deposits, grittiness, or areas of thickening or induration is noted. If gross tumor involvement is evident, the size of the largest tumor deposit should be noted as this has implications for substaging of stage III ovarian carcinoma.
- If the uterus is present, it should be measured in three dimensions and weighed. The serosa should be examined for tumor involvement. If a synchronous endometrial tumor is identified, this should be dealt with as per the uterine carcinoma protocol (Chap. 24).
- If the appendix is submitted, it should be measured and examined for gross tumor involvement, either mucosal or serosal.

Blocks for histology (Fig. 22.2):

- As stated previously, ovaries and fallopian tubes removed prophylactically in those with a hereditary predisposition to develop ovarian cancer are examined in their entirety. The fimbria of the fallopian tube should be transected and sectioned longitudinally, while the remainder of the tubes should be sectioned transversely.
- A single section through the long axis of the ovary suffices for grossly normal ovaries removed as part of a hysterectomy specimen for benign disease or uterine or cervical cancer.

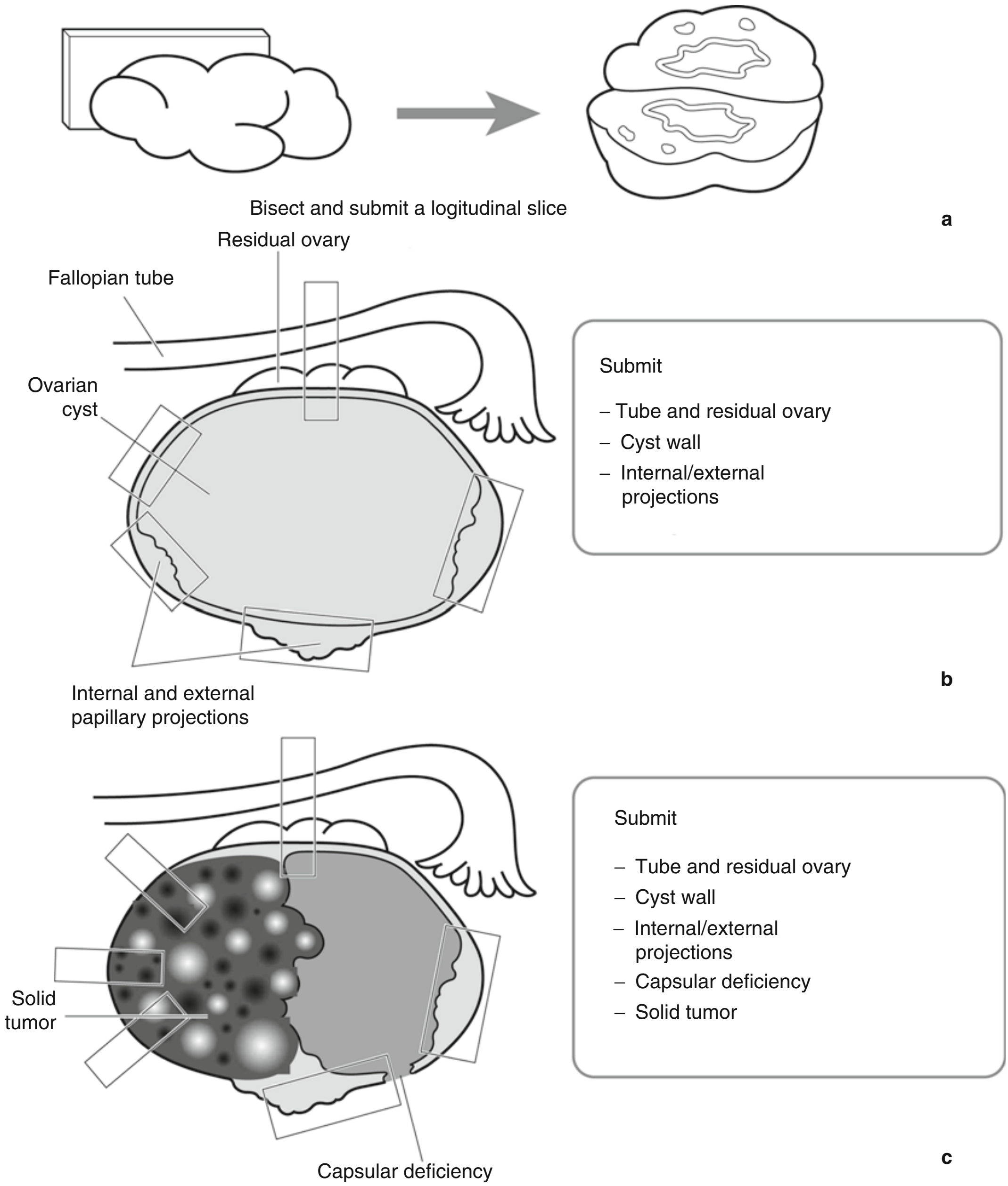

Fig. 22.2 Blocking of ovarian tissues: (**a**) normal; (**b**) cystic; (**c**) mixed solid/cystic (Reproduced, with permission, from Allen and Cameron (2004))

- For suspected benign cystic lesions (thin-walled unilocular or multiloculated cysts) without thickenings or papillary excresences on the external or internal surfaces, representative sampling suffices. With multiloculated mucinous lesions, extensive blocking is required as malignant areas may be focal and coexist with benign and borderline areas. The degree of sampling necessary is controversial, but one block per cm of lesions up to 10 cm in maximum dimension and 2 blocks per cm of lesions greater than this have been suggested.

- For those lesions with papillary excrescences on the internal or external surface, multiple blocks are taken, especially from the papillary areas. This is important since these areas often represent borderline foci.
- For grossly malignant neoplasms, representative sections are taken, usually one section per cm of tumor.
- Special attention should be given to the sampling of areas of capsular breech or infiltration by tumor and a significant number of blocks should include the capsule.
- In neoplasms with a variegated appearance, grossly different areas are blocked.
- Paraovarian lesions should be blocked similarly.
- Representative sections should be taken from the fallopian tubes, including the fimbrial end.
- Representative sections of omentum are taken. If the omentum is grossly normal, three or four blocks suffice. Any tumor deposits or areas of thickening, grittiness, or induration are preferentially sampled. If histological examination reveals implants or borderline lesions, then multiple additional sections may have to be examined.
- If the uterus is present, any serosal abnormality should be sampled. If a synchronous endometrial carcinoma is present, this should be sampled as per the uterine carcinoma protocol. If it appears grossly normal, representative samples from the cervix, endometrium, and full thickness of myometrium should be taken.

Histopathology report:

- Side of tumor – Right/left or bilateral.
- Dimensions of tumor – Measure in three dimensions (cm).
- Gross appearance – Solid/cystic, color, and consistency, presence of hemorrhage or necrosis.
- Tumor type – It is stressed that a wide range of benign and malignant tumors may arise within the ovary. The ovary is also a relatively common site for metastatic carcinomas.
- Tumor grade – There is no universally agreed grading system for ovarian adenocarcinomas and different systems exist. It is recommended in the Royal College of Pathologists Cancer Dataset in the UK that serous carcinomas be graded as either low grade or high grade, a distinction based primarily on assessment of nuclear atypia in the worst area of the tumor; it is stressed that low-grade and high-grade serous carcinomas are two distinct tumor types with a different underlying pathogenesis rather than low-grade and high-grade variants of the same neoplasm. Clear cell carcinoma is regarded as grade 3. Endometrioid carcinoma should be graded using the FIGO grading system used to grade endometrial endometrioid carcinomas into grades I, II, or III, and mucinous adenocarcinomas are graded in a similar manner.
- Capsule – It should be stated whether the capsule is intact, deficient, or breeched by tumor.
- Lymphovascular invasion – Present/not present.
- Lymph nodes – Mention sites and presence or absence of tumor involvement.
- Omentum – Involved/not involved by tumor and maximum size of tumor deposits (cm).
- Other organs (fallopian tube, uterus, cervix) – Involved/not involved by tumor.
- Peritoneal washings/ascitic fluid – Involved/not involved by tumor.
- Other pathology – The presence of coexistent pathology should be mentioned. Endometrioid and clear cell carcinomas may arise in endometriosis.

FIGO stage for borderline and malignant ovarian epithelial/stromal neoplasms

Primary tumor cannot be assessed.

No evidence of primary tumor.

I		Tumor limited to the ovaries
	IA	Tumor limited to one ovary, capsule intact, no tumor on ovarian surface; no malignant cells in ascites or peritoneal washings
	IB	Tumor limited to both ovaries, capsule intact, no tumor on ovarian surface; no malignant cells in ascites or peritoneal washings
	IC	Tumor limited to one or both ovaries with any of the following: Capsule ruptured, tumor on ovarian surface; malignant cells in ascites or peritoneal washings
II		Tumor involves one or both ovaries with pelvic extension
	IIA	Extension and/or implants on uterus and/or tube(s); no malignant cells in ascites or peritoneal washings
	IIB	Extension to other pelvic tissues; no malignant cells in ascites or peritoneal washings
	IIC	Pelvic extension (2a or 2b) with malignant cells in ascites or peritoneal washings
III		Tumor involves one or both ovaries with microscopically confirmed peritoneal metastases outside the pelvis and/or regional lymph node metastasis
	IIIA	Microscopic peritoneal metastasis beyond pelvis
	IIIB	Macroscopic peritoneal metastasis beyond pelvis, 2 cm or less in greatest dimension
	IIIC	Peritoneal metastasis beyond pelvis more than 2 cm in greatest dimension and/or regional lymph node metastasis
IV		Distant metastasis (excludes peritoneal metastasis)

Regional nodes: obturator, common iliac, external iliac, lateral sacral, para-aortic, inguinal.

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23 Fallopian Tube

Oison Houghton and W. Glenn McCluggage

23.1 Anatomy

The fallopian tubes are paired structures that extend from the uterine cornu to the medial pole of the ovary. They are generally 8–12 cm in length. There are four segments to the fallopian tube, which, from medial to lateral, are the intramural segment, the isthmus, the ampulla, and the infundibulum (Fig. 23.1). The lateral aspect of the infundibulum is fimbriated and opens into the pelvic cavity. Microscopically the fallopian tube consists of mucosa, submucosa, muscularis, and serosa, which is covered by a single layer of mesothelial cells.

Lymphovascular drainage:

The fallopian tubes are supplied both by a branch of the ovarian artery and a branch of the uterine artery. The venous drainage is similar. The lymphatic channels draining the fallopian tube descend within the mesosalpinx behind the ovary where they form part of the subovarian plexus.

O. Houghton (✉) • W.G. McCluggage
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: oisin.houghton@belfasttrust.hscni.net; glenn.mccluggage@belfasttrust.hscni.net

23.2 Clinical Presentation

Most fallopian tubes submitted as surgical pathology specimens are a component of larger specimens, and symptomatology specifically related to pathology in the fallopian tube is relatively rare. However, ectopic pregnancies occur in the fallopian tube and usually present with abdominal pain. If there is associated hemoperitoneum, the pain is severe and associated with signs of peritonism. Other fallopian tube pathologies may result in abdominal pain or patients can present with infertility. With neoplasms or other pathological lesions causing enlargement of the fallopian tube there may be a palpable abdominal mass with or without associated ascites. Fallopian tube pathology is occasionally discovered incidentally during abdominal or pelvic imaging.

23.3 Clinical Investigations

- Serum CA-125 measurements: An increase in serum CA-125 may be found with primary fallopian tube carcinoma.
- Abdominal USS scan: Ectopic pregnancies may be seen on ultrasound scan and there is usually a positive pregnancy test. Primary fallopian tube malignancies may also be identified on scanning as well as conditions such as hydrosalpinx or pyosalpinx.
- Laparoscopy: This is undertaken in order to directly visualize the fallopian tube.
- Fine needle aspiration cytology: This can be performed either at laparoscopy or under

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_23, © Springer-Verlag London 2013

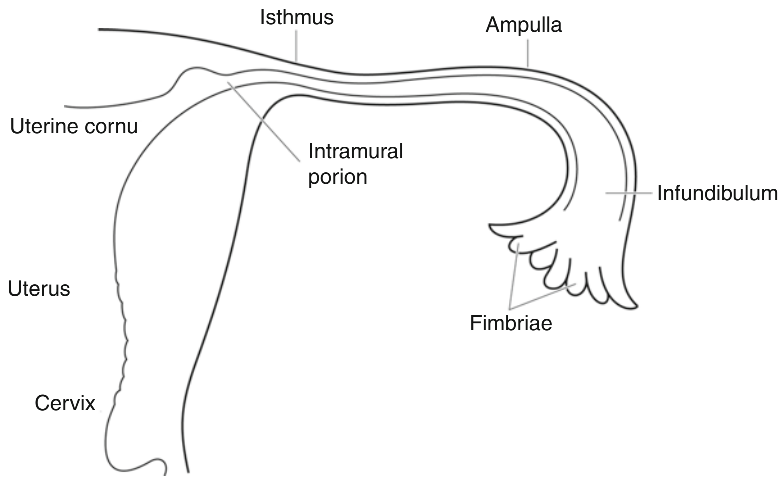

Fig. 23.1 Fallopian tube anatomy (Reproduced, with permission, from Allen and Cameron (2004))

ultrasound guidance. Paratubal cysts or cystic lesions of the fallopian tube, such as hydrosalpinx, are especially liable to be aspirated.

23.4 Pathological Conditions

23.4.1 Non-neoplastic Conditions

Paratubal or fimbrial cysts: These are extremely common, especially paratubal cysts. They are thin-walled cysts usually containing serous fluid. They can be multiple and histologically are lined by a single layer of bland, usually ciliated, epithelial cells. The epithelial lining may be attenuated. Cystic Walthard's rests, lined by transitional epithelium, are also extremely common with a paratubal location.

Hydrosalpinx and pyosalpinx: Hydrosalpinx is dilatation of the fallopian tube. Although rarely of unknown etiology, it is usually secondary to obstruction of the tube with subsequent dilatation. One of the most common causes is pelvic inflammatory disease. Other causes include endometriosis, tumor, or a lesion within the uterus. Grossly the fallopian tube is dilated, sometimes massively so. There may be associated hemorrhage within the lumen, resulting in hematosalpinx. With superimposed infection, pus can accumulate within the lumen and wall of the tube, resulting in pyosalpinx. Histology shows marked dilatation of the lumen of the tube with edema within the wall and numerous polymorphs in the case of pyosalpinx.

Ectopic pregnancy: With tubal ectopic pregnancies, the fallopian tube is generally dilated, sometimes with an area of rupture. Sectioning the tube reveals blood clot and sometimes grossly visible placental tissue within the lumen. Histology shows blood clot, decidua, chorionic villi, and a placental site reaction. Fetal parts may be identified.

23.4.2 Neoplastic Conditions

The fallopian tube has traditionally been considered a relatively rare primary site of neoplastic lesions, both benign and malignant. However, there is now growing evidence and an increasing realization that many high-grade serous carcinomas of the ovary and peritoneum actually arise from the fimbria of the fallopian tube, although these rarely result in a dominant tubal mass.

Benign tumors: Benign tumors of the fallopian tube are rare. They include adenomatoid tumors that are usually firm grey-white or yellow well-circumscribed nodules that involve part of the wall of the tube. A variety of other benign neoplasms, similar to those found within the ovary, may rarely involve the fallopian tube, the most common being serous cystadenofibroma.

Malignant tumors: Most primary malignant neoplasms of the fallopian tube are high-grade serous carcinomas, similar to those that occur in the ovary, although endometrioid carcinomas also occur and a variety of other morphological subtypes have been described. Primary carcinomas of the fallopian tubes have traditionally been considered uncommon. However, it is increasingly recognized that many ovarian and peritoneal high-grade serous carcinomas originate in the fimbrial end of the fallopian tube from a precursor in situ lesion known as serous tubal intraepithelial carcinoma (serous TIC). Fallopian tube carcinomas usually occur in the middle-aged or elderly and are more common in nulliparous women. There is an increased incidence of fallopian tube malignancies in women with BRCA1 and BRCA2 mutations.

Treatment: Treatment of malignant tubal neoplasms is usually total abdominal hysterectomy, bilateral salpingo-oophorectomy, and omentectomy. Surgical staging is similar to that performed for ovarian cancer, including peritoneal washings. Postoperative chemotherapy is often necessary since tumors are frequently advanced.

Prognosis: The prognosis of fallopian tube carcinoma is generally poor and is related to the advanced tumor stage at presentation.

23.5 Surgical Pathological Specimens: Clinical Aspects

23.5.1 Biopsy Specimens

Segments of fallopian tube are removed during sterilization procedures (tubal ligation) performed either via an open approach, usually during Caesarean section, or at laparoscopy. Tubal or paratubal cysts may be aspirated at laparotomy or laparoscopy or under ultrasound guidance and the fluid sent for cytological examination. Such cysts may also be removed leaving the fallopian tube intact.

23.5.2 Resection Specimens

Many fallopian tubes submitted for pathological examination are part of a hysterectomy and bilateral salpingo-oophorectomy performed for either benign or malignant disease. Sometimes just the fallopian tube and ovary are removed. The fallopian tube alone may be resected in tubal ectopic pregnancy or where benign cysts or indeterminate fallopian tube nodules are present. When a malignant neoplasm of the fallopian tube is suspected, hysterectomy and bilateral salpingo-oophorectomy with full staging, including omentectomy, peritoneal washings with or without pelvic and/or para-aortic lymphadenectomy, is usually performed. Fallopian tubes may be removed prophylactically, along with the ovaries, in those with a hereditary predisposition to developing ovarian cancer, e.g., those with BRCA1 or BRCA2 gene mutations.

23.6 Surgical Pathology Specimens: Laboratory Protocols

23.6.1 Biopsy Specimens

FNA specimens are centrifuged and the specimens examined cytologically, usually with Giemsa and Papanicolaou stains. Tubal ligations are measured, the presence of any gross abnormality noted, and a single transverse section is examined histologically to document that the fallopian tube is present. If the tubal fimbria is present, this should be submitted for histological examination.

23.6.2 Resection Specimens

Specimen:
The specimen may consist of fallopian tube only or, more commonly, both fallopian tubes are present as part of a hysterectomy and bilateral salpingo-oophorectomy. In other cases, only one ovary and fallopian tube are removed. If malignancy is suspected, omentectomy and pelvic and/or para-aortic lymphadenectomy may be performed.

Initial procedure and description:

- The length of each fallopian tube is recorded.
- The presence or absence of a fimbriated end is noted.
- If a sterilization clip is present this is documented.

- Note the presence of any gross external abnormality, such as rupture, cyst, nodule, or tumor.
- If the fallopian tube is dilated, the diameter is measured.
- If necessary any gross lesion may be photographed.
- If tumor is present, note the presence or absence of serosal involvement or breech.
- If grossly normal, the fallopian tube is serially sectioned at 3–5 mm intervals.
- The presence of any gross abnormality seen on sectioning, e.g., luminal occlusion, pus, placental tissue, or hemorrhage, is noted.
- If an ectopic pregnancy is suspected, note the presence or absence of tubal rupture.
- If a tumor is present, its location, the size in three dimensions (cm), the color, and consistency are noted, as is the presence or absence of hemorrhage and necrosis.
- If a cyst is present, it is measured and documented as unilocular or multilocular. The relationship to the fallopian tube should be stated. The character of the internal and external surfaces is noted as is the consistency of the fluid.
- Fallopian tubes removed prophylactically in those with a predisposition to developing ovarian cancer (BRCA1 or BRCA2 mutation or positive family history of ovarian cancer) are serially sectioned transversely at 2–3 mm intervals. The entire fallopian tubes and ovaries should be examined histologically since small neoplasms that are not visible grossly may be present, especially involving the fimbria. Amputation of the distal 2 cm and longitudinal sectioning of the infundibulum and fimbrial end allow for maximal visualization of the tubal fimbrial epithelium.

Blocks for histology:

- As stated, fallopian tubes removed prophylactically in those with a hereditary predisposition to develop ovarian cancer should be examined in their entirety.
- A single transverse section is examined in cases of tubal ligation.
- If the fallopian tube is grossly normal, one or two transverse sections and a section from the fimbria are examined in a single cassette.

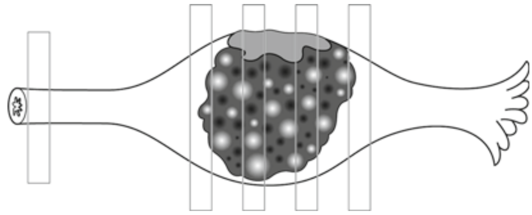

Fig. 23.2 Blocking the fallopian tube in an ectopic pregnancy (Reproduced, with permission, from Allen and Cameron (2004))

- Any gross lesion, e.g., cyst or nodule, is blocked to show its relationship to the tube.
- In cases of suspected ectopic pregnancy, several sections should be taken (Fig. 23.2). Blood clot and placental tissue identified grossly are sampled as is any site of tubal rupture. A section should also be taken from an area of grossly normal proximal tube. If trophoblastic tissue is not identified in initial sections, then extra blocks are taken.
- For malignant fallopian tube neoplasms, at least one block per cm of tumor is submitted for histology. These are taken preferentially from any gross areas of serosal involvement to show the most extensive tumor infiltration. Blocks are also taken to demonstrate origin of the tumor from the fallopian tube epithelium. Blocks of uninvolved fallopian tube should also be submitted.
- In neoplasms with a variegated appearance, grossly different areas are blocked.

Histology report:

- Side of tumor – right/left or bilateral.
- Dimensions of tumor – measure in three dimensions (cm).
- Gross appearance – solid/cystic, color, and consistency, presence of hemorrhage or necrosis.
- Tumor type – most primary fallopian tube malignancies comprise high-grade serous carcinomas.
- Presence of adjacent in situ carcinoma.
- Tumor grade – grading is identical to the approach used for ovarian carcinomas.

- Extent of local tumor spread – involvement of mucosa, submucosa, muscularis, serosa, surrounding structures.
- Lymphovascular invasion – present/not present.
- Lymph nodes – sites, number identified, and number involved by tumor.
- Involvement of other organs, e.g., ovary, omentum.
- Peritoneal washings/ascitic fluid – involved/not involved by tumor.

The British Association of Gynaecological Pathologists, British Gynaecological Cancer Society, and gynecological clinical reference group of the National Cancer Intelligence Network recommend that FIGO staging be used for gynecological cancers rather than TNM.

FIGO stage for fallopian tube carcinoma

Primary tumor cannot be assessed

No evidence of primary tumor

I		Tumor confined to fallopian tube(s)
	IA	Tumor limited to one tube, without penetrating the serosal surface
	IB	Tumor limited to both tubes, without penetrating the serosal surface
	IC	Tumor limited to one or both tube(s) with extension onto or through the tubal serosa, or with malignant cells in ascites or peritoneal washings
II		Tumor involves one or both fallopian tube(s) with pelvic extension
	IIA	Extension and/or metastasis to uterus and/or ovaries
	IIB	Extension to other pelvic structures
	IIC	Pelvic extension (2a or 2b) with malignant cells in ascites or peritoneal washings
III		Tumor involves one or both fallopian tube(s) with peritoneal implants outside the pelvis and/or positive regional nodes
	IIIA	Microscopic peritoneal metastasis beyond pelvis
	IIIB	Macroscopic peritoneal metastasis beyond pelvis, 2 cm or less in greatest dimension
	IIIC	Peritoneal metastasis beyond pelvis more than 2 cm in greatest dimension and/or regional lymph node metastasis
IV		Distant metástasis[a] (excludes peritoneal metastasis)

Notes:

[a]Liver capsule metastasis is stage III; liver parenchymal metastasis is stage IV; pleural effusion must have positive cytology for stage IV

[b]Regional lymph nodes are: obturator, common iliac, external iliac, lateral sacral, para-aortic, inguinal nodes

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24 Uterus

Oisin Houghton and W. Glenn McCluggage

24.1 Anatomy

The uterus is situated in the pelvic cavity between the rectum posteriorly and the urinary bladder anteriorly. The body of the uterus (uterine corpus) comprises the superior part and this is joined to the cervix, which comprises the inferior portion of the uterus (Fig. 24.1). The length of the uterus varies widely depending on the parity and the menopausal status but generally ranges from 5 cm to 15 cm in those uteri which are not involved by any specific pathologic process. The weight of the uterus also varies widely between 20 g and 120 g. Multigravid uteri are considerably larger than nulligravid uteri. The uterus is lined by an inner endometrium composed of endometrial glands and stroma. Most of the wall is composed of myometrial smooth muscle. The lumen of the uterus is connected to the lumen of the fallopian tubes. The part of the uterine body above the origin of the fallopian tubes is the fundus. The lower portion of the uterus which merges with the cervix is known as the isthmus. The anterior surface of the uterus is covered by peritoneum which reflects forward onto the bladder. The peritoneal surface extends lower posteriorly before being reflected onto the rectosigmoid. The lower peritoneal reflection on the posterior aspect can be used as a means to distinguish the anterior and posterior surfaces of the uterus. The anterior and posterior peritoneal linings merge laterally to form the broad ligaments which extend to the pelvic side wall.

Lymphovascular drainage:

The uterus is supplied by the uterine arteries. These are bilateral paired arteries which arise from the internal iliac arteries. The veins of the uterus drain into the uterovaginal venous plexus which is located within the broad ligament. These veins ultimately open into the internal iliac veins. Uterine lymphatics drain into the pelvic and periaortic lymph nodes (Fig. 24.2).

24.2 Clinical Presentation

Clinical features related to uterine pathology are most commonly those of abnormal uterine bleeding. In premenopausal patients, this may take the form of menorrhagia (heavy periods), dysmenorrhea (painful periods), or a variety of other forms of abnormal uterine bleeding. In postmenopausal patients, the most common symptomatology is postmenopausal bleeding. This should always be taken seriously and uterine malignancy excluded.

O. Houghton (✉) • W.G. McCluggage
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: oisin.houghton@belfasttrust.hscni.net; glenn.mccluggage@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_24, © Springer-Verlag London 2013

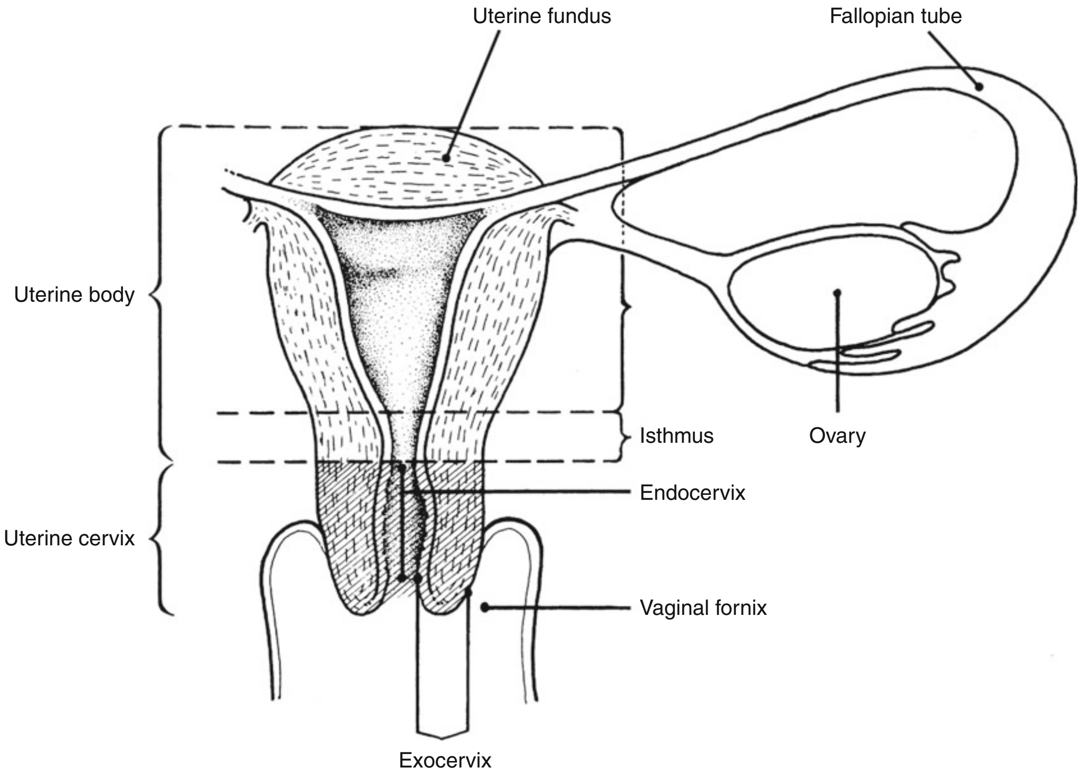

Fig. 24.1 Uterine anatomy (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

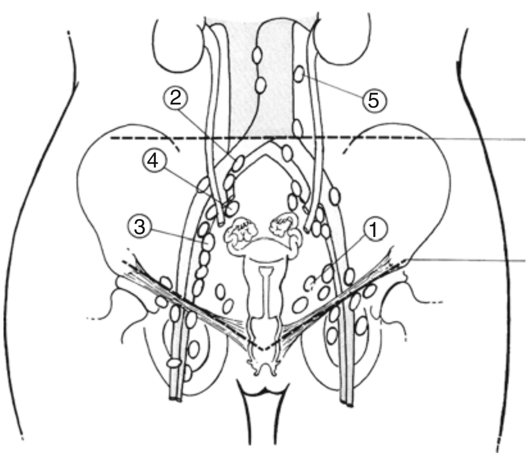

Fig. 24.2 Uterus – regional lymph nodes (*1*) Hypogastric internal iliac. (*2*) Common iliac. (*3*) External iliac (*4*) Lateral sacral. (*5*) Para-aortic (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

Other symptomatologies related to uterine pathology include a palpable abdominal or pelvic mass, pain within the pelvis or abdomen (often deep seated), a feeling of fullness within the abdomen, and uterine prolapse. Uterine pathology may also be associated with symptoms such as constipation, urinary frequency, or infertility.

24.3 Clinical Investigations

- Ultrasound scan: Transvaginal ultrasound scan is often performed in patients with abnormal uterine bleeding and other symptomatology related to the uterus. This may show focal lesions or diffuse thickening of the endometrium or myometrium. The endometrial thickness and endometrial stripe can be measured and related to the menopausal status of the patient.

- Endometrial sampling: Usually sampling of the endometrium to provide material for histology is necessary for a definitive pathological diagnosis, especially in cases of abnormal uterine bleeding such as postmenopausal bleeding. In some centers, endometrial brushings with cytological examination is carried out, but this is rare. Previously the most common means of sampling the endometrium was by dilatation and curettage (D&C). This requires a general anesthetic and is performed as an inpatient procedure. However, pipelle endometrial biopsies can now be performed as an outpatient procedure without the need for a general anesthetic. Histological sampling of the endometrium may also be performed in women who are being treated with tamoxifen or other hormonal agents, or those who are infertile.
- Hysteroscopy: In many cases, hysteroscopy with direct visualization of the endometrium is performed and biopsies are taken at this procedure.
- MRI scanning: In cases where endometrial sampling confirms a malignancy, radiological staging procedures are carried out, and this usually comprises MRI scanning.

24.4 Pathological Conditions

24.4.1 Non-neoplastic Conditions

Cyclical or noncyclical endometrium: Endometrial sampling may reveal normal proliferative, secretory, or menstrual endometrium or postmenopausal atrophic endometrium. Endometrial appearances can also reflect the use of exogenous hormones or the presence of abnormal levels of endogenous hormones. Noncyclical endometria may be a manifestation of conditions such as anovulatory cycles or luteal phase deficiency.

Endometrial polyps: Endometrial polyps are benign endometrial lesions which often result in abnormal uterine bleeding and are most common postmenopausally. They may be single or multiple. They are often removed piecemeal and should be sampled in their entirety when small. With larger lesions selective pathological sampling is performed. Occasionally carcinoma arises in a preexisting polyp. There is an association between endometrial polyp formation and the use of tamoxifen.

Endometritis: This is an inflammatory condition of the endometrium which may result in abnormal uterine bleeding. Generally the presence of plasma cells is required for a definitive pathological diagnosis.

Products of conception: Products of conception may be submitted for histological examination from therapeutic or spontaneous abortions.

Adenomyosis: Adenomyosis is a common condition characterized by extension of endometrial glands and stroma into the underlying myometrium. Usually this results in diffuse uterine enlargement, although occasionally well-circumscribed nodular masses are formed – so-called adenomyomas. Typically adenomyosis results in a trabeculated appearance to the myometrium because of the associated smooth muscle hypertrophy around the pale or hemorrhagic adenomyotic foci. Adenomyosis is most common in the reproductive age group and is thought to develop under estrogenic influence. It is a common cause of irregular uterine bleeding.

24.4.2 Neoplastic Conditions

A range of benign and malignant neoplasms can involve both the endometrium and myometrium.

Endometrial hyperplasias: These are a spectrum of preneoplastic conditions which confer an increased risk of subsequent development of endometrial adenocarcinoma of endometrioid type. The WHO classification of endometrial hyperplasias is used with hyperplasias categorized as simple or complex in type based on architectural features. These are further subdivided into typical and atypical forms, depending on the presence or absence of cytological atypia.

Endometrial carcinomas: A variety of different carcinomas may arise from the endometrium. There are two main types (Type I and Type II), although not all neoplasms fall neatly into either category with mixed tumors being not uncommon. The prototype *type I endometrial carcinoma is endometrioid adenocarcinoma* and the prototype *type II is serous carcinoma*. Type I and Type II neoplasms have different clinicopathologic characteristics, although it is emphasized that there may be overlap.

In general, Type II carcinomas behave in a much more aggressive manner. Endometrial carcinomas (especially Type I) are associated with estrogen excess and obesity, hypertension, diabetes, and unopposed estrogen hormone therapy. There may also be an association with estrogen-secreting ovarian tumors, mainly those within the sex cord-stromal group. Type II carcinomas usually arise in an older age group and are not associated with estrogen excess. Endometrial carcinomas are increasing in incidence and are more common in women of low parity, high socioeconomic status, and in the postmenopausal age group. Occasionally there is a familial predisposition to developing endometrial cancer. Endometrial carcinoma is the second most common neoplasm to arise in patients with hereditary nonpolyposis colorectal cancer syndrome (Lynch syndrome); in fact, the incidence of endometrial cancer in women with Lynch syndrome approximates that of colonic cancer.

Type I endometrial carcinomas usually arise in a background of endometrial hyperplasia. This association is not apparent with Type II endometrial cancers which usually arise within an atrophic endometrium from a precursor known as serous endometrial intraepithelial carcinoma (serous EIC).

Endometrial carcinomas may be polypoid in appearance and project into the endometrial cavity. Conversely some tumors diffusely infiltrate the underlying myometrium.

Uterine carcinosarcomas (Malignant Mixed Mullerian Tumors) are highly aggressive neoplasms composed of carcinomatous and sarcomatous elements. Although traditionally regarded as a subtype of uterine sarcoma, there is now ample evidence that these are in fact metaplastic carcinomas. They are usually bulky neoplasms in elderly patients, often with a polypoid appearance and exhibiting deep myometrial infiltration and vascular invasion.

Uterine leiomyomas: Uterine leiomyomas (fibroids) are benign smooth muscle tumors and one of the most common benign neoplasms to occur in women, especially within the reproductive and early postmenopausal age group. They are often multiple but may be solitary. Uterine leiomyomas can be submucosal, intramural, or subserosal. Occasionally they may separate from the uterus and lie within the pelvic cavity – so-called parasitic leiomyomas. They are usually well circumscribed, white in color with a typical firm whorled appearance and bulge above the surrounding myometrium. Degeneration may result in a variety of different gross appearances.

Malignant uterine mesenchymal lesions: Malignant uterine mesenchymal lesions comprise endometrial stromal sarcomas, leiomyosarcomas, undifferentiated endometrial or uterine sarcomas, and a variety of rare sarcomas, such as rhabdomyosarcoma. Endometrial stromal sarcomas usually cause diffuse uterine enlargement due to infiltration of the myometrium by irregular tongues of neoplastic endometrial stromal cells. There is often marked vascular permeation and the tumor may extend beyond the uterus. Leiomyosarcomas and undifferentiated sarcomas are generally high-grade malignant neoplasms, usually comprising a dominant mass with or without satellite nodules. Grossly areas of hemorrhage and necrosis are common.

Other uterine neoplasms: Endometrial stromal nodules are benign well-circumscribed proliferations of endometrial stroma. Histologically they are identical to endometrial stromal sarcomas and are differentiated from the latter due to their circumscription and lack of infiltrative myometrial permeation or vascular invasion. They may involve both the endometrium and myometrium or may be predominantly located within the myometrium.

Treatment: Treatment of malignant uterine lesions (carcinomas, sarcomas, carcinosarcomas) usually comprises total hysterectomy, either using an abdominal, vaginal, or laparoscopic approach, and bilateral salpingo-oophorectomy. Peritoneal washings are usually performed as part of the staging procedure. Pelvic and/or para-aortic lymph node resection and omentectomy may be performed, especially when preoperative endometrial biopsy shows a high-grade endometrioid carcinoma or a Type II carcinoma or when radiological investigations suggest cervical invasion or extra-uterine spread. Preoperative staging comprises

MRI scanning to assess the extent of tumor spread. Postoperative radiotherapy or chemotherapy may be administered depending on the histological subtype and the stage. This is especially so with high-grade or Type II endometrial carcinomas or where there is cervical involvement, deep myometrial penetration, or extrauterine spread. The FIGO staging system for endometrial carcinoma is used. The British Association of Gynaecological Pathologists, British Gynaecological Cancer Society, and gynecological clinical reference group of the National Cancer Intelligence Network recommend that FIGO staging be used for gynecological cancers rather than TNM. Occasionally with advanced tumors, surgical resection is not feasible and primary treatment is radiotherapy or chemotherapy. All cases should be discussed at a multidisciplinary gynecological oncology meeting.

Uterine leiomyomas may be treated by hysterectomy or, in those who wish to preserve their fertility, medical treatment or myomectomy (simple removal of the fibroids). Adenomyosis is often not expected clinically and is only diagnosed on a hysterectomy specimen performed for menorrhagia.

Troublesome menorrhagia can in many cases be managed by hormonal agents or endometrial ablation (balloon dilatation, laser ablation, or hysteroscopic resection) with resort to simple hysterectomy in a minority of cases with persistence or recurrence of symptoms.

Prognosis: The prognosis of low-grade, early-stage (Stage IA) endometrial adenocarcinoma of endometrioid type is excellent, but overall survival decreases with increasing tumor stage. Prognosis is poor with Type II endometrial adenocarcinomas, especially uterine serous carcinoma. Leiomyosarcoma, undifferentiated uterine sarcoma, and carcinosarcoma usually have a poor prognosis, especially if large and of advanced stage. Endometrial stromal sarcomas are low-grade neoplasms. The overall prognosis is favorable, although there is a significant risk of late recurrence after many years and subsequent metastasis with these tumors. Adjuvant progesterone therapy may be indicated, since these tumors are commonly hormone responsive and estrogen and progesterone receptor positive.

24.5 Surgical Pathology Specimens: Clinical Aspects

24.5.1 Biopsy Specimens

Endometrium pipelle biopsies may be performed blind or under hysteroscopic visualization. Endometrial D&C is performed under general anesthetic. Tissue from therapeutic abortions (performed at D&C) or spontaneous abortions may also be received.

24.5.2 Resection Specimens

The endometrium and superficial myometrium may be removed as multiple chippings at transcervical resection of the endometrium (TCRE). Most uterine specimens comprise a hysterectomy, performed either abdominally or vaginally. Occasionally the cervix is left behind. This is known as a subtotal hysterectomy. The ovaries and fallopian tubes may also be removed with the uterus and cervix. If the uterus is being removed as part of a tumor operation, then peritoneal washings are often sent. The omentum may also be removed as well as pelvic and/or para-aortic lymph nodes. Sometimes for uterine fibroids, myomectomy is performed, where the fibroid is removed, but the uterus is left in situ. A radical hysterectomy is often indicated for cervical cancer or for endometrial cancer involving the cervix. This involves removing a segment of the upper vagina and the parametrium on both lateral aspects of the uterus.

24.6 Surgical Pathology Specimens: Laboratory Protocols

24.6.1 Biopsy Specimens

Endometrial pipelle or curettage biopsies are weighed and processed intact for histopathogical examination. Very scanty pipelle samples may need to be filtered from the fixative. Similarly, TCRE chippings are weighed and all examined. If histology of these samples shows the presence of fat, it may be omental in origin and implies potential perforation of the uterine wall. The clinician should be alerted without delay. For suspected products of conception, the specimen is weighed and the tissue is examined grossly with particular reference to the presence of blood clot, decidua, placental tissue, and fetal parts. Small specimens can be examined in their entirety, but with larger specimens representative sections are taken in order to confirm the presence of products of conception. When vesicles are identified, note their maximum diameter – if 2–3 mm or more, this raises the possibility of a molar pregnancy and extensive sampling is required in order to confirm the presence of a hydatidiform mole and distinguish a partial from a complete mole. Flow cytometric examination or image cytometry of such specimens may be indicated since partial moles are often triploid, whereas complete moles and hydropic abortions are usually diploid. A small minority of molar gestations leads to persistent trophoblastic disease and rarely choriocarcinoma or placental site trophoblastic tumor.

24.6.2 Resections Specimens

The uterine corpus is usually removed together with the cervix as part of a hysterectomy. Occasionally the cervix is left in situ and a subtotal hysterectomy is performed. Myomectomies may also be performed, especially for uterine fibroids.

Initial procedure and description:

- The specimen is weighed.
- The specimen is measured in three dimensions (cm), i.e., superior to inferior, medial to lateral, anterior to posterior.
- The specimen is orientated. The peritoneal reflection extends lower on the posterior aspect of the uterus than anteriorly.
- Ovaries and tubes, if present, are inspected and dealt with as described in Chap. 22.
- The presence of any external abnormality is noted, e.g., tumor on the serosa, serosal adhesions.
- The os of the cervix and the uterine cavity are entered using a probe.
- Cutting along the probe, the uterus is opened longitudinally either along the lateral axis or along the anteroposterior axis from the external os to the cornu.
- The nature of the endometrium is commented on. The thickness can be measured and the presence of tumor, polyp, or any focal lesion described and measured (cm).
- The presence or absence of uterine fibroids is noted. These are counted and the dimensions of the largest stated. Usually uterine fibroids have a typical white whorled appearance and bulge above the surrounding myometrium. The presence of any grossly abnormal areas such as hemorrhage, necrosis, abnormal coloration, calcification, or cystic degeneration is recorded.
- Any cervical abnormalities are noted as described in Chap. 25.
- If a tumor is grossly visible, the dimensions are measured. If this comprises an endometrial carcinoma (usually known from a previous biopsy specimen), then the depth of myometrial invasion is ascertained (inner or outer half) as is the presence or absence of gross cervical involvement. Assessment of the depth of myometrial invasion can be difficult as myometrial invasion may be subtle and these uteri are often atrophic with a thin, compressed myometrium. Any obvious spread to the ovaries or fallopian tubes is documented.
- The presence of tumor infiltrating to the serosal surface of the uterus is also noted and in those tumors which do not extend to the serosal surface the minimum thickness of uninvolved myometrium is measured (mm).
- The presence of grossly visible foci of adenomyosis is recorded.
- The uterus is then sliced either transversely or longitudinally (depending on personal preference) at 3–5 mm intervals. During this procedure, the presence of previously unidentified leiomyomas and the depth of invasion of endometrial carcinomas into the myometrium can be better assessed.
- Photography may be undertaken.
- Myomectomy specimens are enumerated, weighed, measured, and described.
- The number of lymph nodes (if removed) from each site should be documented.
- The omentum (if removed) should be measured and weighed. It should be carefully sliced and the presence of any tumor nodules or any other gross lesion documented.

Blocks for histology:

- When the hysterectomy was performed for benign disease, two representative sections showing the endometrial–myometrial junction and, if possible, the full-wall thickness, are examined. Two blocks of cervix showing the transformation zone (one from the anterior and one from the posterior lip) are also taken (Fig. 24.3).
- When grossly visible adenomyosis is present, this is sampled.

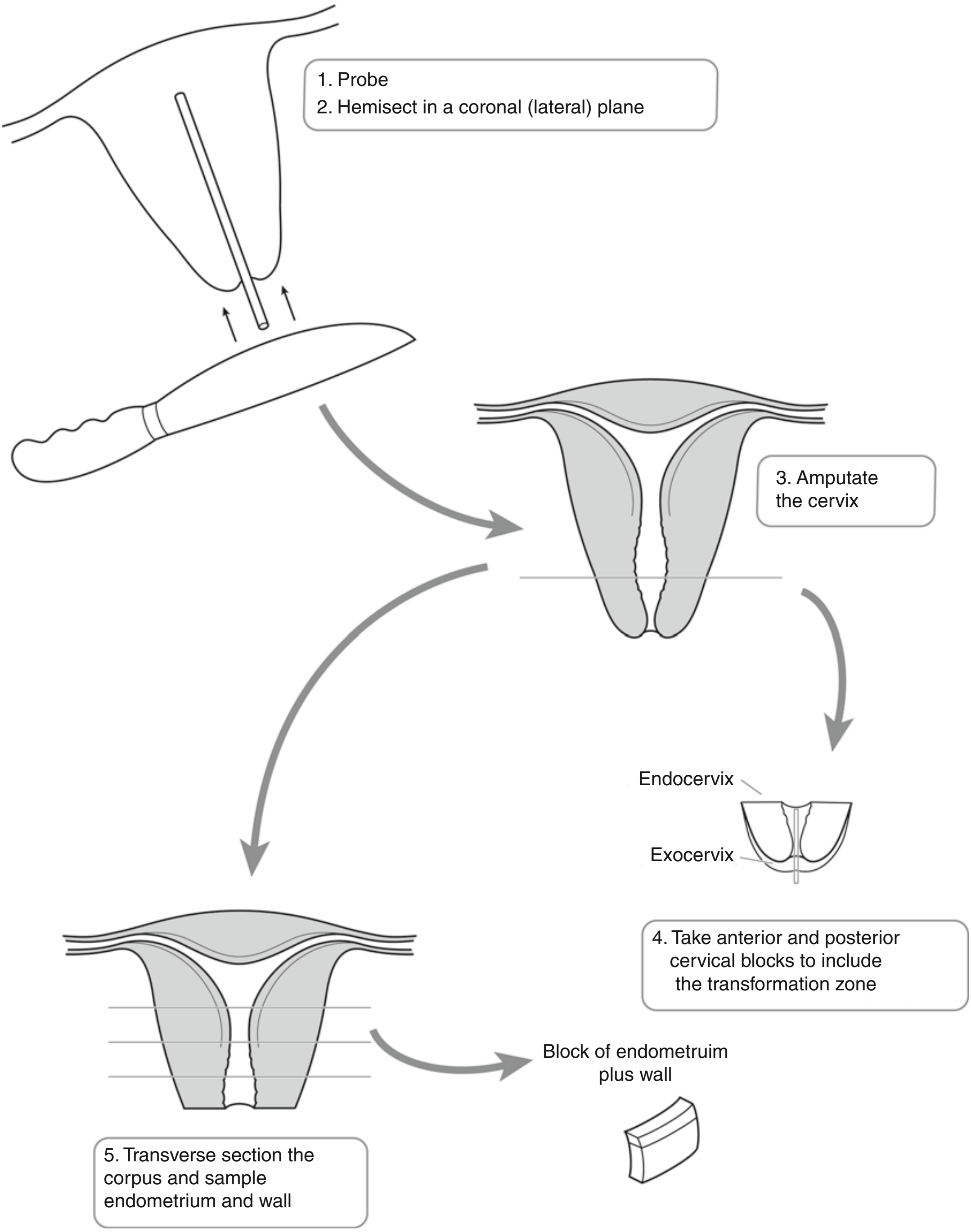

Fig. 24.3 Blocking a routine hysterectomy specimen (Reproduced, with permission, from Allen and Cameron (2004))

- If leiomyomas are present and these are grossly typical, one or two representative sections suffice. If there are multiple leiomyomas, not all need to be examined microscopically.
- If there are areas of hemorrhage or necrosis within a leiomyoma or if any unusual gross findings are present, then extensive sampling should be undertaken, especially from the periphery of the lesion.
- With endometrial carcinomas multiple sections are examined (Fig. 24.4). They are taken to show the deepest point of myometrial infiltration, and also from uninvolved endometrium to assess the presence of coexistent hyperplasia. If no macroscopic tumor is evident in a patient with a biopsy-proven endometrial carcinoma, the entire endometrial cavity may need to be blocked.
- Sections are taken from the cervix from any gross areas of cervical involvement. When this is not seen, take three or four representative sections of the lower uterine segment and cervix.
- Any grossly visible endometrial polyps are sampled.

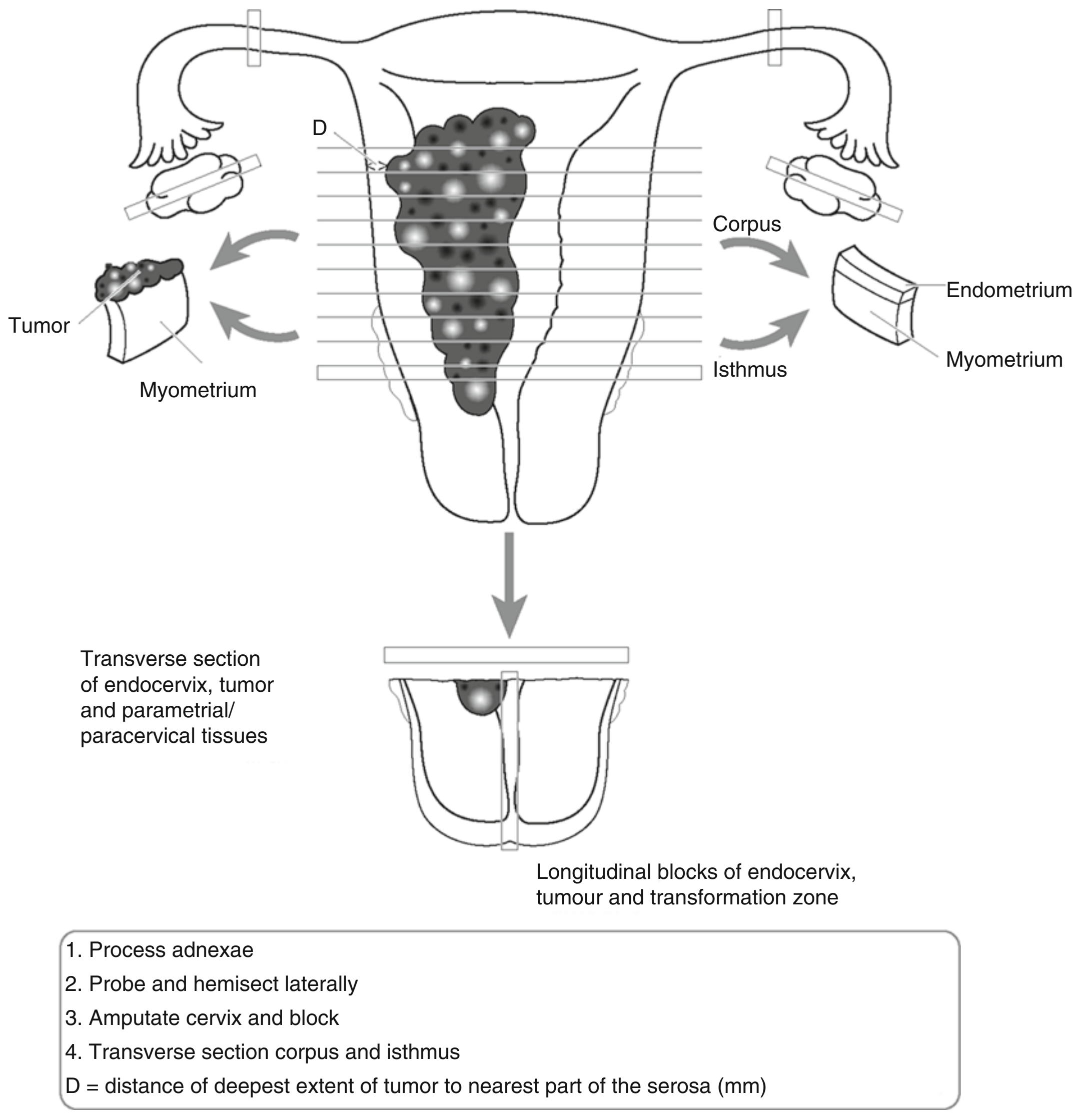

Fig. 24.4 Blocking a hysterectomy for uterine carcinoma (Reproduced, with permission, from Allen and Cameron (2004))

- When there is a history of endometrial hyperplasia and no grossly visible lesion is present, the endometrium should be extensively sampled to assess the worst degree of hyperplasia and to evaluate whether a coexistent adenocarcinoma is present.
- Ovaries and tubes, when grossly normal, are examined as per a benign protocol. Sections of the fallopian tube should include the fimbrial end. If coexistent ovarian tumor is present, this should be blocked as outlined in Chap. 22.
- All lymph nodes should be submitted for histological examination.
- Any parametrial tissue should be submitted.
- If grossly normal, one or two blocks of omentum should be submitted. If tumor nodules are identified grossly, one or two blocks should be submitted.

Histopathology report:

- Site of tumor within the uterus – Fundus, body, lower uterine segment
- Size of tumor – Measure in three dimensions (cm)
- Gross appearance of tumor – Polypoid or infiltrative. Color and consistency. Presence or absence of hemorrhage and necrosis.
- Tumor type – A variety of different adenocarcinomas arise in the endometrium. It is not acceptable to simply render a diagnosis of adenocarcinoma. The type of the adenocarcinoma should be stated.
- Tumor differentiation – Endometrial adenocarcinomas of endometrioid and mucinous types are graded as Grade I–III (FIGO grading system). This depends on architectural and cytological features. The more uncommon morphological subtypes such as serous carcinoma and clear cell carcinoma are not graded since these are automatically high-grade (Grade III) tumors.
- Myometrial invasion – Presence or absence of myometrial invasion. If present – confined to inner half or involves outer half.
- Lymphovascular invasion – Present/not present.
- Lymph nodes – Mention sites, number identified, and number involved by tumor.
- Cervical involvement – Presence or absence of involvement of the endocervical glands and/or stroma. Cervical stromal involvement is associated with a worse prognosis than involvement limited to the endocervical surface or crypt epithelium.
- Serosal involvement – Present/not present.
- Measure minimum distance (mm) from the deepest point of myometrial infiltration by tumor to the serosa.
- Surrounding endometrium – Presence or absence and type of hyperplasia.
- Omental involvement – Present/not present.
- Parametrial involvement – Present/not present.
- Other pathology – The presence of coexistent pathology should be mentioned.
- Peritoneal washings – Presence or absence of tumor cells.
- Ovary and fallopian tube – Presence or absence of tumor metastasis. Note that, especially with endometrioid tumors, synchronous neoplasms may be present within both the ovary and endometrium.

FIGO Stage for carcinoma of endometrium

I	Tumor confined to the corpus uteri
IA	No or less than half myometrial invasion
IB	Invasion equal to or more than half of the myometrium
II	Tumor invades cervical stroma, but does not extend beyond the uterus
III	Local and/or regional spread of the tumor
IIIA	Tumor invades the serosa of the corpus uteri and/or adnexae
IIIB	Vaginal and/or parametrial involvement
IIIC	Metastases to pelvic and/or para-aortic lymph nodes
IIIC1	Positive pelvic nodes
IIIC2	Positive para-aortic lymph nodes with or without positive pelvic lymph nodes
IV	Tumor invades bladder and/or bowel mucosa, and/or distant metastases
IVA	Tumor invasion of bladder and/or bowel mucosa
IVB	Distant metastases, including intra-abdominal metastases and/or inguinal lymph nodes

2009 FIGO staging systems for uterine sarcomas

Uterine leiomyosarcoma and endometrial stromal sarcoma

I	Tumor limited to uterus
IA	≤5 cm
IB	>5 cm
II	Tumor extends beyond the uterus, within the pelvis
IIA	Adnexal involvement
IIB	Involvement of other pelvic tissues
III	Tumor invades abdominal tissues (not just protruding into the abdomen)
IIIA	One site
IIIB	>one site
IIIC	Metastasis to pelvic and/or para-aortic lymph nodes
IV	
IVA	Tumor invades bladder and/or rectum
IVB	Distant metastasis

Uterine adenosarcoma

I	Tumor limited to uterus
IA	Tumor limited to endometrium/endocervix with no myometrial invasion
IB	Less than or equal to half myometrial invasion
IC	More than half myometrial invasion
II	Tumor extends beyond the uterus, within the pelvis
IIA	Adnexal involvement
IIB	Involvement of other pelvic tissues
III	Tumor invades abdominal tissues (not just protruding into the abdomen)
IIIA	One site
IIIB	>one site
IIIC	Metastasis to pelvic and/or para-aortic lymph nodes
IV	
IVA	Distant metastasis
IVB	Distant metastasis

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Cervix

25

Oisin Houghton and W. Glenn McCluggage

25.1 Anatomy

The cervix is joined to the body of the uterus and usually measures 2.5–3 cm in length. The bladder is situated anteriorly and is separated from the cervix by loose connective tissue. On the posterior aspect the upper cervix is covered by peritoneum. Part of the cervix lies within the vagina and is surrounded by a reflection of the vaginal wall called the fornix. The ectocervix (outer cervix) is covered by non-keratinizing stratified squamous epithelium and the endocervix (inner cervix) is lined by a single layer of mucin-secreting epithelial cells. The junction between the two is known as the transformation zone (see Fig. 24.1).

Lymphovascular drainage: The blood supply to the cervix is from the uterine artery. As the uterine artery approaches the cervix, it divides into ascending and descending branches. The descending branch supplies the cervix and upper vagina. The veins of the cervix drain to the uterovaginal plexus in the base of the broad ligament. Cervical lymphatics drain into small perforating lymphatic vessels, which eventually leave the cervix via two main vessels which are closely opposed to the uterine arteries. These drain into pelvic lymph nodes. The pelvic lymph nodes which the cervical lymphatics drain into are the external iliac nodes, the internal iliac nodes, and the common iliac nodes (Fig. 25.1).

25.2 Clinical Presentation

A variety of dysplastic preinvasive lesions, of both squamous and glandular types, are commonly encountered within the cervix. These are usually picked up because of an abnormal cervical smear, performed in the UK as part of the NHS Cervical Screening Programme. These abnormalities are often associated with and due to infection by human papilloma virus (HPV). Other symptoms related to cervical pathology include watery vaginal discharge, and postcoital and intermenstrual bleeding. With advanced cervical tumors invading the bladder or rectum, there may be urinary or bowel symptoms. Large tumors can protrude through the external cervical os into the vagina. Small cervical tumors may be asymptomatic. With advanced tumors, the ureters can become obstructed with resultant hydronephrosis and renal failure – lymphedema and deep venous thrombosis may also occur.

O. Houghton (✉) • W.G. McCluggage
Histopathology Laboratory,
Institute of Pathology, Royal Victoria Hospital,
Belfast Health and Social Care Trust, Belfast, UK
e-mail: oisin.houghton@belfasttrust.hscni.net;
glenn.mccluggage@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_25, © Springer-Verlag London 2013

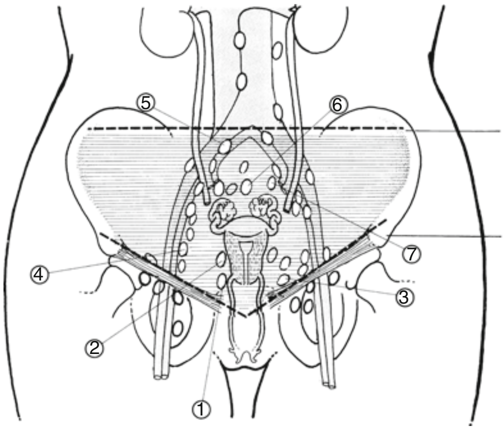

Fig. 25.1 Cervix – regional lymph nodes. (*1*) Paracervical nodes. (*2*) Parametrial nodes. (*3*) Hypogastric (internal iliac) including obdurator nodes. (*4*) External iliac nodes. (*5*) Common iliac nodes. (*6*) Presacral nodes. (*7*) Lateral sacral nodes (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

25.3 Clinical Investigations

Most preinvasive dysplastic lesions are picked up because of an abnormal cervical smear. When a significant cytological abnormality is identified, patients are referred to a gynecologist for colposcopy. This involves looking at the cervix under a special microscope (colposcope) and often taking a biopsy or performing local excision of an abnormal area of cervix (loop or cone biopsy). These areas are identified by their lack of uptake of iodine stain (acetowhite epithelium – AWE) and abnormal surface appearances (e.g., vascular punctation or a mosaic pattern). HPV testing may also be undertaken and involves molecular testing of material taken at a cervical smear. In patients with cervical discharge, material may be sent for microbiological investigations. In cases of cervical tumor, radiological investigation, usually in the form of MRI, is carried out for staging purposes.

25.4 Pathological Conditions

25.4.1 Non-neoplastic Conditions

A variety of benign non-neoplastic conditions occur within the cervix. The chief importance of these is their potential for misdiagnosis as cervical intraepithelial neoplasia (CIN) or cervical glandular intraepithelial neoplasia (CGIN). These conditions include polyps, reserve cell hyperplasia, immature and mature squamous metaplasia, inflammatory induced atypia, tubal and tuboendometrial metaplasia, endometriosis, and microglandular hyperplasia. Mesonephric remnants, when present within the cervix, may also lead to diagnostic problems.

25.4.2 Neoplastic Conditions

The two main malignant neoplasms to occur within the cervix are invasive squamous carcinoma and adenocarcinoma. Mainly due to the advent of organized screening programs, precursor lesions (CIN and CGIN) are identified much more commonly by cytology and the incidence of invasive cervical tumors, especially squamous carcinoma, is decreasing.

Cervical intraepithelial neoplasia (CIN): CIN is the preferred designation in the UK for the spectrum of dysplastic preinvasive squamous lesions which are associated with an increased risk of the subsequent development of cervical squamous carcinoma. These usually arise at the transformation zone of the cervix and are divided into CINI, CINII, and CINIII (previously known as mild, moderate, and severe dysplasia, respectively). Morphological changes associated with HPV infection are termed koilocytosis. In some countries, koilocytosis and CINI are collectively termed low-grade squamous intraepithelial lesion (LSIL), while CINII and CINIII are designated high-grade squamous intraepithelial lesion (HSIL). The transition from CINI to CINIII may take many years and all grades of CIN may revert to normal, especially CINI. The aim of cervical screening is to pick these lesions up in the preinvasive stage. Treatment then reduces the risk of development of squamous carcinoma.

Cervical Glandular Intraepithelial Neoplasia (CGIN): Similar to the situation with CIN, preinvasive glandular lesions may be encountered. These are much rarer than the corresponding squamous lesions and are less likely to be picked up on cytological examination. They often coexist with squamous lesions. In the UK, the preferred designation is cervical glandular intraepithelial neoplasia (CGIN). These are divided into low-grade and high-grade CGIN. The WHO classification uses the terms glandular dysplasia and adenocarcinoma in situ, corresponding to low-grade and high-grade CGIN, respectively. Many of these lesions are associated with HPV infection.

Invasive tumors: Approximately 70–80% of invasive carcinomas of the cervix are squamous cell in type. Most of the remainder are adenocarcinomas; most of these are of the usual or endocervical type, although uncommon variants such as adenoma malignum, gastric, mesonephric, clear cell, and serous also occur. Rarer morphological subtypes of cervical carcinoma include adenosquamous carcinoma and small-cell and large-cell neuroendocrine carcinoma. A variety of other malignant tumors occur within the cervix, but these are rare.

The main risk factor in the development of both squamous carcinoma and adenocarcinoma of the cervix is infection with HPV, although some of the uncommon types of adenocarcinoma are not usually HPV related. There may be an association with oral contraceptive use and cervical adenocarcinoma. Other factors implicated in the pathogenesis of cervical cancer, including early age at first intercourse, multiple sexual partners, etc., are not independent of HPV infection. Smoking is also a risk factor for the development of cervical squamous carcinoma.

Treatment: Following referral because of an abnormal cervical smear (or occasionally a clinically suspicious cervix or symptoms such as postcoital bleeding), colposcopic examination is performed. In general, low-grade lesions (koilocytosis and CINI) are treated by local ablative procedures or cytological follow-up, while high-grade lesions (CIN II and CIN III) are treated by local excision, as is CGIN. Usually this is in the form of diathermy large loop excision of the transformation zone (LLETZ) of the cervix. Occasionally cold knife cone biopsies may be performed, especially if a small invasive carcinoma is suspected or if a cervical glandular lesion is suggested on cytology. With more advanced cervical tumors (usually greater than stage Ia1), radical hysterectomy is usually carried out. This involves removing the uterus and cervix with a cuff of vagina. The surrounding parametrium on both sides is also removed and pelvic lymph node resection is undertaken. The FIGO staging system for cervical cancer is used. With advanced cervical cancers (greater than stage IIa and sometimes with bulky Ib2 tumors), the initial treatment may be chemoradiation. This may be followed by salvage hysterectomy at a later date. Whether chemoradiation is given postsurgery for cervical carcinomas depends on a variety of pathological factors.

LLETZ or cone biopsies with careful assessment of margins and cytological follow-up may be performed in patients with early (stage Ia1) tumors. Occasionally in young patients with 1a2 or small Ib1 cancers (usually less than 2 cm) and who wish to preserve their fertility, a trachelectomy may be performed followed by the insertion of a suture into the cervix. Trachelectomy involves a local excision of the cervix with the upper vagina and the surrounding parametrium. Pelvic lymph nodes are usually removed laparoscopically during this procedure. Careful pathological examination is required to ascertain whether further, more radical, surgery is needed. All cases should be discussed at a multidisciplinary gynecological oncology meeting.

Prognosis: Following ablation or local excision of premalignant cervical lesions, close cytological follow-up is carried out for a period of 5–10 years, depending on the diagnosis.

The prognosis of invasive cervical cancers is largely dependent on the tumor stage. Stage 1a1 carcinomas have an excellent prognosis and these may be treated by conservative local excision therapies, usually in the form of loop or cone biopsies. The prognosis of more advanced cervical cancers, as already stated, largely depends on the stage of the tumor and the lymph node status, especially the presence of extracervical spread, with an overall 5-year survival of about 55%.

25.5 Surgical Pathology Specimens: Clinical Aspects

25.5.1 Biopsy Specimens

Many biopsy specimens of cervix submitted to the pathology laboratory comprise small punch biopsies performed at colposcopic examination.

Cervical polyps are removed following direct visualization of the cervix. Loop and cone biopsies are local excisions of the cervix, usually performed at colposcopic examination. Wedge biopsies are taken with grossly visible neoplasms, in order to confirm the presence of tumor.

25.5.2 Resection Specimens

In general, with the exception of Ia1 carcinomas and young women who wish to preserve their fertility, surgical treatment of cervical carcinoma comprises radical hysterectomy with removal of pelvic lymph nodes. Occasionally simple hysterectomy is performed in women with CIN or CGIN who have other benign uterine pathologies or symptoms related to the uterus or who do not wish to be subjected to regular cytological follow-up.

25.6 Surgical Pathology Specimens: Laboratory Protocols

25.6.1 Biopsy Specimens

Small cervical colposcopic punch biopsies are examined intact at multiple histological levels. Loop and cone biopsies are measured (each individual fragment is measured in three dimensions), carefully sectioned into multiple serial blocks (Fig. 25.2), and the entire tissue is examined histologically. No more than two slices of tissue should be put into a single cassette. Levels are not routinely needed for loop or cone biopsies but may be necessary for a variety of reasons such as if histology shows the presence of an area suspicious of invasion, incomplete correlation with the preceding cytology, or if the full face of the tissue is not represented in the initial sections. Wedge

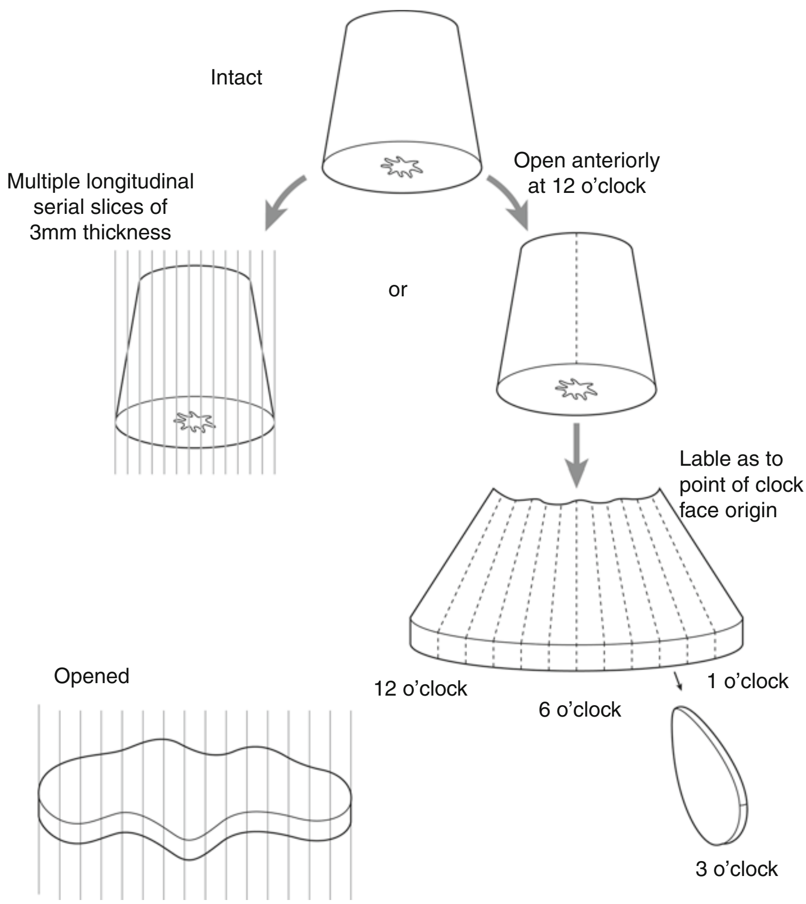

Fig. 25.2 Blocking a cervical cone or loop biopsy (Reproduced, with permission, from Allen and Cameron (2004))

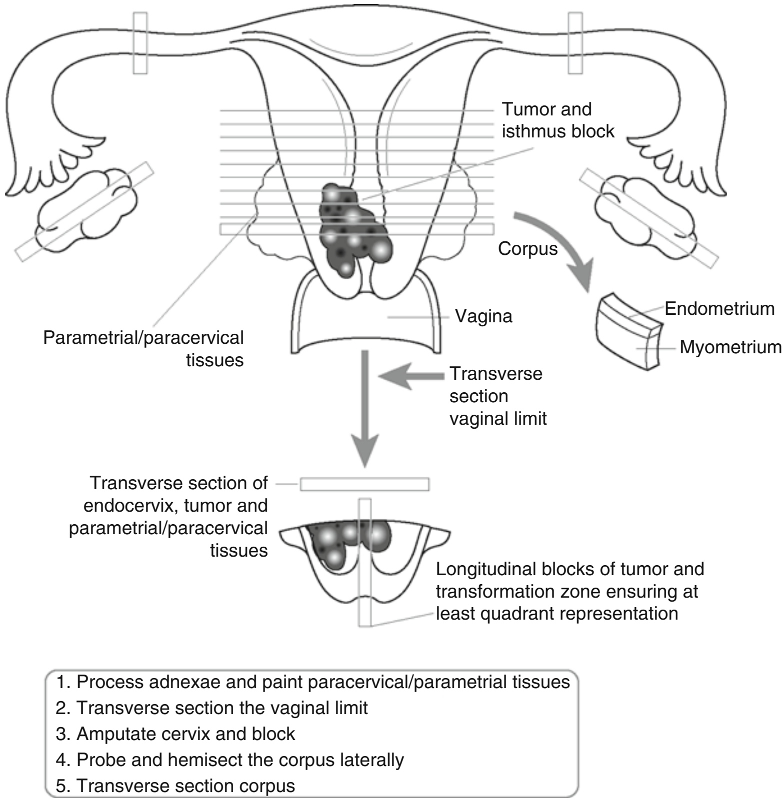

Fig. 25.3 Blocking a radical hysterectomy for cervical carcinoma (Reproduced, with permission, from Allen and Cameron (2004))

biopsies are measured in three dimensions, sectioned thinly, and examined in their entirety.

25.6.2 Resection Specimens

In cases of cervical cancer (usually greater than stage Ia1), the operation of choice is radical hysterectomy together with pelvic lymph node removal. Radical (Wertheim's) hysterectomy involves removal of the uterus and cervix together with a cuff of vagina and the surrounding parametrium. Both ovaries and fallopian tubes are also usually removed, although in young women they may be left behind in order that ova may be available for those who wish to have children and hormonal function may be preserved.

A trachelectomy may be performed in young women with cervical cancer who wish to preserve their fertility. This operation is usually undertaken for early stage Ib carcinomas, the carcinoma measuring less than 2 cm in maximum diameter. During the process of trachelectomy, local excision of the cervix is undertaken together with the upper vagina and the surrounding parametrium and pelvic lymph nodes are removed.

Sometimes simple hysterectomy is carried out for extensive or recurrent CIN or CGIN, or in patients with CIN or CGIN who are symptomatic for other reasons, e.g., dysfunctional uterine bleeding. No uterus should be dissected or reported without full knowledge of any prior endometrial sampling or cervical cytology results.

25.6.2.1 Radical hysterectomy

Procedure, description, and blocks for histology in a radical hysterectomy (Fig. 25.3):

The specimen is weighed and the combined length of the uterus and cervix measured.

The external surfaces of the uterus and cervix are carefully evaluated to ascertain whether there is any tumor infiltration.

At this stage, the serosal surface of the uterus and the external surface of the cervix together with the vaginal resection margin can be inked. Different colors of ink may be used to designate right and left lateral, anterior and posterior. Care should be taken so that the ink does not contaminate other surfaces, especially on sectioning.

- The vaginal limit is sectioned in its entirety and processed for histological examination. Scissors are useful for obtaining these blocks.
- The cervix is detached from the uterus by a complete transverse cut. A parallel slice from the proximal limit of the amputated cervix provides blocks of right and left parametrium which should be inspected for the presence or absence of tumor and lymph nodes.
- The cervix is opened longitudinally and the presence of any gross tumor noted.
- If a tumor is apparent, it is measured in three dimensions (cm) and its site stated (anterior, posterior, left lateral, right lateral, etc.).
- If a gross tumor is identified, representative longitudinal sections are examined, a minimum of one from each quadrant depending on the tumor location and distortion of the cervical anatomy. These are taken to show the deepest point of infiltration into the underlying cervical stroma and the relationship of the tumor to the closest margins. Blocks are labeled as to their site of origin.
- If no tumor is seen grossly, then the entire cervix should be sectioned and examined histologically. Sections are labeled as to what part of the cervix they are taken from, e.g., 1–12 o'clock, with 12 being from the anterior lip of the cervix.
- Two sections are taken from the lower uterine segment to assess the presence or absence of spread of tumor into the lower uterus.
- The uterus is carefully examined and, if unremarkable, sampled as per a benign protocol.
- The ovaries and tubes are carefully sectioned and, if unremarkable, sampled as per a benign protocol. It is usually convenient to dissect and block the adnexae prior to the handling of the main specimen.
- Photography can be undertaken at any stage in the cutting process.
- Lymph nodes are carefully sectioned and labeled as to their site of origin. The number of lymph nodes from each site is recorded. These are usually dissected and submitted to the laboratory by the surgeon in separately labeled pots.

25.6.2.2 Trachelectomy specimens

Procedure for dealing with trachelectomy specimens:

- The specimen is orientated, weighed, and measured in three dimensions (cm).
- The parametrial surface is inked.
- The entire vaginal limit is submitted for histological examination.
- If no tumor is identified grossly, the upper limit is submitted as a transverse section.
- If a tumor is seen grossly, this is measured in three dimensions.
- If a tumor is seen grossly, a section is taken to show the relationship between the tumor and the proximal margin.
- The tissue is serially sliced longitudinally at 3–4 mm intervals and examined in its entirety. Lymph nodes are dealt with as above.

25.6.2.3 Hysterectomy for CIN/CGIN

Procedure for hysterectomy with CIN and CGIN:

- The cervix is amputated and longitudinally sectioned to give good junctional zone representation. The number of blocks obtained depends on the local cervical anatomy and distortion/stenosis as a result of previous procedures, e.g., LLETZ. Block numbers may therefore range from 3 to 4 (quadrants) right up to 12. They are labeled as to their site of origin. It is better to take fewer blocks with good junctional zone representation rather than many blocks with poor representation.
- These specimens often contain a vaginal cuff and the limit of this is submitted in its entirety for histological examination.

Histopathology report:

- The number of blocks of tumor examined and the site of the tumor are stated.
- The tumor is measured in three dimensions (cm). If this is not possible grossly, then it is done histologically. The maximum depth and maximum horizontal dimension are

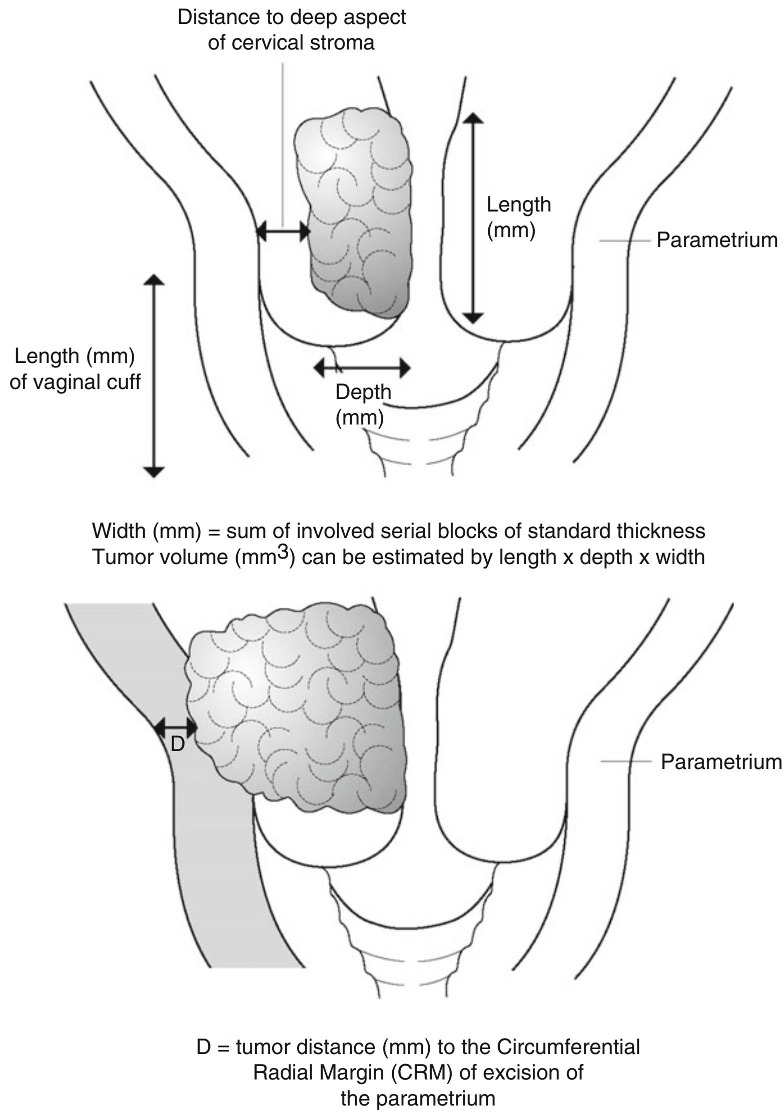

Fig. 25.4 Cervical carcinoma – tumor dimensions and margins (Reproduced, with permission, from Allen (2000))

measured on the slide. It should be remembered that there is a third dimension and this is calculated by taking into account the presence of tumor in adjacent tissue blocks. If a block is taken as measuring 3 mm in thickness, then the total third dimension can be calculated on this basis.

- Tumor type – Most tumors within the cervix are either squamous carcinomas or adenocarcinomas.
- Tumor differentiation – Both squamous carcinomas and adenocarcinomas are classified as being well, moderately, or poorly differentiated. For squamous carcinomas, the prognostic significance of grading is controversial. Squamous carcinomas can also be classified as large-cell keratinizing, large-cell non-keratinizing, and small-cell non-keratinizing.

The presence or absence of the following is noted:

- Adjacent CIN, CGIN, or signs of HPV infection
- Vaginal, paracervical, or parametrial soft tissue tumor involvement
- Tumor at the circumferential limit (state anterior, posterior, right or left lateral), or if clear, the minimum distance (mm) from it (Fig. 25.4)
- Vaginal limit involvement

- Lymphovascular permeation
- Lymph node involvement (site, number of nodes identified and number involved, intraparenchymal or extracapsular spread)
- Response to preoperative chemoradiation
- Uterine involvement, although this does not affect the staging of cervical cancer
- Coexisting pathology in other organs, e.g., vaginal HPV or VAIN.

For tumors confined to the cervix note the minimum distance (mm) from the tumor to the external cervical surface (Fig. 25.4) and state which aspect of the cervix.

When local excision is performed for a small invasive cancer, record the tumor distance (mm) and the distance of CIN or CGIN to the ectocervical, endocervical, and deep limits.

The British Association of Gynaecological Pathologists, British Gynaecological Cancer Society, and gynecological clinical reference group of the National Cancer Intelligence Network recommend that FIGO staging be used for gynecological cancers rather than TNM with recording of the lymph node status for cervical cancer. This may be done by providing a TNM stage for this cancer type only or by recording the lymph node status at the multidisciplinary team meeting.

TNM and FIGO pathological staging of cervical carcinoma

T1/I	Cervical carcinoma confined to the uterus (extension to the corpus should be disregarded)
T1a/IA	Invasive carcinoma, diagnosed only by microscopy, with deepest invasion ≤5.0 mm and ≤7.0 mm
T1a1/IA1	Measured stromal invasion ≤3.0 mm and largest extension of ≤7.0 mm
T1a2/IA2	Measured stromal invasion of >3.0 mm and not >5.0 mm with an extension of not >7.0 mm
T1b/IB	Clinically visible lesion limited to the cervix uteri or preclinical cancers greater than stage IA[a]
T1b1/IB1	Clinically visible lesion ≤4.0 cm in greatest dimension
T1b2/IB2	Clinically visible lesion >4.0 cm in greatest dimension
T2/II	Cervical carcinoma invades beyond the uterus but not to the pelvic wall or to lower third of the vagina
T2a/IIA	Without parametrial invasion
T2a1/IIA1	Clinically visible lesion ≤4.0 cm in greatest dimension
T2a2/IIA2	Clinically visible lesion >4.0 cm in greatest dimension
T2b/IIB	With obvious parametrial invasion
T3/III	The tumor extends to the pelvic wall and/or involves lower third of the vagina, and/or causes hydronephrosis or nonfunctioning kidney[b]
T3a/IIIA	Tumor involves lower third of vagina, with no extension to the pelvic wall
T3b/IIIB	Extension to the pelvic wall and/or hydronephrosis or nonfunctioning kidney
T4/IV	The carcinoma has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum. A bullous edema, as such, does not permit a case to be allocated to stage IV
T4a/IVA	Spread of growth to adjacent organs
M1/IVB	Spread to distant organs

[a]All macroscopically visible lesions – even with superficial invasion – are allotted to stage IB carcinomas. Invasion is limited to a measured stromal invasion with a maximal depth of 5.00 mm and a horizontal extension of not >7.00 mm. Depth of invasion should not be >5.00 mm taken from the base of the epithelium of the original tissue – superficial or glandular. The depth of invasion should always be reported in mm, even in those cases with "early (minimal) stromal invasion" (~1 mm).
The involvement of vascular/lymphatic spaces should not change the stage allotment.

[b]On rectal examination, there is no cancer-free space between the tumor and the pelvic wall. All cases with hydronephrosis or nonfunctioning kidney are included, unless they are known to be due to another cause.

Regional lymph nodes (N)[a] (TNM staging system)

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis

[a]Regional lymph nodes include paracervical, parametrial, hypogastric (internal iliac, obturator); common and external iliac; presacral and lateral sacral nodes. Para-aortic nodes are not regional.

Distant metastasis (M) (TNM staging system)

M0	No distant metastasis
M1	Distant metastasis (includes inguinal lymph nodes and intraperitoneal disease except metastasis to pelvic serosa). It excludes metastasis to vagina, pelvic serosa, and adnexa

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26 Vagina

Oisin Houghton and W. Glenn McCluggage

26.1 Anatomy

The vagina lies anterior to the rectum and posterior to the bladder. It measures 6–12 cm in length normally. The upper aspect terminates in a circular fold around the cervix to form the vaginal fornices. The vaginal surface is lined by non-keratinizing stratified squamous epithelium and the wall comprises fibromuscular connective tissues (see Fig. 22.1).

Lymphovascular drainage:

The descending branch of the uterine artery supplies the upper vagina. The lower vagina is supplied by branches of the internal pudendal artery. The veins of the vagina drain to the uterovaginal plexus, which eventually drains to the internal iliac veins.

The lymphovascular supply of the vagina is closely related to that of the cervix and vulva. Superiorly lymphatics drain along the uterine artery into the external iliac lymph nodes. In the mid-vagina, the lymphatic drainage terminates in the hypogastric nodes, while the inferior aspect of the vagina terminates in the inguinal lymph nodes.

26.2 Clinical Presentation

Primary pathology of the vagina is relatively rare. Symptomatology related to primary vaginal disease may include a mass or feeling of discomfort, vaginal bleeding or discharge, dyspareunia (painful coitus), or postcoital bleeding. Occasionally primary vaginal disease is discovered at colposcopic examination. Many of the signs and symptoms experienced by women with malignant vaginal lesions are similar to those encountered with cervical cancer.

26.3 Clinical Investigations

Usually vaginal tumors can be directly visualized. Dysplastic squamous lesions (known as vaginal intraepithelial neoplasia – VAIN) may be seen at colposcopic examination. With suspected primary vaginal malignancies, pelvic MRI is used to assess the stage of the tumor and the presence or absence of pelvic or inguinal lymphadenopathy. Exfoliative cytology with microscopic examination of cells obtained by aspiration of the vaginal pool is occasionally used in the diagnosis of vaginal lesions, but this practice is not widespread.

O. Houghton (✉) • W.G. McCluggage
Histopathology Laboratory,
Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: oisin.houghton@belfasttrust.hscni.net; glenn.mccluggage@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_26, © Springer-Verlag London 2013

26.4 Pathological Conditions

26.4.1 Non-neoplastic Conditions

Non-neoplastic vaginal lesions are uncommon. A variety of benign cysts may be encountered, usually involving the subepithelial tissues. These include epithelial inclusion cysts, Mullerian derived cysts, and mesonephric cysts. Fibroepithelial stromal polyps are relatively common. Grossly these are polypoid lesions covered by unremarkable or hyperkeratotic surface squamous epithelium. Occasionally following vaginal hysterectomy, the fallopian tube may prolapse and present as a nodule within the vagina. Vaginal granulation tissue may also occur post vaginal hysterectomy. Endometriosis occasionally presents within the vagina and macroscopically is seen as small hemorrhagic areas.

26.4.2 Neoplastic Conditions

Primary neoplastic conditions of the vagina are relatively rare.

Benign tumors: Benign vaginal tumors include squamous papilloma and a variety of benign mesenchymal tumors, the commonest of which is leiomyoma.

Malignant tumors: The most common primary malignant tumor by far to arise within the vagina is squamous carcinoma. However, primary squamous carcinomas of the vagina are rare and much less common than spread from a primary tumor arising elsewhere, e.g., cervix, uterus, urinary bladder, or rectum. If the tumor also involves the cervix, then it is most likely to be of cervical origin. Preinvasive vaginal squamous lesions also occur. They often coexist with CIN lesions within the cervix and with dysplastic lesions elsewhere in the lower female genital tract, e.g., vulva. They are categorized as vaginal intraepithelial neoplasia (VAIN) and graded I to III, similar to the grading system used for CIN. These may be identified at colposcopic examination during investigation of an abnormal cervical smear. Adenocarcinomas rarely arise as a primary lesion within the vagina. A type of vaginal adenocarcinoma (clear cell carcinoma) may be associated with in utero exposure to diethylstilboestrol. Other malignant tumors of the vagina include adenosquamous carcinoma, malignant melanoma, and a variety of malignant mesenchymal lesions. The etiological factors in the pathogenesis of vaginal squamous carcinoma are broadly similar to those implicated in the pathogenesis of the corresponding cervical lesions. Previous pelvic irradiation and a history of preinvasive or invasive cervical squamous lesions are predisposing factors to squamous carcinomas of the vagina.

Treatment: Benign vaginal lesions such as cysts and fibroepithelial polyps are usually removed by biopsy. Benign mesenchymal tumors should be excised preferably with a rim of uninvolved tissue in order to avoid local recurrence. Surgical treatment of early-stage malignant vaginal tumors is radical hysterectomy. Further treatment, usually in the form of radiotherapy or chemoradiation, is then dependent on staging and these cases should be discussed at a multidisciplinary gynecological oncology meeting. With advanced vaginal tumors, radiotherapy or chemoradiation may be the initial treatment. Occasionally recurrent endometrial tumors within the vagina may be managed by vaginectomy (colpectomy).

Prognosis: The prognosis of malignant vaginal tumors largely depends on the FIGO staging. Tumors are staged by a combination of clinical and pathological parameters. Clinical assessment includes speculum examination, bimanual pelvic and rectal examinations, cystoscopy, and proctosigmoidoscopy. Overall 5-year survival is in the region of 40%. Other prognostic factors such as tumor grade, patient age, and tumor localization are of less prognostic significance.

26.5 Surgical Pathology Specimens: Clinical Aspects

26.5.1 Biopsy Specimens

Small benign polypoid lesions are often removed by biopsy following direct visualization. In cases of suspected malignancy, punch or wedge biopsies are performed to confirm the diagnosis. Cystic lesions and submucosal benign mesenchymal lesions are usually removed with a small rim of surrounding uninvolved tissue.

26.5.2 Resection Specimens

As already stated, with malignant vaginal disease, the preferred surgical treatment is radical hysterectomy. This is similar to that performed for cervical cancer.

26.6 Surgical Pathology Specimens: Laboratory Protocols

26.6.1 Biopsy Specimens

Small biopsies are examined in their entirety. The number of biopsy fragments is counted and the entire specimen is submitted and examined at multiple levels.

26.6.2 Resection Specimens

Radical or Werdheim's hysterectomy involves removal of the uterus and cervix together with the upper vagina. This operation, which generally also involves pelvic lymphadenectomy, is usually performed for stage I disease located in the upper part of the vagina. Otherwise many vaginal cancers are primarily treated by chemoradiation. Occasional vaginectomy (colpectomy) specimens are encountered.

Procedure and description for radical hysterectomy (Fig. 26.1):

- The specimen is weighed and the length of the uterus, cervix, and vagina measured (cm). The serosal surface of the uterus and the external surface of the cervix and vagina are inked. Care should be taken so that the ink does not contaminate other surfaces.
- The distal vaginal limit is transversely sectioned in its entirety and processed for histological examination. Scissors are useful for obtaining these blocks.
- On opening the vagina the size (cm) and site of the tumor and its relationship to the cervix is assessed and described.
- The distance of tumor to the distal vaginal limit of excision is measured (cm).
- The tumor is carefully transversely sectioned and the minimum distance from tumor to the circumferential limit measured (mm). The nearest circumferential limit should be stated.
- The deep soft tissue paravaginal margin is sampled for histological examination.
- The presence or absence of cervical involvement is noted grossly.
- Sections are taken from the cervix to show its relationship with the vaginal tumor if possible.
- The uterus is sampled as per a benign protocol.
- The ovaries and tubes, if present, are sampled as per a benign protocol. It is often convenient to do this prior to handling of the main specimen.
- Photography may be undertaken at any stage.
- Colpectomy specimens: weigh, measure, paint externally, open longitudinally, describe the tumor (its dimensions and distance to the specimen limits), and transverse section into multiple serial slices.

Blocks for histology (Fig. 26.1):

- Multiple representative blocks of tumor are submitted for histopathological examination. These may be taken either transversely or longitudinally but should show the relationship with both the cervix and the nearest circumferential margin.
- The vaginal distal limit is blocked in its entirety for histological examination.
- Any lymph nodes submitted are enumerated, sampled for histology, and their site of origin noted.
- As already stated, the uterus, ovaries, and tubes are examined as per a benign protocol.

Histopathology report:

- The site of the tumor (upper, mid, or lower; anterior or posterior; left side or right side) within the vagina is stated.
- The tumor measurement is given in three dimensions (cm) if possible.

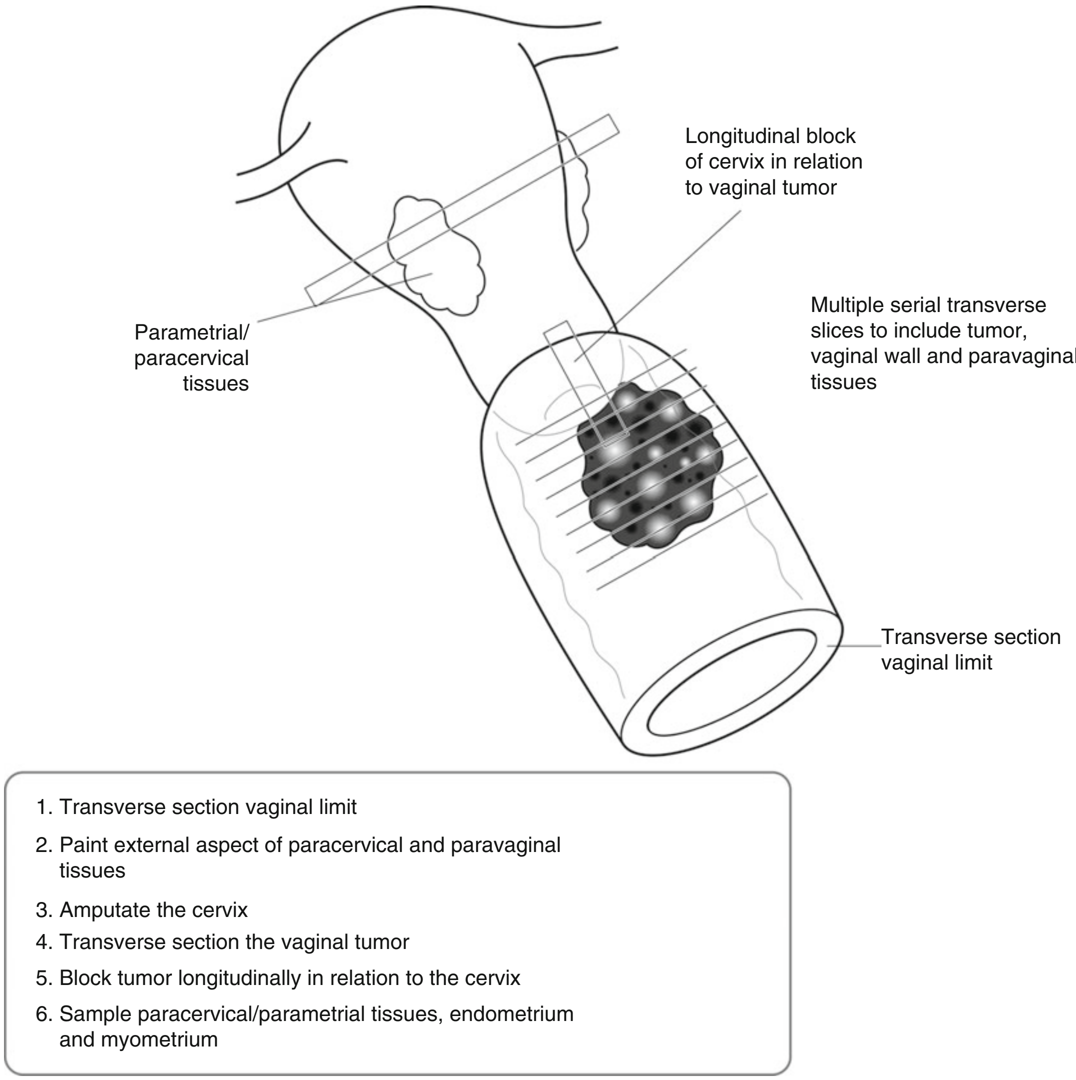

Fig. 26.1 Blocking a radical hysterectomy specimen for vaginal carcinoma (Reproduced, with permission, from Allen and Cameron (2004))

- Tumor type – most tumors arising primary within the vagina are squamous carcinomas. Adenocarcinomas are rarer although they do occur. With an adenocarcinoma, secondary spread from elsewhere should always be excluded, e.g., bladder, uterus, rectum.
- Tumor differentiation – squamous carcinomas and adenocarcinomas are classified as well, moderately, or poorly differentiated.
- The presence or absence of the following are noted:
  – Adjacent VAIN or signs of HPV infection
  – Lymphovascular permeation
  – Lymph node involvement (number from each site and number involved, intraparenchymal or extracapsular spread)
  – VAIN or tumor at the distal vaginal limit, or if clear, the minimum distance (mm) from it

- Paravaginal soft tissue (paracolpium) extension
- Tumor at the circumferential limit (state which one), or if clear, the minimum distance (mm) from it
- Response to preoperative chemoradiation
- Coexistent pathology in other organs, e.g., CIN

FIGO/TNM stage for vagina carcinoma

pT1	Tumor confined to the vagina
pT2	Tumor invades paravaginal tissues but does not extend to pelvic wall
pT3	Tumor extends to pelvic wall
pT4	Tumor invades mucosa of bladder or rectum, and/or extends beyond the true pelvis

Regional nodes: upper two-thirds – pelvic nodes; lower third – inguinal nodes

pN0	No regional lymph node metastasis
pN1	Metastasis in regional lymph node(s)

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27 Vulva

Oisin Houghton and W. Glenn McCluggage

27.1 Anatomy

The vulva is lined by skin. Posteriorly it is limited by the anus, laterally by the inguinal folds, and anteriorly by the mons pubis. The hymen is the medial aspect of the vulva. The vulva comprises the outer hair-bearing labia majora and inner labia minora, the clitoris, and the urethral meatus (Fig. 27.1). Mucous glands, including Bartholin's glands, open into the vulva.

Lymphovascular drainage:

The internal pudendal artery gives off a branch that provides part of the blood supply to the vulva, which is also contributed to by branches from the femoral artery. The venous drainage follows the arterial blood supply.

The lymphatic drainage of each side of the vulva is largely to the ipsilateral inguinal and femoral lymph nodes although some contralateral drainage occurs. Most of the lymphatic drainage is to the superficial inguinal lymph nodes and therefore these are usually the first lymph nodes involved by metastatic tumor in vulval carcinoma. The lymphatic drainage from the clitoris and the midline perineal area is bilateral.

27.2 Clinical Presentation

The vulva may be involved by a variety of dermatological disorders. Often, the main presenting symptom with these is itch (pruritis) or redness. Preinvasive vulval squamous lesions (known as vulval intraepithelial neoplasia – VIN), vulval dystrophies, especially lichen sclerosis, and Paget's disease commonly present in this way. Malignant lesions often present with a mass, itching, bleeding, or an area of ulceration. There may also be discharge, dysuria (painful micturition), and a foul smell. The most common sites of tumors are the labia majora, the labia minora and clitoris, in order of frequency. When tumor has spread to the inguinal lymph nodes, a palpable mass may be present and this can ulcerate through the skin and discharge. Lymphedema and deep venous thrombosis may occur with advanced tumors.

27.3 Clinical Investigations

Most benign dermatological disorders and VIN are diagnosed by a punch biopsy. Colposcopic examination may be used to directly visualize lesions. Radiological investigations, usually MRI, are indicated in staging of vulval cancers,

O. Houghton (✉) • W.G. McCluggage
Histopathology Laboratory,
Institute of Pathology, Royal Victoria Hospital,
Belfast Health and Social Care Trust, Belfast, UK
e-mail: oisin.houghton@belfasttrust.hscni.net;
glenn.mccluggage@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_27, © Springer-Verlag London 2013

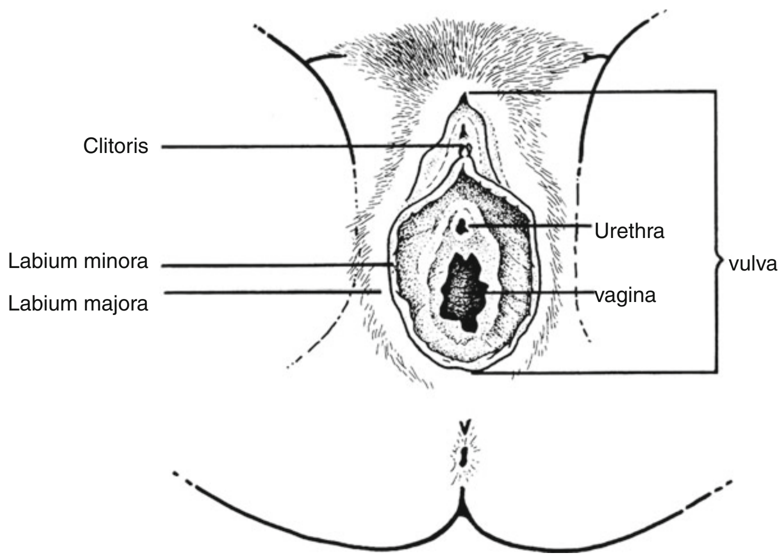

Fig. 27.1 Vulval anatomy (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is, from Wittekind et al. (2005))

in order to ascertain whether regional lymph nodes are enlarged. In some places, exfoliative cytology has been applied to the evaluation of vulval lesions but this is not in widespread use. Fine needle aspiration biopsy is of value in assessing enlarged inguinal lymph nodes and confirming a diagnosis of metastatic cancer.

27.4 Pathological Conditions

27.4.1 Non-neoplastic Conditions

A variety of benign dermatoses may affect the vulval region as with other areas of skin. Non-neoplastic vulval dystrophies (although non-neoplastic, they may be associated with vulval squamous carcinoma) comprise lichen sclerosis and squamous hyperplasia.

27.4.2 Neoplastic Conditions

Similar to the cervix, preinvasive dysplastic squamous lesions occur on the vulva. These are known as *vulval intraepithelial neoplasia* (VIN). This can be categorized into a more common HPV-related type known as classic (undifferentiated, Bowenoid) VIN and a more uncommon non-HPV-related type referred to as differentiated (simplex) VIN. Classic VIN is further categorized as VIN I, II, or III; differentiated VIN is not graded. Most malignant tumors arising on the vulva are *squamous carcinomas*. Rarer primary tumors include basal cell carcinoma, adenocarcinoma, Paget's disease, adenoid cystic carcinoma, small cell carcinoma, malignant melanoma, and aggressive angiomyxoma. A variety of benign epithelial neoplasms, similar to those occurring elsewhere on the skin, can occur on the vulva. A variety of mesenchymal lesions also occur at this site.

Vulval intraepithelial neoplasia (*VIN*): As stated, there are two clinicopathological types of VIN, although there is some overlap. Classic VIN occurs in a younger age group and is often associated with similar lesions in the cervix and elsewhere in the lower female genital tract and with HPV infection. Differentiated VIN may be associated with lichen sclerosis or squamous cell hyperplasia. It occurs in an older age group and is usually not associated with HPV infection or lesions elsewhere in the lower female genital tract.

Vulval squamous carcinoma: Similar to the situation with VIN there are two well-defined clinicopathological types of vulval squamous carcinoma, although there is some overlap. One type occurs in a younger age group and is often associated with adjacent classic type of VIN III. There may be concurrent lesions elsewhere in the female genital tract and there is an association with HPV infection. The other more common type of invasive squamous carcinoma is usually not associated with VIN, although sometimes there may be adjacent differentiated VIN. It occurs in an older age group and there is usually no association with HPV infection or with lesions elsewhere in the female genital tract. Some cases arise in lichen sclerosis.

Treatment: Treatment of VIN is usually wide local excision with careful follow up. There is a risk of multicentricity and the development of further lesions, especially with the classic type of VIN. Colposcopic examination may be useful in follow up, and patients with classic VIN should be investigated for lesions elsewhere in the female genital tract, e.g., CIN. Especially when there are multiple lesions and where invasive carcinoma has been excluded by multiple biopsies, laser ablation and other local ablative techniques may be used for treatment of VIN. This preserves the normal vulval anatomy. With extensive VIN, total or partial vulvectomy may be necessary.

For early low-stage carcinomas of the vulva, the usual treatment is wide local excision with ipsilateral lymph node dissection. For very superficially invasive squamous carcinomas (less than 1 mm invasion – stage Ia), lymph node dissection may not be necessary. Local excision of a carcinoma can comprise *simple excision*, *hemivulvectomy*, *or radical vulvectomy*. In those undergoing radical vulvectomy (usually with centrally located tumors), bilateral inguinal lymph node dissection is usually performed.

Prognosis: The prognosis of VIN is good and is largely determined by the risk of subsequent development of squamous carcinoma. Careful follow up is therefore necessary. With very superficially invasive squamous carcinomas (less than 1 mm – stage Ia), the prognosis is extremely good and the risk of lymph node metastases is close to zero.

With invasive carcinomas of the vulva, survival is primarily related to the stage of the disease. The most important prognostic factor is the presence or absence of lymph node involvement. Patients with stage I carcinoma have a mean 5-year survival of 85%. For stage IV tumors, this drops to approximately 10%.

27.5 Surgical Pathology Specimens: Clinical Aspects

27.5.1 Biopsy Specimens

Small vulval punch biopsies are usually taken for confirmation of the presence of specific dermatoses, vulval dystrophies, VIN, or invasive carcinomas.

27.5.2 Resection Specimens

Resection specimens are usually for VIN or tumors and include wide local excisions, hemivulvectomies, and radical (total) vulvectomies. With a total vulvectomy, all the perineum surrounding the vagina is removed. Inguinal lymph nodes from one or both sides are usually submitted with tumor resections. Ipsilateral lymph node dissection is undertaken for lateral tumors and bilateral lymph node dissection for midline tumors or tumors close to the midline.

27.6 Surgical Pathology Specimens: Laboratory Protocols

27.6.1 Biopsies Specimens

Small vulval punch biopsies are counted and examined in their entirety at multiple histological levels.

27.6.2 Resection Specimens

27.6.2.1 Wide Local Excisions

These are treated like a skin ellipse. The deep and lateral margins are inked and representative blocks taken to show the lesion in relation to them. Especially with VIN, it may be difficult to assess the presence of a lesion grossly as this can be very subtle. The presence of a previous biopsy site may assist in this regard. *Marking of the previous biopsy site by the surgeon with tattoo ink or a suture may be helpful*. Lymph nodes may be submitted separately. These are carefully dissected from the fat and the number retrieved from each site documented.

27.6.2.2 Hemivulvectomy and Radical Vulvectomy

- Hemivulvectomies (or partial vulvectomies) require orientation by the surgeon. If this is not done, it may be necessary to contact the surgeon before sectioning is performed.
- A total vulvectomy looks like an ellipse of skin with a central defect corresponding to the vaginal opening. The specimen is orientated. The clitoris is present superiorly and in the midline. The hair-bearing labia majora are present laterally. Inguinal fat, when present, is on the superior aspect of the specimen and to both sides (Fig. 27.2).
- If a gross lesion is seen this may be photographed.
- The specimen is inked including the free lateral and deep margins.
- The length, width, and depth of the specimen is measured (cm). Diagrams may be necessary and help in reporting.
- If inguinal fat is present on one or both sides, this is carefully sectioned looking for lymph nodes. These are separated, enumerated, and labeled as from the right or left side.
- The presence of any gross lesion is noted and measured in three dimensions (cm). The distances to the nearest resection margins are measured and detailed.
- The presence of any other smaller lesions is documented similar to the main lesion.

  Blocks for histology (Fig. 27.2):

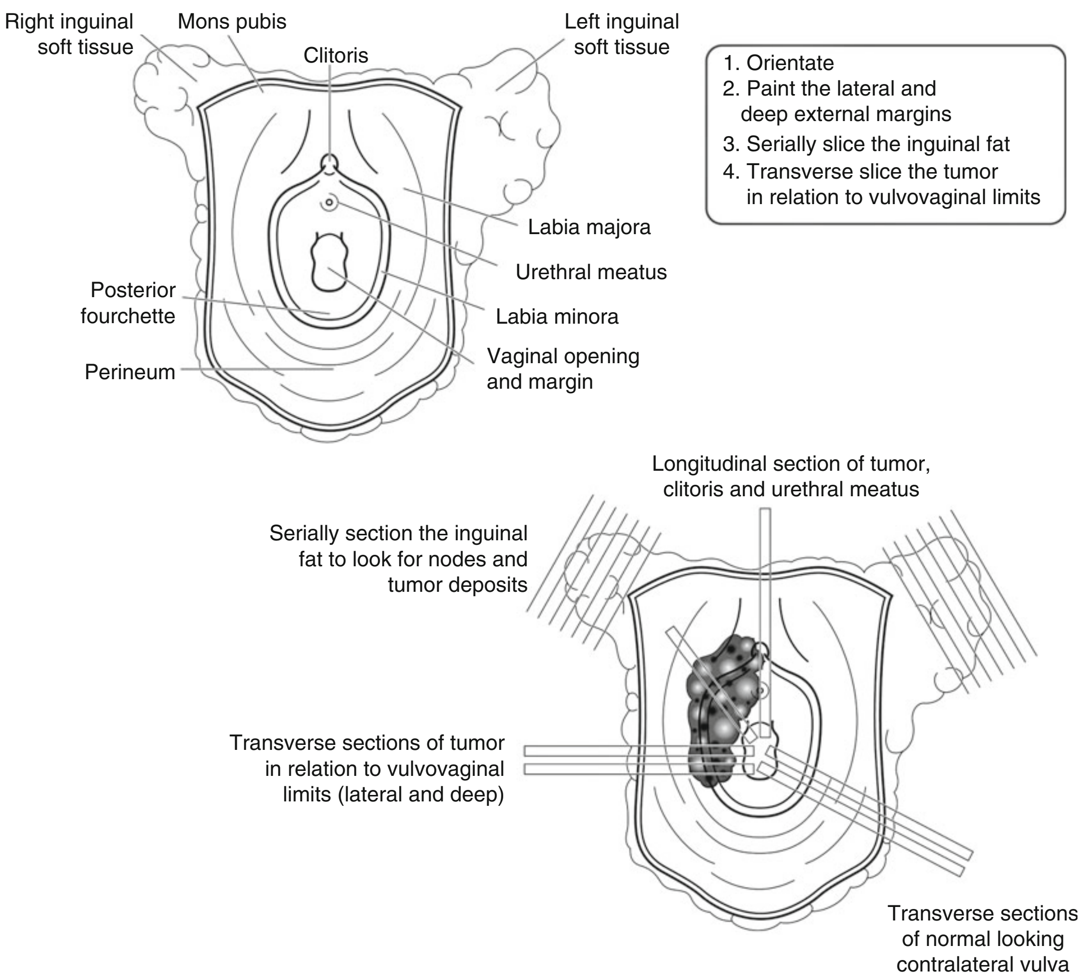

Fig. 27.2 Orientation and blocking of a radical vulvectomy specimen (Reproduced, with permission, from Allen and Cameron (2004))

- Multiple blocks are taken of all lesions seen grossly.
- Transverse blocks are taken to show the relationship of the lesion to the nearest margins including the *lateral*, *deep* (*soft tissue*), *vaginal*, *urethral*, *and anal margins*. The margins are labeled on the slide.
- Also submit representative blocks of grossly normal skin.
- Longitudinal sections of clitoris and any relevant lesion are taken.
- All lymph nodes are serially sectioned and completely submitted. The right- and left-sided lymph nodes are separated.

Histopathology report:

- The site (right, left, labia majora/minora, clitoris) and gross characteristics of the tumor are stated, e.g., polypoid, ulcerated.
- Tumor measurements – the width of tumor in two dimensions (cm) is given.
- Tumor type – the vast majority of malignant tumors are squamous carcinomas, although rarer morphological subtypes may occur.
- Tumor differentiation – squamous carcinomas are classified as well, moderately, or poorly differentiated.
- Depth of invasion – the depth of invasion is measured from the epithelial–stromal junction of the adjacent most superficial dermal papilla to the deepest aspect of the tumor.
- The nature of the invasive component – whether the invasive squamous carcinoma is confluent or exhibits a "finger-like" growth pattern may be of prognostic importance.
- The presence or absence of the following are noted:
  - Adjacent VIN (its type and grade), signs of HPV infection, associated vulval dystrophy or Paget's disease.
  - Lymphovascular permeation.
  - Lymph node involvement (site, number involved, intraparenchymal or extracapsular extension). For small tumor deposits, the size of the largest (mm) should be given.
  - VIN or tumor at the skin or vaginal margins, or, if clear, the minimum distance (mm) from them.
  - Tumor at the deep margin, or, if clear, the minimum distance (mm) from it.
  - Involvement of other structures such as the vagina or anus.

The British Association of Gynaecological Pathologists, British Gynaecological Cancer Society, and gynecological clinical reference group of the National Cancer Intelligence Network recommend that FIGO staging be used for gynecological cancers rather than TNM.

2009 FIGO staging of vulval carcinoma

I	Tumor confined to the vulva
IA	Tumor confined to the vulva or perineum, ≤2 cm in size with stromal invasion ≤1 mm[a], negative nodes
IB	Tumor confined to the vulva or perineum, >2 cm in size or with stromal invasion >1 mm, negative nodes
II	Tumor of any size with extension to adjacent perineal structures (1/3 lower urethra, 1/3 lower vagina, anus), negative nodes
III	Tumor of any size with or without extension to adjacent perineal structures (1/3 lower urethra, 1/3 lower vagina, anus) with positive inguino-femoral lymph nodes
IIIA	
(i)	One lymph node metastasis (≥5 mm)
(ii)	One to two lymph node metastasis(es) (<5 mm)
IIIB	
(i)	Two or more lymph node metastases (≥5 mm)
(ii)	Three or more lymph node metastases (<5 mm)
IIIC	Positive nodes with extracapsular spread
IV	Tumor invades other regional structures (2/3 upper urethra, 2/3 upper vagina) or distant structures
IVA	Tumor invades any of the following:
(i)	Upper urethra and/or vaginal mucosa, bladder mucosa, rectal mucosa, or fixed to pelvic bone
(ii)	Fixed or ulcerated inguino-femoral lymph nodes
IVB	Any distant metastasis including pelvic lymph nodes

[a]The depth of invasion is defined as the measurement of the tumor from the epithelial–stromal junction of the adjacent most superficial epithelial papilla to the deepest point of invasion.

Vascular space involvement, either venous or lymphatic, does not alter the staging.

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28 Placenta

Caroline Gannon

28.1 Clinical

Routine examination of all placentas is unnecessary: there are maternal, fetal, and placental indications for examination, which include hypertension, diabetes, infection, placental abruption, prematurity, and fetal death. In general, around 10–15% of pregnancies are complicated by maternal, fetal, or placental disease and these are the cases that should be examined.

28.2 Anatomy

The average placenta weighs 400–500 g and measures approximately 20 cm in diameter at term: there are considerable variations in size, shape, and form. The placental tissue itself is composed of chorionic villi lined by cytotrophoblast and syncytiotrophoblastic cells. The fetal surface of the placenta is the chorionic plate: the umbilical cord inserts into this. The umbilical cord consists of two arteries and one vein embedded in a gelatinous matrix, and is covered by amnion. The placental (peripheral) membranes are continuous with the chorionic plate and comprise two layers, the chorion and the amnion. The maternal surface of the placenta is divided into lobules or cotyledons.

28.3 Surgical Pathology Specimens: Laboratory Protocols

The placenta can be examined fresh or formalin-fixed (microbiology and karyotyping is possible from fresh placentas).

These measurements should be made initially:

- Trimmed weight (following removal of the membranes) in gm
- Size of disc in three dimensions in cm
- Length and diameter of umbilical cord in cm

Note the following:

- Placental form: normal, succenturiate/accessory lobe, circumvallate, circummarginate
- Cord: presence of knots (false, true), cord spiralling (increased, decreased, normal), cord insertion (central, eccentric, marginal, velamentous), edema, number of vessels
- Membranes: completeness, translucency/opacity, meconium staining
- Maternal surface: completeness, crater, adherent hemorrhage
- Chorionic plate: distribution of vessels, meconium staining, translucency/opacity, dullness, amnion nodosum

The disc is then sliced at 0.5–1.0 cm intervals and simultaneous palpation carried out to identify infarcts and other anomalies. Infarcts should be assessed as old or recent, the size of the largest

C. Gannon
Histopathology Laboratory, Institute of Pathology,
Royal Victoria Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: caroline.gannon@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_28, © Springer-Verlag London 2013

recorded, and an estimation of the volume of the disc affected made.

Blocks for histology:

Usually 4–5 blocks are sufficient, including

- Three representative sections of placental parenchyma
- Three cross sections of the umbilical cord (taken from at least 2 cm above cord insertion, at the midpoint, and at fetal end of cord)
- A membrane roll is prepared by cutting a strip of membranes from the site of rupture to the margin of the disc, grasping the edge of the disc with forceps, rolling the membranes around, and sliding the roll from the forceps; a cross section of the roll is taken
- Any grossly abnormal area is sampled

28.3.1 Multiple Gestations

Twins account for a significant proportion of perinatal morbidity and mortality: placentas from twins demonstrate the same pathology as singleton placentas, as well as pathology related to twinning (twin–twin transfusion, asymmetry, vanishing twin).

Determination of Chorionicity

This is the most important step in the examination of twin placentas. A dichorionic placenta means that two placentas have formed, but these may be separate or fused together. A monochorionic placenta indicates a shared disc: monochorionic twins are monozygotic ("identical"), but dichorionic twins can be monozygotic or dizygotic, depending when the fertilized egg divided into two.

Twin placentas are one of four types:

1. Diamniotic, dichorionic separated twin placentas. The two discs are completely separate.
2. Diamniotic, dichorionic, fused twin placentas.
3. Diamniotic, monochorionic, fused twin placentas.

In 2 and 3, there is a single placental disc with two umbilical cords. Common outer membranes are present. The dividing membrane between the two placental territories must be examined to determine chorionicity.

4. Monoamniotic, monochorionic, twin placentas.

There are two umbilical cords but no dividing membrane. The two cords are usually positioned closely together.

Additional examination for twin placentas: The dividing membrane comprises two amnions (in monochorionic twins), or two chorions and two amnions (in dichorionic twins with fused discs). Monochorionic membranes divide easily and are thin and transparent, whereas dichorionic membranes are opaque, thicker, and difficult to separate. There is often a distinct ridge between the territories of dichorionic twins on the fused common disc; in monochorionic twins there is no ridge. The common membrane is studied histologically from both a non-separated area and the T zone point of attachment to the chorionic plate. Vascular anastomoses between the two territories can lead to discrepancies in size and viability of the infants. The type of anastomosis should be described (artery–artery, artery–vein, vein–vein).

In triplets and higher multiples, similar principles apply with the examination of the dividing membranes between placental territories (Fig. 28.1).

Histopathology report:

The macroscopic description should include

- The trimmed weight and dimensions of the disc
- The length, diameter, coiling index, and insertion of the umbilical cord
- A description of the maternal surface, chorionic plate, and membranes

The microscopic description should include

- An assessment of villous maturation (accelerated, delayed, dysmature)
- An assessment of villous morphology (dysmorphism, hydrops, molar, oedema, villous inflammation, infarction)
- An assessment of the intervillous space (intervillositis, intervillous fibrin)
- A description of any hematoma (extent, position-intervillous, maternal, subchorionic)
- An assessment of the chorionic plate (presence of chorioamnionitis, chorionic plate vasculitis)
- An assessment of the cord (presence of inflammation, number of vessels involved).

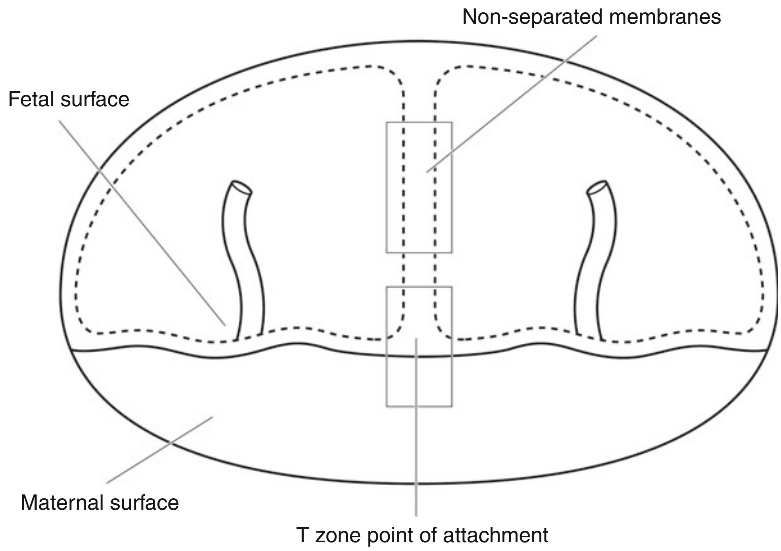

Fig. 28.1 Examination of membrane distribution in twin pregnancy (Reproduced, with permission, from Allen and Cameron (2004))

28.3.2 Placenta Accreta

This is defined as abnormal adherence of the placenta to the uterine wall. There is considerable maternal morbidity and mortality associated with the condition, which is the leading cause of peripartum hysterectomy.

Predisposing factors include advanced maternal age, previous caesarean section delivery, placenta previa, previous placental retention, multigravidity, and high parity.

Pathologically, placental villi are present adjacent to myometrium with no intervening decidual layer. Placenta accreta is classified according to the depth of infiltration through the uterus. In placenta accreta vera the villi embed directly onto superficial myometrium; in placenta increta, the villi are found deeper in the body of myometrium; and in placenta percreta, the villi penetrate the full thickness of myometrium with the risk of uterine perforation and hemoperitoneum. There may be variations in the depth of penetration and the condition may be focal or diffuse.

Examination of a hysterectomy specimen in which placenta accreta is suspected involves careful sampling of the placental bed. The larger radial and arcuate arteries of the uterus may show pregnancy-induced changes, a feature usually only seen in the smaller spiral arteries, and this may be responsible for the severity of hemorrhage usually seen in placenta accreta.

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Part VI

Urological Specimens

29 Kidney, Renal Pelvis, and Ureter

Declan M. O'Rourke and Derek C. Allen

29.1 Anatomy

The kidneys are situated in the retroperitoneum and lie between the upper border of the twelfth thoracic and third lumbar vertebrae. Each kidney has a convex lateral border and a concave medial border which merge at the poles (superior and inferior portions). Surrounding each organ is the fibrous renal capsule, which is loosely adherent to it. Adipose tissue (perirenal fat) encases the capsule and is in turn surrounded by the Gerota's fascia, which secures the kidney to the posterior abdominal wall. Much of the medial border comprises an indentation, the hilum, through which the renal vessels, nerves, lymphatics, and the renal pelvis enter or leave the renal sinus, the space enclosed by the renal parenchyma. The right kidney is usually slightly lower than the left on account of the liver. Each kidney is about 11–12 cm in length, 5–7.5 cm in breadth, and 2.5–3.5 cm in thickness and weighs between 115 and 175 g (Fig. 29.1). The persistence of distinct fetal lobes is common and is a normal anatomical variant.

The relative position of the main structures in the hilum is generally as follows: the vein is in front, the artery in the middle, and the ureter behind and directed downward. The renal capsule is easily stripped off, revealing a smooth and even surface. The parenchyma consists of cortex and medulla that are grossly distinct. The cortex forms a 1-cm layer beneath the renal capsule and extends down between the renal pyramids forming the columns of Bertin. The medulla consists of renal pyramids and is divided into an outer medulla and inner medulla or papilla. The papilla protrudes into the minor calyx. Its tip has between 20 and 70 openings of the papillary collecting ducts (Bellini's ducts).

The renal pelvis is the sac-like expansion of the upper ureter. Two or three major calyces extend from the pelvis and divide into minor calyces, into which the papillae protrude. The ureters measure approximately 30 cm in length equally divided between retroperitoneum and pelvis. They are usually placed on a level with the spinous process of the first lumbar vertebra and run downward and medially in front of the psoas major, and enter the pelvis, reaching the lateral angle of the bladder. Finally, the ureters run obliquely for about 2 cm through the wall of the bladder and open by slit-like apertures into the cavity at the lateral angles of the trigone. Owing to their oblique course, the upper and lower walls of the terminal portions of the ureters become closely applied to each other when the bladder is distended, and, acting as valves, prevent regurgitation of urine from the bladder.

Lymphovascular drainage:

There is a dual lymphatic drainage system. The major lymphatic drainage follows the blood

D.M. O'Rourke (✉) • D.C. Allen
Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: declan.orourke@belfasttrust.hscni.net; derek.allen@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*, DOI 10.1007/978-0-85729-673-3_29, © Springer-Verlag London 2013

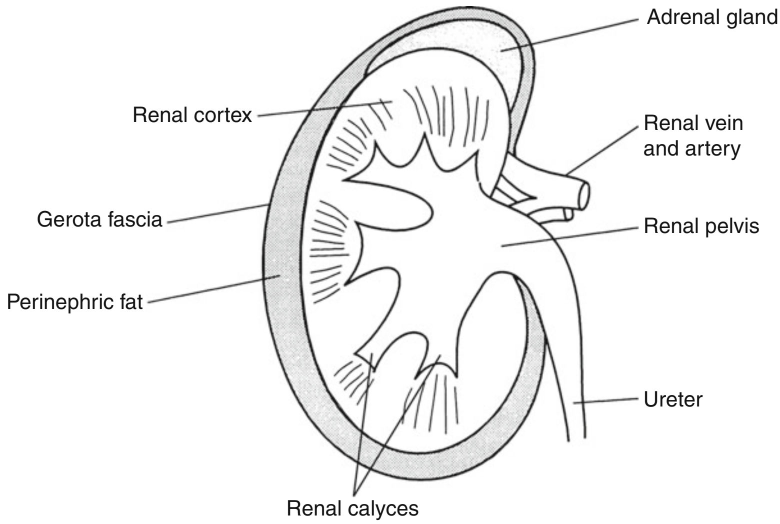


Fig. 29.1 Anatomy of the kidney (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

vessels from the parenchyma to the renal sinus, then to the hilum, and terminating in the para-aortic lymph nodes. There is also a capsular lymphatic drainage from the superficial cortex extending from the capsule to the hilum and joining the major lymphatic flow. When tumor spreads from the kidney, it is initially to the hilar and then to the para-aortic lymph nodes (Fig. 29.2).

29.2 Clinical Presentation

Presentations of medical renal diseases are best grouped as nephrotic syndrome, nephritic syndrome, acute renal failure, and chronic renal failure. Nephrotic syndrome (NS) is defined as the excretion of more than 3.5 g protein/24 h associated with hypoalbuminemia, hypercholesterolemia, and edema. Nephritic syndrome is usually characterized by the relatively sudden onset of hematuria with red blood cell casts and proteinuria accompanied by hypertension, and a reduced glomerular filtration rate. Acute renal failure (ARF) is the loss of renal function occurring rapidly, usually over days. It is characterized by a rapid increase in creatinine and oliguria. Chronic renal failure (CRF) is the progressive loss of renal function, occurring over a period of several years as a result of glomerular or tubulo-interstitial pathology.

Diseases of the kidney and upper urinary tract present with a variety of symptoms including gross hematuria, loin pain, fever, or a mass lesion. Causes can be divided generally into congenital (cysts, tumors) and acquired. Of the acquired group these include medical (acute and chronic pyelonephritis) and surgical (stones) causes.

Renal cell carcinoma (RCC) may remain clinically occult for most of its course. The classic triad of flank pain, hematuria, and flank mass is infrequent (10%) and is indicative of advanced disease. RCC accounts for approximately 2% of all cancers with almost 7,000 new cases of kidney and renal pelvic cancer in the UK in 2009. The incidence of RCC has increased substantially over the last two decades, at least in part, as a result of improved diagnostic techniques, and most cases of RCC are now incidentally detected. Up to 30% of patients with RCC present with metastatic disease, and recurrence develops in 40% of patients treated for localized tumor.

Other signs and symptoms include

- Weight loss (33%)
- Fever (20%)
- Hypertension (20%)
- Hypercalcemia (5%).

Compared to renal cortical tumors, carcinomas of renal pelvis and ureter are relatively uncommon, accounting for 4–5% of all urothelial tumors. Gross hematuria (67%) is the most common presenting symptom of renal pelvis and ureteric tumors. Pain is the next most common symptom, either in the flank or in conjunction with gross hematuria and, therefore, due to clot colic. Other presentations include pyuria, weight

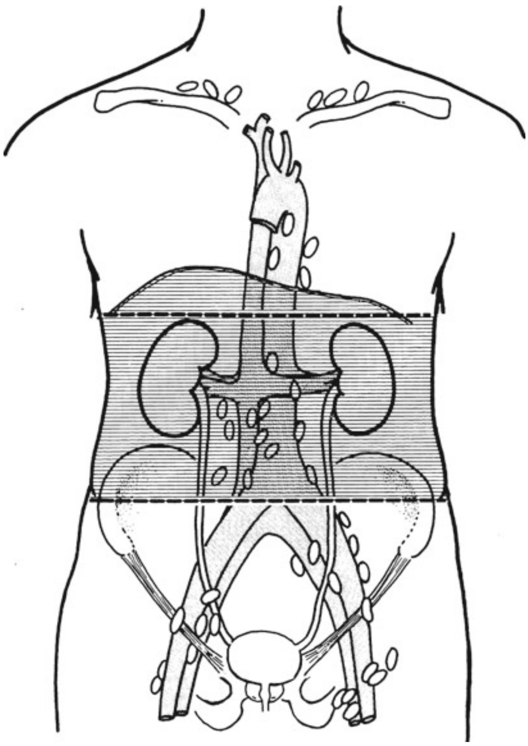


Fig. 29.2 Kidney – regional lymph nodes. The regional lymph nodes are the hilar, abdominal para-aortic, and paracaval nodes. Laterality does not affect the N categories (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

loss, anemia, unexplained fever, hypertension, renal failure, and calculus disease.

29.3 Clinical Investigations

- Biochemical studies in medical renal disease (i.e., urea and electrolytes, creatinine clearance, 24 h urinary protein, plasma protein electrophoresis, and Bence Jones urinary protein).
- Intravenous urogram (IVU) – Not frequently used in the initial evaluation of renal masses because of its low sensitivity and specificity.
- Intravenous pyelogram (IVP) – In pelviureteric junction (PUJ) obstruction reveals a delayed nephrogram that may persist for 24 h or more. Ultrasound has superceded IVP in children.
- Retrograde pyelography – This tends to be used in two circumstances to confirm the presence of a constant filling defect, either in the ureter or renal pelvis, and to investigate patients with a nonfunctioning kidney.
- Cystoscopy and ureteropyeloscopy – This procedure is used increasingly for the diagnosis of upper tract urothelial tumors as biopsy forceps or cytology brushings can be used to collect tissue.
- Cytopathology – Voided urine samples obtained for cytopathology lack sensitivity, especially for low-grade tumors, but this increases for high-grade tumors that tend to shed more tumor cells.
- Contrast-enhanced CT scanning – Has become the imaging procedure of choice for diagnosis and staging of renal cell cancer. In most cases, CT imaging can differentiate cystic from solid masses and supplies information about lymph nodes, renal vein, and inferior vena cava involvement.
- Ultrasonography – Can be useful in evaluating questionable cystic renal lesions if CT imaging is inconclusive. Large papillary renal tumors are frequently undetectable by renal ultrasound.
- Renal arteriography – Is not used in the evaluation of suspected renal mass as frequently now as it was in the past.
- MRI – Useful in suspected inferior vena cava involvement.
- DMSA/DPTA scans for renal function (more use in pediatric nephrology).
- Doppler scans following renal transplantation to look at renal arterial and venous flow.
- A bone scan is recommended for patients with bone pain or an elevated alkaline phosphatase level.

29.4 Pathological Conditions

29.4.1 Non-neoplastic Conditions

Renal stones: Nephrolithiasis affects 2–10% of the population and symptoms (renal colic) arise as these calculi become impacted within the ureter as they pass toward the urinary bladder. Types of renal calculi include calcium stones (75%), magnesium ammonium phosphate stones (15%), and cystine stones (2%). Plain abdominal film may diagnose and locate the stone. Treatments include extracorporeal lithotripsy, ureteroscopy, endoscopic laser lithotripsy, percutaneous nephrolithotomy/nephrostomy, open nephrostomy, and, less commonly, nephrectomy (chronic pain, large staghorn calculi, poorly functioning).

Chronic pyelonephritis: Refers to renal injury induced by recurrent renal infection in patients with urinary tract obstruction, renal dysplasia, or, most commonly, vesicoureteral reflux (VUR). It is associated with progressive renal scarring, which can lead to end-stage renal disease (ESRD). Intravenous urogram establishes the diagnosis of pyelonephritis, and nephrectomy is required in cases with significant morbidity or loss of function.

PUJ obstruction: One of the most common congenital abnormalities of the urinary tract and associated with a number of anomalies. The etiologies are numerous and classified on an anatomic basis as either extrinsic (scars, VUR) or intrinsic (developmental). Treatment is primarily surgical (pyeloplasty), which now can be performed laparoscopically.

Hydronephrosis and hydroureter: Common clinical conditions and major causes include calculi, reflux (children), prostate enlargement, pregnancy, and cervical cancer. Although patients usually present with some signs or symptoms, hydronephrosis can be an incidental finding, e.g., found on CT scan done for other reasons. Grossly the pelvi-calyceal system is dilated with compression of the papillae and parenchymal thinning, progressing to the point at which only a thin rim of parenchyma is present. IVP is probably the most useful imaging study for identifying both the presence and cause of hydronephrosis and hydroureter. Treatments include ureteral stenting, nephrostomy tube, and, ultimately, may require nephrectomy for pain or loss of function.

Pyonephrosis: Refers to infected purulent urine in an obstructed collecting system (e.g., stones, tumors, PUJ obstruction) and may develop from ascending infection of the urinary tract or the hematogenous spread of a bacterial pathogen. Similar to an abscess, it typically is associated with fever, chills, and flank pain. The diagnosis is usually confirmed with ultrasound. This disorder is relatively uncommon and treatment options include percutaneous nephrostomy and antibiotic therapy. In cases with marked obstruction (± staghorn calculi) and loss of function, nephrectomy may be required.

Xanthogranulomatous pyelonephritis (*XGP*): A chronic inflammatory disorder of the kidney characterized by a mass originating in the renal parenchyma. It resembles a true neoplasm in terms of its radiographical appearance and ability to involve adjacent structures or organs. Occurring in late middle-aged females the exact etiology of XGP is unknown, but it is generally accepted that the disease process requires long-term renal obstruction (stones, frequently of staghorn proportions) and infection (Proteus or Escherichia coli). The gross appearance of XGP is a mass of yellow tissue with regional necrosis and hemorrhage, superficially resembling that of a renal cell carcinoma. The pathognomonic microscopic feature is the lipid-laden "foamy" macrophage accompanied by both chronic and acute phase inflammatory cells. Nephrectomy is the treatment of choice.

Renal cysts: Occur in one third of people older than 50 years. While the majority are simple cysts, renal cystic disease has multiple etiologies. Broad categories of cystic disease include the following: congenital, genetic, acquired, cysts associated with systemic disease (von Hippel-Lindau), and malignancy (renal cell carcinoma).

The most common larger cysts are acquired cysts, simple cysts, and cysts with ADPKD.

Autosomal dominant polycystic kidney disease (*ADPKD*): One of the most common inherited disorders in humans, and the most frequent genetic cause of renal failure in adults. ADPKD is a multisystem and progressive disorder characterized by formation and enlargement of cysts in the kidney and other organs (e.g., liver, pancreas, spleen). Clinical features usually begin in the third to fourth decade of life, with the major cause of morbidity being progressive renal dysfunction, resulting in grossly enlarged kidneys. The kidneys sometimes require removal when complicated by infection, hemorrhage, or when they reach a huge size.

Congenital anomalies: Anomalies in form, position, mass, and number; parenchyma maldevelopment.

Vascular diseases: Hypertension, thrombotic microangiopathy, renal artery stenosis, dissection and aneurysm, renal emboli and infarcts, renal atheroemboloism, renal vein thrombosis, vasculitis.

Miscellaneous: Other tubulointerstitial diseases, malakoplakia, tuberculosis, and metabolic and miscellaneous conditions.

29.4.2 Neoplastic Conditions

29.4.2.1 Adult Tumors

Benign tumors

Oncocytoma: Represents 4% of renal tumors and usually occurs in adults over 50 as an incidental finding. It has a benign behavior if strict diagnostic criteria are followed. Grossly it is circumscribed, brown-yellow, with a stellate central scar in larger lesions. It may be bilateral or multifocal and can invade the renal capsule. Histologically it has a sheeted or nested pattern of uniform cells with intensely eosinophilic and coarse granular cytoplasm. Patterns not allowed include papillary areas, clear/spindle cells, and vascular or fat invasion. It is CD117-positive, with occasionally focal positive cells for CK7. Electron microscopy shows the cytoplasm packed with mitochondria.

Angiomyolipoma (*AML*): Represents less than 1% of renal tumors. It is a mesenchymal tumor believed to originate from the so-called perivascular epithelioid cell (PEComa) and is closely related to other PEC group tumors (e.g. clear cell tumors of lung, pancreas, and uterus). Multifocality and bilaterality are often associated with tuberous sclerosis. Grossly the cut surface is variable, reflecting the amounts of fat, smooth muscle, or vessels in the tumor. It comprises adipose tissue, smooth muscle, and dystrophic vessels in variable proportions. It is positive for melanocytic markers (HMB-45, melan-A). The majority are benign, but retroperitoneal hemorrhage is a rare complication that can be fatal (usually >4 cm). Rare aggressive behavior is associated with the predominant epithelioid cell type. Generally asymptomatic with characteristic CT scan appearances, surgical removal is performed only when they exceed 4 cm due to the risk of rupture and hemorrhage.

Other benign tumors: Papillary adenoma, metanephric adenoma, adenofibroma, and cystic nephroma represent other less common tumors.

Benign mesenchymal tumors: Renomedullary interstitial cell tumor, adult mesoblastic nephroma, leiomyoma, hemangioma, lymphangioma, and solitary fibrous tumor.

Malignant tumors

Renal cell carcinoma: More than 90% of tumors in the kidney that come to surgery are renal cell carcinomas and these cause approximately 2.4% of cancer deaths. The age is usually >50 years old with an M/F ratio of 2:1. A number of cellular, environmental, genetic, and hormonal factors have been studied as possible causal factors, including cigarette smoking, obesity, hypertension, unopposed estrogen therapy, occupational exposure to petroleum products, heavy metals, solvents, and asbestos. The risk of renal cell carcinoma is increased with the abuse of phenacetin-containing analgesics, acquired cystic kidney disease associated with chronic renal insufficiency, renal dialysis, tuberous sclerosis, von Hippel-Lindau disease, and renal transplantation with its associated immunosuppression. Prognostic factors for RCC include tumor size, stage, nodal/distant metastases, histological subtype, nuclear grade (in clear cell RCC), sarcomatoid features, and tumor necrosis. Performance status and presence or absence of systemic symptoms are also relevant. Partial, total, or radical nephrectomy (laparoscopic) is the mainstay of treatment options for renal cortical tumors. Surgical resection of primary tumor is often performed to decrease tumor load even in patients with metastatic disease. In situ tumor ablations such as radiofrequency ablation (RFA) are becoming more common, particularly for smaller tumors. RFA is a less invasive treatment option that may be preferable in patients at high surgical risk, but it is associated with a higher risk of local tumor recurrence compared with surgical excision. Biopsy is recommended for all patients undergoing RFA.

Active surveillance may be an acceptable approach to delay or avoid further intervention in the patient at high surgical risk. Immunotherapy using IL-2 and interferons was the treatment of choice with metastatic disease but now targeted therapies (multikinase inhibitors) are preferred with promising results. As well as their use in metastatic RCC, these therapies are currently also being evaluated in the adjuvant setting in high-risk tumors. The overall 5-year survival is 45% (all types), 70% if node-negative, and 15% if there is renal vein or perinephric fat involvement. Excision of solitary metastases has been found to be effective.

WHO classification (2004)

- Clear cell renal cell carcinoma
- Papillary renal cell carcinoma
- Chromophobe renal cell carcinoma
- Collecting duct carcinoma
- Renal medullary carcinoma
- Tubulocystic carcinoma
- Family translocation-associated carcinoma (Xp11.2, t(6;11))
- Mucinous tubular and spindle cell carcinoma
- Thyroid-like follicular carcinoma of kidney
- Clear cell papillary renal cell carcinoma
- Renal cell carcinoma, unclassified
- Tumors associated with end-stage kidney disease

Renal cell carcinoma, *clear cell type*: Derived from the proximal convoluted tubule, it accounts for 70% of renal tumors. Cytogenetic abnormality includes 3p deletion in 98% of cases and this is considered the initial mutation. It is characterized by a multinodular tumor mass often elevating the renal capsule and compressing the adjacent renal parenchyma. It has a predominantly yellow cut surface and additional brown and white foci. Most are solid, but some are composed of multiple cysts varying in size up to 2–3 cm in diameter. Histologically it has compact, tubulocystic, alveolar or rarely papillary architecture. The cells have clear cytoplasm (from glycogen/lipid) and distinct but delicate cell boundaries. A chicken-wire/delicate sinusoidal vasculature is common.

Papillary renal cell carcinoma (*PRCC*)/ *chromophil renal cell carcinoma*: PRCC is more often bilateral and multifocal compared to other common renal cell tumors, and surrounded by a fibrous pseudocapsule on gross evaluation. Multifocality is present in >45% of cases, but in some, this is reported as only a microscopic finding (papillary adenomas). The majority of PRCCs have a broad morphology, including papillary, tubular, and solid patterns. Areas containing papillary differentiation are seen in most cases. Cores of papillae are mostly loose and fibrovascular, often containing variable numbers of foamy macrophages. Psammoma bodies, hemosiderin-laden macrophages, hemosiderin deposition within tumor cells are often seen. WHO divides papillary PRCC into type 1 and type 2 and this has also been supported by molecular studies. Trisomies 7 and 17 are more often reported in type 1 than type 2 PRCC. Many believe the Fuhrman grading system is well suited for PRCC, but this has not been substantiated in studies. Immunohistochemistry shows diffuse positivity for CK7 more often in type 1 than type 2 tumors. AMACR, RCC-Ab, CD10, and pax-2 are also positive. The prognosis overall is better than clear cell RCC (80% 5-year survival), and possibly worse than chromophobe RCC.

Chromophobe renal cell carcinoma: Origin from the intercalated cells of cortical collecting ducts and represents 5% of renal tumors. They are slightly better in behavior than clear cell when sorted by grade/stage. Cytogenetic studies show multiple monosomies. The cut surface of the fresh specimen appears homogeneously orange and turns tan or sandy after formalin fixation. They have a solid pattern of growth with a mixture of pale and eosinophilic cells with "raisinoid" nuclei, perinuclear halos, and prominent cell membranes. There is variable granular diffuse cytoplasmic staining with Hale's colloidal iron in a majority of cases. Immunohistochemistry shows diffuse membranous positivity for CK7. CD117, E-cadherin, MOC-31, CAM 5.2, and BER-EP4 are also positive. Electron microscopy shows abundant mitochondria with tubulocystic cristae.

Collecting duct carcinoma: Rare (<1% of malignant renal cell tumors), high-grade renal cell carcinoma, likely arising from cells of the collecting ducts of the renal medulla. It is predominantly centered in the medulla of the kidney and although responses to therapy have been very limited, targeted therapies against tyrosine kinase receptors of VEGF-related molecules have shown some promise. It has variable architectural patterns including tubular, solid, and tubular/acinar. The prognosis is poor and 50% of patients die of disease within 2 years. Frequent metastatic sites at presentation include lymph, lungs, and bones. It is positive for 34βE12, EMA, CK7, and CEA immunomarkers.

Translocation carcinoma: Renal carcinomas defined by translocations involving MiTF/TFE family genes (*TFE3* or *TFEB*). They are uncommon, but constitute a larger proportion of renal cell carcinomas in pediatric age groups. In children, they usually present at an advanced stage. They are also more aggressive in adults. These carcinomas are high nuclear grade, with prominent papillary and/or alveolar growth patterns, and composed of clear cells. Psammomatous calcifications are often present. The carcinomas are negative or only focally positive for epithelial markers and vimentin. TFE3 and TFEB are highly sensitive and specific positive immunohistochemical markers for translocation carcinomas.

Mucinous tubular and spindle cell carcinoma: Low-grade biphasic carcinoma usually associated with favorable prognosis. It has multiple chromosomal losses and the majority of cases are in females. It is a well-circumscribed mass and histology shows tightly packed elongated tubules with variable low-grade spindle cell areas and nuclei similar to epithelial areas. Immunohistochemistry shows a significant overlap with papillary renal cell carcinoma (CK7, AMACR-positive).

Renal cell carcinoma, *unclassified*: These include renal cell carcinomas that do not readily fit into one of the usual categories including a combination of features of more than one recognized subtype, or sarcomatoid morphology without identifiable epithelial component. By definition, this category not only includes high-grade, aggressive tumors but also low-grade, indolent tumors, including some oncocytoma-like features. Prognosis depends on tumor type, pathologic stage, and metastatic status.

Sarcomatoid carcinoma: Represent 1% of renal tumors, and not a distinct histological entity but due to progressive transformation of different subtypes of renal cell carcinoma. However, most tumors are clear cell carcinomas since they are more common. It is very aggressive with a median survival of 19 months. Grossly it is fleshy, grey-white with infiltrative margins and composed of atypical spindle or tumor giant cells with marked nuclear pleomorphism. It must have an epithelial component (may need generous sampling) and should have sarcomatoid overgrowth of at least one low-power field. It is positive with cytokeratins (focal) and vimentin.

Urothelial carcinoma: Upper tract urothelial tumors of the renal pelvis and ureters are relatively rare. Tumors of the renal pelvis account for approximately 10% of all renal tumors and 5% of all urothelial tumors. Ureteral tumors are even more uncommon. Transitional cell carcinoma (TCC) accounts for more than 90% of upper tract urothelial tumors. The mean age of occurrence is 65 years with a male-to-female ratio of 3:1. Etiological factors are similar to those of bladder cancer. A majority are papillary or exophytic distending and blocking the pelvi-ureteric system, but if infiltrative, the firm, grey-white tumor can involve renal parenchyma causing confusion with other renal cancers in particular sarcomatoid carcinoma or collecting duct carcinoma. Squamous carcinoma and adenocarcinoma are rare but may form a component of high-grade TCC. The distribution of upper tract transitional cell carcinoma is: renal pelvis – 58%; ureter – 35% (73% of which are located in the distal ureter); both renal pelvis and ureter – 7%, and bilateral involvement – 2–5%. Approximately 30–75% of patients also develop bladder tumors at some point in their cancer course.

Laparoscopic nephroureterectomy with excision of the bladder cuff is indicated in patients with renal pelvis TCC, regionally extensive disease, and high-grade or high-stage lesions. Segmental ureterectomy coupled with ureteral reimplantation is a procedure indicated for tumors located in the distal ureter. Renal-sparing surgery, including segmental ureterectomy and endoscopic therapy is used in patients with small, lower-grade superficial lesions. This approach is used more frequently in patients with one kidney, bilateral disease, and compromised renal function. Medical treatment of upper tract urothelial tumors involves the instillation of chemotherapeutic agents, mitomycin C or bacille Calmette-Guérin (BCG). It is most appropriate for patients with multiple superficial disease or carcinoma in situ who also have bilateral disease and/or limited renal function. Although this appears to be safe as adjuvant therapy, its efficacy is not firmly established. Thus, it should be considered second-line therapy. Various chemotherapies, similar to those in bladder tumors, are used for metastatic tumors. Recently, targeted therapies against molecules of multiple pathways active in urothelial cancer are also being implemented. Tumor stage is the most important prognostic factor. Others include age, tumor site, grade, and non-transitional cell histology. The 5-year survival ranges from 91% for stage pT1 to 23% for pT3.

Other cancers: Leiomyosarcoma, liposarcoma, hemangiopericytoma, malignant fibrous histiocytoma, and metastatic neoplasms (melanoma, lung, other kidney, GIT, breast, ovary, and testes).

29.4.2.2 Pediatric Tumors

Wilms' tumors: Comprise more than 80% of renal tumors of childhood usually in children 2–4 years old. There is a slight preponderance of females. Associations with congenital anomalies: cryptorchidism, hypospadias, other genital anomalies, hemihypertrophy, and aniridia are well recognized. Wilms' tumors are usually large masses; more than 5 cm and solid. They are composed of variable admixtures of blastema, epithelium, and stroma. The epithelial component usually consists of small tubules or cysts lined by primitive columnar or cuboidal cells. The stroma may differentiate along the lines of almost any type of soft tissue.

They are divided into two categories: favorable and unfavorable histology, based on the absence or presence of cellular anaplasia, which is found in approximately 6% of Wilms' tumors and can be associated with an adverse outcome. Thus, it is important to sample Wilms' tumor specimens extensively. The usual treatment approach in most patients is nephrectomy followed by chemotherapy (vincristine, dactinomycin, doxorubicin) with or without postoperative radiotherapy (>pT2). Some give neoadjuvant chemotherapy followed by surgery and radiotherapy added postoperatively in the presence of cellular anaplasia. With current strategies, survival rates are approaching 90%.

Other pediatric renal tumors: Cystic nephroma, mesoblastic nephroma, clear cell sarcoma of the kidney, rhabdoid tumor, metanephric adenofibroma, ossifying renal tumor of infancy, lymphangioma, intrarenal teratoma, and uncommon tumors – renal cell carcinoma, lymphoreticular and hematopoietic tumors.

29.5 Surgical Pathology Specimens: Clinical Aspects

29.5.1 Biopsy Specimens

Fine needle aspiration cytology (*FNAC*): Less often performed now but usually in association with a renal core biopsy in the investigation of a mass lesion.

Percutaneous needle biopsy: This is more often in the investigation of medical renal disease but also used for the evaluation of a renal mass. The latter is to obtain a tissue diagnosis of malignancy for treatment options other than curative intent radical surgery, e.g., RFA or oncological drug therapy. This technique obtains a core of fresh renal tissue using a biopsy gun under radiological (ultrasound or CT) guidance.

Medical renal biopsies require special collection procedures and should be done only in centers with appropriate facilities and after consultation with the pathologist. Two to three cores are taken with fresh tissue for immunofluorescence (IF), fixed tissue for light microscopy (some laboratories use special fixatives, e.g., Bouins) and electron microscopy (3% glutaraldehyde). Surgical renal biopsies are routinely fixed in 10% formalin.

Indications for renal biopsy include glomerular hematuria, some cases of proteinuria, suspected renal neoplasm, and, following renal transplantation, to distinguish rejection from other causes of deterioration in renal function. Interpretation of findings requires expertise in the categorization of glomerulonephritis and other glomerulopathies (e.g., diabetes mellitus, amyloid, hereditary renal disease), interstitial nephritis and renal vascular disease, monitoring transplant rejection, diagnosis of drug toxicity, and systemic disease affecting the kidneys (e.g., vasculitis).

Open renal biopsy: Performed under general anesthesia if core biopsy is not possible and more often in the transplant situation (donor and recipient) when there is uncertainty about the state of the kidney. They often consist only of superficial cortex.

Pelvi-ureteric junction obstruction specimen: The specimen may be funnel-shaped if unopened and triangular if opened. The length, diameter at both ends, and thickness of the wall are measured and the presence and size of any strictures described. The specimen is opened along the main axis. The mucosal surface is examined for lesions and irregularities in texture. The outer surface is examined for mass lesions and fibrosis. Multiple sections taken along the long axis are submitted.

29.5.2 Resection Specimens

Simple nephrectomy, radical nephrectomy, and partial nephrectomy. Laparoscopic nephrectomy is now routine in experienced hands.

Simple nephrectomy: Is indicated in patients with an irreversibly damaged kidney because of symptomatic chronic infection, obstruction, calculus disease, or severe traumatic injury. It is also indicated to treat severe unilateral parenchymal damage from nephrosclerosis, pyelonephritis, reflux or congenital cystic dysplasia of the kidney.

Radical nephrectomy: Is the treatment of choice for patients with RCC. Radical nephrectomy encompasses ligating the renal artery and vein, removing the kidney outside the Gerota's fascia, and the ipsilateral adrenal gland, and performing a complete regional lymphadenectomy from the crus of the diaphragm to the aortic bifurcation. The surgical approach includes either a transperitoneal incision (extended or bilateral subcostal and thoracoabdominal) or an extraperitoneal incision, depending on the size and location of the tumor and the patient's condition. The surgical approach is guided more by individual preference than by necessity. Laparoscopic nephrectomy is the preferred approach. Benefits conferred by laparoscopic nephrectomy include decreased patient analgesic requirements, shorter hospitalization, and improved cosmetic results.

Removal of the adrenal gland has been advocated because the gland is enclosed within Gerota's fascia and because ipsilateral adrenal metastasis occurs in 2–10% of most reported series. The risk of adrenal metastasis is related to the malignant potential of the primary tumor, its size, and position. Patients with large tumors or tumors high in the upper pole are probably better served by a standard radical nephrectomy that includes adrenalectomy.

The role of regional lymphadenectomy in patients with localized kidney cancer is controversial. Because no widely effective treatments are available for metastatic RCC, regional lymphadenectomy may benefit a small number of patients. Extensive nodal involvement is associated with a poor prognosis.

Partial nephrectomy (*nephron-sparing surgery* (*NSS*)): Recent advances in preoperative staging, specifically modern imaging techniques, and improvements in surgical techniques have made NSS an attractive alternative to nephrectomy in select patients. It allows for optimal surgical treatment and, at the same time, obviates overtreatment and nephron loss. Whenever possible this is done laparoscopically. Indications include synchronous bilateral tumors, tumors in a solitary kidney, or the presence of a poorly functional contralateral kidney (e.g., chronic pyelonephritis). Recently, indications for NSS for RCC have been expanded to include a normal contralateral kidney in younger patients with an incidental, localized, single, small (<4 cm) RCC.

29.6 Surgical Pathology Specimens: Laboratory Protocols

29.6.1 Biopsy Specimens

Core needle biopsy (*renal tumor*): This is usually one or two cores, which are counted, measured (mm), and processed for initial histological examination through three levels. Careful handling is necessary to avoid crush artifact. When sectioning levels, intervening unstained sections may be usefully kept for ancillary immunohistochemical studies if required.

Closed core needle biopsy (*medical renal*) *and open wedge biopsy* (*donor renal transplant*): These small biopsies (10–15 mm long) are all handled in a similar fashion and embedded in total. A minimum of two core biopsies, each containing renal cortical tissue is recommended to provide adequate material for light microscopy(LM), immunofluorescence (IF), and electron microscopy (EM). Using a microscope, renal tissue can easily be discriminated from fat or muscle. The cores are divided and in certain cases it is reasonable to omit IF or EM to save material for LM. If immediate transfer of biopsies cannot be accomplished, one unfixed portion for IF is sent in phosphate-buffered saline or on crushed ice and samples for LM and EM fixed immediately. For LM, fixation in buffered formalin is common practice particularly if immunohistochemistry rather than immunofluorescence is required. Fixation with Bouin's fluid is also used for kidney biopsies due to a superior preservation of morphological details. For allograft biopsies and if diagnosis is urgently needed in native kidney biopsies, a frozen section or rapid embedding procedure should be available. Serial 2–3 μm sections are prepared for use in histological and immunohistochemical staining procedures. Stains employed include Hematoxylin and Eosin (H&E), Periodic-Acid-Schiff (PAS), silver-methenamine and the Masson trichrome stain.

Immunohistochemistry/immunofluorescence: For native kidney biopsies, the following antibodies should always be used: IgG, IgM, IgA, complement factors (C3, C4 and C1q), and fibrin. Additional antibodies can be necessary (kappa and lambda light chains for light chain deposition disease and AA for amyloid) and for allografts, antibodies against BK viral antigens, and C4d (humoral rejection). EM is an important diagnostic role in more than 50% of cases and is essential for a correct diagnosis in up to 25%.

29.6.2 Resection Specimens

These specimens include radical nephrectomy, nephrouretrectomy, ureterectomy, simple nephrectomy, partial nephrectomy, and transplant nephrectomy. They are handled similarly by the laboratory with some notable exceptions as detailed below.

Initial procedure:

- Palpate and locate the tumor through the perinephric fat.
- If there seems to be a penetrating tumor, ink the surface prior to opening the perirenal fat/capsule. This helps to distinguish true tumor penetration of the perirenal fat and margins from the relatively common finding of elevation of the capsule by a protruding lobulated tumor margin. Ink also the parenchymal kidney resection margin in partial nephrectomy for renal tumors.
- The initial incision should pass through the midline of the kidney in the coronal plane.
- Remove the perirenal fat (Gerota's fascia) with blunt dissection from the capsule and examine the surface for adenomas, adrenal rests, and other subcapsular lesions.
- In tumors of adults, if parts of the capsule are adherent to the tumor, dissect around them leaving them in place so that they can be taken for histological examination.
- In pediatric tumors, the renal capsule and perirenal fat should not be dissected from the kidney and tumor as the capsule retracts when the first cut is made and this may obscure the relationship of tumor, pseudocapsule, renal capsule, and perirenal tissue.

- In partial nephrectomy specimens for renal cortical tumors, inking of parenchymal resection margin is a must. Inking the external surface is usually not required but should be performed in cases with apparent extrarenal involvement or bulging on gross evaluation.
- The specimen should be bisected in a coronal plane into anterior and posterior halves. Dissection may be done from lateral border toward the hilum but should be from medial to lateral if large vessels are noted in the hilum.
- Measurements:
  - Kidney – length (cm), breadth (cm), depth (cm), and weight (g)
  - Ureter – length (cm) and diameter (cm)
  - Tumor

  Length × width × depth (cm) or maximum dimension (cm)

  Distances (mm) from perinephric fat, ureteric and parenchymal surgical margins
- Photograph the bisected kidney.
- Next make a series of parallel slabs in the coronal plane at 1–1.5 cm intervals. Alternatively serial horizontal slices that correlate with CT cross-sectional images.
- Use the first cut surface to collect tumor and kidney tissue for special purposes (EM, imprints, flow cytometry, cytogenetics, tissue culture, snap freezing etc.).
- Place the entire specimen in a large container of buffered formalin for fixation overnight (24–36 h).

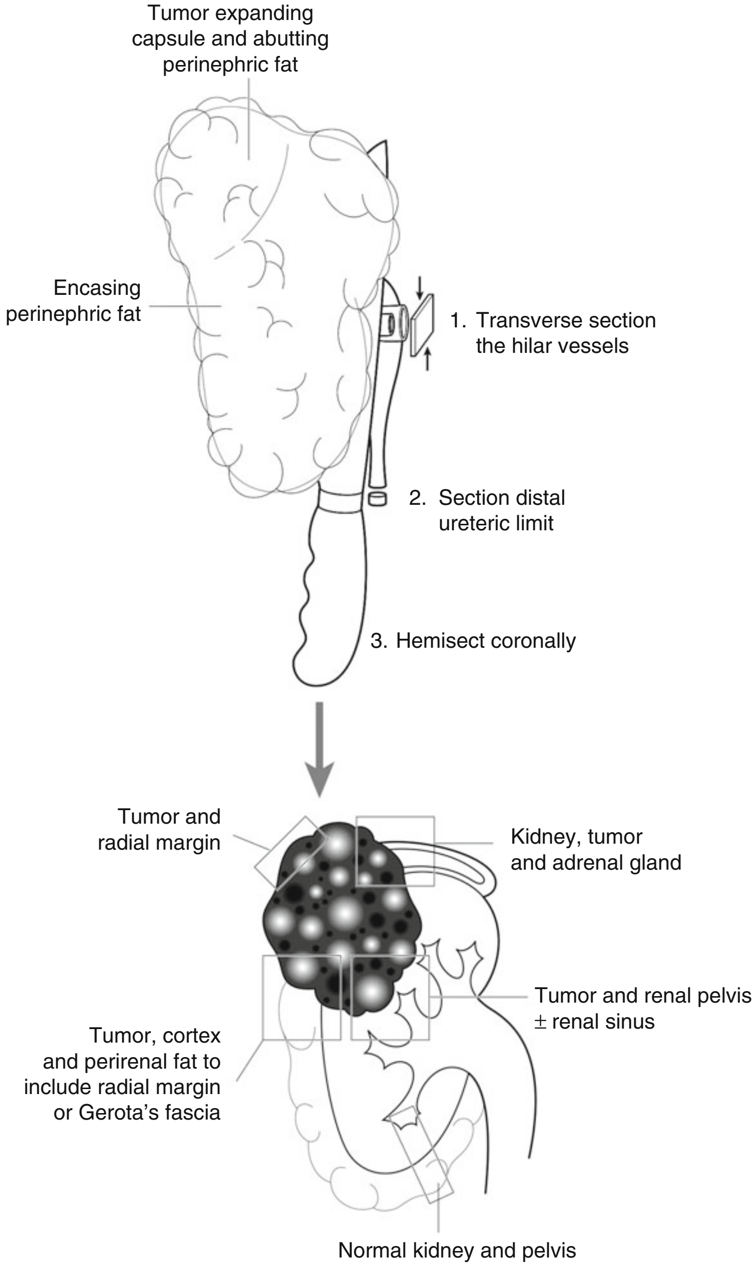
Description:

- Tumor
  - Ball-shaped, uni- or multinodular, uni- or multifocal
  - Border: sharpness of margins, pseudocapsule
  - Color: yellow, grey-white, brown, tan-brown, beige
  - Features of the tumor: homogeneous, solid, cystic, papillary, whorls
  - Regression: necrosis, hemorrhage, scars (central), pseudocysts
- Surrounding kidney – nodules, other tumors, scars.
- Extent of spread – consider the staging criteria (restricted to the kidney, infiltration of the perirenal adipose tissue or the hilar region [renal sinus], macroscopic invasion of hilar veins or pelvis).
- Other – look for lymph nodes and dissect the adrenal gland.

Blocks for histology (Fig. 29.3 – radical nephrectomy):

- Separately label each block and clearly document the exact site of origin.
- Sample margin blocks (perinephric fat, ureter, renal vein, and artery). Ureteric, vessel, and renal sinus blocks may be conveniently taken prior to dissection of the main specimen.
- Sample at least one block/cm diameter of tumor.
- Sample at least two to three blocks from tumor–renal sinus interface and an appropriate/variable number of blocks to identify extension into perirenal fat.
- Sample one block from renal vein, renal artery, and ureter margin.
- Multiple blocks from identifiable or suspected venous or collecting system invasion.
- Sample blocks from all other identifiable renal abnormalities.
- Sample at least one block from macroscopically normal renal parenchyma, away from tumor.
- Sample to show relationships between tumor and the radial margin; tumor and adrenal gland (upper pole tumors); tumor and renal pelvis and surrounding renal parenchyma.
- Sample one block from each tumor area that differs in color.
- Count and sample all lymph nodes.
- Sample the adrenal gland when present (one block).
- In transplant nephrectomies, blocks should be taken serially (from without in) of the hilar vessels to examine the nature of the renal vein and artery.
- In nephrectomies for benign disease, samples to be taken include any abnormal area and one random from otherwise normal parenchyma.

Fig. 29.3 Blocking of a nephrectomy specimen for upper pole renal carcinoma (Reproduced, with permission, from Allen and Cameron (2004))



Blocks for histology (Fig. 29.4 – nephroureterectomy/ureterectomy):

- Separately label each block, and clearly document the exact site of origin.
- Take one block from each tumor area that differs in color.
- One block of tumor/cm of tumor diameter is probably sufficient (minimum of four blocks).
- Sample to show the relationships between tumor and renal pelvis; tumor, renal pelvic wall, and peripelvic fat; tumor, cortex, and perirenal fat.

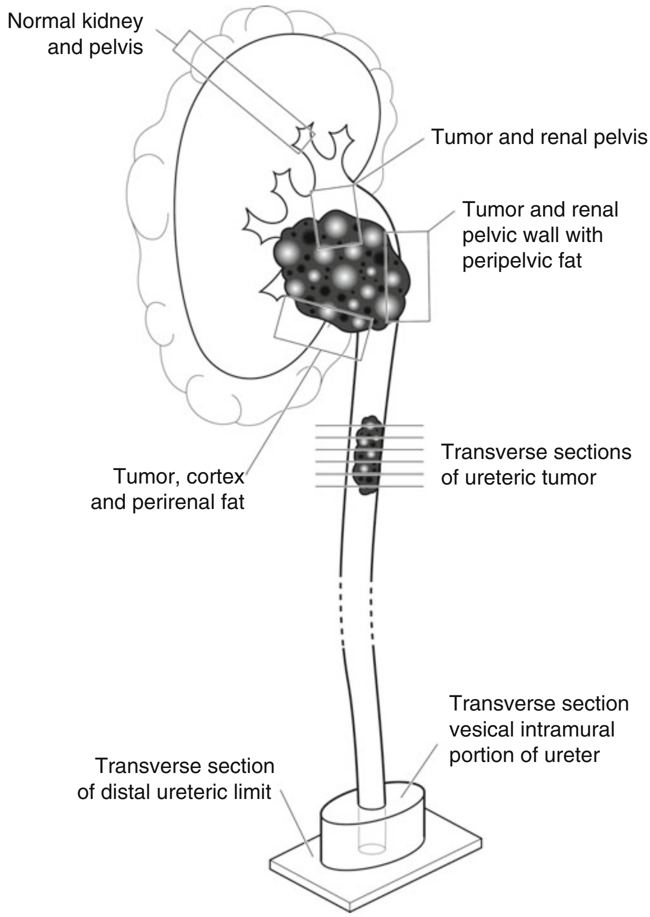


Fig. 29.4 Blocking of a nephroureterectomy for multifocal ureteropelvic transitional cell carcinoma (Reproduced, with permission, from Allen and Cameron (2004))

- In renal pelvic tumors, sample areas of unremarkable and abnormal mucosa away from the tumor in the renal pelvis and ureter.
- With ureteric tumors, serially section the tumor transversely at 3-mm intervals and sample a minimum of three blocks to assess the deepest point of invasion.
- If ureteric tumor is not seen grossly, sample and correspondingly label unremarkable and abnormal mucosal areas.
- Transverse sections of the distal ureteric/bladder cuff margin.
- Count and sample all lymph nodes.
- Additional sections should include renal pelvis, renal artery, renal vein, and ureter.
- Sample the adrenal gland if present (one block).
- Sample the surrounding kidney.

Histopathology report renal cell carcinoma:

- Tumor type – clear cell/papillary/chromophobe/collecting duct/unclassified/other
- Tumor differentiation/grade (Fuhrman) – sarcomatoid?

  Fuhrman grade

  Grade 1 Nuclei round, uniform, approximately 10 μm in diameter; nucleoli inconspicuous or absent

  Grade 2 Nuclei slightly irregular, approximately 15 μm in diameter; nucleoli evident

  Grade 3 Nuclei very irregular, approximately 20 μm in diameter; nucleoli large and prominent

Grade 4 Nuclei bizarre and multilobated, 20 µm or greater in diameter, nucleoli prominent, chromatin clumped

- Tumor edge – infiltrative/pushing/ lymphocytic infiltrate
- Extent of local tumor spread:

pT0	No evidence of primary tumor
pT1	Tumor ≤7 cm in greatest dimension, limited to the kidney
pT1a	Tumor ≤4 cm in greatest dimension, limited to the kidney
pT1b	Tumor >4 cm but ≤7 cm in greatest dimension, limited to the kidney
pT2	Tumor >7 cm in greatest dimension, limited to the kidney
pT2a	Tumor >7 cm but ≤10 cm in greatest dimension, limited to the kidney
pT2b	Tumor >10 cm in greatest dimension, limited to the kidney
pT3	Tumor grossly extends into major veins or perinephric tissues but not into the ipsilateral adrenal gland and not beyond Gerota's fascia
pT3a	Tumor grossly extends into the renal vein or its segmental (muscle-containing) branches or tumor invades perirenal and/ or renal sinus fat but not beyond Gerota's fascia
pT3b	Tumor grossly extends into the vena cava below the diaphragm
pT3c	Tumor grossly extends into vena cava above diaphragm or invades the wall of the vena cava
pT4	Tumor invades beyond Gerota's fascia (including contiguous extension into the ipsilateral adrenal gland)

- Lymphovascular invasion – present/not present. Note perineural invasion
- Regional lymph nodes

pN0	No regional lymph node metastasis
pN1	Metastasis in a single regional lymph node
pN2	Metastasis in more than one regional lymph node

- Excision margins: Perinephric fat, ureter, renal vein, and in partial nephrectomy, the renal parenchymal margin of tumor clearance (mm)
- Other pathology: Multifocal papillary, synchronous tumors, amyloid in tumor, adrenal rests, adenomas, tumor regression, cystic disease
- Transplant nephrectomy: Comment on hilar vessels, presence or absence of acute vascular and/or cellular rejection, donor-related changes

Histopathology report renal pelvis and ureter carcinoma:

- Tumor type – transitional/squamous/ adenocarcinoma/other
- Tumor differentiation – WHO grades I – III
- Pattern of growth
  - (1) Noninvasive (pure) – papillary/flat CIS/ papillary and flat CIS
  - (2) Invasive (pure)
  - (3) Mixed, noninvasive, and invasive
  - (4) Indeterminate
- Extent of invasion:

pTa	Noninvasive papillary carcinoma
pTis	Carcinoma in situ
pT1	Tumor invades subepithelial connective tissue
pT2	Tumor invades the muscularis
pT3	(For renal pelvis only) Tumor invades beyond muscularis into peripelvic fat or the renal parenchyma
pT3	(For ureter only) Tumor invades beyond muscularis into periureteric fat
pT4	Tumor invades adjacent organs, or through the kidney into the perinephric fat

- Lymphovascular invasion – present/not present. Note perineural invasion
- Regional lymph nodes – hilar, abdominal para-aortic, and paracaval nodes, and, for ureter, intrapelvic nodes

pN0	No regional lymph node metastasis
pN1	Metastasis in a single lymph node, ≤2 cm in greatest dimension
pN2	Metastasis in a single lymph node, >2 cm but ≤5 cm in greatest dimension, or multiple lymph nodes, none >5 cm in greatest dimension
pN3	Metastasis in a lymph node >5 cm in greatest dimension.

- Margins: Ureteral, bladder neck, Gerota's fascia (perinephric fat margin), hilar soft tissue, renal parenchyma (partial nephrectomy), tumor clearance (mm)

- Additional pathologic findings, if present: Carcinoma in situ (focal/multifocal), dysplasia, inflammation/regenerative changes, therapy related (BCG, mitomycin)
- Other pathology: Cystitis cystica glandularis, keratinizing squamous metaplasia, intestinal metaplasia

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30 Bladder

Declan M. O'Rourke and Derek C. Allen

30.1 Anatomy

The empty bladder is a pyramidal-shaped organ that lies entirely within the pelvic cavity. Upon filling (capacity approximately 500 ml), the bladder assumes a more ovoid shape, rises out of the pelvis, and separates the peritoneum from the anterior abdominal wall. The bladder has an apex (anteriorly), a base (posteriorly), a superior surface (the dome), and two inferolateral surfaces. The apex is anchored to the anterior abdominal wall by the urachus, a fibrous embryological remnant which, during development, connects the bladder to the allantois. The superior bladder surface is covered by peritoneum, which reflects onto the anterior abdominal and lateral pelvic walls. The base is triangular in shape, limited superolaterally by the entrances of the ureters into the bladder, and inferiorly by the urethral orifice. The area immediately adjacent to the urethral orifice is known as the bladder neck. The seminal vesicles and vasa deferentia lie immediately posterior to the bladder base (Fig. 30.1).

The mucosal surface lining the bladder base is known as the trigone. It is distinct in that, because of firm adherence to the underlying muscle coat, its surface is always smooth, in contrast to the remainder of the mucosa which, when the bladder is empty, assumes an undulated appearance.

The bladder is lined by transitional epithelium or urothelium, usually six cell layers thick. This rests on a thick layer of fibroelastotic connective tissue, allowing considerable distention. Below this are the ill-defined muscularis mucosae, composed of wispy irregular bundles of smooth muscle. The main muscular coat of the bladder, the muscularis propria or detrusor muscle, is composed of interlacing bundles of larger smooth muscle fibers loosely arranged into inner longitudinal, middle circular, and outer longitudinal layers. At the bladder neck, the circular layer is thickened, forming a preprostatic sphincter, which is responsible for maintaining urinary continence. This muscle is richly ennervated by sympathetic nerve fibers.

Lymphovascular drainage:

The blood supply to the bladder is from the superior and inferior vesical arteries and venous drainage is to the internal iliac veins via the vesical venous plexus. Most of the lymphatic drainage is to the external and internal iliac lymph nodes (Fig. 30.2).

30.2 Clinical Presentation

Bladder tumors most commonly present with the painless passage of blood in the urine (hematuria), sometimes with amorphous clots. This is a

D.M. O'Rourke (✉) • D.C. Allen
Histopathology Laboratory, Belfast City Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: declan.orourke@belfasttrust.hscni.net;
derek.allen@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_30, © Springer-Verlag London 2013

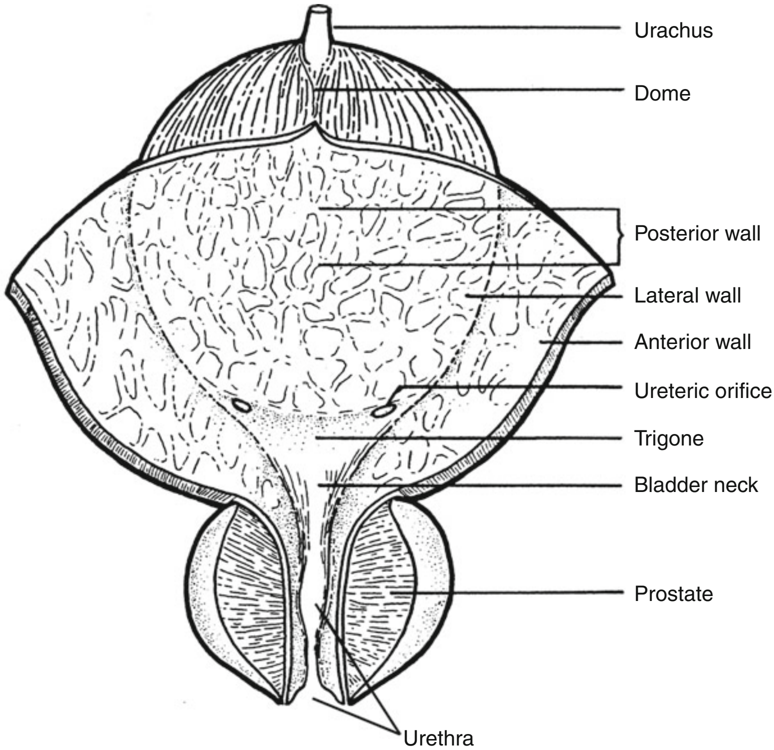

Fig. 30.1 Anatomy of the bladder (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

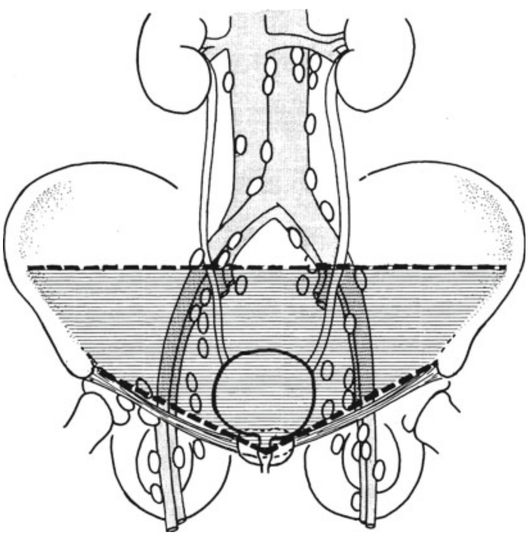

Fig. 30.2 Bladder – regional lymph nodes (pelvic nodes below the bifurcation of the common iliac arteries) (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

serious symptom necessitating immediate urologic investigation, particularly in the adult. Hematuria occurring at the end of micturition (terminal hematuria) points specifically to pathology in the bladder neck region. There are usually no other symptoms unless there is secondary urinary obstruction and/or infection. Advanced bladder cancer may present with symptoms related to a pelvic mass, lower limb edema due to lymphatic obstruction, or metastatic disease.

Acute urinary retention is more commonly due to prostatic enlargement than bladder disease. Overdistention causes a constant suprapubic pain relieved instantaneously by the passage of urine. Occasionally, with slowly progressive urinary obstruction and bladder distention, e.g., neurogenic (flaccid) bladder of diabetics, there is no associated pain.

Inflammatory conditions of the bladder including bacterial infection and interstitial cystitis often present with intermittent suprapubic discomfort or irritative symptoms of painful urination (dysuria), increased number of episodes of micturition daily (frequency), and a sensation of sudden, strong impulse to void (urgency). Diffuse carcinoma in situ of the bladder may also present in this way.

Bladder calculi may be asymptomatic or present with hematuria. There may be pain associated with intermittent bladder outlet obstruction or symptoms related to secondary infection.

Rarer conditions such as diverticula or urachal remnants are usually asymptomatic, although predisposing to stones and infection. Vesicocolic fistula due to colonic diverticulitis, Crohn's disease, or malignancy can present with the unusual symptoms of passage of gas (pneumaturia) or fecal material (fecaluria) in the urine. Vesicovaginal fistula due to malignancy may result in the passage of fecal material vaginally.

30.3 Clinical Investigations

- Urinalysis – A 'dipstick' test will detect microscopic hematuria (not visible grossly) which can indicate bladder disease, or pick up other substances such as protein or sugar in the urine which may flag up bladder infection or an underlying medical condition such as diabetes mellitus.
- Midstream sample of urine (MSSU) culture – Will confirm the presence of bacterial infection.
- Intravenous urogram (IVU) – Of limited value in the diagnosis of bladder disease. Tumors may present as filling defects.
- Cystoscopy and cytology/biopsy.
- Cystography – Occasionally indicated to demonstrate vesicocolic or vesicovaginal fistulae, to evaluate bladder diverticula or post-bladder surgery to look for an anastomotic leak.
- Micturating cystourethrography – assesses the pathophysiology of micturition as well as the lower urinary tract anatomy. In bladder disease, useful for evaluating neurogenic bladder, diverticula, and vesicoureteral reflux.
- Loopography – Occasionally performed to examine reconstructed urinary reservoirs or conduits after resection of the native bladder, e.g., to look for obstruction in an ileal conduit.
- USS – Can be used to detect radiolucent bladder stones or diverticula or to confirm the presence of bladder tumor in suspicious filling defects on IVU. Endoluminal ultrasound (ELUS) is used to stage bladder cancer in some specialized centers.
- CT scanning of the abdomen and pelvis with contrast, with pre-infusion and post-infusion phases. This evaluation is ideally performed with CT urography.
- MRI – Used to stage bladder cancer, primarily in looking for metastatic disease in regional lymph nodes and other organs.

30.4 Pathological Conditions

30.4.1 Non-neoplastic Conditions

Bacterial cystitis: This, the most common cause of cystitis, is usually due to coliform organisms (e.g., *E. coli*) ascending the urethra. Underlying structural (diverticula, fistulae, malformations, stones) or medical (diabetes mellitus, chronic renal failure, immunosuppression) conditions predispose. Recurrent infections, especially in men, should trigger investigation for an underlying cause.

Malakoplakia: Is caused by a defect in the host macrophage response to bacterial infection and can affect practically any organ in the genitourinary system or indeed elsewhere. It is seen primarily in middle-aged women and presents as multiple soft, yellow mucosal plaques on cystoscopy, sometimes mistaken for carcinoma. Biopsy reveals collections of granular histiocytes in the lamina propria, some with characteristic intracytoplasmic concentrically laminated inclusions (Michaelis–Gutmann bodies).

Polypoid/papillary cystitis: These closely related conditions describe localized nonspecific inflammation and edema of the bladder mucosa commonly seen in association with indwelling urinary catheters and less often with vesical fistulae. They may be difficult to differentiate endoscopically and microscopically from papillary urothelial carcinoma, which tends to have finer stromal papillary cores, more urothelial atypia, and less associated inflammation.

Nephrogenic adenoma: Often associated with previous surgery, stones, or infection, these are small, usually polypoid lesions of metaplastic origin and, although most commonly found in the bladder (75%), can be seen anywhere in the urinary tract.

Interstitial cystitis: Usually in middle-aged women, the etiology is obscure and the diagnosis essentially one of exclusion. Symptoms may be extremely severe. On cystoscopy, the typical appearances of diffuse punctate hemorrhage with or without ulceration can closely mimic carcinoma in situ. The histological appearances are nonspecific, with lamina propria congestion, edema, and inflammation featuring lymphocytes, plasma cells, and variable numbers of mast cells (best seen histologically if sample submitted in alcohol rather than formalin). Urine cytology to exclude malignancy and culture for infection are other important investigations. The diagnosis of interstitial cystitis requires close clinical (i.e., history, cystoscopy, and voiding studies) and pathological correlation. Treatment is initially medical for symptom relief (amitriptyline, antihistamines, analgesics) with intravesical therapy an alternative and eventually surgical intervention, in the form of urinary diversion with or without cystourethrectomy, as a last resort.

Bladder stones: Most commonly seen in men with bladder outlet obstruction, and associated with renal or ureteric stones. Rarely result in surgical material.

Diverticula: Most are seen in elderly males and attributed to increased luminal pressure secondary to prostatic enlargement causing outlet obstruction. Few cause symptoms or require surgical treatment. Most are located close to the ureteric orifices. Possible complications include ureteric obstruction, infection, stone formation, and rarely malignancy (urothelial, adeno- or squamous cell carcinoma).

Urachal-related lesions: Persistence of the urachus can result in a completely patent tract from bladder to umbilicus, a blind-ended sinus opening onto the bladder mucosa or umbilical skin, or an enclosed sinus blind at both ends. The lining epithelium may be of urothelial or columnar type. Presentation is usually in childhood. Stasis of urine and epithelial debris predispose to infection, abscesses, and rarely stones. Cysts may occur at any point within the urachal remnant.

Neurogenic bladder: A wide range of neuromuscular conditions (e.g., cerebrovascular accident, multiple sclerosis, spinal cord trauma, diabetes mellitus) can cause voiding dysfunction by interfering with bladder wall compliance, detrusor muscle activity, or sphincter function, resulting eventually in either a tightly contracted or flaccid bladder. These are usually treated by behavioral, pharmacological, or electrophysiological means, but occasionally surgical intervention may be indicated, e.g., augmentation cystoplasty to increase capacity in a contracted bladder, where a segment of stomach or intestine is isolated and anastomosed to the native bladder. Rarely, adenocarcinoma may supervene later in the augmented bladder.

Tumor-like conditions:

Postoperative necrobiotic granulomas: Seen following transurethral surgery with diathermy. Microscopy reveals central necrosis with peripheral palisading of histiocytes and occasional giant cells.

Myofibroblastic proliferations: Different names have been used for identical, cytologically benign myofibroblastic proliferations (pseudosarcomatous myofibroblastic proliferation, pseudosarcomatous fibromyxoid tumor, inflammatory myofibroblastic tumor [IMT], postoperative spindle cell nodule, inflammatory pseudotumor). This is rare with a 10% local recurrence rate. In some there is a history of trauma/prior instrumentation. Histology shows a loose fascicular architecture, with spindle cells in an edematous or myxoid stroma and admixed inflammatory cells. They express smooth muscle actin and cytokeratins and many are ALK1-positive (IHC or FISH).

Others: Fibroepithelial polyp, Müllerian lesions, prostatic-type polyps, and florid von Brunn's nests.

Miscellaneous: Other causes of cystitis include pelvic radiotherapy, intravesical BCG immunotherapy (granulomatous) or chemotherapy, oral drugs (cyclophosphamide), viral infection (CMV, HSV), and parasite infestation (schistosomiasis). Amyloidosis may present as a localized, nodular bladder mass ("amyloid tumor").

30.4.2 Neoplastic Conditions

Benign tumors: Inverted urothelial papilloma, villous adenoma, paraganglioma, leiomyoma, hemangioma, and granular cell tumor of the bladder are occasionally encountered. Benign urothelial papilloma is a rarely made diagnosis.

Keratinizing squamous metaplasia: May be associated with chronic irritation (catheters, stones, parasitic infection). There is a risk of malignant transformation to squamous dysplasia/carcinoma and cystoscopic follow up is advised.

Urothelial dysplasia/carcinoma: Many carcinogenic agents are known to predispose to urothelial malignancy. These include cigarette smoke, industrial aniline dyes (aromatic amines), petrochemicals, cyclophosphamide, and the analgesic phenacetin. Most invasive tumors are associated with urothelial dysplasia or flat carcinoma in situ.

Urothelial carcinoma in situ (*CIS*): Occurs rarely in the absence of invasive tumor, when it can closely mimic interstitial cystitis both clinically and cystoscopically, presenting with irritative bladder symptoms and appearing as multifocal red, velvety patches. More often seen in association with prior or synchronous invasive malignancy which can be multifocal. Cellular pleomorphism involves either full or partial thickness of the urothelium with marked nucleomegaly and loss of polarity to basement membrane common. CIS is strongly positive for CK20 and p53 but negative for CD44 and this is useful in distinction from non-neoplastic reactive atypia. CIS is usually treated with an induction course of BCG, and if tolerated, followed by maintenance therapy. Patients with widespread field change (which may involve the bladder, ureters, urethra, prostatic ducts, and seminal vesicles) and those in whom initial intravesical therapy fails are at high risk of progression to invasive disease, and cystectomy may be in their best interest. The behavior of CIS is somewhat unpredictable, with 50% of patients developing invasive carcinoma within 5 years. Careful follow-up with urine cytology and biopsy is advocated following conservative management to monitor recurrence or progression. Primary (de novo) CIS seems to have lower progression rate than secondary or concomitant CIS.

Urothelial carcinoma: Urothelial (or transitional cell) carcinoma (TCC) accounts for over 90% of primary bladder tumors, most commonly presenting in elderly males as a cystoscopic mass showing an exophytic or endophytic growth. Diagnosis is confirmed by biopsy which commonly shows a papillary or solid growth pattern. Urine cytology is of limited value in the initial evaluation of low-grade bladder tumors and is more useful in CIS and high-grade urothelial carcinoma, industrial screening, and follow-up after treatment. Fluorescent in situ hybridization (FISH) is considerably more sensitive and only slightly less specific than cytology and is a useful initial diagnostic tool in patients suspected of both new and recurrent bladder cancer. Note that noninvasive urothelial carcinoma (TCC, stage pTa) is also classified as carcinoma to avoid confusion with flat CIS (stage pTis). The 1973 WHO classification probably remains the most widely used classification for bladder tumors, particularly with respect to grading. The 1998 WHO/ISUP classification, subsequently published as the WHO 2004 classification, has not been universally endorsed by clinicians. The evidence available for its value is scanty and based only on retrospective data with some inconsistencies in results. Although the 1973 WHO classification is not without its faults and limitations, it has been validated in the literature and it is incorporated into routine clinical practice. Both classifications should be reported in parallel. The WHO 2004/ISUP has a lowered threshold for diagnosis as high grade and this category includes a subset of tumors previously considered grade 2 by WHO 1973. These grades cannot be simply translated.

Pathological staging is extremely important for prognostic and treatment purposes and is determined by the extent of local tumor spread. Assessment of small bladder biopsies is crucial and they must be carefully examined. Muscularis mucosae (MM – pT1) versus muscularis propria (MP – pT2) invasion may be difficult as in some bladders, the junction of MP and lamina propria is not well defined. The MP has large confluent aggregates of thick muscle and of note, is prominent at the trigone where there is minimal submucosa. Smoothelin immunohistochemistry may be helpful in distinguishing MM from MP. There is weak, patchy staining in MM and strong diffuse reactivity in the MP. Cytokeratin stains may be useful in identifying subtle foci of invasive carcinoma but should not be confused with cytokeratin-positive myofibroblasts. In bladder biopsy material, distinction is not made between invasion of the inner (superficial, pT2a) and outer (deep, pT2b) MP due to problems of orientation (reported as "at least" stage pT2a). Biopsies should also be examined closely for coexistent CIS. Separate biopsies may be submitted to assess prostatic involvement. There is a correlation between tumor stage and grade, based on the degree of nuclear atypia, in that more poorly differentiated tumors (WHO grade III) show a much higher rate of concurrent or subsequent muscle invasion. High-grade tumors commonly show focal squamous or glandular differentiation.

The standard treatment of patients with stage T2–T4 (and sometimes grade III, pT1) disease is radical cystoprostatectomy for men and anterior pelvic exenteration for women. Partial cystectomy is reserved for solitary tumors (particularly at the dome – urachal) with no previous history of bladder tumors and no CIS, bladder neck or trigone involvement. Bilateral pelvic lymphadenectomy (PLND) should be performed in conjunction with radical cystoprostatectomy and anterior pelvic exenteration. PLND adds prognostic information by appropriately staging the patient and may confer a therapeutic benefit. Neoadjuvant chemotherapy prior to either radical cystectomy or external beam radiotherapy is controversial but is thought to confer a treatment benefit compared with surgery alone. Chemotherapy may also make potentially curative cystectomy possible. Radiotherapy is used mostly for palliation (unfit for surgery).

Prognostic factors: Depth of invasion in bladder wall determines stage and prognosis and some histological variants have a worse prognosis (small cell carcinoma, micropapillary carcinoma, sarcomatoid carcinoma, undifferentiated carcinoma, carcinoma with rhabdoid features, plasmacytoid carcinoma). Lymphovascular invasion is a controversial prognostic factor and is used for management decisions in some centers. Associated urothelial CIS and/or tumor multifocality carry a higher risk of separate new occurrences. Lymph node and distant metastasis carry a poor prognosis. Overall prognosis depends largely on stage, with a 70% 5-year survival rate for stages pTa and pT1 and 50% for pT2b. Within the pT1 group, grade III decreases the 5-year survival to 60%.

Variants of urothelial carcinoma are: nested (mimics benign von Brunn's nests – look for deep invasion and cytological atypia); microcystic (cysts or tubules containing proteinaceous debris); inverted (architecturally like inverted papilloma but with marked atypia); also giant cell, clear cell, lymphepithelioma-like, and micropapillary variants.

Squamous cell carcinoma: Accounts for less than 5% of bladder tumors in the UK. Chronic irritation from stones, long-term indwelling catheters, diverticula, chronic urinary infections, prolonged cyclophosphamide treatment, and, in particular, schistosomiasis predispose, hence a much higher incidence of bladder squamous cell carcinoma in countries where the latter is endemic, e.g., Egypt. Verrucous carcinoma is more specifically associated with s*chistosomal* infection. HPV association is very rare but may be seen in cases associated with condyloma. High-grade urothelial carcinoma showing squamous differentiation (look for urothelial CIS) and secondary involvement by primary cervical carcinoma should be excluded. Disease is often of advanced stage at presentation and prognosis therefore poor (overall 5-year survival 15%).

Adenocarcinoma: Gland-forming carcinoma of urinary bladder not associated with urothelial or squamous carcinoma component. It is a rare primary bladder neoplasm (<2% of bladder cancers) associated with bladder exstrophy, chronic irritation, diverticula, and nonfunctioning bladder. May occur within a urachal remnant or cyst (dome). Usually shows pure glandular differentiation, although varying patterns have been described (enteric, mucinous/colloid, signet ring cell, clear cell, hepatoid) and associated adenocarcinoma in situ may be seen. It is CK20-positive, CK7-negative, and may express villin and CDX-2 but does not express nuclear β-catenin. PAP reactivity is reported, but PSA is typically negative. The differential diagnoses include direct invasion by prostatic adenocarcinoma or colorectal adenocarcinoma. It has a poor prognosis (5-year survival rate varies from 18% to 47%) due to advanced stage at presentation.

Other cancers: Spindle cell carcinoma, small cell carcinoma, malignant melanoma, leukaemia/malignant lymphoma, leiomyosarcoma, rhabdomyosarcoma, choriocarcinoma, yolk sac tumor, and metastases (direct spread – prostate, cervix, uterus, rectum; distant spread – breast, malignant melanoma, lung, stomach).

30.5 Surgical Pathology Specimens: Clinical Aspects

30.5.1 Biopsy Specimens

Rigid or flexible cystoscopy allows direct visualization of macroscopic bladder pathology for evaluation and biopsy of small lesions using either "cold" cup forceps or a small diathermy loop. The latter may cause significant heat artifact, reducing the value of histologic assessment. Rigid cystoscopy employs a larger lumen, allowing superior visualization (better optics and water flow), greater versatility in the passage of accessory instruments, and easier manipulation. It also provides suitable access for transurethral resection of superficial bladder tumors with diathermy (TURBT). Flexible cystoscopy is more comfortable for the patient, may be easier to pass, and allows a range of angles of visualization within the bladder. Cystoscopy should be avoided during active urinary tract infection as instrumentation can exacerbate the condition. CIS may be invisible to the endoscopist and necessitate random biopsies to make the diagnosis. Distinction from interstitial cystitis may require multiple biopsies as the surface can be extensively denuded. In the presence of an overt tumor, it is important to sample abnormal mucosa (red, velvety) distant from the lesion to look for in situ malignancy. Sampling normal looking mucosa adjacent to tumor is not advised due to the potential risk of tumor reimplantation. Deep biopsies (including muscularis propria) are essential to provide important staging information in invasive tumors.

30.5.2 Resection Specimens

Obviously there are important surgical differences between the sexes. Radical surgery for bladder cancer in the male comprises cystoprostatectomy, with urethrectomy if there is prostatic urethra involvement, and in the female an anterior exenteration (bladder, uterus and adnexae – see Chap. 35). With surgical and anesthetic advances, operative mortality from radical cystectomy has fallen from 20% to <1%.

In the male, the bladder is approached through a midline lower abdominal incision. The urachus and vasa deferentia are identified and ligated. A pelvic lymphadenectomy is performed and the ureters identified and divided close to the bladder. Ureteric margins are ideally submitted separately from the main resection specimen for pathological assessment. The bladder, prostate, and seminal vesicles are separated from the rectum and the puboprostatic ligaments divided. The urethral sphincter is then divided unless a urethrectomy is being considered.

Simple cystectomy is quite a rare operation, typically performed for benign conditions such as interstitial cystitis or neurogenic bladder complicated by chronic infection. It involves bladder removal with maintenance of the urethra in women or the prostate and seminal vesicles in men.

The need for an alternative urinary drainage system following cystectomy has raised difficulties of acceptance for many patients. However, new developments in surgical techniques mean several options are now available:

(1) Urinary diversion and intestinal conduit formation; an isolated segment of small or large intestine (usually ileum) is anastomosed to both ureters and a stoma formed on the anterior abdominal wall. Drainage is continuous into a worn device.
(2) Continent cutaneous diversion (e.g., Indiana pouch, which uses the ileocecal valve as a continence mechanism); requires intermittent self-catheterization.

(3) Continent orthotopic reservoir; a "neobladder" is formed from an ileal or ileocolonic segment and sutured directly onto the urethra; usually confined to men but also possible in women with an intact urethra.
(4) Ureterosigmoidostomy (sigma rectum pouch); the ureters are anastomosed directly onto a detubularized segment of sigmoid colon still in continuity, i.e., remains in contact with faeces. This avoids the need for a stoma or self-catheterization but results in the frequent passage of liquid feces.
(5) Rarely, cutaneous ureterostomy.

Long-term complications following these procedures include stenosis, adenomatous polyps, and tumor formation (usually adenocarcinoma). These may necessitate subsequent resection.

Partial cystectomy is infrequently performed, but may be indicated for a solitary urothelial carcinoma at the bladder dome or for tumor arising in a urachal remnant or diverticulum. Excision of a benign bladder diverticulum may be performed intravesically, extravesically, or, if small, transurethrally.

30.6 Surgical Pathology Specimens: Laboratory Protocols

30.6.1 Biopsy Specimens

Tiny pieces of tissue (several mm) retrieved using either "cold" cup forceps or a small diathermy loop are counted, measured, processed intact, and examined histologically through three levels. TURBT specimens contain larger fragments, possibly recognizable as papillary tumor grossly. There is no evidence concerning sampling policies of large TURBTs for the detection of invasion. Small resections are usually all embedded, and larger specimens can be sampled (up to 3–4 blocks), attempting to include larger fragments with muscle wall. If detrusor muscle is not present in the initial sections, it is worth considering further sampling for accurate staging. In particular, if the initial sections show invasion into the lamina propria (pT1), complete embedding of the remaining specimen may reveal muscle invasion, leading to clinically important upstaging to pT2. Occasionally, levels in selected blocks will clarify stage. A separate biopsy may be sent from the base of the lesion (including muscle) to assess invasion of deep tissues. Random biopsies from red areas or from cystoscopically normal urothelium may be sent to determine whether dysplasia or CIS are present. Complete submission of all tissue to rule out pT1/pT2 disease is recommended if high grade (WHO III).

30.6.2 Resection Specimens

Specimen:

- Most bladder resections are for biopsy-proven malignant tumors: cystourethrectomy, cystoprostatectomy (including seminal vesicles), cystoprostatourethrectomy, anterior exenteration (including uterus and adnexae), simple cystectomy, partial cystectomy.

  Initial procedure (Fig. 30.3):

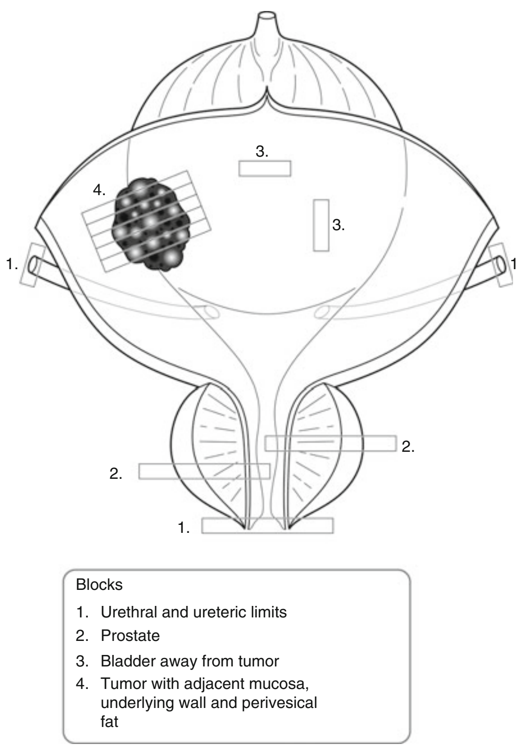

Fig. 30.3 Blocking a cystoprostatectomy specimen for bladder cancer (Reproduced, with permission, from Allen and Cameron (2004))

- Orientate the specimen with the help, if present, of attached pelvic organs (uterus and seminal vesicles are posterior to the bladder) or the peritoneal reflection, which descends further on the posterior bladder wall than anteriorly.
- Locate both ureters in the lateral perivesical fat (may be marked with sutures).
- Place a probe in the bladder via the urethra and open the specimen anteriorly (through the prostate if present) with a sagittal cut using a knife or scissors, trying if possible to avoid cutting into any localized tumor. Keep the posterior aspect of the specimen intact to maintain orientation. Some pathologists prefer to inflate the bladder with 10% formalin and allow fixation prior to opening. Others like to divide the specimen into anterior and posterior halves.
- If there is an obvious tumor, paint the nearest deep perivesical soft tissue margin; if no tumor is grossly obvious (e.g., following preoperative treatment), paint all peripheral soft tissue margins, using different colored inks for orientation. Paint the prostate, if present.
- Measurements:
  - Dimensions (cm) of bladder and, if present, prostate, seminal vesicles, female pelvic organs
  - Lengths (cm) of ureters (limits may be submitted separately) and urethra, if attached
  - Tumor
    Dimensions (cm)
    Distances to urethral and ureteric margins (cm)
- Photograph.
- Fix by immersion in 10% formalin for at least 24–36 h preferably pinned out or using a wick to fully expose the mucosal surface.
- Locate the ureteric orifices at the trigone and open the ureters along their full length with small scissors.
- Make 3–5-mm parallel, transverse sections through the tumor to demonstrate its deepest point of invasion and its relationship to the ureters, prostate, or any other adjacent structures.
- Look for and measure lymph nodes in perivesical fat (usually none found).
- If not involved by the bladder tumor, serially section the prostate perpendicular to the urethra looking for occult primary tumor.
- If not involved by the bladder tumor, process female pelvic organs.
- Photograph suitable slices.
- Partial cystectomies are processed in a similar manner, although orientation may be more difficult (or impossible). The mucosal edges should be treated as surgical margins, i.e., inked and measurements given from tumor (cm).
- If a tumor is identified as arising from the urachal tract (usually in a partial cystectomy specimen comprising dome of bladder, urachal tract and umbilicus), the bladder portion is processed as before, soft tissue margins surrounding the urachal tract are painted and the tract serially sectioned transversely up to the umbilicus.
- Conduits, augmentation cystoplasty, and neobladder specimens containing tumors are processed as before, opening along the urethra and ureters if possible, painting the nearest deep soft tissue margin, and noting the relationship of the tumor to the enteric, bladder, or ureteric mucosa. It may be best to serially section the tumor perpendicular to lines of anastomoses.

Description:

- Tumor
  - Site (trigone, lateral walls, dome, neck, ureteric orifices)
  - Single/multifocal
  - Appearance (papillary/sessile/ulcerated/mucoid/keratotic)
  - Edge (circumscribed/irregular)
- Mucosa
  - Red, velvety CIS away from tumor
- Wall
  - Tumor confined to lamina propria, into muscle wall or through wall into perivesical fat
- Other
  - Fistula, diverticulum, stones, urachal remnant

Blocks for histology (Fig. 30.3):

- Transverse sections the urethral and ureteric limits.
- If bilateral ureteric limits are submitted separately, measure the two lengths and sample the proximal surgical margin of each (if orientated by the surgeon), then serially section the remainder and process separately.

- Sample at least four blocks of tumor to demonstrate depth of invasion, distance to perivesical soft tissue margins, and relationship to adjacent mucosa, ureters, prostate, or other organs.
- Sample any suspicious background mucosa or take at least two random mucosal sections.
- If tumor is not seen grossly, sample and carefully label all bladder mucosal surfaces including the trigone, dome, lateral, anterior and posterior walls.
- Sample any suspicious ureteric mucosa or submit random ureteric transverse sections.
- If not suspicious of harboring malignancy on serial sectioning, sample each lobe of the prostate and the prostatic urethra. If suspicious, multiple site orientated blocks are taken to include the prostatic capsule and relevant surgical margins.
- Sample the seminal vesicles and vasa deferentia.
- Sample any attached female pelvic organs.
- Count and sample all lymph nodes identified.
- In a partial cystectomy, it is important to take perpendicular blocks of tumor that include the nearest lateral mucosal margins.
- A tumor arising in the urachus should be sampled as before, but also to include blocks of the soft tissue margins surrounding the urachus and the skin margin surrounding the umbilicus.
- Conduits, augmentation cystoplasty, and neobladder specimens containing tumor should be sampled as before, also taking tumor blocks to demonstrate the relationship with enteric/urothelial mucosa and anastomotic lines.

Histopathology report:

- Tumor type – Urothelial/squamous/adenocarcinoma/other
- Tumor growth pattern – Papillary/invasive/flat in situ
- Tumor differentiation – Use WHO grades I–III (1973 and 2004 WHO) based on cytological atypia
- Tumor edge – pushing/infiltrative/lymphoid response
- Extent of local tumor spread

pTis	Flat carcinoma in situ: "Flat tumor"
pTa	Papillary noninvasive
pT1	Invasion of subepithelial connective tissue
pT2a	Invasion of superficial muscle (inner half)
pT2b	Invasion of deep muscle (outer half)
pT3	Invasion of perivesical fat
	a. Microscopically b. Macroscopically
pT4	Tumor invades any of the following; prostatic stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall
pT4a	Tumor invades prostatic stroma or uterus or vagina
pT4b	Tumor invades pelvic wall or abdominal wall

- Lymphovascular invasion – present/not present
- Regional lymph nodes

Pelvic nodes below the bifurcation of the common iliac arteries

pN0	No lymph node metastasis
pN1	Single regional lymph node metastasis in the true pelvis (hypogastric, obturator, external iliac, or presacral lymph node)
pN2	Multiple regional lymph node metastasis in the true pelvis (hypogastric, obturator, external iliac, or presacral lymph node metastasis)
pN3	Lymph node metastasis to the common iliac lymph nodes.

- Excision margins
  Distances (mm) to the ureteric, urethral, and nearest perivesical soft tissue margins
  Presence/absence of dysplasia/CIS at ureteric/urethral limits
- Cystoprostatectomy: Presence or absence of prostatic adenocarcinoma, and, if so, extent, grade, stage, and margin status
- Other pathology: Adenocarcinoma of prostate (use protocol for carcinoma of prostate), urothelial (transitional cell) carcinoma involving urethra, prostatic ducts and acini ± stromal invasion

(use protocol for carcinoma of urethra), urothelial dysplasia/CIS, inflammation/regenerative changes, therapy-related changes, cystitis cystica glandularis, keratinizing squamous metaplasia, intestinal metaplasia

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31 Prostate

Declan M. O'Rourke and Derek C. Allen

31.1 Anatomy

The normal prostate weighs 20 g by early adulthood and is best thought of as having an inverted pyramid shape, with anterior, posterior, and lateral surfaces, a narrow apex anteroinferiorly, and a broad base superiorly which lies against the bladder neck. It is related anteriorly to the symphysis pubis, laterally to the anterior fibers of the levator ani muscle, and posteriorly to the seminal vesicles and rectum, separated from the latter by Denonvilliers' fascia. The prostate is surrounded by an ill-defined fibrous capsule which blends with the pelvic fascia. Numerous neurovascular bundles are found within this connective tissue. At the apex, skeletal muscle fibers of the urethral sphincter are admixed with occasional benign prostatic glands and, at the base, fibers from the bladder detrusor muscle blend imperceptibly with the prostate capsule. At these points, the boundaries of the organ are particularly obscure, rendering difficult in resection specimens the interpretation of capsular penetration by carcinoma and capsular incision during surgery. Adipose tissue is occasionally found just inside the prostatic capsule.

The prostate is composed of branching tubuloalveolar glands lined by cuboidal or columnar epithelium and invested and surrounded by fibromuscular stroma which is continuous with the prostatic capsule. The urethra transverses the full diameter of the prostate in a curved fashion, entering at the center of the prostate base and exiting just anterior to the apex. Prostatic ducts empty into the prostatic urethra. The ejaculatory ducts, formed at the juncture of the vasa deferentia and seminal vesicle, also secrete into the prostatic urethra.

The glandular prostatic tissue has been divided into four distinct zones, characterized by differing embryological origin, location, and pathologies (Fig. 31.1a). The anterior fibromuscular stroma, composed mainly of fibromuscular tissue with very few glands, merges with the bladder neck superiorly and the external sphincter at the apex inferiorly. The preprostatic zone surrounds the urethra proximal to the ejaculatory ducts and comprises the periurethral ducts and the larger transition zone. This region commonly gives rise to benign prostatic hypertrophy and approximately 25% of adenocarcinomas. The central zone, surrounding the ejaculatory ducts, is felt to differ embryologically from the remainder of the gland and is least commonly affected by pathological abnormality. Glands in the central zone may show complex papillary infoldings and a cribriform architecture on histology. Lack of cytological atypia distinguishes them from prostatic intraepithelial neoplasia (PIN). The peripheral zone occupies approximately 70% of the normal prostate in a horseshoe shape around the posterior and lateral aspects of the organ. Glands are normally small and

D.M. O'Rourke (✉) • D.C. Allen
Histopathology Laboratory, Belfast City Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: declan.orourke@belfasttrust.hscni.net;
derek.allen@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_31, © Springer-Verlag London 2013

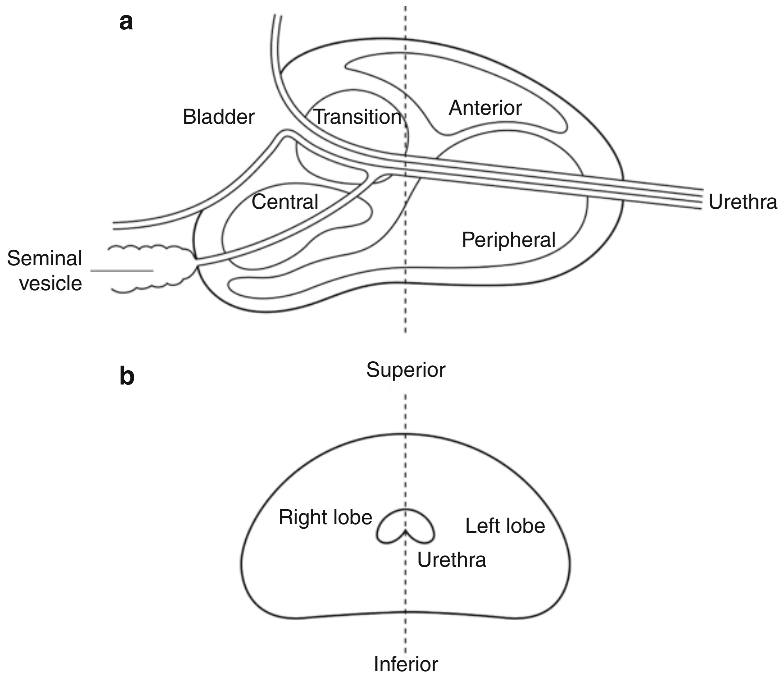

Fig. 31.1 (**a**) Prostatic zones (lateral view); (**b**) prostatic lobes (anterior view) (Reproduced, with permission, from Allen and Cameron (2004))

simple, but this zone is the main site of origin for prostatic adenocarcinomas (70%).

To simplify the concept of zones, the prostate may be considered to have significantly differing inner (transition zone) and outer (peripheral and central zones) regions.

Clinically the prostate gland is often described as having right and left lateral lobes, a central sulcus, and a middle lobe. These do not equate to any anatomically defined structures but rather relate to palpable masses on rectal examination, usually enlargement of the transitional zone laterally and periurethral glands centrally.

For the purposes of TNM staging, the prostate gland is simply divided into right and left lobes (Fig. 31.1b).

Lymphovascular drainage:

Lymphatic drainage from the prostate is to the obturator, hypogastric, and external iliac nodes, i.e., pelvic nodes below the bifurcation of the common iliac arteries (see Fig. 30.2 in Chap. 30).

The seminal vesicles are paired, convoluted glands measuring approximately 5 cm in length and lying on the posterior wall of the prostate. Their function is to add a significant volume of alkaline secretion to the ejaculate, which promotes sperm motility and survival. Cowper's (bulbourethral) glands are paired, pea-sized, tubular glands found periurethrally immediately distal to the prostate.

31.2 Clinical Presentation

Benign prostatic enlargement, usually affecting the periurethral glands, causes urinary tract obstructive symptoms of delayed start to micturition (hesitancy), decreased force of urination, intermittency of the urinary stream and post-micturition dribbling. Secondary changes in bladder compliance later lead to irritative symptoms of frequency (increase in daily episodes of micturition), urgency (sudden, strong desire to micturate), and nocturia (nocturnal frequency), the latter being the most common presenting complaint. Patients may also present in acute urinary retention. These symptoms are often referred as LUTS (lower urinary tract symptoms) but do not point specifically to a definitive diagnosis. Digital rectal examination (DRE) usually reveals a rubbery, smoothly enlarged prostate, although there is poor correlation between symptoms and gland size. Alternatively, an enlarged asymptomatic benign prostate gland may be detected incidentally on performing DRE to investigate lower gastrointestinal symptoms or on screening for prostate cancer. This is a very common finding in men over age 50 years and per se is not a reason to precipitate further urologic investigation.

In contrast, carcinoma of the prostate, usually involving the periphery of the gland, rarely causes urinary symptoms and is often clinically silent. Indeed, presentation with obstructive urinary symptoms implies advanced disease. Patients present most commonly with advanced metastatic disease in lymph nodes or bone. Early cancer may be picked up by DRE, which frequently reveals a firm, indurated mass. This may be performed as part of a routine physical examination, to investigate urinary, gastrointestinal, or generalized symptoms or as part of a screening procedure along with serum prostatic specific antigen (PSA) (see Clinical Investigations). DRE alone is of limited accuracy and cannot reliably differentiate malignancy from prostatic stones and granulomas. Early-stage, commonly low-grade prostatic carcinoma may be detected incidentally in transurethral resection specimens from men with concomitant benign hyperplasia.

Acute or chronic inflammation of the prostate (prostatitis) usually associated with bacterial infection may cause perineal pain, which can be referred to the back, inguinal region, or testes. It is frequently associated with irritative urinary symptoms of frequency and dysuria. DRE may reveal a tender, fluctuant, or boggy prostate but is often extremely uncomfortable for the patient.

31.3 Clinical Investigations

- Serum prostate specific antigen (PSA) – a highly organ-specific biochemical marker used as a diagnostic tool in those suspected as having prostatic cancer clinically and to screen for prostatic cancer in asymptomatic men. The upper limit of normal for PSA is 4 ng/mL. With a PSA level of 4–10 ng/mL, the likelihood of prostate cancer is about 25%; with a level above 10 ng/mL, the likelihood is over 50%. Some advocate age-related PSA cut-offs which are more specific. To increase the specificity of the PSA assay for cancer, adjuvant tests (PSA velocity and free PSA) have been recommended. The velocity of PSA level increase and the percentage of free PSA can be helpful to differentiate mildly elevated PSA levels due to cancer from elevated levels due to benign prostatic hyperplasia. A rise by more than 0.75 ng/mL per year shows a 90% specificity of cancer. Free PSA is calculated as a percentage of total PSA and the lower the percentage of free PSA (<15%), the higher the likelihood of cancer. This information is more useful in men with very large glands (PSA density) or in those who have had a negative biopsy result. Following prostatectomy, PSA should fall to undetectable levels. Persistent or subsequent elevation provides a sensitive indicator of residual or recurrent disease.
- Transrectal ultrasound (TRUS) and biopsy – US is more sensitive and accurate than DRE and may detect prostate cancer as peripheral hypoechoic regions. However, many prostate cancers are not detected on US appearances, and most hypoechoic lesions are not cancer (may represent PIN, benign hyperplasia, atrophy, infarction, or infection). TRUS is, therefore, mainly used to guide needle biopsy sampling for definitive diagnosis after an abnormal DRE or elevated serum PSA. As regards staging, locally extensive disease will be easily detected but not unexpectedly US will understage microscopic capsular penetration. TRUS biopsies can be taken to monitor progression following non-surgical treatment.
- CT/MRI – to determine pre-treatment tumor stage by assessing capsular penetration and regional lymph node metastases (by size), although both are of limited sensitivity and specificity.
- Radiolabeled isotope bone scan – investigation of choice to detect distant skeletal metastases.

31.4 Pathological Conditions

31.4.1 Non-neoplastic Conditions

Benign nodular hyperplasia (*BNH*): An extremely common androgen-dependent disorder (castration is protective) caused by hormonal imbalance affecting the stromal-epithelial relationship. No other clear risk factors apart from aging have been identified. Benign prostatic hypertrophy (BPH) is variably defined by the presence of urinary obstructive symptoms, macroscopic prostate enlargement, or histological hyperplasia. The average weight of the prostate with histologically confirmed BPH is 33 g but weights of over 800 g have been recorded. Incidence increases with age (approximately 50% in the fifth decade and 75% in the eighth decade). BPH first develops in the transition and periurethral (preprostatic) zones, giving rise to enlargement of the clinical lateral and middle lobes respectively. Grossly the appearances are those of multiple, variably-sized, gray to yellow central nodules, compressing the urethra and the peripheral zone. Histology shows hyperplasia of both prostatic glandular and stromal elements (benign prostatic *hypertrophy* is pathologically incorrect). This can adopt various forms, some reminiscent of benign breast disease such as fibroadenoma – like hyperplasia or sclerosing adenosis. Pure stromal nodules composed almost entirely of smooth muscle may also be seen. Treatment may be initially medical in the form of $alpha_1$-adrenergic blockers, which ease obstruction by relaxing prostatic smooth muscle or androgen suppression. Aromatase inhibitors are a newer approach. Patients suffering serious complications of BPH such as recurrent urinary retention or infections, renal insufficiency, or bladder stones are not suitable for medical therapy and should be offered surgery. Similarly, patients whose quality of life is significantly affected by their urinary symptoms are appropriate surgical candidates. Transurethral resection of prostate (TURP) has been the gold standard for surgical treatment of BPH for many years against which new treatments are compared. High-volume disease is best treated by enucleation of the entire gland using a retropubic or suprapubic approach (open prostatectomy). Newer, less invasive and less morbid, alternatives to TURP include the following:

- Transurethral incision of the prostate
- Transurethral balloon dilatation of the prostate
- Intraurethral stenting
- Hyperthermia and thermotherapy
- High-intensity focused ultrasound (HIFU)
- Laser therapy
- Transurethral electrovaporization of the prostate
- Transurethral needle ablation of the prostate

Prostatitis: Acute bacterial prostatitis is associated with urinary tract infection (UTI) and responds to the same antimicrobial therapy so is rarely seen in surgical pathology practice. Chronic bacterial prostatitis is characterized by recurrent UTIs caused by the same pathogen and is less responsive to medical treatment. Large infected prostatic stones predispose and resection by TURP may be attempted, providing surgical material. On histological examination, reactive glandular atypia may mimic carcinoma. The presence of inflammatory cells and glandular atrophy, obvious on low power, should prevent misdiagnosis. Often in prostatic tissue showing chronic inflammation, no organisms are cultured (non-bacterial prostatitis), hence it is better to report the histology as chronic inflammation rather than chronic prostatitis.

Abscess: Most commonly seen with bladder outlet obstruction as a complication of urinary tract infection or, less often, following biopsy. DRE and TRUS are diagnostic. Treatment is transurethral drainage and antimicrobial therapy.

Infarction: Often found in prostates with significant BNH. Histology shows coagulative necrosis of glands and stroma with often prominent surrounding squamous metaplasia, not to be confused with squamous cell carcinoma (exceptionally rare in prostate).

Granulomatous prostatitis: Seen following BCG therapy for bladder cancer (look for suburethral distribution of granulomas) with prostatic involvement in systemic mycobacterial or fungal infection (in an immunocompromised host), and in association with eosinophilia and possibly systemic vasculitis (allergic granulomatous prostatitis). Non-specific granulomatous prostatitis, due to an immune response to extravasated prostatic secretions, is the most commonly diagnosed non-infectious granulomatous prostatitis and clinically can closely mimic prostate cancer (abnormal DRE and elevated PSA).

Postoperative necrobiotic granuloma: May be identified years following TURP and has a characteristic histological appearance of central fibrinoid necrosis with palisading histiocytes.

Adenosis (*atypical adenomatous hyperplasia*): Small- to medium-sized acinar proliferation, which does not fulfill the cytological criteria of carcinoma. There is little evidence to suggest that adenosis represents a preneoplastic entity. It is seen as a prostate cancer mimic in needle biopsy or TURP. The lesion is comprised of a well-circumscribed proliferation of small- to medium-sized glands in the transition zone, usually mixed with hyperplastic nodules. There is sometimes a dilated centrally located gland. Basal cell markers frequently show a discontinuous basal cell layer and AMACR is often focally positive.

Miscellaneous: Malakoplakia, stones, pseudosarcomatous fibromyxoid tumor (inflammatory myofibroblastic tumors), postoperative spindle cell nodule (following TURP), although all more commonly seen in the bladder, may be found in the prostate.

31.4.2 Neoplastic Conditions

Benign tumors: Extremely rare, e.g., leiomyoma, cystadenoma.

Malignant tumors: Prostate cancer is the second leading cause of cancer-related death in men after lung cancer. It is androgen-dependent and risk factors include aging, positive family history, black race, and high dietary fat. Fruits and vegetables (rich in lycopenes) may have a protective effect. Overall, most of the data for chemoprevention and dietary modulation has either been negative or only possibly promising. However, one chemopreventive, finasteride, has been found to be successful. It decreases the detection rate of prostate cancer, but increases the detection of high-grade, more clinically significant prostate cancer.

Prostatic intraepithelial neoplasia (PIN) represents a precancerous condition confined to prostatic ducts and acini.

Prostatic intraepithelial neoplasia (*PIN*): Non-invasive neoplastic transformation of the lining epithelium of existing prostatic ducts and acini characterized by marked nuclear atypia. Although low-grade PIN is recognized, the diagnostic reproducibility is very low and significance is uncertain. High-grade PIN (HGPIN) is present as isolated diagnosis in 4–16% of needle core biopsies and <5% of transurethral resection specimens but present in over 80% of prostate glands containing adenocarcinoma. There are four major architectural patterns; tufted, micropapillary, cribriform, and flat. The risk of cancer following diagnosis of HGPIN is around 21% rising to 60% if present in more than three cores. HGPIN does not elevate the PSA. A preserved or discontinuous basal cell layer may be readily identified on H&E or with basal cell specific immunohistochemistry (p63/34βE12/CK5–6). AMACR stains acinar cells and can be useful in the distinction from benign glands. Detection in TURP chippings necessitates processing all of the tissue. Management is dependent on the extent of HGPIN and re-biopsy considered in association with PSA and other clinical features.

Prostatic adenocarcinoma: Of acinar/proximal duct origin accounts for over 95% of primary prostate cancers. 70% arise in the peripheral zone and most cause few symptoms initially, often presenting insidiously in elderly men. DRE or serum PSA may raise suspicion and diagnosis is usually confirmed on TRUS-guided needle biopsy. Alternatively, cancer may be an incidental finding in prostatic chippings following TURP. This may either have arisen in the transition zone (20% of cancers – often low volume and grade) or represent spread from a larger, often high-grade peripheral tumor. Prostatic carcinoma can be difficult to identify grossly, even on radical resection specimens. It may be visible as solid, firm, pale yellow foci found peripherally in the gland.

Histology shows small acini arranged in a variety of architectural patterns (acinar, papillary, cribriform, comedo, solid) with cytological atypia, at least focal nucleolar prominence and absence of surrounding basal cell layer on high power (negative for basal cell specific immunohistochemistry (p63/34βE12/CK5–6)). Ancillary features which may be seen in carcinoma include perineural invasion and intraluminal wispy secretions or crystalloids. These features should help distinguish carcinoma from PIN and from the many benign small acinar proliferations which, especially on needle biopsy, may cause misdiagnosis (e.g. atypical adenomatous hyperplasia, basal cell hyperplasia, post-atrophic hyperplasia, sclerosing adenosis). Insufficient diagnostic criteria for malignancy present in a limited focus of acinar proliferation on needle core biopsy may lead to a report of "suspicious but not diagnostic of malignancy", usually prompting re-biopsy.

The Gleason grading system for prostate cancer is the predominant grading system used and is based on glandular architecture. The primary and secondary patterns, that is, the most prevalent and the second most prevalent patterns are added to obtain a Gleason score. Gleason grading of prostate cancer has changed over the years in an effort to incorporate new understanding of some features of prostate cancer and to adapt to the widespread use of needle biopsies, which were unavailable at the time of the originally proposed system.

Following a diagnosis of carcinoma on needle biopsy or TURP chippings, the Gleason score, clinicopathological stage (any evidence of extracapsular spread) and tumor volume (or length/proportion of needle core/tissue involved), together with the patient's age, serum PSA, general health and personal preferences, will direct treatment. The presence of a few prostatic glands in skeletal muscle does not necessarily imply extraprostatic extension (EPE), as toward the apex benign glands are intimately associated with skeletal muscle. Growth within adipose tissue generally indicates EPE as intraprostatic fat is rare. Some studies suggest strong correlation of perineural invasion (PNI) in a biopsy with EPE on radical prostatectomy (RP). PNI present in fat is considered as EPE. Lymphovascular invasion (LVI) is an independent predictor of disease progression. Seminal vesicle (SV) invasion is defined as carcinoma involving the muscular wall. The portion of SV may be intraprostatic. Only extraprostatic SV involvement should be considered. In needle biopsy, do not over interpret ejaculatory duct involvement as SV invasion. The SV has a thick muscular wall, which is not present in the ejaculatory duct.

At present, there are five main treatment options available for prostate cancer with others controversial at present:

- Active surveillance (watchful waiting)
- Radical prostatectomy
- Radiotherapy
- Androgen deprivation therapy
- Brachytherapy
- HIFU
- Cryoablation

The most appropriate treatment for prostate cancer is highly controversial and truly valid analytical comparisons between options are lacking.

Active surveillance, or watchful waiting, is a program of regular examinations used to monitor symptoms. Watchful waiting is typically recommended or considered in patients of advanced age or those who have significant comorbid conditions with a life expectancy of less than 10 years. For patients with a longer life expectancy, active surveillance is considered in those with T1/2a disease, a Gleason score of <7, and a PSA level below 10 ng/mL. They have PSA measurement every 3 months and repeat biopsy at two yearly intervals or if the PSA velocity rises. Biopsy findings are the most important factor in deciding whether to pursue treatment. Radical prostatectomy should be reserved for men with curable disease (i.e., organ-confined), who will live long enough to benefit from the cure (at least 10 years). Clinicopathological index of suspicion of EPE is the main determinant in a man of suitable age and health contraindicating this major operation. The ablative therapies of HIFU and cryotherapy are promising but remain experimental as first-line modalities.

Positive surgical margins following radical prostatectomy, although not shown to decrease long-term disease control or survival, are usually treated with pelvic radiotherapy, as is EPE (stage pT3) in the resection. Palliative radiotherapy may also be appropriate in metastatic disease, to treat bone pain. Disease which is locally advanced or metastatic at diagnosis is best treated with androgen-deprivation therapy, which blocks the hormonal drive that sustains tumor cells. This was achieved previously with surgical castration (bilateral orchidectomy) but now much more commonly with "medical castration" using luteinizing hormone-releasing hormone (LHRH) agonists or estrogens. Anti-androgens are an alternative endocrine therapeutic option and can be administered with an LHRH agonist (maximal androgen blockade). Cytotoxic chemotherapy is reserved for androgen-resistant prostate cancer, i.e., when symptoms recur following endocrine therapy.

Prognosis: Grade (Gleason score), pathological stage (notably EPE), and tumor volume are the most important prognostic indicators. Other factors are positive surgical margins following radical prostatectomy, perineural and lymphovascular invasion, and serum PSA level, which is an indirect measure of tumor volume and spread. Overall 10-year survival is approximately 50% with a 90% 10-year survival in organ-confined (pT1/pT2) disease, 60% in pT3/pT4 disease, and only 10% in disease with bone metastases.

Variants of prostatic adenocarcinoma are: Mucinous adenocarcinoma (exclude colorectal or bladder primary), signet ring cell adenocarcinoma (exclude gastric, bladder, or colorectal primary), periurethral duct adenocarcinoma (syn. endometrioid carcinoma), adenoid basal carcinoma, and clear cell adenocarcinoma.

Other carcinomas: Rarely, urothelial carcinoma arises in periurethral prostatic ducts, small cell carcinoma, squamous/adenosquamous carcinoma, sarcomatoid carcinoma.

Other cancers: Prostate is a common site for rhabdomyosarcoma (usually embryonal) in children; leiomyosarcoma is the commonest prostatic sarcoma in adults; leukemia/malignant lymphoma (especially secondary involvement by chronic lymphocytic leukemia); metastases (direct spread – bladder, colorectum; distant spread – lung, malignant melanoma).

31.5 Surgical Pathology Specimens: Clinical Aspects

31.5.1 Biopsy Specimens

Transrectal ultrasound (*TRUS*) *biopsy*

Prostate biopsy is indicated for histological diagnosis of prostate cancer and evaluation of a mass lesion or hypoechoic area. It is performed for elevated serum PSA level with or without an abnormal DRE and via TRUS guided using an 18-gauge needle as an outpatient procedure. However, it may also be performed perineally or transurethrally. There are differing biopsy schemes from sextant biopsy (6 cores each lobe) from base, midgland, and apex, extended biopsy which increases the cancer detection rate and saturation biopsy (≥20 cores), which is considered in men with persistently elevated PSA and prior negative biopsies. This includes biopsies of the transition zone as some of these have central gland tumors. If random, the biopsies should be carefully labeled to direct further biopsies in the event of a non-diagnostic histology report.

Transurethral resection of the prostate (*TURP*)

Transurethral Resection of Prostate (TURP) is the surgical treatment of choice for benign prostatic hyperplasia (BPH). This procedure is performed via a cystoscope using a diathermy loop for resection (resectoscope) and bladder irrigation to wash out the resected chippings. Hemostasis is controlled using electrocoagulation. The bladder neck may be incised following resection. Rarely dilutional hyponatremia due to absorption of bladder irrigation fluid causes confusion, nausea, and vomiting post-operatively (transurethral resection syndrome). The TURP specimen consists of pale rubbery fragments called prostate chippings and includes the transition zone and areas around the proximal prostatic urethra.

31.5.2 Resection Specimens

Open (*simple*) *prostatectomy*

This operation is reserved for BPH where the prostate weighs over 50–75 g. It is also appropriate where there is concomitant benign bladder disease requiring treatment such as a symptomatic diverticulum or a large stone. Potential risks are urinary incontinence, erectile dysfunction, retrograde ejaculation, and urinary tract infection. The advantages over TURP are complete removal of the gland (therefore no recurrence) and no risk of dilutional hyponatremia. However, there is an increased risk of intraoperative hemorrhage and a longer hospital stay. Previous prostatectomy, prior pelvic surgery, and prostate cancer are contraindications to the operation.

There are two possible approaches to enucleation of the prostate gland via open prostatectomy:

- Retropubic – through a direct incision of the anterior prostatic capsule.
- Suprapubic – through an extraperitoneal incision of the lower anterior bladder wall.

The retropubic approach allows excellent exposure and visualization of the prostate and prostatic fossa during enucleation ensuring complete removal and control of bleeding sites. There is minimal trauma to the bladder and precise transection of the urethra distally to preserve urinary continence. The suprapubic approach allows direct access to the bladder and bladder neck and is suited to patients with bladder pathology (diverticulum, stone) or a large "middle" lobe of prostate protruding into the bladder.

Radical prostatectomy

The three aims of this operation are cancer control, preservation of urinary continence and of sexual function. Two approaches are available: The perineal approach was pioneered first and has the advantages of usually less blood loss and greater exposure of and access to the apex of the prostate, thus optimizing removal of tumor from this critical margin and allowing precise transection of the urethra. However, its main disadvantage is that it does not allow access to perform a pelvic lymphadenectomy. Furthermore, a greater understanding of periprostatic anatomy and developments in surgical technique over the years have reduced blood loss and improved tumor clearance using the retropubic approach, to the extent that currently the perineal procedure is seldom performed. It may be indicated for small, low-grade tumors when pelvic lymphadenectomy can be safely omitted.

Surgery should be deferred for at least 6 weeks following needle biopsy and 12 weeks following TURP to allow any inflammatory adhesions or hematoma to resolve. In retropubic prostatectomy, a midline extraperitoneal lower abdominal incision is made from pubis to umbilicus, and after appropriate dissection, a bilateral pelvic lymphadenectomy is performed. This is a staging rather than a curative procedure, and in some centers, the surgeon may ask for a frozen section lymph node analysis, halting the operation should tumor be detected in the node. Prostatectomy proceeds with dissection of the periprostatic fascia, division of the puboprostatic ligaments, dorsal vein complex, urethra and bladder neck and excision of the seminal vesicles. Newer nerve-sparing surgical techniques are possible, with the aim of maintaining erectile function post-operatively. These involve preserving the neurovascular bundles, which run between two layers of periprostatic fascia (prostatic and levator). This is most successful in young patients with organ-confined disease but involves a higher risk of positive surgical margins. An option is to remove one neurovascular bundle, on the side of the palpable lesion or positive biopsy, leaving the other intact. Alternatively, if there is a high probability of capsular extension on pre-operative assessment, or if the patient is impotent, the bundles should be widely excised. Over the past decade, laparoscopic radical prostatectomy (LRP) has developed into an accepted surgical approach for the treatment of localized prostate cancer. In many high volume centers, LRP with or without robotics has become the surgical therapy of choice. Potential advantages of the da Vinci robot for prostatectomy include three dimensional magnified vision, tremor filtering, and instruments that replicate human wrist movements. LRP appears to be equivalent to robotic radical prostatectomy (RRP) in experienced hands. Advantages of LRP include shorter stays, less blood loss and postoperative pain, and earlier recovery. Current disadvantages of LRP include a lack of availability at all centers and excess cost which is likely to diminish over time.

This major operation has surprisingly low postoperative mortality (0.2%) or serious morbidity. Urinary incontinence, possibly due to distal urethral sphincter dysfunction or bladder neck contracture, is often the most troublesome side effect. Loss of erectile function is now less of a problem, thanks to modern surgical alternatives.

31.6 Surgical Pathology Specimens: Laboratory Protocols

31.6.1 Biopsy Specimens

TRUS needle biopsy: The wide-bore needle cores (18 gauge) are counted and measured (in mm), submitted separately if labeled accordingly and processed for initial histological examination through three levels. Careful handling is necessary to avoid crush artifact. Cores may be painted with alcian blue so that they are easily visible on facing the paraffin block. Most of the tissue in the block from superficial to deep should be included in sections. When sectioning, intervening ribbons of unstained sections are usefully kept for ancillary immunohistochemical studies if required.

TURP chippings: Weighed and sampled according to laboratory protocol. 14% of specimens will reveal an unexpected carcinoma (stage pT1), and the more tissue processed, the higher the detection rate. The availability of serum PSA now means that the chances of missing a clinically significant carcinoma are reduced. The potential management of such a detected cancer should ideally be known to avoid a substantial waste of resource. If aggressive treatment (radical prostatectomy) might be considered, for example in a younger patient, all tissue is embedded and examined histologically, as is also the case if there is any clinical suspicion of malignancy.

Otherwise, initial sampling of TURP specimens is recommended along the following Royal College of Pathologists guidelines:

For 12 g or less of tissue, all is processed; for over 12 g, 12 g plus an extra 2 g for every 5 g of tissue in excess of 12 g should be processed. This will normally equate to approximately six to eight cassettes for the average case. The suggestion of scrutinizing chippings for suspicious (yellow or indurated) areas is felt to be impractical. One level is examined from each block. If carcinoma (or high-grade PIN) is found, all tissue should be processed to give an accurate stage (pT1a tumor in ≤5% of tissue resected; pT1b tumor in >5% of tissue resected).

If there is a previous diagnosis of carcinoma of the prostate, only a small amount of tissue, say 6 g or four cassettes, need be embedded.

31.6.2 Resection Specimens

Specimen:

- Most prostate resections are retropubic radical prostatectomy specimens (including seminal vesicles) for biopsy-proven adenocarcinoma.
- Occasionally simple (retropubic/suprapubic) prostatectomy is performed for BPH.

  This is an enucleation procedure and usually produces an intact nodule with a wedge-shaped cut in one side. Orientation is not usually possible, although two distinct lobes should be identifiable. The specimen is weighed and measured (three dimensions, mm), then serially sectioned at 3–4 mm intervals. These sections are carefully examined for areas suspicious of carcinoma (yellow, firm) and six to eight cassettes of tissue processed, labeling the two lobes separately.

31.6.2.1 Radical prostatectomy

Initial procedure:

- Orientate the specimen using the seminal vesicles (situated on the posterior aspect) and by placing a probe (sometimes a catheter is in situ) into the prostatic urethra. This will allow identification of the flat base superiorly (proximal, bladder, base margin) and the more conical apex anteroinferiorly (distal, urethral, apical margin).
- Weigh the entire specimen, measure the prostate in three dimensions (mm) and give the lengths (cm) of the attached seminal vesicles and vasa deferentia.
- Paint the right, left, anterior, posterior, superior, and inferior surfaces of the prostate using six different-colored inks, including the soft tissue around the base of the seminal vesicles but not the seminal vesicles themselves. Make a note of any areas where the prostatic tissue has been disrupted by the surgical knife, as this may lead to a false positive surgical margin.
- Fix the specimen by immersion in 10% formalin for at least 24–36 h. Microwave-assisted technique to facilitate fixing may be used.
- Dissect off the seminal vesicles and vasa deferentia and serially section these.
- The proximal and distal margins are then removed. One option is to perform a very thin (1 mm) shave and submit these intact, ensuring they are embedded such that the true margin is sectioned. Shaved (en face) margin is generally not recommended as it may lead to a false-positive margin. In respect of the distal margin, one is interested in the prostatic tissue surrounding the distal urethral limit, rather than the urethra itself, which often seems to retract into the fixed specimen. An alternative (preferred) method involves amputating the proximal and distal 5 mm of the prostate

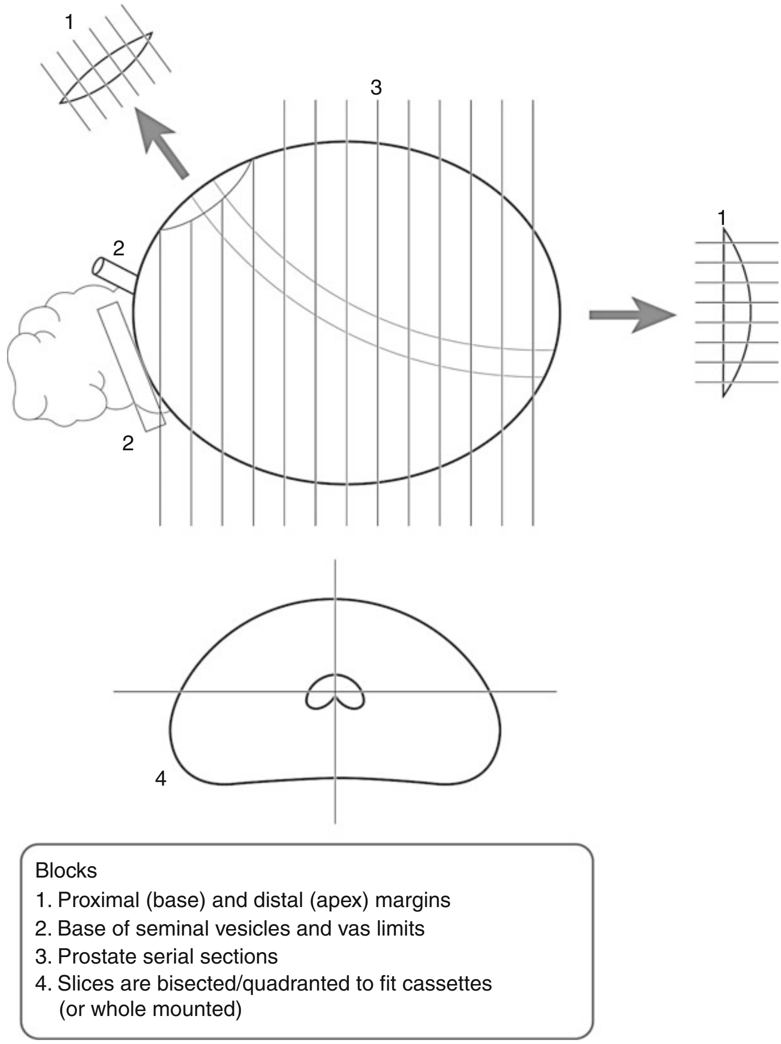

Fig. 31.2 Blocking a radical prostatectomy specimen (Reproduced, with permission, from Allen and Cameron (2004))

(corresponding to the bladder and urethral margins respectively) and serially sectioning these at 3 mm intervals perpendicular to the amputating cut (i.e. parallel to the urethra – Fig. 31.2). This technique allows a more accurate assessment of how close the tumor extends to these margins but, as only one section is examined for each 3 mm slice, the entire margin will not have been sampled.

- After removal of the margins, the prostate is serially sectioned at 3–4 mm intervals in a coronal plane from anterior to posterior. Some pathologists prefer to section the prostate in a horizontal plane. The slices are laid out sequentially and carefully examined, maintaining orientation with the help of the colored inks. Malignancy is often not obvious macroscopically but may appear as multifocal, peripheral, usually posterior, solid, gray to yellow nodules, contrasting with the central, spongy, non-neoplastic tissue. Asymmetry between lobes may be another clue.

Description:

- Tumor
  - Site (right/left, peripheral/central, anterior/posterior)
  - Size (mm)
  - Multifocality
  - Appearance (soft/firm, pale/yellow/granular)

- Edge (circumscribed/irregular)
- Extension beyond capsule/into seminal vesicles

- Non-neoplastic tissue
  - Appearance (color, consistency, nodularity)

Blocks for histology (Fig. 31.2):

- Sample proximal (base) and distal (apical) margins as described.
- Sample the seminal vesicles at their bases (junction with the prostate) and the vasa deferentia at their limits. It is not necessary to submit the entire seminal vesicle.
- Each serial section is bisected into right and left halves (and if necessary into superior and inferior quadrants) to fit into routine cassettes and the entire gland processed for histological examination, labeling each block carefully to aid microscopic interpretation.
- Alternatively, some pathologists prefer to partially sample the prostate initially, concentrating on suspicious areas and random sections from the circumferential margin. If partial sampling is used, this should be systematic to allow for assessment of orientation, volume, and multifocality.
- Whole mount sectioning may be available in some centers, greatly facilitating histological interpretation, but is usually reserved for teaching and research purposes. It is cumbersome and requires more space filing and storage.
- All pelvic lymph nodes (usually submitted separately) should be counted and sampled.

Histopathology report:

- Tumor type – acinar (proximal duct) adenocarcinoma/other.
- Tumor differentiation – use the Gleason grading system. Each tumor is assigned two grades, based on the most predominant of five different architectural patterns present, ranging from grade 1 (well-differentiated) to grade 5 (undifferentiated). The two grades are summed to give the Gleason score (maximum 10). If only one grade is present, the grade is doubled to give the score e.g. 3 + 3 = 6. The International Society of Urological Pathology (ISUP) 2005 consensus conference proposed several modifications and guidelines. In radical prostatectomy, provide the Gleason score (primary and secondary pattern) and separately mention the tertiary pattern. Also assign separate Gleason scores to dominant tumors in radical prostatectomy.
- Tumor volume – the tumor is outlined microscopically on each glass slide and the area involved measured (mm^2). The areas for all sections are summated, and the overall tumor volume (mm^3) is derived from multiplying by the average slice thickness (3–4 mm). This may be expressed as a proportion of the total volume of the prostate, to give the percentage gland involvement.
- Tumor edge – pushing/infiltrative/lymphoid response.
- Extent of local tumor spread.

pT1		Clinically inapparent tumor not palpable or visible by imaging
	T1a	Incidental finding in ≤5% of tissue resected
	T1b	Incidental finding in >5% of tissue resected
	T1c	Identified by needle biopsy
pT2		Tumor confined within the prostate
	T2a	Involves ≤ one half of one lobe
	T2b	Involves > one half of one lobe but not both lobes
	T2c	Involves both lobes
pT3		Tumor extends through the prostatic capsule
	T3a	Extracapsular extension (unilateral or bilateral) including microscopic bladder neck involvement
	T3b	Invades seminal vesicle(s)
pT4		Tumor is fixed or invades neighboring structures other than seminal vesicles: external sphincter, rectum, levator muscles, and/or pelvic wall.

Note a positive surgical resection margin at a point lacking extraprostatic tissue can be reported as pT2+, i.e., extracapsular extension cannot be accurately assessed.

- Lymphovascular invasion
  - Perineural and lymphovascular space
  - Present/not present
  - Inside/outside capsule
- Regional lymph nodes

Pelvic nodes below the bifurcation of the common iliac arteries

pN0	No regional lymph node metastasis
pN1	Metastasis in regional lymph node(s)

- Excision margins

  Proximal (base), distal (apical), circumferential margins involved/uninvolved.

  Distances (in mm) to nearest margins. The margin is positive if tumor cells touch the ink. Report the extent of positive margin in mm and the location. Some also report the Gleason score at the involved margin.
- Other pathology

  High-grade PIN, inflammation (specify type), atypical adenomatous hyperplasia (adenosis), nodular prostatic hyperplasia, effects of radiotherapy or androgen-deprivation therapy (glandular atrophy, apoptosis, vacuolation, stromal fibrosis).

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32 Urethra

Declan M. O'Rourke and Derek C. Allen

32.1 Anatomy

The urethra extends from the internal urethral orifice at the bladder neck to the external meatus. In the male, it is approximately 15–20 cm long and is divided into three sections (Fig. 32.1).

The prostatic urethra is 3–4 cm long, traversing the prostate in a curved manner. Throughout its length, a midline ridge on the posterior wall known as the urethral crest projects into the lumen causing it to appear crescentic on transverse section. The most prominent part of this ridge, close to the mid-point, is called the verumontanum. Here lies the orifice of the prostatic utricle, a short, blind-ended vestigial sac. The openings of the ejaculatory ducts lie on either side of the verumontanum. Prostatic ducts empty into the urethral sinuses, gutters flanking the urethral crest.

The membranous urethra extends from the prostatic apex to the bulb of the penis and measures 1 cm approximately. Small bulbourethral or Cowper's glands lie on either side and secrete into it.

The penile urethra measures 10–15 cm and is surrounded by the corpus spongiosum throughout most of its length. It includes the bulbous urethra proximally and the pendulous urethra distally. Scattered mucus-secreting (Littre's) glands are present periurethrally. The distal portion within the glans penis is dilated to form the fossa terminalis before narrowing at the external meatus.

The female urethra is approximately 4 cm long and extends from the bladder neck to the external urethral meatus, embedded throughout its length in the adventitial coat of the anterior vaginal wall. Like the male counterpart it has a posterior midline ridge, the urethral crest, which gives a crescentic shape on sectioning, and periurethral mucus-secreting (Skene's) glands.

The urethra is lined proximally by urothelium and distally by non-keratinizing stratified squamous epithelium, and, in males, the intervening membranous urethra (and part of the penile urethra) by pseudostratified columnar epithelium. However, it should be noted that most urethral tissue submitted for pathological examination is diseased or altered by instrumentation and hence highly susceptible to metaplastic change.

Lymphovascular drainage:

The lymphatics of the proximal urethra drain to the external iliac, obturator, and hypogastric nodes (see Fig. 30.2 in Chap. 30), while the distal urethra drains to the superficial and then deep inguinal nodes.

32.2 Clinical Presentation

Urethral lesions may be asymptomatic or, if causing obstruction, can present with hematuria, urinary retention, or symptoms of infection. Urethral

D.M. O'Rourke (✉) • D.C. Allen
Histopathology Laboratory, Belfast City Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: declan.orourke@belfasttrust.hscni.net;
derek.allen@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_32, © Springer-Verlag London 2013

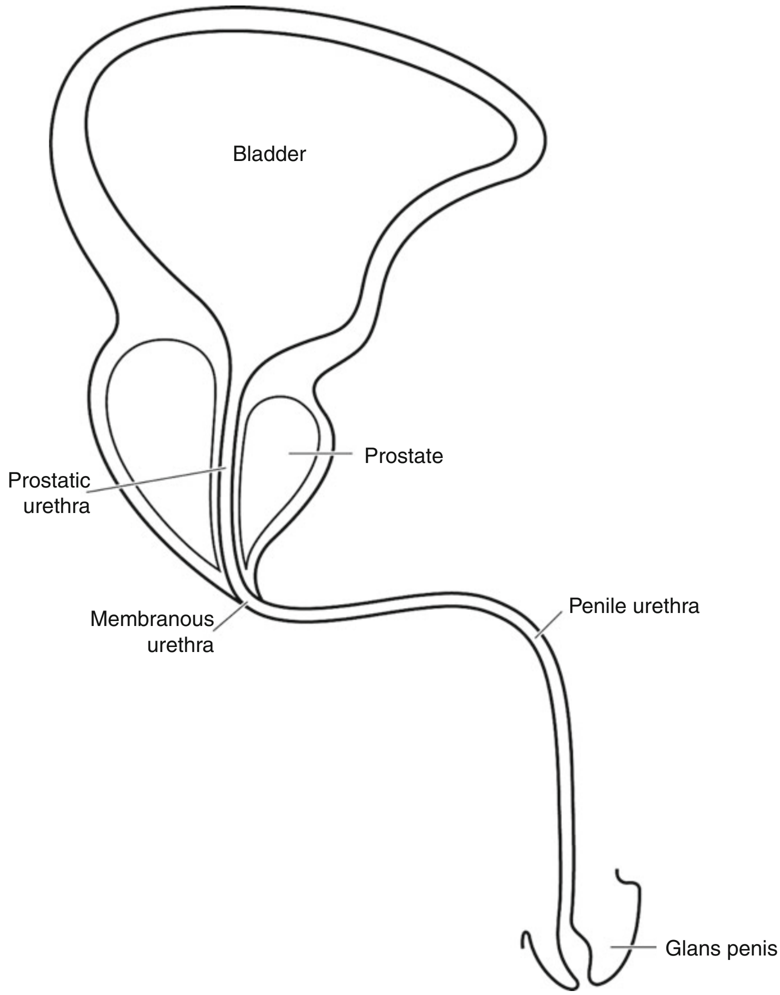

Fig. 32.1 Anatomy of the urethra (Reproduced, with permission, from Allen and Cameron (2004))

stricture can mimic benign prostatic enlargement, presenting with obstructive symptoms in the absence of hematuria. Urethral cancer tends to invade locally and metastasize to adjacent soft tissues and, therefore, most of these tumors are locally advanced at the time of diagnosis. Primary urethral malignancy usually presents at a late stage when arising in the proximal urethra. Urethral diverticula may cause irritative symptoms or dribbling.

32.3 Clinical Investigations

- Urethroscopy and biopsy.
- Urethrography (micturating and retrograde) – the best method for evaluation of urethral strictures, diverticula and, less often, neoplasms.
- CT/MRI – to determine tumor stage.
- Positron emission tomography (PET) is useful in the evaluation of suspected metastatic disease.

32.4 Pathological Conditions

32.4.1 Non-neoplastic Conditions

Urethritis: Usually a sexually transmitted infection due to Chlamydia trachomatis, Neisseria gonorrhoea, or Gardnerella vaginalis. Usually symptomatic in females, but not in males, with urethral smear diagnostic. Occasionally seen in young males as part of Reiter's syndrome (urethritis, arthritis, and conjunctivitis). Rarely provides surgical biopsy material.

Polypoid urethritis: The urethral equivalent of polypoid cystitis. This is an edematous, inflammatory growth which may be confused with a papillary neoplasm. It is most commonly seen in the prostatic urethra near the verumontanum. An association with indwelling catheters has not been shown.

Caruncle: A polypoid, fleshy, and friable lesion seen near the meatus exclusively in women. Irritative urinary symptoms are common. Histology reveals a hyperplastic urothelial lining with prominent stromal inflammation and vascularity. Scattered bizarre stromal cells may cause diagnostic confusion with sarcoma.

Benign stricture: May result from previous inflammation or trauma, e.g., catheterization, and can closely mimic malignancy, resulting in biopsy.

Diverticula: Usually seen in women and may be palpated through the vagina. Most are acquired, following infection, obstruction, and dilatation of a periurethral gland. Histology often fails to reveal an epithelial lining. An infected diverticulum may be the source of recurrent urinary tract infections. Other possible complications include stones, bladder outlet obstruction and, rarely, malignancy (most commonly adenocarcinoma). Diverticulectomy is the recommended treatment.

Urethral valves: Folds of mucosa that project into the urethral lumen. They are rarely seen in surgical pathology material unless they cause obstruction, when they may be associated with bladder neck hypertrophy.

Urethral polyps: Prostatic urethral polyps are small, papillary growths seen in the prostatic urethra of adult males. They are usually asymptomatic but may cause hematuria. Histology shows a lining of benign prostatic acinar epithelium. These polyps are often seen incidentally at cystourethroscopy and sometimes biopsied. Congenital urethral polyps, seen in young males most commonly in the prostatic urethra, are rare growths lined by urothelium which can occasionally cause obstructive symptoms.

Miscellaneous: Inverted urothelial papilloma, villous adenoma, nephrogenic adenoma, malakoplakia, amyloidosis, and condylomata acuminata are infrequently encountered within the urethra.

32.4.2 Neoplastic Conditions

Benign tumors: The benign urothelial neoplasms of papilloma and inverted papilloma share the same morphological features as their bladder counterparts and rarely arise primarily in the urethra. Hematuria is the commonest symptom. Both should be managed by transurethral resection alone. Leiomyomas and hemangiomas are seen only rarely.

Malignant tumors: The urethra is much more commonly involved secondarily by urothelial carcinoma of the bladder than by primary carcinoma. As with bladder cancer, secondary urethral involvement is more common in males with a reported incidence of approximately 10–20%. This may take the form of papillary urothelial carcinoma, flat carcinoma in situ (which may extend into periurethral ducts) or prostatic stromal invasion. Distinction is important as the latter has a worse prognosis. The same diagnostic histological criteria apply as in the bladder. In females, total urethrectomy is usually performed as part of the cystectomy procedure but, in males, urethrectomy is only performed when separate biopsies show prostatic urethra involvement. Recurrence of urothelial carcinoma in the urethral stump following a urethra-sparing cystectomy may be treated by instillation of BCG immunotherapy or, if there is stromal invasion, transurethral resection or urethrectomy.

Primary urethral carcinoma is more common in females than males and affects mainly those over 50 years of age. Etiological factors have not been clearly elucidated, although chronic inflammation may play a role. Most tumors arising proximally in the urethra are urothelial in type whereas distal lesions are more often squamous, reflecting the normal epithelial linings at these sites. Adenocarcinoma is seen in association with diverticula, prostatic adenocarcinoma or, in women, arising in periurethral glands. The clear cell variant should be distinguished from nephrogenic adenoma and spread of malignancy from the female genital tract or kidney. In males, primary urothelial carcinoma is usually treated by surgical excision, the extent of which depends on the location and stage of the tumor. Prior to any surgery, the extent of local invasion must be accurately assessed to ensure en bloc resection of all involved structures. Radiotherapy has the advantage of preserving the penis but has a higher rate of tumor recurrence and may result in urethral stricture. Primary urethral carcinoma in the female usually involves the proximal urethra and is locally advanced at presentation. Radiotherapy has generally been reserved as an adjunct to definitive surgery in more advanced disease. Combination chemotherapy with radiotherapy or neoadjuvant chemotherapy with radiotherapy prior to surgery are other treatment options. Local excision is often adequate for distal urethral carcinoma in the female.

Prognosis: Prognosis relates to anatomical location and pathological stage. Distal carcinomas have a better prognosis as they are often well-differentiated squamous cell or verrucous types and present earlier. Proximal tumors are more frequently high-grade and present at a later stage, hence prognosis is worse. In men, overall 5-year survival rates are 60–70% for penile urethral carcinomas and only 20% for membranous/prostatic urethral lesions.

Other cancers: Rare but include adenosquamous carcinoma, small cell carcinoma, malignant melanoma, lymphoma/leukemia, embryonal rhabdomyosarcoma (in children), aggressive angiomyxoma (in women), and metastatic carcinoma.

32.5 Surgical Pathology Specimens: Clinical Aspects

32.5.1 Biopsy Specimens

Urethroscopy may be undertaken in isolation or, more commonly, in tandem with cystoscopy. Small urethral lesions are snared using "cold" cup forceps or resected with a small diathermy loop. Staging biopsies of the prostatic urethra are frequently undertaken at the time of cystourethroscopy for evaluation of bladder cancer. Follow-up after cystectomy may require biopsy from the urethral stump in the event of positive urethral washings.

32.5.2 Resection Specimens

Urethrectomy is performed in one of three situations:

- For bladder cancer in continuity with cystoprostatectomy.
- For recurrence of bladder cancer in the urethral stump (secondary urethrectomy).
- For primary urethral carcinoma.

In women, up until recently, the urethra was routinely resected as part of a radical cystectomy procedure for bladder cancer. However, with careful pre-operative evaluation, it is now sometimes possible to preserve the urethra for orthotopic functional reconstruction of the urinary tract using a neobladder.

In men with bladder cancer, the standard surgical procedure is a radical cystoprostatectomy. Carcinomatous involvement of the urethra (usually prostatic) assessed on pre-operative biopsies is an indication for concomitant urethrectomy.

This is performed in two stages. Prior to the cystoprostatectomy, the membranous urethra is dissected from the urogenital diaphragm and transected. This facilitates the subsequent perineal dissection and preservation of the neurovascular bundle. Cystoprostatectomy is completed and then the remainder of the urethra is resected from a perineal approach, dividing it distally and dissecting the bulbar urethra up to the urogenital diaphragm.

Secondary urethrectomy is indicated if urethral washings or biopsy following previous cystoprostatectomy for bladder cancer reveal recurrent tumor. This involves perineal dissection as described for primary urethrectomy but, because of scarring and proximity of small bowel to the urogenital diaphragm, it is a much more difficult operation. Complete excision of the membranous urethra proximally is less certain, but frozen section may offer reassurance that a negative margin has been attained.

The best treatment of primary urethral carcinoma in the male is surgical excision. Distal tumors may be treated by transurethral resection, local excision, partial or radical penectomy depending on the extent of tumor infiltration. Carcinoma of the bulbomembranous urethra usually requires radical cystoprostatectomy, pelvic lymphadenectomy, and total penectomy. This procedure may be extended to include in-continuity resection of the pubic rami and adjacent urogenital diaphragm to improve the margin of resection. Primary prostatic urethral carcinoma may be treated by transurethral resection if superficial but otherwise requires cystoprostatectomy and total urethrectomy.

32.6 Surgical Pathology Specimens: Laboratory Protocols

32.6.1 Biopsy Specimens

Tiny pieces of tissue (several mm) retrieved using either "cold" cup forceps or a small diathermy loop are counted, measured, processed intact, and examined histologically through three levels. Transurethral specimens should be weighed collectively, the number of fragments counted, and all tissue embedded if possible.

32.6.2 Resection Specimens

Specimen:

- Most urethrectomy resection specimens are for neoplasia as part of a cysto(prostato)urethrectomy. Occasionally isolated urethrectomy is performed.

Initial procedure:

- The specimen may be in several tubular fragments labeled separately or with attached sutures to aid orientation. In the absence of such markers, definitive orientation may not be possible, although the distally resected urethra may be identifiable, having a smaller diameter.
- Weigh (g) and measure (cm) each fragment; record the number of fragments.
- Paint the external circumferential radial margin comprising adventitial connective tissue.
- Fix the specimen by immersion in 10% formalin for at least 24–36 h.
- Remove the proximal and distal surgical resection limits (Fig. 32.2) by taking circumferential transverse sections (rings) from the ends of the appropriate fragments. If separate fragments are not labeled, take sections from both ends of all fragments for later possible correlation with clinical information.
- After removal of the limits, the remaining urethra is serially sectioned transversely throughout its length at 3 mm intervals, and the sections laid out sequentially for examination and photography, if desired. Alternatively, if a grossly obvious tumor is identifiable on one luminal surface of the urethra, the specimen is opened longitudinally with small scissors along the opposite surface taking care not to disturb the tumor. A combination of both approaches often provides the best histological material.

Description:

- Tumor
  - Site (prostatic/membranous/bulbar/pendulous urethra, meatus)
  - Length × width × depth (mm)
  - Multifocality
  - Appearance (papillary/polypoid/verrucous/sessile/ulcerated/color)
  - Edge (circumscribed/irregular)

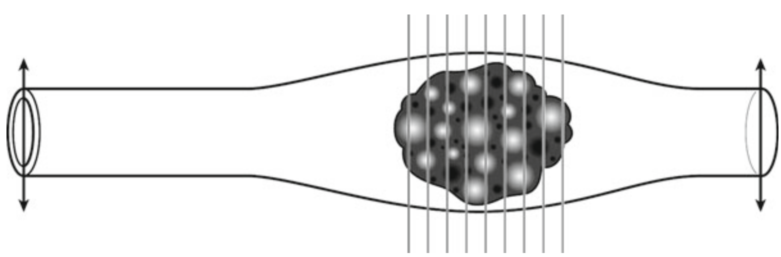

Fig. 32.2 Blocking a urethrectomy specimen. Transverse section the limits and the tumor at 3 mm intervals (Reproduced, with permission, from Allen and Cameron (2004))

- Mucosa
  - Carcinoma in situ away from tumor may appear red and velvety
- Wall
  - Tumor confined to mucosa or infiltrative
- Other
  - Stricture, dilatation, diverticulum

Blocks for histology (*Fig*. 32.2):

- Sample the proximal and distal limits of surgical resection as complete circumferential rings; more than one fragment may need to be sampled.
- Sample at least three blocks of tumor, in the form of transverse or longitudinal sections or both, to show the deepest point of circumferential invasion and the relationship to the painted circumferential margin and the adjacent mucosa.
- Sample at least one random block of background mucosa to look for carcinoma in situ.
- Count and sample all lymph nodes (usually submitted separately).

Histopathology report:

- Tumor type – squamous/urothelial/adenocarcinoma/other
- Tumor growth pattern – papillary/invasive/flat in situ
- Tumor differentiation – use WHO grades I–III (based on cytological atypia)
- Tumor edge – pushing/infiltrative/lymphoid response
- Extent of local tumor spread

  Urethra (male and female)

pTa	Non-invasive papillary, polypoid, or verrucous carcinoma
pTis	Carcinoma in situ
pT1	Invasion of subepithelial connective tissue
pT2	Invasion of any of: Corpus spongiosum, prostate, periurethral muscle
pT3	Invasion of any of: Corpus cavernosum, beyond prostatic capsule, anterior vagina, bladder neck (extraprostatic extension)
pT4	Invasion into other adjacent organs (invasion of the bladder)

Urothelial carcinoma of prostatic urethra

pTis pu	Carcinoma in situ, involvement of prostatic urethra
pTis pd	Carcinoma in situ, involvement of prostatic ducts
pT1	Invasion of subepithelial connective tissue
pT2	Invasion of any of: Prostatic stroma, corpus spongiosum, periurethral muscle
pT3	Invasion of any of: Corpus cavernosum, beyond prostatic capsule, bladder neck (extraprostatic extension)
pT4	Invasion into other adjacent organs (invasion of the bladder)

- Lymphovascular invasion – present/not present
- Regional lymph nodes

  Inguinal/pelvic

pN0	No regional lymph node metastasis
pN1	Metastasis in a single regional node ≤2 cm
pN2	Metastasis in a single regional node >2 cm or multiple regional nodes

- Excision margins

  Distances (in mm) to the nearest longitudinal and circumferential periurethral resection limits

  Presence/absence of carcinoma in situ at longitudinal limits
- Other pathology

  Urothelial carcinoma in situ, diverticulum

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33 Testis, Epididymis, and Vas

Declan M. O'Rourke and Derek C. Allen

33.1 Anatomy

The testes are suspended in the scrotum by the spermatic cords, the left testis hangs somewhat lower (Fig. 33.1). The average size is from 4 to 5 cm in length, 2.5 cm in breadth, and 3 cm in the antero-posterior diameter. Weight varies from 10.5 to 14 g. Within the scrotum the testis is covered on its anterior, medial, and lateral surfaces by tunica vaginalis, the remnant of a developmental connection with the peritoneal cavity. Each testis is covered by a tough fibrous coat, the tunica albuginea. The substance of the testis is subdivided by septa which run inwards from the tunica albuginea. The glandular structure consists of numerous lobules (~400) contained in one of the intervals between the fibrous septa. They consist of from one to four seminiferous tubules, between which lie the interstitial or Leydig cells, responsible for the production of testosterone. In the apices of the lobules, the tubules become less convoluted and unite together to form 20 to 30 larger ducts (tubuli recti). The tubuli recti enter the fibrous tissue of the mediastinum forming a close network of anastomosing tubes (rete testis). The rete testis perforates the tunica albuginea and carries the seminal fluid from the testis to the epididymis.

The epididymis lies on the posterior surface of the testis. It consists of a central portion or body, an upper enlarged extremity (the head), and a lower pointed end (the tail), which is continuous with the ductus (vas) deferens. The head is intimately connected with the upper end of the testis by means of the efferent ductules of the gland; the tail is connected with the lower end by cellular tissue and a reflection of the tunica vaginalis. The epididymis is connected to the back of the testis by a fold of the serous membrane. On the upper extremity of the testis, just beneath the head of the epididymis, is a minute oval, sessile body, the appendix of the testis (hydatid of Morgagni). It is the remnant of the upper end of the Müllerian duct. On the head of the epididymis is a second small stalked appendage (appendix epididymis) usually regarded as a detached efferent duct.

The vas deferens consists of a muscular tube, formed of three layers, which connects the tail of the epididymis to the ejaculatory duct at the prostate. The vas passes up in the spermatic cord through the superficial inguinal ring, inguinal canal, and deep ring to reach the posterior surface of the bladder. At the ejaculatory duct it is joined by the duct from the seminal vesicles. The vas is lined by a tall columnar epithelium.

Lymphovascular drainage:

The lymphatic vessels of the testes form from four to eight collecting trunks which ascend with the spermatic veins in the spermatic cord. The

D.M. O'Rourke (✉) • D.C. Allen
Histopathology Laboratory, Belfast City Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: declan.orourke@belfasttrust.hscni.net;
derek.allen@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_33, © Springer-Verlag London 2013

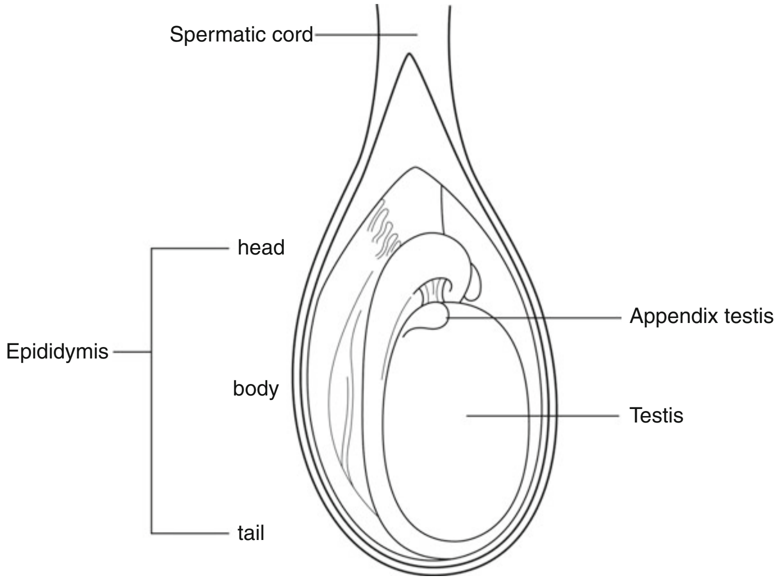

Fig. 33.1 Anatomy of the testis, epididymis, and spermatic cord (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

testicular lymphatics drain to the periaortic and pericaval abdominal nodes. Intrapelvic (common and external iliacs) and inguinal nodes are considered regional after scrotal or inguinal surgery (Fig. 33.2).

33.2 Clinical Presentation

A painless testicular mass is pathognomonic of a primary testicular tumor, occurring in only a minority of patients. The majority present with diffuse testicular pain, swelling, hardness, or some combination of these findings. Since infectious epididymitis or orchitis is more common than tumor, a trial of antibiotic therapy is often undertaken. If testicular discomfort does not abate or findings do not revert to normal within 2–4 weeks, a testicular ultrasound examination is indicated. Delays in patients seeking definitive treatment after recognition of the initial lesion are frequent (3–6 months) and correlate with development of metastasis. Trauma to the testis can sometimes lead to confusion in diagnosis. Endocrine manifestations such as gynecomastia (2–4% of patients) are sometimes seen in association with sex cord-stromal tumors and choriocarcinoma. Exophthalmos has also been reported with choriocarcinoma (due to high HCG levels). In 5–10% of patients, symptoms result from metastasis, e.g., back pain.

Undescended testicular tumors present with a suprapubic lump, urinary or bowel complaints. Development of torsion in an undescended testicle can sometimes be the warning sign of testicular tumor. Rarely testicular tumors may present with metastasis and symptoms of an abdominal lump, chronic cough, or as a "neck node with unknown primary."

33.3 Clinical Investigations

33.3.1 Radiological Evaluation

- Scrotal ultrasound – an important non-invasive diagnostic tool. Testicular tumors appear as a hypoechoic lesion.
- Chest X ray – preliminary evaluation of pulmonary involvement.
- CT scan – imaging of the abdomen and pelvis is required. CT or MRI of the brain is performed in patients with neurologic signs or symptoms, and MRI is also useful in the evaluation of bone metastasis.
- Both PET (Positron Emission Tomography) and PET-CT provide superior staging accuracy to conventional imaging, particularly for

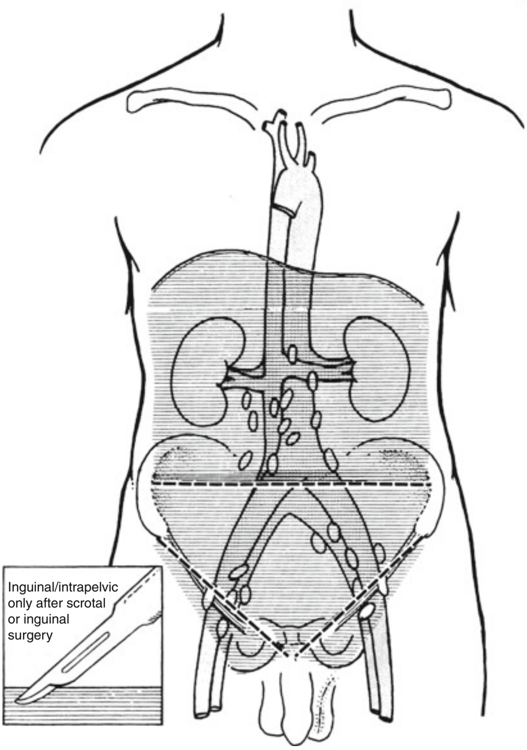

Fig. 33.2 Testis – regional lymph nodes. The regional lymph nodes are the abdominal para-aortic (periaortic), preaortic, interaortocaval, precaval, paracaval, retrocaval, and retroaortic nodes. Nodes along the spermatic vein should be considered regional. Laterality does not affect the N classification. The intrapelvic nodes and the inguinal nodes are considered regional after scrotal or inguinal surgery (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

seminoma and early stage disease. PET, however, is not as reliable in mature teratoma differentiated.

- FNAC of testicular tumors is generally not recommended as it is useful only if it is positive, and there is a theoretical risk of needle tract recurrence. FNAC is valuable in the investigation of possible metastatic lesions (seminoma versus non-seminoma).

33.3.2 Serum Tumor Markers

Serum tumor marker concentrations are determined before, during, and after treatment and throughout long-term follow-up. Increased or rising concentrations of alpha-fetoprotein (AFP), human chorionic gonadotrophin (HCG), or both, without radiographic or clinical findings, imply active disease and are sufficient reason to initiate treatment if likely causes of false positive results have been ruled out.

Alpha-fetoprotein: Production is restricted to non-seminomatous germ cell tumors, specifically embryonal carcinoma and yolk sac tumors.

HCG: May be observed in both seminomas and non-seminomatous tumors.

Lactate dehydrogenase (*LDH*): Less specific but has independent prognostic value in patients with advanced germ cell tumors.

33.4 Pathological Conditions

33.4.1 Testis

33.4.1.1 Non-neoplastic Conditions

Pyogenic epididymo-orchitis: Usually due to E. coli and presents as a painful mass, often clinically confused with testicular cancer. Generally differentiated by ultrasound scanning. Histology resembles a granulomatous orchitis. This can be complicated by venous thrombosis and septic testicular infarct.

Granulomatous orchitis: Etiology and pathogenesis unknown, but there is speculation that the disease may have an autoimmune basis. It presents in middle-aged males with painful unilateral testicular mass and associated fever. Some cases are associated with urinary tract infections, history of prostatectomy, inguinal hernia repair, and trauma. Grossly the testis is enlarged and the cut surface is vaguely nodular, yellowish, and hard. Testicular involvement may be total or partial. Histologically there is a mixed chronic inflammatory infiltrate, fibroblasts, and scattered multinucleated giant cells.

Other infections: Syphilis, tuberculosis, mumps.

Cysts: Epidermoid cysts (below), cysts of tunica albuginea, rete testis, efferent ducts, or testicular parenchyma have been described. Cystic dysplasia is a rare congenital disorder with numerous irregular cystic spaces in the mediastinum testis.

Epidermoid cyst: May represent monodermal teratoma, but no metastasis if no adnexal structures or other tissue types are found after thorough sampling. It usually affects ages 10–39 years and grossly consists of an intraparenchymal lesion containing white grumous keratin debris, with a keratinized squamous epithelial lining. There is no intratubular germ cell neoplasia.

Hydrocele: Accumulation of clear serous fluid between the visceral and parietal layers of the tunica vaginalis, associated with trauma and epididymitis. Histology shows loose to fibrotic connective tissue with a mesothelial lining.

Rare lesions: Include malakoplakia, inflammatory pseudotumor.

33.4.1.2 Neoplastic Conditions

Testicular neoplasms represent less than 1% of all malignancies in males although their incidence is rising. They are highly curable even if advanced; 95% are germ cell tumors and 5% sex cord-stromal tumors. The rest are rare but include mixed tumors not specific to the testis and metastases. Predisposing factors include cryptorchidism, genetic factors, testicular dysgenesis, Li-Fraumeni syndrome, prior testicular germ cell tumor or intratubular germ cell neoplasia.

Germ cell tumors: The British and American histological classification of teratoma differ, but the terminology can be correlated (Table 33.1). For management and prognostic purposes, the most important distinction is between seminomatous and non-seminomatous tumors. However, the WHO classification (Table 33.2) is recommended as it correlates better with the pathology. Pathological staging has minor clinical significance as therapy is largely dependent on clinical staging (TNM and the modified Royal Marsden systems) based on imaging techniques (for abdominal/pulmonary/cerebral metastases) and levels of serum tumor markers.

Intratubular germ cell neoplasia (*ITGCN*): In situ stage of germ cell neoplasia seen in 90–100% of testes adjacent to germ cell tumors. There is an association with infertility (0.4–1.0%), cryptorchidism (2–8% of patients), and in the contralateral testis in patients with prior testicular tumor (5%), 50% progress to germ cell tumor in 5 years.

Table 33.1 Malignant teratoma classification

British (BTTP)	American/WHO
Teratoma differentiated (TD)	Mature teratoma Immature teratoma
Malignant teratoma intermediate (MTI)	Teratoma with embryonal carcinoma and/or yolk sac tumor
Malignant teratoma undifferentiated (MTU)	Embryonal carcinoma
Malignant teratoma trophoblastic (MTT)	Choriocarcinoma (only element)

Table 33.2 World Health Organization (WHO) histological classification of testicular germ cell tumors

Germ cell tumors
Intratubular germ cell neoplasia (ITGCN), unclassified
Other types
Tumors of one histologic type (pure forms)
Seminoma
Seminoma with syncytiotrophoblastic cells
Spermatocytic seminoma (SS)
Embryonal carcinoma (EC)
Yolk sac tumor (YST)
Trophoblastic tumors: choriocarcinoma, monophasic choriocarcinoma, placental site trophoblastic tumor
Teratoma: mature/immature and variants - dermoid cyst, monodermal teratoma (carcinoid tumor), teratoma with somatic-type malignancies
Tumors of more than one histologic type (mixed forms, mixed NSGCT)

Histology shows large seminoma-like cells present along a thickened/hyalinized tubular basement membrane. Spermatogenesis is usually absent. The cells are PAS positive but sensitive to diastase. Immunohistochemistry shows positive staining for CD117, podoplanin, OCT3/4, and PLAP. Cytokeratins, α-fetoprotein, and CD30 are negative. It can be treated with low-dose radiation but watchful waiting (clinical, ultrasound examination, and serum markers) is advocated by some.

Seminoma: Represents 30–50% of testicular germ cell tumors with a mean age at diagnosis of 40 years. 40% have increased serum PLAP and 70% of patients have stage I disease. Metastases are to lymph nodes or bone. The presence of elevated serum HCG does not change the classification and has no clinical significance. However, elevated AFP indicates a non-seminomatous germ cell component (or liver disease), even if not seen histologically. The gross appearance is that of a well-circumscribed pale lobulated fleshy mass with a bulging cut surface. Histology shows uniform cells with abundant clear cytoplasm, well-defined cell borders, and vesicular nuclei with prominent nucleoli. A lymphoplasmacytic infiltrate is always present. Granulomatous inflammation, trophoblastic giant cells, and Pagetoid spread to the rete are seen in a minority of cases. The tumor cells are PAS positive with diastase sensitivity, and positive for OCT3/4 (nuclear), podoplanin (D2–40), CD117, SALL4, and PLAP. Cytokeratin may be focal or weak. Treatment options depend on TNM stage and whether the tumor is seminoma or non-seminomatous. Stage I seminoma is managed by radical inguinal orchidectomy followed by surveillance protocol (serum markers, chest radiographs, and CT scan), single-dose carboplatin adjuvant therapy, or radiation therapy. Surveillance is a safe option for patients who will cooperate with long-term follow-up and who are regarded as low risk for relapse (primary tumor size less than 4 cm and with no invasion of rete testis). Stage II seminoma is treated by radical inguinal orchiectomy followed by radiation or cisplatin-based adjuvant therapy. As a result chemotherapy is preferred for patients with bulky stage II seminoma or if patients relapse following radiotherapy. Stage III seminoma is managed by radical inguinal orchidectomy followed by multidrug chemotherapy.

The prognosis is excellent with 98% cure rate for stage I or II seminoma. Prognostic factors include stage, tumor size (>4 cm), rete testis invasion, and intertubular growth. The term "anaplastic" seminoma (>3 mitoses per high-power field) is not accepted as a separate entity and is not an adverse prognostic factor.

Spermatocytic seminoma: Extremely rare germ cell tumor composed of three cell types of variable size. It forms a well-circumscribed, soft, friable, pale mass with a mucoid or gelatinous, bulging cut surface. Histology shows a diffuse sheet pattern of small lymphocyte-like cells, intermediate cells, and giant cells with an edematous stroma. It is distinguished from seminoma by the absence of stroma, lymphocytes, glycogen, granulomas, and ITGCN. It is negative for most germ cell-associated markers (OCT3/4, podoplanin (D2–40), PLAP, α-fetoprotein, glypican-3, HCG, and CD30). It may be positive for SALL4 and CD117. Radical orchidectomy alone is curative. It has an excellent prognosis with rare malignant behaviour (less than 1%). Sarcomatoid transformation is rare, but when present is associated with distant metastasis.

Non-seminomatous germ cell tumors (*NSGCT*): In general, more aggressive and metastasize earlier than seminomas. The metastases may not resemble the primary tumor and are radioresistant. 80% have elevated AFP or HCG at diagnosis. The prognosis is good with 95% cure rate if there is no lymph node or metastatic involvement but ranges from 40% to 95% with metastases. There is a poor prognosis if extensive pulmonary disease is present. Traditionally the treatment for stage I non-seminomatous germ cell tumors has been orchidectomy followed by retroperitoneal lymph node dissection (RPLND) to eradicate the disease while confined to the local lymph nodes. It is now believed, however, that most patients with such tumors do not benefit from this dissection, a procedure which is not without complications. More recently, stage I non-seminomatous germ cell tumors have been managed by surveillance (regular serum tumor markers and CT scanning). Stage I tumor with lymphovascular invasion and more advanced disease are best treated with chemotherapy. Stage II NSGCT is treated by radical inguinal orchidectomy followed by RPLND, RPLND and chemotherapy, or chemotherapy and delayed RPLND. Salvage chemotherapy can be initiated on detection of relapse. If the concentrations of tumor markers fall after chemotherapy and residual retroperitoneal masses are seen on CT, then lymph node dissection is appropriate as 20% of such nodes will harbor residual tumor. When the tumor markers do not fall to normal concentrations after chemotherapy, opinion on treatment is divided between lymph node dissection and further chemotherapy. Although organ-sparing surgery is not indicated it can be attempted in certain cases with precaution. Indications include synchronous bilateral testicular tumors, metachronous contralateral tumors, or a tumor in a solitary testis with normal preoperative testosterone levels. Organ-preserving surgery can be performed when the tumor measures less than 2 cm. In those cases, the rate of associated ITGCN is high (up to 80%) and can be treated with radiotherapy. This option has to be carefully discussed with the patient and surgery performed in a center with experience.

Teratoma: Represents 5% of germ cell tumors and contains cellular components derived from two or three germ layers. It is the second most common testicular tumor after yolk sac in children (age <3), is not associated with intratubular germ cell neoplasia and almost never metastasizes. In adults, there is a presumption of malignant behaviour regardless of tumor differentiation. Grossly large (5–10 cm), multinodular, and heterogeneous (solid, cartilaginous, cystic). Histologically mature teratomas contain differentiated tissues including cartilage, nerve, and various epithelia, whereas immature teratomas have foci resembling embryonic or fetal structures including primitive neuroectoderm, poorly formed cartilage, neuroblasts, loose mesenchyme, and primitive glandular structures (amount important). Occasional cases undergo malignant transformation with focal malignancy such as squamous cell carcinoma, adenocarcinoma, or sarcoma (adults).

Mixed germ cell tumors: Mixed forms are common accounting for one third of germ cell tumors and 70% of non-seminomatous tumors of the testes. Common combinations include embryonal and teratoma; embryonal and seminoma; embryonal, yolk sac tumor and teratoma. Clinical presentation and management are the same as non-seminomatous germ cell tumor, and the prognosis is usually that of the worst component.

Embryonal carcinoma (*EC*): Pure tumors represent 2% of germ cell tumors, but 85% of NSGCTs have an embryonal carcinoma component. Histologically solid, alveolar, tubular, or papillary patterns of large, epithelioid, anaplastic cells. Immunohistochemistry shows positive staining for cytokeratin, CD30, OCT3/4, podoplanin (D2–40), SALL4, and PLAP. Treatment is similar to other non-seminomatous germ cell tumors depending mainly on clinical stage. The prognosis is the poorest among all germ cell tumors.

Yolk sac tumor (*YST*): Most common germ cell tumor of infants and young children (accounts for 75% of all childhood testicular neoplasms) but pure form is rare in adults where it usually presents as a component of mixed germ cell tumors. More than 95% patients have elevated serum AFP, which is valuable in diagnosis and monitoring treatment. YST has multiple usually mixed growth patterns. Reticular or microcystic patterns are the most frequent (80%). Others are: Endodermal sinus (Schiller Duval bodies), solid, papillary, and glandular. The cells can look very pleomorphic and difficult to separate from EC. They are positive for cytokeratin, AFP, PLAP (variable), SALL4, glypican-3, and negative for CD30, podoplanin (D2–40), OCT3/4, and HCG. For infants and children with pure YST, radical inguinal orchidectomy with close follow-up is the recommended treatment of choice. For adult YST (usually mixed with other germ cell tumor) treatment is as for NSGCTs.

Choriocarcinoma: 0.3–1% of germ cell tumors are pure choriocarcinoma, but mixed tumors are more common. It may present initially with early hematogenous metastasis (liver, lung, mediastinum, retroperitoneum) and a normal testis or small tumor but with increased serum HCG. Patients may have gynecomastia or hyperthyroidism. It is usually fatal if pure. Histologically there is hemorrhage and necrosis with a biphasic arrangement of cytotrophoblast and syncytiotrophoblast cells.

It is positive for cytokeratins, HCG, HPL, EMA (only syncytiotrophoblast), and SALL4. Treatment is radical orchidectomy and systemic chemotherapy. The level of HCG correlates with prognosis, reflecting tumor load.

Sex cord-stromal tumors: 4% of testicular neoplasms and containing epithelial elements of sex cord origin (Sertoli and granulosa cells) admixed with mesenchymal components (Leydig and theca-lutein cells) in varying combinations and degrees of differentiation. Specific types of sex cord-stromal tumor may be associated with genetic syndromes. e.g., large cell calcifying Sertoli cell tumor in Peutz-Jeghers syndrome and testicular feminization syndrome for Sertoli cell tumors. Almost all are immunoreactive for inhibin.

Leydig (*interstitial*) *cell tumors*: 1–3% of testicular tumors (age 20–60 years) with 3% bilateral. They secrete sex hormones and symptoms include gynecomastia with virilism, precocious puberty, and a testicular mass. In adults, 10% have malignant behaviour with metastasis to lymph nodes, lung, and liver. They are positive for inhibin-α, calretinin, melan-A(MART-1), WT1, androgenic hormones, and vimentin. They are typically negative for cytokeratins, OCT3/4, SALL4, S100, PLAP, and HMB-45. Features suggesting malignancy include large size (>5 cm), necrosis, vascular invasion, nuclear atypia, infiltrative margins, older patients, aneuploidy, atypical and numerous mitoses (>3/10 high-power fields) and high MIB-1 (Ki67) activity. Mean survival when malignant is 4 years. Grossly they are solid brown tumors and 10% have extratesticular extension. Histology reveals sheets of large, round/polygonal cells with eosinophilic cytoplasm and round central nuclei. Reinke crystals are present in 25% of cases. Treatment includes orchidectomy and/or lymph node dissection if malignant.

Granulosa cell tumor: Resembles the analogous ovarian tumor. The adult form is rare with an age range 20–53 years, usually non-functional and rarely associated with gynecomastia. It is usually benign but metastasis occur in 10% (associated with size >7 cm, hemorrhage, necrosis, lymphovascular invasion). The juvenile form is the most common neonatal testicular tumor with an average age of onset less than 1 month or even congenital. There is an association with trisomy 12 and sex chromosome mosaicism if abnormal external genitalia. There is no association with endocrine manifestations. It has a benign behaviour following orchidectomy.

Sertoli cell tumors: One-third present with gynecomastia without virilism and 10% are malignant (to local lymph nodes) indicators being nuclear pleomorphism, size >5 cm, mitoses (>5/10 high power fields), necrosis, and lymphovascular invasion.

Grossly firm small well–circumscribed yellow-white nodules. Histology shows trabeculae lined by Sertoli-like cells. They are positive for AE1/AE3, EMA, vimentin, α-inhibin, melan-A (MART-1), WT1, CD99, calretinin, S100 (weak), and synaptophysin. They are typically negative for PLAP, OCT3/4, SALL4, α-fetoprotein and CD30. Electron microscopy shows Charcot-Böttcher filaments which are pathognomonic of Sertoli cell differentiation. Treatment is orchidectomy – radiation and chemotherapy have little effect.

Mixed germ cell-sex cord stromal tumors: (gonadoblastoma)

Other tumors not specific to testis:

Leukemia: Testis may be the first site of relapse, e.g., ALL in children.

Lymphoma: 50% of testicular neoplasms in men aged 60+ years, 20% bilateral.

Granulocytic sarcoma: 20–35% patients involved.

Carcinoid: Presumed to be a monodermal teratoma, although 20% have other teratomatous elements. It is rare and 10% have clinical carcinoid syndrome.

Metastasis to testes: Rarely the first clinical sign of disease. Lung, prostate, and skin (Merkel cell tumors, melanoma) are the usual primary sites. Immunohistochemistry may help in distinguishing the primary site.

33.5 Epididymis

33.5.1 Non-neoplastic Conditions

Epididymitis: Primary cause of epididymal obstruction and usually related to cystitis, prostatitis or urethritis that spreads through the vas deferens or lymphatics. It may cause testicular ischemia and necrosis. Causes include Chlamydia trachomatis, Neisseria gonorrhoea, E. coli, pseudomonas, other urinary tract infection organisms, and rarely tuberculosis and brucellosis.

Cysts of epididymal appendix and epididymal cysts: The former can twist, necrose, and present with pain, while the latter form an epididymal mass separate from the testis. Treatment is resection of the necrotic appendix and cyst aspiration respectively, or if persistent, epididymectomy.

Spermatic granuloma/epididymitis nodosa: Inflammation or trauma damage to the epithelium or basement membrane, causing spillage of spermatozoa into the interstitium (similar to vasitis nodosa). It consists of a nodule up to 3 cm in the head of the epididymis with histological features of non-caseating granulomas around spermatozoa.

Spermatocele: Cystic dilation of efferent ducts lined by ciliated columnar cells with thin connective tissue wall, no smooth muscle. The cysts are usually translucent and contain spermatozoa and proteinaceous fluid.

33.5.2 Neoplastic Conditions

Adenomatoid tumor: Benign paratesticular tumor of mesothelial cell origin (similar to the tumor in spermatic cord, fallopian tube, and uterus) found mostly in the epididymis but also in the tunica vaginalis, albuginea, and rete testis. Grossly forms a circumscribed white mass up to 5 cm. Histology shows a variety of growth patterns including glands, cysts, tubules, and cords (32%). Lymphoid aggregates may be prominent within or at the periphery of the tumor. It is immunopositive for cytokeratin, calretinin, podoplanin, CK5/6, thrombomodulin, and WT1. Resection is curative.

Papillary cystadenoma: Familial, unilateral, or bilateral (40%) with a mean age of 36 years. Associated with von Hippel-Lindau disease.

Carcinoma of epididymis: Rare, with a poor prognosis. It usually presents as a scrotal mass. It is large and often hemorrhagic or necrotic.

33.6 Rete Testis

33.6.1 Non-neoplastic Conditions

Adenomatous hyperplasia: Solid/cystic mass in testicular hilus and usually an incidental microscopic finding.

Cystic dilation (*transformation*): Due to obstruction of epididymis or intratesticular excretory ducts and also seen after hemodialysis.

Cystic dysplasia: Presents as testicular mass in infants and children. Consists of cystic dilation of rete testis with compression/atrophy of seminiferous tubules. It is thought to be a developmental anomaly sometimes associated with ipsilateral renal agenesis.

33.6.2 Neoplastic Conditions

Rete testis adenocarcinoma: Very rare and resembles mesothelioma of the tunica vaginalis. Wide age range with a poor prognosis.

Sertoliform cystadenoma of rete testis: Extremely rare benign tumor (ages 34–62) usually presenting as a unilateral painless testicular mass.

33.7 Spermatic Cord and Paratesticular Region

33.7.1 Non-neoplastic Conditions

Torsion: May cause testicular infarct if not treated quickly. This usually occurs in the first year of life or also towards puberty due to trauma. It is associated with incomplete descent, absent scrotal ligaments, absent gubernaculum testis, or testicular atrophy causing the testis to be abnormally mobile. Torsion must last at least 6–24 h to cause an infarct. Treatment consists of untwisting and fixing the testis to dartos muscle or orchidectomy. The opposite testis should be fixed to dartos muscle as a preventive measure.

Vasitis nodosa: Granulomatous condition of the vas deferens, which resembles spermatic granuloma of the epididymis. It is usually post vasectomy or herniorrhaphy and occasionally associated with recanalization. Histology shows proliferating ductules and dilated tubules containing spermatozoa in the wall of the vas deferens with hyperplastic smooth muscle. May see perineural or vascular invasion by the proliferating ductules.

Varicocele: Abnormal dilation and tortuosity of veins in the pampiniform plexus of the spermatic cord probably due to insufficiency of venous valves. It is often associated with infertility. 90% are on the left and 10% bilateral. Treatment consists of ligation or occlusion of the left spermatic vein, and after treatment, 40–55% are fertile.

33.7.2 Neoplastic Conditions

Benign tumors are usually lipomas. Fat collections around a hernia sac are not true lipomas. Non-neoplastic masses include mesothelial and dermoid cysts.

Aggressive angiomyxoma: More usual in the vulva with local recurrence common, but no metastasis. Grossly non-encapsulated with bland spindle cells in myxoid stroma containing prominent thick-walled/hyalinized vessels.

Angiomyofibroblastoma: Benign soft tissue tumor, which is well circumscribed consisting of alternating hypocellular and hypercellular zones.

Embryonal rhabdomyosarcoma: Most common childhood malignant tumor of the spermatic cord. The peak age is 9 years with an 80% overall survival. It is a fleshy white to tan tumor, 4–6 cm, and may be mucoid. Small cells, eosinophilic cells, and spindle cells with variable cross striations. Immunopositive for desmin, sm-actin, myoglobin, myoD-1, myogenin, and vimentin.

Sarcomas: Adults – most common tumors are liposarcoma, malignant fibrous histiocytoma, leiomyosarcoma, and fibrosarcoma. Treatment is orchidectomy with high ligation of the cord and radiation therapy.

Liposarcoma: Usually well-differentiated or sclerosing types. There is a 20% recurrence rate following local removal.

Mesothelioma: Cystic/solid/nodular masses lining a hernial sac. They have an aggressive behaviour.

Papillary serous carcinoma, *Mullerian subtype*: Malignant Müllerian-type epithelial tumor with serous differentiation. It is extremely rare, and histologically identical to the ovarian counterpart. It shows positive staining for CK7, CA125, S100, EMA/MUC1, BER-EP4, CD15, and CEA. Treatment includes radical orchidectomy and Mullerian type chemotherapy.

Desmoplastic small round cell tumor: Occurs in young men and is an aggressive primitive neuroectodermal tumor with a 3–4 cm gray-white, firm mass, often near the epididymis. Comprises small cells in a desmoplastic stroma, and the prognosis is very poor.

Lymphoma: Rare to involve paratesticular regions without testicular involvement.

Metastasis to paratesticular region: Tumors secondarily involving paratesticular structures by hematogenous metastasis or intraperitoneal spread from distant sites and include prostate, lung, kidney, and gastrointestinal tract as the commonest sites of origin.

33.8 Surgical Pathology Specimens: Clinical Aspects

33.8.1 Biopsy Specimens

1. Inguinal exposure with testicular isolation and biopsy.
   Testicular biopsy is standard management in patients at high risk of ITGCN as it is thought to progress to invasive tumor in 50–100% of cases, and therapy should be considered. It is also useful in the management of the contralateral testis in patients with germ cell tumors, approximately 5% of whom have ITGCN of the opposite testicle. A high incidence of ITGCN (35%) is found in young (<30 years) patients where the contralateral testis is small (<16 ml) and of poor quality (soft). These patients constitute a high-risk group, in whom it is appropriate to recommend biopsy at initial presentation. Biopsy should be 0.3–1.0 cm in maximum dimension and removed atraumatically without squeezing the tissue or handling it with forceps.

Open biopsy is considered the normal procedure, but needle biopsy may be adequate.

2. Transscrotal open or needle biopsy.
   Rarely performed due to the presumed risk of wound seeding and lymphovascular spread to inguinal lymph nodes.

33.8.2 Resection Specimens

Radical inguinal orchidectomy is performed when a testis tumor is appreciated on examination and/or preoperative imaging studies. This is accomplished via an inguinal incision in order not to alter the lymphatic drainage pattern of the testicle (to the retroperitoneal lymph nodes) by violating the scrotal wall (drainage to the superficial inguinal lymph nodes). Radical orchidectomy also allows ligation of the vas deferens and testicular vessels at the internal inguinal ring, so that should subsequent surgical removal of the spermatic cord and retroperitoneal lymph nodes be required (for therapy or staging) the inguinal canal need not be explored again.

33.9 Surgical Pathology Specimens: Laboratory Protocols

33.9.1 Biopsy Specimens

Testicular biopsy (*0.3–1.0 cm*): Placed in Bouin's fixative for a minimum of 2 h (smaller biopsies) and a maximum of 24 h. Paraffin sections are cut through levels and stained routinely with hematoxylin and eosin. Comment should be made on the presence or absence of ITGCN, the degree of spermatogenesis, and evidence of atrophy of seminiferous tubules. Immunohistochemical assessment of PLAP, CD117, OCT3/4 (present in ITGCN cells) is helpful in a biopsy showing equivocal morphology.

Epididymectomy: Weigh (g) and measure (cm), bisect or serially slice noting any focal lesions (abscess/adenomatoid tumor), and submit representative blocks. Cysts are submitted in total, or if large, blocks of the wall are sampled. Fluid contents can be examined microscopically for sperm to distinguish from a hernial sac.

Appendix epididymis (*Hydatid of Morgagni*): Measure (mm), process intact.

Vasectomy: Measure (cm) the segments of right and left vas and submit two complete transverse sections of each. Lengths vary from 0.5 to 5 cm – small specimens are often distorted by surgical clamping and care needs to be taken with blocking and embedding to obtain a representative cross section. Levels or reembedding may be required.

Hydrocele wall: Weigh (g), measure (cm), and submit representative blocks. Note any contents, e.g., blood/fibrin.

33.9.2 Resection Specimens

Specimen:

- Most radical orchidectomy specimens are for malignant tumors, but the diagnosis usually cannot be definitively made preoperatively.

Initial procedure:

- Testes are sometimes received having been incised prior to receipt, and this may make assessment of invasion through the tunica albuginea difficult and accurate staging impossible. Urologists should be encouraged to send the intact testis to the laboratory rapidly to allow bisection by the pathologist.
- Some tumors spread to involve the cord, and this should be looked for and sampled prior to opening the testis to minimize the risk of contamination by tumor.
- The testis is incised in a plane that bisects the epididymis and rete testis such that invasion of these structures can be recognized (Fig. 33.3).
- Fix by immersion in 10% formalin for 24–36 h.
- Cuts parallel to the incised plane to examine the entire testis are then performed.
- Photograph tumor and individual slices if appropriate.
- Measurements:
  - Dimensions (cm) of testis and length (cm) of spermatic cord.
  - Weight of specimen in total (g).
  - Tumor – length × width × breadth or maximum dimension (cm).
- Identify different tumor appearances looking particularly for areas of hemorrhage and necrosis.

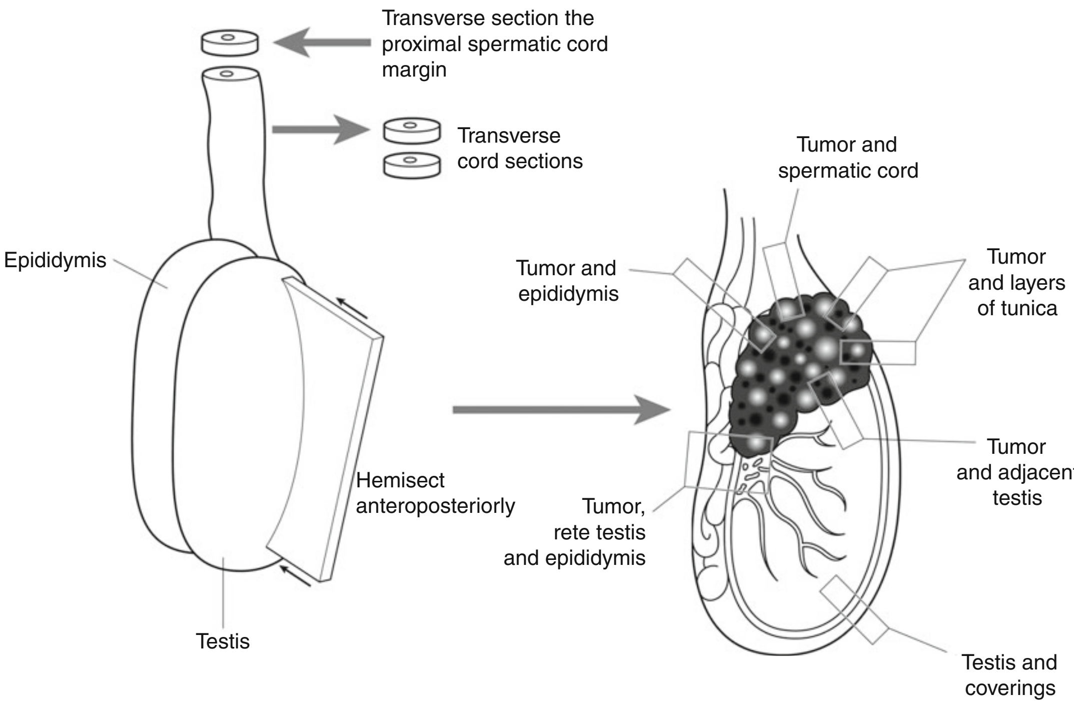

Fig. 33.3 Blocking of an orchidectomy specimen for tumor (Reproduced, with permission, from Allen and Cameron (2004))

- All areas of different macroscopic appearances should be sampled in order to identify all the histological patterns present (seminomatous versus NSGCT).
- Count and submit any lymph nodes with the main specimen.
- Examine the cord and surrounding tissue for abnormality.

Description:

- Tumor
  - Location (testis, rete, epididymis, tunica albuginea, spermatic cord)
  - Single/multifocal – mixed germ cell
  - Colour (pale/uniform – seminoma or lymphoma)
  - Necrosis (embryonal – MTU)
  - Hemorrhage (choriocarcinoma)
  - Cysts/cartilage (teratoma – MTD).
- Surrounding testis
  - Normal, scar, calcification.
- Other
  - Epididymal cysts, scarring, cord or paratesticular involvement, other lesions.

Blocks for histology (*Fig*. 33.3):

- All areas of different macroscopic appearances are sampled in order to identify all the histological patterns present.
- One block of tumor per centimeter diameter is taken.
- Submit tumor entirely if small (<2 cm).
- Sample areas demonstrating the relationship of tumor, rete, and epididymis, and of any area where tumor appears to invade these structures or the tunica (Fig. 33.3).
- Sample, where possible, adjacent non-tumorous tissue to look for vascular invasion.
- More samples may be required in pure seminoma to rule out other germ cell components, especially if there are areas of hemorrhage and necrosis or elevated serum AFP levels.
- Sample the surrounding normal testis to look for ITGCN and status of the normal testis, i.e., scar,

inflammation, regressional changes, other tumors, calcification.

- Sample a transverse section of the cut end of the spermatic cord and ideally this should be done before opening the testis to minimize the risk of contamination by tumor.
- Sample the cord in one or two other areas.
- Samples for special studies (e.g. electron microscopy, cytogenetics, molecular biologic studies, flow cytometry, image analysis).

Histopathology report:

- Tumor location: testis/rete/epididymis/cord involvement.
- Tumor type – seminomatous/non-seminomatous/sex cord-stromal tumor.
- Tumor classification (WHO/BTTP).
- Estimate percentage of each component for mixed tumors.
- Intratubular, invasive, or both.
- Extent of invasion:
  - Invasion or penetration of tunica albuginea (specify).
  - Involvement of paratesticular structures.

pT0	No evidence of primary tumor (i.e. scar in testis)
pTis	Intratubular germ cell neoplasia (carcinoma in situ)
pT1	Tumor involves testis and epididymis or tunica albuginea, no lymphovascular invasion
pT2	Tumor involves testis and epididymis or tunica vaginalis with lymphovascular invasion
pT3	Tumor invades spermatic cord ± lymphovascular invasion
pT4	Tumor invades scrotum ± lymphovascular invasion.

- Lymphatic/blood vessel invasion (specify if in testis or paratestis/spermatic cord).
- Regional lymph nodes – abdominal, periaortic, pericaval, and those along the spermatic veins. Generally, not removed at surgery but retroperitoneal lymph node dissection occasionally performed – usually following orchidectomy and chemo/radiotherapy.

pN0	No regional lymph node metastasis.
pN1	Regional lymph node metastasis ≤2 cm but ≤5 positive nodes.
pN2	Regional lymph node metastasis >2 cm but ≤5 cm or >5 positive nodes or extranodal extension.
pN3	Regional lymph node metastasis >5 cm.

- Other tissue(s) – involved/uninvolved by tumor.
- Results of special studies (immunohistochemistry) – AFP, HCG, PLAP, OCT3/4, CD117, SALL4, Glypican-3, podoplanin, SOX2, HPL, CD30, CAM 5.2, EMA (germ cell tumors), inhibin (sex cord stromal).
- Comments
  - Correlation with other specimens, as appropriate
  - Correlation with clinical information, as appropriate
  - Presence/absence of embryonal carcinoma, yolk sac tumor, and lymphovascular invasion are prognostically significant
- Resection margin(s) including spermatic cord.
- Additional pathologic findings, Leydig cell hyperplasia (correlated with HCG), scarring, the presence of hemosiderin-laden macrophages and intratubular calcification (tumor regression), testicular atrophy, and abnormal testicular development (e.g., dysgenesis).

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34 Penis

Declan M. O'Rourke and Derek C. Allen

34.1 Anatomy

The penis (Fig. 34.1) comprises the body or shaft and the two ends, anterior and posterior (root). The anterior portion is composed of the glans, coronal sulcus, and foreskin (prepuce). There is a vertical cleft, the meatus, in the apex 5 mm in length, and this is attached to the foreskin by a triangular piece of mucosa, known as the frenulum. The base of the cone is represented by the corona, an elevated ridge surrounding the glans. The coronal sulcus below the corona separates the glans from the foreskin.

The glans is composed of the following layers: epithelium, lamina propria, corpus spongiosum, tunica albuginea, and corpus cavernosum. The stratified epithelium is thin and nonkeratinized in uncircumcised males but keratinized in circumcised males. The lamina propria is loose, 1–4 mm thick, and separates the epithelium from the corpus spongiosum. The corpus spongiosum is the main component of the glans and consists of specialized erectile tissues with numerous anastomosing venous sinuses. It is 8–10 mm in thickness. The tunica albuginea is a very dense white fibrous membrane which terminates in or near the glans separating the corpus spongiosum from the corpora cavernosa and constitutes an important barrier to the spread of cancer to the latter. The coronal sulcus is a narrow and circumferential "cul de sac" located just below the glans corona. It is a common site for recurrence of carcinoma or of a positive margin in cases of foreskin carcinoma. The foreskin is a double membrane which encases the glans and from which it is separated by a potential space.

The shaft comprises three cylindrical masses of cavernous erectile tissue bound together by the fibrous tunica albuginea and encased in Buck's fascia. These cylinders are the ventral corpus spongiosum with a centrally located urethra and two corpora cavernosa separated by a median raphe.

The posterior part (root) of the penis is deeply embedded in the perineum. It is fixed to the anterior wall of the pelvis by a ligamentous insertion of the corpora cavernosa to the ischium and pelvic bones.

Lymphovascular drainage:

A rich network of lymphatics in the glans and corpora cavernosa courses along the dorsal vein and drains into superficial and deep inguinal lymph nodes. The foreskin and shaft skin lymphatics drain also to the superficial inguinal lymph nodes. The sentinel group of the superficial inguinal lymph nodes is the most common site for lymph node metastasis. Within the deep inguinal nodes, the node of Cloquet (highest) is significant as the next level of drainage is the iliac nodes.

D.M. O'Rourke (✉) • D.C. Allen
Histopathology Laboratory, Belfast City Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: declan.orourke@belfasttrust.hscni.net;
derek.allen@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_34, © Springer-Verlag London 2013

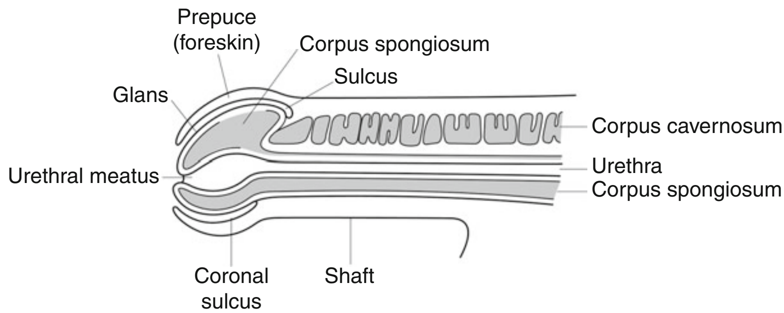

Fig. 34.1 Anatomy of the penis (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

34.2 Clinical Presentation

Infectious lesions present with an area of induration or erythema on the glans which is often painful and itchy. Biopsy or microbiological culture is usually confirmatory, and treatment is decided depending on the nature of the lesion.

Early symptoms of penile cancer include the appearance of a painless nodule, warty growth, or ulcer, especially on the glans or foreskin, or swelling at the end of the penis. Any abnormality including warts, blisters, sores, ulcers, white patches, rash, or bumps should be evaluated. Most penile cancers do not cause pain, but can result in ulceration and bleeding in later stages. Adult circumcision offers little or no protection but penile cancer is almost never observed in individuals who are circumcised in the neonatal period. A number of benign conditions, such as genital warts or infections, can have similar symptoms.

34.3 Clinical Investigations

- Full blood picture (low Hb).
- Biochemistry – raised calcium in bone metastasis in 20%.
- Swab if suspected local infection – candida etc.
- Chest X-ray.
- MRI and CT are useful for local cancer staging and for assessing the inguinal lymph nodes.
- PET/CT imaging may be helpful in assessing metastatic disease, but some studies have suggested limitations when disease is micrometastatic.
- Bone scan if indicated.

34.4 Pathological Conditions

34.4.1 Non-neoplastic Conditions

Paraphimosis: Forceful retraction of phimotic foreskin over the glans may cause marked swelling which blocks replacement of the foreskin. This is often painful and associated with constriction and urinary retention. Treatment consists of circumcision.

Phimosis: The foreskin orifice is too small to permit its retraction, usually due to scarring from repeated infection. Smegma (desquamated epithelial cells, sweat, debris) accumulates and causes secondary infections and possibly carcinoma.

Tumor-like/Inflammatory lesions

Balanitis circumscripta plasmacellularis (*Zoon's balanitis*): Occurs in uncircumcised men with an unknown etiology (possibly autoimmune). It consists grossly of well-defined brown/red plaques, solitary or multiple, and clinically resembles erythroplasia of Queyrat.

Balanitis xerotica obliterans (*BXO*): This is the male equivalent of lichen sclerosus et atrophicus of vulva. It may cause narrowing of the urethral meatus or phimosis. There is a weak association with carcinoma of the foreskin. The gross appearances are that of gray-white foci of atrophy in the foreskin or perimeatal glans.

Balanoposthitis: Infection of the glans and foreskin, usually due to candida, anaerobes, gardnerella, or pyogenic bacteria. It is common in uncircumcised newborns or uncircumcised men with poor hygiene and accumulation of smegma and due to the propensity of pathogenic bacteria to adhere to the mucosal surface of the foreskin. It causes phimosis.

Peyronie'*s disease*: Fibrous dermal and fascial thickening causing curvature toward the side of the lesion and restricting movement during erection. There is an association with Dupuytren's contracture and it is considered a form of fibromatosis. It may regress spontaneously but responds to small amounts of irradiation, steroids, and excision.

Fournier'*s gangrene*: Necrotizing fasciitis of the genitalia due to bacterial infection. Risk factors include trauma, burns, anorectal disease, diabetes, leukemia, and alcoholic cirrhosis.

Nicorandil-induced penile ulceration: Several reports in the literature have implicated nicorandil as a cause of penile ulceration. This is essentially a diagnosis of exclusion, and biopsy is necessary to rule out malignancy. The ulcers are located on the foreskin or shaft and characteristically form a deep, punched out, and well-circumscribed lesion. Histology shows granulation tissue and acute inflammatory changes with occasional granulomas.

Sexually transmitted disease: These include granuloma inguinale (Calymmatobacterium granulomatis), herpes simplex virus, lymphogranuloma venereum (Chlamydia trachomatis), candida, molluscum contagiosum, scabies, and syphilis.

Others: Inflammatory pseudotumor, lentiginous melanosis, papillomatosis of glans corona, penile cyst, pseudoepitheliomatous keratotic and micaceous balanitis, and verruciform xanthoma.

34.4.2 Neoplastic Conditions

Condyloma accuminatum: Benign tumor caused by HPV 6 or 11 and related to verruca vulgaris (common wart). It is usually sexually transmitted and seen near the coronal sulcus and inner surface of the foreskin. It has a propensity for recurrence but does not evolve into invasive cancer. The gross features are of papillary, wart-like, often multiple lesions, 1 mm or larger. Treatment involves local preparations (podophyllin).

Giant condyloma accuminatum: Very rare, benign, exophytic papillary growth of penis (Buschke-Löwenstein tumor). Grossly usually involves the foreskin and coronal sulcus, 5–10-cm cauliflower-like verruciform tumor. Histology resembles that of a condyloma.

Penile intraepithelial neoplasia (*PeIN*): Synonymous with erythroplasia of Queyrat, Bowen's disease, squamous cell carcinoma in situ, and squamous intraepithelial lesion (SIL). Differentiated (simplex) PeIN is unrelated to HPV. It is seen in older patients, frequently affects foreskin, and usually arises in a setting of chronic inflammation/scarring. It comprises thickened epithelium with atypical basal cells and hyperchromatic nuclei. Basaloid (undifferentiated), warty, or mixed warty/basaloid PeIN is seen in younger patients, usually affects the glans and perimeatal region, and there may be a history of condyloma. It is HPV related, especially HPV 16. Treatment of PeIN includes local excision/Mohs' excision with reconstructive surgery, laser therapy, electrodessication and curettage, cryosurgery, and topical 5-fluorouracil.

Bowen'*s disease*: Clinical designation of carcinoma in situ located on the shaft skin.

Erythroplasia of Queyrat: Clinical designation of carcinoma in situ located on the glans, usually erythematous.

Bowenoid papulosis: Multifocal HPV-related papular condition affecting the anogenital region in young adults with benign looking papules of the penile shaft skin. It may also affect the glans and coronal sulcus. It is often related to HPV 16, and histology shows atypical basaloid and koilocytic cells either singly or involving the full thickness of the epithelium. A minority of lesions evolve to invasive squamous cell carcinoma.

Extramammary Paget'*s disease* (*EMPD*): Presents as erythematous patches on the glans. It consists of round large pale cells in all levels of the epidermis which in primary EMPD are positive for CK7 and negative for CK20. In secondary EMPD, they have a more variable immunohistochemical profile and more often associated with urothelial carcinoma (CK7 and CK20 positive).

Squamous cell carcinoma of penis: This is relatively rare in the UK (<1% of carcinomas in men versus 10–20% in Asia, Africa, South America), affecting ages 40–70 years. It is particularly rare with early circumcision (at birth). There is an association with paraphimosis, phimosis, HPV 16, smoking, psoriasis, and patients treated with UVB radiation. One third of non-HPV cases are associated with balanitis xerotica obliterans.

Most tumors arise from the glans or inner foreskin near the coronal sulcus as a slowly growing, irregular mass. Patients occasionally present with inguinal nodal metastases and occult penile cancer due to severe phimosis or a very small primary tumor. Metastases to inguinal lymph nodes, lung, liver, or bone occur and are present in 15% of cases at diagnosis. Lymph nodes may be enlarged at clinical presentation due to infection alone.

The gross appearance is papillary or flat (ulcerated papule). The cut surface shows a white solid irregular tumor with superficial or deep penetration. The microscopy is classified according to growth patterns as superficial spreading, vertical growth, verruciform, multicentric, or mixed. They are graded on differentiation as well, moderate or poor (G1,G2,G3) depending on the extent of keratinization. Tumor grading should be performed considering the highest grade component regardless of its proportion. Undifferentiated carcinomas are rare. Most cases have associated carcinoma in situ and/or squamous hyperplasia.

Prognosis: Dependent on histologic grade, nodal status, and depth of penetration into the various anatomical compartments. Poor prognostic factors are lymphovascular invasion, vertical growth pattern, basaloid, sarcomatoid, solid, anaplastic, and pseudoglandular subtypes. A Prognostic Index is useful for allocation of patients into risk groups for inguinal metastases and survival. The histological grade, involved anatomical levels, and perineural invasion allow a designated score to be assigned guiding subsequent therapy. The average 5-year survival is 70–80%.

Basaloid carcinoma: An aggressive high-grade and deeply invasive tumor in which 50% have enlarged inguinal nodes (due to metastasis) at diagnosis. It is usually associated with HPV and represents 5–10% of penile cancers.

Sarcomatoid carcinoma: Is a rare, aggressive, large tumor with a predominance of anaplastic spindle cells. It usually involves the glans and there are frequent recurrences due to inadequate surgery.

Verrucous carcinoma: This is a slow-growing, extremely well-differentiated variant of squamous cell carcinoma (5–10%) with low malignant potential. It is locally invasive, one third recurs (inadequate surgery or multifocal tumor) but rarely/never metastasizes. It is associated with HPV 6 and 11. The tumor involves all penile compartments (glans most common) and penetrates through lamina propria with a broad base and pushing borders. It is prone to local recurrence if incompletely excised and may dedifferentiate with radiotherapy.

Papillary carcinoma: Low-grade malignant tumor representing 5–15% of all penile squamous cell carcinomas with a typical exophytic pattern of growth. It is frequently associated with lichen sclerosus and usually not HPV related. It is less aggressive than usual squamous cell carcinoma and well differentiated with architecturally complex papillae and an irregular jagged base.

Warty carcinoma: Large, slow-growing cauliflower-like tumor with cobblestone appearance usually affecting the glans. It is HPV related in the majority of cases (HPV 16) and represents 5–10% of penile squamous cell carcinomas. The prognosis is intermediate between that of low-grade verruciform tumors and squamous cell carcinoma of usual type. It may be associated with inguinal nodal metastasis.

Pseudohyperplastic carcinoma: Rare tumor involving foreskin and strong association with lichen sclerosus et atrophicus. It can be multifocal but has an excellent prognosis.

Metastatic tumors: Bladder urothelial carcinoma and prostatic adenocarcinoma account for most cases, with the corpora cavernosa the most frequently affected site.

Malignant melanoma: Is the most common tumor after squamous cell carcinoma, but is still rare (<1%). It is similar to melanoma at other sites but shows propensity for lymph node spread (50%).

Sarcomas: Extremely rare but include Kaposi's sarcoma, leiomyosarcoma, epithelioid sarcoma, and rhabdomyosarcoma.

Others: Nevi and melanocytic proliferations, hemangioma, glomus tumor, angiokeratoma, fibrous histiocytoma, neurofibroma, granular cell tumor, myointimoma, and leiomyoma.

34.5 Surgical Pathology Specimens: Clinical Aspects

34.5.1 Biopsy Specimens

Macules, papules, nodules, and ulcers from the glans are biopsied to exclude neoplasia or confirm the diagnosis particularly if these lesions have been long-standing. Specimens are either punch biopsies (3–5 mm) or excision skin ellipses.

Circumcision specimens consisting of the foreskin are removed more often in the context of benign penile conditions (BXO, Zoon's, phimosis, and paraphimosis). Occasionally a small cancer is removed in this fashion, and margins in this case will be important. These are dealt with below.

For carcinoma in situ of the glans with or without adjacent skin involvement, therapeutic options include local applications of fluorouracil cream, microscopically controlled surgery, cryosurgery, electrofulguration, and laser ablation for smaller lesions. Wide local excision with circumcision may be adequate therapy for control of lesions limited to the foreskin.

34.5.2 Resection Specimens

The goal of treatment in invasive penile carcinoma is complete excision with adequate margins. For lesions involving the prepuce, this may be accomplished with simple circumcision. For infiltrating tumors of the glans, with or without involvement of the adjacent skin, the choice of therapy is dictated by tumor size, extent of infiltration, and degree of tumor destruction of normal tissue. The options include penile amputation (partial or total penectomy) and irradiation. Stage I and II penile cancer is most frequently managed by penile amputation for local control. Whether the amputation is partial, total, or radical will depend on the extent and location of the neoplasm. Radiation therapy with surgical salvage is an alternative approach. There is no standard treatment which is curative for stage IV penile cancer. Therapy is directed at palliation, which may be achieved either with surgery or radiation therapy.

34.5.2.1 Glansectomy

This procedure involves removing the foreskin and glans and although not commonly performed, is indicated for localized tumors and carcinoma in situ of the glans. There is a higher risk of incomplete removal and therefore tumor recurrence.

34.5.2.2 Partial Penectomy

Successful local control by partial penectomy depends on division of the penis 2 cm proximal to the gross tumor extent. During the operation, the skin is incised circumferentially and the cavernous bodies are divided sharply to the urethra. The dorsal vessels are then ligated and the urethra is dissected proximally and distally to attain a 1-cm redundancy. After a dorsal urethrotomy, a skin to urethra anastomosis is performed and the redundant skin approximated dorsally to complete the closure.

34.5.2.3 Modified Partial Penectomy

When the penile stump after partial penectomy is too short for directing the urinary stream, releasing the corpora from the suspensory ligament, dividing the ischiocavernosus muscle, and partially separating the crura from the pubic rami can obtain further length. The scrotum is incised and skin flaps fashioned for penile coverage.

34.5.2.4 Total Penectomy

If the size/site precludes partial penectomy, then as part of penile amputation the proximal urethra is dissected and transposed to the perineum with an indwelling catheter placed for an adequate urinary stream.

34.5.2.5 Radical Surgery

This is rarely performed but involves penectomy including removal of the scrotum, testes, spermatic cords, and ilioinguinal lymph node dissection.

34.5.2.6 Ilioinguinal Lymph Node Dissection

Inguinal lymphadenopathy in patients with penile cancer is common but may be the result of infection rather than neoplasm. If palpable enlarged lymph nodes persist 3 or more weeks after removal of the infected primary lesion and a course of antibiotic therapy, lymphadenectomy should be considered.

In cases of proven regional inguinal lymph node metastasis (fine needle aspiration cytology or biopsy) without evidence of distant spread, bilateral ilioinguinal dissection is the treatment of choice. Radiation therapy may be an alternative in patients who are not surgical candidates. Postoperative irradiation can decrease the incidence of inguinal recurrences. Because of the high incidence of microscopic node metastases, elective adjunctive inguinal dissection of clinically uninvolved (negative) lymph nodes in conjunction with amputation is often used for patients with poorly differentiated tumors. PET/CT may be useful in case selection but there are limitations with micrometastatic disease. However, lymphadenectomy can carry substantial morbidity, such as infection, skin necrosis, wound breakdown, chronic edema, and even a low, but finite, mortality rate. The impact of prophylactic lymphadenectomy on survival is not known. For these reasons, there are varying opinions on its use.

Few histopathologists in the UK have had the opportunity to see and deal with large numbers of penile tumors. The Urology Cancer Improving Outcome Guidance proposed the setting up of dedicated multidisciplinary teams in Supraregional Penile Cancer Centres serving a population base of four million or more and expecting to manage a minimum of 25 new patients each year. Developments include local excision with organ preservation for low-grade, early-stage tumors. The surgical margins are often close and checked by intraoperative frozen section.

34.6 Surgical Pathology Specimens: Laboratory Protocols

34.6.1 Biopsy Specimens

Diagnostic punch and incisional biopsies: Count, measure (mm), process intact, and cut through three levels. PAS stain for fungi if suspected.

Elliptical excisions: Measure (mm), ink the deep and lateral (circumferential) margins, and cut into multiple transverse serial slices.

Foreskin

- Measure, inspect, and orientate.
- Ideally pin the four corners of the specimen with the mucosa oriented on one side and the skin on the other.
- Identify the coronal sulcus and ink the mucosal and cutaneous margins of resection with different colors.
- Fix the specimen in 10% buffered formalin overnight.
- Specimen photography may be necessary.
- Cut serial transverse sections (to its longest axis) clockwise.
- Include any obvious areas of surface scarring or raised lesions.
- Submit entirely if <3–4 cm and if greater, sample at least one block/cm.

34.6.2 Resection Specimens

34.6.2.1 Penectomy (Partial/Total) and Glansectomy

Initial procedure:

The various anatomical components of the penis should be examined as any of these may be the site of involvement.

- Photograph the specimen, before and after longitudinal sectioning, with emphasis on tumor invasion of the various anatomic levels.
- Fix in 10% formalin for 24–36 h.
- Measurements:
  – Dimensions (cm) of the specimen and individual components (foreskin, shaft, and glans).
  – Tumor
    Length × width × depth (cm) or maximum dimension (cm)
    Distances (cm) to the urethral and surgical resection margins
- Identify the shaft and glans.
- Remove the foreskin, leaving a 2–3-mm redundant edge of skin around the sulcus. This permits better evaluation of the coronal sulcus. Proceed with foreskin as above. If the foreskin is affected by tumor, do not remove.
- Ideal sectioning is longitudinal, centered along the urethra, with additional parallel sections on both sides. With a probe as a guide, the urethra is opened along the ventral aspect where it is closest to the surface and the cut is then continued to bisect the penis.
- Involvement of the foreskin, frenulum, glans, meatus, corpora cavernosa, urethra, and corpus spongiosum is recorded.
- A transverse section of the urethral margin should include the mucosal surface, surrounding lamina propria, and corpus spongiosum. This is usually long in partial penectomy specimens because the surgical technique uses a long urethra stump for reconstruction.
- Shaft margin: usually a large specimen. Divide it in two, from dorsal to ventral along the central septum, and submit the cut surface entirely. Each half should be labeled left or right. If the specimen has a long shaft, cut two or three additional sections distal to the margin.
- Examination of the cut surface of the glans represents the best approach for surgical pathology evaluation.
- **Glans (glansectomy):** several longitudinal sections should be taken. Cut the specimen into two halves labeling them left and right, going from meatus to proximal urethra. Cut three to six serial sections, 2–3 mm in width from each half.
- Photograph suitable individual slices.
- If accompanying lymphadenectomy specimens, fix in 10% buffered formalin, preferably overnight.
- Identify the number and size of all lymph nodes.
- If orientated, record the location of lymph nodes as upper inner quadrant, superficial, and deep inguinal nodes.
- Submit all lymph nodes for histological examination.

Description:

- Tumor
  – Site (urethral meatus/glans/prepuce/coronal sulcus/shaft – dorsal, ventral, lateral)
  – Single/multifocal
  – Appearance (verrucous/warty/exophytic/sessile/ulcerated)
  – Edge (circumscribed/irregular)
- Foreskin
  – Ulcerated/thickened/papule/warty
- Glans
  – Erythematous/ulcerated/macule/papule/warty
- Others
  – BXO, scars of previous surgery/biopsy

Blocks for histology (Fig. 34.2):

- Shave section from the shaft margin (including skin, erectile bodies, and urethra).
- Samples of foreskin to include associated conditions.
- Sample four sections of tumor to demonstrate depth of invasion and relationships to the adjacent surface epithelium, corpora cavernosa, corpus spongiosum, and urethra.
- Sample two to three transverse sections through the shaft at different levels.
- Sample longitudinal sections through the glans to include the urethra.
- In larger specimens, it is important to submit two to three additional sections of the more distal urethral cylinder to ensure adequacy of the resection margin.
- Count and sample all lymph nodes accompanying the specimen.

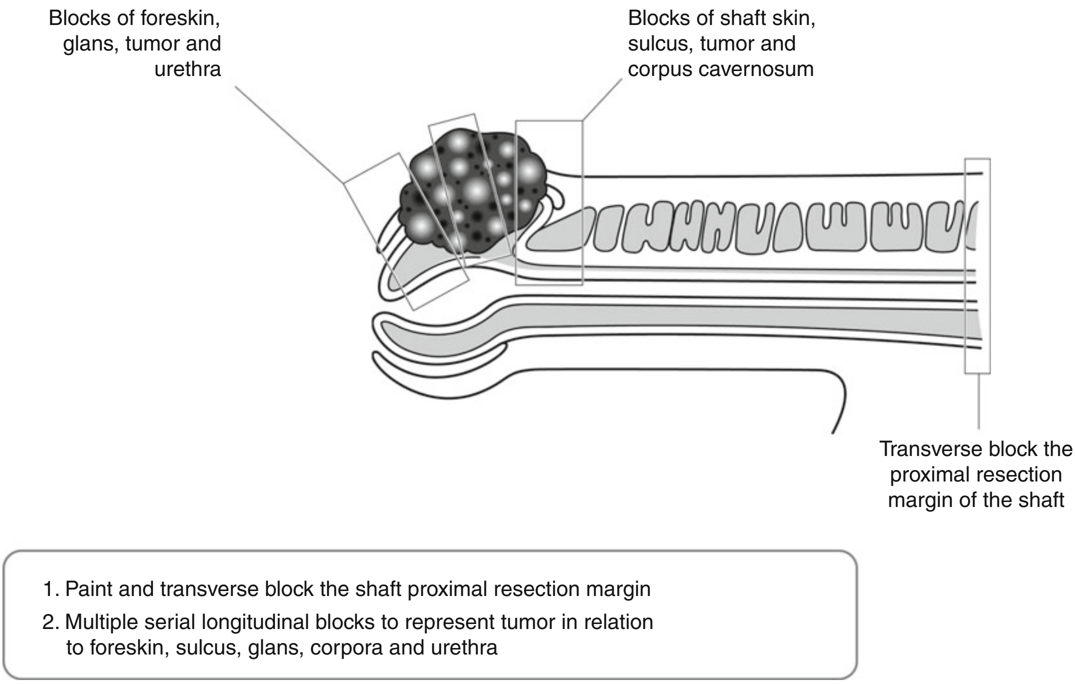

Fig. 34.2 Blocking a penectomy specimen (Reproduced, with permission, from Allen and Cameron (2004))

Histopathology report:

- Tumor site (urethra, foreskin, glans, shaft)
- Tumor size and depth (mm)
- Patterns of growth and histological type
- Tumor grade (well, moderately, poorly differentiated, or verrucous)
- Tumor extension: subepithelial connective tissue, tunica albuginea, corpus spongiosum, corpus cavernosum, urethra
- In situ component (present/absent/extent, multifocal)

  TNM Staging

pT0	No evidence of primary tumor
PTis	Carcinoma in situ
PTa	Noninvasive verrucous carcinoma
pT1a	Tumor invades subepithelial connective tissue without lymphovascular invasion and is not poorly differentiated
pT1b	Tumor invades subepithelial connective tissue with lymphovascular invasion or is poorly differentiated
pT2	Tumor invades corpus spongiosum or cavernosum
pT3	Tumor invades urethra
pT4	Tumor invades other adjacent structures

- Lymphovascular space invasion (present/absent).
- Perineural space invasion (present/absent).
- Regional lymph nodes

  These are the superficial and deep inguinal nodes and the pelvic nodes

pN0	No regional lymph node metastasis
pN1	Metastasis in a single inguinal lymph node
pN2	Metastasis in multiple or bilateral inguinal lymph nodes
pN3	Extranodal extension of lymph node metastasis or pelvic lymph node(s) unilateral or bilateral

- Excision margins: urethra, corpora, skin – distances (mm)
- Ancillary studies: immunohistochemistry and molecular studies for HPV typing
- Prognostic index: histological grade/anatomical level and perineural invasion
- Other pathology: status of nonneoplastic epithelium (PeIN, squamous hyperplasia, condyloma, BXO, Zoon's, inflammatory process)

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Part VII

Pelvic and Retroperitoneal Specimens

35 Pelvic Exenteration Specimens

Maurice B. Loughrey and Damian T. McManus

35.1 Anatomy

The relevant anatomy is discussed in other sections pertaining to the various organs in which cancers originate. Specimens may be classified into one of three groups (Fig. 35.1):

Anterior pelvic exenteration: Bladder, lower ureters, reproductive organs, draining lymph nodes, and pelvic peritoneum.

Posterior pelvic exenteration: Rectum, distal colon, internal reproductive organs, draining lymph nodes, and peritoneum. Such procedures are also known as composite resections.

Total pelvic exenteration: Bladder, lower ureters, rectum, distal colon, reproductive organs, draining lymph nodes, and peritoneum.

35.2 Clinical Presentation

Pelvic exenteration is performed for locally advanced or recurrent malignant tumors within the pelvis. Whilst locally advanced (stage IV) cervical carcinoma was formerly the commonest indication, this is now much rarer as a result of earlier detection of cervical cancer by screening programs. Exenteration is now performed with increasing frequency for pelvic recurrence of rectal adenocarcinoma or anal carcinoma. Patients undergoing this procedure for these cancers will often have been treated by radiotherapy pre-operatively.

More detailed discussion of the symptoms and clinical signs of the various malignant tumors that might result in a pelvic exenteration are found in the relevant chapters relating to gastrointestinal tract and gynecological specimens. Locally advanced malignancies may produce fistula between viscera such as the rectum and vagina. Specific symptoms and signs result, e.g., fistula between the rectum and urinary bladder may result in pneumaturia (gas bubbles in the urine) and contamination of the urine by feces (fecaluria).

35.3 Clinical Investigations

Exenterations are performed for advanced or recurrent pelvic malignancy in the absence of extra-pelvic metastatic spread. Patients will usually have been staged by one or more radiological techniques:

- *CT scanning*: This is particularly useful in the evaluation of pelvic and retroperitoneal lymphadenopathy and metastatic disease outside the pelvis. Magnetic resonance imaging has largely replaced CT scanning in the evaluation of the T stage of cervical, endometrial, and

M.B. Loughrey (✉)
Histopathology Laboratory, Institute of Pathology,
Royal Victoria Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: maurice.loughrey@belfasttrust.hscni.net

D.T. McManus
Histopathology Laboratory, Belfast City Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: damian.mcmanus@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_35, © Springer-Verlag London 2013

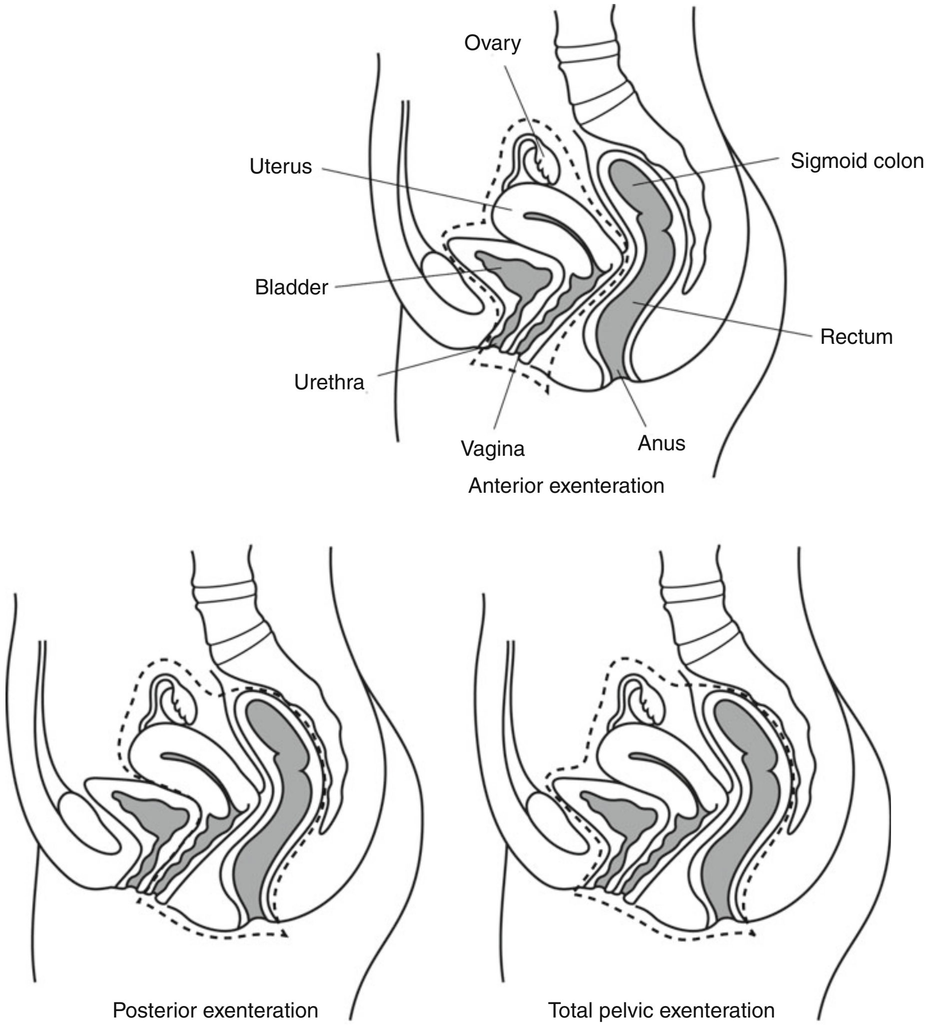

Fig. 35.1 Pelvic exenterations (Reproduced, with permission, from Allen and Cameron (2004))

rectal tumors. Pelvic exenteration is a major surgical procedure and carries with it considerable morbidity and mortality. Although occasionally it might be performed as a palliative procedure, it is contraindicated if there is evidence of widespread distant metastases.

- *Magnetic resonance imaging* (*MRI*): This is used to clinically stage cervical, endometrial, and rectal cancers pre-operatively.
- *Positron emission tomography* (*PET scanning*): PET scanning detects metabolic activity in malignant tumors and in combination with CT (CT-PET) is particularly useful in identification and localization of recurrent or metastatic disease.

35.4 Pathological Conditions

Pelvic exenteration may be performed for:

- Advanced stage IV cervical carcinoma.
- Locally advanced rectal adenocarcinoma.
- Recurrent cervical, rectal, or anal carcinoma with no evidence of distant metastasis.
- Certain sarcomas or locally invasive tumors such as aggressive angiomyxoma. The pelvis is a common site of such tumors, which may be associated with advanced pelvic disease without distant metastasis elsewhere.
- Pelvic exenteration may be used occasionally for advanced endometrial adenocarcinoma with involvement of the vagina but is

generally not recommended in ovarian carcinoma as there is usually peritoneal disease outside the pelvis. Advanced vaginal or vulval squamous carcinoma with involvement of the rectum or urinary bladder may rarely be treated by pelvic exenteration but such locally advanced disease is frequently accompanied by pelvic side wall involvement or nodal metastasis.
- Aggressive muscle invasive transitional carcinoma can be treated by cystoprostatectomy or variants of pelvic exenteration. Prostatic carcinoma may be treated by radical prostatectomy in certain circumstances, but pelvic exenteration has no role in the management of locally advanced prostatic carcinoma, as such disease is almost invariably accompanied by distant metastatic spread.

35.5 Surgical Pathology Specimens: Clinical Aspects

Advanced (stage IV) cervical carcinoma is usually treated pre-operatively by radiotherapy or neoadjuvant chemotherapy with radiotherapy. Locally advanced rectal cancer may be treated by long course or short course (chemo-)radiotherapy to downstage tumors prior to resection. Cervical carcinoma can show an excellent response to radical radiotherapy treatment, and it is not uncommon to find no evidence of residual disease. There can be a similar downstaging of rectal carcinoma, in some instances obviating the need for composite resection, and macroscopically the tumor may only be represented by a small area of ulceration. Similarly local lymph nodes hyalinize becoming difficult to identify and harvest.

Contraindications to pelvic exenteration include significant comorbidity or distant metastatic disease (except perhaps for isolated resectable liver metastasis from rectal carcinoma). Involvement of major pelvic vessels or nerves by carcinoma is generally felt to represent a contraindication to surgery for carcinomas but not necessarily sarcomas. Involvement of pelvic side walls or sacral bone are also relative contraindications, although *en bloc* resection of the sacrum can be used for locally advanced primary and recurrent rectal carcinoma.

35.6 Surgical Pathology Specimens: Laboratory Protocols

A general protocol is described. This can be modified according to the type of specimen received (anterior, posterior, or total pelvic exenteration) and the primary site of the tumor.

Specimen:
- Anterior exenteration
- Posterior exenteration/composite resection
- Total pelvic exenteration

Initial procedure:
- Identify and measure each organ that is present in the resection.
- Identify and take a limit block from:
  - Ureters.
  - Urethra.
  - Vagina.
  - Proximal and distal bowel.
  - Painted circumferential or radial fascial margins and serosal surface of rectum above peritoneal reflection if present. The plane of the mesorectal excision should also be graded for advanced rectal tumors as discussed in Chap. 6.
- Fixation can be problematic in such a large specimen. The bladder can be inflated with formalin from without. The bowel may be partially opened avoiding disruption of the serosa and radial margin in the vicinity of the tumor.
- After fixation, the specimen can be bisected in the sagittal plane to give roughly equal left and right halves. This should allow visualization of the anatomical relationship between cervical tumors and the rectum posteriorly, the vagina inferiorly, and the urinary bladder anteriorly. Rectal tumors may spread anteriorly to involve the vagina and also the urinary bladder. Fistulae may be present and can be demonstrated by exploration with a probe.

Description:
- List the organs present and dimensions (cm).
- Presence or absence of tumor.
- Site of tumor.

- Size (cm) of tumor.
- Extent of tumor; relationship of tumor to adjacent organs and resection margins.
- Fistulous tracts involving tumor or perforation.
- Describe anatomical location, size, and number of harvested lymph nodes.
- List of any separately submitted lymph node groups.
- Other pathology, e.g., dilatation of ureter.

Blocks for histology:

- It may be helpful to use a labeled digital photograph or diagram such as Fig. 35.2 to identify the origin of blocks for histology.
- Longitudinal limit blocks of colon, rectum, ureters, urethra, and distal vagina.
- The remaining blocks taken for histology depend on the type and origin of the tumor if a tumor can be identified:

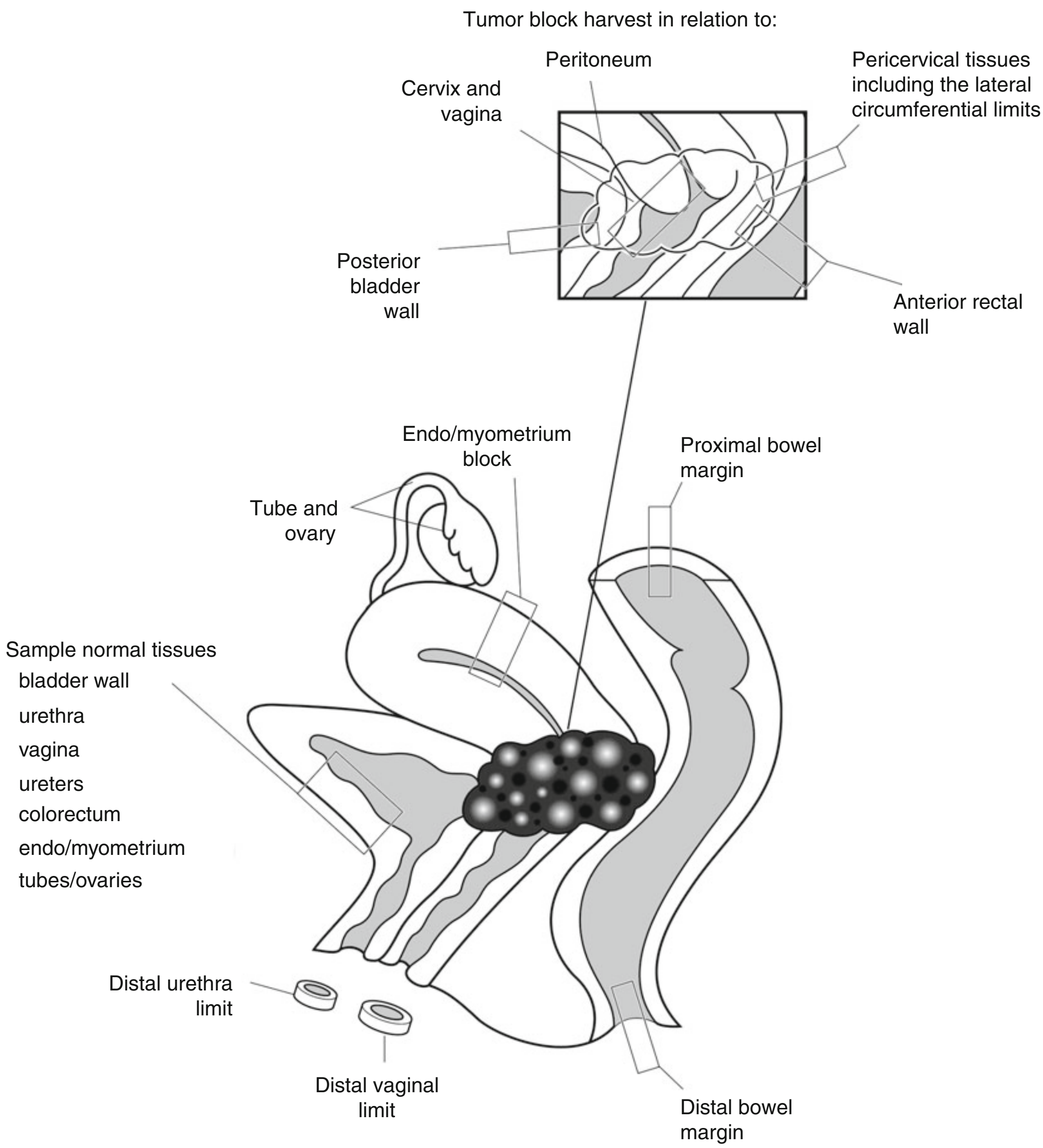

Fig. 35.2 Blocking of total pelvic exenteration specimen for a pT4 cervical carcinoma (Reproduced, with permission, from Allen and Cameron (2004))

Cervical carcinoma

- Blocks of tumor and tumor in relation to:
  - Anterior rectal wall.
  - Posterior bladder wall.
  - Vagina.
  - Pericervical tissues with lateral circumferential margins.
  - Ureters.
  - Peritoneum.
  - If no cervical tumor is apparent post-radiotherapy, then the cervix must be blocked to identify residual disease histologically. It may be "clockfaced" submitting the entire cervix for histology, or more pragmatically, four quadrants taken from the transformation zone.
- Representative sections are also submitted from non-neoplastic tissues:
  - Bladder wall
  - Urethra
  - Ureters
  - Vagina
  - Endometrium/myometrium
  - Fallopian tubes and ovaries
  - Colorectum
- Pelvic lymph node dissections will often be submitted separately and individually labeled and are described later.

Rectal carcinoma

- Blocks of tumor and tumor in relation to:
  - Mucosa
  - Posterior vaginal wall
  - Dome of urinary bladder
  - Prostate and seminal vesicles (in males)
  - Circumferential margin of mesorectum
  - Peritoneum
- The mesorectal fat must be dissected to identify lymph nodes.
- Representative sections of non-neoplastic tissues are also submitted.

Histopathology report:

- The specific features that should be included in pathology reports of cervical, rectal cancers, and soft tissue tumors are detailed in other relevant chapters. The purpose of pelvic exenteration is one of complete local excision, and in view of this, the status of longitudinal and circumferential resection margins and peritoneum in relation to the tumor must be documented.

Sarcomas and tumor recurrence in soft tissues

- The dissection and precise blocking protocol must be adapted to suit the individual specimen.
- In general terms, blocks should be taken from tumor (1/cm), tumor and involved pelvic structures, tumor and resection margins, and representative blocks of uninvolved structures.
- Tumor stage; use the TNM system for soft tissue sarcomas where pelvis is a specific topographical site and pelvic tumors are classified as deep tumors.

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Retroperitoneum

36

Maurice B. Loughrey and Damian T. McManus

36.1 Anatomy

The retroperitoneal space may be defined as that part of the lumboiliac region, which is bounded anteriorly by the parietal peritoneum, posteriorly by the posterior abdominal wall, superiorly by the 12th rib and vertebra, and inferiorly by the iliac crest and the base of the sacrum. The lateral borders are formed by the quadratus lumborum muscles.

This space contains the kidneys, adrenal glands, ureters, aorta and inferior vena cava and their tributaries, and many lymph nodes. Numerous nerves, the lumbosacral nerve plexus and ganglia from both the sympathetic and parasympathetic autonomic nervous system, are also present.

The retroperitoneal and pelvic lymph nodes are found around the aorta and its branches and may be divided into the following groups (Fig. 36.1):

1. Para-aortic
2. Inferior mesenteric
3. Common iliac
4. Internal iliac
5. External iliac
6. Superficial and deep inguinal
7. Sacral
8. Pararectal

The testes drain to the para-aortic lymph nodes and the prostate to the sacral and internal iliac nodes. The uterus drains to the external and common iliac nodes.

The genitourinary system is considered in detail in Chaps. 29–34. This section will consider tumors of the retroperitoneum and retroperitoneal lymph node dissections. Diseases of the adrenal gland are discussed in Chap. 37.

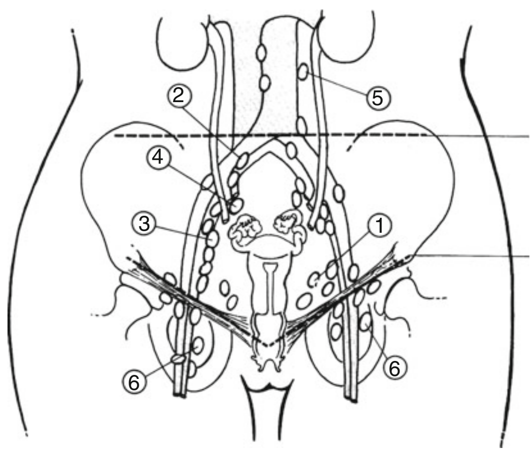

Fig. 36.1 Retroperitoneal and pelvic lymph nodes. (*1*) Hypogastric (internal iliac); (*2*) common iliac; (*3*) external iliac; (*4*) lateral sacral; (*5*) para-aortic; (*6*) inguinal (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

M.B. Loughrey (✉)
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: maurice.loug1hrey@belfasttrust.hscni.net

D.T. McManus
Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: damian.mcmanus@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_36, © Springer-Verlag London 2013

36.2 Clinical Presentation

The retroperitoneum is rather inaccessible, and because of its anatomical location, tumors can grow to a large size before becoming clinically apparent. Symptoms and signs of a retroperitoneal tumor may be vague and only manifest late in the course of the disease because of obstruction/displacement of adjacent structures such as the ureter.

36.3 Clinical Investigations

Plain abdominal X-ray, barium enema, or an intravenous pyelogram may suggest the presence of a retroperitoneal tumor due to distortion of normal structures. However, the investigations of choice are ultrasonography, CT scanning, or magnetic resonance imaging (MRI). Arteriography may also be useful, particularly if resection of a large tumor is contemplated. Historically, lymphangiography was the investigation of choice in the evaluation of lymphadenopathy in the retroperitoneum. Now CT scanning is more commonly performed. Whilst CT is very good at detecting large nodal masses associated with malignant lymphoma, it is less effective in the assessment of metastatic disease to the pelvic and retroperitoneal nodes. The status of the pelvic and retroperitoneal lymph nodes is particularly important in patients with stage 1 non-seminomatous malignant germ cell tumors of the testis for reasons discussed later, and positron emission tomography (PET) scanning can occasionally give supplementary information when the CT scan is equivocal.

36.4 Pathological Conditions

A variety of tumors, both benign and malignant may arise within the retroperitoneum. The commoner lesions are discussed here.

Liposarcoma:

Arising within the retroperitoneal fat, well-differentiated liposarcoma, particularly the sclerosing variant, represents one of the commonest histological subtypes. There is a very low risk of metastasis with this type of lesion, which indeed has been described as an atypical lipoma at other sites. However, such lesions can be difficult to excise from the retroperitoneum and may prove lethal because of pressure effects on adjacent organs or local recurrence if incompletely excised. These tumors may also contain or develop a dedifferentiated spindle cell component, resembling a high-grade spindle cell sarcoma. Careful macroscopic examination and adequate sampling are important. Lipomas also arise at this site; careful evaluation is necessary to distinguish from well-differentiated liposarcoma as described above.

Other sarcomas:

These include malignant fibrous histiocytoma and leiomyosarcoma. Careful consideration must be given before rendering a diagnosis of malignant fibrous histiocytoma as it is increasingly recognized that a proportion of such lesions represent anaplastic forms of other malignant tumors. At this site, lesions such as sarcomatoid renal cell carcinoma should also be excluded. Both benign and malignant smooth muscle tumors may occur. Leiomyoma is reported as very rare and is to be distinguished from leiomyosarcoma and renal angiomyolipoma. Leiomyosarcoma may arise from the wall of the inferior vena cava or its tributaries.

Peripheral nerve tumors:

Relatively common at this site, although not as frequent as in the mediastinum. Schwannomas may be quite large and show cystic degeneration; neurofibromas and malignant peripheral nerve sheath tumors are also described. Rarer tumors include lesions such as paraganglioma (chemodectoma/aortic body tumor), ganglioneuroma, neuroblastoma, and other small round blue cell tumors such as Ewing's sarcoma/PNET and intraabdominal desmoplastic small cell tumor.

Solitary fibrous tumor, hemangiopericytoma and carcinoid tumors:

Although hemangiopericytoma-like areas may be seen in various soft tissue tumors, the retroperitoneum remains a typical site for true hemangiopericytomas in middle-aged female patients. The histogenesis of retroperitoneal carcinoid tumors is uncertain. Some may represent a form of germ cell tumor, which also occur at this site either as primary tumors or more commonly as lymph node metastasis from a testicular or ovarian primary.

Malignant lymphoma and metastatic disease: Lymphoma may involve the retroperitoneal lymph nodes and lead to massive enlargement. Diffuse large B cell lymphoma and follicle center cell lymphomas are among the commonest. Pelvic and retroperitoneal nodes are a common site for metastatic disease from malignant germ cell tumors of the testis, prostatic carcinoma, or gynecological malignancy.

Miscellaneous:

Abdominal aortic aneurysms are only rarely biopsied. Idiopathic retroperitoneal fibrosis is an uncommon reactive, inflammatory condition that may simulate a tumor at laparotomy – it strictures and distorts the ureters resulting in hydronephrosis. Most cases are of unknown etiology, possibly related to IgG4 systemic sclerosing disease, a minority being drug-related or associated with inflammatory type aortic aneurysms.

36.5 Surgical Pathology Specimens: Clinical Aspects

36.5.1 Biopsy Specimens

Percutaneous CT-guided needle core biopsy or fine needle aspiration may be performed for retroperitoneal tumors or if there is evidence of lymphadenopathy suggestive of lymphoma. It is not commonly used in the investigation of suspected metastatic disease at this site.

36.5.2 Resection Specimens

Retroperitoneal tumors:

These may be very large and structures such as the kidney enveloped by the tumor. A smaller wedge biopsy obtained at laparotomy may also be submitted if it is not possible to excise the whole tumor or if a needle core biopsy has proven inconclusive.

Retroperitoneal and pelvic lymph node dissections:

Nodal dissections are frequently performed in association with cervical carcinomas unless these fall into the micro-invasive category and/or are being managed by a non-radical surgical approach. Nodal dissection is also indicated for late stage and high-grade endometrial cancers, radical prostatectomy and cystectomy, but the situation for testicular germ cell tumors is more complex.

Metastatic seminoma is generally treated with radio/chemotherapy. Retroperitoneal lymph node dissection (RPLND) may be performed as prophylaxis against abdominal recurrence in clinical stage 1 non-seminomatous germ cell tumors or in the context of a residual mass post-chemotherapy. Prophylactic RPLND is generally not performed in the United Kingdom for clinical stage 1 non-seminomatous germ cell tumors, in contrast to Europe or the United States, where such operations are more common.

Clinical stage 1 non-seminomatous testicular germ cell tumors may be managed conservatively by surveillance with CT scanning and serial serum tumor markers or by chemotherapy. The prognostic factors influencing the administration of chemotherapy are considered in more detail in Chap. 33. However, about 25% of patients managed by surveillance will relapse with abdominal nodal disease being the most frequent site. Chemotherapy will then be administered and if a residual mass persists this will be excised. Such specimens frequently show widespread necrosis and fibrosis, but there may be residual areas of viable tumor. This can range from differentiated, mature tissues that are insensitive to chemotherapy and form cystic masses that press on local structures (growing teratoma syndrome) to mixed solid/cystic lesions containing immature/undifferentiated teratoma (10–25% of cases). The factors influencing an increased risk of progression are the presence of MTU (embryonal carcinoma), yolk sac tumor or trophoblastic tumor, and incomplete resection (as judged by the surgeon). These criteria will influence the decision to give further chemotherapy, and this should be borne in mind when the pathologist is examining these specimens so that sufficient blocks are sampled and margins inked.

36.6 Surgical Pathology Specimens: Laboratory Protocols

36.6.1 Retroperitoneal Tumors

36.6.1.1 Needle Core Biopsy Specimens

These are counted, their length recorded (in mm) and embedded for histological examination through multiple levels. Fine cores may be painted with alcian blue to allow visualization when facing the paraffin block at section cutting.

36.6.1.2 Excision Biopsy

These are weighed (g) and their dimensions (cm) recorded. The lesion is serially sectioned, and either representative sections taken for histology or all the tissue is processed. If the biopsy is received unfixed and depending on the clinical differential diagnosis, material may be triaged for appropriate ancillary methods including DNA extraction for PCR and clonality or sequence analysis, touch imprints for FISH, and if available, glutaraldehyde-fixed tissue for electron microscopy.

36.6.1.3 Resection Specimens

Initial procedure:

- The specimen is weighed (g) and measured (cm). The relationship of tumor to any recognizable organs such as the kidney that are present in the resection is noted.
- The surface of the specimen is painted with ink.
- If the specimen is received unfixed, then consideration should be given to the use of ancillary techniques as described above.
- The specimen is fixed in formalin for 24–36 h. It may be advantageous to cleanly bisect large specimens after a few hours to allow adequate fixation in the center.
- The specimen is serially sectioned at intervals of 1–2 cm.

Description:

- Weight (g), dimensions (cm) of specimen and constituents (fat, connective tissue, kidney, lymph nodes, etc.).
- Tumor size (maximum diameter or three dimensions – cm).
- Edge of tumor (well circumscribed, encapsulated, or infiltrative) and relationship to surrounding structures.
- Appearance of cut surface of tumor (hemorrhage, necrosis, cystic degeneration, etc.).

Blocks for histology:

- Representative samples of tumor (approximately one block per centimeter to include any macroscopically different looking areas).
- Tumor and adjacent structures.
- Tumor and inked circumferential margin of specimen.
- Lymph nodes.
- Uninvolved organs/tissues.

Histopathology report:

- Tumor type.
- Maximum diameter of tumor.
- Tumor grade (if applicable).
- Tumor stage; use the TNM system for soft tissue sarcomas where retroperitoneum is a specific topographical site and retroperitoneal tumors are classified as deep tumors.
- Completeness of excision.

36.6.2 Retroperitoneal and Pelvic Lymph Node Dissections

- Such specimens will often be submitted in multiple parts, each representing a specific anatomical nodal group, and it is important that this information is preserved in the final histology report.
- Weigh (g) each specimen and dissect out recognizable lymph nodes. The maximum dimension (cm) of the largest node should be recorded. Smaller lymph nodes may be submitted intact; larger nodes can be bisected or serially sectioned and then submitted in a separate tissue block. It is important to record on the final histology report the number of nodes identified.
- RPLNDs post-chemotherapy for testicular germ cell tumors present particular challenges. There may be a recognizable tumor mass present. The circumferential margin is linked to assess the adequacy of excision. Multiple representative sections are taken to ensure that any

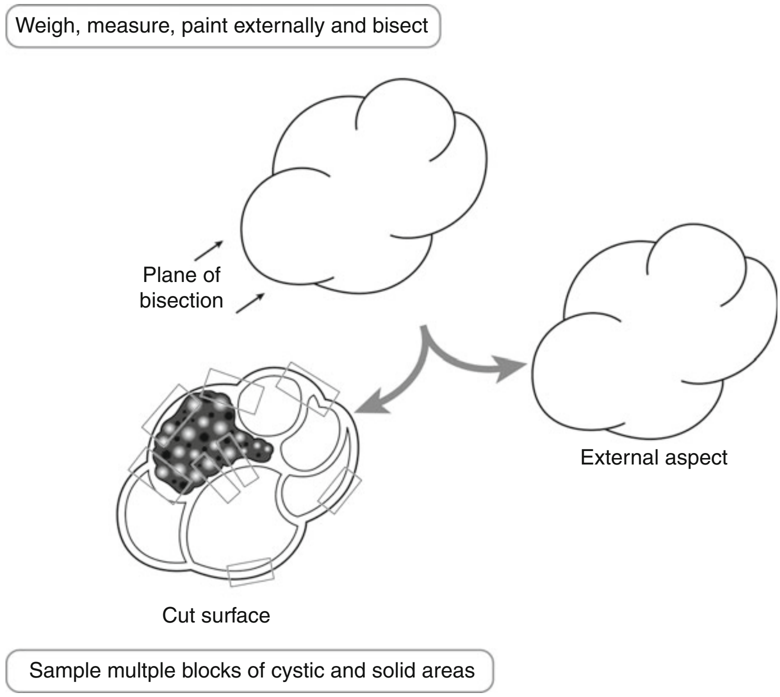

Fig. 36.2 Blocking of a retroperitoneal lymph node dissection (RPLND) specimen (Reproduced, with permission, from Allen and Cameron (2004))

residual viable areas of embryonal carcinoma or yolk sac tumor are detected (Fig. 36.2).

- Pelvic lymph node dissections (PLND) are usually for the staging and treatment of urological and gynecological malignancies.

Histopathology report:

- Anatomical location of lymph node groups.
- Weight (g) of tissue. Number of nodes identified. Number of lymph nodes involved by metastatic disease. Maximum diameter of largest involved node. Presence or absence of extra-nodal spread.

RPLNDs for non-seminomatous germ cell tumors post-chemotherapy.

- Presence of fibrosis, tumor necrosis, or other effects of chemotherapy.
- Presence or absence of residual viable tumor. Mature cystic teratomatous components, immature elements, malignancies of somatic components, i.e., carcinoma, sarcoma, or neuroectodermal malignancies.
- Presence or absence of residual viable embryonal carcinoma, yolk sac tumor, or choriocarcinoma.
- Relationship to the inked circumferential margin.

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37 Adrenal Gland

Maurice B. Loughrey, Damian T. McManus,
and Moyra Gray

37.1 Anatomy

The adrenal glands are paired, yellowish, retroperitoneal organs that lie close to the upper poles of the kidneys (see Fig. 29.1 in Chap. 29). They are surrounded by renal fascia but separated from the kidneys by perirenal fat. The left adrenal is almost crescentric in shape whereas the right is more pyramidal. In adults they average 5 cm in length and 5 g in weight, being proportionately larger at birth.

Each gland is divided into an outer cortex and inner medulla, the former being of mesodermal origin and the latter neuroectodermal. Both layers have important physiological roles in hormone secretion, which is the basis for the most common clinical presentations of adrenal pathological conditions. The cortex is divided into three zones (the glomerulosa, fasciculata, and reticularis) and is under direct control from pituitary secretion of adrenocorticotrophic hormone (ACTH) via a negative feedback system. The adrenal cortex secretes the mineralocorticoid hormone aldosterone which is responsible for maintaining fluid and electrolyte balance (mainly under influence of the renin-angiotensin axis), the glucocorticoid hormone cortisol (important in control of metabolism), and small amounts of sex hormones. The outer cortex is lipid laden and golden yellow in color whereas the inner cortex (zona reticularis) is brown due to high lipofuscin content. The gray-white medulla secretes the catecholamines noradrenaline and adrenaline.

Lymphovascular Drainage

Arterial blood supply is from adrenal branches of the aorta and renal and inferior phrenic arteries. The right adrenal vein drains directly into the inferior vena cava whereas the left drains into the left renal vein. Lymphatics pass to the lateral aortic nodes.

37.2 Clinical Presentation

Because of their location, adrenal tumors seldom present with symptoms due to mass effect. Rarely, a large adrenal carcinoma may cause abdominal pain, low-grade fever (due to tumor necrosis), and a palpable mass. Instead, presentation is more often as an incidental finding on CT scanning of the abdomen or as a result of symptoms related to hormones secreted by the adrenal tumor. These hormones give rise to characteristic constellations of symptoms and signs (clinical syndromes), and the initial diagnosis may be

M.B. Loughrey (✉) • M. Gray
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: maurice.loughrey@belfasttrust.hscni.net; moyra.gray@belfasttrust.hscni.net

D.T. McManus
Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: damian.mcmanus@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_37, © Springer-Verlag London 2013

confirmed by appropriate biochemical investigation, after excluding an exogenous cause of hormone excess.

Hypercortisolism (Cushing's syndrome): central weight gain, "moon face," thin skin, striae, bruising, hirsutism, hypertension, osteoporosis, proximal myopathy.

Hyperaldosteronism (Conn's syndrome): urinary frequency, weakness, hypertension, hypokalemia, hypernatremia, metabolic alkalosis.

Hypoadrenalism/adrenal insufficiency (Addison's disease): anorexia, weight loss, fatigue, cutaneous pigmentation, orthostatic hypotension, hyponatremia, hyperkalemia, metabolic acidosis.

Virilizing adrenal tumors: hirsutism and primary amenorrhea.

Pheochromocytoma: headaches, sweating, anxiety, chest pain, tachycardia, tremor, (paroxysmal) hypertension.

37.3 Clinical Investigations

37.3.1 Biochemical Assessment

Hypercortisolism

- Morning and evening serum cortisols (diurnal variation is lost in hypercortisolism)
- 24-h urinary cortisol
- Dexamethasone suppression test (cortisol normally suppressed)
- Plasma ACTH (differentiates primary hypercortisolism (ACTH low) from pituitary-dependent hypercortisolism or ectopic ACTH production (ACTH high))
- Petrosal venous sinus sampling (to localize source of ACTH production)

Hyper/Hypoaldosteronism

- Serum sodium, potassium, bicarbonate
- Serum aldosterone and plasma renin (primary/secondary hyperaldosteronism)
- Erect and supine aldosterone:renin ratios (normally vary with posture)
- Saline suppression tests (saline infusion normally suppresses aldosterone)
- Adrenal vein sampling of aldosterone (for lateralization of lesion in primary hyperaldosteronism)

Hypoadrenalism

- ACTH stimulation (Synacthen) test (low cortisol response indicates hypoadrenalism)
- Adrenal autoantibodies (+ve in autoimmune hypoadrenalism)

Virilizing Tumor

- Serum adrenal androgen profile

Pheochromocytoma

- 24-h urinary catecholamines
- Clonidine suppression test if above result ambiguous (normally suppresses catecholamine production)

37.3.2 Radiological assessment

- CXR – to look for lung tumor as a possible ectopic source of ACTH in hypercortisolism
- CT/MRI – to assess size, margins, multifocality, density, homogeneity, and presence of necrosis or calcification in adrenal lesions. Often poor, however, at distinguishing benign from malignant apart from on size. In overt adrenal carcinoma used to assess staging and resectability preoperatively: contralateral adrenal and pituitary assessment may be helpful in distinguishing adrenal hyperplasia from adenoma
- Radioisotope metaiodobenzylguanidine (MIBG) scan – images medullary tissue and is particularly useful for detecting extra-adrenal pheochromocytomas
- Core biopsy and FNA

37.4 Pathological Conditions

37.4.1 Cortex

Congenital adrenal hyperplasia: This autosomal recessive disorder is caused by an enzyme deficiency in the cortisol biosynthetic pathway, most commonly 21-hydroxylase. ACTH levels are elevated by negative feedback, causing adrenal enlargement. Symptoms are caused by diversion of cortisol precursors into androgenic steroid pathways, leading to virilization. A spectrum of severity exists, and the condition may go unrecognized in males. Affected females may present at birth with sexual ambiguity and adrenal failure, or later with hirsutism or primary amenorrhea. Treatment consists of steroid replacement, and surgical pathology specimens are seldom seen.

Addison's disease: Chronic adrenal cortical insufficiency is most commonly of autoimmune etiology but other causes include tuberculosis, malignant infiltration, fungal infection, and sarcoidosis. Treatment is aimed at the underlying cause, in addition to steroid replacement. Surgical resection is seldom indicated.

Acquired hyperplasia: Is usually due to overproduction of ACTH either by the pituitary gland (usually an adenoma) or an ectopic source, most commonly bronchogenic small cell carcinoma. Both cause bilateral (may be asymmetrical) adrenal enlargement, which may be diffuse or nodular. Histology shows thickening of and lipid depletion within the zona fasciculata of the adrenal cortex. Occasionally pigmented cortical nodules are seen, possibly as part of Carney's syndrome. Pituitary-dependent adrenal cortical hyperplasia (Cushing's disease) accounts for 60–70% of adult cases of Cushing's syndrome. It is treated by transsphenoidal pituitary surgery and irradiation if this fails. Bilateral adrenalectomy is now rarely performed. Ectopic ACTH production accounts for 15% of adult cases of Cushing's syndrome and treatment is aimed at the primary tumor. Conn's syndrome is due to bilateral adrenal hyperplasia in approximately 25% of cases. Treatment is primarily medical in the form of spironolactone, an aldosterone antagonist (may see spironolactone bodies on histology), but unilateral adrenalectomy is curative in some cases.

Adenoma: These may be incidental findings or present with hormone-related symptoms. They are usually solitary, sharply circumscribed, and weigh less than 50 g.

Sectioning reveals a golden-yellow appearance, possibly with irregular mottling. Histology usually shows lipid-rich cells resembling those of the zona fasciculata, arranged in cords or nests. Focal, mild to moderate nuclear enlargement and pleomorphism is common and not an indication of malignancy. The best indication of hormone functionality is to look for cortical atrophy in the adjacent adrenal tissue (indicates Cushing's syndrome). Conn's syndrome caused by an aldosterone-secreting adenoma is significant as it represents a curable form of systemic hypertension. Regardless of functionality, most adrenal adenomas are surgically excised, although radiological monitoring (by CT or MRI) for size increase may be acceptable for small (<5 cm), nonfunctioning masses.

Carcinoma: Adrenal carcinoma is rare, affecting both sexes equally at an average age of 50. Because the hormone-producing capability is often deleted in tumor cells, clinical manifestations of hormone secretion usually only become apparent when the tumor has reached a large size. Symptoms are often mixed, e.g., Cushing's syndrome plus virilization, and, together with patient age, give some indication as to the likelihood of underlying malignancy. Adrenal carcinoma rarely causes pure hyperaldosteronism. The tumors are usually large (almost all weigh over 100 g) with a variegated appearance showing focal hemorrhagic, necrotic, or cystic change. A capsule may be seen, often with obvious tumor infiltration. Histology most characteristically shows a trabecular architectural arrangement of cells with small nuclei and eosinophilic cytoplasm but cytological atypia is highly variable. The best histological predictors of behavior are mitotic index, a diffuse growth pattern, fibrous bands, and vascular invasion. Distinction from metastatic renal cell carcinoma is important. Spread is via hematogenous and lymphatic routes to liver, lung, and lymph nodes. In addition, there is often local invasion into kidney and possibly inferior vena cava. Treatment is aimed at complete surgical removal of the tumor, if possible. Adjuvant mitotane (o,p'-DDD) therapy may control endocrine symptoms and tumor size. The overall 5-year survival for these aggressive tumors is only 35%.

37.4.2 Medulla

Pheochromocytoma: Induces all its clinical manifestations through the production of catecholamines, which may be intermittent and life-threatening. Known as the 10% tumor (approximately 10% are bilateral, 10% are extra-adrenal, up to 10% are malignant), there is a strong association with multiple endocrine neoplasia (MEN) type 2A, von Hippel-Lindau disease, and neurofibromatosis type 1. Extra-adrenal pheochromocytomas, or paragangliomas, are morphologically identical and are most commonly found in the retroperitoneum, mediastinum, carotid body, and urinary bladder. These have a higher incidence of malignant behavior. Pheochromocytomas average 3–5 cm in diameter and 75–150 g in weight and are therefore usually easily seen on radiographic imaging with CT or MRI. They are soft, pale to tan-colored often with mottled areas of congestion, hemorrhage or necrosis, focal cystic degeneration, and a fibrous pseudocapsule. Histology characteristically shows well-defined nests of cells ("Zellballen") separated by a delicate fibrovascular stroma. Nuclear enlargement and pleomorphism are common and are not an indication of malignancy, which is notoriously difficult to predict histologically. In fact, the presence of distant metastases is the only reliable criterion. Favored metastatic sites include ribs and spine. Treatment is primarily surgical excision of the tumor, sometimes solely as a debulking procedure in the presence of advanced malignant disease. The overall 5-year survival rate for pheochromocytomas is under 50%. Background medullary hyperplasia is an indicator of familial disease.

Neuroblastoma: A pediatric tumor (80% occur <4 years of age) of the sympathetic nervous system belonging to the family of "small round blue cell" tumors. Most present with an intra-abdominal mass. Forty percent arise in the adrenal glands, most of the remainder being retroperitoneal or intrathoracic. Ganglioneuroblastoma and ganglioneuroma represent better differentiated counterparts which are seen in an older age group and less commonly involving the adrenal gland. The clinical and laboratory aspects of these highly specialized pediatric tumors will not be discussed further.

Miscellaneous conditions: Chronic adrenalitis is usually secondary to inflammation in adjacent organs, e.g., chronic pyelonephritis; adrenal hemorrhage (secondary to sepsis, shock, coagulopathy), cysts, myelolipoma (composed of fat and hematopoietic tissue), lipoma, angioma, schwannoma, and adenomatoid tumor (of mesothelial origin) are all occasionally encountered in surgical pathology practice.

Other malignant neoplasms: Sarcomas (most commonly leiomyosarcoma) are very rare in the adrenal gland. Malignant melanoma and malignant lymphoma/leukemia usually secondarily involve the adrenals but may rarely be primary. Metastatic carcinoma is the commonest pathological lesion and can closely mimic primary adrenal carcinoma (lung, breast, and kidney are the most common primary sites).

37.5 Surgical Pathology Specimens: Clinical Aspects

37.5.1 Biopsy Specimens

Biopsies of adrenal masses, in the form of fine needle aspiration or needle core, are taken under CT guidance, most often to distinguish primary and secondary malignancy. Biochemical investigations often render biopsy of an adrenal mass unnecessary. Because of the potentially serious risk of hypertensive crisis, suspected pheochromocytoma has been regarded as a contraindication to needle biopsy.

37.5.2 Resection Specimens

There are numerous surgical approaches to the adrenal gland, and the choice is determined by the underlying pathology, the size of the adrenal lesion, patient habitus, and personal preference of the operating surgeon. Each case should be assessed individually.

Advances in laparoscopic surgery now mean laparoscopic adrenalectomy is commonly offered as treatment of choice, especially for the excision of small, possibly incidentally found, adrenal tumors.

A posterior (or modified posterior) approach is traditional for small, well-localized lesions. This approach was also used for bilateral adrenal exploration in primary hyperaldosteronism, but as preoperative radiographic localization is now mandatory, the bilateral posterior approach is reserved for bilateral adrenalectomy.

A solitary large pheochromocytoma or a large adrenal adenoma or carcinoma may be best approached through a thoracoabdominal (ninth or tenth rib) incision. Multiple pheochromocytomas necessitate an abdominal approach to allow careful exploration for metastases. Children and those with MEN or a positive family history of pheochromocytoma are considered at high risk for multiple lesions.

Surgical manipulation of a pheochromocytoma causes extreme cardiovascular instability due to fluctuation in catecholamine release. Careful pre- and perioperative medical control of blood pressure with adrenergic blockade is essential and requires expert anesthetic technique.

Partial adrenalectomy may be occasionally performed, usually in cases of bilateral neoplasms to leave some functional cortical tissue, or in rare patients with a solitary adrenal gland.

37.6 Surgical Pathology Specimens: Laboratory Protocols

37.6.1 Biopsy Specimens

Wide-bore needle cores are counted, measured (in mm), and embedded in entirety for histological examination through multiple levels. Careful handling is necessary to avoid crush artifact.

37.6.2 Resection Specimens

Specimen:
- Adrenalectomy specimens are usually complete gland resections to remove an adrenal tumor, or part of an en bloc radical nephrectomy. Partial and bilateral adrenalectomy specimens are rare. Laparoscopic resection may result in a fragmented specimen. Extra-adrenal pheochromocytomas (paragangliomas) are handled in a similar manner to their intra-adrenal counterparts, obviously with some variation depending on their location.

Initial procedure:
- Weigh the specimen (g) and measure in three dimensions (mm). If the gland appears grossly normal, attached soft tissue may be dissected off and the naked gland reweighed.
- Identify and measure (cm) any adjacent organs or structures such as the adrenal vein, if attached.
- If the specimen is infiltrated by obvious tumor, paint the entire surrounding connective tissue; if there is a localized mass, paint its outer surface.
- Fix the specimen by immersion in 10% formalin for at least 24 h.
- Serially section the entire specimen at 3-mm intervals perpendicular to the longest axis of any localized mass (Fig. 37.1) and lay the sections out sequentially for examination and photography.
- Look for lymph nodes in any attached soft tissue.

Description:
- Tumor
  - Size in three dimensions (mm)
  - Color (yellow, pale, white, tan, red-brown, mottled)
  - Site (relationship to cortex/medulla)
  - Appearance (hemorrhagic/necrotic/cystic/calcified areas)
  - Edge (capsule/circumscribed/irregular/invasion into soft tissue)
- Nonneoplastic tissue
  - Nodularity, color, atrophy
- Others
  - Cysts, hemorrhage

Blocks for histology:
- Sample tumor according to size (at least 2 blocks plus 1 for each 5 cm tumor diameter) taking blocks to show all grossly different areas, capsule and the relationship to adjacent adrenal tissue, other structures if attached (e.g. the adrenal vein) and the painted circumferential soft tissue margin.
- One representative section should be submitted from grossly normal adrenal tissue.
- Count and sample any lymph nodes identified.

Histopathology report:
- Tumor type – cortical adenoma/cortical carcinoma/pheochromocytoma/other.

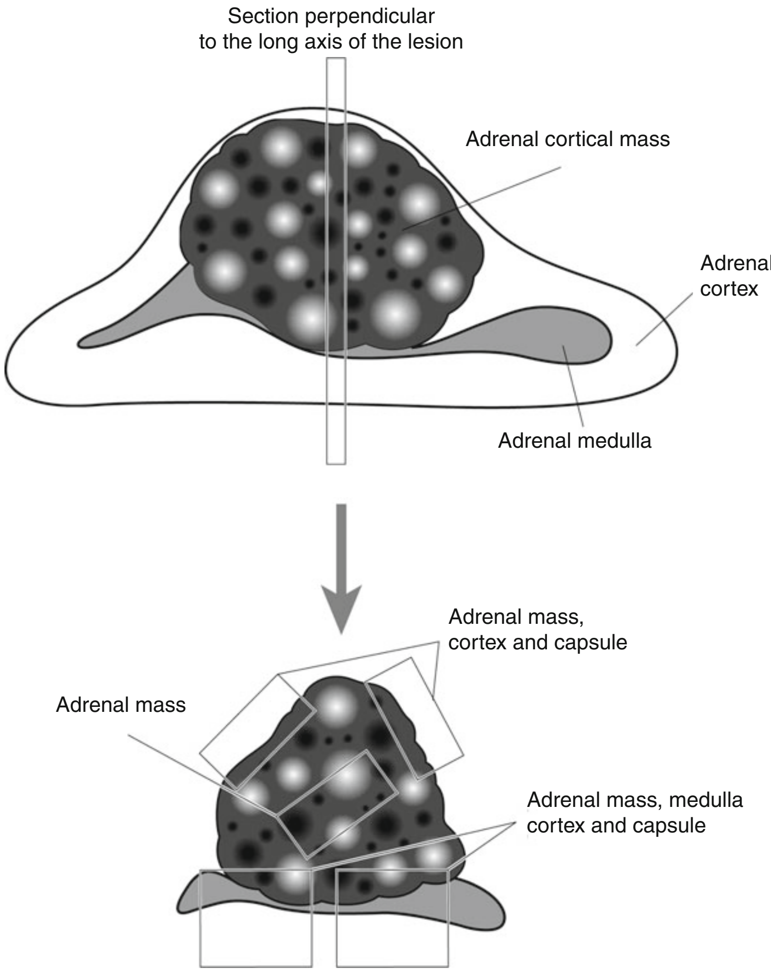

Fig. 37.1 Sectioning an adrenal gland mass (Reproduced, with permission, from Allen and Cameron (2004))

- Features of malignancy – extremely difficult to predict using histological criteria alone, but the following may be important and are usually reported:
  Nuclear pleomorphism, mitotic index, MIB 1/Ki 67 (proliferation index), atypical mitotic figures, architecture, presence of necrosis, capsular penetration, and broad fibrous bands.
- Tumor edge – circumscribed/irregular/capsule/pseudocapsule.
- Extent of local tumor spread – TNM 7 includes a staging system for adrenal cortical tumors.

pT1	≤5 cm, no extra-adrenal invasion
pT2	>5 cm, no extra-adrenal invasion
pT3	Any size, locally invasive but not invading adjacent organs[a]
pT4	Any size with invasion of adjacent organs[a]

[a]Including kidney, diaphragm, great vessels, pancreas, and liver

- Lymphovascular invasion – present/not present
- Regional lymph nodes

pN0	No regional lymph node metastasis
pN1	Regional lymph nodes involved
pNX	Cannot assess regional nodes.

- Excision margins
  Distances (in mm) to the nearest circumferential soft tissue limit
- Other pathology
  Nodularity, atrophy, spironolactone bodies, medullary hyperplasia

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Part VIII

Skin Specimens

38 Skin

Maureen Y. Walsh

38.1 Anatomy

The skin is the largest organ in the body. There are regional variations in the structure and function of skin between different sites in the body, and this is reflected in the microscopic appearance of the skin. The skin at all sites consists of three layers: (a) epidermis which provides a protective waterproof covering, (b) dermis which gives structural support and contains skin appendages, and (c) subcutaneous fat.

Epidermis: The epidermis is a keratinizing stratified squamous epithelial layer. The cells arise from the basal layer and divide to form the spinous cell layer. At the granular layer, cell death occurs, and the dead cells form the keratin (horny) layer, which is shed from the body. The epidermis also contains two other cell types: (a) melanocytes, which produce melanin pigment. These cells are scattered individually along the basal layer of the epidermis and (b) Langerhans cells which are located within the epidermis. They have a role in the immunoresponse of the body.

Dermis: The dermis is the layer of connective tissue and elastic tissue containing blood and lymphatic vessels, nerves and nerve endings with skin appendage structures. The dermis is divided into the papillary dermis which is the superficial structure that folds between the rete pegs of the epidermis and the reticular dermis (deeper dermis).

Skin appendages: The skin appendage structure is derived from the epidermal cells which flow down into the dermis. These may form hair follicles and sebaceous glands which are closely associated with each other, forming a pilosebaceous unit. There are also eccrine and apocrine sweat glands. The skin appendage structures often extend into the subcutaneous fat.

Subcutaneous fat: Beneath the dermis is a layer of adipose tissue with an associated fibrovascular stroma. Hair follicles and sweat gland structures extend into it.

Hair and nails: The hair and nails are specialized structures formed from keratin. They are located at specific specialized sites in the body.

Lymphovascular drainage: Drains to the locoregional lymph nodes of the body site at which the skin lesion occurs.

38.2 Clinical Presentation

Clinical dermatology can be divided into two broad categories: (a) skin rashes and (b) tumors/tumor-like lesions.

Skin rashes: Skin rashes present with a wide range of clinical appearances and include blistering disorders, skin manifestations of systemic disease, congenital, and genetic syndromes. The

M.Y. Walsh
Histopathology Laboratory, Institute of Pathology,
Royal Victoria Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: maureen.walsh@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_38, © Springer-Verlag London 2013

dermatologist usually makes a diagnosis based on the history and clinical appearance including the distribution of the rash. Pathologists dealing with specimens from these lesions need to have good clinicopathological correlation and a knowledge of clinical dermatology to ensure that the appropriate and best diagnosis is arrived at for the patient.

Tumors/tumor-like lesions: The second category of dermatology involves removal of a vast array of "lumps and bumps" by the clinician. These can range from benign cysts and tumors through to malignant skin tumors. Once again, the clinical background, appearance, site, and distribution of the lesion may aid in the diagnosis.

38.3 Clinical Investigations

In skin diseases, the clinical history and examination of the patient will usually assist the dermatologist or plastic surgeon in making the correct diagnosis. Diagnostic biopsy for histological examination is often the next stage in the management process. Particularly in inflammatory disorders, the skin may be involved in systemic disease and full clinical examination and investigation of the patient are required. Similarly, patients with congenital anomalies often have multiple abnormalities emphasising the need for full clinical assessment.

38.4 Pathological Conditions

Inflammatory disease biopsies require histology and close correlation with clinical details as do tumorous lesions which can arise from all the structures in the three skin layers resulting in a range of benign and malignant conditions.

Cysts: There are a variety of benign epithelial cysts that usually occur in the dermis and present as a dermal swelling. The type of cyst is determined by microscopic examination of the cell lining. Common examples are pilar and epidermal inclusion cysts – clinically termed sebaceous cysts.

Melanocytic nevi (*moles*): Most Caucasians have several benign moles or nevi on their body, the number relating to sun exposure and to the age of the patient. Nevi vary both in size and colour. They may be the patient's skin tone, white, or red through to shades of brown to blue/black in colour. Melanocytic nevi are removed for various reasons. They may have changed in appearance or developed symptoms suspicious clinically of malignant change requiring excision for histological examination. Nevi are also removed for cosmetic reasons, because they are being traumatized, occur at a hidden site on the body, or constitute a newly formed nevus in an adult. There are a variety of histological types of benign nevi that are dependent on microscopic examination for correct diagnosis.

Malignant melanomas: Malignant melanomas, like benign nevi, are derived from melanocytes. They may arise *de novo* or from within an existing melanocytic nevus. Changes in a pre-existing mole that cause concern include (a) asymmetry; (b) irregular borders; (c) change or variation in colour; (d) size > 6 mm; (e) elevation and also itching, bleeding or symptoms associated with nevus. When the clinician is suspicious of a diagnosis of malignant melanoma, the lesion is removed in total, usually with an ellipse of normal skin around it. Depending on the degree of certainty of the clinical diagnosis, a wide excision may or may not be done at that time. Melanomas are the third most common malignant skin tumor. Their incidence is rising, and they are the primary skin tumor most likely to metastasize and cause death. Malignant melanomas typically occur after puberty, and their incidence increases with advancing age.

Actinic keratosis, *Bowen's disease*, *basal cell carcinoma*, *squamous cell carcinoma*: Most skin cancers and pre-cancerous lesions of the skin are related to chronic sun exposure in white skin, and their incidence is increasing. Other etiological factors include a genetic pre-disposition and immunosuppression. Patients who have had organ transplants are at greater risk of developing skin neoplasia.

Actinic (*solar*) *keratosis*: Actinic keratoses present usually as multiple red, scaly lesions on sites of chronic sun exposure, particularly the head and neck, back of hands and forearms. The lesions are usually removed and submitted for pathology when the clinician is concerned that there may be malignant change, and particularly invasive malignancy. Often patients with actinic (solar) keratosis have multiple lesions, which are treated by a variety of topical agents and are not submitted for histological examination. Various biopsy techniques may be used to remove actinic keratoses including curettage, shave, punch and excision biopsies.

Bowen's disease (*carcinoma in situ*): Bowen's disease is a pre-invasive or *in situ* malignancy of the skin usually presenting as a red scaly patch. Most of these lesions present in a background of solar damage although it can occur in areas of non-sun damaged skin, where it may be associated with a higher incidence of internal malignancy. Bowen's disease is often treated by dermatologists with topical agents and may be biopsied to confirm the diagnosis and to exclude invasive malignancy. Occasionally there will be a biopsy to remove the lesion. Depending on whether the biopsy is excisional or diagnostic in intent, the laboratory will receive either a curettage, shave, punch, or elliptical specimen.

Basal cell carcinoma: Basal cell carcinoma is the commonest malignant tumor of the skin, overall in humans. The vast majority are associated with chronic sun exposure and occur in the head and neck area of fair-skinned people. A few occur at sites of scarring in the skin and a small number of patients with a genetic predisposition develop multiple basal cell carcinomas. These patients often present at an early age. Basal cell carcinomas have a variety of clinical appearances from a nodular lesion to an ulcer or scarred areas, and they may also be multifocal. The colour of the tumors can vary. The cell of origin of basal cell carcinoma is thought to be either the basal cell layer of the epidermis or hair follicle. Basal cell carcinomas are locally aggressive tumors, often infiltrating and destroying adjacent tissue. They do not, however, metastasize to other sites. The treatment of choice is surgical removal. The clinician may submit a variety of specimen types to the laboratory depending on the surgical technique used. These may be curettage, shave, punch, or excision. Based on clinical need, Mohs micrographic surgery is used in the treatment of a small number of cases. Occasionally basal cell carcinomas may be treated by radiotherapy following a confirmatory diagnostic biopsy.

Squamous cell carcinoma: Squamous cell carcinoma is the second most common malignant tumor of the skin typically at sun exposed sites in patients with fair skin. Increasing numbers of patients who are immunosupressed including renal transplant patients are at increased risk of developing squamous cell carcinoma. A small number of squamous cell carcinomas occur in patients with predisposing genetic disorders or at sites of chronic scarring. These tumors arise from the surface epithelium. They have a variety of clinical appearances including nodules and ulcers, and they also can vary in colour. These tumors do have the potential to metastasize although the vast majority are cured by adequate local treatment. The treatment of choice is surgical, and the clinician will submit various specimens including curettage, shave, punch, and excision biopsies. Mohs micrographic surgery may be used in selected cases. Some cases are treated with radiotherapy following a pathological diagnosis.

38.4.1 Other Skin Tumors

Merkel cell tumors: Merkel cell tumors are tumors of neuroendocrine origin that occur in elderly patients usually presenting as a rapidly growing nodule often in the head and neck area. They may present with skin involvement and lymph node spread. Prognosis in these tumors is poor. Secondary spread from small cell carcinoma of lung must be excluded.

Paget's disease of nipple: Paget's disease of the nipple presents as an eczematous area on the nipple or areola. It is associated with underlying malignancy in the breast.

Extramammary Paget's disease: Extramammary Paget's disease occurs at the vulva, perineum, scrotum, penis, anus, and axilla. It presents as a red velvety area and on histological examination is an *in situ* carcinoma. It may or may not be associated with underlying carcinoma in the sweat glands of the skin or visceral malignancy in the gastrointestinal, urinary, or gynecological tracts.

Skin appendage tumors (*benign and malignant*): The hair follicle and sweat gland structures are capable of giving rise to a wide variety of skin appendage tumors. If multiple, they may be associated with clinical syndromes. Most of these lesions present as nodules in the skin and correct diagnosis is dependent on histological examination. The majority of lesions are benign, although a small number are malignant and may metastasize and cause death in the patient.

Benign epithelial tumors and tumor-like lesions: Seborrhoeic keratosis is a benign epithelial tumor arising in the skin of middle-aged and elderly patients, presenting usually as a stuck-on, warty type of lesion. They are often pigmented and may be mistaken by the patient and clinician for a melanoma.

Viral warts: Most viral warts are treated with topical agents and are not submitted for histological diagnosis, unless the diagnosis is unclear.

Benign mesenchymal tumors: The mesenchymal tissue in the dermis and subcutis can give rise to various tumors. Most present as nodules in the skin and may be biopsied or excised by the clinician using curettage, shave, punch, and elliptical excision.

Malignant mesenchymal tumors (*sarcomas*): Malignant mesenchymal tumors are rare. These lesions are often large and may have a history of growth or change. They may be biopsied to establish the diagnosis or have a wide surgical excision to remove the lesion.

Leukemia and lymphoma: Leukemias and lymphomas may affect the skin in two main ways: (a) as an inflammatory skin rash as a consequence of the underlying malignancy and (b) as a lymphoma/leukemia involving the skin, either as a primary skin lesion or spread to the skin as part of systemic disease. Lymphoma and leukemia involvement of the skin may present as a skin rash, plaques, or nodules of tumor. Usually a small diagnostic biopsy is taken in such cases, either as a punch or an ellipse.

Secondary tumors: Secondary tumors may involve the skin, either as directly from an underlying tumor or as metastatic spread. A small biopsy is usually used for diagnostic purposes. FNA also has a role to play (see below).

38.5 Surgical Pathology Specimens: Clinical Aspects

38.5.1 Biopsy and Excision Specimens

A variety of biopsies are submitted depending on the clinical diagnosis and the type of information the clinician wants.

Curettage: A curetted specimen is used to remove or sample small warty type lesions, which are usually benign or small basal or squamous cell carcinomas. This can be associated with cautery to the lesion base (C + C). Occasionally a basal cell carcinoma, actinic keratosis, or squamous cell carcinoma may be removed by curettage, and then formal surgical excision is carried out of the curetted area. The laboratory in this case will receive two specimens from one patient: a curettage and the excision biopsy. This combined technique is used to give a good cosmetic result. The curettage removes the bulk of the tumor, and the excision results in a neat scar.

Shave biopsy: Shave biopsies are used to remove polypoid or raised lesions on the skin. Usually the clinician thinks the lesion is benign, and a shave will give a good cosmetic result.

Diagnostic punch biopsy: A diagnostic punch biopsy is usually done to assist in the diagnosis of inflammatory diseases, or to establish the diagnosis of a tumor before formal wider excision is carried out.

Punch excision: A punch excision biopsy is used to remove completely the lesion on the skin such as a small mole or nevus. The lesion is removed with a rim of normal tissue surrounding it.

Diagnostic elliptical biopsy: An ellipse of skin may be removed to establish the diagnosis in skin rashes. This may involve lesional skin and surrounding normal skin, or only lesional skin. Where the biopsy is taken depends on the clinical diagnosis, and where the most likely diagnostic pathology is to be found. The dermatologist on the advice of the dermatopathologist must take the most appropriate site for diagnosis. Diagnostic elliptical biopsies are also done for skin tumors before, if

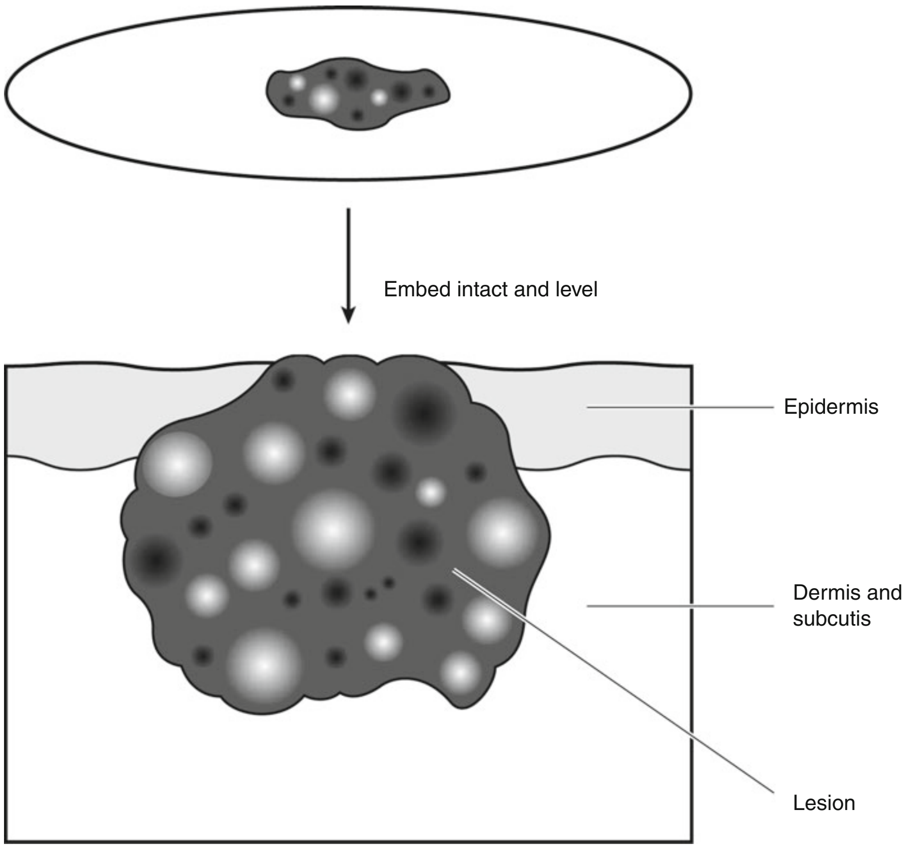

Fig. 38.1 Punch, shave, or ellipse biopsy embedded intact (Reproduced, with permission, from Allen and Cameron (2004))

necessary, formal excisions are carried out. They are not recommended for lesions where malignant melanoma is a suspected diagnosis clinically.

Elliptical excisions: Elliptical excisions are carried out to remove skin tumors, both benign and malignant. The dermatological surgeon will usually remove the lesion with a surrounding rim of normal skin.

Pigmented lesions: Where the clinician suspects that he is dealing with a possible malignant melanoma, the biopsy should be an excision biopsy with a rim of normal surrounding skin. Only in exceptional circumstances should a diagnostic biopsy of a suspected melanoma be carried out, e.g., a pigmented lesion on a digit where full excision would result in an amputation.

Fine needle aspiration biopsies (*FNA*): FNAs are used to diagnose subcutaneous lumps in the skin and to establish the diagnosis in secondary carcinoma. The role of FNA in primary tumors of the skin is limited because the diagnostic biopsy often comprises surgical removal of the lesion.

38.6 Surgical Pathology Specimens: Laboratory Protocols

A variety of biopsies are submitted and received.

Curettage: A curetted specimen is usually received in multiple fragments which are all submitted for histological diagnosis. The pathologist, based on the curette, makes a diagnosis of the lesion but cannot comment on adequacy of excision. Deeper levels are employed as appropriate.

Shave: Shave biopsies are measured, i.e., the length, breadth, and depth, in millimetres. If a lesion is noted grossly, this is also measured in millimetres. Depending on the size of the shave, it is submitted in total (Fig. 38.1), but if greater

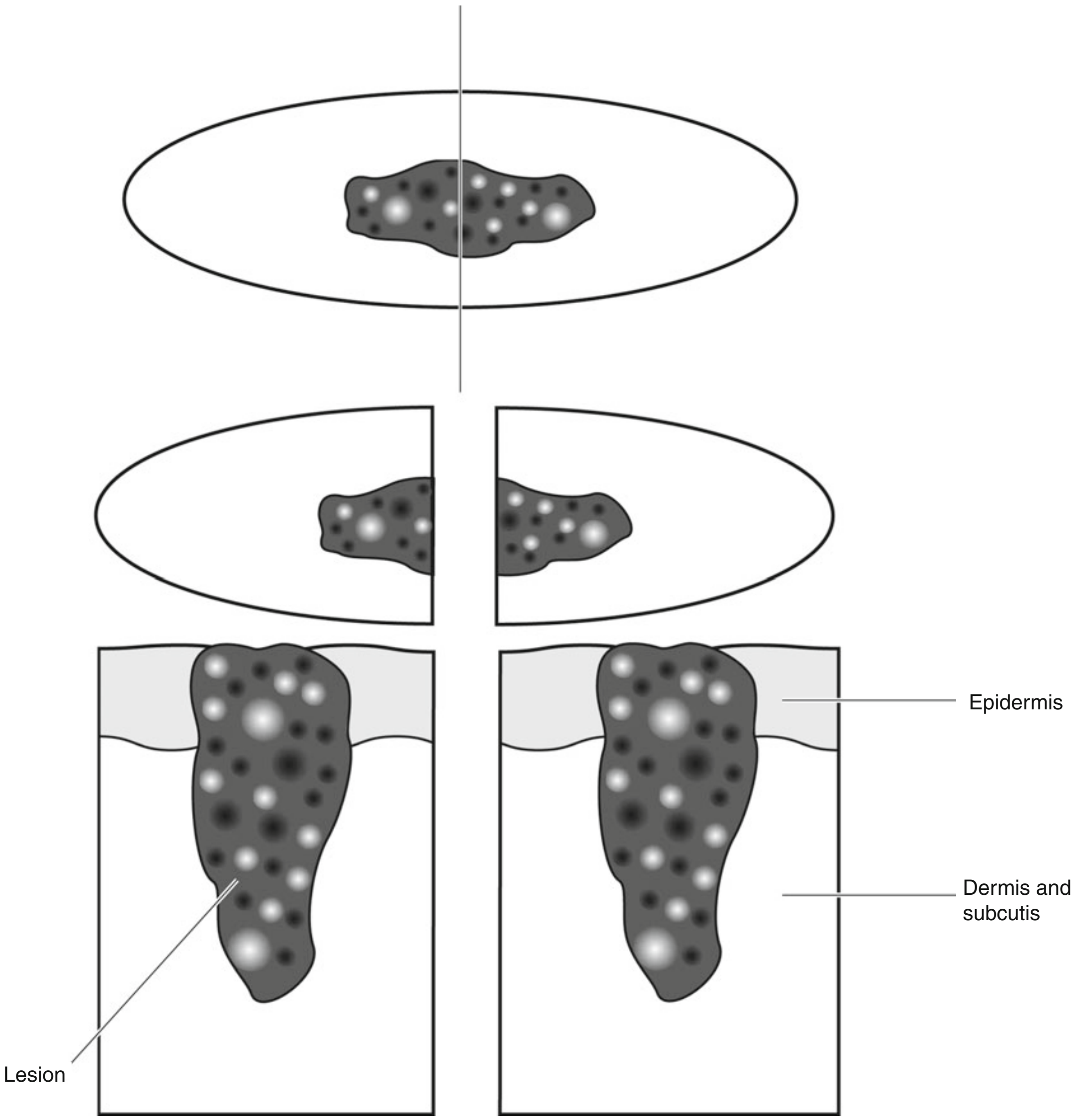

Fig. 38.2 Punch, shave, or ellipse bisected and embedded (Reproduced, with permission, from Allen and Cameron (2004))

than 6 mm, it is first bisected (Fig. 38.2). Shave biopsies are not excisional biopsies, and the lesion often extends to the deep margin. Excision margins are not commented on in benign lesions in a shave biopsy.

Diagnostic punch biopsy: Diagnostic punch biopsies come in a variety of sizes ranging from 2 to 8 mm. The smaller-sized punch biopsies are usually for diagnostic purposes. The size of the punch is recorded and a description of any lesion seen. Small punches less than 4 mm are submitted in total and will require examination of multiple levels (Fig. 38.1). Punch biopsies 4 mm and above are bisected and then submitted in total. Bisecting the specimen through the center of the lesion results in its representation in the initial levels.

Punch biopsy for alopecia: Punch biopsies are taken to establish the cause of alopecia and are embedded in the usual manner. In some centers,

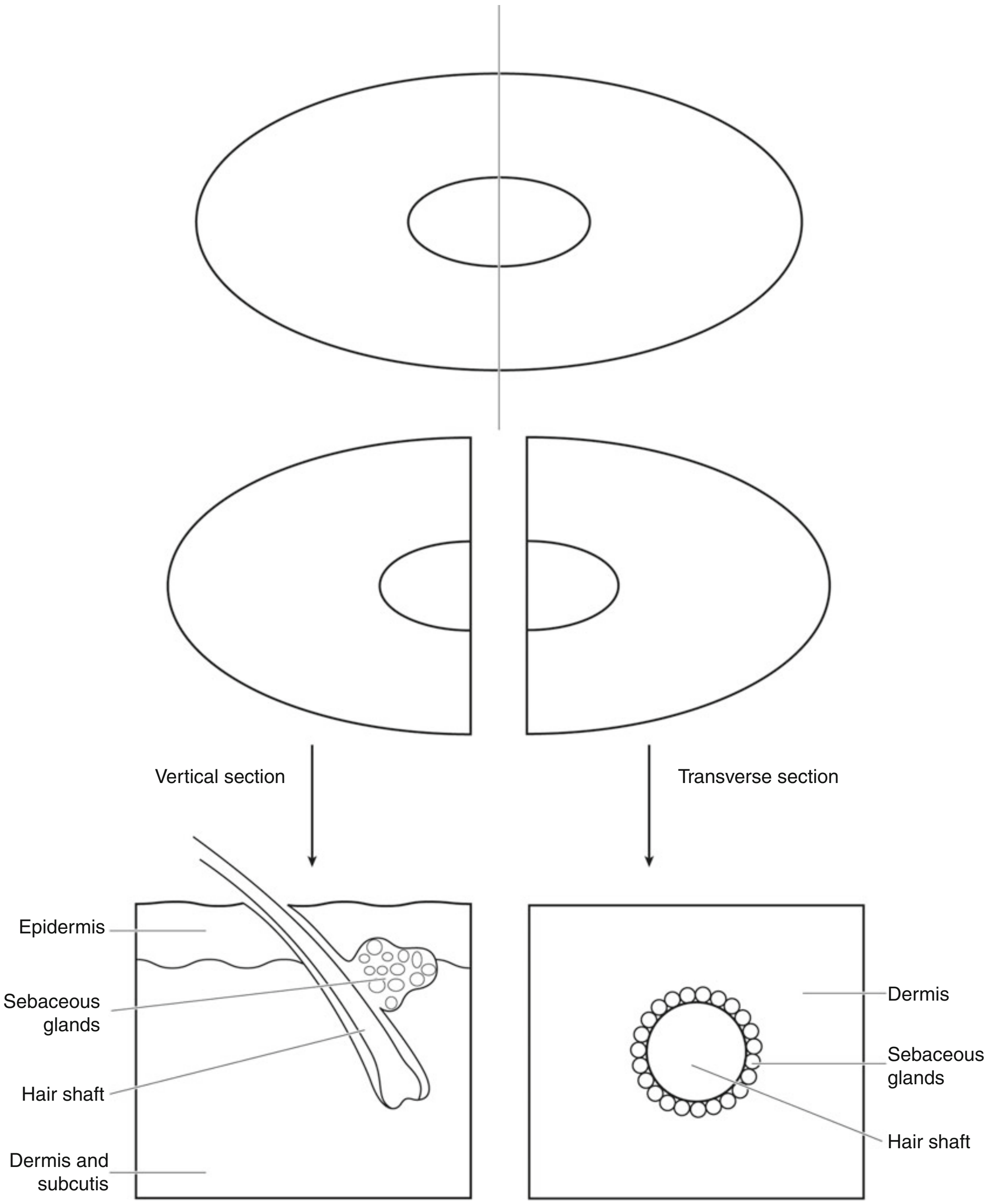

Fig. 38.3 Vertical and horizontal sections of a punch biopsy for the diagnosis of alopecia (Reproduced, with permission, from Allen and Cameron (2004))

depending on the experience of the dermatopathologist, the punch biopsy may be bisected, with one half embedded and sectioned in the usual vertical fashion and the other half sectioned transversely. This is thought to give a better view of the hair follicle structures and assist in the diagnosis of alopecia (Fig. 38.3).

Punch excision: Punch excisions, like diagnostic punch biopsies, come in a variety of sizes, usually 4 mm and greater. The size of the punch

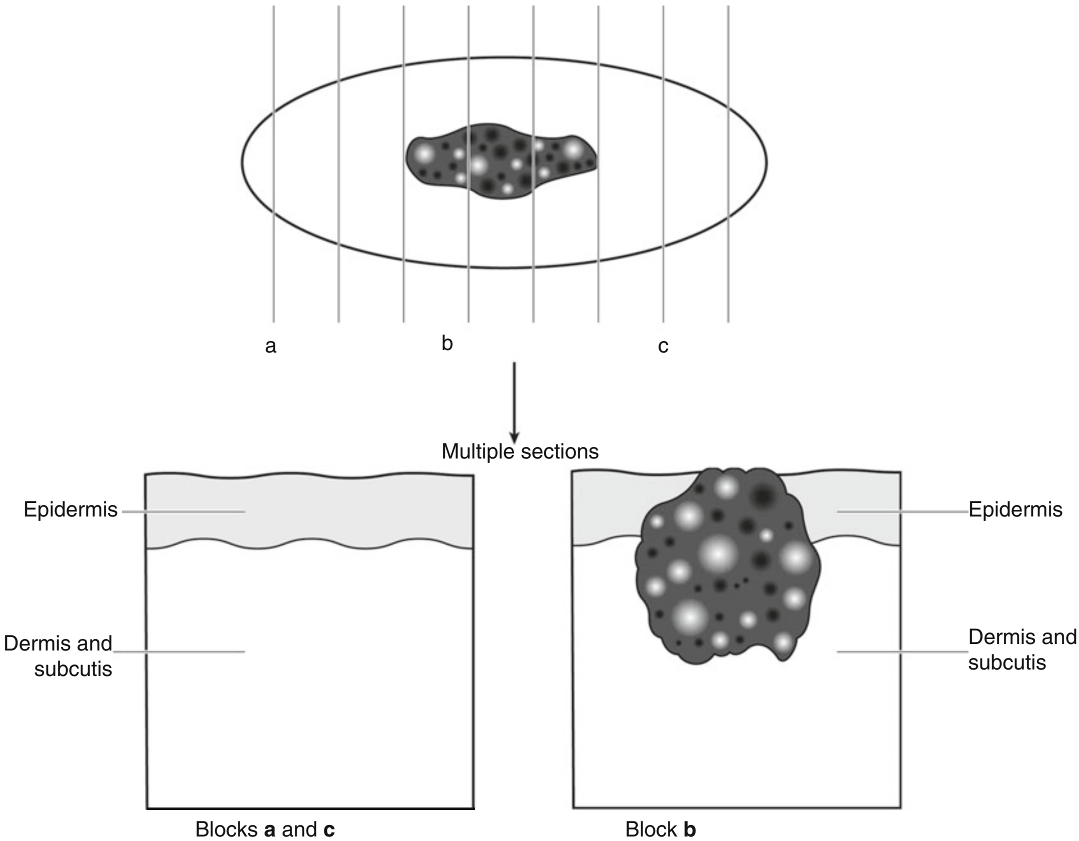

Fig. 38.4 Serial section of a skin ellipse (Reproduced, with permission, from Allen and Cameron (2004))

is measured and the edges inked. Depending on the size, the punch may be embedded intact and adequate sections cut to see the full face of the lesion (Fig. 38.1). Larger punch biopsies are bisected (Fig. 38.2) or sliced through to examine the lesion (Fig. 38.4). All punch excision biopsies and lesions present are described and measured in millimetres.

Elliptical biopsy: Small ellipses of skin may be removed for diagnosis. They are usually processed intact or bisected longitudinally and examined through multiple levels (Figs. 38.1 and 38.2). Biopsies are measured in millimetres and any lesion seen described and measured. They may have their edges inked.

Elliptical excision: Skin ellipses are used to remove tumor with a rim of normal tissue around the lesion. The pathologist needs to see the full face of the lesion and examine for adequacy of excision. All skin ellipses are measured and described. Any sutures and pins etc. placed by the clinician for orientation are noted and if any specific questions are asked on the request form regarding the excision, these are considered when sectioning the skin ellipse. Most elliptical skin excisions are not photographed unless the gross appearance is unusual when often it will have been photographed by the clinician before surgical removal. Photography or a photocopy of the lesion surface may be useful if sampling of the lesion is complex to indicate where blocks have been taken, but usually a diagram is adequate. The edges of the ellipse are inked to indicate the true surgical margins.

Elliptical excisions are dealt with in the laboratory in a variety of ways:

(i) If small (<6 mm), they can be processed intact and cut along the long axis. Multiple levels need to be examined to see the full face of the lesion (Fig. 38.1).

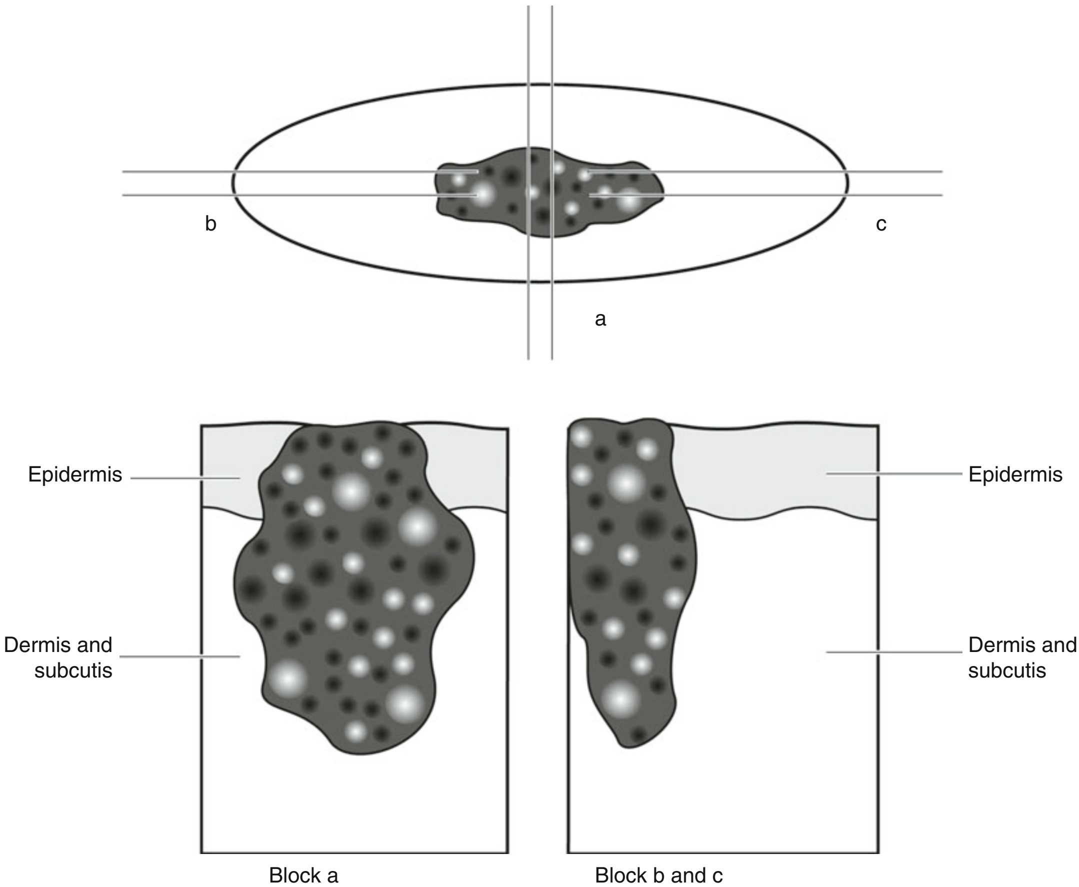

Fig. 38.5 Quadrant blocks of a skin ellipse (Reproduced, with permission, from Allen and Cameron (2004))

(ii) Small ellipses may be bisected across the short axis and embedded to show the center of the lesion. This provides information on the deep limit and nearest peripheral margins at the short axis but not the long axis (Fig. 38.2).

(iii) Quadrant blocks of the lesion. A block is taken through the center of the lesion across the short axis and two lateral blocks are taken across the long axis. This gives the full face of the lesion and margins on four quadrants (Fig. 38.5).

(iv) Skin ellipses may be serially sectioned or sliced like a loaf of bread through the lesion at 2–3 mm intervals. This ensures that the whole of the lesion is examined and is useful in melanocytic lesions of the skin (Fig. 38.4).

Wedge excisions: Wedge excisions are used to remove skin from the eyelid, lip, ear, and vulval areas. These and any gross lesions are described and measured. The surgical limits are the outer margins of the wedge, and these are sampled for histology. A section is then taken through the center of the tumor (Fig. 38.6).

38.7 Special Techniques and Considerations

Immunofluorescence: Immunofluorescent examinations are required for the diagnosis of chronic blistering diseases and are useful in connective tissue diseases. The site of biopsy is important for immunofluorescence, particularly in the blistering disorders. In dermatitis herpetiformis, a

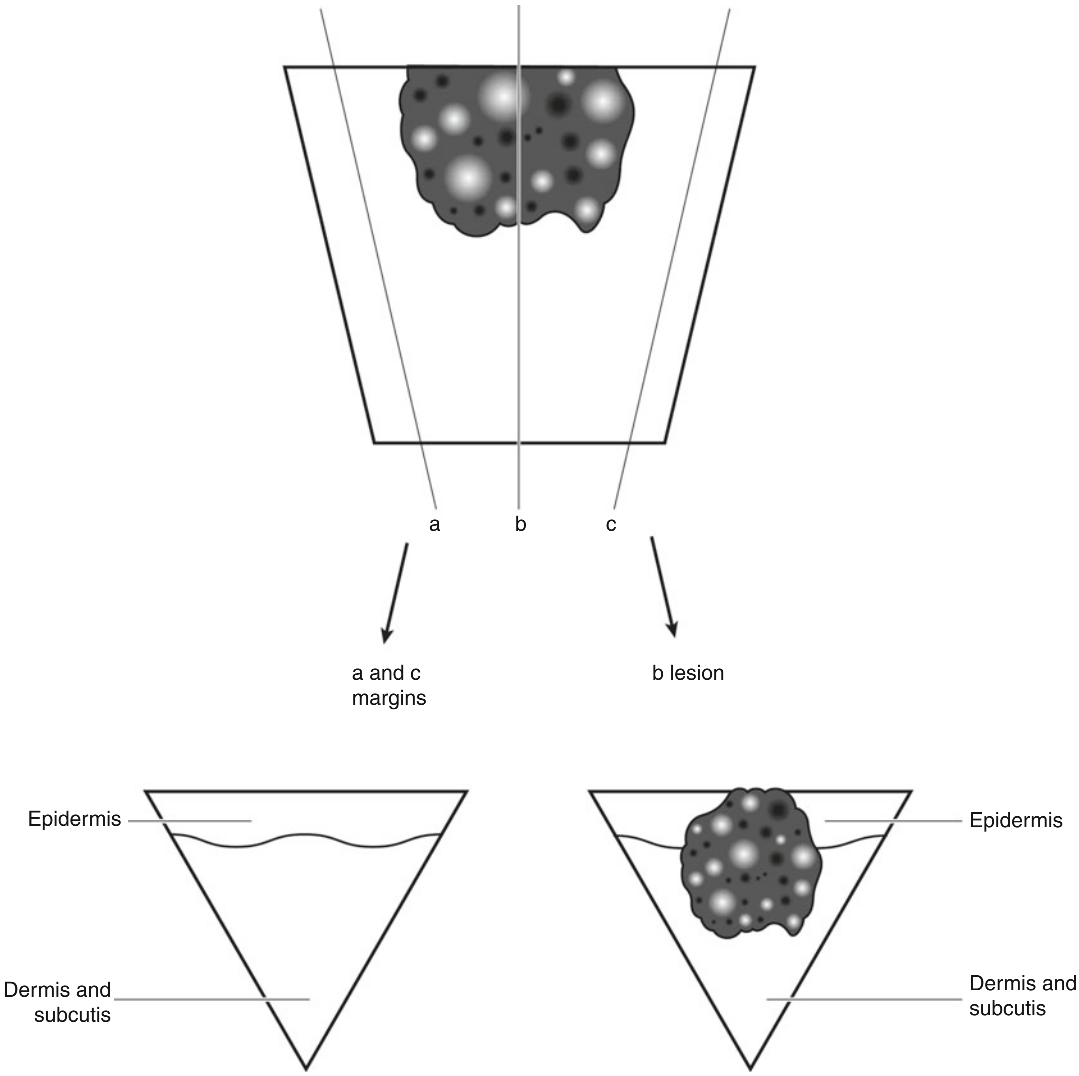

Fig. 38.6 Wedge resection of skin (Reproduced, with permission, from Allen and Cameron (2004))

biopsy for immunofluorescence should be taken from clinically normal skin away from the area of blistering. In the other blistering disorders, perilesional skin is submitted. The skin should have an intact epidermal/dermal junction. In most of the connective tissue disorders, lesional skin is submitted for immunofluorescence except for the lupus band test, where normal non-sun exposed skin is used. Most patients having skin submitted for immunofluorescence should also have a serum sample sent to the laboratory for examination for autoantibodies related to the disease process. Skin biopsy specimens for immunofluorescence are either snap frozen in liquid nitrogen or sent in Michel's transport medium to the laboratory. Most laboratories doing immunofluorescence will supply the clinical area with the appropriate transport medium. Formalin fixation is inappropriate for immunofluorescence and will render the specimen unsuitable for examination. Specimens for immunofluorescence are either punch or elliptical biopsies.

Mohs micrographic surgery: Mohs micrographic surgery is the surgical removal of the tumor under microscopic control. The aim of the technique is to remove all the tumor with the minimum of surrounding normal tissue. This is a time consuming and slow procedure for the patient, dermatological surgeon, and laboratory staff, but it is useful in a small number of cases. Mohs micrographic surgery is used primarily for the treatment of basal cell carcinoma but may be used for squamous cell carcinoma, some sarcomas of the skin, especially dermatofibrosarcoma protuberans and, rarely, some types of desmoplastic melanoma and other malignant skin appendage tumors. It is especially useful for tumors occurring on the face around the eyelids, nose, and mouth where a good cosmetic result is required. The technique involves examination of frozen sections of surgical margins with the patient and the surgeon awaiting the results. If limits are involved, a further excision of this area is carried out and examined by frozen section. This is repeated until the margins are clear. The defect is then repaired by the surgeon on the same day. In some units, the tissue is fixed, processed to paraffin, and margin sections examined the next day. If the margins are involved, further tissue is removed, processed to paraffin, and sections examined. Only when the margins are clear is repair carried out. This is a slower procedure over a period of days in which the patient has a defect which has to wait for confirmation of clearance before repair can take place. This technique is useful for rarer types of tumor where there may be an infiltrate of single spindle cell such as a desmoplastic malignant melanoma or where immunocytochemistry is required to identify tumor cells.

Mohs laboratory procedure: It is essential that there is clear communication between the surgeon and the pathologist examining the specimen by Mohs micrographic surgery. The specimen should be laid out flat on a dish or board, and the margins indicated either by sutures or pins of different colour. It is useful if the surgeon also draws a diagram of the lesion and its location on the patient with appropriate landmarks. The pathologist ensures that the complete surgical margins are examined. It is often necessary to divide the specimen into smaller blocks to be examined microscopically. They may need to be marked so that the area involved by tumor can be clearly pinpointed. On an ellipse skin margins can be marked in relation to the clockface or to compass points. The surgical margins are marked with different coloured inks to aid locating the correct area with tumor involvement (Fig. 38.7).

Surgical margins: On excision biopsies the pathologist should comment on the adequacy of excision, and in line with protocols, measure the tumor distance from the margins. In punch biopsies, the specimen is either embedded intact or bisected and embedded. The pathologist can comment on two lateral and deep margins. The edge of the punch biopsy can be inked to indicate microscopically the true excision margins which are also often associated with red blood cells.

Similarly, in an elliptical biopsy, the margin status is documented by the pathologist. Quadrant blocks result in four lateral margins and a deep margin being examined. Bread-loaf slicing through the ellipse results in all the margins being seen microscopically but this is only suitable for relatively small ellipses. The margins can be inked to assist microscopic identification, although usually red blood cells are present. To examine all the surgical margins in a large skin biopsy, the best approach is a modified Mohs technique. The pathologist sections the margins, and these are marked with different coloured inks to aid identification.

Sutures and markers: The surgeon will often mark margins with sutures to help orientate the specimen and an accompanying diagram is also useful. Techniques of margin sampling in large excisions may need to be modified in the light of attached sutures or clinical request form information.

Grafts: Tumors may recur under and around an area of skin grafting. These specimens are dealt with in the usual manner for a skin ellipse.

Re-excisions: The most common cause for re-excision is when a malignant tumor is incompletely excised, or in the case of a malignant melanoma, despite complete primary excision, the margins are not wide enough to follow standard guidelines. Re-excision biopsies are sampled as for primary excisions. Tumor, if present, is usually at the edge of the previous biopsy scar. Again, margins of excision are commented on.

Diagrams: Diagrams are also useful in orientating specimens.

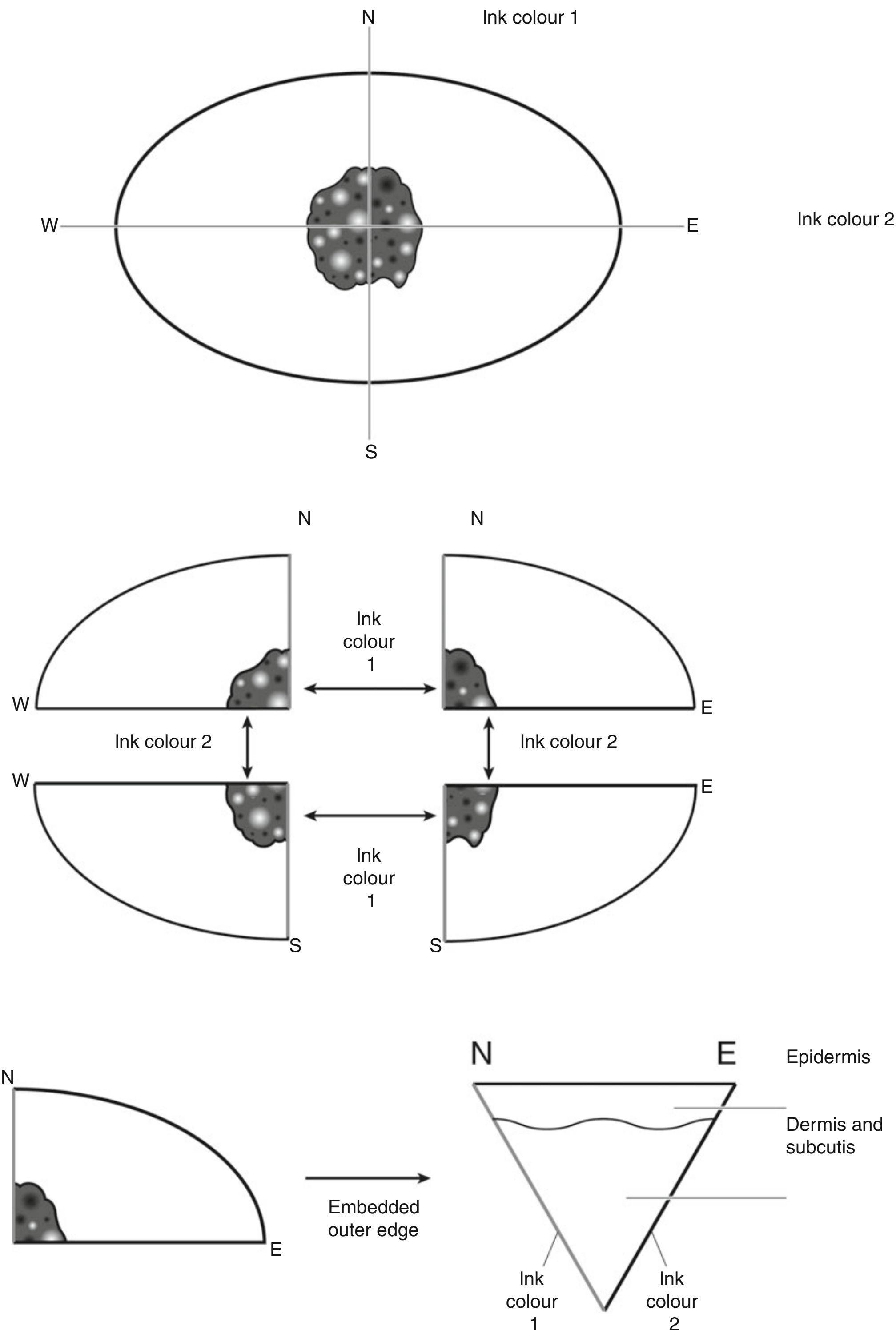

Fig. 38.7 Sections for Mohs micrographic surgery (Reproduced, with permission, from Allen and Cameron (2004))

Transmission electron microscopy (*TEM*): As in other branches of pathology, the role of diagnostic TEM is declining. Immunocytochemistry has reduced the need for it in diagnosing undifferentiated tumors and viral infections, although it is still useful for inborn errors of metabolism. All such samples of skin should be placed in glutaraldehyde fixative. Other indications for TEM are in the diagnosis and subclassification of: (1) congenital anomalies, e.g., ichthyosis, (2) blistering diseases as in the epidermolysis bullosa group (it may be necessary to obtain a fresh blister by rubbing up with an eraser), and (3) acquired blistering disorders (in conjunction with immunohistochemistry).

Scanning electron microscopy (*SEM*): Scanning electron microscopy is useful in the diagnosis of hair shaft anomalies. The hair sample should be sent unfixed to the laboratory.

Microprobe analysis: A microprobe attached to the scanning electron microscope can be useful to detect small amounts of elements present in the skin that may be causing increased abnormal pigmentation.

Skin scrapings: Scrapings from the skin surface can be examined for fungal particles or scabies mites. This may be a wet preparation by putting the scrapings in potassium hydroxide or fixing the tissue and processing it. This is a useful way to make a diagnosis without a full surgical biopsy.

Fixation: The usual fixation for skin biopsies for histology is 10% formalin. Mast cells are easier to demonstrate if the skin biopsy has been fixed in alcohol. Where a mast cell lesion is suspected, the biopsy should be divided and halves placed in formalin and alcohol. However, if mast cells are present in large numbers, they can still be seen in formalin fixed tissue. Similarly, the urate crystals in gout dissolve in formalin. It is still possible to diagnose gout on formalin fixed tissue, but it is easier to demonstrate the crystals if the tissue has been placed in alcohol fixative.

38.8 Special Sites

Hair: Hair samples should be plucked, not cut, from the patient and sent unfixed to the laboratory. The hair is mounted unfixed on glass slides and examined for hair shaft anomalies or to look at the hair roots and count the telogen:anagen ratio—this requires a minimum of 50 hairs. Scanning electron microscopy provides more information in patients with hair shaft anomalies and picks up more subtle changes than those seen at light microscopy.

Nails: Fragments of nails may be submitted for examination either to detect fungi or the cause of nail pigmentation. The fragments are softened in phenol and then processed in the usual way for histology. For pigmented lesions or growths beneath the nail, the nail must be removed by the surgeon before skin biopsy of the nail bed is taken. Nails may be involved in several skin diseases, but usually a biopsy of skin involved elsewhere is taken to confirm the diagnosis.

Digits: Pigmented lesions beneath nails often cause diagnostic problems in distinguishing between benign lesions, trauma, and malignant melanoma. Trauma to the nail which bleeds grows outwards as the nail grows, whereas nevi and melanomas do not. If melanoma is suspected, the clinician must first remove the nail and biopsy the lesion on the nail bed. Excision biopsy is ideal but if this is not possible then a diagnostic biopsy is permitted. This is allowed in the nail bed as treatment for melanoma is amputation of the digit. Because of this the pathologist should only diagnose melanoma when there is a high degree of certainty, otherwise another biopsy is requested. Digits are measured and described in the usual manner including which joints have been disarticulated. The tumor is measured and described. The surgical margin of excision is blocked and the tumor sampled through its deepest area.

Eyelid: The eyelid margins can be involved in a variety of benign and malignant tumors. Benign tumors are dealt with in the usual manner. In malignant tumors, especially basal cell carcinomas, squamous cell carcinomas, and melanomas, the surgeon's aim is to remove all the tumor with as little normal tissue as possible. The surgeon may use a modified Mohs technique to do this or orientate the specimen with pins and sutures. This will then be treated in the laboratory as a wedge excision and the margins carefully marked.

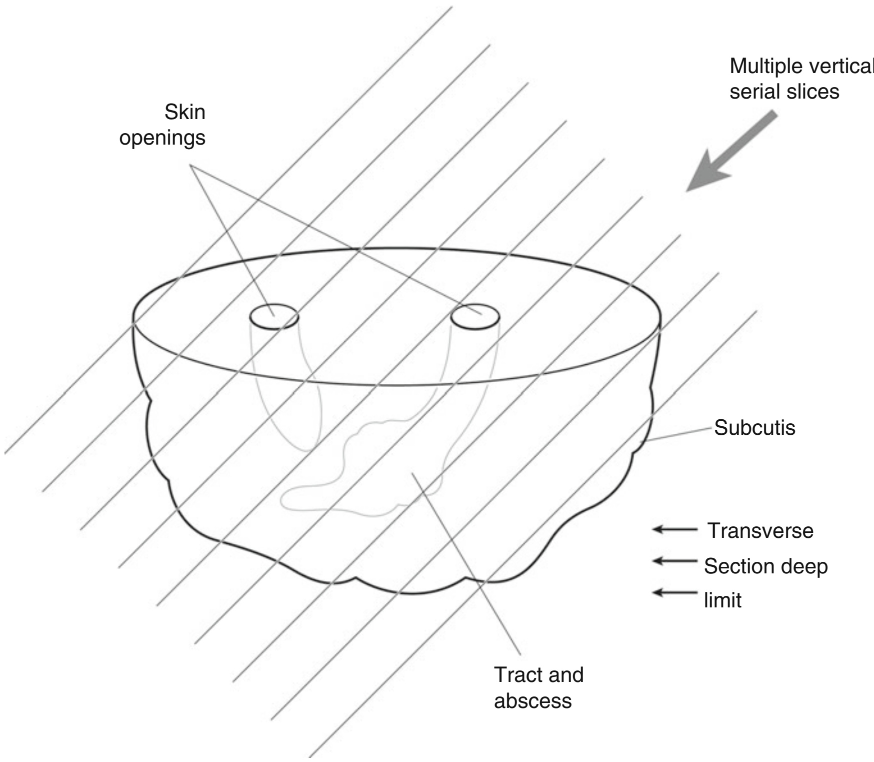

Fig. 38.8 Blocking a pilonidal sinus specimen (Reproduced, with permission, from Allen and Cameron (2004))

Ear: The ear may be involved in skin rashes, benign and malignant tumors. Skin rashes rarely only involve the ear, and skin from elsewhere should be sampled. Benign lesions will have a variety of biopsy samples which are dealt with in the usual way. Tumors are often removed as a wedge and dealt with accordingly (Fig. 38.6).

Lip: Lip biopsies from benign lesions are treated as other biopsies, but malignant tumors are removed as a wedge and dealt with accordingly (Fig. 38.6).

Pilonidal sinus: Occurs in the natal cleft of young to middle-aged males due to insinuation of hair shafts into the dermis and subcutis forming a tract variably lined by epidermis and/or granulation tissue. It is associated with serous discharge and potentially infection with pain and abscess formation. There may be several tracts present and communication points with the surface epidermis. Treatment involves wide elliptical excision of the skin and subcutis down to the level of the sacral fascia. The specimen is measured, and the presence of opening(s)/tract(s) noted. A horizontal transverse block of the deep limit allows microscopic assessment of tract extension to the deep margin. The tract is demonstrated by serial vertical slices (Fig. 38.8).

38.9 Histopathology Reports

Inflammatory disease:
- Specimen site, type, and size (mm)
- Description of histological findings
- Diagnosis or differential diagnosis with most likely diagnosis

Benign tumors:
- Specimen site, type, and size (mm).
- Tumor type and brief description of lesion.
- If excision biopsy, is the lesion excised?

Malignant tumors:

38.9.1 Basal cell carcinoma

- Specimen site:
- Specimen type:
- Specimen size:
  Length _________ mm
  Breadth ________ mm
  Depth __________ mm
- Size of lesion:
  Length _________ mm
  Breadth ________ mm
  Depth __________ mm
- Growth pattern:
  Nodular/superficial/infiltrative (morphoeic)/micronodular/other
- Differentiation:
  Severely atypical or malignant squamous component present—yes/no
- Invasion:
  Lymphatic/vascular invasion:
  Yes/no/uncertain
  Perineural invasion:
  Yes/no/uncertain
- Excision margins:
  Nearest peripheral:
  Not involved ________ mm/involved
  Nearest deep:
  Not involved ________ mm/involved
- Extent of local tumor spread (see squamous cell carcinoma)

38.9.2 Squamous cell carcinoma

Specimen site:
- Specimen type:
- Specimen size:
  Length ________ mm
  Breadth _______ mm
  Depth _________ mm
- Maximum diameter of lesion:
  _________ mm
- Histology suptype:
  Classical/spindle cell/acantholytic/verrucous/desmoplastic/other
- Differentiation
  Grade I (over 75% differentiated)
  Grade II (between 25 and 75% differentiated)
  Grade III (under 25% differentiated)
  Grade IV (no differentiation)
- Tumor thickness:
  _________ mm
- Clark level:
  I/II/III/IV/V
- Invasion:
  Lymphatic/vascular invasion:
  Yes/no/uncertain
  Perineural invasion:
  Yes/no/uncertain
- Excision margins:
- Nearest peripheral:
  Clear _______ mm/involved (*in situ*/ invasive)
- Nearest deep:
  Clear _______ mm/involved (*in situ*/ invasive)
- Extent of local tumor spread:

pTis	Carcinoma *in situ*
pT1	Tumor ≤20 mm in greatest dimension
pT2	Tumor between 21 and 50 mm
pT3	Invasion into deep structures, e.g., muscle, bone, cartilage, jaws, or orbit
pT4	Direct or perineural invasion of skull base or axial skeleton
pN0	No regional lymph node metastasis
pN1–3	Metastasis in regional lymph node(s)

38.9.3 Malignant melanoma

- Specimen site:
- Specimen type:
- Specimen size:
  Length _______ mm
  Breadth ______ mm
  Depth ________ mm
- Lesion size:
  Length _______ mm
  Breadth ______ mm
  Depth ________ mm
- Lesion margins:
  Regular/irregular
- Profile:
- Histogenic type:
  Lentigo maligna/superficial spreading/nodular/acral lentiginous/desmoplastic/neurotropic
- Clark level:
  I/II/III/IV/V

- Breslow's depth:
  _________ mm
- Mitoses/mm^2:
- Growth phase:
  Radial/vertical
- Ulceration:
  Yes—diameter _______ mm/no
- Microscopic satellitosis:
- Excision margins:
  Nearest peripheral:
  Clear _____ mm/involved (*in situ*/invasive)
  Nearest deep:
  Clear _____ mm/involved (*in situ*/invasive)
- Extent of local tumor spread:

pTis	Melanoma *in situ*
pT1	Tumor ≤1.0 mm
	(a) Without ulceration and mitoses <1/mm^2
	(b) With ulceration or/and mitoses ≥1/mm^2
pT2*	Tumor 1.01–2.0 mm
pT3*	Tumor 2.01–4.0 mm
pT4*	Tumor > 4.0 mm
	*(a) Without ulceration
	(b) With ulceration
pN1*	1 lymph node involved
pN2*	2–3 lymph nodes involved
pN3*+	≥4 lymph nodes involved
	*(a) Micrometastasis
	(b) Macrometastasis
	+(c) In-transit metastasis/ satellites without metastatic lymph nodes
pM1a	Distant skin or nodal metastasis
pM1b	Lung metastasis
pM1c	All other visceral metastases any distant metastases with elevated serum LDH

Lymph nodes: Lymph nodes may be removed where there is tumor involvement in patients with squamous cell carcinoma or malignant melanoma. If the lymph node is subcutaneous, a fine needle aspiration will be carried out to confirm the diagnosis prior to surgical removal. The node(s) should be weighed, measured, counted, and submitted for histological examination

Sentinel node biopsy: Sentinel node biopsy is used in some centers in patients with biopsy-proven malignant melanoma of the skin. The sentinel node is the first drainage node at the site of the excised malignant melanoma. This is removed and examined in the laboratory for microscopic tumor using multiple step sections through the node and both hematoxylin and eosin and immunocytochemistry markers including S100, Melan-A, and HMB45 to confirm small microscopic deposits of malignant melanoma. Subsequent regional lymph node block dissection may then be carried out.

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Part IX

Cardiothoracic Specimens and Vessels

39 Lung

Kathleen M. Mulholland

39.1 Anatomy

The combined weight of both lungs is approximately 850 g in the male, 750 g in the female. The apex of each lung is situated in the root of the neck, the base of the lung on the diaphragm. The mediastinum is medial to the lungs, and ribs are present laterally. The hilum of each lung contains a main bronchus, pulmonary artery, two pulmonary veins, pulmonary nerve plexus, and lymph nodes.

The lungs are separated into lobes by invaginations of pleura along fissures (Fig. 39.1). The right lung is divided into three lobes by the oblique and horizontal fissures, the left into two lobes by the oblique fissure. The inferior part of the left upper lobe (the lingula) is the homologue of the right middle lobe.

The trachea branches at the level of T4 and T5 into two main bronchi. The right bronchus enters the lung behind the right pulmonary artery, the left bronchus crosses behind the left pulmonary artery and enters the lung below it. The main bronchi divide to give five lobar bronchi. These then divide into segmental bronchi supplying the 19 bronchopulmonary segments. Segments are roughly wedge shaped with their base at the pleural surface. Each segment is supplied by a segmental artery and bronchus. Veins draining segments often anastomose with those from adjacent segments. Bronchopulmonary segments can be resected with little hemorrhage or leakage of air from adjacent raw surfaces. Further divisions of the bronchi produce bronchioles. Glands and cartilage are present in the walls of bronchi but not in bronchioles.

Bronchioles have a diameter of less than 1 mm. Terminal bronchioles lead to respiratory bronchioles, which branch to produce alveolar ducts, alveolar sacs, and then alveoli. The lung may be

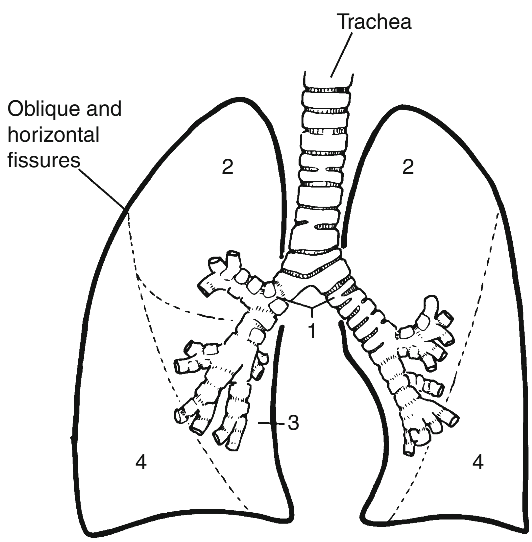

Fig. 39.1 Anatomy of the lungs. *1* Main bronchus, *2* upper lobe, *3* middle lobe, *4* lower lobe (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

K.M. Mulholland
Histopathology Laboratory, Altnagelvin Hospital, Western Health and Social Care Trust, Londonderry, UK
e-mail: kathleen.mulholland@westerntrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*, DOI 10.1007/978-0-85729-673-3_39, © Springer-Verlag London 2013

divided into lobules, areas that measure 1–2 cm across and are poorly demarcated by incomplete fibrous septa. Each lobule is made up of 3–10 acini. An acinus or terminal respiratory unit is that portion of the lung supplied by one terminal bronchiole. Pulmonary arteries are found at the centers of each acinus alongside bronchioles, whereas pulmonary veins run in the interlobular septa. Flattened type 1 pneumocytes and occasional rounded type two pneumocytes line the alveoli.

Lymphovascular Drainage

Lymphatic drainage is by a superficial subpleural lymph plexus and a deep plexus of vessels accompanying the bronchi. Both groups drain through hilar (bronchopulmonary) lymph nodes into tracheobronchial nodes around the bifurcation of the trachea and from there into mediastinal lymph trunks.

Blood supply to the lungs is by a dual arterial supply – the pulmonary arteries and the bronchial arteries. Blood returns to the heart via the pulmonary veins or travels in the bronchial veins to the azygos or accessory hemiazygos veins.

39.2 Clinical Presentation

Respiratory symptoms include cough, sputum production, dyspnea (undue respiratory effort), orthopnea (breathlessness on lying down), wheeze due to airway obstruction, hemoptysis (coughing up blood), and chest pain. The commonest cause of hemoptysis is acute infection. Other causes include tuberculosis and pulmonary infarction. Tumor, e.g., carcinoid or bronchial carcinoma, may cause hemoptysis due either to ulceration of the expanding tumor or secondary infection caused by obstruction. Chest pain can be localized and pleuritic, due to infection or infarction or constant, severe, and dull due to chest wall invasion by carcinoma. Lung cancer can produce secondary pneumonia, bronchiectasis, pleural effusions, hoarseness (laryngeal nerve involvement), and paralysis of the diaphragm (phrenic nerve involvement). Tumors in the apical part of the upper lobe (Pancoast's tumors) may result in unilateral Horner's syndrome due to involvement of sympathetic ganglia. Pancoast's tumors may also produce pain and weakness in the shoulder and arm due to invasion of the brachial plexus. Mediastinal disease may lead to superior vena cava syndrome with congestion and edema of the face, neck, and chest associated with dyspnea.

Paraneoplastic syndromes are systemic effects of tumors not mediated by tumor spread. They include Cushing's syndrome, inappropriate antidiuretic hormone secretion, and acromegaly. Weight loss may occur in chronic inflammatory diseases such as TB or secondary to tumors.

39.3 Clinical Investigations

- Chest x-ray: primary technique for imaging the thorax
- CT scan – the only cross sectional imaging technique that adequately evaluates the lung parenchyma and is equivalent to MRI in the evaluation of mediastinum, pleura, and chest wall. CT has a sensitivity of 80–94% compared with results obtained by mediastinoscopy and lymph node sampling. CT scans are used to stage carcinoma of bronchus and may be extended to include liver, adrenal glands, and brain.
- High Resolution (thin section) CT scan (HRCT) – slices are 1–3 mm thick. HRCT is of value for the investigation of sarcoidosis, lymphoma, cryptogenic and extrinsic alveolitis, occupational lung disease, bronchiectasis, and lymphangitis carcinomatosa. It is also used to distinguish emphysema from interstitial lung disease or pulmonary vascular disease.
- Spiral CT scan and Ventilation/Perfusion (V/Q) scan – used in detection of pulmonary emboli.
- MRI – less useful than CT because of poorer imaging of lung parenchyma. MRI may be used in assessing disease near the lung apex, the spine, the thoracoabdominal regions, and the mediastinum. It can detect invasion into spinal cord, vertebral bodies, brachial plexus and chest.
- PET scan – used to distinguish between benign and malignant conditions. If the lesion does not demonstrate high radiation activity, it is interpreted as having a low metabolic rate and is likely to be benign. Conversely it demonstrates FDG–avid primary cancers and their local and distant metastases.

- Respiratory function tests – include peak expiratory flow rate (PEFR), forced expiratory volume in 1 s (FEV1), vital capacity (VC), forced expiratory ratio (FEV/VC) and carbon monoxide transfer (Tco). These tests are helpful in differentiating between lung disease due to airways obstruction, restrictive conditions, or respiratory muscle weakness.
- Measurement of blood gases – important in the diagnosis of respiratory failure.
- Full blood picture (FBP) – hemoglobin: Anemia, PCV: Secondary polycythemia.
- Biochemistry – alpha-1-antitrypsin levels, autoantibodies, *Aspergillus* antibodies, IgE to specific allergens. Hypercalcemia occurs in one in five patients with sarcoidosis, as a paraneoplastic syndrome notably in association with squamous cell carcinoma and secondary to bony metastases. Hyponatremia is seen in association with small cell carcinoma.
- Sputum – cytological examination of sputum can detect between 60% and 90% of malignancies if multiple specimens are examined. Gram stain and culture are of value in pneumonia, TB, and *Aspergillus*.
- Transthoracic needle aspiration and biopsy (TTNA and TTNB)/percutaneous needle aspiration and biopsy – performed under fluoroscopic or CT guidance. They successfully diagnose lung cancer with at least 85–90% accuracy. The most common indication for aspiration is to evaluate a solitary peripheral lung nodule suspicious of carcinoma. Biopsy is more appropriate when lymphoma or sarcoidosis is suspected. Needle core biopsies may be obtained from lesions close to the chest wall, while more central lesions require fine needle aspiration.
- Bronchial brushings and washings – fiberoptic bronchoscopy (FOB) can reach and sample up to 90% of malignancies.
- Transbronchial fine needle aspiration – at the time of FOB makes submucosal and paratracheal lesions accessible.
- Endobronchial and transbronchial biopsy – lead to a histological diagnosis in 95% of central lung carcinomas, but in only 50–75% of peripheral lesions. Transbronchial biopsy is of particular use in the diagnosis of sarcoidosis and lymphangitis carcinomatosa.
- Bronchoalveolar lavage (BAL) – using a flexible fiberoptic bronchoscope small volume lavages (up to 300 ml) are performed. BAL may be used to monitor progression of interstitial lung disease. It is useful in eosinophilic pneumonia, eosinophilic granuloma, pulmonary alveolar proteinosis, and in the diagnosis of opportunistic infections, e.g., in the immunosuppressed.
- Mediastinoscopy – allows access to, and biopsy of, lymph nodes. Cervical mediastinoscopy accesses paratracheal and subcarinal mediastinal nodes for diagnostic and staging purposes. Anterior mediastinoscopy is used primarily to sample enlarged nodes or tumor in the left aortopulmonary window region.
- Frozen sections – sent intra-operatively to distinguish between inflammatory and neoplastic parenchymal lesions, as a prequel to a cancer resection operation or a lung-sparing wedge resection. These specimens should be handled with care in a microbiological safety cabinet. If there is any suspicion of tuberculosis, frozen sections are inappropriate and should not be performed. Such tissue needs thorough formalin fixation.
- Open/closed lung biopsy – used to evaluate pleural/peripheral lesions and interstitial lung disorders.

39.4 Pathological Conditions

39.4.1 Non-neoplastic Conditions

Bacterial pneumonia: Lobar pneumonia can be of rapid onset in otherwise healthy patients, and entire lobes are involved by neutrophilic infiltrates. Bronchopneumonia affects older, debilitated patients and is characterized by more circumscribed infiltrates.

Lung abscess: An area of infection with parenchymal necrosis. Primary lung abscess occurs more often on the right side as the right main bronchus leads more directly off the trachea and aspiration can occur more easily. Secondary abscesses occur when there are predisposing factors such as carcinoma, foreign body, or bronchiectasis.

Viral infection: May occur in the lungs due to respiratory viruses such as influenza or in an immunocompromised patient (cytomegalovirus, respiratory syncytial virus, varicella zoster, or herpes simplex). Histological examination shows alveolar cell injury with a mononuclear cell interstitial infiltrate.

Tuberculosis: The characteristic histological lesion is the caseating granuloma. Primary TB presents with a solitary parenchymal nodule and hilar lymph node involvement. Secondary TB may present as miliary TB, tuberculous pneumonia, or cavitary TB.

Mycotic infections: Tangled masses of fungal hyphae and debris may be found in lung cavities and are known as fungal balls. These are usually non-invasive unless the patient is immunocompromised. *Aspergillus fumigatus* is the most common cause, the fungal balls being called aspergillomas. Surgery may be needed for diagnosis and treatment of disease resistant to medical treatment.

Pneumocystis jiroveci: A fungal organism, which occurs in immunocompromised patients. Classically there is an acellular intra-alveolar exudate. However, *Pneumocystis jiroveci* can produce any pattern of lung injury. Silver stains or antibody techniques demonstrate the organism.

Chronic bronchitis and *emphysema*: Often occur together. Emphysema is characterized by an increase in the size of airspaces distal to the terminal bronchioles. It is classified into three types depending on the part of the lung involved by the process – centrilobular, panlobular (panacinar), and paraseptal. Chronic bronchitis results from hypersecretion from bronchial mucous glands.

Bronchiectasis: Permanent abnormal dilatation of the bronchi with infection of the bronchial wall and obliteration of distal airways. Cystic fibrosis is the most common predisposing factor.

Endogenous lipoid pneumonia: May occur distal to a lung tumor and is secondary to breakdown of lung parenchyma. The alveoli contain lipid-laden macrophages.

Pneumoconiosis: Defined as permanent alteration of lung structure due to inhalation of mineral dusts and tissue reactions, which follow this. Included in this group are *silicosis*, *asbestosis*, *coal worker's pneumoconiosis*, *hard metal disease*, and *berylliosis*. *Asbestosis* is a form of interstitial fibrotic lung disease secondary to asbestos exposure. Fibrosis is characteristically found in the lower lobes, especially in the subpleural areas. Asbestos bodies are present in the lung parenchyma. Asbestosis may be graded depending on the amount of lung substance involved and the severity of the fibrosis. Other asbestos related conditions include benign pleural plaques, diffuse pleural thickening, and malignant mesothelioma. The incidence of carcinoma of the lung is increased in those with a history of asbestos exposure.

Interstitial pneumonia/cryptogenic fibrosing alveolitis/pulmonary fibrosis: Chronic inflammatory disease, which shows thickening of the alveolar walls, initially by lymphocytes and plasma cells, later by fibroblastic proliferation. Eventually "honeycomb lung" is produced with scarring and multiple air-filled spaces. The need to assess both spatial and temporal distribution of the pathology means that open or thoracoscopic lung biopsies from different zones are usually required. Of clinical value is the sub classification of interstitial pneumonia as prognosis and response to treatment varies between subgroups. These include *usual interstitial pneumonia* (*UIP*), *desquamative interstitial pneumonia* (*DIP*), *respiratory bronchiolitis-associated interstitial lung disease* (*RBILD*), and *non-specific interstitial pneumonia* (*NSIP*). Prognosis and response to treatment are worse for UIP than other subgroups (5-year survival 55%).

Immune-mediated lung diseases: *Extrinsic allergic alveolitis* is a chronic granulomatous disease of the lungs due to inhalation of organic dusts, e.g., farmer's lung, bird – fancier's lung, mushroom worker's lung. Upper lobes are more severely affected than basal portions with fibrotic changes occurring in advanced disease.

Wegener's granulomatosis: In the lungs, it is characterized by vasculitis and granulomas. Isolated pulmonary or associated upper respiratory or renal disease. Serum c-ANCA positive.

Sarcoidosis: Occurs most often in the lungs though lymph nodes, skin, eyes, liver, and spleen may also be affected. Characteristically, sharply circumscribed non-caseating epithelioid granulomas are present, and 25% of cases show marked interstitial fibrosis.

Pulmonary vascular disease: *Emboli* that lodge in peripheral arteries cause pulmonary infarcts in patients, whose pulmonary circulation is already compromised.

Pulmonary hypertension: Primary or secondary. Changes in the arteries may be graded according to the Heath-Edward's classification.

Lung transplantation: The most common indication for lung transplantation is emphysema, e.g., secondary to alpha-1-antitrypsin deficiency. Other indications include chronic obstructive pulmonary disease, septic disease such as cystic fibrosis, fibrotic lung disease, and primary pulmonary hypertension. Surveillance involves transbronchial biopsy to look for rejection, which is graded according to the 1995 working classification.

Rare conditions of variable neoplastic potential include *Langerhans cell histiocytosis*, *lymphomatoid granulomatosis*, and *pulmonary lymphangioleiomyomatosis*.

39.4.2 Neoplastic Conditions

39.4.2.1 Benign Tumors

Most benign tumors are identified incidentally on chest X-ray as solitary lung nodules. The use of minimally invasive surgery such as video-assisted thoracic surgery has lowered the threshold for early referral and surgical excision.

Chondroid hamartoma: The most common benign lung tumor. It consists of a mass of cartilage with entrapped epithelial structures. Other connective tissue elements such as bone, adipose tissue, and fibrous tissue may be present.

Other benign tumors include *lipoma*, *sclerosing hemangioma*, and *hemangiopericytoma.*

39.4.2.2 Malignant Tumors

Lung cancer causes approximately 40,000 deaths annually in the UK and has a strong association with cigarette smoking. Two thirds of lung cancers are inoperable at the time of diagnosis. Traditionally lung cancer is classified into either small cell carcinoma (SCLC) or non-small cell carcinoma (NSCLC). The behaviour and treatment of the two groups differs. In general, SCLC is treated by chemotherapy, whereas NSCLC, after appropriate clinical staging, is either resected (stage pT2 N1 disease or less) or treated with radiotherapy or chemotherapy. Few patients with SCLC survive longer than 12–19 months. Patients with NSCLC have an average 5-year survival of 10–15%.

Increasingly, non-small cell lung carcinomas are being further sub-classified by specific cell type, e.g., squamous cell carcinoma, adenocarcinoma, using ancillary techniques such as immunohistochemistry if morphology is unhelpful. Molecular testing for targeted therapies has been introduced, e.g., epidermal growth factor receptor (EGFR) status testing.

Squamous cell carcinoma: The commonest carcinoma of the lung (30–45%). It is a malignant epithelial neoplasm showing at least one of the following: keratinization as single-cell keratinization or keratin pearls or intercellular bridges. Squamous cell carcinomas mainly occur centrally in a main or lobar bronchus and can reach a considerable size with central necrosis. Histological variants include *papillary*, *clear cell*, *small cell*, and *basaloid carcinoma.*

*Adenocarcinoma***:** A malignant epithelial neoplasm showing glandular differentiation. It is the lung malignancy, which occurs most frequently in non-smokers, females, and in the young. It often involves the upper lobes and may present peripherally as a subpleural mass or nodule, with retraction of the pleura. Central scarring in the tumor is quite common.

Secondary adenocarcinomas may come from pancreas, colon, ovary, or kidney and show various patterns. If there is involvement of the hilar nodes and significant scarring, then the tumor is more likely to be a primary; if multiple tumors are present, it is more likely to be secondary. Lung adenocarcinoma is classified into the following subtypes.

Bronchioloalveolar/lepidic carcinoma (*BAC*): A histological variant of adenocarcinoma, which has low-grade malignant behaviour. It is an adenocarcinoma in situ (AIS), which grows in a lepidic manner along alveolar septa without invasion into the interstitium. BACs are most frequently non-mucinous but can be mucinous. It may be multifocal or diffuse, when it can mimic pneumonia.

Other variants of adenocarcinoma include *papillary carcinoma*, *acinar adenocarcinoma*, *solid carcinoma with mucin formation* and *mixed subtypes*. *Adenosquamous carcinoma* shows areas of both squamous cell and adenocarcinomatous differentiation, with the minor component accounting for at least 10%. Prognosis is worse than for pure squamous cell carcinoma or adenocarcinoma.

Large cell carcinoma: Accounts for 5–10% of all lung malignancies and early metastasis is common. Histological examination shows sheets of large tumor cells with no glands or keratinization. Variants include *large cell neuroendocrine carcinoma*, *basaloid large cell carcinoma*, *lymphoepithelioma-like carcinoma*, *clear cell carcinoma*, and *large cell carcinoma with rhabdoid phenotype.*

Typical carcinoid tumors: Account for 90% of bronchial carcinoids. The vast majority are cured by complete excision with more than 90% 10-year survival. They are of low malignant potential – only 10–15% spread to local lymph nodes and distant metastases are rare. Typical carcinoids may occur either centrally or peripherally. Grossly, they are yellowish or pale tan and may be "dumbbell"-like, as they extend into the lumen of the bronchus and the lung parenchyma. They are composed of a uniform cell population arranged in ribbons, cords, or islands. In peripheral carcinoids, the cells are often spindle shaped. They have fewer than two mitoses per ten high power fields and show no necrosis. It is recommended that resection margins should be to within 5 mm of the tumor.

Atypical carcinoids: Metastasize in 50–70% of cases with a 5-year survival of 60%. Necrosis, which is usually focal, and increased mitotic activity (>2–10/10 high power fields) are the most reliable indicators of malignant behaviour.

Small cell carcinoma (*SCLC*): Accounts for 20% of all lung cancers. SCLC is most often located centrally and tends to metastasize early. It is the lung carcinoma most frequently associated with paraneoplastic syndromes. It is primarily treated with chemotherapy, the role of surgery usually being limited to obtaining a definitive tissue diagnosis and for staging. Histological examination shows small- or medium-sized cells with scanty cytoplasm, arranged in nests, ribbons, or strands but often showing a lack of an architectural pattern. A variant of small cell carcinoma is a combined tumor where other tumor elements such as squamous or adenocarcinoma are present.

Salivary gland tumors: *Adenoid cystic carcinoma* is most commonly located in the trachea and major bronchi. The tumor shows a cribriform pattern with tubular and solid areas. Perineural infiltration is common. It is generally slow growing. *Mucoepidermoid carcinoma* arises from minor salivary gland lining the tracheobronchial tree.

Other cancers: *Pleomorphic carcinoma*, *spindle cell carcinoma*, and *giant cell carcinoma* contain spindle cells and/or giant cells. *Carcinosarcomas* contain both malignant epithelial and sarcomatous elements. *Blastomas* have a biphasic pattern consisting of epithelial tubules or cords in an undifferentiated stroma.

Malt lymphoma is the commonest primary lung lymphoma. It may be solitary or multifocal. Histological examination shows a monomorphic population of centrocyte-like cells. Most malt lymphomas are low grade but can transform to high grade. Lymphoma may also present in the lung secondary to nodal or systemic disease.

Hodgkin's disease is usually secondary to spread from mediastinal disease. *Malignant melanoma* is usually due to metastasis from another site.

Tracheal tumors: Primary tracheal tumors are rare, secondary tumors being more common. In adults, most primary tracheal tumors are malignant and include s*quamous cell carcinoma*, which is usually locally advanced at the time of presentation, and *adenoid cystic carcinoma*.

Chest wall tumors: *Malignant small cell tumor of the thoracopulmonary region* (*Askin Tumor)* occurs in the first two decades of life. It is composed of sheets of undifferentiated, small, hyperchromatic cells, which may form rosettes around a central tangle of fibrillary cytoplasmic processes. Other chest wall tumors include *extra-abdominal desmoid tumors*, *elastofibroma dorsi*, and primary tumors of muscle, fat, blood vessels, nerve sheath, or bone.

39.5 Surgical Pathology Specimens: Clinical Aspects

39.5.1 Biopsy Specimens

Percutaneous/transthoracic needle biopsy: Performed under X-ray guidance, an 18-gauge needle is inserted with the aid of a spring-loaded firing device. The biopsy is rinsed directly into the fixative. Occasional cases require fresh tissue to be sent for microbiological culture. Fresh frozen or glutaraldehyde fixed tissue may be needed for special investigations (specialized immunohistochemistry, electron microscopy).

Endobronchial/transbronchial biopsies: Taken by rigid or flexible bronchoscopy. The rigid bronchoscope ranges from 3 to 9 mm in diameter and may be used for straight ahead viewing or at 30° or 90° for visualization of the upper lobe bronchi. It is essential for complete examination of the trachea as a flexible bronchoscopy may miss lesions. It may also be used for brush cytology, biopsy, and to trap sputum for cytology and culture.

However, it allows visualization of major lobar orifices only and is usually performed under general anesthetic. Flexible bronchoscopes have outer diameters ranging from 3 to 6 mm. Light is transmitted through fiberoptic bundles. The bronchoscope can be attached to a video camera for large screen display. The working channel allows insertion of various diagnostic and therapeutic accessories. Biopsy forceps are inserted to obtain bronchial or transbronchial biopsies. Lesions not accessible to direct biopsy can be approached with a brush to obtain specimens for cytological or microbiologic analysis. Needles may also be used for aspiration and biopsy. Flexible bronchoscopy is the endoscopic procedure of choice as it is simple, quick to use, and is performed under local anesthetic.

Open lung biopsies: Obtained by thoracotomy for the assessment of peripheral lung disease.

39.5.2 Resection Specimens

Video-assisted thoracoscopic surgery (*VATS*): Direct thoracoscopy is being replaced by video-assisted thoracoscopic surgical (VATS) technique. VATS allows access to peripleural lung nodules, biopsy, and sampling of mediastinal nodes, especially in the aortopulmonary window, examination of the pleural space for tumor, wedge resection of lung for diagnosis of diffuse lung infiltrates, or peripheral nodules, and resection of apical pleural blebs for spontaneous pneumothorax.

Wedge/segmental resections: Obtained via open lung biopsy or video-assisted closed chest biopsy. They are used to sample focal areas that are suspicious, e.g., pleural-based nodules or to resect tumors if a patient cannot tolerate a more extensive procedure. Recurrence rates of tumor are higher than with more radical surgery.

Bullectomies: Used to excise bulla to improve lung function via a median sternotomy approach, posterolateral thoracotomy, or VATS.

Bronchoplastic or sleeve resections: Used as an alternative to pneumonectomy. They are lung sparing and are typically used to resect proximal endobronchial lesions at, or adjacent to, the carina in order to preserve distal, uninvolved lung.

Sleeve lobectomy: Excision of a lobe with the associated lobar bronchus and subsequent anastomosis of the distal bronchial tree to the proximal airway.

Bronchial sleeve resection: Resection of either mainstem bronchus with anastomosis of the distal airway to the carina or lower trachea.

Sleeve pneumonectomy: Resection of the carina with pneumonectomy and anastomosis of the contralateral distal bronchus to the distal trachea.

Lobectomy/bilobectomy: Resection of one or two lobes of lung and includes complete resection of hilar (N1) lymph nodes draining the primary tumor.

Pneumonectomy: Resection of the whole lung and accounts for 20% of all lung resections. It is indicated when tumor invades hilar structures such as the mainstem bronchus or the main pulmonary artery. It is also indicated when tumor crosses the oblique fissure or when there is lymph node involvement along the mainstem bronchus proximal to the upper lobe take-off. In the majority of cases, it is for the purpose of resecting tumors, an exception being recipient pneumonectomy performed prior to lung transplant.

Extended pulmonary resection: Extension of the limits of conventional pulmonary resection with an en bloc excision of contiguous intrathoracic structures involved by tumor.

Extrapleural pneumonectomy: Indications include resection of malignant mesothelioma or rarely carcinoma of the lung, which is restricted to the lung, pleura, and local lymph nodes.

Surgical incisions: The most versatile approach used by the thoracic surgeon is the *posterolateral thoracotomy*. The incision is 8 cm lateral to the 6th thoracic spinous process and curves forward, 2 cm below the tip of the scapula to the mid-axillary line in the line of the ribs. It is used in unilateral lung resection, chest wall tumor resection, unilateral lung volume reduction surgery, bullectomy, and tumors of the posterior mediastinum.

Anterolateral thoracotomy and axillary thoracotomy are used with decreasing frequency. *Median sternotomy* is used in mediastinal tumor resection, bilateral lung volume reduction surgery or bullectomy, resection of multiple pulmonary lesions (e.g., metastasis) and transpericardial access to the trachea or bronchus, e.g., carinal tumors.

The thoracoabdominal approach is used in procedures where access is needed to both pleural and peritoneal cavities. Bilateral anterior thoracotomies (also known as the clamshell incision) provide maximum exposure of both hemithoraces and mediastinal structures. This approach is used in bilateral lung resections such as lung transplant and lung reduction surgery.

Lung volume reduction surgery (*LVRS*): A palliative surgical procedure for patients with end-stage emphysema in which 20–30% of each upper lobe is resected.

39.6 Surgical Pathology Specimens: Laboratory Protocols

39.6.1 Biopsy Specimens

Small biopsies are promptly put into 10% formalin fixative.

Endobronchial/transbronchial biopsies:

- Count the number of fragments.
- Record the greatest dimension of the largest fragment (mm).
- Note the color and consistency of the fragments.
- Place the fragments in cassettes between foam pads or wrapped in filter paper or in molten agar for processing.
- Cut through multiple levels, keeping intervening spares for further stains.

Open lung biopsy:

- Re-inflate the lung using a formalin injection.
- Record the size (mm) and weight (g)/number of fragments.
- Describe the color and consistency.
- Serially transverse section at 3 mm intervals and sample representative blocks.

Histopathology report:

- Nature of biopsy.
- Size of biopsy (mm), number of fragments.
- Bronchial wall – epithelium, thickness of wall, goblet cell hyperplasia, malignancy.
- Alveoli – count approximate number, type I or II pneumocyte proliferation, inflammation, macrophages in lumen, consolidation.
- Nature of inflammatory cells if present.
- Fibrosis if present: type – interstitial, intra-alveolar (BOOP), grade of fibrosis – mild, moderate, severe.
- Other pathology – vascular, asbestos bodies, doubly refractile material.
- If transplant, grade rejection according to the 1995 working formulation.

39.6.2 Resection Specimens

Larger specimens are put into dry containers and brought promptly to the laboratory.

39.6.2.1 Wedge resections (or Cornish pastie)

- Consist of a triangular segment of lung and pleura with two staple lines at the margin (Fig. 39.2).
- Palpate the specimen to locate the lesion.
- Record the dimensions (cm).
- Describe the pleura.
- Inflate with a syringe of formalin. A disadvantage of inflation fixation is that free cells may

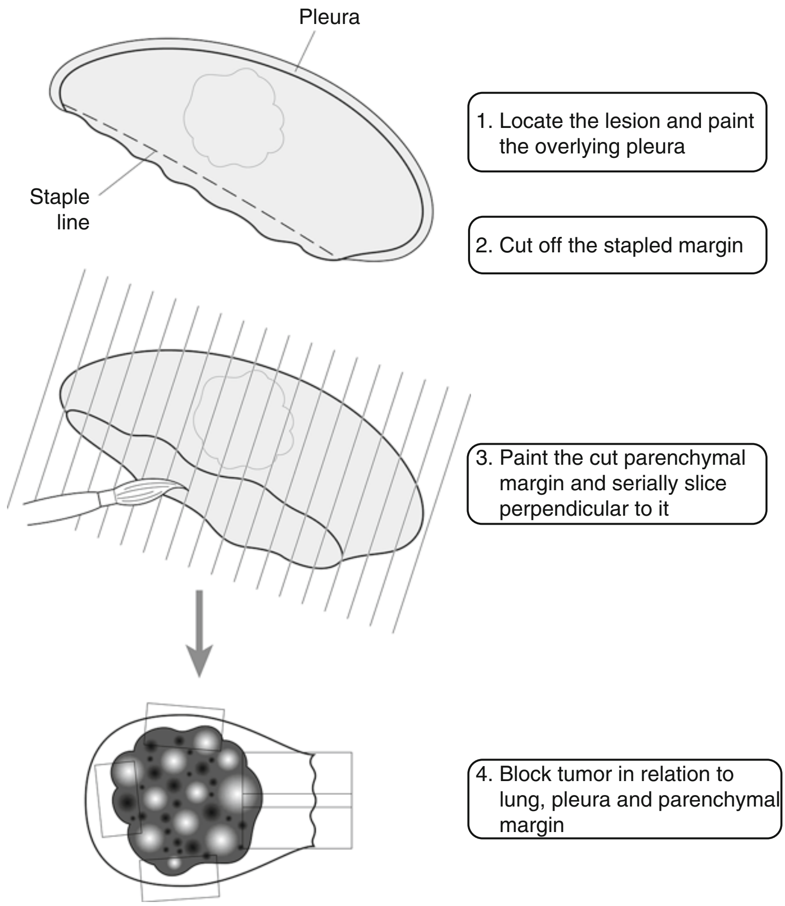

Fig. 39.2 Blocking a wedge resection of lung (Reproduced, with permission, from Allen and Cameron (2004))

be cleared from consolidated alveoli so that diagnoses such as desquamative interstitial pneumonia (DIP) are obscured. Measure the length of the margin. Cut off the staple line as closely as possible. The cut surface of the lung can be taken *en face* or perpendicularly. The open surface is inked.

- Ink the pleural surface over the lesion.
- Serially transverse section at 3 mm intervals.
- Describe the lesion – size, color, pleural involvement, distance (mm) from margin.
- Describe the remainder of the lung.
- Take representative sections of any lesion, of its relationship to the pleura and uninvolved lung, and the closest margin.
- If the lung disease is diffuse, submit the vast majority of the specimen for histology.

39.6.2.2 Lung Resection for Tumors

Most resections are for tumor: lobectomy, bilobectomy, and pneumonectomy.

Initial procedure:

- Palpate to locate tumor or areas of abnormality.
- Specify which lung or lobe.
- Record the weight (g) and dimensions (cm). Ink the pleura overlying the tumor.
- Remove the bronchial margin by sectioning transversely, before inflation fixation (Fig. 39.3). Sample the hilar nodes.
- Inflation: If the specimen is intact, instill fixative from a height of about 25 cm via tubing that terminates in a nozzle wedged into the supply bronchus or bronchi. Continue until the pleural surface is smooth. Immerse in a container of fixative overnight with a covering of lint or filter paper to prevent drying. If the specimen is not intact, inflate with a syringe. Remember to culture, if appropriate, before fixation.
- Allow to fix for 24–36 h.
- To access airways open from the hilum, pass a probe down to the tumor and then cut along it.

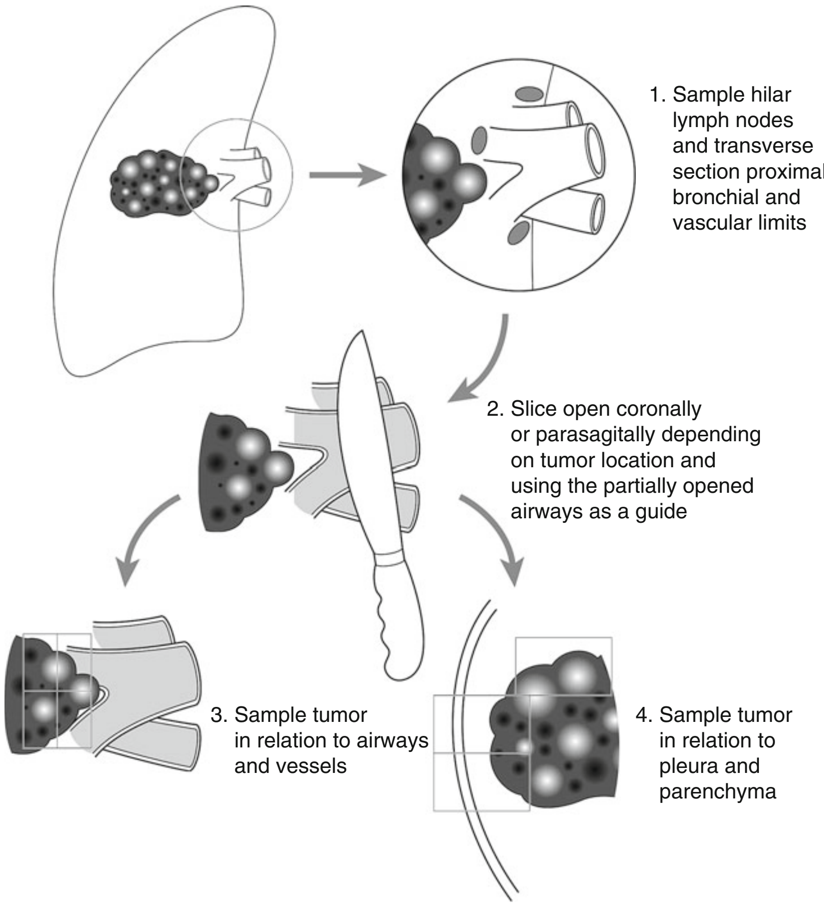

Fig. 39.3 Blocking a pneumonectomy specimen (Reproduced, with permission, from Allen and Cameron (2004))

- Serially slice the tumor at 3 mm intervals in the plane that best demonstrates its relationship to the anatomical structures. In general, mid-zone and peripheral lesions are sliced parasagittally, hilar lesions coronally.
- With vascular lesions such as pulmonary emboli, approach laterally within fissures cutting towards the hilum until the pulmonary artery is entered.
- Photograph.
- Ribs – decalcify.

Description:

- Lesion site – central/peripheral, main/segmental bronchus.
  - Endobronchial/bronchial/extrabronchial/extrinsic compression.
  - Distances (mm) to the bronchial or parenchymal resection margins/pleura.
- Lesion size – length × width × depth or maximum dimension (cm).
- Lesion appearance – color/consistency/necrosis/hemorrhage/cavitation.
- Lesion edges – circumscribed/infiltrative.
- Lung – emphysema/fibrosis/bullae/bronchiectasis/mucus plugging/post-obstructive pneumonia.
- Hilar lymph nodes – number/size/color/consistency.

Blocks for histology (Fig. 39.3):

- Transverse section the proximal resection margin and the pulmonary staple margin if the specimen is a lobectomy.
- Submit all hilar lymph nodes. The surgeon for staging purposes often also submits other separate named lymph node stations, and these are processed separately.

- Sample four sections of tumor showing relationships to uninvolved lung, adjacent bronchi, and vessels and the nearest aspect of the pleura.
- Sample uninvolved lung (one or two blocks – more if there is suspected asbestosis).
- Sample the margins of any attached parietal pleura, chest wall soft tissue, or ribs – represent the deepest point of rib invasion.

Histopathology report:

- Type of procedure – wedge resection, lobectomy, bilobectomy, pneumonectomy.
- Tumor type – squamous carcinoma/adenocarcinoma/small cell carcinoma/large cell carcinoma/neuroendocrine tumors/salivary gland type adenocarcinoma/others.
- Tumor differentiation – well/moderate/poor.
- Tumor edge – pushing/infiltrative/lymphoid response.
- Extent of local tumor spread. Elastin stain may be helpful in recognizing visceral pleural invasion.

Small cell carcinomas: Staging via TNM 7 is now recommended for those with limited disease

Limited disease – disease confined to one hemithorax including involvement of ipsi and/or contralateral hilar, mediastinal, or supraclavicular lymph nodes. Patients with ipsilateral pleural effusion, regardless of pleural cytology, should be included in this group.

Extensive disease – any disease beyond the definition of limited stage.

Carcinoid tumors: Staging via TNM 7 is now recommended for all cases

Non-small cell carcinoma

pTis	Carcinoma in situ
pT1a	Tumor ≤20 mm diameter
pT1b	Tumor >20–≤30 mm
pT2	Tumor≥20 mm from the carina, invades visceral pleura, partial atelectasis
pT2a	>30 to ≤50 mm
pT2b	>50 to ≤70 mm
pT3	Tumor >70 mm; involvement of parietal pleura, mediastinal pleura, chest wall, pericardium or diaphragm; tumor <20 mm from the carina; atelectasis/obstructive pneumonitis involving whole lung; separate nodule(s) in the same lobe
pT4	Involvement of great vessels, mediastinum, carina, trachea, esophagus, vertebra, or heart. Separate tumor nodule(s) in different ipsilateral lobe
pN0	No regional node involvement
pN1	Ipsilateral hilar/intrapulmonary nodes (node stations 10–14)
pN2	Ipsilateral mediastinal/subcarinal nodes (node stations 1–9)
pN3	Contralateral mediastinal, hilar, ipsilateral, or contralateral scalene, supraclavicular nodes
pM0	No distant metastasis
pM1	Distant metastasis
pM1a	Separate tumor nodule(s) in a contralateral lobe, pleural nodules or malignant pleural or pericardial effusion.
pM1b	Distant metastasis

- Excision margins – distances (mm) to the proximal bronchial, vascular and mediastinal limits and pleura.
- Other pathology – atelectasis/bronchiectasis/lipid or suppurative pneumonia.

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40 Pleura

Kathleen M. Mulholland

40.1 Anatomy

The visceral pleura covers the surface of the lungs, and the parietal pleura covers the inner surface of the chest wall, mediastinum, and diaphragm. Under normal circumstances, the cavity contains 5–20 ml of fluid. The lining of the pleura is composed of a continuous layer of flat or low cuboidal cells, the mesothelium.

Lymphovascular Drainage

Branches of systemic arteries supply the parietal pleura. The bronchial circulation supplies the visceral pleura. Venous blood from the visceral pleura drains into the pulmonary veins, and lymph from the visceral pleura passes to a superficial plexus in the lung and then to the hilar nodes. Lymph leaves the pleural cavity mainly via the parietal lymphatic system. The parietal pleura drains to the parasternal, diaphragmatic, and posterior mediastinal nodes.

K.M. Mulholland
Histopathology Laboratory, Altnagelvin Hospital,
Western Health and Social Care Trust,
Londonderry, UK
e-mail: kathleen.mulholland@westerntrust.hscni.net

40.2 Clinical Presentation

Pleural disease may present with pain and breathlessness. Nonspecific symptoms such as weakness, anorexia, and fever may be present in 25% of cases of malignant mesothelioma. Paraneoplastic syndromes occasionally arise causing immunosuppression, thrombocytosis, cachexia, amyloidosis, or hypoglycemia.

40.3 Clinical Investigations

- Chest X-ray – to detect pleural effusions and calcified pleural plaques.
- CT scan – may identify an effusion undetectable by conventional radiography. It will show pleural thickening and calcification due to asbestos exposure. It is important in detecting invasion of chest wall, ribs, and mediastinum by malignant mesothelioma.
- Ultrasound – used to localize pleural effusions during thoracentesis.
- Thoracentesis – aspiration of pleural fluid using a sterile technique. 50–100 ml is sufficient for diagnosis, but more may be removed if the thoracentesis is therapeutic.
- Pleural fluid analysis (cytology, biochemistry) – total protein, lactate dehydrogenase, amylase, glucose, pH, lipids, complement and antibodies, Gram stain, and culture.

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_40, © Springer-Verlag London 2013

- Pleural needle biopsy – percutaneous closed needle biopsy with thoracentesis. A diagnosis of malignancy is achieved in 40–70% of cases.
- Thoracoscopy and pleural biopsy – thoracoscopy, especially if guided by CT findings, should improve the diagnostic yield to over 95%. Minimally invasive approaches such as video-assisted thoracoscopy (VATS) lead to earlier diagnosis.
- Open pleural biopsy (with or without decortication) – occasionally rigid (open tube) pleuroscopy or even minithoracotomy is required to obtain an adequate pleural biopsy.

40.4 Pathological Conditions

Pleural disease is either primary or secondary, e.g., to an underlying lung lesion or systemic disorder such as SLE.

Pleural effusions: over 90% of effusions are secondary to one of four conditions – congestive heart failure, pneumonia, malignancy, or pulmonary emboli. Transudates are effusions containing low concentrations of proteins (<3 g/dl), and the majority are due to congestive heart failure. Exudates contain higher concentrations of protein (>3 g/dl), and over 80% are due to pneumonia, neoplasm, or pulmonary emboli. Depending on the etiology, effusions can be serous, fibrinous, serofibrinous, purulent, or hemorrhagic.

Empyema is the presence of frank pus in the pleural cavity. Noninfective processes such as pulmonary infarction, rheumatoid disease, SLE, and uremia may present with a pleural effusion.

Asbestos-related pleural effusion develops in 3% of asbestos workers. In most, it will resolve in 1–2 years, but 20% progress to massive pleural fibrosis and 5% develop malignant mesothelioma. Carcinoma of the lung is the most common malignancy to invade the pleura and produce pleural effusions, followed by carcinoma of the breast.

Pneumothorax: primary spontaneous pneumothorax occurs most commonly in 30- to 40-year-old tall, thin males. They are most often due to rupture of blebs or bullae on the apical parts of the upper lobes. Rate of recurrence is 25%. Secondary pneumothorax occurs in chronic obstructive pulmonary disease, cystic fibrosis, asthma, tuberculosis, idiopathic pulmonary fibrosis, lymphangioleiomyomatosis, Langerhans histiocytosis, and *Pneumocystis jiroveci* pneumonia (PJP). Catamenial pneumothorax is associated with menstruation and may be due to focal endometrial deposits on the pleura. Traumatic pneumothorax can be iatrogenic, e.g., secondary to biopsy, or otherwise, e.g., penetrating chest trauma.

Pleural plaques: usually but not always associated with asbestos. Histological examination shows hyalinized fibrous tissue with basket weave collagen fibers. Plaques are usually present on the parietal pleura mainly in the intercostal spaces on the anterior and posterolateral aspects of the chest wall and on the dome of the diaphragm.

Diffuse pleural thickening: involves the visceral pleura and is associated with asbestos exposure.

Asbestos-induced mesothelial hyperplasia: consists of a papillary proliferation of the surface mesothelium with cores of connective tissue and a surface lining of regular mesothelial cells. It may be difficult to distinguish from well-differentiated malignant mesothelioma on pleural biopsy.

40.4.1 Neoplastic Conditions

Metastatic tumors, e.g., lung and breast cancer, are the most common tumors of the pleura.

Malignant mesothelioma: most common primary malignant tumor of the pleura. There is a proven relationship with asbestos exposure, although 10–20% appear to be unrelated. Rarely, they may be associated with therapeutic irradiation or intrapleural thorium dioxide (Thorotrast). In industrialized countries, malignant mesothelioma accounts for about 1% of all cancer deaths. There is a latency period of 20 years between exposure to asbestos and development of the tumor.

Malignant mesothelioma occurs usually in the lower half of the hemithorax on the right side more often than the left. It encases the lung, and direct extension into the subpleural lung is common. If nodular masses are present within the lung parenchyma, a primary lung carcinoma with pleural spread is more likely.

Histological patterns include epithelial, sarcomatoid (or fibrous), and biphasic, a combination of both. The epithelioid pattern appears to be commonest in most series, with tubules, papillae, and sometimes psammoma bodies being seen. Spindle cells set in varying amounts of collagenized stroma are seen in the sarcomatoid pattern.

Differential diagnosis includes adenocarcinoma, reactive mesothelial hyperplasia, and fibrosis. Immunohistochemistry, assessment of radiological findings, and disease course are correlated to reach a final diagnosis.

Distant metastases occur late if, at all, the sarcomatous form showing metastatic spread more commonly. Malignant mesotheliomas are rarely operable. Few patients survive longer than 2 years, and the outlook is not significantly affected by current therapy.

Occasional patients are suitable for adjuvant chemotherapy with local resection of limited disease. Symptomatic relief is gained by multiple paracentesis of malignant effusions supplemented by intracavitary injection of chemotherapeutic or sclerosant agents.

Solitary fibrous tumor: origin is from the subpleural mesenchyme, from fibroblasts or myofibroblasts, and is unrelated to asbestos exposure. It more often arises from the visceral pleura and is often attached by a pedicle. Histological examination shows a low-grade spindle cell neoplasm of variable cellularity with tumor cells dispersed in a collagenous stroma.

The most important prognostic factor is the completeness of excision and particularly the presence or absence of a pedicle. Tumor size and cellularity correlate with malignancy. Prognosis is generally good with a minority showing local recurrence.

Calcifying fibrous pseudotumor: occurs in young adults and may be a late stage of inflammatory myofibroblastic tumor.

Pyothorax- associated lymphoma: non-Hodgkin's lymphoma of B cell phenotype. It is associated with Epstein-Barr virus (EBV).

Body cavity-based lymphoma: presents as a mass lesion. It is associated with EBV, human herpes virus 8, and HIV. It is a high-grade lymphoma of null cell phenotype.

40.5 Surgical Pathology Specimens: Clinical Aspects

40.5.1 Biopsy Specimens

A number of procedures can be undertaken to obtain pleural biopsies.

A special needle, usually an Abrams or Cope needle, may be used during thoracentesis, both to drain fluid and obtain a pleural biopsy. A pleural needle biopsy often provides insufficient tissue for diagnosis. When this is the case, thoracoscopy and a visually directed pleural biopsy may be required. In some cases, an open pleural biopsy is undertaken with or without decortication. *Decortication* is a procedure to remove constricting visceral pleural peel in order to expand the underlying lung. It is of use in very few patients as the morbidity and mortality usually outweigh any benefit.

Approximately 10% of cases of malignant mesothelioma that have a biopsy will have seeding of the biopsy tract by tumor with subsequent chest wall recurrence. To prevent this happening, radiation therapy is used on the biopsy site.

40.5.2 Resection Specimens

Surgery may be used to treat pneumothorax. The preferred approach is using minimally invasive techniques (VATS), but thoracotomy can be used with an axillary, muscle sparing approach. Resection of blebs or bullae is achieved using mechanical stapling devices.

Pleurectomy is a procedure used to debulk a malignant mesothelioma or for diagnosis. Multiple fragments or strips of pleural membrane are obtained.

An *extrapleural pneumonectomy (EPP)* is the en bloc resection of visceral and parietal pleura, lung, ipsilateral hemidiaphragm and pericardium. It has an operative mortality of 5–15%. Combined with postoperative chemotherapy and adjuvant radiotherapy, it may improve survival.

40.6 Surgical Pathology Specimens: Laboratory Protocols

40.6.1 Biopsy Specimens

As for lung biopsies, see Chap. 39.

40.6.2 Resection Specimens

Pleurectomy (Fig. 40.1)

- Record the number of fragments.
- Measure the dimensions (cm) of the fragments unless there are more than three, then note dimensions of the smallest and largest, and weigh the fragments (g).
- Describe any lesions – color, consistency, and sizes.
- Record the presence of other structures such as muscle, pericardium, or fat and any involvement of these by tumor.
- Ink margins and note the distances (cm) of any lesion from them.

  Submit one section for each centimeter of tumor to include the margins.

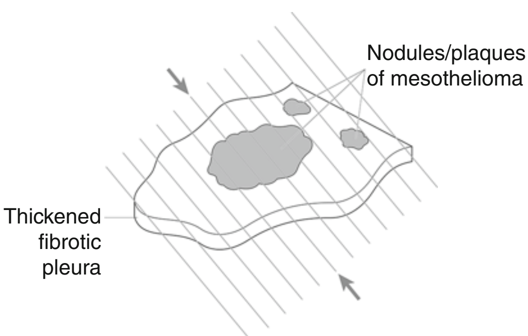

Fig. 40.1 Blocking a pleurectomy specimen (Reproduced, with permission, from Allen and Cameron (2004))

Extrapleural pneumonectomy

- Tissue may be taken for electron microscopy before fixation.
- Weigh the specimen (g) and record its dimensions (cm).
- Inflate and fix the lung.
- Take the bronchial margin and remove hilar lymph nodes (number/size).
- Examine the pleura – determine the percentage involvement by tumor.
- Examine the pericardium for tumor.
- Ink margins close to the tumor.
- Serially section the specimen coronally at 1-cm intervals.
- Describe involvement of the diaphragm by tumor – distance from the anterior, posterior, medial, and lateral margins; depth of invasion into diaphragm; and involvement of the peritoneal surface of the diaphragm.
- Describe involvement of visceral pleura – extent of fusion of visceral pleura to parietal pleura and size of nodules of tumor.
- Invasion of lung – usually, tumor invades along interlobar fissures (Fig. 40.2). Describe parenchymal disease such as pneumonia or fibrosis.
- If rib is attached, describe the dimensions and any tumor involvement seen. If lesions are seen in the ribs, X-ray.

Blocks for histology

- Take sections perpendicular to the pleura from the apex of the lung; the anterior, posterior, medial, and lateral pleura at one level; and the anterior, lateral, medial, posterior, and inferior margins of the diaphragm.
- Take sections of rib if involved.
- If chest wall is attached to the specimen, take margins.

Histopathology report

- Type of specimen – biopsy/pleurectomy/extrapleural pneumonectomy.
- Size (cm) and weight (g).
- Tumor site – visceral/parietal/parenchymal.
- Tumor size – length × width × depth or maximum dimension (cm).

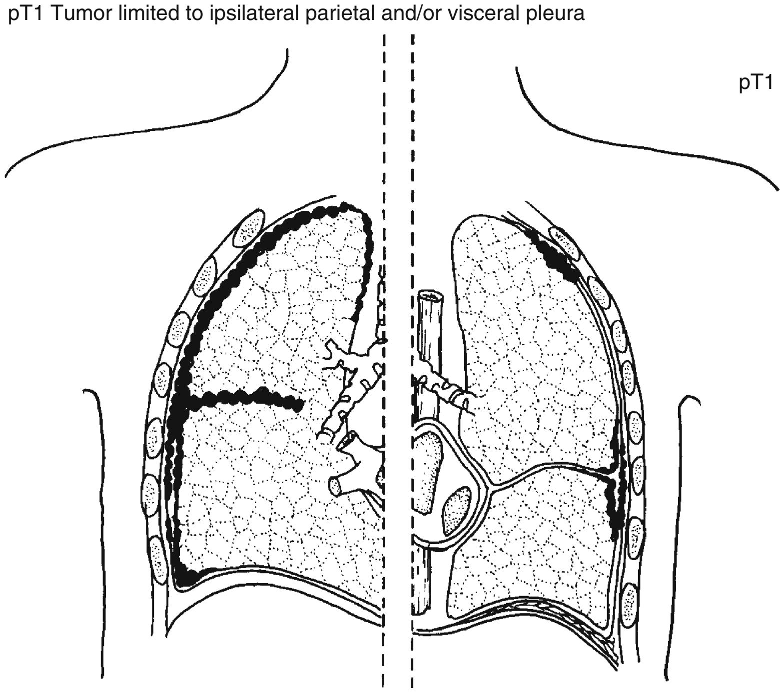

Fig. 40.2 Pleural and interlobar spread of malignant mesothelioma (Used with the permission of the Union for International Cancer Control (UICC), Geneva, Switzerland. The original source for this material is from Wittekind et al. (2005))

- Tumor appearance – localized/diffuse/nodular/plaque/infiltrative/cystic change.
- Tumor histological type – malignant mesothelioma: biphasic, epithelioid, or sarcomatoid.
- Extent of local tumor spread. TNM 7 applies to pleural mesothelioma.

pT1	Tumor limited to ipsilateral parietal and/or focal visceral (pT1b) pleura.
pT2	Tumor invades any of the following: ipsilateral lung, diaphragm, and/or confluent visceral pleura.
pT3	Tumor invades any of the following: endothoracic fascia, mediastinal fat, focal chest wall, and/or non-transmural pericardium.
pT4	Tumor directly extends to any of the following: contralateral pleura, peritoneum, rib, extensive chest wall, mediastinum, myocardium, brachial plexus, spine, transmural pericardium, and/or malignant pericardial effusion.

- Lymphovascular invasion – present/not present.
- Regional lymph nodes – intrathoracic, internal mammary, scalene, or supraclavicular.

pN0	No regional lymph node metastases
pN1	Metastasis in ipsilateral bronchopulmonary and/or hilar lymph nodes
pN2	Metastasis in ipsilateral internal mammary, mediastinal, and/or subcarinal lymph nodes
pN3	Metastasis in contralateral mediastinal, contralateral internal mammary or hilar, and/or ipsilateral or contralateral scalene or supraclavicular lymph nodes

- Excision margins – distance (mm) to the nearest inked margin of local resection of limited disease.
- Other pathology – pleural plaques, asbestosis, bronchogenic carcinoma, fibrosis, or emphysema.

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41 Mediastinum

Kathleen M. Mulholland

41.1 Anatomy

The mediastinum is that part of the thoracic cavity located centrally between the pleural cavities. It extends anteroposteriorly from the inner aspect of the sternum to the spine and superoinferiorly from the thoracic inlet to the diaphragm. It can be subdivided arbitrarily into anterior, superior, middle, and posterior compartments (Fig. 41.1).

The anterosuperior compartment contains the thymus gland, lymph nodes, vessels, and fat. The thymus is large at birth but atrophies after puberty and in the adult is variable in size. It can extend down beyond the aortic arch and lie in front of the brachiocephalic veins and left common carotid artery. Parathyroid tissue may be embedded in it. The great vessels, the aorta, the superior vena cava, and the azygos vein lie in the anterosuperior compartment.

The middle compartment contains the heart, pericardium, trachea, major bronchi, pulmonary vessels, and phrenic and vagus nerves.

The posterior (paravertebral) compartment contains the sympathetic chain, vagus nerves, esophagus, thoracic duct, descending aorta, azygos and hemiazygos veins, and lymph nodes.

K.M. Mulholland
Histopathology Laboratory, Altnagelvin Hospital,
Western Health and Social Care Trust,
Londonderry, UK
e-mail: kathleen.mulholland@westerntrust.hscni.net

Lymphovascular Drainage

Lymphatic drainage is to tracheobronchial lymph nodes situated at the carina.

41.2 Clinical Presentation

Almost half of patients with mediastinal cysts or tumors are asymptomatic. Lesions are often discovered incidentally on X-ray.

Local symptoms may result from compression or invasion of mediastinal structures and include cough, dysphagia, recurrent pulmonary infection, dyspnea, pain, and rarely hemoptysis.

Most bronchial, gastric, and gastroenteric cysts are asymptomatic, although the latter can be life threatening because of gastric secretion leading to hemorrhage, peptic ulcer, and perforation. Superior vena cava (SVC) syndrome, due to compression or invasion of the superior vena cava, usually indicates the presence of malignancy but can be caused by benign fibrosing mediastinitis.

Myasthenia gravis is present in one third of patients with thymomas. Symptoms include fatigability affecting the proximal limb muscles, extraocular muscles, and muscles of mastication, speech, and facial expression. Respiratory difficulties may occur. Other associated conditions in 5–10% of cases are red cell aplasia with severe anemia, hypogammaglobulinemia resulting in bacterial infections and diarrhea, and pemphigus foliaceus producing skin blisters.

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_41, © Springer-Verlag London 2013

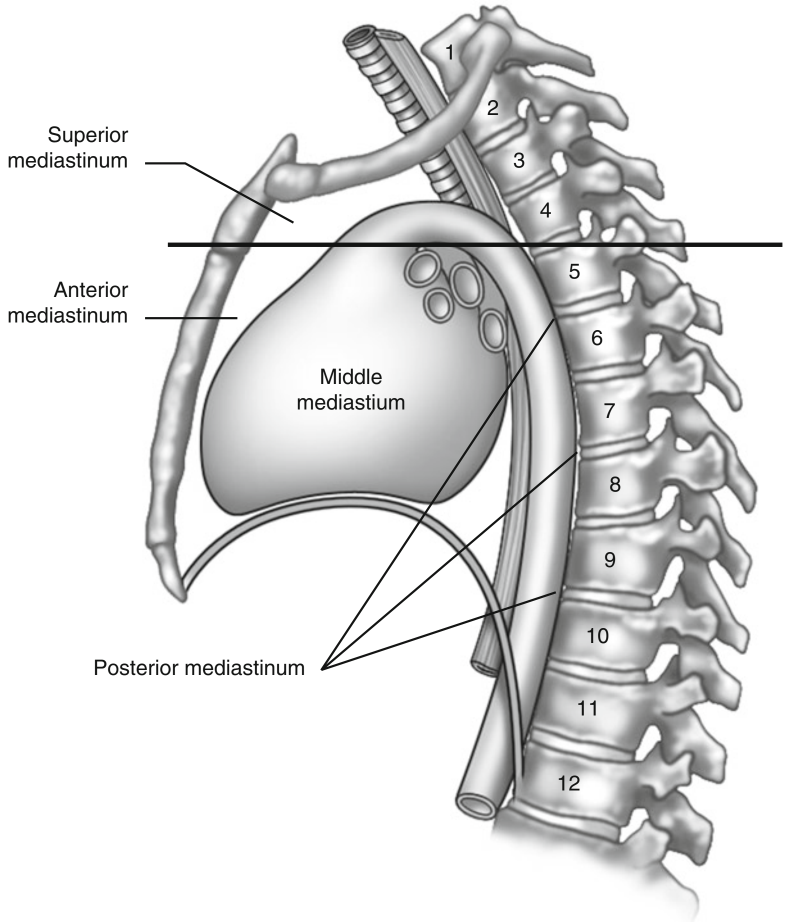

Fig. 41.1 Compartments of the mediastinum

41.3 Clinical Investigations

- Chest X-ray – mediastinal masses commonly present on chest X-ray obtained for other purposes or in patients with cancer undergoing CT scan staging of the chest and abdomen
- CT scan – has limitations when distinguishing between cystic and solid structures
- MRI scan – particularly useful in determining tumor invasion of vascular or neural structures, when coronal or radial body sections are necessary, or when contrast material cannot be given intravenously due to renal disease or allergy
- Angiography – used in vascular lesions
- Barium swallow – used to investigate posterior mediastinal lesions
- Gallium scan – of value in the diagnosis of lymphoma
- Blood tests:
  - Mediastinal germ cell tumors: AFP (alpha-fetoprotein), β-HCG (beta-human chorionic gonadotropin)
  - Lymphoma/seminoma: LDH (lactate dehydrogenase)
  - Parathyroid tumors: serum calcium and alkaline phosphatase
  - Myasthenia gravis: acetylcholine receptor antibody
- Blood count – normochromic normocytic anemia (red cell aplasia)
- Single fiber EMG (electromyography) and tensilon test – for suspected myasthenia gravis
- Percutaneous or thoracoscopic fine-needle aspiration – performed under radiological guidance

- Percutaneous or thoracoscopic core biopsy – using a larger gauge or cutting needle under radiological guidance
- Esophagoscopy – for dysphagia or for any mass close to the esophagus
- Bronchoscopy – used when there is hilar or peritracheal lymphadenopathy

41.4 Pathological Conditions

41.4.1 Infections

Acute mediastinitis: potentially fatal, may affect all three compartments and arise from an adjacent pneumonia or as a complication of esophageal perforation.

Fibrosing mediastinitis: characterized by fibrosis causing a variety of symptoms depending on which structures are constricted. Etiological factors include histoplasma capsulatum and tuberculosis.

41.4.2 Mediastinal Masses

See Table 41.1.

41.4.2.1 Anterior Mediastinal Masses

Unilocular thymic cysts: of developmental origin and occur more often in the neck than the mediastinum. The lining may be flattened, cuboidal, columnar, or (rarely) squamous epithelium with thymic tissue in the wall.

Multilocular thymic cysts: acquired and thought to be secondary to inflammation. Some cases are seen in HIV infection. They can mimic an invasive thymic tumor and occur in about half of thymuses with nodular sclerosing Hodgkin's disease or seminoma. They also occur in other tumors such as thymoma and large cell lymphoma, though less frequently. Exceptionally, true squamous cell carcinoma arises from these cysts.

Thymic hyperplasia: strongly associated with autoimmune disease especially myasthenia gravis. There is extreme variability in size and weight of the thymus with formation of germinal centers, principally in the medulla that expand and cause cortical atrophy.

Thymoma: the most common primary neoplasm of the mediastinum. 75% present in the anterior mediastinum, but they can also occur in other compartments (neck, thyroid, pulmonary hilum, lung parenchyma, pleura). It is a mixture of neoplastic thymic epithelial cells and nonneoplastic lymphocytes. Tumors are evaluated on the basis of the morphology of the neoplastic epithelial cells (spindle, plump) and the relative number of these cells, compared with the nonneoplastic lymphocytic component.

Medullary thymomas: composed of epithelial cells that resemble those of the medulla and are elongated or spindle shaped. They are benign.

Mixed thymomas: show a mixture of spindle cells and plumper, rounder, cortical-type epithelial cells. They act in a benign fashion.

Predominantly cortical (organoid) thymomas: have a less prominent epithelial component with lymphocyte-rich organoid corticomedullary areas. Local invasion is common.

Cortical thymomas: have a lesser component of lymphocytes with large round or polygonal epithelial cells. They are frequently locally invasive.

Well-differentiated thymic carcinoma: composed predominantly of epithelial cells with mild nuclear atypia and few lymphocytes. It is locally invasive.

Thymic carcinoma: an epithelial tumor exhibiting cytological features of malignancy. Cytoarchitectural features are no longer specific to the thymus but are analogous to those seen in carcinomas of other organs. No immature lymphocytes are present. Microscopic types of thymic carcinoma are, in 90% of cases, squamous cell carcinoma, nonkeratinizing squamous cell carcinoma, or lymphoepithelioma-like carcinoma.

Lymphoma: 10–14% of mediastinal masses in adults and is the commonest primary neoplasm of the middle mediastinum. Lymphoma of any type may occur, generally as part of widespread disease.

Mediastinal Hodgkin's disease: the nodular sclerosing variety occurs most frequently with mediastinal involvement in 80% of cases. There

Table 41.1 Mediastinal masses

Anterior/superior compartment	Middle compartment	Posterior compartment
Thymomas	Bronchogenic cyst	Neurogenic tumors – neurofibroma, neurilemmoma (schwannoma), ganglioneuroma, ganglioneuroblastoma, malignant schwannoma, neuroblastoma, paraganglioma
Thymolipomas	Enteric cyst	Malignant lymphoma
Carcinoid tumors	Pericardial cyst	Gastroenteric cysts
Thymic cyst	Malignant lymphoma	
Germ cell tumors	Primary cardiac tumors	
Malignant lymphoma	Metastatic carcinoma	
Teratomas		
Metastatic carcinoma		
Thyroid/parathyroid lesions		
Mesenchymal lesions –lipoma, hemangioma, lymphangioma		
Aberrant thyroid		
Thyroid goiter		

is a nodular growth pattern, collagen bands, and lacunar cells.

Non-Hodgkin's lymphoma: usually high-grade; T-lymphoblastic (young patients) or large B-cell and occasionally low-grade (MALToma).

Mediastinal large B-cell lymphoma: thought to be of thymic B-cell origin. Histological examination shows a diffuse proliferation of cells, which is compartmentalized into groups by fine bands of sclerosis. There may be thymic remnants. There is an association with nodular sclerosis Hodgkin's lymphoma (composite lymphoma).

Biopsy samples are often small and may be obscured by profuse sclerosis with associated cellular crush artifact.

Germ cell tumors: make up 20% of mediastinal masses.

Mature cystic teratoma: the most common type of mediastinal germ cell neoplasm comprising a disorganized mixture of derivatives of the three germinal layers – ectoderm, mesoderm, and endoderm.

Immature teratoma: a germ cell tumor similar to mature teratoma but also containing immature epithelial, mesenchymal, or neural elements.

Seminoma: the most common malignant germ cell tumor to occur in the mediastinum. These arise almost always within the thymus.

Nonseminomatous germ cell tumors: include malignant teratomas, teratocarcinomas, yolk sac tumors, choriocarcinomas and embryonal carcinomas.

Malignant germ cell tumors are usually treated with chemotherapy and radiotherapy. If a residual mass is left, it is usually a benign teratoma or necrotic tumor mass that can potentially degenerate and redevelop malignancy. Excision may be carried out.

41.4.2.2 Middle Mediastinal Masses

Pericardial cysts: benign cysts, the inner surface of which is lined by a single layer of mesothelium and contain clear watery fluid.

Bronchial (bronchogenic) cysts: make up 60% of all mediastinal cysts and occur along the tracheobronchial tree commonly posterior to the carina. They are usually lined by ciliated columnar epithelium, but there may be focal or extensive squamous metaplasia. The wall can

contain hyaline cartilage, smooth muscle, bronchial glands, or nerve trunks.
Esophageal cysts: usually in the wall of the lower half of the esophagus. The lining may be squamous, ciliated, or columnar epithelium, and there is a double layer of smooth muscle in the wall.

41.4.2.3 Posterior Mediastinal Masses

Gastric and *enteric cysts*: located in the posterior mediastinum in a paravertebral location and nearly all are associated with vertebral malformations. The gastric type has the same coats as the stomach and the enteric type similar to the wall of the small intestine. Combined forms of cysts are termed gastroenteric cysts.

Neurogenic tumors: the most common posterior mediastinal masses. Most are asymptomatic. MRI scan may be necessary to rule out intraspinal extension along the nerve roots (dumbbell tumors).

Nerve sheath tumors account for 65% of all mediastinal neurogenic tumors and include *neurilemmoma* (schwannoma) and neurofibromas. 25–40% of patients with nerve sheath tumors have multiple neurofibromatosis (von Recklinghausen's disease). Malignant tumors such as neurogenic sarcomas and malignant schwannomas may occur, and other tumors include neuroblastomas and paragangliomas.

41.5 Surgical Pathology Specimens: Clinical Aspects

41.5.1 Biopsy Specimens

Percutaneous or thoracoscopic fine-needle or *core biopsy*: used to obtain a tissue diagnosis, e.g., malignant lymphoma. The role of needle biopsy for diagnosis of thymoma is controversial. Diagnostic accuracy is 59%, but the differentiation between benign and malignant thymoma is difficult. There is also an intraoperative risk of seeding tumor cells in the mediastinum or pleural space.

Open biopsy: in some cases, invasive mediastinal incisional biopsy may be required. Surgical approaches include cervical mediastinoscopy, subxiphoid mediastinoscopy, anterior mediastinoscopy, and videothoracoscopy.

Cervical mediastinoscopy is performed through a small incision in the suprasternal notch. It is used to sample masses in the superior mediastinum or lymph nodes in the subcarinal and paratracheal area. *Anterior mediastinotomy* (*Chamberlain procedure*) is performed through a small incision over the second or third rib on either side. It is used to sample lymph nodes in the para-aortic position or anterior mediastinal masses. Biopsy of the thymus may cause seeding of tumor into the operative site and violate the tumor capsule. Diagnostic accuracy for thymoma by open biopsy is 81%.

41.5.2 Resection Specimens

Thymectomy: performed for benign or malignant thymic tumors, treatment of myasthenia gravis, or may be incidental during thoracic surgery such as open-heart surgery. If the thymus is not very large, thymectomy may be carried out through a transcervical route. The usual surgical approach is through either partial or complete sternotomy. Median sternotomy involves the use of an incision in the midline from the suprasternal notch to just below the xiphoid process with division of the sternum longitudinally. Ideally there should be complete removal of the thymus with surrounding margins of normal tissue. Alternatively tumor debulking may be undertaken. The clinical ease of excision and the tumor circumscription or degree of spread into adjacent tissues are strong indicators of potential for future local recurrence and invasion.

41.6 Surgical Pathology Specimens: Laboratory Protocols

41.6.1 Biopsy Specimens

- Count the number of fragments and measure their length (mm).
- Describe – color, consistency.

- Place in cassettes between foam pads or wrapped in filter paper.
- Examine histologically through multiple levels, and keep intervening sections for stains.

41.6.2 Resection Specimens

Initial procedure and description:

- Measure and record size – length × width × depth (cm) and weight (g).
- Appearance – cystic/hemorrhagic/necrosis.
  - Capsule/soft tissue invasion
  - Adherence/pleura/pericardium
- Photograph.
- Fixation by immersion in 10% formalin for 48 h.
- Ink the outer surface.
- Serially section the specimen transversely at 3–5 mm intervals.
- Describe lesions – size (cm), color, whether lobulated or smooth, relationship to the capsule and surrounding structures, edges (encapsulated or infiltrating), the presence of calcification, necrosis, or hemorrhage.
- Describe uninvolved tissue, e.g., thymus – color, consistency, proportions of fat and parenchyma.
- Dissect out and submit lymph nodes in any attached tissue.

Blocks for histology:

- Sample four or five blocks of the lesion and its relationship to the capsule if present and to the rest of the tissue.
- Block the margins.
- Sample blocks from uninvolved tissue (at least two).
- Sample lymph nodes.
- Block pleura and/or pericardium if present.

Histopathology report:

- Tumor type – metastatic carcinoma, malignant lymphoma, germ cell tumor, neurogenic tumor, thymoma, sarcoma
- Tumor differentiation:
  - Metastatic carcinoma: well/moderate/poor
  - Malignant lymphoma: low-grade (MALToma)/high-grade (diffuse large cell lymphoma, lymphoblastic lymphoma)
  - Germ cell tumor (seminoma/nonseminomatous): mature, immature, malignant-embryonal carcinoma, yolk sac tumor, choriocarcinoma
  - Neurogenic tumors: small round blue cell/neuroblastoma component
  - Sarcoma – low-grade/high-grade
  - Thymoma – classify according to morphology (see above)
- Tumor edge – pushing/infiltrative/lymphoid response
- Extent of local tumor spread

  All tumors:
- Confined to mediastinal nodes
- Confined to the thymus
- Into mediastinal connective tissues
- Into other organs: pleura, lung, pericardium, main vessels

  Thymoma (WHO):
- *Encapsulated*: thymoma completely surrounded by a fibrous capsule of varying thickness which is not infiltrated by tumor; it may infiltrate into but not through.
- *Minimally invasive*: thymoma surrounded by a capsule which is focally infiltrated by tumor growth or which invades mediastinal fat.
- *Widely invasive*: thymoma spreading by direct extension into adjacent structures such as pericardium, large vessels, and lung (may appear invasive to the surgeon – excision may be incomplete).
- *With implants*: thymoma in which tumor nodules separate from the main mass are found on the pericardial or pleural surface.
- *With lymph node metastasis*: a tumor that involves one or more lymph nodes anatomically separate from the main mass (most commonly mediastinal and supraclavicular).
- *With distant metastases*: tumor accompanied by embolic metastases to a distant site (lung, liver, skeletal system).
- Encapsulated thymomas with no implants, no lymph node metastases, or no distant metastases are benign. All other combinations are malignant.
- Lymphovascular invasion – present/not present. Note perineural invasion.
- Regional lymph nodes – intrathoracic, scalene, supraclavicular nodes.

Thymoma

pN0	No regional lymph nodes involved
pN1	Metastasis to anterior mediastinal lymph nodes
pN2	Metastasis to intrathoracic lymph nodes other than the anterior mediastinal lymph nodes
pN3	Metastasis to extrathoracic lymph nodes.

- Excision margins – comment on adequacy of excision.

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42 Heart

Kathleen M. Mulholland

42.1 Anatomy

The heart is a muscular pump weighing approximately 300 g. It consists of four chambers, the right and left atria and the right and left ventricles (Fig. 42.1). The right side of the heart pumps venous blood through the pulmonary circulation for oxygenation, the left heart oxygenated blood through the systemic circulation for distribution to the tissues.

The superior and inferior vena cavae enter the right atrium. There is a small projection, the right auricle, which overlaps the beginning of the ascending aorta. The atrioventricular valves, the mitral and tricuspid valves each consist of a valve ring or annulus, leaflets, anchoring chordae tendineae, and papillary muscles. The semilunar valves, the aortic, and pulmonary valves comprise three cusps, each with a sinus. The cusps meet at three commissures. The thickness of the wall of the right ventricle is normally 0.25–0.3 cm and the left ventricle 0.9–1.5 cm.

The heart is surrounded by the pericardial sac, which is composed of two layers of connective tissue (visceral and parietal pericardia), each covered by a layer of mesothelial cells.

The coronary arteries supply blood to the heart (Fig. 42.1). The right coronary artery arises from the anterior coronary sinus and runs over the anterior surface of the heart before crossing the posterior surface, where it finally anastomoses with the left coronary artery. Its major branches are the marginal artery and the posterior interventricular artery. There are also atrial and ventricular branches. The left coronary artery arises from the left posterior aortic sinus.

Major branches include the anterior interventricular artery and the circumflex artery. The anterior interventricular artery descends towards the apex and anastomoses with the posterior interventricular artery. A diagonal branch runs to the left ventricle. The circumflex artery anastomoses with the terminal branch of the right coronary artery. It gives off the marginal artery, which runs along the left border of the heart.

The heart is composed of three layers, the epicardium (serous pericardium), the muscular myocardium, and the endocardium.

Lymphovascular Drainage

Lymphatic drainage of the heart is to the tracheobronchial lymph nodes.

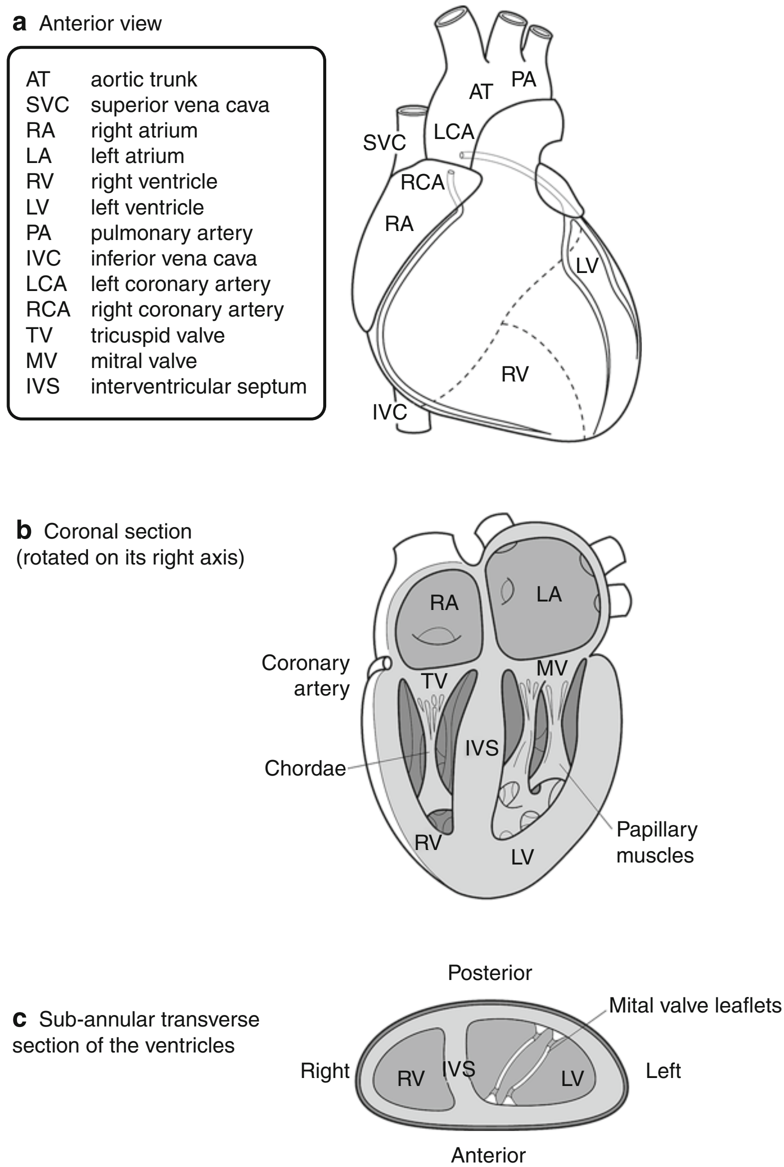
42.2 Clinical Presentation

Dyspnea is an awareness of breathlessness and a symptom of congestive cardiac failure, the end-stage of many cardiac conditions. Orthopnea and paroxysmal nocturnal dyspnea are shortness of

K.M. Mulholland
Histopathology Laboratory, Altnagelvin Hospital, Western Health and Social Care Trust, Londonderry, UK
e-mail: kathleen.mulholland@westerntrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_42, © Springer-Verlag London 2013

Fig. 42.1 Anatomy of the heart (Reproduced with permission from Allen and Cameron (2004))



breath, which arise when the patient has been recumbent due to collection of fluid in the pulmonary circulation (pulmonary edema).

Wheezing (cardiac asthma) is due to swelling of the bronchial lining, and ankle swelling is secondary to congestive cardiac failure with systemic venous congestion.

Angina commonly presents as central gripping chest pain radiating to the jaws, neck, or arms provoked by exercise and relieved by rest. It is due to cardiac hypoxia.

Myocardial infarction is similar but is not relieved by rest.

Pericarditis presents with severe, sharp, central chest pain, aggravated by movement, posture, respiration and coughing, and myocarditis with mild pleuritic chest pain and lethargy.

Sudden unexplained death may be the presentation of acute cardiac failure due to ischemic heart disease, and syncope (fainting episodes) may occur in aortic stenosis, both of which can also be caused by cardiac dysrhythmia.

Infective endocarditis presents with fever, weight loss, malaise, splenomegaly, and splinter hemorrhages of the fingernails due to embolic infarcts of the skin. Abdominal pain may be secondary to renal or splenic infarcts. Chest pain due to pulmonary infarcts can occur in tricuspid valve endocarditis.

In hypertrophic obstructive cardiomyopathy, the patient may present with atrial fibrillation, ventricular arrhythmias, or sudden death.

Cardiac myxoma can present with symptoms of mitral stenosis and embolization of fragments of the tumor or of overlying thrombus. Fever, cachexia, and malaise also occur.

Cardiac rhabdomyomas may cause stillbirth or death within the first few days of life.

42.3 Clinical Investigations

- Echocardiography/Doppler flow studies – used to study valvular heart disease, congenital abnormalities, cardiac tumors, and pericardial effusions.
- Transesophageal echocardiography (TEE) – used to investigate lesions of the left atrium, the ascending aorta, the aortic valve, and septal defects.
- Chest X-ray – used to assess heart size and to identify calcification or fluid in the pericardium.
- Electrocardiogram (ECG) – a resting ECG is useful in the diagnosis of myocardial infarction, cardiac hypertrophy, or abnormalities of rhythm. An exercise ECG is useful in patients with angina, and an ambulatory ECG made over 24 h may be used when heart rhythm disturbances occur only intermittently.
- Nuclear cardiology – assesses the function of cardiac muscle. Radioactivity from substances injected intravenously into the patient are measured and allow evaluation of cardiac function and assessment of ischemia and infarction.
- Cardiac catheterization and angiography – catheters are advanced into the right and left sides of the heart and pressure and oxygen saturation studies performed. During coronary angiography, radio-opaque contrast medium is injected through the catheter into the coronary artery ostia.
- Magnetic Resonance Imaging (MRI) – synchronized with the ECG gives systolic and diastolic images.
- Endomyocardial biopsies – taken via cardiac catheter.

42.4 Pathological Conditions

42.4.1 Non-neoplastic Conditions

42.4.1.1 Disorders of the Endocardium

Infective endocarditis: A disorder affecting the endocardial surface of the heart as a result of infection. The characteristic lesion is the vegetation, which consists of thrombus containing microorganisms. In acute bacterial endocarditis, large friable vegetations are found on the valves, which can cause erosion or perforation of the underlying tissue. Predisposing factors to endocarditis are valvular anatomical abnormalities (congenital or acquired, e.g., rheumatic heart disease), sepsis, immunosuppression, or IV drug abuse.

Non-bacterial thrombotic endocarditis (*NBTE*): Produces small, bland vegetations attached to the valve surface at the lines of closure. They are seen in cachetic patients, e.g., disseminated tumor. Systemic lupus erythematosus can be associated with *Libman-Sacks endocarditis* with small bland vegetations located on both surfaces of the valves or cords.

Rheumatic fever: An inflammatory disease, which in the acute phase produces pathognomonic Aschoff bodies in the heart. Chronic rheumatic heart disease is characterized by organization of the endocardial inflammation with subsequent fibrosis, particularly affecting the valves.

42.4.1.2 Disorders of the Valves

In valvular disease, assessment of the gross appearance often contributes to the final diagnosis more than microscopic examination.

Mitral valve stenosis: Most commonly due to rheumatic fever with commissural fusion, cusp scarring, and dystrophic calcification.

Mitral valve regurgitation: Due to floppy mitral valve shows valve cusps, which are increased in area, and dome shaped with myxoid change. Other causes include rheumatic fever, rupture of a papillary muscle or chordae tendineae, ventricular enlargement, or infective endocarditis.

Aortic valve stenosis: Due to calcification of a congenitally bicuspid valve, senile calcific aortic stenosis, or post-inflammatory scarring.

Aortic regurgitation: Secondary to post-inflammatory scarring, infective endocarditis, or abnormalities of the cusps and commissures.

Pulmonary valve abnormalities: Consist of stenosis, insufficiency, or a combination of the two. 95% of cases are due to congenital heart disease, tetralogy of Fallot being the most common. A bicuspid pulmonary valve is the most common anomaly.

Tricuspid valve abnormalities: Most commonly pure insufficiency and caused by post-inflammatory scarring, congenital abnormalities, infective endocarditis, or dilatation of the valve ring in cardiac failure.

42.4.1.3 Disorders of the Myocardium

Myocarditis: Viral, bacterial or fungal. In developed countries, viral infections predominate. Parasitic etiologies include toxoplasmosis and the protozoan Trypanosoma cruzi. Granulomatous myocarditis can occur due to tuberculosis or sarcoidosis. Myocarditis occurs secondary to collagen vascular disease, especially rheumatic fever, and may also be drug or radiation induced.

Idiopathic hypertrophic obstructive cardiomyopathy (*HOCM*): Massive myocardial hypertrophy, and classically, there is asymmetric ventricular septal hypertrophy. Histological examination shows myofiber disarray with hypertrophy and interstitial fibrosis. A scoring system may be used to quantitatively assess the degree of myocardial abnormality.

Idiopathic dilated cardiomyopathy (*congestive cardiomyopathy – DCM*): The clinical presentation of DCM is progressive cardiac failure. There is hypertrophy but also marked dilatation of all chambers. Histological examination shows non-specific abnormalities with hypertrophy and degenerative changes in the myocardial fibers. A significant number of cases are thought to be post-viral.

Restrictive cardiomyopathy (*RCM*): The least common of the three types of cardiomyopathy in developed countries. The ventricles are of approximately normal size or slightly enlarged, and the cavities are not dilated. Histology shows patchy or interstitial fibrosis.

Infiltrative cardiomyopathies: May be due to amyloidosis, hemochromatosis, hemosiderosis, glycogenosis, or mitochondrial myopathies.

Right ventricular dysplasia: A familial idiopathic cardiomyopathy involving mainly the right ventricle. Histological examination shows infiltration of the right ventricular myocardium by adipose and fibrous tissue.

Drug-induced cardiomyopathy: Caused by drugs such as adriamycin and cyclophosphamide, which cause characteristic subcellular changes seen on electron microscopy.

Heart transplant rejection: Graded using the International Society for Heart and Lung Transplantation (ISHLT) grading system.

42.4.1.4 Disorders of the Pericardium

Acute pericarditis: Due to infection caused by viruses or bacteria. Viruses include coxsackie B, echoviruses, influenza, mumps, and Epstein-Barr virus. Bacterial pericarditis may be due to Staphylococcus aureus, Streptococci, or Haemophilus influenza. Tuberculous pericarditis usually becomes chronic.

Acute pericarditis can also be secondary to acute rheumatic fever, myocardial infarction, connective tissue disorders such as systemic lupus erythematosus and rheumatoid disease, uremia, renal transplantation, irradiation, or following cardiac trauma.

Chronic pericarditis: Can lead to *constrictive pericarditis* where the heart is encased in a thick layer of fibrous tissue. Surgical removal of pericardium is the only effective means of treatment.

42.4.2 Neoplastic Conditions

Myxoma: Accounts for 50% of primary tumors of the heart. Familial or sporadic, they usually occur in the left atrium (86%). Familial cases can be multicentric and have extra-cardiac abnormalities (Carney's syndrome). Histological examination shows round, polygonal, or stellate cells in an abundant loose stroma. Mitoses, pleomorphism, and necrosis are minimal to absent. Myxomas may show ossification (petrified myxoma), cartilaginous tissue, extramedullary hematopoiesis, and thymic or foregut remnants. When myxoma is excised with a partial atrial septectomy, it rarely recurs.

Rhabdomyoma: The most common primary tumor of the heart in children. It is often congenital and has a close association with tuberous sclerosis. Most rhabdomyomas are multiple, occur in the ventricles and regress spontaneously.

Other benign conditions include mesothelial/monocytic incidental cardiac excrescences (MICE), papillary fibroelastoma, hemangioma, lipomatous hypertrophy (of the atrial septum), mesothelioma of the atrioventricular node, fibroma, paraganglioma, granular cell tumor, lymphangioma, and schwannoma.

Primary malignant tumors: Very rare and more commonly found in the right side of the heart, benign tumors in the left. Angiosarcoma is probably the commonest, others being leiomyosarcoma, rhabdomyosarcoma, and Kaposi's sarcoma. Primary malignant lymphoma of the heart is very rare, most being diffuse large cell, especially those occurring in AIDS. Secondary involvement of the heart by systemic lymphoma is more usual.

The pericardium is involved in approximately 8.5% of cases of disseminated malignancy, but primary neoplasms are very rare, e.g., mesothelioma of the pericardial sac, germ cell tumor, and angiosarcoma.

42.5 Surgical Pathology Specimens: Clinical Aspects

Endomyocardial biopsies: Right heart biopsies (and occasionally left) are taken via cardiac catheter. They are used to evaluate graft status in cardiac transplant patients and to diagnose cardiomyopathies and intracavitary or myocardial tumors. The auricular appendage may also be sampled at the same time as a mitral valve correction. One third of these biopsies, as well as showing myocardial hypertrophy, have necrobiotic Aschoff nodules. Their presence does not correlate with clinical evidence of the activity of the rheumatic process or with the postoperative course.

Cardiac valves: Native aortic valves are generally resected because of calcific degeneration and are often bicuspid. Mitral valves are usually replaced because of rheumatic valve disease or because the valve is myxomatous. Valves are also resected due to the sequelae of bacterial endocarditis e.g. perforation. Prosthetic valves may be removed because of infection, thrombosis, anastomotic or valvular leakage, hemolysis, obstructive fibrous tissue overgrowth, or mechanical failure e.g. fracture.

Open heart surgical procedures: Used in the repair of ventricular aneurysms, septal resection in HOCM and in the removal of atrial myxomas or other tumors. In resection of a myxoma, the tumor and site of origin such as the atrial septum segment or atrial wall segment is removed.

Heart transplant: Performed in patients with end-stage cardiac failure due to ischemic heart disease or idiopathic cardiomyopathy. The resected specimen usually consists of atria and the upper parts of the ventricles.

42.6 Surgical Pathology Specimens: Laboratory Protocols

42.6.1 Biopsy Specimens

- Count the number of fragments.
- Measure their size (mm).
- Describe – color, consistency.
- Place in cassettes between foam pads or wrapped in filter paper.
- Examine histologically through multiple levels and keep intervening sections for stains, e.g., Masson trichrome (fibrous tissue), Congo Red (amyloid), Perl's Prussian Blue (iron), or immunohistochemistry (CMV antibody, B/T lymphocytes).
- Occasional cases may require fresh frozen tissue or glutaraldehyde fixation for specialist techniques (immunohistochemistry, electron microscopy).

42.6.2 Cardiac Valves

Native valves: Most are received in fragments though some may be submitted intact.

- Identify and document the type of valve – aortic, congenital bicuspid aortic, mitral, tricuspid.
- Photograph if intact.
- X-ray to document calcification.
- Culture.
- Measure the dimensions (cm) of the valve and the valve orifice.
- Describe the leaflets or cusps.
  - Number, sizes (mm), consistency.
  - Abnormalities, e.g., myxoid changes, fibrosis, calcifications, thrombi, perforations.
- Describe vegetations if present – distribution, location, consistency, presence of destruction of the valve leaflet or cusp.
- Describe the commissures – relationship to each other, fused or not, completely or partially.
- Describe the chordae tendineae – length, status – normal/shortened/thickened/stretched/fused/ruptured.
- Describe the papillary muscles – hypertrophy, elongation, scarring – evidence of recent or past myocardial infarction.
- Decalcification may be needed.

  Blocks for histology:
- Representative sections are taken from the free edge of the valve to the annulus.

Mechanical heart valves:

- Culture.
- Photograph.
- Document the type of valve.
- Measure the diameter of the external sewing ring.
- Check function – ability to open and close fully.
- Describe the presence of calcifications, mechanical degeneration, cracks in any of the components.
- Describe the presence of tissue overgrowth.
- Check for vegetations – color, site, size, consistency, presence of underlying destruction.

  Blocks for histology:
- In most cases, it is not possible to submit any tissue for histology unless vegetations are present.

Bioprosthetic heart valve:

- Culture.
- Photograph.
- X-ray – aids type identification, shows calcification (grade 1 to 4) and ring or stent fracture.
- Measure the diameter of the external sewing ring.
- Inspect leaflets for thrombi, vegetations, calcifications.
- Check for fibrous overgrowth.
- Check the valve leaflets for tears or perforations. Document the location and size of any lesions and the effect these appear to have on valve function.

  Blocks for histology:
- A portion of the valve cusp is submitted for histological examination. Vegetations are also submitted if present.

42.6.3 Resection Specimens

Specimen:

May consist of atrial myxomas and other tumors, portions of heart removed during open-heart surgical procedures such as repair of ventricular aneurysms or septal resection in hypertrophic cardiomyopathy.

- Measure the specimen (cm) and weigh (g).
- Document the presence of scarring and if transmural.
- Document any inflammation and its pattern.
- Note the presence of necrosis, calcification, mural thrombus, hemorrhage.
- Describe the endocardium – color, thickness (mm).
- Describe the epicardium – color, thickness (mm).
- Section transversely at 3-mm intervals.
- Sample representative blocks for histology.

Resection for tumor:

- Measure the specimen (cm) and weigh (g).

- Describe the appearance – myxoid, hemorrhage, necrosis, site of origin – atrial wall/ventricular wall/atrial septal wall, infiltration into wall.
- Photograph.
- Fix in 10% formalin for 48 h.
- Ink limits – underlying wall.
- Section lesion, noting appearance and attachment to the wall.

Blocks for histology:

- Sample representative blocks of tumor, tumor and adjacent myocardium, and specimen limits.

Heart transplants:

- Weigh (g).
- Describe the epicardial surface – fat, petechiae, adhesions.
- Fix in 10% formalin for 48 h.
- Use method of cutting appropriate to the specimen:
  – *Apical four chamber cut* – cut longitudinally from apex to base bivalving both ventricles and bisecting the tricuspid and mitral valves. This method is useful in cutting specimens showing dilated cardiomyopathy.
  – *Serial sectioning* – cut heart transversely beginning at the apex and extending to the level of the mitral valve at approximately 1- to 2-cm intervals. The base of the heart may be cut longitudinally or opened according to the lines of flow. This method is useful in ischemic heart disease.
- Describe each ventricle – hypertrophy, dilatation, fibrosis, infarcts, trabeculation, papillary muscles, mural thrombus.
- Measure the thickness of the ventricular walls (mm).
- Describe the atria and any endocardial lesions.
- Describe the valves (as above).
- Dissect atherosclerotic coronary arteries, fix and decalcify them, section transversely at 3- to 5-mm intervals.
- Describe coronary arteries – presence of right or left dominance, thrombi, atheroma, locations.
- Describe bypass grafts if present – type, location, presence of thrombus, atheroma.

Blocks for histology:

- Take sections from the left and right ventricular walls, the ventricular septum, native coronary arteries, bypass grafts, other lesions.

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43 Vessels

Kathleen M. Mulholland

43.1 Anatomy

Vessels consist of three layers – the lining intima, the musculo-elastic media, and the connective tissue adventitia.

Coronary arteries: see Chap. 42.

Aorta: The main trunk of the systemic circulation. It arises from the left ventricle and ascends as the ascending aorta, becomes the arch, the descending aorta, and then the abdominal aorta. The arch supplies the main vessels of the head and neck, the abdominal aorta the viscera of the abdomen and pelvis and the legs via the femoral arteries.

Temporal artery: The external carotid artery ends behind the neck of the mandible by dividing into the maxillary and superficial temporal arteries. The latter ascends over the posterior end of the zygomatic arch on the lateral aspect of the scalp, where it divides into anterior and posterior branches. It supplies the face, the auricle, and the scalp.

43.2 Clinical Presentation

Peripheral vascular disease presents with "intermittent claudication," cramp-like pain in the calf and thigh muscles on exercise, which disappears on resting for a few minutes. It is caused by atherosclerosis which is responsible for multiple symptoms depending on the affected arterial supply–ischemic heart disease leading to angina or myocardial infarction, stroke, transient ischemic attacks (TIA) and intestinal ischemia.

Internal carotid artery atheroma may lead to a transient ischemic attack or cerebrovascular accident (CVA). A TIA is a transient loss of function in one region of the brain lasting less than 24 h. The patient can present with aphasia, hemiparesis, hemisensory loss, hemianopic visual loss, and amaurosis fugax (transient loss of vision in one eye). A CVA produces similar symptoms lasting longer than 24 h.

Aortic abdominal aneurysm presents with epigastric or back pain exacerbated by rupture and may be associated with a palpable, pulsatile abdominal mass.

Thoracic aortic aneurysms cause chest pain or evidence of pressure on other organs such as the superior vena cava or esophagus.

Dissecting aortic aneurysm causes severe central chest pain radiating into the back, arms, and neck. There may be neurological signs due to involvement of spinal vessels. Rupture of an aneurysm is a surgical emergency.

Temporal arteritis presents with a headache, which is often localized to, and tender over, the temporal area. Involvement of the ophthalmic artery may lead to blindness, and prompt treatment with systemic steroids is necessary.

Vasculitides produce a wide spectrum of symptoms depending on the location of the affected

K.M. Mulholland
Histopathology Laboratory, Altnagelvin Hospital, Western Health and Social Care Trust, Londonderry, UK
e-mail: kathleen.mulholland@westerntrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_43, © Springer-Verlag London 2013

vessels including skin rashes, fever, myalgia, arthralgia, malaise, abdominal pain, and renal failure.

43.3 Clinical Investigations

- Arteriography – used to localize the site and extent of vessel blockage and presence of collaterals.
- Doppler studies – used to determine the site of blockage and flow rates in vessels.
- Duplex ultrasound – used to measure pressure in small arteries.
- X-ray – plain X-ray of abdomen may show an aortic aneurysm if the wall is calcified.
- Ultrasound – used to diagnose abdominal aortic aneurysm or deep venous thrombosis.
- CT scan – a sensitive imaging method that allows precise measurement of size, e.g., abdominal aortic aneurysm.
- Transesophageal echocardiography (TEE) – used to diagnose aortic root dissection.
- Ventilation/Perfusion scan (V/Q scan) – detects pulmonary emboli.
- Blood tests:
  - Erythrocyte Sedimentation Rate (ESR) – characteristically over 100 mm in the first hour in temporal arteritis.
  - c-ANCA–associated with Wegener's granulomatosis and some cases of polyarteritis nodosa.
  - p-ANCA–associated with polyarteritis nodosa.

43.4 Pathological Conditions

43.4.1 Non-neoplastic Conditions

Atherosclerosis: Very common and affects the elastic arteries (aorta, carotid, iliac) and large and medium-sized muscular arteries (coronary and popliteal). The vessels show intimal thickening and lipid accumulation producing atheromatous plaques, which may become complicated by calcification, focal rupture, or gross ulceration. Debris can be discharged into the bloodstream forming microemboli. Hemorrhage may occur into a plaque or a thrombus may form on the surface potentially occluding the vessel. With the formation of atheromatous plaques the adjacent media atrophies and aneurysmal dilatation may occur.

Hyaline arteriolosclerosis: Occurs in the elderly with an increased incidence in hypertension and diabetes. There is thickening of the walls with deposition of pink homogenous material and narrowing of the lumen. *Hyperplastic arteriolitis* is a characteristic of, but not limited to, malignant hypertension.

Vasculitis: Inflammation of the walls of blood vessels due to immune-mediated inflammation or to invasion of the wall by pathogenic organisms.

Giant cell arteritis (*temporal arteritis*): A granulomatous arteritis affecting the aorta and major branches, especially the extracranial branches of the carotid artery (temporal, vertebral, and ophthalmic arteries). Most commonly there is a granulomatous inflammation of the inner half of the media centered on the internal elastic lamina. Up to 40% of patients with good clinical evidence of cranial arteritis have a negative temporal artery biopsy. The diagnostic histological findings are often also only found focally within an involved segment.

Takayasu arteritis (*pulseless disease*): A rare granulomatous vasculitis of the aortic arch, its branches, and the pulmonary arteries. Morphological changes may be indistinguishable from giant cell arteritis, but the clinical profile differs with patients usually being female and under the age of 40 years but elderly in giant cell arteritis.

Polyarteritis nodosa: A relatively uncommon condition causing necrotizing fibrinoid vasculitis of small- to medium-sized arteries particularly in the kidneys, heart, liver, and gastrointestinal tract. Vessel necrosis, thrombosis, rupture, and aneurysms occur with fibrous repair resulting in mural nodularity.

Microscopic polyarteritis (*leukocytoclastic vasculitis*): Involves arterioles, capillaries, and venules. It affects skin, mucous membranes, lungs, brain, heart, gastrointestinal tract, kidneys, and muscle in isolation or various combinations. It is much more common than polyarteritis nodosa and may be precipitated by drugs or infections.

Kawasaki syndrome: A rare arteritis, which affects the large, medium, and small arteries (often coronary arteries). Eighty percent are less than 4 years old and 20% develop cardiovascular sequelae.

Wegener's granulomatosis: A focal necrotizing or granulomatous vasculitis involving small- and medium-sized vessels, most prominent in the lungs or upper airways and associated with focal or necrotizing (often crescentic) glomerulitis.

Aneurysm: An abnormal widening of a blood vessel wall. In a true aneurysm, the walls make up the boundary; in a false aneurysm, the boundary is made of hematoma or fibrous tissue.

Abdominal aortic aneurysms: The most common site for atherosclerotic aneurysms, usually below the renal arteries, above the bifurcation of the aorta. Aneurysms less than 5 cm diameter rarely rupture, while about 50% of those more than 5 cm suffer fatal rupture within a 10-year period. Operative mortality after rupture is approximately 50% but 5% prior to it. A small minority are inflammatory in type, with a thick cuff of surrounding fibrous tissue, and associated with obstruction of the ureters.

Dissecting aneurysms: Blood enters the wall of the aorta and dissects between layers. It affects two groups of patients – males predominantly between the age of 40–60 years with a history of hypertension and a younger group with an abnormality of the connective tissue, e.g., Marfan's syndrome. Histological examination shows cystic medial degeneration with elastic tissue fragmentation. Surgery involves plication of the aortic wall (65–75% of patients with dissection survive).

Syphilitic aneurysms: Obliterative endarteritis affects the vasa vasorum leading to a thoracic aortitis and subsequent aneurysmal dilatation of the thoracic aorta and the aortic annulus. These are now rare in developed countries.

Other aneurysms: *Berry aneurysms* occur in the circle of Willis of the brain, due to congenital defects in the vessel wall, and are an important cause of sudden subarachnoid hemorrhage in young adults.

Mycotic aneurysm: Occurs in the arterial wall secondary to damage caused by sepsis. They are rare in developed countries. *Polyarteritis nodosa* may be associated with multiple microaneurysms. *Kawasaki disease* causes arteritis and aneurysm of the coronary arteries.

Varicose veins: Abnormally dilated, tortuous veins due to prolonged intraluminal pressure or loss of support of the vessel wall. They affect a wide range of patients but particularly obese females over 50 years of age. There is also a familial tendency. Varicosities also occur in the esophagus secondary to portal hypertension in association with liver cirrhosis. Hemorrhoids are varicose dilatations of the hemorrhoidal plexus of veins at the anorectal junction.

43.4.2 Neoplastic Conditions

Benign

- Hemangioma – capillary, cavernous, pyogenic granuloma (lobular capillary hemangioma).
- Lymphangioma.
- Glomus tumor.
- Vascular ectasia.
- Bacillary angiomatosis is a reactive vascular proliferation.

Intermediate Grade Neoplasms

- Kaposi's sarcoma
- Hemangioendothelioma

Malignant Neoplasms

- Angiosarcoma
- Hemangiopericytoma

These specimens are discussed in the skin and soft tissue chapters.

43.5 Surgical Pathology Specimens: Clinical Aspects

Dissecting aortic aneurysm: A section of aorta is excised which shows a medial hematoma with an associated intimal flap entrance site and often either an intimal re-entrant or adventitial rupture site.

Abdominal aortic aneurysm: Surgical repair involves opening the aneurysm and removing the clot. The graft is sewn inside the aorta, and the wall of the aorta is closed. The specimen may consist of clot only, or clot with media.

Internal carotid endarterectomy: Considered in symptomatic patients who have carotid artery stenosis that narrows the arterial lumen by more than 70%. The specimen may retain the shape of the bifurcation. It consists of luminal plaque with portions of intima and media attached.

Atherectomy: The removal of atherosclerotic plaque by cardiac catheterization. Open thrombectomy or embolectomy of peripheral vessels, e.g., femoral artery, is also undertaken for the acutely ischemic limb.

Vascular grafts: Removed because of thrombosis, fibrous obstruction, or infection.

Coronary artery bypass graft (*CABG*): During the second CABG, the saphenous vein or internal artery mammary grafts are occasionally removed.

Temporal artery: A biopsy of approximately 2–10 mm length is taken.

Varicose veins: Usually inverted during the procedure and not submitted for histology.

43.6 Surgical Pathology Specimens: Laboratory Protocols

Vessel specimens:

- Measure the length, internal and external diameter (mm) of the vessel.
- Examine the lumen for thrombi.
- Estimate the percentage of luminal narrowing caused by any lesions.
- Examine the media – check for aneurysm formation, fibromuscular hyperplasia, calcification, and rupture.

Blocks for histology:

- Sample multiple representative transverse sections of the vessel.

Aortic dissection:

- Measure (cm) and weigh (g).
- Describe the location of the dissection, intimal flap entrance, intimal re-entrant site, or adventitial rupture site.
- Take sections from areas of medial separation and from grossly normal tissue. One section should be stained for elastin.
- When embedding, orientate the specimens on edge to ensure the entire thickness can be assessed.

Aortic aneurysm:

- Measure (cm) and weigh (g).
- Describe – thrombus only or aortic wall also present – color, consistency, organization (lines of Zahn), and calcification.
- Serially section the thrombus to look for tumor or mycotic aneurysm.

Atherectomy specimens:

- Count and measure the fragments (mm).
- Decalcify plaques.
- Submit all fragments.

Endarterectomy:

- If intact, open longitudinally.
- Measure (cm).
- Describe – shape, color, calcification, stenosis.
- Decalcification may be needed.
- Sample representative transverse blocks.

Embolectomy specimens:

- Measure (mm).
- Examine for tumor fragments.
- Serially slice transversely and submit representative blocks.

Temporal artery:

- Measure – length and diameter (mm).
- Describe – color, presence of thrombus, wall thickening.
- Cut into 2–3 mm cross-sections after processing and before embedding.
- Embed each piece on end.
- Examine histologically through multiple levels and keep intervening sections for stains, e.g., elastin.

Vascular grafts:

- Culture.
- Measure – length and diameter (cm).
- Describe – type of graft – saphenous vein, Gore-tex grafts, Dacron grafts; color, tears or holes, thrombus.

- Sections of graft may be submitted – saphenous vein and Gore-tex are easy to cut; Dacron is difficult.

Coronary artery bypass grafts:
- Measure – length and diameter (mm).
- Describe – atherosclerosis, thrombus.
- Take multiple cross sections of the graft.

Varicose veins:
- Measure – length and diameter (cm).
- Describe – thrombus, wall thickening, nodularities.
- Take one transverse section.

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Part X

Osteoarticular and Soft Tissue Specimens

44 Joint Space, Bone, Soft Tissues, and Special Techniques

Richard I. Davis

44.1 Joint Space

44.1.1 Anatomy

Most joints are *synovial* joints formed by a thin lining of synovium which secretes fluid into the joint (Fig. 44.1). The joint is covered by a capsule. The synovium not only forms the lining of joints but also covers tendon sheaths and bursae.

The synovial membrane consists of an intimal layer and the subintimal supportive layer of fibrofatty tissue. The intima is 1–2 cell layers thick and composed of synoviocytes. About 90% are fibroblast-like, but the other 10% have ultrastructural features of macrophages.

The space between the two articulating bone surfaces is occupied by articular hyaline cartilage (Fig. 44.1). It is firm pliable tissue and resists compressive forces. In young people, it is bluish-white and translucent, but in later life, it becomes opaque and yellow. Cartilage is avascular and devoid of nerves and lymphatics, obtaining nutrients by diffusion from the surrounding synovial fluid.

Cartilage is rather poorly cellular tissue composed of chondrocytes laid down within a matrix or ground substance composed of collagen fibers and proteoglycans. The latter are complex biopolymers consisting of a central protein core with attached chains of carbohydrates. These proteoglycans include chondroitin sulfate and keratan sulfate and can absorb large volumes of water to form gels.

44.1.2 Clinical Presentation

Symptoms of joint disease generally tend to be nonspecific, and accurate diagnosis depends on detailed clinical history noting the number of joints involved, sites, and specific patterns of joint disease. Typical symptoms include pain, stiffness, swelling, and reduced range of movement. Severe acute inflammation such as in septic arthritis results in a red, hot, swollen joint. Effusions due to increased fluid, blood, or pus may also be seen.

44.1.3 Clinical Investigation

- Routine blood tests such as white cell count, ESR, and C-reactive protein to detect active inflammation. Checking the blood for the presence of increased quantities of antibodies (such as rheumatoid factor) is very important in rheumatology. These tests are generally not specific and need to be interpreted carefully in the light of the patient's clinical history and pattern of joint involvement.
- Plain X-ray and tomography are performed in order to detect evidence of arthritis such as loss of joint space (due to destruction of cartilage), osteophytes (bone irregularities), bone

R.I. Davis
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: richard.davis@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_44, © Springer-Verlag London 2013

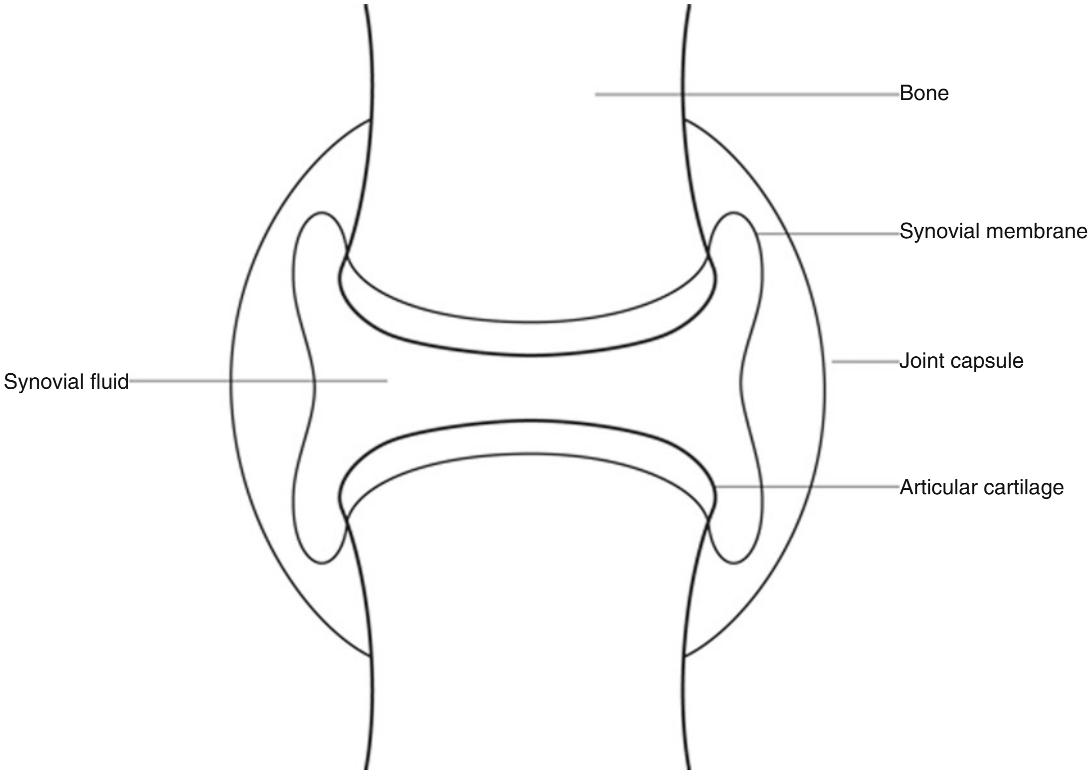

Fig. 44.1 Synovial joint (Reproduced, with permission, from Allen and Cameron (2004))

sclerosis (thickening), and localized osteoporosis (thinning).

- Effusions may be aspirated and investigated for the presence of blood, pus, cell content, and crystals. This really constitutes *synovial fluid analysis*. In order to detect the presence of crystals, the fluid is examined using polarized light with a red filter. The needle-shaped crystals associated with gout exhibit a strong negative birefringence, whereas the rhomboid-shaped crystals associated with pseudogout exhibit weak positive birefringence.
- In general, biopsy has very little role to play in the investigation and diagnosis of joint disease. Accurate clinical diagnosis seldom depends on histological analysis of the synovium.

44.1.4 Pathological Conditions

44.1.4.1 Non-neoplastic Conditions

Synovitis: the majority of patients have nonspecific synovitis characterized by hyperplasia of the synovium and chronic inflammation. There are really no specific features which can be regarded as pathognomonic of any particular type of synovitis.

Purulent synovitis: the synovium contains large numbers of neutrophil polymorphs and is consistent with septic arthritis.

Rheumatoid arthritis: a rheumatoid nodule may be seen and is highly suggestive of this condition, but only a small number of biopsies (less than 5%) display this feature.

Granulomatous inflammation: the presence of granulomata can indicate infection due to tuberculosis, atypical TB, fungi, sarcoid, or reaction to foreign body material such as prosthetic wear products.

Prosthetic reactions: patients who have had knee or hip arthroplasty may suffer from joint loosening years later due to foreign body reaction and inflammation caused by breakdown of the prosthetic materials. A foreign body reaction composed of giant cells and macrophages with doubly refractile material can be seen.

Crystal arthropathy: this includes *gout* and *pseudogout*. A giant cell reaction to doubly refractile material may be seen. Synovial fluid analysis can be helpful in this diagnosis. Gout can also be associated with extra-articular soft tissue lesions or tophi, e.g., in the skin overlying the elbows or ears.

Synovial chondromatosis: islands of metaplastic cartilage in the synovium characterized clinically by the presence of loose bodies and reduced joint movement.

Pigmented villonodular synovitis: the presence of hemosiderin pigment with a collection of macrophages and giant cells may indicate a reaction to *hemarthrosis* (hemophilia or trauma) or pigmented villonodular synovitis.

44.1.4.2 Neoplastic Conditions

Very rarely a benign giant cell tumor of tendon sheath may occur in a joint space. This is seen when the tumor arises within an intra-articular tendon such as the knee. Primary or secondary malignancy is exceedingly rare in the joints.

44.1.5 Surgical Pathology Specimens: Clinical Aspects

44.1.5.1 Biopsy Specimens

In general, biopsy has very little role to play in the investigation and diagnosis of joint disease. Synovium is difficult to biopsy, and sufficient material is only reliably obtained at arthroscopy or at open joint exploration. Most inflammatory arthritides have similar histological features, and therefore, biopsy will not discriminate one type of arthritis from another. For these reasons, synovial biopsy is not routinely performed.

44.1.5.2 Resection Specimens

Resection of the synovium is seldom undertaken except for a florid synovial chondromatosis or pigmented villonodular synovitis. Partial or total synovectomy is technically difficult and may often lead to damage to the articular cartilage in later life.

44.1.6 Surgical Pathology Specimens: Laboratory Protocols

44.1.6.1 Biopsy and Resection Specimens

The tissue is submitted in formalin. However, if the clinician strongly suspects the presence of gout, the biopsy should be sent in alcohol and not formalin so as to better preserve the crystals. The size of the biopsy should be recorded and is usually submitted in toto. There are usually no distinctive gross features to note except for the tan coloration associated with a hemarthrosis or pigmented villonodular synovitis or the presence of cartilage as in synovial chondromatosis.

Histopathology Report:

- Presence of hyperplasia
- Inflammation – intensity (mild/moderate/severe), diffuse/focal, and acute/chronic/granulomatous
- Presence of hemosiderin/crystals/prosthetic wear products/cartilage

44.2 Bone

44.2.1 Anatomy

Bones may be classified as long (femur, humerus, radius, ulna), tubular (small bones of hands and feet), or flat (scapula, pelvis, rib, vertebrae). The shell of the bone is called the *cortex*, and the interior is known as the *medulla* which consists of interconnecting bars of bone called *trabeculae*. This trabecular bone is also referred to as *cancellous* bone. The thickness of the cortex varies considerably along the length of a given bone and especially between different bones. The proportion of a bone occupied by cortical and cancellous bone also varies between bones and with age. The trabecular bone is set in a fatty marrow containing hemopoietic tissue. The cortex is covered by a thin tough mesenchymal layer known as *periosteum*. The ends of a bone are known as the *epiphysis*. The *metaphysis* is the region immediately adjacent to the epiphysis, and the *diaphysis* is the shaft (area between the two metaphyses) (Fig. 44.2).

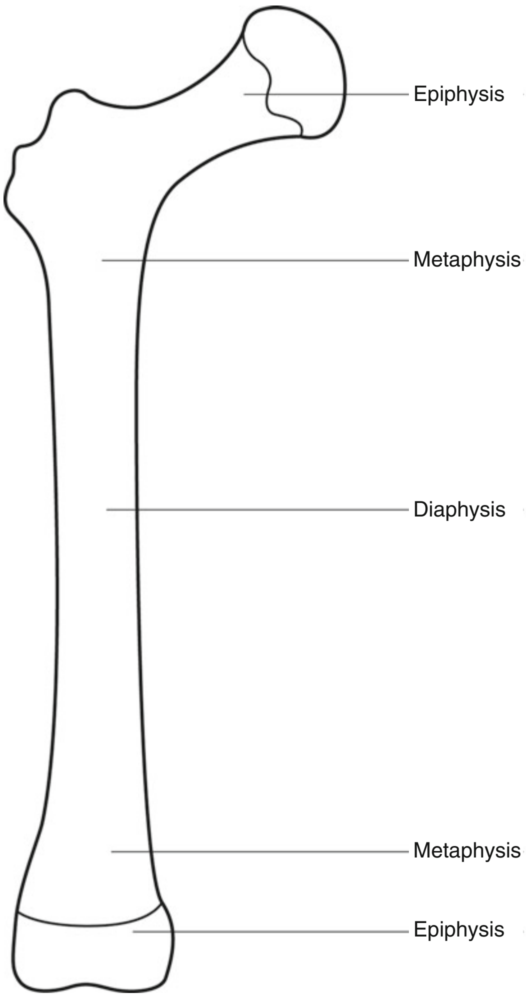

Fig. 44.2 A typical long bone (Reproduced, with permission, from Allen and Cameron (2004))

44.2.2 Clinical Presentation

The symptoms of bone disease are few and rather nonspecific. The most common symptom is pain which may be of variable intensity. Severe unremitting pain continuing at night in bed is insidious and suspicious of malignancy. Pain relieved by nonsteroidal anti-inflammatory agents is suggestive of a benign osteoid osteoma. Remember that sometimes pain felt in one bone may be *referred* pain, that is, disease originating elsewhere. A swelling is indicative of a primary bone tumor. Pathological fracture, a fracture occurring due to low-impact trauma, is indicative of a diseased bone and suggests osteoporosis, multiple myeloma, or metastatic disease.

44.2.3 Clinical Investigations

- Routine blood tests include white cell count, ESR, and C-reactive protein for the presence of inflammation. A *calcium profile* consists of serum calcium, phosphate, and alkaline phosphatase. This test is particularly useful in endocrine or metabolic bone disease. *Hypercalcemia* is seen in primary hyperparathyroidism and metastatic disease. A raised alkaline phosphatase can be indicative of increased osteoblast activity and if very high suggests Paget's disease. The alkaline phosphatase results need to be interpreted in the light of the age of the patient as a child or adolescent during a growth spurt will have very elevated values. Plasma protein electrophoresis will detect a monoclonal gammopathy indicative of myeloma.
- Urinary hydroxyproline – a useful measure of osteoclast activity and is elevated in Paget's disease.
- Good quality plain X-ray – using two views is still the mainstay of skeletal radiology. X-rays should be examined carefully for a periosteal reaction, indicative of osteomyelitis or a tumor.
- Isotope bone scan – measures osteoblast activity. A positive bone scan confined to one bone suggests fracture, infection, or tumor. A positive result in several long bones indicates metastatic disease, growth spurt, or generalized arthritis.
- CT scan – extremely valuable in the diagnosis of a primary bone tumor.

44.2.4 Pathological Conditions

44.2.4.1 Non-neoplastic Conditions

Fracture: a fracture is not routinely biopsied unless there is nonunion, delayed healing or is thought to be pathological. In the former, there is little or no new bone formation and only loose fibrous tissue. Sometimes there may be evidence of accompanying infection. Pathological fracture is most often due to metastatic carcinoma or myeloma although occasionally a primary bone tumor such as malignant fibrous histiocytoma, dedifferentiated chondrosarcoma, or a benign bone cyst may be the cause.

Osteomyelitis: in the acute stages, this disease is not routinely biopsied, but when a biopsy is submitted, material will be routinely sent to microbiology for culture as well. Infection is most commonly due to S*taphylococcus aureus*, but increasingly, low virulence organisms are being implicated as in drug addicts or the immunosuppressed. Chronic osteomyelitis can result in marked bone deformity and usually is characterized by prominent bone formation with quite minimal inflammation. The pathologist should always be aware of tuberculosis which is increasing in incidence.

Metabolic bone disease: osteoporosis is very effectively diagnosed using DEXA (dual energy X-ray absorptiometry) and is almost never routinely biopsied. Osteomalacia is most uncommon in the UK but may rarely be seen in renal failure or in patients taking long-term phenytoin therapy. Paget's disease is easily diagnosed using plain X-ray, serum alkaline phosphatase, and urinary hydroxyproline so it is almost never biopsied.

Avascular necrosis: seen in some fractures (neck of femur, scaphoid, talus), chronic steroid therapy, alcohol abuse, sickle cell anemia, Caisson disease (dysbarism).

44.2.4.2 Neoplastic Conditions

Benign tumors: benign tumors are relatively rare, and the most common is the *osteochondroma*, a bony polyp with a cap of hyaline cartilage seen usually in long bones. *Enchondromas* are cartilaginous tumors occurring in the medullary cavity of long bones. *Osteoid osteoma* is a painful lesion occurring in the cortex of a long bone, with a central lytic nidus and a margin of sclerotic bone. A *giant cell tumor* of bone is seen in people aged 20–40 years of age and characteristically represents a lytic lesion occurring in the epiphysis of long bones. Other benign tumors include osteoblastoma, chondroblastoma, and chondromyxoid fibroma, but these are very rare.

Tumorlike conditions: these include cysts, reparative granulomas, fibrous dysplasia, benign fibrous histiocytoma, and eosinophilic granuloma.

Primary malignant tumor: these are also rare and most commonly seen in young people and children. O*steosarcoma* is a high-grade sarcoma producing malignant osteoid, typically seen in the metaphysis of long bones and people aged 10–25 years old. It is very rare in older people but can be associated with previous radiation exposure and Paget's disease. It is treated by a combination of chemotherapy and surgery. *Ewing's sarcoma* is a poorly differentiated small round blue cell tumor seen in the pelvis and long bones of children and young adults. It is treated with a combination of chemotherapy, radiotherapy, and surgery. *Chondrosarcomas* are usually low-grade sarcomas occurring in long bones and flat bones in middle-aged and older people. They have a tendency for recurrence rather than metastasis. They do not respond to chemotherapy, and treatment is surgical removal. The *dedifferentiated* chondrosarcoma is a high-grade tumor, typically large in size occurring in the pelvis and proximal femur of older people. These tumors metastasize early and have an extremely bad prognosis.

Multiple myeloma: this is really a hematological tumor but sometimes classified as a bone tumor. It is a tumor arising from the plasma cells and occurs in multiple skeletal sites. It is treated with chemotherapy.

Metastatic carcinoma: this is by far the most common malignant tumor in bone and presents as bone pain or pathological fracture. The most common primary sites are lung, kidney, breast, prostate, and thyroid. Most metastatic tumors produce lytic lesions, but prostatic secondaries are often sclerotic. The usual skeletal sites are proximal long bones, rib, pelvis, and vertebrae. Metastatic disease is distinctly uncommon in the skeleton distal to the elbow or knee.

44.2.5 Surgical Pathology Specimens: Clinical Aspects

44.2.5.1 Biopsy Specimens

Fine needle aspiration (FNA) cytology has only a very limited role in the diagnosis of primary bone tumors. Bones are obviously deep-seated and due to the hard nature of the tissue do not avail themselves to aspiration cytology. Although this technique is very well established in breast and head and neck pathology, it has almost no role to play in the diagnosis of primary bone tumors. Occasionally radiologically guided fine needle aspiration can be performed where metastatic disease is suspected.

Needle biopsy (Jamshidi needle or Surecut) under radiological control is the preferred method used to obtain a biopsy. Often the radiologist performs the biopsy. This method has the advantage of saving valuable theater time, requires minimal anesthesia, and is much less invasive for the patient and for planning future treatment. It is most important that good radiological imaging is available to ensure that the needle is in the right place. Occasionally needle biopsy fails to obtain a good sample to allow definitive diagnosis to be made and then open biopsy is required.

44.2.5.2 Resection Specimens

Femoral heads removed at hip replacement for arthritis or repair of hip fracture are not routinely submitted for pathology. They are submitted in cases of pathological fracture, avascular necrosis, or in rapidly progressive arthropathy occurring in young people.

Malignant tumors need to be removed completely with a tumor-free margin or else they will recur. Characteristically malignant bone tumors also permeate the soft tissues adjacent to the bone. These soft tissues must also be removed. Detailed preoperative planning and careful examination of MRI scans is required to determine the appropriate dissection. An *intralesional* excision occurs when the surgeon cuts through the tumor. A *marginal* excision is where the tumor is completely removed without any significant margin of normal tissue. A *wide* excision is where the surgeon removes the tumor completely with a cuff of normal tissue. A *radical* excision is where the entire muscle compartment of bone is removed, and it usually implies the removal of the joint proximal to the tumor. These are often disarticulations.

44.2.6 Surgical Pathology Specimens: Laboratory Protocols

44.2.6.1 Biopsy Specimens

Needle biopsy specimens are submitted directly in formalin. If gritty or firm, they are put in 4% acetic acid/formalin for 1–4 h and then in EDTA overnight. Decalcification is clearly important so as to obtain good sections, but gentle decalcification is necessary in case immunohistochemistry is required. Vigorous decalcification using formic acid can destroy the tumor cells and expressed antigens rendering subsequent immunohistochemistry unhelpful. The number of cores and lengths in millimeter should be recorded.

Small bony fragments, curettings, or reamings from a primary bone tumor, infection, or metastatic disease are placed into cassettes and decalcified in 10% formic acid after adequate fixation. If the curettings are from a suspect bone cyst or primary tumor, it is advisable to use gentler methods of decalcification such as 4% acetic acid/formalin and overnight treatment with EDTA.

Bone biopsy for osteomalacia requires undecalcified sections cut by a sledge microtome, plastic embedding, and use of trichrome/toluidine blue stains. Fluorescent tetracycline labeling is also used for the assessment of bone turnover.

The diagnosis of osseous tumors may also be aided by the use of alkaline phosphatase stains. Briefly, air-dried imprints are made from fresh tissue and stained for alkaline phosphatase demonstrated by the naphthol ASBI phosphoric acid method. The alkaline phosphatase appears as bright red intracytoplasmic granules.

44.2.6.2 Resection Specimens

Femoral heads or large chunks of bone require adequate fixation and are then cut coronally with a junior hacksaw or vibrating table saw. The diameter of the specimen should be recorded in centimeters. The thin sawn slices are placed in 10% formic acid for decalcification. The decalcified slices are then serially blocked, labeled, and submitted in toto.

Amputation specimens are examined to:

- Evaluate the resection margins including vessel involvement
- Determine the extent of bone involvement
- Evaluate the tumor necrosis, a measure of response to prior chemotherapy

Initial Procedure:

- Ideally, amputation specimens should be properly fixed before attempting dissection, but for large specimens especially above-knee amputations, it is not practicable, and therefore, extreme care must be taken.
- The hand (left or right) and limb (upper or lower) are recorded.
- Palpate for any obvious tumor masses or soft tissue involvement.
- Look for tumor satellite lesions.
- Note any previous scars including length and location.
- Locate the proximal limit of all major nerves and vessels.
- Confirm that the proximal limit is free of tumor.
- Cut through the skin and soft tissues to determine if soft tissue spread has occurred; the soft tissue should be incised to the bone.
- Locate and trace major nerves and vessels to ensure they are free and not encased by tumor.
- The bone itself is bivalved with a bandsaw or mortuary skull saw. Extreme safety precautions and care must be taken when using the bandsaw and only specifically named personnel, specially trained in its use, should be allowed to use this apparatus.
- Measurements:

  Amputated bone
  - Length (cm)

  Tumor
  - Length, width, and depth (cm)
  - Distance (cm) to proximal resection margin
  - Depth of overlying skin and soft tissues free of tumor
  - Extent of extraosseous tumor involvement in soft tissues

Description:

- Tumor
  - Location (bone surface, cortex, intramedullary canal)
  - Site (epiphysis, metaphysis, diaphysis)
  - Relationship to nearby joints or joint involvement
  - Gross features (osseous, cartilaginous, cystic change, necrosis)

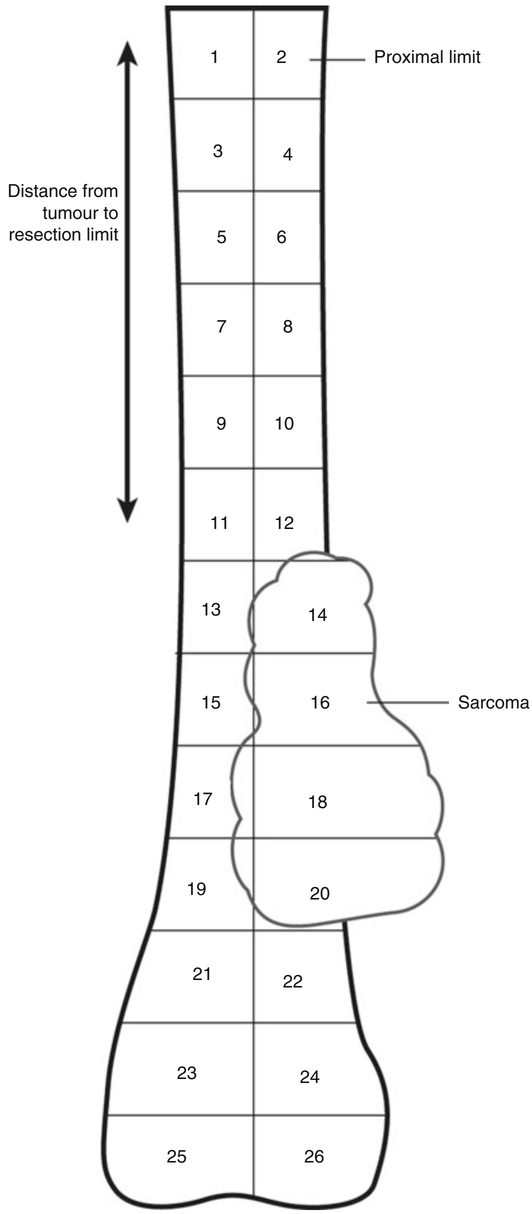

Fig. 44.3 Taking blocks from a bone slab containing sarcoma (Reproduced, with permission, from Allen and Cameron (2004))

Blocks for Histology (Fig. 44.3):

- Vessel limits.
- Marrow and proximal margin of resection.

- Any scars related to previous surgery or open biopsy.
- Representative blocks of involved soft tissues.
- Representative blocks of tumor obviously around or in major vessels.
- Blocks to evaluate the tumor characteristics and extent of necrosis for assessing response to chemotherapy.
- The extent of tumor necrosis is roughly estimated by calculating the tumor necrosis present in a whole bone slab 0.4 cm thick. Having bivalved the affected bone, this 0.4-cm longitudinal slab is obtained by cutting along the plane of maximum tumor diameter. The slab is drawn out and the whole slab cut into blocks for histology. These blocks are individually labeled and are correspondingly noted and labeled on the written diagram. About 20–40 blocks are required to achieve this properly (Fig. 44.3).
- The blocks are properly fixed in formalin prior to decalcification. It is most important that blocks, especially these relatively large bone blocks, are well fixed as the acid used in decalcification can destroy the cell morphology. Often about 48 h, fixation is required.
- Decalcification is obtained using 10% formic acid. This may take several days.

  Histopathology Report:
- Type of specimen, that is, core, reamings, resection, and amputation.
- Tumor type – osteosarcoma/Ewing's sarcoma/chondrosarcoma.
- Tumor size, if relevant.
- Tumor subtype – parosteal/small cell osteoblastic/chondroblastic, fibroblastic, and dedifferentiated.
- Tumor grade – osteosarcoma and Ewing's (nearly all are high grade), chondrosarcoma (grades I–III), and dedifferentiated.
- Tumor necrosis – response as a percentage for an entire slab (applies only to osteosarcoma).
- Extent of local tumor spread – medullary cavity/cortex/extraosseous soft tissues/proximal marrow involvement.
- A TNM system is available to help with staging bone tumors. It is rather complicated, and full details are beyond the scope of this book. Moreover, unlike carcinomas, it will be recalled that most sarcomas seldom spread to the lymph nodes and tend to metastasize directly to the lungs. However, for practical purposes, a simplified summary of the results of the TNM system is given below.

  Stage Grouping

Stage 1A	Low grade, small, superficial, and deep
Stage 1B	Low grade, large, and superficial
Stage 11A	Low grade, large, and deep
Stage 11B	High grade, small, superficial and deep
Stage 11C	High grade, large, and superficial
Stage III	High grade, large, and deep
Stage IV	Any metastasis

- Many surgeons tend to use a surgical staging system devised by Enneking.

  Enneking System

Stage	*Grade*	*Site*
1A	G_1 (low)	T_1
1B	G_1 (low)	T_2
2A	G_2 (high)	T_1
2B	G_2 (high)	T_2
3	G_1 or G_2	Metastases

T_1 intracompartmental
T_2 extracompartmental

- Lymphovascular invasion including vessel limits
- Excision margins – proximal limit, skin, and overlying scars
- Results of any cytogenetic/molecular pathology investigations

44.3 Soft Tissues

44.3.1 Anatomy

The soft tissues refer to nonepithelial extraskeletal tissue of the body, but not including the hemopoietic or brain tissue. It really consists of muscle, fat, and fibrous tissue along with blood vessels and peripheral nerves.

44.3.2 Clinical Presentation

Most soft tissue lesions present as a lump or swelling and are usually painless. The lump may reach quite a size before the patient is aware of its existence, particularly if the lump is deep-seated or in the retroperitoneum. From a clinical point, the most important aspect is the possibility that the soft tissue lump could be malignant, that is, a sarcoma. In general, any lump which is more than 5 cm in size, deep-seated, painful, or growing rapidly should be considered as a possible sarcoma and investigated accordingly. Early diagnosis improves patient outcomes.

44.3.3 Clinical Investigations

- Routine blood tests have no useful role to play in diagnosing a soft tissue lump.
- Plain X-rays also yield very little information unless there is calcification or ossification within the lump. This may sometimes be seen in synovial sarcomas.
- Ultrasound imaging can rapidly triage benign from more suspicious lesions.
- The most useful investigation in assessing soft tissue lumps is magnetic resonance imaging (MRI), but the interpretation of the scans is complicated and depends on a highly trained and experienced radiologist. MRI can define the composition of a lump, identify the location and extent of the mass, provide information on involvement of nearby structures such as nerves and vessels, may help differentiate between benign and malignant tumors, and can stage a malignant tumor locally.
- CT scan is used for detection of bony involvement and preoperative staging of pulmonary metastases.

44.3.4 Pathological Conditions

44.3.4.1 Non-neoplastic Conditions

Ganglion: this is a fibrous-walled cyst typically arising from a tendon in the hands, wrist, or feet. They are regarded as herniations of synovium, usually have no recognizable lining, and a focally myxoid fibrotic wall with gelatinous contents.

Bursa: this is a swelling which may be painful, arising from the synovial lining between muscles, tendons, and bones. They are especially common near joints. They include Baker's cyst at the back of the knee and housemaid's knee.

Rheumatoid nodule: this is an irregular swelling seen in the soft tissues or in the organs in patients with rheumatoid arthritis. However, not every patient has well-established rheumatoid arthritis. It forms part of the necrobiotic collagen disorders.

44.3.4.2 Neoplastic Conditions

Benign tumor: there are many different benign soft tissue lesions including lipomas, chondromas, neural tumors, leiomyomas, and a range of fibromatoses and fasciitis conditions. *Lipomas* are by far the most common and are usually less than 2–3 cm in size, mobile, and superficial. Some lipomas can be quite large and deeply located and may contain small numbers of atypical cells. It may be difficult to determine if these lesions are benign or malignant. *Fibromatoses*, e.g. (Dupuytren's contracture), are irregular, poorly defined lesions which can be difficult to remove surgically and have a high rate of recurrence.

Nodular fasciitis: a rapidly growing lump which clinically and microscopically can mimic malignancy.

Sarcomas: these are malignant soft tissue tumors and include such lesions as liposarcomas, fibrosarcomas, synovial sarcoma, malignant fibrous histiocytoma, and leiomyosarcomas. They are quite rare, and benign soft tissue lumps outnumber sarcomas by a ratio of 50–100:1. The precise classification of sarcomas is very complicated but clinically and prognostically is of limited value. Surgery remains the mainstay of treatment and chemotherapy, except in a few specific tumors such as extraskeletal Ewing's sarcoma or childhood rhabdomyosarcoma, has little role to play as the toxicity of the therapy outweighs any benefits in increased survival. The most important features are size, grade, and stage. Grading in turn depends on differentiation, necrosis, and mitotic rates. The 5-year survival for most sarcomas is about 40%.

44.3.5 Surgical Pathology Specimens: Clinical Aspects

44.3.5.1 Biopsy Specimens

Fine needle aspiration cytology has only a limited role to play, but in some large national centers, with good clinicopathological correlation and highly experienced operators, it can be used to reliably distinguish most benign and malignant soft tissue lumps. If FNA is to be relied on, it is most important that the cytopathologist is well experienced in dealing with soft tissue lumps. The technique is quick, relatively painless, and requires no anesthesia. It is essential that any soft tissue swelling is properly assessed clinically and radiologically prior to FNA. Many lesions may be too deeply located to rely on FNA. Sometimes only necrotic tissue is obtained. Some benign lesions can contain atypical cells such as nodular fasciitis, and some malignant tumors such as synovial sarcoma or well-differentiated liposarcoma have rather bland cytology. Immunohistochemistry and where available cytogenetic analysis may be performed on fine needle aspiration specimens. FNA is also useful for assessing recurrence or metastases in patients with previously diagnosed sarcoma.

In most UK centers, needle core biopsy (Jamshidi or Surecut) performed under radiological control is the preferred method of obtaining a tissue diagnosis. More than 90% of these lesions are diagnosed using this technique. It must be emphasized that FNA and needle biopsy are only used to determine if a swelling is benign or malignant. Biopsy is not a reliable means for precise subdiagnosis. Some soft tissue tumors have a variety of patterns, and needle biopsy may result in sampling error.

Open biopsy is used when FNA or closed needle biopsy have failed. It is performed by a surgeon, and general anesthesia is required. Only experienced surgeons specifically trained to deal with soft tissue tumors should perform this procedure and not by general surgeons. A wedge of tissue is removed, preferably along the long axis of the tumor and directly over the tumor.

44.3.5.2 Resection Specimens

Treatment aims to provide complete local excision with an acceptable resection margin and, if possible, limb salvage surgery with retention of a functional limb. Large pelvic or retroperitoneal tumors impacting on several organ systems may require a multidisciplinary surgical approach. Postoperative radiotherapy improves local control and is used for intermediate to high-grade tumors, deep-seated tumors, and those with a close or incomplete resection margin.

Intracapsular excision: these are performed inside the tumor and are often piecemeal in nature, and local recurrence is almost 100%.

Marginal excision: this refers to removal of the lesion but without any significant margin of normal tissue. Sometimes the excision biopsy is referred to as shelling out. This technique is adequate for superficial tumors or smaller tumors less than 3 cm in diameter. In larger lesions, it can leave satellite nodules in the surrounding zone of reactive tissue with a high local recurrence rate.

Wide excision: these are excisions through the normal tissue beyond the reactive zone associated with the tumor but still within the muscle compartment of origin. The tumor is never visualized during the procedure. Low recurrence rates are achieved.

Radical excision: this is the en bloc removal of the tumor and entire muscle compartment of origin. A radical amputation usually requires disarticulation of the joint proximal to the involved compartment.

44.3.6 Surgical Pathological Specimens: Laboratory Protocols

44.3.6.1 Biopsy Specimens

Biopsies are usually in the form of needle-shaped pieces of tissue. They will usually be submitted directly in formalin. The biopsies are examined through levels. Special techniques such as immunohistochemistry and cytogenetics can be helpful (see later).

44.3.6.2 Resection Specimens

Most resections for soft tissue tumors will consist of the tumor with a margin of uninvolved soft tissue. It is very unusual to submit any attached bone, and actual amputations are extremely rare.

Initial procedure:

- Palpate the soft tissue to locate the tumor.
- Paint the outer surface in toto or selectively to assist the assessment of margins.

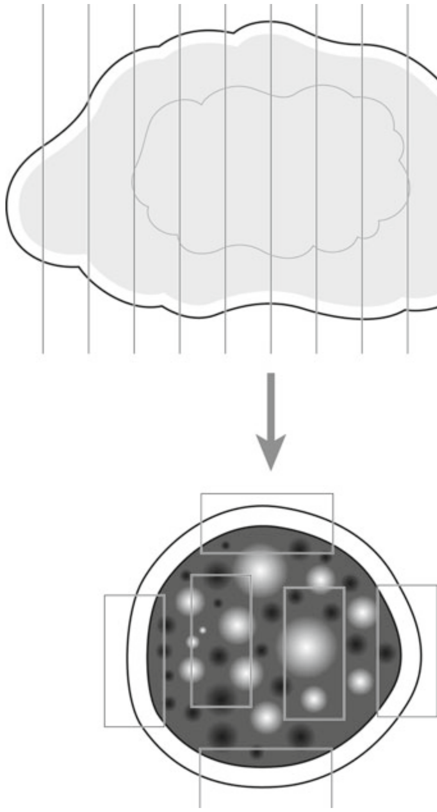

Fig. 44.4 Blocking a wide excision of a soft tissue mass (Reproduced, with permission, from Allen and Cameron (2004))

- Note the presence of attached skin ellipse and any scars present.
- Serially cut through the specimen making a series of transverse parallel cuts at 0.5-cm intervals (pan-loafing) (Fig. 44.4).
- Measurements:

  Specimen – length × width × depth (cm), weight (g)

  Tumor
  – Length × width × depth (cm) or maximum dimension (cm)
  – Distance (cm) from overlying skin
  – Distance (cm) from nearest margins

  Skin
  – Length × width (cm)
  – Length (cm) of scar

  Description:
- Tumor
  – Character of cut surface.
  – Margins (infiltrating or circumscribed).
  – Presence of necrosis, hemorrhage, cystic change, and mucinous change.
  – Ulceration of overlying skin.
  – Relationship to any major vessels or nerves.
- Other
  – Note tissues included in the specimen (muscle, fat, major vessels, bone).

Blocks for Histology:
- Limits of any major nerves or vessels included in the resection
- Representative blocks of margins
- Sufficient blocks to adequately sample the tumor including different macroscopic appearances such as cystic and necrotic areas (about one block per centimeter of greatest dimension)
- Any bone present
- Overlying skin and scars

Histopathology Report:
- Tumor type/subtype (often requires immunohistochemistry or cytogenetics)
- Tumor size
- Tumor grade – Trojani grade (I, II, III) based on tumor differentiation, necrosis, and mitoses

- Tumor edge – circumscribed or infiltrating
- Extent of local tumor spread:

pT1	Tumor less than or equal to 5 cm in greatest dimension:
	(a) Superficial
	(b) Deep
pT2	Tumor greater than 5 cm in greatest dimension:
	(a) Superficial
	(b) Deep

- Note ulceration of overlying skin.
- Lymphovascular invasion
- Excision margins – distance (cm) from nearest excision margin

44.4 Special Techniques

44.4.1 Frozen Section

This is rarely required and is dependent on close cooperation between surgeon, radiologist, and pathologist. The decision on frozen section should be at the discretion of the reporting pathologist. Moreover, frozen section of hard tissues is impracticable.

It may provide information on the adequacy of excision margins, adequacy of a biopsy, or nature of the lesion.

44.4.2 Immunohistochemistry

Immunohistochemistry can be useful in diagnosing bone and soft tissue sarcomas. In bone tumors, the decalcification process can destroy antigens in the tumor cells, and this can limit the usefulness of the technique. As with all tumors, the following general points must be emphasized:

- Antibodies are not specific to a particular type of tumor, and there is often overlap with several other types.
- Immunohistochemistry will not directly determine if the tumor is benign or malignant.
- Beware of interpretation in the presence of extensive tumor necrosis.
- Be careful of edge artifact.
- Know whether the antibodies you use should stain on the membrane, within the cytoplasm, or nucleus of the cell.
- Always use a panel of antibodies.
- The use of immunohistochemistry in bone and soft tissue sarcomas is a huge subject, and good standard textbooks of soft tissue tumor pathology should be consulted. However, the list below illustrates some diagnostically useful antibodies:

CD45 (leucocyte common antigen), CD20 (B cell), and CD3 (T cell)

- Lymphomas and chronic inflammation

Cytokeratins

- Metastatic carcinoma, synovial sarcoma, and epithelioid sarcoma

PSAP/PSA

- Metastatic prostate carcinoma

S100

- Neural, lipomatous, and cartilaginous tumors. Also metastatic melanoma (with melan-A and HMB-45)

Smooth muscle actin

- Muscle tumors and fibroblastic and myofibroblastic soft tissue lesions

Desmin and h-caldesmon

- Smooth muscle tumors

Desmin, myogenin, and myo-D1

- Rhabdomyoma and rhabdomyosarcoma

CD99 (MIC-2)

- Ewing's sarcoma (but it can be positive in many other tumors)

CD34

- Positive in vascular tumors and some fibrous lesions such as solitary fibrous tumor. Also dermatofibrosarcoma protuberans and epithelioid sarcoma

Others

- TLE 1 (synovial sarcoma), DOG 1 (GIST), and INI 1 (epithelioid vascular tumors)
- FLI 1 (Ewing's sarcoma), MDM-2 and CDK 4 (atypical lipomas/liposarcomas)
- β-Catenin (deep fibromatoses)

44.4.3 Cytogenetics

Specific chromosomal abnormalities have been identified in some bone and soft tissue sarcomas. The presence of these chromosomal abnormalities can support a particular diagnosis, help to subclassify a lesion, and may provide information associated with a poor prognosis. However, this technology is expensive, available to only a minority of laboratories, depends on successful cell culture of the tumor cells, requires the immediate dispatch of fresh tumor tissue from the operating theater to the pathology laboratory, and in most cases does not provide any additional information that cannot be obtained by histology and immunohistochemistry. Nevertheless, cytogenetics is an interesting technique, and major developments are taking place. Increasingly many of these molecular tests can be performed on paraffin-based tissue. Furthermore, there is a tendency for oncological protocols and clinical trials to require cytogenetic confirmation where relevant, e.g., Ewing's sarcoma or rhabdomyosarcoma.

44.4.4 Electron Microscopy

Electron microscopy has a very limited role in routine diagnostic practice.

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Part XI

Haemopoietic Specimens

45 Lymph Nodes, Spleen, and Bone Marrow

Lakshmi Venkatraman and Damian T. McManus

45.1 Lymph Nodes

45.1.1 Anatomy

The lymph node is an ovoid encapsulated structure situated at regular intervals along the lymphatic channels. It neutralizes, degrades, or modifies the antigens that are presented to it by the lymphatics before returning them to the blood. The immune response to antigens after birth determines the structure and composition of the node. Each lymph node has a fibrous capsule from which trabeculae extend into the parenchyma. The non-stimulated/minimally stimulated lymph node is composed of a reticulum meshwork supported by fibroblastic dendritic cells. The broad functional and anatomical divisions within a lymph node are the outer cortex and the inner medulla, which are sometimes visible on naked eye examination (Fig. 45.1). The cortex or the B zone contains pale staining, densely packed aggregates of lymphocytes called primary follicles. These are separated from each other and the sinuses by smaller lymphocytes forming a mantle of darkly staining cells—the mantle zone and yet another zone of paler cells—the marginal zone. Deep to the cortex and between follicles is the paracortex or T zone as it is composed mostly of T cells mixed with histiocytes, interdigitating reticulum cells, and Langerhan's cells. The paracortex also has the characteristic high endothelial venules that are involved in lymphocyte trafficking.

With cognate help from T cells, antigenic stimulation of the cells of the primary B follicles takes place. The primary follicular B cells become larger, acquire multiple nucleoli, divide, and die. The germinal centers of the secondary follicles thus formed contain immunoblasts, centroblasts, centrocytes, few T cells, and tingible body macrophages. The paracortical T cell response also consists of increased proliferation and transformation to blast cells. This is necessary for primary immune response by B cells. The medulla contains large numbers of plasma cells, which are the terminally mature B cells.

The afferent lymphatics enter the lymph node through the convex surface on the cortex and drain in to the subcapsular venous sinuses. The lymph is conveyed to the efferent lymphatics in the hilum by the intermediate and medullary sinuses.

Three lineages of lymphocytes are recognized in the lymph node, the B, T, and NK (natural killer) cells. The B lymphocytes express surface and cytoplasmic immunoglobulins, which mediate humoral immunity. Large quantities of immunoglobulin are produced by the plasma cells. The T cells including the helper and suppressor

L. Venkatraman (✉)
Histopathology Laboratory, Institute of Pathology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: lakshmi.venkatraman@belfasttrust.hscni.net

D.T. McManus
Histopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, UK
e-mail: damian.mcmanus@belfasttrust.hscni.net

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_45, © Springer-Verlag London 2013

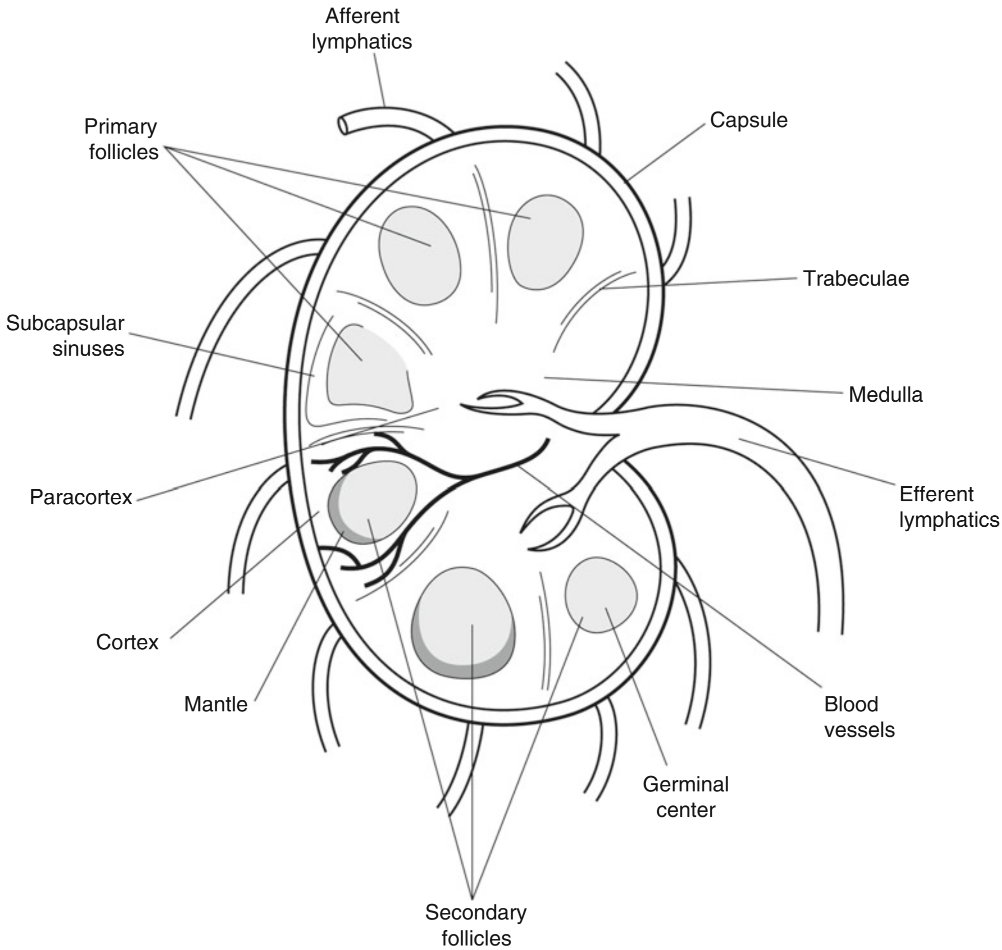

Fig. 45.1 Architecture of lymph node (Reproduced, with permission, from Allen and Cameron (2004))

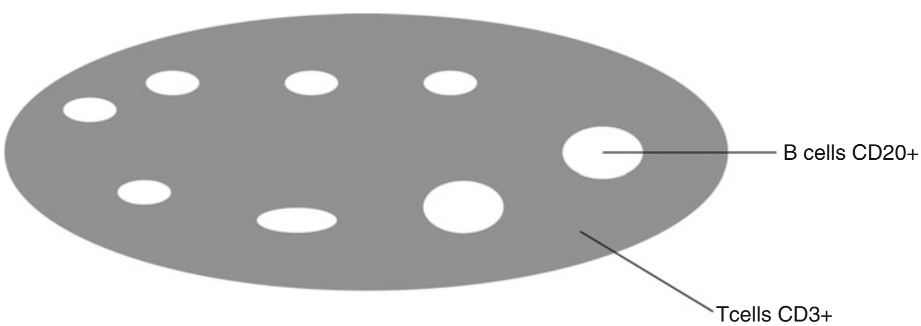

Fig. 45.2 Normal immunoarchitecture of lymph node (Reproduced, with permission, from Allen and Cameron (2004))

subsets mediate cellular immunity. The two mechanisms of immunity are interdependent.

Immunophenotyping is essential in characterization of lymphoid diseases, and it is important to be familiar with the normal immunoarchitecture of the lymph node (Fig. 45.2).

In general, the follicles stain strongly with B cell markers (CD19, CD20, CD 79a).

The interfollicular and paracortical regions express CD3 predominantly. The developmental stages and immunophenotype of B and T lymphocytes are shown in Fig. 45.3.

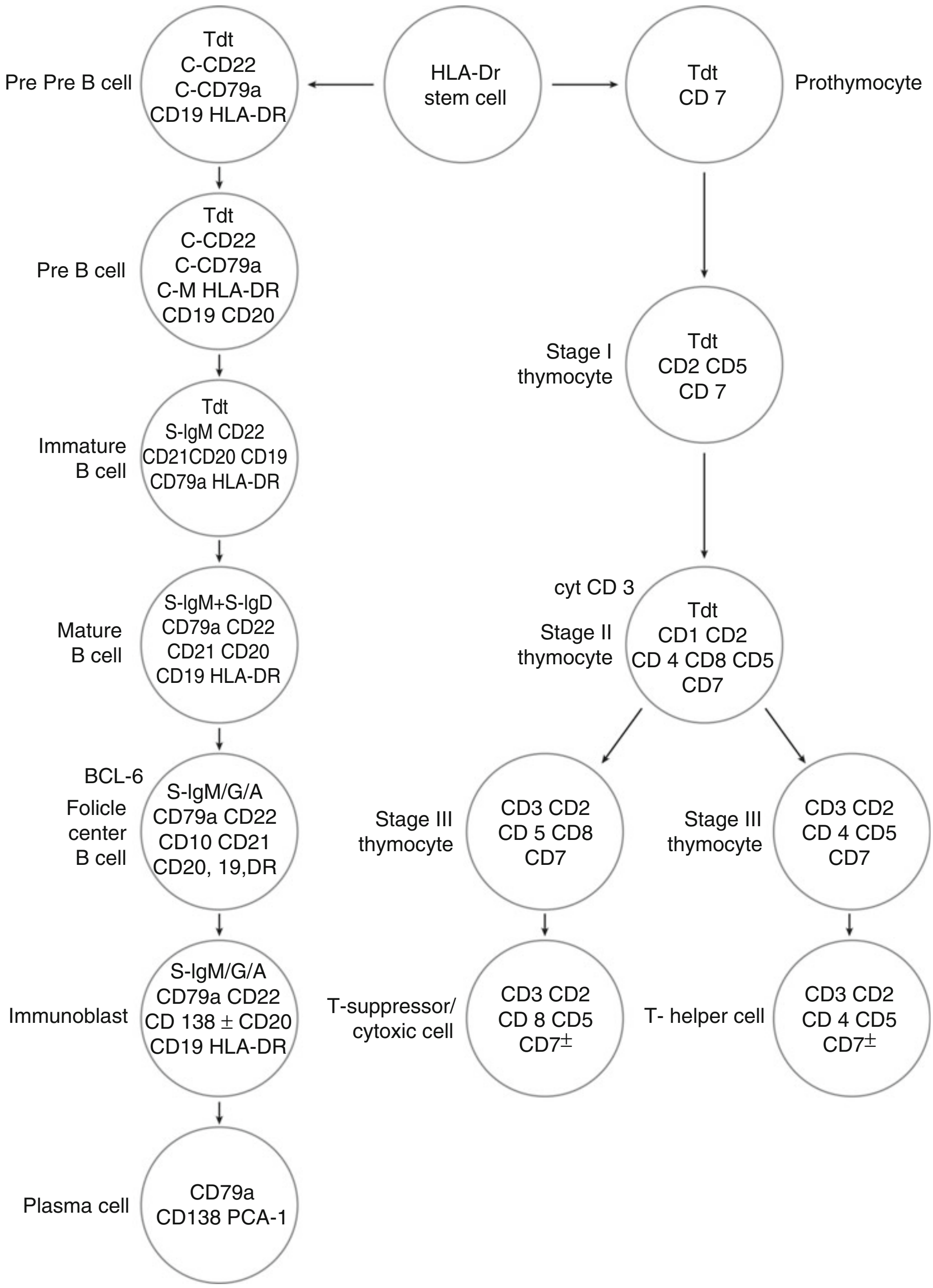

Fig. 45.3 Development of B and T lymphocytes (Reproduced, with permission, from Allen and Cameron (2004))

Lymphovascular drainage:

The artery enters the lymph node at the hilum where it divides into numerous branches. These follow the trabeculae and reach the cortex to form a capillary network. Some arterioles reach the medulla through the trabeculae. The post capillary venules draining the cortex and paracortex coalesce to form collecting veins that leave the hilum of the lymph node. The intra-nodal lymphatic flow is detailed above.

45.1.2 Clinical Presentation

Lymphadenopathy may be the presenting sign or symptom of illness or an incidental finding. Up to two-thirds of patients have non-specific causes or upper respiratory illness. Patients may present with sore throat, cough, fever, night sweats, and fatigue or weight loss. There are many diseases associated with lymphadenopathy. The major categories are listed in Table 45.1.

Pain is usually secondary to inflammation. However, rapid enlargement and pain may be present in lymphomas and leukemias. Lymphadenopathy can be localized or generalized. The site of enlargement may provide a clue to the cause. Some of the common sites of lymphadenopathy and causes of enlargement are listed in Table 45.2.

Table 45.1 Causes of lymphadenopathy

Infectious diseases
Viral: Infectious mononucleosis, hepatitis, herpes simplex, HIV, measles, varicella zoster, rubella
Bacterial: Streptococci, brucellosis, tuberculosis, other mycobacterial infection, plague, primary and secondary syphilis
Fungal: Histoplasmosis, cryptococcosis
Chlamydia: Lymphogranuloma venereum
Parasitic: Toxoplasmosis, leishmaniasis
Rickettsial: Rickettsial pox, scrub typhus
Immunologic disorders
Rheumatoid arthritis, lupus erythematosus, dermatomyositis, Sjogren's syndrome, primary biliary cirrhosis
Drug hypersensitivity: Diphenylhydantoin, hydralazine, allopurinol, gold, carbamazepine
Graft versus host disease
Malignancy
Hematological
Metastasis: From various sites
Lipid storage disorders
Nieman Pick's, Gaucher's
Endocrine diseases
Hyperthyroidism
Others
Castleman's disease, sarcoidosis, dermatopathic lymphadenitis, Kikuchi's disease, sinus histiocytosis with massive lymphadenopathy, inflammatory pseudotumor

Small size (<1 cm diameter) usually indicates a benign lymph node.

Malignant lymphoma: Large, discrete, symmetric, mobile, rubbery, non-tender lymph nodes.

Metastatic carcinoma: Hard, non-tender lymph nodes fixed to surrounding tissues.

Patients with lymphadenopathy may have splenomegaly as seen in chronic lymphocytic leukemia, lupus erythematosus, toxoplasmosis, and some hematological disorders.

Non-superficial lymphadenopathy: Thoracic or abdominal. Thoracic lymph nodes may be secondary to lung diseases and identified on routine work up chest X-ray. Other symptoms are cough and wheezing from airway compression, hoarseness from recurrent laryngeal nerve involvement, dysphagia from esophageal compression or swelling of the face due to superior vena cava compression. Abdominal/retroperitoneal lymph nodes if enlarged are usually malignant. However, tuberculosis can also cause mesenteric lymphadenopathy.

Table 45.2 Sites of lymphadenopathy and the related causes

Occipital: Scalp infection
Pre-auricular: Conjunctival infection
Neck: Oral, dental and respiratory infections, viral diseases, e.g., infectious mononucleosis
Malignant neck nodes: Drain thyroid, head and neck, breast and lung carcinomas
Scalene and supraclavicular (Virchow's nodes): Always abnormal if enlarged as these drain lung and retroperitoneum
Causes: Infection, lymphomas, or other malignancies. Tuberculosis, sarcoidosis, and toxoplasmosis are the commonest causes of non-neoplastic enlargement at this site
Virchow's node: Associated with a gastrointestinal primary. Metastasis from the lung, breast, testes, and ovaries may present as lymphadenopathy at this site
Axillary: Non-neoplastic: Trauma, infection of the ipsilateral upper extremity
Neoplastic: Metastasis from malignant melanoma, breast cancer, or lymphoma
Inguinal: Non-neoplastic: Trauma, infection of the lower extremities, or venereal diseases
Neoplastic: Metastasis from cancers of lower rectum, anal canal, genitalia, and melanoma of the lower extremities

45.1.3 Clinical Investigations

Investigations are done to find the cause suspected from the history and physical findings.

- ENT examination: Essential in the work-up of persistent cervical lymphadenopathy.
- Full blood picture (FBP): Raised WBC count in acute/ chronic leukemias and pyogenic infections.
- Serology: Raised immunoglobulin titers in EBV, CMV, HIV, toxoplasmosis, and brucellosis. ANA (anti-nuclear antibody), anti-dsDNA: raised in rheumatoid arthritis and lupus erythematosus.
- Chest X-ray: If there is an abnormality suggestive of tuberculosis, sarcoidosis, or cancer, further investigations are necessary.
- USS (ultra sound scan): Long axis (L)/short axis (S) ratio <2 has 95% specificity and sensitivity to detect metastatic disease.
- CT (computed tomography) and MRI (magnetic resonance imaging): 65–90% accurate in the diagnosis of metastatic malignancy.
- FDG-PET and PET/CT scans are the most sensitive and specific techniques in pre-treatment assessment of patients with lymphoma (and other cancers) but not routinely used for diagnosis.
- FNA (fine needle aspiration): >90% sensitivity and specificity in diagnosis of metastatic cancer and even lymphoma in some centers. It is extremely useful in selecting patients for lymph node excision biopsy and sometimes can help focus further evaluation.
- Indications for lymph node excision biopsy:
  1. To make a diagnosis in cases of unexplained, persistent lymphadenopathy.
  2. To confirm an FNA or needle core biopsy of malignant lymphoma or any clinical diagnosis when adequate information for therapy is not available from an FNA.
  3. As part of the diagnostic work-up of a systemic disease with lymphadenopathy, e.g., rheumatoid arthritis and lupus erythe matosus.
  4. Staging protocol for cancers.
  5. To monitor progress in a previously diagnosed malignant lymphoma.

45.1.4 Pathological Conditions

45.1.4.1 Non-neoplastic Conditions

These include various patterns of hyperplasia all of which show morphological and immunohistochemical preservation of the nodal architecture. The main patterns are:

Follicular hyperplasia: This is characterized by prominent hyperplastic follicles in the cortex. It is a common non-specific reaction. It is a striking feature of lymph nodes in progressive transformation of germinal centers, HIV-associated lymphadenopathy, rheumatoid arthritis, and syphilis.

Mantle zone hyperplasia: The reactive follicles have thick mantles. This is best seen in Castleman's disease and is a common pattern in reactive mesenteric lymph nodes.

Marginal zone hyperplasia: Characterized by monocytoid B lymphocyte proliferation within sinuses. It is seen in toxoplasmosis, HIV-associated lymphadenopathy, B cell-associated granulomatous diseases such as cat scratch disease, lymphogranuloma venereum, and CMV lymphadenitis.

Granulomatous inflammation: May be due to infections such as tuberculosis, brucellosis, foreign body reaction, sarcoidosis, and in response to malignancy as in lymph nodes draining carcinomas or in patients with Hodgkin's disease.

- Caseating granulomas: Seen in tuberculosis.
- Suppuration and granulomata: Seen in non-tuberculous mycobacterial infections and fungal infections due to histoplasmosis, cryptococcosis, aspergillosis, mucormycosis, and candidiasis.
- Microgranulomata within germinal centers: Seen in toxoplasmosis.

Suppurative lymphadenitis with/without granulomata: Seen in cat scratch disease, tularemia, and lymphogranuloma venereum.

Necrotizing lymphadenitis (*Kikuchi's disease*): Occurs in young women, is of unknown etiology and shows pale patches of large lymphoid cells with karyorrhectic debris, crescentic histiocytes, and plasmacytoid monocytes. It may be confused with a large cell lymphoma.

Paracortical hyperplasia: Commonly a nonspecific response but is typical of dermatopathic lymphadenitis, which occurs in generalized exfoliative dermatitis.

Immunoblastic proliferation: Characteristic of infectious mononucleosis and prominent in other viral infections, hypersensitivity reactions, post vaccinial, and Kikuchi's disease.

Sinus proliferation: Frequently present in lymph nodes draining carcinomas. Other disorders that show prominent sinus involvement include Rosai-Dorfman disease (sinus histiocytosis with massive lymphadenopathy), Langerhan's histiocytosis, Whipple's disease, and virus-associated hemophagocytic syndrome.

45.1.4.2 Neoplastic Conditions

Malignant lymphomas: Hodgkin's lymphoma and non-Hodgkin's lymphoma (NHL) are the two broad categories of malignant lymphoid neoplasms in the WHO consensus classification. The NHLs are further subclassified as neoplasms of the B, T, and NK cells. The disease entities are recognized on the basis of available information using morphology, immunophenotyping, genetics, and clinical features.

The WHO classification includes both lymphomas and leukemias since both solid and circulatory phases as well as a normal cellular counterpart in lymphoid development are recognized in many hematological neoplasms. The practical approach to diagnosis is based on morphology and immunophenotype, which correlate with clinical features and response to treatment. The clinical course is variable; both cellular characteristics and phenotype in any given lymphoma may change over time. Hence the WHO classification no longer relies on grade to stratify lymphoid neoplasms.

The prognosis strongly relates to stage of disease, treatment protocols, and biological features.

Up to 90% of the NHLs are B cell neoplasms. The clinical and pathological features of the most frequent "*indolent*" *small B cell lymphomas* are listed in the Table 45.3.

Marginal zone lymphoma: Accounts for 7–8% of lymphoid neoplasms. It is rare in the nodes but nearly always presents at extranodal sites as MALTomas, e.g., stomach, salivary gland, and thyroid.

"*Aggressive*" *diffuse large B cell lymphomas*: Aggressive and present as nodal, extranodal, localized, or disseminated disease. Distinctive clinical variants are mediastinal large B cell lymphoma, primary effusion lymphoma, and intravascular lymphoma. Molecular subgroups, i.e., germinal center and non-germinal center cell of origin predict survival and have largely replaced the morphologic variants such as centroblastic, immunoblastic, plasmablastic, T cell rich, and anaplastic. The widespread use of immunochemotherapy (R-CHOP) has dramatically improved survival.

Burkitt's lymphoma: A tumor of medium size rapidly proliferating B cells that may present as a lymphoma or acute leukemia. The major clinical subtypes include endemic, sporadic, and immunodeficiency related. The c-myc translocation is characteristic of this lymphoma but not specific for it hence the diagnosis is based on a combination of morphology, immunophenotype, and genetic analyses.

T/NK cell neoplasms: Relatively uncommon and account for approximately 12% of the lymphoid neoplasms in the West. The clinical features are important for subtyping, as the morphology, immunophenotype, and genetics are not absolutely specific. The commonest subtypes are peripheral T cell lymphoma, not otherwise specified, angioimmunoblastic T cell lymphoma, and anaplastic large cell lymphoma.

Peripheral T cell lymphoma, *NOS*: A heterogeneous group of neoplasms with a broad cytological spectrum. The tumor cells are small to medium sized with irregular nuclei and pleomorphic cells that may be Reed-Sternberg like. CD30 and T cell antigens are positive but aberrant antigen expression is common. TCR genes are rearranged, but there are no markers of monoclonality. Angioimmunoblastic lymphoma is a type of peripheral T cell lymphoma characterized by systemic disease and immunological abnormalities. In lymph nodes, it is composed of a polymorphous infiltrate of neoplastic T cells admixed with B cells that are often EBV driven,

Table 45.3 Low grade small B cell non-Hodgkin's lymphomas

	CLL	MCL	FCL	LPL
Clinical	≈6% of NHL	≈6% of NHL	≈22% of NHL	Disseminated at presentation
	Rare under 40 years	M:F ratio 5:1	Rare under 20 years	M protein, Hyperviscosity, cryoglobulinemia
	Disseminated disease at presentation	Disseminated at presentation	Disseminated at presentation	
		Biologically aggressive		
		Median survival 2–5 years		
Architecture	Pale staining pseudofollicles	Diffuse, less commonly nodular proliferation	Closely packed follicles, absent mantles, loss of polarity	Diffuse
	Background diffuse small lymphocytes			Interfollicular
Cytology	Paraimmunoblasts	Small- to medium-sized lymphocytes with slight nuclear irregularity. Scattered histiocytes and hyalinized vessels common. Blasts in blastoid variant	Centroblasts, centrocytes. May have signet ring cells	Small B lymphocytes
	Prolymphocytes. Small lymphoid cells with clotted chromatin minimally larger than mature lymphocytes			Plasmacytoid lymphocytes
IHC	CD5, CD20, CD23, CD43 positive	CD5, cyclin D1, CD43 positive	CD20, CD10, bcl2, bcl6 positive	CD20,VS38positive.CD43+/–
	CD10, cyclin D1-negative	CD10, CD23, bcl6 negative	CD5/43 negative. CD23+/–	CD5,CD10, CD23 negative
Cytogenetics	13q deletion in 50%	t (11:14)	t (14:18)	t (9:14)

CLL chronic lymphocytic leukemia, *MCL* mantle cell lymphoma, *FCL* follicle center cell lymphoma, *LPL* lymphoplasmacytic lymphoma, *IHC* immunohistochemistry a proliferation of high endothelial venules and follicular dendritic meshwork.

Anaplastic large cell lymphoma: Most frequently occurs in the first three decades of life. The tumor cells have pleomorphic horseshoe shaped nuclei, abundant cytoplasm, and are referred to as "hallmark cells". T cell antigens, EMA, cytotoxic granule proteins, ALK and CD30 are positive. 90% cases have clonal rearrangement of TCR genes. ALK expression is due to t (2:5) (q23; 35). Variant translocations involving ALK and other partner genes on chromosomes 1, 2, 3, and 17 also occur. Overall 5-year survival rate is 80% in ALK positive patients while ALK negativity is prognostically adverse.

Hodgkin's lymphoma: A lymphoma of "crippled" B cells and accounts for 30% of all lymphomas. The new WHO classification divides Hodgkin's lymphoma into two major subtypes.

- Classic Hodgkin's lymphoma (HL)
  - HL, nodular sclerosis, grades I & II
  - HL, lymphocyte-rich
  - HL, mixed cellularity
  - HL, lymphocyte depleted
- Nodular lymphocyte predominant HL (NLPHL).

The clinical and pathological features are detailed below (Table 45.4)

Plasma cell neoplasms: Include myeloma and its variants, plasmacytoma, immunoglobulin deposition diseases, osteosclerotic myeloma, and heavy chain diseases, all of which have a clonal proliferation of immunoglobulin secreting terminally differentiated B cells, i.e., plasma cells and plasmacytoid lymphocytes.

Metastatic tumors: Lymph nodes are the commonest site of tumor metastasis which may be the presenting feature. Nodal spread is common in carcinomas, malignant melanomas, and germ cell tumors, rare in mesotheliomas and uncommon in sarcomas and brain tumors.

Lymphomas mimicking carcinomas: Anaplastic large cell lymphoma, diffuse large B cell lymphoma with sclerosis, large cell lymphoma with sinusoidal growth pattern, nodular sclerosing Hodgkin's lymphoma, and signet ring lymphoma.

Metastatic carcinoma mimicking lymphoma: Nasopharyngeal/lymphoepithelial carcinoma, small cell carcinoma, and lobular carcinoma breast.

Cystic metastases in cervical lymph nodes: Commonly due to papillary thyroid carcinoma and squamous cell carcinoma.

45.1.5 Surgical Pathology Specimens: Clinical Aspects

45.1.5.1 Excision Biopsy of Lymph Node

The general principle is to remove a representative lymph node and submit it for histology with minimal tissue distortion. It may be possible to select the lymph node for excision and appropriate handling by doing a pre-operative FNA.

Some lymph node groups are always pathological, i.e., Virchow's/supraclavicular. Inguinal lymph nodes usually show non-specific lymphadenitis or scarring and are unlikely to be informative except when markedly enlarged or the patient has a previous history of malignancy.

Obtaining biopsies from deep lymph nodes is difficult, and it may not be possible to distinguish lymphadenopathy from visceral or soft tissue malignancies. In such situations, FNAs and needle core biopsies are taken under radiological guidance. Note that interpretation may be hindered by handling artifact and cell size/lymphoma grade underestimated.

45.1.6 Surgical Pathology Specimens: Laboratory Protocols

45.1.6.1 Lymph Node Biopsy

- Usually received intact and fresh soon after excision.
- After assigning a laboratory number, dissect the lymph node free from surrounding fat/connective tissue.
- Count the number of nodes and measure their size (length × width × depth—mm).
- Make parallel cuts along the transverse axis at 2–3 mm intervals with a sharp blade.
- A small portion is submitted for microbiological investigations if infectious disease is suspected

Table 45.4 Subtypes of Hodgkin's lymphomas

	Nodular sclerosis	Lymphocyte rich	Mixed cellularity	Lymphocyte depleted	NLPHL
Clinical	Most common subtype. Peripheral/mediastinal LN. Usually Stage II	Stage I/II in peripheral nodes. Rare B symptoms[a]. Mediastinal disease uncommon	High stage at presentation. B symptoms[a] common. Spleen involved in 30%. Usually peripheral node involvement	Rarest subtype. Frequently associated with HIV. Involves abdominal organs, retroperitoneal LN, and bone marrow. Presents as high stage disease	Occurs in young, often single cervical node involved. Stage I common. Frequent relapses, usually chemosensitive
Architecture	Prominent nodularity. Collagen bands at least around one nodule	Commonly nodular, rarely diffuse	Obliterated architecture. No fibrous bands	May have diffuse fibrosis	Nodular/ nodular and diffuse
Cytology	Lacunar RS cells. Grade I: >75% nodules contain few R-S cells in a lymphocyte rich, mixed cellularity or fibrohistiocytic background. Grade II: at least 25% nodules are lymphocyte depleted and have increased RS cells	Scattered R-S cells against a nodular background of small lymphocytes	Typical R-S cells against a polymorphous background of cells including eosinophils, neutrophils, histiocytes and plasma cells	Variable numbers of pleomorphic R-S cells and few lymphocytes. Can look anaplastic or fibrohistiocytic	Nodules contain darkly staining small B-lymphocytes, neoplastic "popcorn" L&H cells and rare classic R-S cells
IHC	CD30/15+, PAX5+in 90 % cases CD20−/+. EMA, ALK neg. EBVLMP1+/−	Same as nodular sclerosis	Same as nodular sclerosis. EBVLMP1+ in 75% cases	Same as nodular sclerosis. HIV+patients express EBVLMP1	CD20/CD79a/EMA/bcl6+, transcription factors Oct2/BoB1+in L&H cells. CD15/30 usually negative
Prognosis	Slightly better than mixed cellularity or lymphocyte depleted. Bulky mediastinal disease is an adverse risk factor	As good as NLPHL	Intermediate between nodular sclerosis and lymphocyte depleted but differences not observed with modern chemotherapy	Aggressive in HIV+patients but with modern chemotherapy prognosis similar to other subtypes in immunocompetent patients	Excellent prognosis in Stage I. High stage disease is rare

[a]B symptoms, e.g., weight loss, night sweats, pain
EBVLMP Epstein Barr latent membrane protein

clinically. If not submitted immediately, store at 4°C. Make smears for Gram's/Ziehl-Nielsen stain.

- Make five imprints of the cut surface on coated, alcohol-cleaned slides.
- A small portion is submitted for flow cytometry if lymphoma is in the clinical differential diagnosis and adequate tissue is available for both flow cytometry and histology.
- Submit the slices for histology. Fix in 10% formalin for 24–48 h prior to paraffin processing. Prolonged fixation can bind antigenic sites and hamper immunohistochemistry. Good quality, thin (3–4 μm) sections are required for H & E for morphology. Correlate imprint findings with histology of the slice from which it was obtained.

Imprints: Touch the glass slide gently to the cut surface of the node after ensuring the cut surface is not too wet or bloody. Avoid using force. Dry the slides in air. Heating or blow-drying is unnecessary and creates artifacts. For wet fixation, the smears are dipped in alcohol-based fixative immediately after taking imprints.

Frozen section: Place a 2 × 2 × 1 cm piece or as large a fragment as feasible on moistened filter paper in a petri dish. This is useful for intra-operative staging of cancers and for ensuring that the material submitted is diagnostic. Adequate unfrozen tissue must be available for routine histology.

Cytogenetics/flow cytometry: Place a 0.5–1 cm^3 piece of tissue in a bottle containing a culture medium such as RPMI/DMEM and send to the appropriate laboratory. Snap freeze tissue at −70°C if tissue is not immediately processed.

Immunoglobulin heavy chain and T cell receptor gene rearrangement studies can be carried out using both fresh and paraffin-processed material as determined by local protocols.

45.1.6.2 Needle Biopsy

- Count number of fragments, search the container well for all tissue
- Handle tissue gently, take care not to squeeze or transect the biopsy
- Record the length and diameter (mm) of all cores of tissue
- Note the color and any other distinctive feature
- Submit all tissue for histology, preferably in separate blocks. Cores may be painted with alcian blue prior to processing so that they are readily apparent when facing the block and vital tissue is not lost. Cut initial and deeper sections and keep the intervening ribbons pending morphological assessment and any need for immunohistochemistry.

Description:

- Size (mm) of the node.
- Capsule present/intact.
- Appearance of the cut surface and color—pink or gray in the normal nodes, variegated with distinct nodules in metastatic carcinomas, uniformly whitish with fish-flesh appearance in lymphomas. Can be black in metastatic melanomas.
- Nodularity—prominent in Hodgkin's lymphoma, sometimes follicles are prominent in follicular lymphoma.
- Hemorrhage.
- Necrosis—caseous /cheesy in tuberculosis, pale friable areas in high-grade lymphomas, also seen sometimes in Kikuchi's disease.

Blocks for histology:

- Cross section of the node including capsule.
- One to three slices submitted depending on size and whether the abnormality is focal or diffuse on gross examination.

Histopathology report:

- Indication for investigation—primary diagnosis, staging, relapse/progression, re-staging.
- Type of biopsy—excision, needle biopsy, endoscopic biopsy, bone marrow biopsy, extra-nodal resection, or other biopsy.
- Site and size of—lymph node, skin, bone marrow trephine, and other extra-nodal biopsies.
- Tumor type—lymphoma or others. If lymphoma, specify type using immunohistochemistry and cytogenetics as necessary to characterize entities included in the current WHO classification.
- Bone marrow—involved or not involved.

45.2 Spleen

45.2.1 Anatomy

The spleen is an encapsulated reticuloendothelial organ in the left upper quadrant of the abdominal cavity. Anatomically, it has two compartments—the red pulp and the white pulp with an intervening poorly defined marginal zone. The white pulp comprises T lymphocytes in the periarteriolar lymphoid sheath and B lymphocytes that form primary follicles eccentrically around this sheath. The red pulp consists of cords and a complex network of venous sinuses that contain splenic macrophages. Specialized endothelial cells known as littoral cells line the sinuses. The lining is discontinuous in order to facilitate cell traffic between cords and sinuses. The important physiological roles of the spleen are thought to be removal of abnormal and senescent RBCs, mounting an antibody response to immunogens, removal of antibody-coated bacteria and other antibody-coated particles from the blood. Hemopoiesis occurs in the fetal spleen, stops within 2 weeks after birth and may begin again when hemopiesis in the bone marrow is insufficient to meet the body's needs. Increased normal function of the spleen can cause splenomegaly.

45.2.2 Clinical Presentation

Patients may be symptomatic either as a result of splenic enlargement or the underlying disease causing it.

- Pain in the left upper quadrant of abdomen. Rupture of the spleen may be painless and yet cause intraabdominal hemorrhage, shock, and death. Severe pain due to infarction is common in children with sickle cell disease.
- Early satiety.

Palpable spleen is a major physical sign and may be due to hyperfunction, passive congestion, or infiltration by infectious disease, benign and malignant hematological disorders, metastatic carcinoma (rare), and storage diseases (Table 45.5). Rarely the cause is unknown.

45.2.3 Clinical Investigations

- Clinical methods—palpation and percussion to detect splenomegaly.
- CT scan, MRI, or USS to confirm palpable swelling of spleen and exclusion of other causes. These methods also show alteration in splenic texture.
- Cytopenias: May result from hypersplenism or hyposplenism.
- Hypersplenism: Splenomegaly, cytopenia(s), normal/hyperplastic marrow, responds to splenectomy.
- Hyposplenism: Can be caused by surgical removal, sickle cell disease, and splenic irradiation for neoplastic/autoimmune disease.
- Full blood picture: Red blood cell counts and indices, i.e., MCV, MCH, MCHC, reticulocyte index.
- WBC and platelet counts: May be normal, increased or decreased depending on underlying disorders.

45.2.4 Pathological Conditions

45.2.4.1 Non-neoplastic Conditions

Traumatic rupture of spleen and iatrogenic removal: The most frequent reasons cited for splenectomy, e.g., road traffic accident, and, splenic damage or creating access at abdominal surgery. Spontaneous rupture does occur in diseases such as infectious mononucleosis, infective endocarditis, malaria, lymphoma/leukemia, and primary non-lymphoid splenic neoplasms.

Congestive splenomegaly: Due to portal venous hypertension, commonly secondary to liver cirrhosis but may also result from portal venous thrombosis, inflammation, sclerosis, or stenosis.

Amyloidosis: Secondary involvement of the spleen results in a characteristic gross appearance, i.e. , the sago or lardaceous spleen.

Hypersplenism: Refers to a condition in which the blood cells are culled excessively within the spleen. It usually occurs when the hemopoietic cells are intrinsically abnormal—as in idiopathic thrombocytopenic purpura (ITP), congenital

Table 45.5 Causes of splenomegaly

Hyperfunction: removal of RBCs, e.g., spherocytosis, sickle cell disease, hemoglobinopathies, paroxysmal nocturnal hematuria, nutritional anemias	
Immune hyperplasia:	Viral – infectious mononucleosis, hepatitis, CMV
	Bacterial – infective endocarditis, septicemia, abscess, tuberculosis
	Fungal – histoplasmosis
	Parasitic – malaria, leishmaniasis
Disordered immune regulation: rheumatoid arthritis, lupus erythematosus, immune hemolytic anemias, immune thrombocytopenia, drug hypersensitivity, sarcoidosis	
Extramedullary hemopoiesis: chronic myeloid leukemia, myelofibrosis, marrow failure due to any cause	
Increased splenic blood flow: portal hypertension of any cause – cirrhosis, splenic vein obstruction, portal vein obstruction including due to schistosomiasis, congestive heart failure	
Infiltration: amyloid, Gaucher's, Niemann-Pick's, hyperlipidemias	
Benign and malignant infiltrations: leukemia, lymphoma, myeloproliferative disorders, metastatic carcinoma, angiosarcoma, histiocytosis X	
Unknown	

(congenital spherocytosis), and acquired hemolytic anemias (due to various leukemias, Hodgkin's lymphomas, sarcoidosis, lupus erythematosus, etc.).

45.2.4.2 Neoplastic Conditions

Benign Tumors

Hemangioma: The commonest primary splenic tumor. It may be an incidental finding and is often less than 2 cm in size. It is usually of cavernous type and associated with hemangiomas elsewhere. Rupture and bleeding are common complications.

Littoral cell angioma: A multinodular tumor that resembles splenic venous sinuses histologically.

Other benign tumors include: Hamartoma, epidermoid cysts, inflammatory mycobacterial pseudotumor, lymphangioma, and lipoma.

Malignant Tumors

Malignant lymphoma: The commonest malignant tumor involving the spleen and represents secondary spread in most cases. The gross pattern of involvement often corresponds to the microscopic types: Homogeneous in low-grade small lymphocytic lymphoma, miliary in follicular lymphoma, solitary nodules or multiple masses in large cell lymphoma and Hodgkin's lymphoma.

Primary splenic marginal zone lymphoma: Primarily involves splenic white pulp. It involves the splenic hilar lymph nodes and bone marrow frequently. Lymphoma cells often circulate in peripheral blood as villous lymphocytes. It is composed of small lymphocytes, which overrun the germinal centers in the white pulp and merge with the transformed larger cells in the peripheral marginal zone. The patients have long-term survival after splenectomy but respond poorly to chemotherapy.

Leukemia: Any leukemia can involve the spleen; however, marked splenomegaly is typical of chronic myeloid leukemia, hairy cell leukemia, and myelofibrosis. In the latter, extramedullary hemopoiesis occurs in the spleen.

Systemic mastocytosis: Almost always involves the spleen. The mast cell nodules are seen as fibrotic masses on gross examination.

Other hematolymphoid conditions involving the spleen: Hodgkin's lymphoma, particularly the nodular sclerosis subtype, Langerhan's histiocytosis and follicular dendritic reticulum cell tumor, which is always EBV associated at this site.

Non-hematological malignancies: Angiosarcoma is the commonest non-lymphoid primary splenic malignancy. It may present with spontaneous splenic rupture or mimic hemopoietic disease. Malignant fibrous histiocytoma and carcinosarcoma can also present as primary tumors at this site.

Metastatic carcinoma: Uncommon. Malignant melanoma, lung (small cell) and breast cancers are the commonest primaries to involve the spleen and can be mistaken for hemopoietic disease. Gastric and pancreatic cancers may also show direct spread to the spleen.

45.2.5 Surgical Pathology Specimens: Clinical Aspects

Splenectomy may be a diagnostic procedure. The common indications for splenectomy are: Traumatic rupture, removal of a primary lymphoma, symptom control in massive splenomegaly, e.g., CML and correction of cytopenia in hypersplenism.

Contraindication: Marrow failure, where hemopoiesis in the spleen is a source of circulating blood cells.

Immediately after splenectomy there is an increase in WBC and platelet counts, but this normalizes in 2 or 3 weeks. In the long term there are erythrocyte abnormalities including anisocytosis, poikilocytosis, Howell-Jolly bodies, and Heinz bodies. A major consequence of splenectomy is increased susceptibility to bacterial infections due to *S. pneumoniae*, *H. Influenzae*, and sepsis. This requires pneumococcal vaccination and lifelong antibiotic prophylaxis.

45.2.6 Surgical Pathology Specimens: Laboratory Protocols

45.2.6.1 Splenectomy Specimens

Usually of three types

- Incidental
- Traumatically ruptured
- Diseased spleen

The specimen is received fresh in the laboratory and fixed according to established protocols.

- Measurements:
- Length × width × depth (cm).
- Number/maximum dimensions (cm) of any capsular deficits, infarcts, cysts, or tumor nodules.
- Weight (g) must be taken before slicing, as blood loss from the cut surface reduces the splenic weight considerably.
- Photograph.
- Look for splenic lymph nodes and dissect them off the hilum.

Fixation: Make parallel thin slices 5 mm thick with a sharp knife. Examine each slice for focal lesions. Do not wash in tap water. Submit a 1 × 1 cm (fresh specimen) section for culture if infectious disease is suspected or for flow cytometry if lymphoma is queried. Make imprints for immediate assessment. Fix each slice flat in a container of 10% buffered formalin. For suspected sickle cell disease, fix in formalin immediately after slicing.

Description:

- Hilum: Nature of blood vessels, presence of lymph nodes, and accessory spleen.
- Capsule: Colour, thickness—icing in perisplenitis. If defect/laceration is present, record—location, length, depth. Record any other focal changes.
- Examine the cut surface for:
  - Colour: Infarcts are pale
  - Consistency: Soft/diffluent in sepsis, firm in portal hypertension, wax like in amyloidosis
  - White pulp: If prominent size of individual nodules
  - Fibrous bands: Present/ absent
  - Nodules/masses: Record number, size, color, presence of hemorrhage or necrosis
  - Any diffuse involvement.

Blocks for histology:

- A general recommendation is to sample any nodule larger than the adjacent white pulp.
- No abnormality in an incidental splenectomy: four blocks including samples of the superior, inferior borders, the hilum, and the lateral convex border.
- Focal gross abnormality: Two or three blocks of the abnormal area depending on size and two blocks from uninvolved parenchyma.
- Diffuse abnormality: Four blocks.

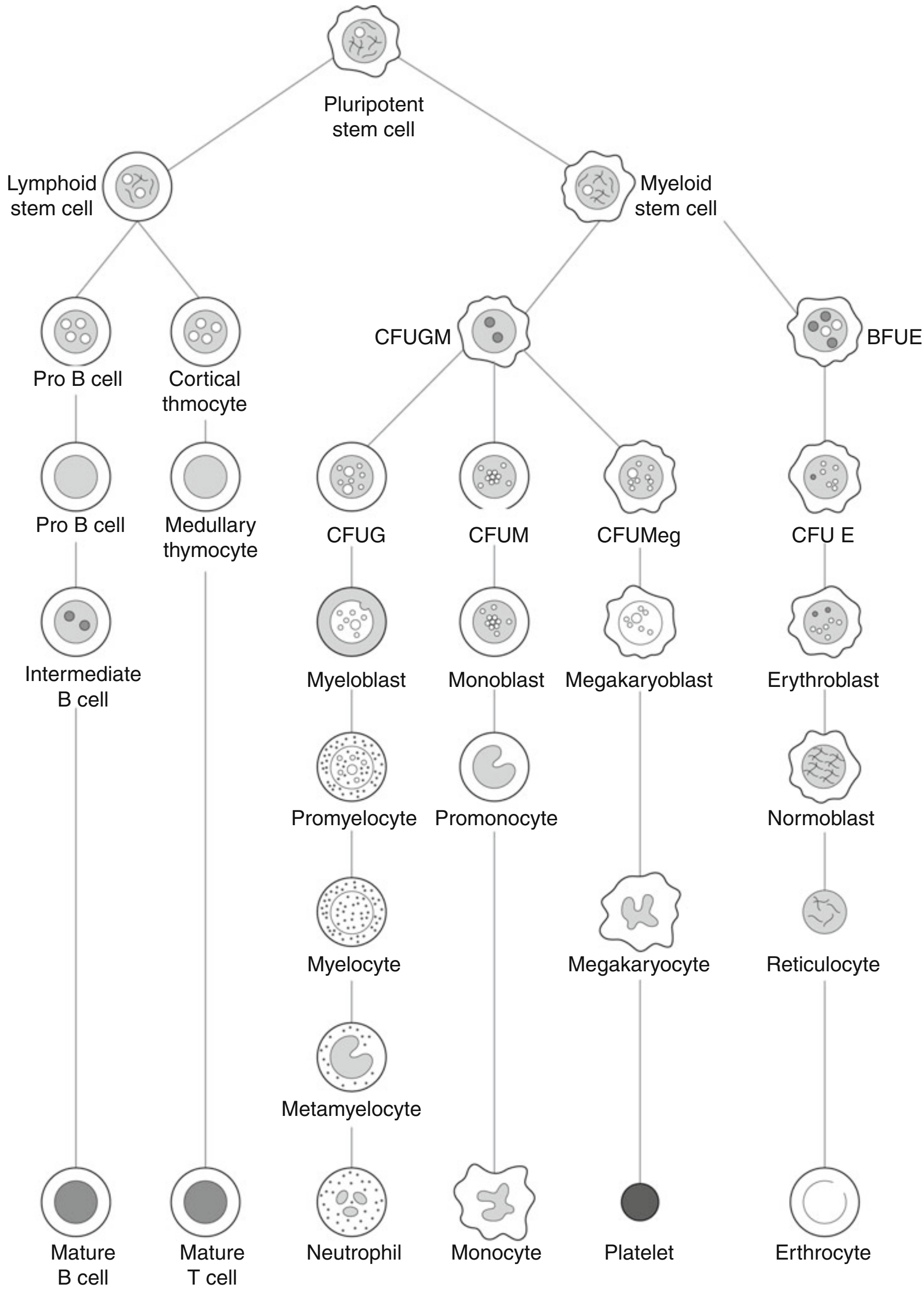

Fig. 45.4 Development and maturation of hemopoietic cells (Reproduced, with permission, from Allen and Cameron (2004))

Histopathology report:

Specify: Type of splenectomy, i.e., laparoscopic, partial, total. Mention reason for splenectomy, i.e., incidental, traumatic, or therapeutic removal of diseased spleen. For incidental and traumatic splenectomies mention the presence or absence of tears/lacerations and any other pathology related to the cause of surgery. For therapeutic splenectomies, confirm the primary diagnosis and exclude additional pathology. Classify lymphoma/leukemia in the spleen according to the current WHO classification.

45.3 Bone Marrow

45.3.1 Anatomy

The bone marrow is a specialized tissue of hematopoietic elements, supporting bony and stromal tissue with definite spatial organization. The supporting stromal tissue consists of a fine reticulin meshwork, fat and blood vessels. The amount of fat varies with age and quantity of hemopoiesis. From 50–80% fat is seen in marrows of adults and elderly patients. Little or no fat is seen in marrows of children and neonates as hemopoiesis occurs throughout the marrow in all bones. In adults, hemopoiesis is limited to certain areas within bone marrow of skull, vertebrae, sternum, ribs, pelvic bones, and proximal long bones. The row of fat cells separating hemopoietic cells from bone trabeculae is called the first fat space. This is lost in leukemic proliferations. The lamellar bone trabeculae contain osteoblasts and multinuclear osteoclasts on the endosteal surface and osteocytes within lacunae. In elderly patients with osteoporosis, the trabeculae are thinned. Thin-walled venous sinuses are seen throughout the marrow and mature hemopoietic cells have a perisinusoidal location. Small muscular arteries and capillaries are also present.

Hemopoietic elements include the myeloid, erythroid, megakaryocytic, and lymphoid cells; all of these have a common precursor stem cell. The committed stem cells give rise to the distinct cell lines. The various stages of maturation are shown in Fig. 45.4. The morphology of hemopoietic cells in general is better appreciated in Giemsa stained thin/semi-thin sections. The different types of cells and some of their features are presented in Table 45.6.

Artifacts including non-hemopoietic cells such as epidermis, skin appendages, muscle, and bone tissue may be introduced into the trephine biopsy inadvertently during the biopsy procedure. These may be confused with metastatic malignancy.

45.3.2 Clinical Presentation

Anemia: Most often detected in routine blood examination. Acute anemia due to blood loss or hemolysis presents with signs of hemodynamic instability. Severe back pain, renal failure, and hemoglobinuria occur in acute hemolysis. Chronic anemia, irrespective of cause, is associated with tiredness, shortness of breath, and tachycardia. Often the symptoms are due to the underlying cause of anemia such as rheumatoid arthritis causing joint pains.

Polycythemia: Defined as an increase in circulating red blood cells. The patients may be asymptomatic, have thrombotic symptoms or neurological symptoms such as vertigo, tinnitus, headache, and visual disturbances.

Bleeding disorders: Usually there is a history of prolonged blood loss during menstruation or following tooth extraction, childbirth, or minor surgery. Bleeding into body cavities, particularly joints, causes deformity and limited mobility.

Thrombotic disorders: Ischemia of critical organs, such as gastrointestinal tract, brain, and myocardium caused by unregulated clotting within blood vessels.

Fever, malaise, weight loss, night sweats, bleeding, fatigue, and increased susceptibility to infections are common features of white blood cell disorders.

45.3.3 Clinical Investigations

- Evaluation of anemia: Directed toward its classification as a hypoproliferative disorder (e.g., aplasia), maturation disorder (e.g., megaloblastic anemia), blood loss, or due to hemolysis such as hemoglobinopathies.
- Full blood picture, red blood cell indices, reticulocyte count, and red cell morphology aid classification. Reticulocyte index <2.5 suggests a maturation disorder or a

Table 45.6 Hemopoietic cells

	Precursors	Usual location within marrow	Numbers	Cytology	Clues to pathological proliferation
Granulocytes including neutrophils and eosinophils	Myeloblasts	Immature – paratrabecular Mature – central	2–4 times the number of erythroid cells	Immature cells – high N/C ratio and granular cytoplasm Mature – lobated nuclei and specialized granules in cytoplasm	Excess of blasts, present in both paratrabecular and central areas
Erythroid	Erythroblasts	Colonies in central intertrabecular areas	1/3–1/4 of the myeloid cells	Immature cells – dark blue cytoplasm, round nuclei, coarse chromatin, and nucleoli attached to nuclear membrane. Mature – dark, densely staining perfectly round nuclei	Paratrabecular proliferation
Megakaryocytes	Megakaryoblast	Perisinusoidal	Variable, usually at least 1/field	Largest cells. Occur singly. Multilobated nuclei with abundant cytoplasm	Clustering, paratrabecular location and nuclear hypolobation
Lymphoid	Lymphoblast	Interstitial infiltrates or <3 nodular aggregates	Up to 50% in children, 5–10% in adult marrows	Mixed population of small mature and larger lymphoid cells ± germinal centers	>3 aggregates/ diffuse heavy interstitial proliferation particularly if monoclonal
Plasma cells	Lymphoblast	Perivascular	Up to 2% in adult marrows	Mature plasma cells – eccentric nuclei with clock face chromatin, no nucleoli Immature plasma cells – nucleolated	Large clusters and nucleolated plasma cells; monoclonality
Mast cells	Myeloblast	Perivascular or paratrabecular	Few	Basophilic coarse granules in cytoplasm, round/oval nucleus	Increased numbers, spindle cells, fibrosis; aberrant phenotype

hypoproliferative disorder. High reticulocyte index is usual in hemolytic anemias.

- Iron levels and iron storage indices: Low in iron deficiency anemia.
- Serum erythropoietin levels and red cell mass: Increased in polycythemia.
- Bleeding time: A sensitive measure of platelet function.
- Platelet count: Normal ranges 150,000–450,000/μL. Decreased platelet count increases risk of bleeding from severe trauma or spontaneously—petechiae in skin or intracranial hemorrhage.
- Prothrombin time, partial thromboplastin time, thrombin time, clot lysis, clot solubility: Tests for detecting coagulation defects.
- White blood cell count and differential leukocyte counts: Valuable in diagnosing acute and chronic leukemias, infections, and inflammatory disorders such as lupus erythematosus.
- Marrow aspirate: For definitive diagnosis of hematological disorders and hematological malignancies.
- Role of the trephine biopsy: Complementary to the aspirate. Main uses are—evaluation of cellularity, particularly if there has been a dry tap due to a packed or empty marrow, spatial relationships between constituent cell types, enumeration and distribution of cells, staging of lymphomas, assessing lymphoid aggregates, staging other malignant disease, assessing fibrosis and post-chemotherapy changes (residual disease/remission/relapse).
- Other investigations are directed by clinical suspicion.

45.3.4 Pathological Conditions

Many hematological diseases can be diagnosed on microscopy of the bone marrow aspirate and the currently available ancillary investigations including flow cytometry and molecular biological techniques. The following paragraphs emphasize those conditions in which the trephine biopsy has a definite diagnostic role.

45.3.4.1 Non-neoplastic Conditions

Reactive hyperplasia: Occurs commonly in a wide range of infections, autoimmune disorders including immune thrombocytopenias, hemolytic anemia, and megaloblastic anemia, and as a non-specific response to systemic malignancy. A variation is the occurrence of reactive/benign lymphoid aggregates. These are non-paratrabecular and polyclonal.

Granulomatous inflammation: The causes and histological features are similar to granulomas that occur elsewhere in the body, e.g., tuberculosis, sarcoidosis, reaction to malignancy.

HIV and AIDS: The marrow can show specific features such as neoplasm, opportunistic infections, absent iron stores, and Parvovirus-induced red cell aplasia.

Aplastic anemia: Diagnosed when marrow cellularity is <25% of normality for the age. It may be congenital or acquired from exposure to toxins, viral infections, or drugs. There is marked reduction in erythroid, myeloid and megakaryocytic series, and increase in fat, perivascular plasma cells and lymphocytes.

The trephine biopsy is useful in diagnosis of megaloblastic anemia, but the features can be mistaken for acute leukemia hence correlation with aspirate findings is mandatory.

45.3.4.2 Neoplastic Conditions

Acute leukemias: The WHO classification has largely replaced the earlier FAB and EGIL systems. Most acute leukemias can be diagnosed as prognostically distinct entities on peripheral blood and bone marrow aspirate examination with immunophenotyping and molecular studies. The trephine is useful when marrow aspiration fails due to a dry tap. The presence of undifferentiated/poorly differentiated blast cells is diagnostic of leukemia even in hypocellular marrows where the differential diagnosis includes aplastic anemia and myelofibrosis. The trephine biopsy is helpful in assessment of overall marrow cellularity, topography, and maturation of the hemopoietic cells. Post-chemotherapy, if the disease is sensitive, the tumor cells die, and the marrow becomes hypocellular. Regeneration of stromal/fat cells occurs followed by restoration of hemopoiesis. Growth factors and chemotherapeutic regimens used can cause alarming changes in the quantity and quality of hemopoiesis. After bone marrow transplantation, the changes are similar.

Myelodysplastic syndromes (*MDS*): A group of clonal hemopoietic stem cell disorders characterized by cytopenias, dysplastic features in one or more marrow cell lineages, ineffective hemopoiesis, and an increased risk of developing acute myeloid leukemia. The WHO classification recognizes seven types: refractory cytopenia with unilineage dysplasia (RCUD), refractory anemia with ring sideroblasts (RARS), refractory cytopenia with multilineage dysplasia (RCMD), refractory anemia with excess blasts (types 1 and 2) (RAEB-1, RAEB-2), MDS-unclassified (MDS-U), and MDS associated with isolated del(5q). The diagnostically helpful features in the trephine biopsy are: ALIPs (abnormally located immature precursors), hypercellularity, trilineage dysplasia particularly striking in megakaryocytes and increased reticulin fibrosis.

Myeloproliferative neoplasms (*MPN*): Include chronic myeloid leukemia BCR-ABL positive, chronic neutrophilic leukemia, polycythemia vera, primary myelofibrosis, essential thrombocythemia, chronic eosinophilic leukemia, mastocytosis, and myeloproliferative neoplasm, unclassifiable. These are characterized by hypercellular and variably fibrotic bone marrows with effective maturation, increased leukocyte, RBC or platelet count, and a disease course that terminates in marrow failure due to fibrosis, ineffective hemopoiesis or transformation to acute leukemia. Testing for Jak2 mutation is part of the diagnostic algorithm. The WHO also recognizes a category of myeloid neoplasms that have features of both MDS and MPN, i.e., the myelodysplastic/myeloproliferative neoplasms(MDS/MPN). This includes chronic myelomonocytic leukemia (CMML), atypical chronic myeloid leukemia, BCR-ABL1 negative, juvenile myelomonocytic leukemia, and MDS/MPN unclassifiable.

Lymphoproliferative malignancies: Include acute and chronic lymphoid leukemias, special types such as hairy cell leukemia and lymphomas secondarily involving the bone marrow.

Hairy cell leukemia is a chronic B cell lymphoproliferative disorder involving the blood, bone marrow, and the spleen simultaneously. It presents with pancytopenia and splenomegaly. The hairy cytoplasmic projections are seen in peripheral blood, but the cells have characteristic haloes and cause increased reticulin fibrosis in the trephine biopsy.

Lymphomatous involvement of the marrow is common in low-grade lymphomas, i.e., follicular lymphoma, mantle cell lymphoma, well-differentiated small lymphocytic lymphoma (diagnosed arbitrarily as chronic lymphocytic leukemia when absolute lymphocyte count in the peripheral blood exceeds 5,000/μl) and some high-grade lymphomas such as peripheral T cell lymphoma and Burkitt's lymphoma. The pattern of involvement may be obvious or subtle, diffuse, focal, non-trabecular, focal paratrabecular, or interstitial. Sometimes appropriate immunostains are required to demonstrate involvement, e.g., CD30 in anaplastic large cell lymphoma and cyclin D1 in mantle cell lymphoma. The cytology and architecture may be similar to the lymph node involved or discordant as in follicular lymphoma. Classic Hodgkin's lymphoma involves marrow in approximately 5% of cases; this is common in the lymphocyte-depleted subtype and requires the same diagnostic criteria as in the lymph node for a definite diagnosis.

Metastases: Most common from breast, thyroid, prostate, lung, stomach, colon, and renal cancers in adults. In children, the most common metastatic tumors in the marrow are neuroblastoma, Ewing's sarcoma, rhabdomyosarcoma, retinoblastoma, and clear cell sarcoma of the kidney.

Myeloma: A monoclonal proliferation of plasma cells that form nodules, large aggregates, and sheets in the trephine biopsy. The diagnosis is based on a combination of clinical, morphologic features, laboratory data, and radiological findings. It is usually incurable with a median survival of 3 years and 10% survival at 10 years.

Miscellaneous: Some bony abnormalities such as osteoporosis and Paget's disease can be diagnosed in a trephine biopsy.

45.3.5 Surgical Pathology Specimens: Clinical Aspects

45.3.5.1 Trephine Biopsy

There are several reusable and disposable commercially available instruments for bone marrow trephine biopsy procedure in adult and pediatric patients. The choice is based on safety, convenience, and quality of the specimen obtained.

Smaller gauge needles are used in children and patients with severe osteoporosis.

The procedure is usually done in an operating theatre under sterile conditions. The patients are given suitable local anesthesia/analgesia and positioned properly for the procedure. The posterior superior iliac spines are the most favorable sites for aspiration. Anterior iliac crest or sternum are rarely aspirated.

The technique: The aim is to obtain a biopsy specimen 1–1.5 cm in length avoiding crushing and excess hemorrhage.

45.3.6 Surgical Pathology Specimens: Laboratory Protocols

All material should be submitted for histology. If the biopsies are obtained from multiple sites, these are submitted separately.

Make imprints prior to fixation.

Description:

- Number and length (mm) of each fragment.
- Colour, consistency, and whether homogeneous.

Procedure:

- Fixation: Routinely fixed immediately in 10% formalin or aceto-zinc formalin (AZF). Fixation time: 20–24 h. Further fixation results in loss of antigenicity and lesser fixation yields poor morphology.
- Decalcification: Should be carefully controlled and limited to softening the bone enough to permit even sectioning. Decalcification in 5–10% formic acid or Gooding and Stewart's decalcification fluid is more rapid compared to calcium chelation, which requires 24–48 h for adequate bone softening.
- Embedding: The choice between plastic resin and paraffin wax largely depends on individual laboratory preference. The morphology is better preserved in plastic embedded semi-thin sections, but many pathologists are unfamiliar with this preparation. The advantage of paraffin embedding lies in familiarity, cost effectiveness and antigen preservation that allows use of immunohistochemistry.
- Sectioning: 2–4 μm sections are ideal for good morphological detail.
- Staining: Both reticulin and H&E are essential for routine interpretation. Additional stains—Giemsa, Perl's stain for iron, Leder's stain for granulopoiesis, and Ziehl-Nielsen stain for acid fast bacilli are commonly used.

Histopathology report:

Should contain all clinical details including aspirate findings as stated on the request form, comment on technical preparation, and whether the sample is representative. A proforma helps in maintaining uniformity and serves as a checklist for all items that need to be mentioned in the report. These include: Length of the trephine, number of intertrabecular spaces, cellularity, number and distribution of erythroid, myeloid, and megakaryocytic series, lymphocytes, plasma cells, other cells including extra medullary cells if present, granulomata, reticulin, iron stores, sinusoids, and immunohistochemistry if used. The report should take into account the results of other hematological investigations, incorporate data from flow cytometry, cytogenetics, and PCR to present a unifying conclusion and/suggest further investigation for appropriate management of the patient.

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Part XII

Miscellaneous Specimens and Ancillary Techniques

46 Miscellaneous Specimens and Ancillary Techniques

Damian T. McManus

46.1 Needle Core Biopsies

Minimally invasive techniques for diagnosis and treatment are increasingly important in many fields of medicine, and this has implications for the types of specimens received by pathologists.

Biopsy needles range in caliber from 22-gauge (skinny core needle) through 18-gauge to the standard 14-gauge Tru-Cut needle. The introduction of automated spring-loaded 18-gauge core biopsy guns has been accompanied by a dramatic increase in the use of core needle biopsy of the breast, prostate, and bone or soft tissue masses. Such biopsies are increasingly performed by a radiologist or specialist clinician using ultrasound guidance. Stereotactic core biopsy is frequently used by radiologists in the investigation of breast lesions such as microcalcification detected by screening mammography.

CT-guided percutaneous biopsy is an invaluable tool in the assessment of deep-seated inaccessible tumors. It may be used to evaluate peripherally located lung lesions, anterior mediastinal masses, or retroperitoneal/mediastinal lymph node masses. Percutaneous liver or kidney biopsy are well-established techniques both in the investigation of tumors and medical conditions.

D.T. McManus
Histopathology Laboratory, Belfast City Hospital,
Belfast Health and Social Care Trust,
Belfast, UK
e-mail: damian.mcmanus@belfasttrust.hscni.net

Core biopsy may establish a specific histological diagnosis of malignancy prior to radical surgical excision/resection or enable radical or palliative radiotherapy and or chemotherapy to be administered by oncologists. It may also confirm metastatic disease, although fine needle aspiration cytology can often represent a less invasive alternative. The increasing use of sophisticated radiological imaging modalities such as PET scanning may be associated with increased use of needle core biopsy to confirm or refute metastatic disease. Core biopsy may also be used to stage lymphoma or to detect recurrent disease. Although excision biopsy of an easily accessible superficial lymph node is still recommended to make a primary diagnosis of lymphoma and for subtyping, it is increasingly acknowledged that core biopsy may be more appropriate for deeply situated lesions or in the elderly and infirm.

Transrectal ultrasound directed biopsy is commonly used in the evaluation of the prostate in patients with a raised serum PSA. Smaller caliber 18-G biopsy needles can reduce the complications associated with this procedure including infection and clot retention without compromising sensitivity and specificity. However, it can be difficult to embed multiple fine tissue cores in a single wax block, and a variety of techniques have been proposed to check that they are fully faced on sectioning including marking the biopsies with ink so that they are more easily seen.

The trend towards small-gauge needle core biopsies has been accompanied by the increasing use of immunohistochemistry and other ancillary

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3_46, © Springer-Verlag London 2013

investigations in the diagnosis and classification of cancer. The conservation of tissue for these techniques must be balanced by the need to examine an adequate number of levels to detect focal areas of involvement by cancer. These two somewhat conflicting requirements may be resolved by carefully leveling into the tissue and storing intervening sections as ribbons. These can be used subsequently for immunohistochemistry if needed, and it is also possible to select the most representative level for the appropriate stains. Such an approach is particularly relevant to prostate needle core biopsies and endoscopic biopsies from the upper gastrointestinal tract.

Concerns have been raised about tumor diagnosis using small biopsies. Clearly, tumor heterogeneity is not uncommon, and small biopsies may not be representative of a large lesion. Correlation with radiological and other clinical findings is important.

Conversely, needle core biopsies are now frequently used for ER, PR, and HER2 testing in breast cancer so that these results are available at multidisciplinarian team meetings prior to resection. The small size and uniformity of fixation can be advantageous for immunohistochemistry. It can be difficult to assess cell size and to accurately subtype malignant lymphomas in needle core biopsies; they are also particularly susceptible to compression artifact.

46.2 Fine Needle Aspirates

Fine needle aspiration cytology (FNAC) may also be used as an alternative to core biopsy. Air-dried slides may be stained immediately with a modified May-Grünwald–Giemsa stain and examined in a small side room in an outpatient setting. Slides may also be fixed in alcohol-based fixatives. This should be done immediately to avoid drying artifact. Papanicolaou and H&E stains are routinely used.

FNAC is particularly useful in the assessment of superficial palpable breast lumps and lesions around the head and neck such as thyroid nodules, salivary gland lesions, or enlarged lymph nodes. FNA may also be performed under radiological guidance and is generally less traumatic than core biopsy. However, the technique has its limitations. Diagnostic accuracy depends on adequate sampling of the lesion under investigation, good preparation, and staining of the slides and careful interpretation by an experienced cytopathologist with due consideration given to the clinical and radiological findings. It is not possible to provide a detailed review here, but in general, best results are obtained when a large volume of aspirates is examined by a limited number of pathologists. If pathologists are not actively involved in aspiration, then ideally rapid review will provide immediate feedback to the aspirator on the quantity of aspirated material and on the preparation.

It can be difficult to obtain sufficient material for extended panels of immunohistochemical markers. It is generally not possible to reliably distinguish between in situ and invasive malignancies in the breast, and often, material from areas of microcalcification detected at screening is scanty. Precise categorization of borderline lesions discovered in breast screening programs can also be problematic. FNA and core biopsy may be performed simultaneously for impalpable screen-detected breast lesions and provide complementary information.

46.3 Cytospins, Liquid-Based Cytology, and Cell Blocks

The interpretation of diagnostic cytology specimens (FNA, cell effusions, and respiratory specimens) may be greatly facilitated by the use of immunocytochemical staining techniques, described in more detail below.

A variety of techniques have been used to prepare such specimens. A cytospin can be used to make slides from cell suspensions obtained from effusions, needle washings, FNAs, or other specimens. The cells may be partially obscured by blood, or proteinaceous debris and the preparation can be improved by lysis of red cells and/or the addition of agents such as polyethylene glycol to the cell suspension. Alternative methods of processing are also available such as liquid-based cytology exemplified by the proprietary ThinPrep system in which vortexing of the cell suspension is followed by transfer to a slide by a membrane using gentle vacuum suction. This is claimed to reduce obscuring debris and to produce a representative cell sample on the slide. Cell blocks may be prepared from effusions or FNAs if a clot forms. This is transferred to formalin for fixation and processed through paraffin wax as a small biopsy, providing complementary morphological information to that of the direct smear preparations and also suitable for conventional immunohistochemistry.

46.4 Specimen Photography

An accurate macroscopic description of a gross specimen is often vital in making the correct diagnosis (e.g., the pattern of involvement in inflammatory bowel disease) and in accurately staging a malignant tumor. With the decline of the autopsy and the controversy surrounding organ retention, macroscopic specimen photographs also play an important role in undergraduate teaching and may be correlated with radiological images in today's integrated courses. Macroscopic specimen photography has also been used to audit the plane of mesorectal excision in rectal cancers, and it is an important communication medium in multidisciplinarian team meetings. Another factor that may contribute to its increasing use includes participation of biomedical scientists in specimen dissection and in the selection of tissue for histology; specimen dissection is inherently destructive, and sequential pictures can be used to record key features of the specimen.

The principles of specimen photography are described in many standard textbooks of surgical pathology and some reviews. It is important that there is a clean, textureless background that is suitably illuminated. Reflective glare and wet highlights should be avoided by switching off the room lights, correct positioning of the illumination system, and blotting of the cut surface of the specimen. The specimen should be properly centered and orientated. In general, the cut surface usually provides more information than the external aspect of a specimen. It is important to trim away fat and other extraneous tissue and to slice the tissue cleanly. It may be advantageous to open ducts, etc., to highlight these structures, but the inclusion of probes and forceps or other objects can be distracting.

The use of a stand is recommended. A 35-mm camera may be used. A smaller lens aperture (higher f-stop) will maximize the depth of field for specimens of a substantial height, and many photographers will use a number of slightly different exposures. Polaroid cameras offer a convenient method to produce specimen pictures rapidly that can then be marked as to the origin of blocks for histology. However, such pictures are often of poor quality, and it is not easy to produce 35-mm slides from the prints. Digital photography can be used to produce high-quality images relatively cheaply and hard copy rapidly. Digital images may be archived easily on CD-ROM, and software systems exist that allow the incorporation of digital images into biopsy files. While nonspecialized equipment can give good results and the use of a simple scanner has been advocated, there are integrated commercial systems available designed to facilitate digital photography at the cut-up bench with archiving of images on laboratory computer systems. While conflicting advice has been published on the use of photomicrographs without patient consent, GMC guidance on "Making and using visual and audio recordings" explicitly states that specific consent does not need to be separately obtained for the use of "images of internal organs and structures" or "images of pathology slides" for ethically legitimate purposes as long as patient confidentiality is not compromised.

46.5 Specimen Radiography

Specimen radiography may be used in a variety of different specimens for a range of purposes:

- Breast: to identify and confirm excision of small impalpable lesions detected by screening mammography or to localize areas of microcalcification in excision and core biopsies

- Bone and joint: to delineate the extent of a tumor involving bone
- Bioprosthetic heart valves: to document the degree of calcification

46.6 Frozen Section

The number of frozen sections appears to be declining in the United Kingdom due in part to improved preoperative diagnosis of breast lumps and many other tumors by FNA, needle core, or endoscopic biopsy. This is in contrast to the situation in North America where frozen sections and intraoperative consultations are very common.

The use of frozen section should be restricted to those cases where the result will change the intraoperative management of the patient.

Frozen sections are used in a wide variety of clinical situations:

- Confirmation of excised tissue, for example, parathyroidectomy versus lymph node or a thyroid nodule
- Evaluation of a suspicious lymph node and liver or lung nodule as part of an operative staging procedure or prequel to consideration of radical surgery
- Determination of a lung, pancreatic, or ampullary mass prior to proceeding to lobectomy or a Whipple's procedure
- Clearance of resection margins, for example, gastrectomy, pulmonary lobectomy, or resections for squamous cell carcinoma of the upper aerodigestive tract
- Diagnosis of suspicious abdominopelvic masses at laparotomy, for example, ovarian tumors

Specimens for frozen section are best examined using a safety cabinet. As the tissue is not fixed, full precautions must be taken against blood-borne Category Three infections. Thin fragments of tissue (no more than 2–3 mm thick and no wider than the diameter of the chuck) should be removed by a scalpel and placed on the surface of a metal chuck in a blob of embedding medium such as OCT compound (Tissue Tek) so that the tissue is covered. The chuck is rapidly cooled by standing it in a small volume of liquid nitrogen or using a proprietary aerosol spray such as CryoSpray Freezer Spray (CellPath Plc). The sections are then cut using a microtome and cryostat and stained routinely by hematoxylin and eosin.

Touch imprints can be made by gently smearing the fresh tissue against a glass slide. This is allowed to air-dry and then stained by Giemsa or proprietary stains such as Diff-Quick. This can be very useful in the evaluation of lymph nodes and many tumors providing complementary cytological detail that cannot be appreciated on frozen section. Immunocytochemistry and FISH may be performed on touch imprints of tumors made onto suitable adhesive-coated slides (e.g., APES).

Relative contraindications to frozen section include certain infections such as suspected tuberculosis or where the frozen section is unlikely to yield a clinically useful result and may compromise the final diagnosis (e.g., an impalpable breast lesion containing microcalcification picked up at screening). Some diagnoses cannot be readily made on frozen section; classical examples being the distinctions between follicular carcinoma and adenoma of the thyroid and lymph node hyperplasia and follicular lymphoma.

46.7 Ancillary Techniques

46.7.1 Immunohistochemistry and Immunofluorescence

The development of antigen retrieval techniques and the increasing range of monoclonal and polyclonal antibodies have been accompanied by widespread application of immunohistochemistry to routinely fixed, paraffin wax-embedded material. Adequate, controlled fixation is still crucial in obtaining the best results from immunohistochemistry, but there is little indication for the use of fixatives other than formalin outside a research setting.

Heat-mediated antigen retrieval techniques (HMAR) are particularly important for the detection of nuclear antigens such as ER (estrogen receptor) and Ki67, low density surface antigens, for example, CD5, and other surface antigens such as CD20. Both microwaving and pressure cooking have been used for HMAR, with citrate or EDTA buffer. HMAR avoids the risk of over-digestion and loss of morphology that may accompany pretreatments with proteolytic enzymes but can lead to loss of adherence of the section to the slide in a significant fraction of cases. This technical problem may be circumvented by the use of slides pretreated with an adhesive material such as APES.

Other technical advances in immunohistochemistry include the development of highly sensitive detection systems and the increasing use of automated immunostainers. New polymer-based detection systems (e.g., Envision, Dako) may give superior sensitivity to existing methods, but many laboratories will continue to use more established ABC techniques (e.g., Duet, Dako) which appear adequate for routine use. The use of immunohistochemistry has been reported to have risen 600% over the last few years in some laboratories, and automation is helpful in managing this rising workload. It may also offer more reproducible staining with less batch to batch variation, particularly important with stains for predictive markers such as ER or HER2 which are assessed in a semiquantitative fashion.

The use of immunohistochemistry continues to increase rapidly. It plays a crucial role in tumor diagnosis in particular and especially in the differential diagnosis of tumor types with similar morphological appearances, for example, in the diagnosis of small round blue cell tumors and soft tissue lesions and in the differentiation of malignant mesothelioma and metastatic adenocarcinoma. The accurate classification of lymphoma subtypes is crucially dependent on the use of appropriate antibody panels and interpretation of the results; indeed, the latest classifications of lymphoma fully incorporate both immunophenotyping and genotyping results. Panels of immunohistochemical stains may also be used to help diagnose anaplastic malignant tumors or to determine the likely origin of metastatic carcinoma where the primary remains occult (Table 46.1).

The increasing use of diagnostic immunohistochemistry can cause problems. Anomalous or unexpected patterns of staining may be potentially confusing or indeed misleading and result in an erroneous diagnosis, unless the pathologist is aware of such a possibility. It is generally better to use panels of markers rather than to rely on one or two isolated stains. The use of very extended antibody panels with little consideration given to a differential morphological diagnosis is likely to confuse and be very expensive. Standardized panels are easier to order and to organize on automated immunostainers and may assist in the calculation of costs. Technical pitfalls may also trap the unwary, and the results of immunohistochemistry should always be considered critically. Judicious use of positive and negative controls and correlation with morphological, clinical, and radiological findings are essential. Reagent costs tend to be particularly high with automated systems, and incorporation of control tissue onto the test slide has been suggested to contain costs while maintaining quality.

Immunohistochemistry has also been used to detect prognostic and predictive biomarkers in malignant tumors and in premalignant conditions. The use of prognostic factors is mainly a research activity with the exception of ER/PR receptors in breast cancer and proliferation indices (Ki67) in lesions such as non-Hodgkin's lymphoma, gastrointestinal stromal tumors, and hemangiopericytoma. In multivariate analysis, many new "prognostic biomarkers" do not show effects on prognosis independent of histological grade or tumor stage.

A tissue microarray consists of an array of small caliber core biopsies of tumors (or other tissues) prepared either prospectively from resected tumor specimens or retrospectively from paraffin-embedded tumor tissue. High-density arrays can have hundreds of cores on a single glass slide. This innovation represents an "industrial revolution," and although it has greatly facilitated large-scale immunohistochemical investigations, it has not seen widespread adoption in diagnostic laboratories.

Predictive biomarkers are used to predict the response of a malignancy to either conventional treatments such as chemotherapy and radiotherapy or novel targeted therapies. Examples include CD 20 and rituximab in certain B cell lymphomas, CD

Table 46.1 Immunoprofile of cancer types

System	Tumor/condition	Marker panel
Head and neck	Salivary gland tumors	Calponin, S100, SmActin, AE1/AE3
	Thyroid tumors	Thyroglobulin, calcitonin, CEA, TTF1, CK19
Gastrointestinal	Esophageal tumors	AE1/AE3, CAM5.2, CK7, CK20, CK5/6, p63
	Barrett's esophagus	Villin, Ki67, AMACR, p53
	Gastric and small bowel	CK7, CK20, CEA
	Colorectal	CK7, CK20, CEA, CDX2, β-catenin
	Hepatocellular carcinoma	α-Fetoprotein, HEPAR1, CEA polyclonal, CD10, CAM5.2
	Pancreaticobiliary carcinoma	CK7, CK20, CEA, CA19.9, CA125, DPC4
	Gastrointestinal stromal tumors	DOG1,CD117, CD34, SmActin, desmin, S100, Ki67, vimentin
	Neuroendocrine carcinoids	Chromogranin, synaptophysin, CD56, Ki67, gastrin, insulin, glucagon
Respiratory	Small cell carcinoma	CD45, CAM5.2, synaptophysin, CD56, TTF1, Ki67
	Non-small cell carcinoma	CK5/6, p63, CK7, TTF1, napsin A
	Malignant mesothelioma	Calretinin, thrombomodulin, HBME1, CK5/6, WT1, EMA, EP4, CEA
	Salivary gland type tumors	Calponin, S100, SmActin,AE1/AE3
Gynecological	Ovarian carcinoma	CK7, CK20, CEA, CA125, WT1
	Sex cord stromal	Calretinin, vimentin, AE1/AE3, inhibin, EMA
	Uterus, mesenchymal	CD10, desmin, h-caldesmon, SmActin, ER, Ki67
	Endometrial carcinoma	CK7, CK20, CD10, ER, p53
	Cervix-GGIN	BCL-2, p16, Ki67
	Cervical adenocarcinoma	ER, CEA, vimentin
Genitourinary	Renal carcinoma	CK7, EMA, vimentin, CD10, RCC Ab
	Prostate	PSA, PSAP, 34βE12, p63, AMACR
	Transitional carcinoma	34βE12 CK7,CK20, p53
	Testicular tumors	PLAP, α-fetoprotein, HCG, CAM5.2, EMA, CD30, CD117, OCT3/4, SALL4, glycipan-3
Breast	Breast carcinoma	ER, PR, HER2, p63, SmActin, CK5/6, CK14, CK8/18, E-cadherin
Soft tissue	Spindle cell sarcomas	Vimentin, CD34, SmActin, desmin, h-caldesmon, CD99, TLE1, CAM5.2, AE1/AE3, S100, Ki67
	Small round blue cell tumors	CD 45, S100, CD99, FLI1,desmin, myogenin, WT1, NB84, vimentin, CD56
Skin	Melanoma	S100, MelanA, HMB45
Hematopoietic	Lymphomas	CD45, CD20, CD3, CD5, CD10, CD 15, CD21, CD23, CD30, CD43, CD56, CD57, ALK, cyclin D1, Ki67, κ&λ, BCL-2, BCL-6, BCL-10, LMP-1, EBER, OCT2, BOB1, granzyme B, TIA1 myeloperoxidase, CD34, CD117

These panels can be adapted and modified to suit individual cases and preferences. It is not really possible to summarize this rapidly expanding and complex area with a simple table. Queries about immunohistochemical staining may be answered by logging onto www.immunoquery.com. An "immunohistochemical vade mecum" is also a useful site accessed at http://e-immunohistochemistry.info/

117 (c-kit) and STI 571 in gastrointestinal stromal tumors and HER2 and trastuzumab in breast cancer. ER/PR and HER2 are good examples of biomarkers that are both prognostic and predictive and that have clinical utility in the choice of treatments for patients with breast

cancer. The expression levels of such markers in tumors vary both within and between tumors, and both technical issues and interobserver variation may affect the validity and reproducibility of the results.

The increasingly important role of immunohistochemistry and the need for standardization of assays for predictive markers such as ER have prompted the development of external quality assurance schemes to ensure acceptable technical standards. Methods have also been developed to improve the reproducibility of scoring, the best examples being the “Histo” and “Quick Score” methods used to score ER expression in breast cancer.

Antibodies to phosphorylated epitopes of receptors and signaling molecules such as pAKT have also become available. Research suggests applicability to rapidly and uniformly fixed small biopsies such as cores or endoscopic biopsies, but uneven staining is seen in larger resection specimens. They have been used to demonstrate changes in phosphorylation (and by implication, activation) in cell lines/tumor samples following treatments with tyrosine kinase inhibitors.

Immunofluorescence continues to be used in many laboratories for the evaluation of renal biopsies and skin biopsies in conditions characterized by the deposition of immune complexes or autoantibody binding, fluorescence providing high resolution and precise localization. Immunofluorescence ideally requires frozen sections and specialized fluorescence microscopy equipment. As fluorescent preparations fade, photomicroscopy is needed to provide a permanent record, and some laboratories have abandoned this technique for conventional immunoperoxidase.

46.7.2 Flow Cytometry

Flow cytometry is a technique that allows the measurement of fluorescence intensity of large numbers of cells in suspension. Cells may be labeled using antibodies conjugated to fluorescent reporter molecules, and it is possible to accurately detect the fraction of cells in a population expressing an antigen. Two or more antigens can also be examined simultaneously. This technique has found an important diagnostic role in leukemia and lymphoma subtyping. Propidium iodide is a fluorescent dye that binds stoichiometrically to DNA. Tumor cell suspensions can be prepared from paraffin blocks, and it is possible to produce DNA histograms based on the analysis of thousands of tumor cells. This can be used to measure S phase fraction or to detect aneuploid DNA content, although they generally do not emerge as prognostic markers independent of stage and grade, and therefore, this technique is not routinely used. Detection of a triploid DNA content is of value in the differentiation between complete and partial hydatidiform mole, but this distinction can often be made on histology and is not vital clinically.

46.7.3 In Situ Hybridization Including FISH

This technique has been regarded as a research tool, but improved technologies (proprietary kits and integrated instruments for automated immunohistochemistry and in situ hybridization) are leading to clinical applications. In situ hybridization may be used to detect viral nucleic acid, examples being the detection of EBV in post-transplant lymphoproliferative disorders or HPV subtyping in cervical biopsies. In situ hybridization for κ and λ light chain mRNA may have advantages over conventional immunohistochemistry. Fluorescence in situ hybridization (FISH) may be used to detect karyotypic abnormalities in the intact interphase nucleus such as HER2 amplification in breast cancer, particularly in patients with equivocal immunohistochemistry, and n-myc amplification in neuroblastoma. It can also be used to detect translocations involving key target genes using dual color break-apart probes. The development of antibodies detecting fusion gene proteins (e.g., NPM–ALK) and the availability of PCR and RT-PCR (reverse transcriptase polymerase chain reaction)-based methods for translocation detection are complementary to such assays (Table 46.2). FISH requires access to a good

Table 46.2 Translocations in cancer types

Translocation	Tumor type	Testing methods	Clinical utility
t(11;22)(q24;q12) EWS–FLI 1 fusion t(21;22)(q12;q12) EWS–ERG fusion also t(2,7,17;22)	Ewing's sarcoma/PNET	Break-apart FISH assay for EWS target; RT-PCR for specific fusion partners	Diagnosis
t(11;22)(p13;q12) EWS–WT1 fusion	Intra-abdominal desmoplastic small round cell tumor	Break-apart FISH assay for EWS target; RT-PCR for specific fusion partners	Diagnosis
t(12;22)(q13;q12) EWS–ATF1 fusion	Clear cell sarcoma	Break-apart FISH assay for EWS target; RT-PCR for specific fusion partner	Diagnosis
t(X;18)(p11;q11) SYT–SSX1 or SYT–SSX2 fusion	Synovial sarcoma	FISH translocation assay or RT-PCR for specific fusion partners	Diagnosis? Prognosis
t(2;13)(q35;q14) PAX-3–FKHR fusion t(1;13)(p36;q14) PAX-7–FKHR fusion	Alveolar rhabdomyosarcoma	FISH translocation assay or RT-PCR for specific fusion partners	Diagnosis
t(12;16)(q13;p11) TLS–CHOP fusion t(12;22)(q13;q12) EWS–CHOP fusion	Myxoid liposarcoma	FISH translocation assay or RT-PCR for specific fusion partners	Diagnosis
t(17,22)(q21;q13) COL1A1–PDGFR β fusion	Dermatofibrosarcoma protuberans	FISH translocation assay or RT-PCR	Diagnosis/predictive of response to imatinib
t(8,14) (q24;q32) and variants C-myc translocated to Ig heavy chain and deregulated expression	Burkitt's lymphoma	Break-apart c-myc FISH assay, Southern blot of hmwDNA	Diagnosis of Burkitt's but also found in subset DLBCL where it has prognostic implications
t(14,18)(q32;q21) and variants BCL2 translocated to Ig heavy chain and deregulated expression	Follicular lymphoma	FISH, PCR BCL2 immunohistochemistry	Diagnosis of follicular lymphoma but also seen in subset DLBCL where it has prognostic implications
t(11,14)(q13;q32) and variants Cyclin D1 translocated to Ig heavy chain and deregulated expression	Mantle cell lymphoma	FISH, PCR Cyclin D1 immunohistochemistry	Diagnosis of mantle cell lymphoma
T(2,5)(p23;q35) NPM–ALK fusion	Anaplastic large cell lymphoma	FISH break-apart ALK probe RT-PCR	Diagnosis of anaplastic large cell lymphoma/inflammatory myofibroblastic tumor
TPM3 clathrin or other gene fusion targets	Inflammatory myofibroblastic tumor	Immunohistochemistry ALK	Potentially predictive of response to crizotinib
TMPRSS2–ERG or other ETS family members	Prostatic adenocarcinoma	FISH translocation assay or RT-PCR	??Prognostic

Reciprocal translocations are particularly associated with lymphomas and sarcomas but more recently have also been detected in some carcinomas as well. Translocations may result in altered/overexpression of gene products (most lymphomas, e.g., cyclinD1 or BCL2) or result in a novel chimeric fusion gene product (most sarcomas, e.g., EWS–FLI 1). Translocations can be detected by dual color interphase FISH assays to a single target gene with break-apart probes designed to span the breakpoint or by using dual target probes to detect fusion signals. Multiplex RT-PCR may be used to detect different fusion gene products and in some instances immunohistochemistry can be employed to detect increased expression (e.g., cyclin D1)/abnormal localization of gene products (e.g., ALK) with appropriate antibodies. Although such techniques are applicable to conventional formalin-fixed paraffin-embedded tissue sections, submission of fresh tissue allows preparation of touch imprints for FISH and extraction of higher molecular weight and better preserved nucleic acid. Translocations are of particular use in diagnosis as detection of such translocations can help corroborate difficult or rare diagnoses in these tumor types. Some translocations are associated with constitutive activation of tyrosine kinases (e.g., ALK) and also have a role as predictive biomarkers for novel targeted therapies.

fluorescence microscope with appropriate filter sets and a low-light CCDTV camera to capture and digitize images. It is a specialized technique only available in a small number of large centers. Routinely fixed and processed paraffin sections may be used, but the technique is equally applicable to touch imprints or similar cytological preparations.

46.7.4 Electron Microscopy

The increasing application of immunohistochemistry and pressures to contain costs have led to a decline in the use of electron microscopy, which is usually only available in large institutions. Nonetheless, EM can still be very useful in the evaluation of renal biopsies and in the differential diagnosis of pediatric small round blue cell tumors and high-grade pleomorphic sarcomas. It can also help in the differentiation of malignant mesothelioma from adenocarcinoma and can be used on cytology specimens. Although paraffin-embedded material can be processed for EM, the best results are obtained when small cubes (~1 mm^3) of fresh tissue are fixed without delay in a solution such as 3% glutaraldehyde in cacodylate buffer.

46.7.5 Cytogenetics

Metaphase cytogenetics has become an established technique in the evaluation of leukemias and other hematological conditions. Although it is not used routinely in solid tumors, the detection of specific translocations or other cytogenetic abnormalities may be useful in pediatric and soft tissue tumors. Metaphase cytogenetics requires fresh tissue which should be taken under sterile conditions. Small cubes of minced tissue are placed in tissue culture medium and sent to the cytogenetics laboratory. More detailed karyotypes can now be gleaned from metaphase spreads due to improved chromosome banding techniques and technical innovations such as chromosome painting and spectral karyotyping. These techniques have an established role in the classification of leukemias, lymphomas, and myeloproliferative disorders in large centers and may be used in solid tumors such as kidney tumors, small round blue cell tumors of childhood, and soft tissue lesions.

46.7.6 Molecular Genetics and Proteomics

Nucleic acid and protein may be extracted from fresh tissue and used as substrate for a wide range of investigative techniques usually performed as part of a research study. However, certain tumor types such as lymphomas, pediatric small round cell tumors, and soft tissue sarcomas harbor tumor-specific genetic abnormalities, usually translocations that may be of diagnostic and prognostic relevance (Table 46.2).

More recently, the use of targeted anticancer therapies has led to demand for predictive tests based on somatic mutation detection within solid tumors (Table 46.3). It is also possible to test for clonality and cell lineage in lymphomas by detecting immunoglobulin heavy chain or T cell receptor gene rearrangements. Detection of somatic hypermutation in the variable region of the immunoglobulin gene can also be used in the classification of B cell lymphomas.

Microsatellite instability (MSI) may be detected in colorectal and other carcinomas by PCR of standardized panels of microsatellite repeats. MSI may be detected in sporadic (often right-sided mucinous) tumors where it is associated with epigenetic silencing of the mismatch repair gene MLH1 promoter by methylation. In the context of a family history (revised Bethesda guidelines), loss of expression of mismatch repair genes by immunohistochemistry is highly predictive of hereditary nonpolyposis colorectal carcinoma.

Many of these assays now routinely use PCR or RT-PCR technology and can be adapted so that the impure, partially degraded DNA and mRNA that is extracted from paraffin-embedded material can serve as a template, although this should not deter prospective collection of fresh/frozen material where the opportunity exists. Even relatively small biopsies can be used for a wide range

Table 46.3 Genetic-based predictive tests in cancer types

Somatic genetic change	Cancer type	Methodology	Clinical relevance
K-ras mutations	Colon cancer	PCR/direct sequencing	Predictive: mutation positive less likely to respond to anti-EGFR treatment
B-RAF mutations	Melanoma	PCR/direct sequencing or tests for V600E mutation	Predictive of response to vemurafenib
	Thyroid carcinoma		Diagnosis?
EGFR mutations	Lung adenocarcinoma	PCR/direct sequencing	Predictive: mutation positive more likely to respond to gefitinib treatment
c-kit/PDGFRA mutations	Gastrointestinal stromal tumors	PCR/direct sequencing	Predictive: exon 11 mutations more likely to respond than exon 9 to imatinib
HER2 overexpression/amplification	Breast carcinoma, gastric carcinoma	Algorithmic IHC/FISH or CISH	Predictive: strong (+++) IHC and moderate IHC(++)/ISH positive more likely to respond to trastuzamab
EML4-ALK translocations	Lung adenocarcinoma	IHC, ALK break-apart FISH assay	Predictive of response to erlotinib
Mismatch repair gene immunohistochemistry	Colorectal carcinoma, Endometrial carcinoma	IHC	In context of family history/fulfillment of revised Bethesda criteria suggests HNPCC
MSI testing		PCR, electrophoresis	Some evidence as prognostic marker (good) and effect on response to conventional fluoropyrimidine-based adjuvant treatment
XP11 translocations	Renal cell carcinoma	FISH for translocation/TFE3 immunohistochemistry	Diagnosis of subtype of renal carcinoma

Carcinomas are often associated with more genetic complexity and heterogeneity than lymphomas and sarcomas. Fewer translocations have been detected. However, the introduction of targeted therapies has led to clinical demand for predictive biomarkers of response. While algorithmic testing by IHC and FISH has been successful in predicting response to trastuzamab, EGFR IHC has been less successful in predicting response to anti-EGFR therapy. Indeed recently, RAS mutations have emerged as a negative predictive marker for response to cetuximab therapy in colorectal carcinoma as it lies "downstream" to the EGFR in the phosphorylation cascade signaling mechanism. Activating point mutations in receptors with tyrosine kinase domains have been associated with response to novel tyrosine kinase inhibitors.

The sharp rise in demand for such predictive tests has not always been accompanied by a concomitant increase in capacity in pathology laboratories, and such assays tend to be performed in larger centers with multiprofessional input and suitable volumes. More targeted therapies (esp. tyrosine kinase inhibitors) are under development/in trials, and this area is set for significant expansion in coming years, acknowledged by initiatives such as CR UK's Stratified Medicine Programme. It is also possible that the falling costs and increased availability of next generation/massively parallel sequencing platforms will permit the development of predictive assays based on activation or disruption of signaling networks rather than individual target genes.

of investigative techniques, if handled carefully. Small biopsies can be bisected. One half can be used to make touch imprints for FISH or immunocytochemistry and further bisected for EM and snap freezing. The other half can be processed through to paraffin wax.

RNA extracted from tumors and tissues can be hybridized to cDNA or oligonucleotide arrays, techniques variously described as transcriptional profiling or molecular fingerprinting. These high-throughput techniques generate enormous quantities of data and have necessitated new bioinformatics approaches. The pattern of gene transcription, "the transcriptome," may be used to predict prognostic or behavioral differences within morphologically homogenous or indistinguishable groups. While expression arrays have been used to refine prognosis within DLBCL and breast cancer (e.g., Oncotype Dx), similar results may be obtained by the use of immunohistochemical markers for algorithmic testing (e.g., the Hans algorithm or variants in DLBCL) or the use of conventional histopathology in conjunction with established prognostic/predictive markers in breast cancer (ER, PR, and HER2).

Protein may also be extracted from tissues and analyzed by 2D PAGE, western blotting, and variants of mass spectroscopy such as SELDI. These are specialized research techniques and require fresh tissue as formalin fixation irreversibly denatures proteins.

46.8 Tissue Banking and the Use of Surplus Tissue for Research

The increasing use of minimally invasive techniques and the advent of screening programs for breast and cervical carcinoma have been accompanied by a reduction in the size and amount of tumor tissue submitted and an increased range of investigative techniques. Radiotherapy and/or neoadjuvant chemotherapy has been used in the treatment of esophageal, rectal, breast, and cervical carcinoma, and when successful, there may be very little evidence of residual tumor. Pathologists have an important role in the triage of tissue and in the selection of the most appropriate ancillary techniques to assist diagnosis. Moreover, pathologists must ensure that the removal of tissue for research projects (which may be led by basic scientists and usually involve exciting cutting-edge technologies as described above) does not compromise the diagnosis, staging, or assessment of resection margins, which remain fundamental to optimal patient care.

The controversy in the United Kingdom related to the use of retained organs and tissues removed at autopsy for research and teaching has led to a reconsideration of the ethical and legal framework surrounding the use of surplus biopsy tissue for research. Detailed guidelines are available from the MRC and the Royal College of Pathologists. Generally, prospective investigations involving the procurement of fresh tissue should have been considered and approved by an official ethics committee. Informed consent from the patient is usually needed. It is not always feasible to obtain consent retrospectively for archived tissue, and suitably anonymized studies may be permitted. Some trusts have convened tissue committees specifically to deal with the ethical and practical issues outlined above.

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Clinical Request Form Abbreviations

General

Adeno	Adenocarcinoma
AIDS	Acquired immune deficiency syndrome
B9	Benign
Bx	Biopsy
Ca	Carcinoma
CIS	Carcinoma in situ
CMV	Cytomegalovirus
C/O	Complaining of
Coronal	At right angles to the sagittal plane dividing into anterior and posterior halves
CRM	Circumferential radial margin
CT scan	Computerized tomography scan
CXR	Chest X-ray
CXT	Chemotherapy
Δ or DD	(Differential) diagnosis
DPB	Diagnostic punch biopsy
DXT	Radiotherapy
FDG	Fluorodeoxyglucose (a radioactive labeled sugar metabolized by cancer cells and used in PET scans)
FH	Family history
FNAB	Fine needle aspiration biopsy
FNA(C)	Fine needle aspiration (cytology)
FUP	Follow up
HIV	Human immunodeficiency virus
H/O	History of
HPE	Histopathology examination
Hx	History of
Ix	Investigation
L (t)	Left
MAI	Mycobacterium avium intracellulare
MDTM	Multidisciplinary team meeting
MRI	Magnetic resonance imaging
N	Normal
NAD	Nothing abnormal detected
NCB	Needle core biopsy
NG	Neoplasm, new growth
O/E	On examination
P/C	Presenting complaint
PET	Positron emission tomography
P(M)H	Past (medical) history
1°	Primary neoplasm
PUO	Pyrexia (fever) of unknown origin
?	Query
R (t)	Right
R/O	Rule out
Rx	Resection or treatment
Sagittal	Anteroposterior plane
SCF	Supraclavicular fossa
2°	Secondary neoplasm
Sx	Surgery
∴	Therefore
Tx	Treatment
U and E	Urea and electrolytes
USS	Ultrasound scan
x/7	X days
x/52	X weeks
x/12	X months
yr	Year

Gastrointestinal Specimens

AFP	Alpha fetoprotein: serum/tissue marker of hepatocellular carcinoma or germ cell tumor
AIH	Autoimmune hepatitis
AIN	Anal intraepithelial neoplasia
AIS	Autoimmune screen

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3, © Springer-Verlag London 2013

ALT	Alanine aminotransferase
AMA	Antimitochondrial antibody
ANA	Antinuclear antibody
AP	Alkaline phosphatase
APC	Adenomatous polyposis coli
AP(ER)	Abdominoperineal (excision of rectum)
AR	Anterior resection
ASMA	Anti-smooth muscle antibody
AST	Aspartate aminotransferase
AXR	Abdominal X-ray
Ba enema	Barium enema
Ba meal	Barium meal
BCS(P)	Bowel Cancer Screening (Programme)
CA19-9	Serum/tissue marker of pancreatic or upper GI malignancy
CBD	Common bile duct
CD	Crohn's disease or celiac disease
CEA	Carcinoembryonic antigen – serum/tissue marker of intestinal malignancy
(C)IBD	(Chronic) inflammatory bowel disease
CLO	Columnar lined (Barrett's) oesophagus
CMV	Cytomegalovirus
CRC	Colorectal cancer
CRM	Circumferential radial margin
D1-4	Parts of the duodenum
DALM	Dysplasia associated lesion or mass
DU	Duodenal ulcer
EATCL	Enteropathy associated (or type) T-cell lymphoma
ECL	Enterochromaffin-like cell
EGC	Early gastric cancer
ELUS	Endoluminal ultrasound
EMA	Endomysial antibody
EMR	Endoscopic mucosal resection
ERCP	Endoscopic retrograde cholangiopancreatography
ESD	Endoscopic submucosal dissection
FAP	Familial adenomatous polyposis
GAVE	Gastric antral vascular ectasia
GFD	Gluten free diet
GIST	Gastrointestinal stromal tumor
GOR(D)	Gastroesophageal reflux (disease)
GU	Gastric ulcer
Hematemesis	Blood in vomitus
HBV, HCV	Hepatitis B, C infection
HH	Hiatus hernia
HNPCC	Hereditary non-polyposis colon cancer (Lynch syndrome)
HP	*Helicobacter pylori*
HSV	Herpes simplex virus
IBS	Irritable bowel syndrome
IRA	Ileo-rectal anastomosis
KS	Kaposi's sarcoma
lap. chole	Laparoscopic cholecystectomy
LFTs	Liver function tests
MALToma	Lymphoma of Mucosa Associated Lymphoid Tissue
Melena	Altered blood passed per rectum
MRC	Magnetic resonance cholangiography
NSAIDs	Non-steroidal anti-inflammatory drugs
OGD	Oesophago-gastro-duodenoscopy
OGJ	Oesophago-gastric junction
PBC	Primary biliary cirrhosis
PD	Pancreaticoduodenectomy
PDT	Photodynamic therapy
PP	Pseudomyxoma peritonei
PR	Per rectum
PSC	Primary sclerosing cholangitis
PTC	Percutaneous transhepatic cholangiogram
PUD	Peptic ulcer disease
RFA	Radiofrequency ablation
RIF	Right iliac fossa
RUQ	Right upper quadrant
Σ or siggy	Sigmoidoscopy
SRUS	Solitary rectal ulcer syndrome
TART	Transanal resection of tumor
TEMS	Transanal endoscopic microsurgery
TME	Total mesorectal excision
TTG	Tissue transglutaminase antibody
UC	Ulcerative colitis
ZE syndrome	Zollinger-Ellison syndrome

Breast Specimens

ANC	Axillary node clearance
ANS	Axillary node sampling
BBR	Bilateral breast reduction
BCS	Breast conserving surgery
DCIS	Ductal carcinoma in situ
ER	Estrogen receptor

FNAC Fine needle aspiration cytology
HER 2 Human epidermal growth factor receptor
LCIS Lobular carcinoma in situ
LVI Lymphovascular invasion
NAC Nipple areolar complex
NCB Needle core biopsy
NPI Nottingham prognostic index
PM Partial mastectomy
PR Progesterone receptor
SNB Sentinel node biopsy
TCB Trucut biopsy
TM Total mastectomy
WLE Wide local excision

Head and Neck Specimens
AG Apical granuloma
apic. Apicectomy or apical
B (tooth) Buccal surface
C/- Upper complete denture
-/C Lower complete denture
C/C Upper and lower complete denture
D (tooth) Distal surface
DIGO Drug-induced gingival overgrowth
DIH Denture-induced hyperplasia
FE Fibrous epulis
FEP Fibroepithelial polyp
EC Ethyl chloride
EPT Electric pulp tester
FOM Floor of mouth
GP Gutta percha
K-cyst Keratocyst
L (tooth) Lingual surface
LA Lymphadenopathy
LL (+ numeral) Lower left (tooth designated by numeral)
LN Lymph node(s)
LP Lichen planus
LR Lichenoid reaction
LR (+ numeral) Lower right (tooth designated by numeral)
M (tooth) Mesial surface
MNG Multinodular goitre
MRND Modified radical neck dissection
NG Tumor
O (tooth) Occlusal surface
OKC Keratocyst
P/- Upper partial denture
-/P Lower partial denture
P/P Upper and lower partial dentures
PE (tooth) Partially erupted
PJC Porcelain jacket crown
P(S)A Pleomorphic (salivary) adenoma
Q Quadrant of jaw
RAS/RAU Recurrent aphthous ulceration
RND Radical neck dissection
RRF Retrograde root filling
RCT Root canal treatment
SCC/SCCa Squamous cell carcinoma
SS Sjögren's syndrome
TTP Tenderness to percussion
UE (tooth) Unerupted
UL (+ numeral) Upper left (tooth designated by numeral)
UR (+ numeral) Upper right (tooth designated by numeral)
WSN White sponge nevus

Tooth Nomenclature
A Deciduous central incisor
B Deciduous lateral incisor
C Deciduous canine
D Deciduous first molar
E Deciduous second molar
1 Permanent central incisor
2 Permanent lateral incisor
3 Permanent canine
4 Permanent first premolar
5 Permanent second premolar
6 Permanent first molar
7 Permanent second molar
8 Permanent third molar

Gynecological Specimens
A = x/52 Amenorrhea = x weeks
AIS Adenocarcinoma in situ
AWE Acetowhite epithelium
(α) **AFP** Alpha fetoprotein – serum marker of yolk sac tumor
BTB Breakthrough bleeding
CA125 Serum/tissue marker of ovarian malignancy

CIN	Cervical intraepithelial neoplasia
CGIN	Cervical glandular intraepithelial neoplasia
D & C	Dilatation and curettage
DUB	Dysfunctional uterine bleeding
EDC	Expected date of confinement
EIN	Endometrial intraepithelial neoplasia
EIC	Endometrial intraepithelial carcinoma
ET	Endometrial thickness
EUA	Examination under anesthesia
HCG	Human chorionic gonadotrophin – serum/tissue marker of pregnancy or trophoblastic tumor
HPV	Human papilloma virus
HRT	Hormone replacement therapy
HSV	Herpes simplex virus
IMB	Intermenstrual bleeding
LAVH	Laparoscopic assisted vaginal hysterectomy
LLETZ	Large loop excision of cervical transformation zone
LMP	Last menstrual period
LMS	Leiomyosarcoma
MMMT	Malignant mixed mesodermal tumor (carcinosarcoma)
NDMCS	No dyskaryotic or malignant cells seen
OCP	Oral contraceptive pill
PCB	Post coital bleeding
PLND	Pelvic lymph node dissection
PMB	Post menopausal bleeding
POC	Products of conception
PV	Per vagina
SIL	Squamous intraepithelial lesion
TAHBSO	Total abdominal hysterectomy with bilateral salpingo-oophorectomy
TBA	Therapeutic balloon ablation of the endometrium
TCRE	Transcervical resection of endometrium
TIC	Tubal intraepithelial carcinoma
TVS	Transvaginal ultrasound scan
UBT	Uterine balloon dilatation therapy
VAIN	Vaginal intraepithelial neoplasia
VIN	Vulval intraepithelial neoplasia

Urological Specimens

ADPKD	Adult polycystic kidney disease
AFP	Alpha fetoprotein – tissue/serum marker of germ cell tumor
AML	Angiomyolipoma (see PEComa)
ARF	Acute renal failure
BCG	Intravesical attenuated tubercle Bacille-Calmette-Guerin
BNH	Benign nodular hyperplasia
BPH	Benign prostatic hyperplasia
BOO	Bladder outlet obstruction
BTTP	British Testicular Tumour Panel
BXO	Balanitis xerotica obliterans
CAPD	Continuous ambulatory peritoneal dialysis
CIS	Carcinoma in situ
CRF	Chronic renal failure
DMSA	Dimercaptosuccinic acid – radionucleotide renal function test
DPTA	Diaminopropanoltetraacetic acid – radionucleotide renal test
DRE	Digital rectal examination
EC	Embryonal carcinoma
ELUS	Endoluminal ultrasound
EM	Electron microscopy
EPE	Extraprostatic extension
ESRD	End-stage renal disease
G1, G2, G3	WHO cytological grade I, II, III (transitional cell carcinoma)
GCT	Germ cell tumor
Hematuria	Blood in the urine
Hb	Hemoglobin
HCG	Human chorionic gonadotrophin – serum/tissue marker for germ cell tumor
HIFU	High intensity focused ultrasound
IF	Immunofluorescence
ITGCN	Intratubular germ cell neoplasia
IVC	Inferior vena cava
IVP	Intravenous pyelogram
IVU	Intravenous urogram
LDH	Lactate dehydrogenase
LHRH	Luteinizing hormone releasing hormone
LM	Light microscopy
LRP	Laparoscopic radical prostatectomy
LUTS	Lower urinary tract symptoms
MP	Muscularis propria

MSSU	Mid stream specimen of urine
MTD	Malignant teratoma differentiated
MTI	Malignant teratoma intermediate
MTU	Malignant teratoma undifferentiated
NSGCT	Non-seminomatous germ cell tumor
NSS	Nephron sparing surgery
PA(N)	Polyarteritis (nodosa)
PAP	Prostatic acid phosphatase
PEComa	Perivascular epithelioid cell tumor
PIN	Prostatic intraepithelial neoplasia
PLAP	Placental alkaline phosphatase – tissue marker of seminoma and carcinoma in situ: also CD 117, OCT 3/4
Pneumaturia	Gas in the urine usually from a gut fistula
post BCG	Following therapy with intravesical attenuated tubercle (Bacille-Calmette-Guerin)
PSA	Prostate specific antigen – tissue/serum marker of prostatic tumor
PU	Pass urine
PUJ	Pelviureteric junction
RBC	Red blood cells
RCC	Renal cell carcinoma
RPF	Retroperitoneal fibrosis
RPLND	Retroperitoneal lymph node dissection
RRP	Robotic radical prostatectomy
SV	Seminal vesicle
TCC	Transitional cell carcinoma
TRUS	Transrectal ultrasound of the prostate
TURB(T)	Transurethral resection bladder (tumor)
TURP	Transurethral resection prostate
UTI	Urinary tract infection
VUR	Vesicoureteric reflux
WHO	World Health Organization
XGP	Xanthogranulomatous pyelonephritis
YST	Yolk sac tumor

Pelvic and Retroperitoneal Specimens

ACTH	Adrenocorticotrophic hormone
MEN	Multiple endocrine neoplasia syndrome
RPF	Retroperitoneal fibrosis
RPLND	Retroperitoneal lymph node dissection

Skin Specimens

AFX	Atypical fibroxanthoma
AK	Actinic (solar) keratosis
BCC	Basal cell carcinoma
BCE	Basal cell epithelioma
BCP	Basal cell papilloma (seborrheic keratosis)
CMN	Congenital melanocytic nevus
C & C	Curettage and cautery
CBCL	Cutaneous B-cell lymphoma
CTCL	Cutaneous T-cell lymphoma
DFSP	Dermatofibrosarcoma protuberans
DH	Dermatitis herpetiformis
DLE	Discoid lupus erythematosus
DMN	Dysplastic (atypical) melanocytic nevus
DPB	Diagnostic punch biopsy
EED	Erythema elevatum diutinum
EM	Erythema multiforme
EPD	Extramammary Paget's disease
GA	Granuloma annulare
GVHD	Graft-versus-host disease
KA	Keratoacanthoma
KP	Keratosis pilaris
LE	Lupus erythematosus
LM	Lentigo maligna
LMM	Lentigo maligna melanoma
LyP	Lymphomatoid papulosis
LP	Lichen planus
LSC	Lichen simplex chronicus
LS et A	Lichen sclerosus et atrophicus
MFH	Malignant fibrous histiocytoma
MF	Mycosis fungoides
MM	Malignant melanoma
MPD	Mammary Paget's disease
MZL	Marginal zone B-cell lymphoma
NLD	Necrobiosis lipoidica diabeticorum
PLC	Pityriasis lichenoides chronica
PLE	Polymorphous light eruption
PLEVA	Pityriasis lichenoides et varioliformis acuta
PLC	Pityriasis lichenoides chronica
PRP	Pityriasis rubra pilaris
PRPPP	Pruritic urticarial papules and plaques of pregnancy
SALE	Subacute lupus erythematosus
SCC	Squamous cell carcinoma

S(eb)K	Seborrheic keratosis
SSM	Superficial spreading melanoma
SLE	Systemic lupus erythematosus
TEM	Toxic epidermal necrolysis

Cardiothoracic Specimens

AAH	Atypical adenomatous hyperplasia
ARDS	Adult respiratory distress syndrome
BAC	Bronchioloalveolar carcinoma
BAL	Bronchoalveolar lavage
BHLN	Bilateral hilar lymphadenopathy
BOOP	Bronchiolitis obliterans pneumonia
CFA	Cryptogenic fibrosing alveolitis
COPD	Chronic obstructive pulmonary disease
CS	Churg-Strauss syndrome
CVA	Cerebrovascular accident
DAD	Diffuse alveolar damage
DIP	Desquamative interstitial pneumonia
EAA	Extrinsic allergic alveolitis
EGFR	Epidermal growth factor receptor
FEV	Forced expiratory volume in one second
FVC	Forced vital capacity
Hemoptysis	Blood in sputum
IPF	Idiopathic pulmonary fibrosis
LVRS	Lung volume reduction surgery
MI	Myocardial infarction
NSCLC	Non–small cell lung cancer
NSIP	Nonspecific interstitial pneumonitis
PCP	*Pneumocystis carinii* (*jirovecii*) pneumonia
PE	Pulmonary embolus
SCLC	Small cell lung cancer
SVCO	Superior vena cava obstruction
TB	Tuberculosis
TOE	Transoesophageal echocardiography
TTNA	Transthoracic needle aspiration
TTNB	Transthoracic needle biopsy
VATS	Video-assisted thoracoscopic surgery
V/Q Scan	Ventilation/perfusion scan
WG	Wegener's granulomatosis

Osteoarticular and Soft Tissue Specimens

ABC	Aneurysmal bone cyst
AKA	Above knee amputation
AS	Ankylosing spondylitis
FD	Fibrous dysplasia
GCT	Giant cell tumor
LCH	Langerhan's cell histiocytosis
MFH	Malignant fibrous histiocytoma
NOF	Neck of femur
OA	Osteoarthritis
PSC	Primary synovial chondromatosis
PVNS	Pigmented villonodular synovitis
RA	Rheumatoid arthritis
THR	Total hip replacement
TKR	Total knee replacement
#	Fracture

Hemopoietic Specimens

AL	Acute leukemia
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
ALCL	Anaplastic large cell lymphoma
BM(T)	Bone marrow trephine
BMTx	Bone marrow transplant
BT	Bleeding time
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
CT	Clotting time
DC	Differential count
DLBCL	Diffuse large B-cell lymphoma
(D)WCC	(Differential) white cell count
ESR	Erythrocyte sedimentation rate
ET	Essential thrombocythemia
FBP	Full blood picture or count (FBC)
HCL	Hairy cell leukemia
HD/HL	Hodgkin's disease/lymphoma
ITP	Idiopathic thrombocytopenic purpura
IVL	Intravascular lymphoma
LD(HD/HL)	Lymphocyte depleted (Hodgkin's disease/lymphoma)
MC(HD/HL)	Mixed cellularity (Hodgkin's disease/lymphoma)
MCTD	Mixed connective tissue disorder
MDS	Myelodysplastic syndrome
MF	Mycosis fungoides

MM Multiple myeloma
NHL Non-Hodgkin's lymphoma
NLPHL Nodular lymphocyte predominant Hodgkin's lymphoma
NS(HD/HL) Nodular sclerosis (Hodgkin's disease/lymphoma)
PV Polycythemia vera
PT Prothrombin time
RA Rheumatoid arthritis
SLE Systemic lupus erythematosus
TCRBCL T-cell rich B-cell lymphoma
TT Thrombin time
WCC White cell count

Resection Specimen Blocking Summary

Gastrointestinal Resection Specimen Blocking

	Blocks
Cancer	
Primary tumor (to serosa/mesentery)	4
Longitudinal limit (if <3 cm)	1
Circumferential limit (if <1 cm)	1
All lymph nodes	*N*
Anastomotic ring(s)	1 each
Other(s) e.g. polyp(s), appendix	*N*
Ischemia	
Longitudinal limits	1 each
Abnormal/normal bowel	2
Mesentery	1
Lymph node sample	1 cassette
Chronic inflammatory bowel disease	
Longitudinal limits	1 each
Sequential samples every 10–20 cm	*N*
Lymph node sample	2 cassettes
Other(s) e.g. polyp(s)/ulcer/stricture/fistula/abscess/appendix	*N*
Diverticular disease	
Transverse sections	2
Others(s) e.g. polyp(s)/abscess/fistula	*N*
Lymph node sample	1 cassette
Volvulus	
Longitudinal limits	1 each
Bowel	2

N variable number of blocks/cassettes

Gynecological Resection Specimen Blocking

	Blocks
Vulvectomy for vin/cancer/soft tissue lesion	
Wide local excision, simple/radical vulvectomy	
Block to lateral cutaneous/medial mucosal/deep soft tissue margins	
Primary lesion	4
Other(s) e.g. ipsi-/contralateral lesion (s)	*N*
Vulva e.g. lichen sclerosis	
Ipsilateral	1
Contralateral	2
All lymph nodes	*N*
Trachelectomy for cervical cancer	
Vaginal cuff limit	2
Proximal transverse limit	2
Trachelectomy	4–6 approx.
Other(s) e.g. vaginal lesion	*N*
All lymph nodes	*N*
Radical hysterectomy for cervical cancer	
Vaginal cuff limit	2
Cervix	4–6 approx.
Paracervix/parametria	1 each R/L side
Endometrium and body/lower uterine segment	2 each
Tubes and ovaries	1 each
Other(s) e.g. vaginal lesion, omentum (2)	*N*
All lymph nodes	*N*
Hysterectomy for uterine cancer/sarcoma	
Vaginal cuff limit	2

D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3, © Springer-Verlag London 2013

	Blocks
Primary tumor (to myometrium/serosa)	4
Endometrium and body	2
Endocervix (endometrial cancer)	2
Cervix	2
Tubes and ovaries	1 each
Other(s) e.g. omentum (2), parametrium (2)	*N*
All lymph nodes	*N*
Hysterectomy	
Endometrium and body	2
Cervix (anterior/posterior)	2
Previous CIN	4 minimum
Fibroids	
Usual/multiple	1–2
Unusual appearance	3–4
Adenomyosis	1
Serosal adhesions	1
Polyp – tip/body, and, base	2
Previous hyperplasia endometrium	2–4
Ovary	
Normal and tube	1 each
BRCA gene and tube	All – *N*
Inflammatory	3
Simple cyst	2–3
Multiloculated cyst (complex/warty/solid areas)	1/cm dia up to 10 cm, or 2/cm dia if >10 cm
Mixed solid/cystic	As above
Solid (to capsule)	As above
Omentum	3–4
Other(s), tube(s), contralateral ovary, endometrium and body, anterior cervix, posterior cervix	1 each

CIN cervical intraepithelial neoplasia

Urology Resection Specimen Blocking

	Blocks
Nephrectomy for cancer	
Primary tumor: to capsule/renal pelvis and sinus/perinephric fat and margin/adrenal gland/parenchymal limit (partial nephrectomy)/Gerota's fascia	4 minimum or 1/cm dia
Other(s) e.g. white/gray areas, satellite nodule(s)	*N*
Renal sinus	2
Kidney and adrenal gland	1 each
Renal vein (RCC)	1
Ureter (TCC)	
Limit	1
Other pelviureteric lesion(s)	*N*
All lymph nodes	*N*
Nephrectomy for non-cancer	
Kidney/renal pelvis	3
Ureter	1
Cystectomy/cystoprostatecomy for cancer	
Primary tumor (to wall and perivesical fat)	4
Other(s) e.g. grossly abnormal areas	*N*
Distal urethral limit	1
Circumferential limit (if <1 cm)	1
Ureteric limit(s) or separately labeled specimens	1 each
Bladder	2
Prostate	2 minimum each R/L lobe
All lymph nodes	*N*
Cystectomy/cystoprostatectomy for non-cancer	
Bladder	3
Prostate	2 minimum each R/L lobes
Urethral/ureteric limits	1 each
Radical prostatectomy	
Proximal bladder limit	1 cassette
Distal urethral limit	1 cassette
Prostate and seminal vesicles	*N*
All lymph nodes	*N*
Testis for cancer	
Primary tumor (to testis/tunica/rete)	1/cm dia – *N*
Other(s) e.g. solid/hemorrhagic/necrotic areas, satellite lesion(s)	*N*
Testis	1–2
Epididymis (if grossly involved)	1–2
Spermatic cord proximal limit	1
Testis for non-cancer	
Testis ± lesion (atrophy/infarct/abscess)	2
Hydrocoele	1
Spermatic cord	1
Penectomy for cancer	
Primary tumor (to corpora/urethra)	4
Other(s) e.g. satellite nodule in penile skin	*N*
Proximal resection limit	1–2
All lymph nodes	*N*

TCC transitional cell carcinoma, *RCC* renal cell carcinoma

Breast Resection Specimen Blocking

	Blocks
Mastectomy for cancer	
Primary tumor (to nearest margin(s))	3
Breast	1
Nipple	1
Skin (only block if involved)	1
Others(s) e.g. DCIS, satellite lesion(s)	*N*
All lymph nodes	*N*
Cavity shave(s) (sample fibrous tissue/partial mastectomy)	1–2 (more if the related specimen margin is involved)
Completion mastectomy (previous cancer)	
Macroscopically identified tumor	2
No tumor – cavity walls	4 minimum
Breast	2
Nipple	1
All lymph nodes	*N* cassettes
Fibroadenoma/phyllodes tumor	
1 block/cm dia (to nearest margin(s))	*N*
Breast reduction	
Representative blocks	4
Other(s) e.g. discrete lesion	*N*
Localization for non-palpable lesion/calcification	
Macroscopic lesion (to nearest margin(s))	3
No macroscopic lesion – representative blocks (sample fibrous tissue)	6 approx. ± EBs, correlate with specimen/slice radiography
Cavity shave(s) (sample fibrous tissue)	1–2 each

EBs extra blocks

Lung Resection Specimen Blocking

Cancer/abscess	Blocks
Proximal bronchial limit	1
Mass lesion (to bronchial limit and/or pleura)	3
Lung	1
All lymph nodes	*N*
Others(s) e.g. satellite lesion/consolidation	*N*

Thyroid Resection Specimen Blocking

Multinodular colloid goiter	4 maximum
Thyroiditis e.g. Hashimotos	3 each lobe maximum
Cyst/solitary nodule/tumor (to capsule/margins(s))	All up to 5 cm dia, or, 1/cm dia – *N*
Other(s) e.g. satellite lesion(s), parathyroid(s)	*N*
All lymph nodes	*N*

Salivary Gland Resection Specimen Blocking

Sialadenitis	3
Cyst/tumor (to capsule/margin(s))	1/cm dia – *N*
Other(s) e.g. satellite lesion(s), lymph node, facial nerve	*N*
All lymph nodes	*N*

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D.C. Allen, R.I. Cameron (eds.), *Histopathology Specimens*,
DOI 10.1007/978-0-85729-673-3, © Springer-Verlag London 2013

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